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1

POSSIBLE FUNCTIONS OF CHANNEL SUBUNIT FAMILIES

B. Sakmann

Functional characterization of membrane channels by patch clamp techniques has revealed great diversity of transmitter or voltage gated channels in native membranes. Concomitantly recombinant DNA techniques revealed a plethora of genes encoding channel subunits. Thus functional diversity within a particular class of channels may be generated by families of genes encoding homologous channel subunits that assemble in various combinations into functionally distinct channel subtypes. For most channels the subunit composition and stoichiometry of a particular functional subtype is not yet established except for the nicotinic acetylcholine receptor of *Torpedo*. One way to identify the subunit composition of channels in native membranes is to compare their functional properties with those of channels expressed in a host membrane, following introduction of subunit coding nucleic acids (cRNA or cDNA) into a host cell. In the case of ligand-gated channels, such as channels gated by acetylcholine (AChR channels) or γ-amino butyric acid (GABAR channels) and voltage gated K^+ channels mediating delayed or transient outward currents (RCK channels) it has been shown that particular functions of the channel can be attributed to particular channel subunits. Examples are the γ- and ε-subunits of skeletal muscle AChR channels or the β- and γ-subunits of GABAR channels, which specify channel subtypes with different pharmacological, kinetic and conductance properties. In the case of voltage gated K^+ channels single RCK subunits specify functionally diverse homomultimeric K^+ channels, which mediate transient and delayed K^+ currents. However, heteromultimeric channels with novel properties can also assemble from different RCK subunits. The constituent RCK subunits specify sensitivity to K^+ channel blockers, gating and conductance properties.

A clear correlation between particular channel phenotypes in the native membrane, their subunit composition and gene regulation of the respective mRNAs has been established for the nicotinic AChR channel in skeletal muscle. Here a developmental switch in the expression of the γ- and ε-subunit genes causes a change in end-plate channel properties from the fetal type, composed of αβγδ-subunits to the adult type subtype composed of αβδε-subunits. Northern blot and *in situ* hybridisation analysis of AChR subunit specific mRNAs in fetal, adult and denervated skeletal muscle indicate that the expression of subunit specific mRNAs is regulated by multiple transcriptional mechanisms. First, in a mechanism which is restricted to the end-plate, subsynaptic nuclei become "imprinted" early during synaptogenesis to express subunit specific mRNAs. The expression then remains independent of nervous or muscular signals. Second, a more generalized mechanism operates on extrasynaptic nuclei and is dependent on the electrical activity of muscle fibres. Each AChR subunit gene is under multiple transcriptional controls each having different importance for each subunit.

The functional diversity of channels may allow control of gene expression by multiple transcription mechanisms. A switch in the expression of genes encoding particular subunits can occur in response to external stimuli causing a change in the channel phenotypes. This may be required for longterm adaptive changes in synaptic efficacy and in electrical excitability of neurones during development or differentiation.

Max-Planck-Institut für medizinische Forschung, Abteilung Zellphysiologie, Jahnstr. 29, D-6900 Heidelberg

2

ROLE OF NITRIC OXIDE (NO) AND ADENOSINE IN THE REGULATION OF CORONARY BLOOD FLOW.

J. Schrader

The oxygen supply via the coronary circulation normally exactly matches the oxygen requirements of the heart so that cardiac energy usage relative to the delivery of oxygen is in equilibrium. Thus, metabolic regulation of coronary blood flow in essence is maintaining cardiac energy metabolism balanced (14). This concept was recently broadened by the demonstration that vasoconstrictory and vasodilatory factors are continuously formed by vascular resistance vessels (1). To what extent the endothelium-derived factors are under metabolic control of the cardiomyocytes is presently not known.

Criteria for the envolvement of any metabolite in the control of coronary flow are 1. Qantitative studies should indicate that the amounts and kinetics of transmitter release is appropriate to give the indicated effect. 2.Exogenously applied transmitter should mimic the physiological response. 2.Rapid mechanism of transmitter inactivation should be present 3.Inhibitors of transmitter action should have effects consistent with the hypothesis.

Palmer et al. (1987) provided the first evidence for a parallelism between the release of endothelium-derived relaxing factor (EDRF) and nitric oxide (15). Using a specific difference-spectrophotometric assay based on the rapid oxidation of oxyhemoglobin to methemoglobin we have measured the formation and release of NO into the coronary effluent perfusate of isolated guinea pig hearts (6-8). Authentic NO applied into the coronary circulation dose-dependently decreased vascular resistance and enhanced coronary release of cGMP. During single passage through the intact coronary system 86% of the infused NO was converted to nitrite ions. Increasing oxygen tension in aqueous solution from 150 to 700 mmHg decreased half life of NO (5.6s) by 32%. During passage of NO through the coronary circulation half life was as short as 130 ms. Isolated hearts constantly release NO and cGMP at a rate of 161 pmoles/min and 342 fmoles/min, respectively. The NO-scavenger oxyhemoglobin and methylene blue increased coronary resistance and decreased cGMP release. L-arginine, a putative precursor of NO (16), slightly increased coronary resistance while release of cGMP was enhanced. L-N^Gmonomethylarginine (10^{-4}M) reduced basal release of NO and cGMP and this was associated with coronary vasoconstriction. Onset of NO release in bradykinin stimulated hearts preceded onset of coronary vasodilation in all cases. Amounts of bradykinin-induced NO release were within the dose-response curve for exogenously infused NO. Similar findings were obtained with acetylcholine, ATP and serotonine. Collectively these findings suggest that basal formation of NO is likely to play an important role in setting the resting tone of coronary resistance vessels. Nitric oxide appears to fulfill all of the above mentioned criteria for a chemical transmitter for the modulation of coronary vascular tone.

Adenosine is another potent coronary vasodilator the physiology and biochemistry of which has been intensively studied over the past twenty years (5) whithout finally defining its physiological role. The reasons for the remaining uncertainties reside in the fact that 1. the transmethylation pathway has been identified as an additional important intracellular route for the adenosine formation in the well oxygenated heart (4, 11). 2. The coronary endothelium constitutes a metabolic barrier for intracoronarily applied adenosine so that the true sensitivity of the coronary vascular smooth muscle might be higher than dose-response curves suggest (10, 13). 3. The coronary endothelium itself is capable of producing adenosine (9). 4. A substantial fraction of the released adenosine is derived from extracellular degradation of adenine nucleotides primarely liberated from isolated hearts and cultured endothelial cells (18) 5. The turnover of plasma adenosine is extremely short (less than 1 s in humans) which precludes any precise quantification of adenosine formation from arteriovenous differences (12).

Assessment of the physiological role of adenosine is further hindered by imprecise knowledge of the interstitial concentration of adenosine, to which vascular smooth muscle cells are exposed. Most of the cardiac adenosine is protein bound (S-adenosylhomocysteinehydrolase (SAH)) and small changes in the fraction of free adenosine may go undetected by conventional tissue extraction procedures. A new approach was recently developed in our laboratory (2, 3) to estimate the free intracellular adenosine concentration that makes use of the kinetic properties of SAH-hydrolase. Since the equilibrium constant of SAH-hydrolase favors synthesis, SAH formation prevails in the presence of increased levels of homocysteine. Enzyme kinetics predict that with saturating concentrations of l-homocysteine the rate of SAH formation is directly proportional to the free intracellular adenosine concentration. Using the principles of the SAH-method and C-11-homocysteine, a positron emitting isotope, cardiac adenosine formation was also assessed with PET. In the dog model, cardiac ischemia and coronary stenosis resulted in the local accumulation of C-11-SAH. Thus, an enhanced regional formation of adenosine can now be measured noninvasively. During pressure autoregulation no additional adenosine is formed by the heart. Only when the autoregulatory reserve is exhausted does the adenosine formation steeply increase. These and additional findings argue for the hypothesis that tissue oxygenation and not energy turnover is the most important stimulus which finally determines cardiac adenosine formation (17). Coronary blood flow must be regulated accordingly, if adenosine is the mediator.

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Physiologisches Institut, Heinrich-Heine-Universität Düsseldorf, Moorenstraße 5, 4000 Düsseldorf 1

3

THE MOLECULAR MECHANISM OF MUSCLE CONTRACTION. CONTROVERSIAL ISSUES AND RECENT IDEAS ABOUT CROSS-BRIDGE ACTION IN MUSCLE

B. Brenner

It is now generally accepted that muscle contraction occurs when the actin and myosin filaments slide past each other. According to the cross-bridge theory, filament sliding, or force generation under isometric conditions, are driven by domains of the myosin molecules, the cross-bridges. In the hypotheses developed up to the mid seventies (e.g. A.F. Huxley, Prog. Biophys. 7, 1957; H.E. Huxley, Science 164, 1969; A.F. Huxley & Simmons, Nature 233, 1971; Lymn & Taylor, Biochem. 10, 1971; A.F. Huxley, J. Physiol. 243, 1974), cross-bridges were considered to act as "independent force generators" which cyclically interact with actin as ATP is hydrolysed. This cyclic interaction was considered to represent some oar-like mechanism involving several key features:
(i) A coordinated cycle of attachment, structural change of the attached cross-bridge (leading to force generation or filament sliding), detachment, and finally a structural change (reverse of the structural change of the attached cross-bridge) which returns the detached cross-bridge to the starting point of the cycle. (ii) With each attachment/detachment cycle one molecule of ATP was assumed to be hydrolysed (1:1 coupling of attachment/detachment to ATP-hydrolysis). (iii) The structural change of the attached cross-bridge was thought to induce filament sliding of some 10nm (1:1 coupling of 10nm of filament sliding to ATP-hydrolysis). (iv) Release of hydrolysis products was assumed to induce the structural change of the attached cross-bridge, while the actual hydrolysis step was thought to induce the large scale structural change returning the detached cross-bridge to its starting point in the cycle. The actual hydrolysis step should only occur while cross-bridges are detached from actin, release of products only while cross-bridges are attached to actin. (v) Regulation of muscle contraction was postulated to occur via control of the number of cycling cross-bridges (recruitment; Podolsky and Teicholz, J. Physiol. 211, 1970), provided by steric blocking and unblocking of attachment prior to force generation (Haselgrove, Cold Spring Harb. Symp. 37, 1973; Huxley, ibid.; Parry & Squire, JBC 75, 1973).

Most of these postulated basic properties, however, are in conflict with recent experimental results or could not yet be confirmed:
(1) Biochemical studies revealed that the actual hydrolysis step does not induce a structural change and occurs both while cross-bridges are attached to or detached from actin (Stein et al., Biochem. 18, 1979). (2) Many experimental approaches failed to reveal a large-scale structural change in attached cross-bridges. Only in EM studies and some X-ray diffraction work evidence for structural differences between attached cross-bridges in various states was found (e.g. Craig et al., PNAS 82, 1985; Yu & Brenner, Biophys. J. 49, 1986). However, it is not at all clear whether these differences arise from an "active" change in the structure of the attached cross-bridge or from a "passive" deformation imposed onto the attached cross-bridge. (3) Under high-speed shortening, ATP-hydrolysis is not several-fold larger than under isometric conditions, as expected from the theories of the seventies (e.g Kushmerick & Davies, Proc. R. Soc. Lond. Ser. B 174, 1969). This finding, and recent in vitro studies (Yanagida et al., Nature 316, 1985) suggest that the sliding distance while a crossbridge passes through the force-generating states(s) is several fold larger than 10nm. (4) For all states it was found that cross-bridges can be attached to or detached from actin. In fact attachment and detachment are very fast reactions on the time scale of a cross-bridge cycle, i.e., a cross-bridge can detach and reattach many times as it completes one cycle, hydrolysing one molecule of ATP (Brenner et al., PNAS 79, 1982; Biophys. J. 50, 1986; Brenner, PNAS 85, 1988). (5) Regulation of muscle contraction was found not to act via recruitment (e.g., by steric blocking of attachment or of a subsequent step in the cycle) but rather through changes in cross-bridge turnover kinetics (Brenner, PNAS 85, 1988). (6) At least at partial activation, force-generating cross-bridges affect the state of the regulatory proteins (Bremel & Weber, Nature New Biol. 238, 1972) which in turn determines the magnitude of the rate constants for active turnover (Brenner, PNAS 85, 1988). Thus, at least at partial activation cross-bridges do not act completely independent of each other.

This summary illustrates that almost all of the key features of the generally accepted scheme for cross-bridge action need reconsideration.

A working hypothesis will be discussed which was developed from the recent findings of mechanical, biochemical, and structural studies (Brenner, in: Molec. Mech. of Muscle Contr., J.M. Squire ed., Macmillan, 1989) . In short, cross-bridges are assumed to (a) cycle between two main groups of states as ATP is hydrolysed; the weak-binding states in which cross-bridges cannot generate active force, and the strong-binding states which represent the main force-generating states. In both groups of states cross-bridges dynamically interact with actin (detach and reattach) more or less rapidly but always fast compared to active turnover. In the strong-binding states upon reattachment cross-bridges rapidly (re)assume a force-generating configuration. In the weak-binding states this conformational change after reattachment does not occur, leaving cross-bridges in a weakly-attached nonforce-generating configuration. As ATP is hydrolysed, cross-bridges cycle between these two main groups of states. (b) Rapid detachment which is necessary during high speed shortening to avoid impedance of filament sliding is provided by (transient) detachment with reattachment to a subsequent site on the actin filament while crossbridges remain in the strong-binding states. Thus, different from the previous hypotheses, no large increase in ATPase activity with shortening velocity is expected and filament sliding while occupying the strong-binding states is not limited to 10nm, the expected range of a permanently attached cross-bridge. (c) Concerning regulation, we propose that P_i-release, the step which leads into the strong-binding states, only occurs when weak-binding cross-bridges are attached to the "turned on" ("activated") form of actin, whereby the equilibrium between the "turned on" and "turned off" form of actin is determined by the Ca^{++} binding to TnC along the actin filament.

We shall discuss the essential differences to previous hypotheses and their implications not only for our understanding of muscle contraction and cell motility but also for resulting new pharmacological approaches for modulation of contractile function, especially in myocardium and smooth muscle.

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Abt. Allgemeine Physiologie, Universität Ulm, Albert-Einstein-Allee 11, D-7900 Ulm, FRG.

4

PTH-dependent inhibition of proximal tubular transport: cellular mechanisms

H. Murer

The kidney is one of the target organs of parathyroid hormone (PTH): PTH stimulates gluconeogenesis, ammoniagenesis as well as the production of $1,25(OH)_2Vit\ D_3$ in cortical (proximal tubular) tissue, has effects on glomerular filtration rate and affects tubular reabsorption. Three 'direct' transport effects of PTH are of main importance: 1) it inhibits proximal tubular phosphate-reabsorption; 2) it inhibits proximal tubular bicarbonate-reabsorption and 3) it stimulates distal tubular calcium-reabsorption (1).

In vivo and *in vitro* micropuncture/microperfusion studies have well documented that proximal tubular phosphate-reabsorption is Na-dependent and secondary active (2, 4, 9). Similarly, it was found, that proximal tubular bicarbonate-reabsorption ist to its large extent Na-dependent and transcellular transport is mediated by a secondary (tertiary?) active transport (3). The elements of transcellular Na-dependent transport of bicarbonate (Zelle A) and phosphate (Zelle B) are summarized in figure 1.

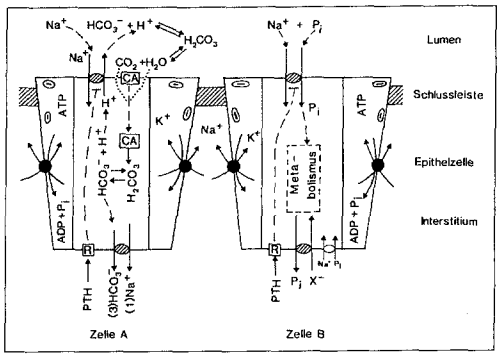

Figure 1: Cellular scheme for bicarbonate-rabsorption (Zelle A) and phosphate-reabsorption (Zelle B).

These models are based on studies on intact cellular preparations as well as on studies with isolated membrane vesicles (2-10). A significant part (30-40%) of bicarbonate reabsorption does not involve Na/H-exchange but is driven by a primary active H-ATPase located in the brush border membrane (3). From studies on physiologically altered bicarbonate- and/or phosphate-reabsorption (PTH; P_i-deprivation/overload; acidosis/alkalosis) it was concluded that altered transport is mainly related to altered transport rates of membrane transport mechanisms located in the apical membrane (2, 3, 4, 9).

As already indicated, increased PTH levels lead to inhibition of bicarbonate- and phosphate-reabsorption; studies with isolated membrane vesicles have suggested that these transport alterations are related to a reduced V_{max} of either Na/P_i cotransport (2, 4, 9) or Na/H exchange (3, 10). It has been generally accepted, that PTH-dependent inhibition is mediated by basolateral hormone/receptor-interactions and stimulation of adenylate cyclase and that protein kinase A mediated phosphorylation reactions at the brush border membrane level lead to reduced transport rates of Na/H exchange and Na/P_i cotransport rates (2, 3, 4, 9, 10). However, there is a gap between PTH concentrations occurring at 'normal' (physiological) conditions ($\approx 10^{-12}$ M) and those required for stimulation of the basolaterally located receptor/adenylate cyclase system (half maximal activation: $\approx 10^{-8}$ M). Thus, the search for a second, cAMP-independent pathway in PTH dependent control of proximal tubular transport was initiated. Indeed, already several years ago evidence for PTH-dependent alterations in phosphatidylinositol-metabolism was presented (e.g. 11); more recently PTH dependent activation of phosphalipase C activity was documented in an enriched proximal tubular basolateral membrane preparation (12). Furthermore, in suspended mouse renal tubules a phorbol ester dependent activation of protein kinase C led to a reduced Na-dependent phosphate uptake (13).

We and others have used over the last several years an established renal cell line derived from opossum kidney as a model system to study PTH regulation of proximal tubular transport functions. This cell line (OK-cells) shows a polar distribution of transport systems: Na/P_i cotransport, Na/H exchange and Na/hexose cotransport in the apical and Na/K-ATPase, Na/P_i cotransport and Na-independent phosphate transport in the basolateral membrane; the apical membrane Na/P_i cotransport is different from that in the basolateral membrane and shows kinetic properties similar to that in the renal proximal tubule; also Na/H exchange is similar to that in the renal proximal tubule and shows allosteric control by intracellular pH (5-8). Furthermore, apical Na/H exchange and Na/P_i cotransport (but not the basolateral transport systems) are inhibited by PTH, half maximal inhibition is observed at extremely low PTH concentrations, i.e. lower then 10^{-11} M (8, 14-17). PTH inhibition of Na/P_i cotransport and Na/H exchange is mimicked by pharmacological activation of either kinase A or kinase C (5, 6, 15). PTH also leads to activation of phosphalipase C and production of diacylglycerol (DAG) and inositoltrisphosphate (IP_3) (12, 17). Dose response relationships (see figure 2) suggest that phospholipase C activation and subsequent protein kinase C activation is of particular importance in control of transport activities at low (physiological) PTH concentrations.

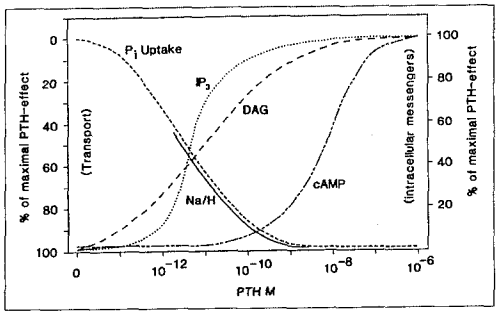

Figure 2: "Correlation" between PTH-dependent production of intracellular messengers and inhibition of Na/H exchange and Na/P_i cotransport.

Although there is a close similarity in the regulatory cascades for PTH-dependent inhibition of Na/H exchange activity and Na/P_i cotransport, we have obtained evidence that the 'final' inhibitory mechanisms may be different: Na/P_i cotransport inhibition, but not Na/H exchange inhibition, seems to involve an endocytotic mechanism (18). The inhibition of Na/H exchange by activation of kinase C is surprising; experiments using a different cell line ($LLC\text{-}PK_1/PK20$), which has apical as well as basolateral Na/H exchange, documented that apical activity ('epithelial' Na/H exchange) is inhibited by kinase C whereas basolateral exchange ('housekeeping') is stimulated (19).

SUMMARY: Proximal tubular brush border membrane Na/H exchange and Na/P_i cotransport are inhibited by PTH initiated regulatory cascades. It is/was generally accepted that cAMP-mediation plays a role in cellular control of these transport activities. However, recent studies suggest that also a cAMP independent pathway, i.e. phospholipase C/protein kinase C, is able to mediate PTH action. Studies on cultured epithelial cells suggest that the cAMP-independent pathway is of particular importance at low PTH concentrations; thus, a dual receptor concept for PTH action on proximal tubular transport has to be envisaged (see figure 3).

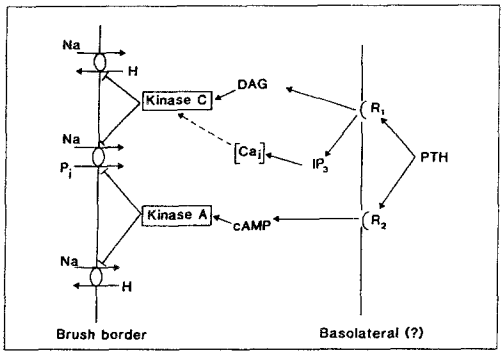

Figure 3: Schematic representation of cellular mechanisms in PTH-dependent control of proximal tubular transport.

Further work should focus 1) on the verification in intact tubular preparations of the model presented in figure 3 (based on observations made in cultured epithelia), 2) on the identification and location of (2) PTH receptor(s) including their cellular location (apical vs basolateral) as well as 3) on the structural identification of the transport systems known to be targets of PTH action.

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Physiologisches Institut der Universität Zürich-Irchel, Winterthurerstrasse 190, CH - 8057 Zürich (SCHWEIZ)

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5

NEUROPHYSIOLOGY OF OLFACTION

J. Boeckh

The indroduction of modern methods and approaches into the area of olfactory physiology and the impact of an increasing number of researchers especially in the US have brought about conniderable progress into this field which has long been notorious for its poor development in comparison to the investigation of the other major sensory pathways. Even if there is no convincing report on the isolation or identification of receptor proteins, odor induced changes in second messenger systems have been detected, and membrane studies have revealed a number of ionic channels as well as receptor currents and other details of electrogenesis. Odor binding proteins have been identified, and the inventory of known cell specific proteins increases permantently. Tracer studies and immunohistochemistry of the developing, regenerating and adult mucosa and central stages resulted in identification of cell types, projection patterns and formation of local circuits and pathways. Electrophysiology and micro-pharmacology have provided more and extended inside into modes of interactions between neuronal elements and have partly confirmed, extended or corrected prior and current hypotheses about modes of coding of quality, quantity, time course and spatial distribution of odor stimuli. A host of recent evidence exists about the neurochemistry and function of centrifugal innervation which casts new light upon modulation of responsivness, specificity and coordination especially of bulbar activity. Ablation experiments, application of metabolic markers as well as fastly working voltage-sensitive dyes have demonstrated specific cooperation of many local circuits and parts of the bulb during processing of odor input. A special contribution on this topic came from comparative studies on vertebrates and invertebrates. Experimental neuroanatomical studies have enlarged our view about secondary and tertiary central connections of the olfactory pathway. Recently, first data have been published on the neurobiology of olfactory learning and imprinting. Such data will further promote our understanding of neurophysiological events underlying odor induced, and odor guided behaviour.

Institut für Zoologie der Universität Regensburg, Universitätsstraße 31, D-8400 Regensburg

6

CURRENT CONCEPTS OF NOCICEPTOR FUNCTIONS

H.O. Handwerker

Two decades ago nociceptors were simply regarded to be slowly conducting afferent units specialized to signal tissue damage to the central nervous system. The method of microneurography opened the possibility to study the stimulus induced activity of single slowly conducting cutaneous afferents in human subjects together with the concomitant pain experiences. The close correlations found in numerous studies supported the notion that pain is mediated by a distinct group of afferents. However, recently the concepts of the function of small primary afferents have been modified under several aspects.

With the advent of neuropeptide histochemistry a subpopulation of small dorsal root ganglion cells with slowly conducting axons has been specified which synthetize tachykinins such as substance P, CGRP, and somatostatin. Initiation and transmission of impulses mediating pain may be but one of several functions of these peptidergic primary afferents. In particular in visceral organs many of them seem to be involved in the local neuroeffector control of the smooth muscles.

Another important function of slowly conducting primary afferents is the stimulus induced release of vaso-active substances, probably tachykinins, from the peripheral nerve endings causing vasodilatation and plasma-extravasation (1). Since unmyelinated afferents cause these effects at frequencies less than 1 Hz, which may not be consciously perceived, it has been proposed that they may often have a pro-inflammatory action without producing any reflex or sensory effects.

In contrast to fast conducting primary afferents most unmyelinated and some thin myelinated units are sensitive to chemical stimuli, in particular to endogeneous agents which are released in inflammation. The sensor and transducer functions underlying the chemosensitivity of slowly conducting afferents are now being studied with newly developed in-vitro techniques. For the study of membrane currents requiring intracellular and patch clamp recording techniques, the cell bodies of dorsal root ganglion cells have been used as models. No unique membrane receptor or second messenger system characteristic of nociceptors has been found hitherto, but rather a multiplicity of agents and potential second messengers (2). Though most of the small primary afferents are more or less sensitive to the whole spectrum of inflammatory agents, qualitative differences have been found between different populations.

Thus, the population mediating itch sensations from the superficial skin layers probably differs from another population mediating sensations of burning pain (3).

Characteristically slowly conducting afferents are sensitized by inflammatory processes for chemical, thermal and mechanical stimuli. Probably this sensitization is a basis of inflammatory pain and hyperalgesia. The membrane mechanisms of the sensitization are still unknown. However, it has recently been shown that in inflammation opioid receptors are expressed in the membrane of nociceptors (4). This may be but one example of membrane plasticity.

Until recently nociceptor sensitization was understood as a merely quantitative phenomenon leading to enhanced input to central neurons and hence to temporal summation. However, there is now increasing evidence from studies in different tissues that a large proportion of these units are virtually unexcitable in normal tissue. These "silent" units may turn to an active state when the tissue is inflamed. Thus, different populations of primary afferents mediate pain in inflammation.

The nature of the neurotransmitter(s) released by slowly conducting primary afferents at their central synapses is not yet clearly established. However, existing evidence does not support the notion that tachykinins like substance P are classical neurotransmitters. Instead, the release of tachykinins from the central endings of primary afferents seems to have conditioning functions on the spinal neurons. This may be the basis of plasticity changes in spinal cord circuitry occurring in the course of prolonged pain states. One sign of plasticity induced by ongoing activity in nociceptive primary afferents is the induction of the proto-oncogene C-fos in those units which are involved in the processing of nociceptive impulses.

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Institut für Physiologie und Biokybernetik, Universität Erlangen, D-8520 Erlangen, Universitätsstr.17

7

ACTIVATOR COMODIFICATION: A NEW METHOD TO MEASURE MICROSCOPIC REACTION DYNAMICS OF SODIUM CHANNEL SITE 2 ACTIVATORS

P. Honerjäger, M. Dugas, and G. Wang

Sodium channel comodification with batrachotoxin or other activators has frequently been used to measure microscopic reaction dynamics of channel blockers like tetrodotoxin, saxitoxin, local anaesthetics or antiarrhythmic drugs. We now show that activator comodification can also be used to detect the reaction dynamics of sodium channel activators, if the two activators act allosterically and induce different conductance states of the sodium channel.

Outside-out patches were obtained from dissociated and cultured ventricular myocytes of late-fetal rats. 1 µM BDF 9145* was added to the extracellular medium containing 140 mM Na, test drugs were added to the intracellular solution containing 148 mM Cs (20°C). BDF 9145 induced "full" activation of the sodium channel by imposing a normal open-channel current of -1.2 pA (at -30 mV) for hundreds of milliseconds after depolarization from -110 mV holding potential. Repetitive pulsing was halted at -30 mV as soon as a single channel became modified by BDF 9145. The additional binding of veratridine, a partial activator, was manifested by a sudden reduction of open-channel current to -0.3 pA, veratridine unbinding by a sudden return to -1.2 pA. Dwell-time histograms of bound and unbound channel states showed exponential distributions, and the rate constant for leaving the unbound state increased linearly with veratridine concentration (0.3-30 µM) while that for leaving the bound state was unaffected by veratridine concentration. The method was also successfully applied to four other ceveratrum alkaloids (veracevine, cevadine, germine-3-acetate, and germitrine) and to grayanotoxin-I. (Supported by DFG and Sandoz-Stiftung für therapeutische Forschung.)

*4-(3-(4-((4-cyanomethoxyphenyl)phenylmethyl)-1-piperazinyl)-2-hydroxypropoxy)-1H-indol-2-carbonitrile

Institut für Pharmakologie und Toxikologie der TU München Biedersteiner Str. 29, D-8000 München 40, F.R.G.

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USE DEPENDENCE OF LOCAL ANAESTHETICS IS CORRELATED WITH EFFECTS ON INACTIVATION OF SODIUM CHANNELS

A. DeLuca, H. Brinkmeier, B. Fakler, T. Pröbstle and R. Rüdel

To investigate why some local anaesthetic-like antiarrhythmic drugs block the amplitude of the action potential the more effective the faster a cell is stimulated, we tested the effects of benzocaine and tocainide on the inactivation of the sodium channels in human myoballs (Pröbstle et al. Pflügers Arch 412:264-269, 1988). The cells were voltage-clamped in the whole-cell recording mode at a holding potential of -85 mV and stimulated with test pulses going to -20 mV for 8 ms. The maximum of the sodium current flowing during single test pulses was reduced to about 50% when the cells were kept in 1 mM benzocaine or 0.5 mM tocainide. When the cells were stimulated at 1 Hz in benzocaine, the reduction went on for only a few % during the first 5-6 stimuli, while in tocainide the additional reduction was pronounced and stabilized at 25% only after 40-50 s, i.e. the action of tocainide is use-dependent, while that of benzocaine is not.

This difference in action is not caused by drug effects on the activation of the sodium current, as both drugs left the time constant of activation, τ_m, unchanged. It is rather reflected in different effects of the 2 drugs on the inactivation of the sodium channels. Benzocaine reduced the time constant of fast inactivation, τ_h, by about 20%, while tocainide did not alter τ_h. The effect of the 2 drugs on steady-state inactivation (the h∞-curve), determined with 200-ms conditioning pulses to -135 mV preceding every prepulse/test pulse sequence (to remove use dependence), with prepulses of variable size, and with test pulses to -20 mV was different depending on whether the prepulse duration was long or short. Both drugs shifted the curve towards more negative potentials. With prepulses of 8 ms duration, this shift was 8.7 ± 2.7 mV (mean ± S.D., n = 4) in 1 mM benzocaine, but only 2.1 ± 1.5 mV (n = 6) in 0.5 mM tocainide. With 512-ms prepulses, the left-shift with benzocaine was 15.6 ± 4.2 mV (n = 11), that with tocainide was 11.1 ± 4.5 mV (n = 11). These results indicate that, in contrast to benzocaine, tocainide has little effect on fast inactivation, and mostly affects intermediate inactivation, and this would explain the use dependence (Fakler et al. Pflügers Arch, in press). A reason for that might be that tocainide reaches equilibrium with its receptor more slowly than benzocaine.

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Abteilung für Allgemeine Physiologie, Universität Ulm, Albert-Einstein-Allee 11, D-7900 Ulm, Federal Republic of Germany

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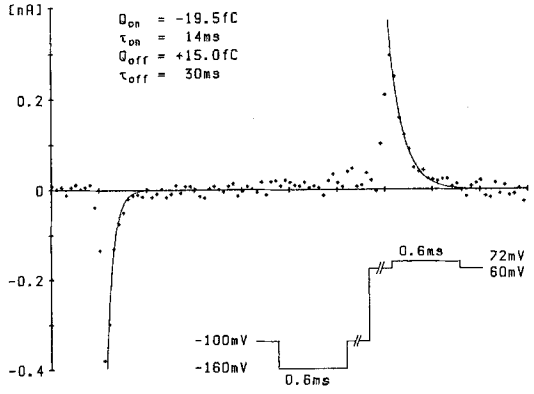
NONLINEAR CHARGE MOVEMENT OVER THE VOLTAGE RANGE -200 TO -100 MV IN FROG NODES OF RANVIER

J.-A. Pohl and H. Meves

In squid axons, the steady-state relation between nonlinear charge, Q, and membrane potential, E, saturates at both ends, near -150 and 50 mV (Armstrong and Gilly, 1979; Bezanilla et al., 1982). For the frog node of Ranvier, saturation of the Q(E) curve at positive potentials is well documented, but little is known about the range E < -100 mV. The figure shows an upside-down gating current recorded with pulses from -100 to -160 mV (P/5 method, control pulses superimposed on 60 mV, 10°C). The charge $|Q_{on}|$ increased linearly over the voltage range -100 to -200 $^{Q_{on}}$mV without any sign of saturation. The charge movement produced by hyperpolarizing pulses was much smaller and faster than that produced by depolarizing pulses.



I. Physiologisches Institut der Universität des Saarlandes, D - 6650 Homburg-Saar

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EXPRESSION OF EPITHELIAL Na^+ CHANNELS IN XENOPUS OOCYTES FROM SIZE FRACTIONATED MRNA OF BOVINE TRACHEAL EPITHELIUM

B. Kroll, S. Bremer, B. Tümmler, E. Frömter

$Poly(A)^+$-RNA was prepared from native bovine tracheal epithelium and size-fractionated by saccharose density gradient centrifugation. A total of 9 fractions spanning the entire gradient was injected into full grown immature oocytes of Xenopus laevis to test for their ability to induce epithelial Na^+ channels. Two days after injection of 25 ng of the fractionated mRNA the current response to bath application of 0.01 mmol/l amiloride was monitored while the oocytes were clamped to -70 mV. Whereas water-injected control oocytes did not respond, mRNA injected oocytes showed amiloride-inhibitable currents of up to 190 nA. Expression maxima were found in oocytes injected with either fraction 0 (mRNA length up to 10 kb) or fraction 5 (up to 4,4 kb). To better identify the ion channels responsible for the amiloride-inhibitable current flow a number of tests was performed. At a holding potential of -100 mV the dose/response curve for amiloride was measured. It followed a single-site inhibition model with half maximal inhibition at 44 nmol/l. This value agrees well with amiloride inhibition of Na^+ channels in native respiratory epithelia. To test for Na^+ over K^+ selectivity, the current-voltage relation of the amiloride-inhibitable current was tested after reducing extracellular Na^+ concentration from 85 to 27, 8.5 and 0.85 mmol/l by substitution with N-methyl-D-glucamine. In these experiments the reversal potential shifted by an average of 55.2 mV (n=9) for a tenfold change in extracellular Na^+ concentration indicating a Na^+ over K^+ selectivity of at least 10. This result was confirmed by replacing all bath Na^+ by K^+ which virtually abolished the current response to amiloride. We conclude that oocytes are able to express highly selective epithelial Na^+ channels in their cell membrane from large size fractions of mRNA of tracheal epithelium.

Zentrum der Physiologie, J.W.Goethe-Universität, Theodor-Stern-Kai 7, D-6000 Frankfurt/Main and Zentrum Biochemie, Medizinische Hochschule, Konstanty-Gutschow Str. 8, D-3000 Hannover

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Na^+-SELECTIVE CHANNELS IN THE APICAL MEMBRANE OF CULTURED PROXIMAL TUBULE CELLS (OK): EVIDENCE FOR VOLTAGE- AND pH SENSITIVITY

J.S. Schwegler, W.Steigner and S.Silbernagl

Sodium ions enter the proximal tubule cells mainly by solute cotransport and Na^+/H^+ exchange. In the present study we give evidence for 2 types of sodium conducting channels in the apical membrane of confluent monolayers of the opossum kidney cell line. Patch clamp experiments were performed in the excised, inside-out oriented configuration at voltage clamped conditions. We found 2 types of sodium permeant channels: (1) an unselective cation channel (P_{Na}/P_K about 1/1) with a single channel conductance of 150 pS at positive pipette potentials (neg. PD_m) that can be activated by membrane stretch; (2) a low-conductive channels (18.8 ± 1.4 pS; n=10) with a selectivity of P_{Na}/P_K = 4.8/1 and no detectable conductivity for chloride. Both channels are inward rectifying, i.e. reduce their conductance at negative pipette potentials. The open probability of the sodium selective channel is low (<0.5%) and not always detectable in the cell-attached configuration (n=6). However, channel opening can be eliceted by reducing the bath pH from 7.8 to 6.4 to 1.1-4.2% (n=3).

This low conductive channel has remarkable similarities to the one found in rabbit proximal tubule cells (Gögelein and Greger, Pflügers Arch. 1986, 406:198ff) and might adjust sodium influx from the luminal side to different states of sodium transport activity.

Dept. of Physiology, University of Würzburg, Röntgenring 9, D-8700 Würzburg, FRG

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DIFFERENTIAL EFFECTS OF EXTERNAL CALCIUM ON GATING AND OPEN-CHANNEL CURRENT OF CLONED NA CHANNELS EXPRESSED IN OOCYTES

Michael Pusch

Macroscopic and single-channel currents through rat brain type II, type III and 'K226Q' (a mutant of type II) Na^+ channels expressed in oocytes have been measured using the patch-clamp technique. Mutant K226Q differs from the wild-type at homologous position 226 where the positive amino-acid lysine (K) is replaced by an uncharged glutamine (Q). Position 226 is located at the C-terminal end of segment S4 of homologous repeat I of the Na^+ channel sequence.

The blocking effect of extracellular Ca^{2+} on single-channel inward-currents was examined for the three channel types. Affinities turned out to be similar for wild-type II and K226Q channels. However, the form of the concentration- as well as the voltage-dependence of the Ca^{2+} block was different for all channel types. In particular the form of the concentration-dependence for wild-type II channels could not be accounted for by a simple block model, suggesting the existence of several Ca^{2+} binding sites.

In addition to the blocking effect, it is known that external Ca^{2+} shifts steady-state activation curves to more positive voltages. This effect was confirmed for wild-type II and K226Q channels. Interestingly, K226Q channels are significantly more sensitive to external Ca^{2+} than wild-type II channels with respect to shifts of steady-state activation.

From these observations it is concluded that mutant K226Q differs from wild-type in a region of the protein which is located close to the extracellular side of the membrane, accessible to external Ca^{2+}.

Max-Planck-Institut für biophysikalische Chemie, Am Faßberg, D-3400 Göttingen

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NON-INACTIVATING SODIUM CURRENT IN NEUROBLASTOMA CELLS AFTER CHLORAMINE-T TREATMENT: A WHOLE-CELL AND SINGLE-CHANNEL STUDY

P. Niemann, J. Schmidtmayer, W. Ulbricht

In voltage-clamped euroblastoma cells (line N1E-115; tested at 15-17°C) decline of I_{Na} due to inactivation during a depolarizing pulse is proportional to $A + B*\exp(-t/\tau_0) + C*\exp(-t/\tau_1)$ where A+B+C = 1. B (mean τ_0 = 1.7 ms, E = 0) dominates over C (mean τ_1 = 7.7 ms) and A ≈ 0 i.e. inactivation is complete. After treatment with chloramine-T (Cl-T) both τ_0 and τ_1 increase by a factor of 2 or more; A increases on average from 0.07 at E = -20 mV to 0.2 at E = 0. Peak I_{Na} is considerably larger than in the control, particularly at -30 < E < 0 mV.

The single-channel behaviour underlying these effects was studied on inside-out patches in the control and with Cl-T applied to the internal face of the membrane. Depolarizing pulses of 40 ms (control) or 80 ms duration (Cl-T) were applied from a holding potential E_H = -80 mV as a rule. Open-time histograms could be fitted by a single exponential both in the control and after Cl-T treatment pointing to a single mean open time which was almost independent of test pulse potential ≥ -10 mV, $\bar{t}_o$ = 0.55 ms in the control and $\bar{t}_o$ = 2.0 ms after Cl-T. In contrast with the single mean open time, inactivation of the ensemble averaged currents was diphasic in either solution with τ_0 always clearly larger than $\bar{t}_o$. After Cl-T treatment inactivation was incomplete due to an increased probability of the channels to reopen as deduced from the bursting behaviour. The mean burst time $\bar{t}_b$ increased with depolarization where t_b is defined as a period of short openings interrupted by closed gaps of duration $t_g \geq$ 1.5 ms. As t_b increased, t_g decreased.

The existence of a) only one level of single-channel conductance which was unaffected by Cl-T (12 pS *vs.* 13 pS in the control) and of b) a single mean open time suggests a homogeneous population of Na channels.

Physiol. Institut d. Univ., Olshausenstr. 40, D-2300 Kiel

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EFFECT OF A SUDDEN CHANGE IN SODIUM AND LITHIUM CONCENTRATION ON SODIUM CHANNEL BLOCK BY TETRODOTOXIN

G. Hansen, W. Ulbricht

We have continued our work on Na^+-tetrodotoxin (TTX) interaction in blocking Na channels in frog nodes of Ranvier (Pflügers Archiv 413:R1; 1989) which showed that in the presence of high $[Na^+]$ (= 216 mM), 3.2 nM TTX blocked less effectively and washed out faster than in 54 mM Na^+ (+ TMA^+ to stay twice hypertonic). We have extended our study to 1.6 and 6.4 nM TTX in either $[Na^+]$ and find the equilibrium fraction, p, of I_{Na} left unblocked to be 0.72 ± 0.01 (mean ± S.E.M; n = 3) and 0.38 ± 0.01 (n = 7) in 216 mM Na^+ but 0.62 ± 0.01 (n = 5) and 0.28 ± 0.03 (n = 6) in 54 mM Na^+. In 216 mM Na^+ we have further tested 0.8 and 12.8 nM TTX yielding p = 0.80 ± 0.01 (n = 5) and 0.22 ± 0.01 (n = 5). Onset and offset of TTX action could be well described by single exponential functions with τ_{on} decreasing with increasing [TTX] but changing little with $[Na^+]$ whereas τ_{off} was independent of [TTX] but faster in 216 than 54 mM Na^+ throughout. If, after equilibration in other low-Na^+ 3.2-nM TTX solutions, $[Na^+]$ is suddenly raised to 216 mM block is partially relieved with a time constant, τ (16-18°C). On stepping from 27 and 7 mM Na^+ τ = 50 ± 3 s (n = 4) and 64 ± 7 s (n = 5), clearly faster than τ_{on} = 96 s in 216 mM Na^+ as reported before. This discrepancy was even more pronounced when Na^+ was substituted by Li^+: a step after equilibration in 3.2 nM TTX from 54 to 216 mM Li^+ resulted in τ = 33 ± 4 s (n = 6) whereas τ_{on} in 216 mM Li^+ was 94 ± 6 s (n = 3). Li^+ was chosen to differentiate between selectivity ($P_{Li} \approx P_{Na}$) and conductance of the Na channel, since I_{Li}/I_{Na} = 0.58 ± 0.01 (n = 12), for 216 mM and E to to yield maximum inward current, a ratio less than reported for isotonic solutions. For 3.2 nM TTX, p in 54 *vs.* 216 mM Li^+, 0.50 ± 0.02 (n = 3) *vs.* 0.59 ± 0.02 (n = 7), is very similar to that in the corresponding $[Na^+]$. $\tau_{off} = f[Na^+]$ is incompatible with a simple cation-TTX competition and $\tau \neq \tau_{on}$ in 216 mM Na^+ or Li^+ is hard to reconcile with simple models of cation-modulated affinity of the channel for TTX.

Physiol. Institut d. Univ., Olshausenstr. 40, D-2300 Kiel

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INTERACTION BETWEEN TTX AND MONOVALENT CATIONS AT NODAL Na CHANNELS

U. Lönnendonker and B. Neumcke

Na currents and Na-current fluctuations were measured in myelinated frog nerve fibres to study interactions between the Na channel blocker tetrodotoxin (TTX) and monovalent cations in the external solution. In hyperosmolar solutions containing 12 nM TTX and various permeant cations the number N of Na channels per node not blocked by TTX was higher than in Ringer + 12 nM TTX. The relief of TTX block depended on the cations in the external solution. N increased by the following factors upon addition of 110 mM chloride salts to TTX Ringer: 1.75 (Na), 1.64 (Li), 1.88 (hydrazine), 1.15 (guanidine), 1.20 (K). No significant changes of N were observed in TTX-Ringer + 110 mM TMA·Cl or 250 mM sucrose. Hence, the efficacy of TTX displacement by Na:Li :hydrazine:guanidine:K:TMA is 1:0.85:1.17:0.20: 0.27:0. This sequence reflects a weaker cation discrimination than the ion selectivity 1:0.93:0.59:0.13:0.086:0 of nodal Na channels (Hille J Gen Physiol (1971) 58: 599, (1972) 59: 637). The results are interpreted by a TTX receptor in a superficial prefilter to the Na channel contributing to cation discrimination at the outer channel region.

I.Physiologisches Institut, Universität des Saarlandes, 6650 Homburg/Saar

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ULTRASLOW SODIUM CHANNEL INACTIVATION PRESENT IN RAT SKELETAL MUSCLE IS ABSENT IN CARDIAC MUSCLE. LOOSE-PATCH MEASUREMENTS.

R. Eickhorn, D. Hornung, H. Antoni

An inactivation process of the fast sodium system developing with a time constant in the range of minutes was described in loose-patch experiments on rat skeletal muscle by Simoncini & Stühmer and by Ruff et al. (J Physiol 383 (1987) 327 and 339). We searched for a similar process in cardiac muscle using right and left ventricular papillary muscles of Wistar rats (n=10). Skeletal muscle fibres from the same animals were used for comparison. The muscles were voltage clamped by means of the loose-patch at 25°C in Krebs-Henseleit solution containing 1.8 mM Ca^{2+} and 5.9 mM K^+.

Steady state fast inactivation from a holding potential of -100 mV with conditioning prepulses of 250 ms showed a midpoint of the Boltzmann distribution at -79.8 ± 1.9 mV with a steepness of 11.1 ± 0.75 per e-fold. If instead of conditioning pulses of only 250 ms those of 10 s were given the peak current at -140 mV increased by 14 ± 10%. The position and steepnes of the inactivation curve were, however, not changed significantly.

In a series of 4 experiments the holding potential was stepped from -100 mV to -140, -120 or -80 mV for 5 minutes during which every ten seconds a test pulse (5 ms, 0 mV) was given. The change of current amplitude was complete within the first 10 s. The peak current elicited 4 minutes after a step from -100 mV to -140 mV differed only by 0.06 ± 0.54 nA from that between 10 and 40 s (not significant). These results in cardiac muscle differ markedly from skeletal muscle, in which the peak sodium current changes with a time constant of 119 ± 50 s at -80 mV. Hence, ultraslow inactivation is absent in cardiac muscle.

Physiologisches Institut der Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg i.Br.

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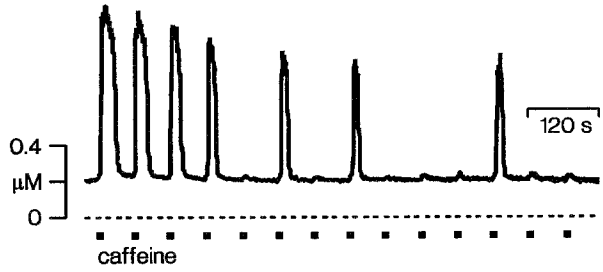
FUNCTIONAL EXPRESSION OF THE CALCIUM RELEASE CHANNEL FROM SKELETAL MUSCLE RYANODINE RECEPTOR cDNA

R. Penner, E. Neher, H.Takeshima*, S. Nishimura* & S. Numa*

Combined patch-clamp and fura-2 measurements were performed to study the calcium release properties of Chinese hamster ovary (CHO) cells transfected with the rabbit skeletal muscle ryanodine receptor cDNA carried by an expression vector.

Both caffeine (1-50 mM) and ryanodine (100 µM) induced release of calcium from intracellular stores of transformed CHO cells but not from control (non-transfected) CHO cells. The calcium responses to caffeine and ryanodine closely resembled those commonly observed in skeletal muscle. Repetitive applications of caffeine produced characteristic all-or-none rises in intracellular calcium:



Inositol 1,4,5-trisphosphate (IP_3) neither activated the ryanodine receptor channel nor interfered with the caffeine-elicited calcium release. These results indicate that functional calcium release channels are formed by expression of the ryanodine receptor cDNA.

Max-Planck-Institute for Biophysical Chemistry, Am Fassberg, D-3400 Göttingen, FRG and *Department of Molecular Genetics, Kyoto, Japan.

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CA CURRENT AND INTRAMEMBRANE CHARGE MOVEMENTS AFTER CONDITIONING DEPOLARIZATION IN FROG SKELETAL MUSCLE.

By W. Melzer, D. Feldmeyer, B. Pohl and P. Zöllner

The activation of the Ca current in skeletal muscle, which is known for its slow kinetics, can be markedly accelerated by a conditioning current activation (*Feldmeyer et al. J.Physiol. 415:115P, 1989*).

We measured Ca inward currents in cut muscle fibres of the frog and determined their voltage dependence in the normal slow and the fast gating mode. Fast gating was established by a large depolarizing pulse causing considerable current activation but no inactivation; it was followed by repolarization to a subtreshold potential of -50 mV for 100 ms and a test pulse to different superthreshold potentials.

Although the conditioning pre-pulse caused a strong increase in the activation speed of the test current at each potential the current peaks nearly coincided. This indicates that not a new channel population with different kinetic characteristics became available but that those channels which normally give rise to the slow Ca current exhibited an altered gating behaviour.

Intramembrane charge movements which have previously been correlated with Ca release are sensitive to Ca antagonists suggesting that at least part of them could be involved in Ca channel gating (*Lamb & Walsh. J.Physiol. 393:595. 1987*). We applied the pulse protocol described above after eliminating the Ca-current by removing Ca from the bathing solution and adding 2 mM Cd. The charge movements measured during the test pulses remained virtually unaffected by the conditioning pulse.

A model in which a fast reaction (possibly accompanied by Ca antagonist-sensitive charge movements) in conjunction with an additional slow reaction leads to channel gating can account for most experimental observations.

Lehrstuhl für Zellphysiologie, Ruhr-Universität Bochum, Universitätsstr. 150, Gebäude ND-4, D-4630 Bochum 1

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CALCIUM CURRENTS IN HYPOTHALAMIC NEURONS OF AN IN VITRO NETWORK

T.H. Müller, D. Swandulla and U. Misgeld

Many neurosecretory cells of the hypothalamus display synchronous phasic activity which appears to be important for the regulation of hormone secretion (D.A. Poulain and J.B.Wakerly, Neurosci. 7:773 1982). Embryonic hypothalamic neurons have been shown to grow synchronously bursting networks when cultured on a glial background monolayer (U. Misgeld and D. Swandulla, Neurosci.Lett. 98:291 1989). While synchronization and desynchronization of phasic activity has been shown to rely on quisqualate-type receptors coupled to channels selective for monovalent cations and $GABA_A$-controlled chloride channels, little is known about voltage-activated currents that may be involved in burst generation and control of transmitter release.

In the present study we recorded voltage-activated currents from embryonic (E14-E15) hypothalamic neurons after up to 56 days in culture (DIC) on a glial monolayer. Both low- and high-threshold calcium currents were found to develop gradually between 7 and 21 DIC. This correlates with the proliferation of dendrites and the formation of complex networks which occurs during this time. By contrast, TTX sensitive sodium currents developed within 5-10 DIC and potassium currents could be observed after as little as 6 hours. Low-threshold calcium currents were drastically reduced by external application of Ni^{2+} in low concentration (70% reduction at 50µM) where high-threshold currents were not affected. Neurons were also redissociated after 21 DIC or later and calcium currents recorded 4 to 8 hours after replating. After this treatment the neurons usually had a spherical shape and lacked dendritic processes. High-threshold calcium currents were still present in almost all of the neurons, whereas transient low-threshold currents could not be observed.

The ability of the in vitro networks to generate synchronous phasic activity parallels the development of calcium currents and is most pronounced after 21 DIC, suggesting that calcium currents are required for bursting or for synchronization of neuronal activity. In particular, low-threshold calcium channels appear to be located primarily in the dendrites where they could be involved in the control of synaptic potentials.

Max-Planck-Institut für Psychiatrie, Abteilung Neurophysiologie,
Am Klopferspitz 18a, D-8033 Martinsried

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FREQUENCY-DEPENDENCE OF T-TYPE CALCIUM CURRENTS IN SINGLE SMOOTH MUSCLE CELLS ISOLATED FROM GUINEA PIG CORONARY ARTERIES

V.Ya. Ganitkevich and G. Isenberg

Myocytes from coronary arteries have two types of Ca-currents which are carried through Ca-channels similar to T-type and L-type. The types of Ca-current can be distinguished by their voltage-dependence. With 10 mM extracellular $BaCl_2$ as charge carrier,the current at -50, -40 and -30 mV is mostly T-current whereas the current at positive potentials is mostly L-current.

We studied the Ca-currents at 35 °C with the whole-cell configuration of the patch-clamp method. The pipettes were filled with EGTA-free CsCl-solution. The holding potential was -90 mV and the depolarizing pulses lasted 200 ms. The amplitude of Ca-current changed when the frequency of stimulation was increased from 0.2 Hz to 3 Hz. With pulses to positive potentials, the current fell along a 'negative staircase' as expected from the long recovery time of the L-current. At negative potentials, where the Ca-current was mostly T-current, 3-Hz pulsing increased the amplitude of Ca-current up to three times. Repriming of T-current was studied with a two-pulse protocol at a holding potential of -100 mV. At pulse-intervals of 100-200 ms, the current was facilitated beyond the control current by a factor of 1.8 -2.8 (n=17). This overshoot in repriming was attenuated at -90 mV, and it was abolished by holding potentials less negative than -80 mV. Frequency-dependent facilitation of T-current does not require Ca-influx, i.e. the overshoot was similar in media with 10 mM $BaCl_2$ or 10 mM $CaCl_2$. The facilitation is unlikely to be mediated by changes in i.c. Ca-concentration since, the overshoot was not modified by caffeine-induced SR-Ca-release of by cell-dialysis with 40 mM EGTA plus 10 mM BAPTA. Preliminary analysis of single T-channel events suggests that the higher frequency facilitates channel openness. That is, the T-channel spends more time in the open state (1.7-1.9 times) when the frequency is increased from 0.2 to 1.6 Hz.

Zentrum für Physiologie, Robert-Koch-Str. 39, 5000 Köln 41, F.R.G.

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REGULATION OF CARDIAC CA^{++} CHANNELS BY THE GTP-BINDING PROTEIN G_S AND PHOSPHORYLATION

A. Cavalié, T.J.A. Allen and W. Trautwein

In heart cells, coupling of ß-adrenergic agonists to their receptor enhances the Ca current via cAMP-dependent phosphorylation of L-type Ca channels and/or via a direct action of the GTP-binding protein G_S. Simultaneous activation of both pathways by 1 µM isoprenaline produces a 3-4 fold increase of the Ca current, but the isoprenaline effect is reduced to 1.7 fold if the cell was previously dialysed with 0.5 mM R_pcAMPS. The latter result supports the suggestion that G_S can modulate Ca channels in intact myocytes when the cAMP-dependent phosphorylation was suppressed. Furthermore, we studied the relationship between these two signalling pathways by stimulating them sequentially. After cell dialysis with 100-200 µM cAMP, bath application of 20-60 µM IBMX produced a 3-4 fold increase of the Ca current, suggesting saturation of the cAMP cascade. Under this conditions 1 µM isoprenaline did not enhance the current further, indicating that G_S cannot modulate maximally phosphorylated Ca channels. However, simultaneous application of 20 µM IBMX and 1 µM isoprenaline increased the Ca current up to 7-8 fold, an effect much stronger than exerted by IBMX or isoprenaline alone. These results suggest that a direct action of G_S may act to prime Ca channels for up-regulation by cAMP-dependent phosphorylation.

II. Physiologisches Institut der Universität des Saarlandes, D-6650 Homburg/Saar

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DIRECT STIMULATION OF HEART CELL CALCIUM CURRENT BY ß-ADRENERGIC SIGNAL-TRANSDUCING, GUANINE NUCLEOTIDE-BINDING G_S PROTEIN

Siegried Pelzer[1,3], Yaroslav M. Shuba[2], Tatsuya Asai[3], Wolfgang Trautwein[1], Arthur M. Brown[4], Lutz Birnbaumer[5], Terence F. McDonald[3] and Dieter Pelzer[1,3]

A several fold increase in whole-cell calcium current (I_{Ca}) is a well-known feature of the maximal ß-adrenergic response of the heart. It is generally ascribed to a stimulation of cAMP-dependent channel phosphorylation triggered by receptor activation of G_S transducing protein and G_S activation of adenylate cyclase. We blocked phosphorylation pathways in guinea pig ventricular cardiomyocytes by dialysing cells with a low-Ca, substrate-free solution (minimal intracellular solution, MICS) containing (in mM) CsCl 80, CsOH 40, $MgCl_2$ 2, EGTA 10, HEPES 10 (pH 7.4) supplemented by phosphorylation-inhibitory agents (5 mM AMP-PNP, 50 µM DNP, 10 mM ADP-ß-S and 100 µM cGMP (PI-MICS)) to unmask other possible I_{Ca}-stimulatory modes. Na-, K-free external solution was used in most experiments to allow pulsing from relatively negative holding potentials and a dual-pipette method was applied in many experiments. This method had two major advantages: (1) "Control" intracellular dialysis could be followed by test dialysis, and (2) dialysis (and probably Ca_i-buffering) could be greatly facilitated by applying pressure across the two pipettes. In cells with blocked adenylate cyclase/cAMP cascade where, by marked contrast to control conditions (MICS, 1 mM GTP, 5 mM ATP), forskolin (1-5 µM) was without effect on I_{Ca}, I_{Ca} increased by approximately 50% during (1) receptor activation of G_S by 0.1 µM isoproterenol, (2) intracellular activation of G_S by 100 µM GTP-γ-S and, less effectively, by 100 µM GMP-PNP, or (3) intracellular application of 6 nM GTP-γ-S-preactivated G_S. We conclude that fast, direct G_S modulation participates in the physiological regulation of cardiac I_{Ca}.

[1]II. Physiologisches Institut, Homburg/Saar, FRG, [2]A.A. Bogomoletz Institute of Physiological Sciences, Kiev, USSR, [3]Dept. of Physiology & Biophysics, Halifax, Canada, [4]Dept. of Physiology & Molecular Biophysics and [5]Dept. of Cell Biology, Houston, USA

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PAST AND SLOW REGULATION OF THE CALCIUM-CURRENT IN NEURO-BLASTOMA x GLIOMA HYBRID CELLS

R. Eckert, *J. Hescheler, *W. Rosenthal, *S. Offermann
*D. Krautwurst, *G. Schultz, W. Trautwein

Previous studies have shown that a variety of agonists binding on inhibitory presynaptic receptors of central and peripheral neurons induce inhibition of voltage-dependent Ca^{2+}-currents (I_{Ca}) that is related to the inhibition of neurosecretion. In neuroblastoma x glioma hybrid cells (108CC15), the synthetic opioid D-Ala^2-D-Leu^5-enkephalin (DADLE), somatostatin and adrenaline (in the presence of propranolol) induced a fully reversible inhibition of I_{Ca} in a dose-dependent manner, whereas bradykinin was not effective. The following inhibition values were obtained: DADLE-74.72 ± 4.43 % (n=41), somatostatin-55.80 ± 3.12 % (n=16), adrenaline-α_2-13.57 ± 3.67 % (n=31), bradykinin-4,88 ± 2.40 % (n=14). Effects of intracellularly applied guanine nucleotides and the sensitivity towards pertussis toxin indicate that a pertussis toxin-sensitive G-protein (G_i-family or G_o) are involved in the inhibitory modulation of I_{Ca}. In order to identify the endogenous G-protein activated via inhibitory receptors, the effects of receptor agonist on photolabeling of G-protein α-subunits with the photoreactive GTP analogue, (α-^{32}P)GTP azido-anilide, were examined in membranes of NxG cells. The data obtained in a series of experiments showed that the ability of receptor agonists to stimulate photolabeling of a protein comigrating with the α-subunit of G_o (39 kDa) correlated well with their ability to induce I_{Ca}-inhibition (DADLE > somatostatin > adrenaline > bradykinin). The data suggest that G_o is involved in the inhibitory control of I_{Ca}. This hypothesis is supported by findings that antibodies against the G_o α-subunit attentuate the adrenaline induced inhibition of I_{Ca} in NxG cells (McFadzean et al, Neuron 3: 177-182, 1989).
Bodewei et al (Gen. J. Physiol. and Biophysics 4: 113-127, 129-141, 1985) have shown that cAMP is not involved in the fast regulation of I_{Ca} in NxG cells, but in the long term modulation of Ca^{2+} current magnitude. Our studies show an exact time course of the development of a fast and slowly inactivating Ca^{2+} current during incubation NxG cells in dBcAMP (1 mM) or forskolin (0.1 μM). Furthermore we found that I_{Ca} disappeared, when Ca^{2+}-channel blockers were included in the culture medium and could be restored by further differentiation with dBcAMP. The dBcAMP induced reappearence of I_{Ca} after Ni^{2+} treatment could be prevented by cycloheximide, a potent proteinsynthesis blocker. The results suggested that the Ca^{2+}-channel protein synthesis may be affected during long term incubation of NxG cells in media containing Ca^{2+}-channel blockers. This hypothesis was supported by binding studies that showed that the number of dihydropyridine (DHP) binding sites was significantly reduced in Ni^{2+}-treated cells (80.1 ± 16.5 fmol/mg protein, n=4) compared with the control cells (203.4 ± 20.9 fmol/mg protein, n=4).

2. Physiologisches Institut der Universität des Saarlandes
6650 Homburg/Saar, FRG
* Pharmakologisches Institut der Freien Universität Berlin
1000 Berlin 33, FRG

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IDENTIFICATION OF TWO CALCIUM CURRENTS IN THE MEMBRANE OF CULTURED GLIAL PRECURSOR CELLS FROM MOUSE CORTEX

BY HELMUT KETTENMANN, *J.TROTTER, AND ALEXEJ N. VERKHRATSKY*

The development of oligodendrocytes from their precursor cells can be studied in vitro in cultures containing cells at different developmental stages.These stages can be correlated with distinct patterns in Na^+ and K^+ channel expression. In this study we demonstrate that glial precursor cells of the oligodendrocyte lineage express two types of Ca^{++} channels which can be distinguished by their peak activation. These currents are similar to the low and the high voltage activated Ca^{++} channel described in neurons. Shift of the holding potential from -75 mV to -40 mV led to a complete disappearance of the low-voltage activated current component. While the majority of precursor cells expressed only one of the two current types respectively, in 30% of cells both types of current were identified. In contrast, we could not detect Ca^{++} channels in oligodendrocytes. In the precursor cells, Ca^{++} channels might play an important role in signal transduction during target recognition of the developing glial cell.

Department of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 364, Heidelberg, West Germany.

* *A.A.Bogomoletz Institute of Physiology, Bogomoletz St. 4, Kiev - 24,GSP 252601, Ukraina, U.S.S.R.*

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OMEGA-CONOTOXIN BLOCKADE OF CALCIUM CURRENTS IN CULTURED NEONATAL RAT CARDIOMYOCYTES.

BY ALEXEJ N.SAVTCHENKO & ALEXEJ N.VERKHRATSKY

Calcium currents from neonatal rat ventricular heart muscle cells grown in primary culture were examined using the "whole-cell" voltage clamp technique. The calcium current (I_{Ca}) was investigated in conditions when other components of membrane permeability were excluded. Only the high-threshold (L) calcium current was found in cultured neonatal ventricular myocytes. An inward current characterized by large amplitude and slow inactivation decay was induced when the extracellular Ca^{2+} concentration was reduced by EGTA. This current was suppressed by extracellular Na^+ removal, or by calcium antagonists, and increased by epinephrine and BAY K 8644. These findings suggest that this current is carried by sodium ions through Ca^{2+} channels. Both Ca and Na currents through calcium channels were irreversibly blocked by omega-conotoxin (10uM). Complete blockade developed 10 - 15 minutes after toxin introduction in the extracellular solution. At the same time the genuine run-down of calcium current in cultured neonatal rat ventricular myocytes takes much more time (40 - 45 minutes for complete current abolishing). Blockade of Na currents through calcium channels was characterized by a transient increase of current amplitude without any changes in its kinetics and voltage-dependent properties. Structural differences between calcium channels in rat and guinea-pig and frog cardiomyocytes were suggested.

A.A.Bogomoletz Institute of Physiology, Bogomoletz St. 4, Kiev-24, GSP252601, Ukraina, U.S.S.R.

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L-TYPE Ca-CHANNELS IN URINARY BLADDER MYOCYTES: SIMILAR Q_{10} OF Ca-, Ba- and Na-CONDUCTANCE POINTS TO THE IMPORTANCE OF ION-CHANNEL INTERACTION

A. Schiefer, U. Klöckner and G. Isenberg

Ion permeation through L-tpye Ca-channels has been described with a 2-site 3-barrier model [1]. The model predicts a high Q_{10} for open channel conductance of Ca- and Ba-ions that bind with high energy, but a low Q_{10} for Na- and Li ions that bind only weakly.
Myocytes were isolated from the urinary bladder of the guinea-pig. Elementary Ca, Ba, Li or Na currents through L-type Ca-channels were recorded from cell attached patches at 22°C or at 35°C. The higher temperature increased the open channel conductance for Ca-ions from 8.5 to 16 pS (Q_{10} =1.63 ± 0.07, mean ± S.D.), for Ba-ions from 24 to 43 pS (Q_{10} =1.55 ± 0.06), for Li from 27 pS to 50 pS (Q_{10} = 1.61 ± 0.08), and for Na from 74 to 131 pS (Q_{10} =1.55 ± 0.09). The differences in the Q_{10}'s are not significant, that is, the predictions of the 3 barrier model with 2 intra-channel binding sites are not fulfilled.
To model our results, we gave up the concept of discrete binding sites. We postulated the Q_{10} to result from multiple ion-channel interactions over a 1.1 nm long channel part. Part of the ionic hydration shell was assumed to be substituted by polar groups of the channel-protein until rehydration in the exit. On this level of negative free energy a permeation barrier was superimposed. The energy of this barrier was calculated from multiple ion-channel interactions that were assumed to occur with rates similar to those describing replacement of water molecules in the inner water shell of the cation [2]. With these theoretical values we calculated Q_{10}'s that approximated the experimental Q_{10}'s for all ions.

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Zentrum für Physiologie, Robert-Koch-Str. 39, 5000 Köln 41, F.R.G.

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CYCLIC AMP AND G-PROTEIN DEPENDENT MODULATION OF CALCIUM-ACTIVATED NONSPECIFIC CATION CURRENTS

D. Swandulla, L.D. Partridge and T.H. Müller

Currents through calcium-activated nonspecific cation (CAN) channels were studied in the fast burster neuron of *Helix aspersa* and *Helix pomatia*. CAN currents were activated by intracellular injections of Ca using a fast, quantitative pressure injection technique (for details see Müller, Swandulla & Lux, J. Gen. Physiol., **94**, 1989).
External application of forskolin (10-25 μM), an activator of adenylate cyclase, caused a transition from endogenous bursting activity of the cells to beating activity. These same concentrations of forskolin reduced CAN currents reversibly to about 50%. External application of the membrane permeable cAMP analogues 8-bromo-cAMP and dibutyryl-cAMP (100-500 μM) almost completely blocked the CAN current. A marked reduction in the CAN current was also observed following quantitative injections of cAMP (internal concentrations up to 50 μM) directly into the cells from a second pressure injection pipette. Similar results were obtained with quantitative injections of the catalytic subunit (C-subunit) of the cAMP-dependent protein kinase (internal concentrations 10^{-4} units of enzyme) directly into the cells.
Injection of the nonhydrolysable GTP analogue, GTP-γ-S (internal concentration 100 μM), which stimulates G-proteins, produced a prolonged increase in CAN current amplitude by as much as 300%.
External application of serotonin at concentrations of 100-200 μM caused a transition from bursting to beating activity of the neurons and mimicked cAMP's effects on CAN currents. Two other neurotransmitters tested, dopamine and acetylcholine, were not significantly effective in reducing CAN currents.
Our results indicate that CAN currents in *Helix* burster neurons are modulated by cAMP-dependent membrane phosphorylation and by G-proteins. The physiological transmitter that induces this second messenger action may be serotonin. The dual control of CAN channels by two second messengers, Ca and cAMP, has functional implications. While Ca activates CAN channels which generate the pacemaker current in these neurons (Swandulla & Lux, J. Neurophysiol. **54**, 1985; Partridge & Swandulla, Pflügers Archiv, **410**, 1987) cAMP-dependent phosphorylation down-regulates them thereby resulting in modulation of neuronal bursting activity.

Max-Planck-Institut für Psychiatrie, Abteilung Neurophysiologie,
Am Klopferspitz 18a, D-8033 Martinsried

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M-CURRENT INHIBITION IN NG108-15 NEUROBLASTOMA X GLIOMA HYBRID CELLS

S. Schäfer and H. Meves

In NG108-15 cells, bradykinin (BK) inhibits the M-current, a voltage dependent K^+-current. Since phorbol dibutyrate (PdBu) and oleolyl acetylglycerol (OAG), activators of protein kinase C (PKC), mimic this effect it has been suggested that M-current inhibition by BK is mediated by the activation of PKC (Brown & Higashida, J. Physiol. 397, 1988).
Using the whole-cell mode of the patch clamp technique we found that even 1μM PdBu only partially depressed the M-current, and that it did not prevent further inhibition by BK. Bath application of H7 (50-70 μM), a relatively unspecific inhibitor of PKC, partially reversed the otherwise irreversible effect of PdBu but did not affect M-current inhibition by BK. Further studies using more potent and more specific PKC inhibitors are required to reveal whether PKC really mediates BK induced M-current inhibition in NG108-15 cells.
The M-current was also depressed by millimolar concentrations of methylxanthines: IBMX was the most effective followed by 1,7-dimethylxanthine, caffeine, and theophylline. The threshold for the caffeine response was at 2 mM and inhibition was nearly complete at 20 mM. It was not prevented by prior application of 1 μM PdBu, nor did it affect M-current inhibition by BK. Adding 10 μM ryanodine to the pipette solution abolished neither the caffeine nor the BK effect. Taken together with the observation that Ca^{2+} injections do not affect the M-current (Brown & Higashida, 1988) it seems unlikely that Ca^{2+} release from intracellular stores plays a role in mediating M-current inhibition in NG108-15 cells.

I. Physiologisches Institut, Universität des Saarlandes,
D-6650 Homburg/Saar, FRG

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INTRACELLULAR ATP AND CA MODULATE TWO K CHANNELS IN MYELINATED NERVE FIBRES

P. Jonas, D.S. Koh, K. Kampe, M. Hermsteiner and W. Vogel

Different types of potential-dependent K channels in myelinated nerve fibres have been demonstrated on the macroscopic level and, more recently, on the single-channel level applying a specific enzymatic treatment and patch-clamp technique (Jonas et al. 1989, PNAS 86: 7238). This new method allows access to the inner side of the membrane. For the first time we present evidence for axonal K channels controlled by cytoplasmic factors in amphibian nerve fibres; one is activated by Ca^{2+} ions, the other is blocked by ATP.

The first channel has a single-channel conductance of 122 pS with 105 mM-K on both sides of the membrane at 15 °C. The reversal potential in Ringer solution is below -50 mV, indicating that this channel is selective for K^+ over Na^+ ions. The maxi K channel is activated both by micromolar concentrations of intracellular Ca^{2+} ions and by depolarization. The second channel has a single-channel conductance of 42 pS, is blocked by internal ATP (IC_{50} = 30 μM) and shows little voltage dependence. The reversal potential depends on extracellular K concentration, indicating that it is also a K channel. Openings of this ATP-sensitive K channel typically occur in bursts. Both channels are blocked by millimolar concentrations of external tetraethylammonium ions. They may link axonal metabolism and excitability and may be part of a complex feedback system regulating membrane potential under physiological and pathophysiological conditions.
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Physiol. Inst. Aulweg 129, D-6300 Giessen, FRG

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TRH MODULATES AN INWARD-RECTIFYING K^+ CURRENT IN RAT PITUITARY CELLS

C.K. Bauer and J.R. Schwarz

Thyrotropin-releasing hormone (TRH) is known to induce a biphasic secretion of prolactin in anterior pituitary cells of the rat (GH_3 cells). It has been postulated that phase II of secretion is mediated by the closing of voltage-dependent K^+ channels (e.g. Ozawa & Sand, Phys Rev 1986).
Using the whole cell configuration of the patch clamp technique we could demonstrate an inward-rectifying K^+ current, activated by hyperpolarizing pulses from a holding-potential of -40 mV in isotonic KCl solution. Peak current amplitudes increased with depolarizing pre-pulses indicating the existence of open K^+ channels at the holding potential. The K^+ currents showed time- and voltage-dependent inactivation at potentials more negative than -60 mV. Recovery from inactivation was slow and voltage-dependent (>10 s at -40 mV). The inward current was reduced by Cs^+ and Ba^{2+}, but not by Ni^+ and Co^{2+} (5 mM). Drugs like quinidine, 4-aminopyridine and TEA blocked the current, in contrast to dendrotoxin. Single channel recordings from inside-out patches revealed an inward-rectifying 46 pS channel (isotonic KCl) which is suggested to underlie the macroscopic inward K^+ current.
Bath application of TRH consistently reduced the inward K^+ current. This reduction could be abolished by GDPβS (400 μM), confirming the involvement of G-proteins in the signal transduction pathway. TRH shifted the voltage dependence of inward current inactivation to less negative potentials. In intact cells, current inactivation in the range of the resting potential would result in a depolarization and could readily explain the TRH-induced increase in action potential firing leading to phase II of secretion.

Physiologisches Institut, Universität Hamburg, Martinistr. 52,
D-2000 Hamburg 20

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HIGH K-MEMBRANE-CONDUCTANCE IN METABOLICALLY EXHAUSTED MUSCLE CORRELATED WITH PROPERTIES OF SINGLE ATP SENSITIVE AND Ca^{++} ACTIVATED K-CHANNELS

R.H.A. Fink and E. Wettwer

Reduction of metabolic energy reserves induces a large increase in K conductance in amphibian skeletal muscle (R.H.A. Fink et al.,J Physiol 336: 211,1983). Recently, it has been suggested that this increase is due to activation of ATP-sensitive K-channels by the lowering of ATP during exhaustion. These channels are thought to be the most abundant K-channels in the sarcolemma (A.E. Spruce et al.,J Physiol 382:213, 1987), though Ca^{++}activated K-channels may also contribute to the high conductance. We have carried out further micro-electrode voltage-clamp experiments in single muscle fibres of frog sartorius muscle poisoned with 2mM CN and 1mM IAA and stimulated to complete loss of twitch force. The macroscopic K-currents declined with time and were followed by 'tail'-currents which increased in amplitude with size and duration of the depolarizing or hyperpolarizing voltage steps. The accompanying shifts in reversal potential and the low temperature dependence of the 'tail'-current kinetics indicate that these effects are mainly due to accumulation or depletion of K^+ in the vicinity of the membrane. The instantaneous I-V curves are mostly linear showing only some saturation with larger voltage steps. The features of these macroscopic currents can well be explained with the characteristics of single ATP-sensitive K-channels (see also P.R. Stanfield, TINS 10:335, 1987) but also with those of Ca^{++}activated K-channels found in native sarcolemma vesicles of differentiated mammalian skeletal muscle (R.H.A. Fink et al., Proc IUPS 17:187, 1989).

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II.Physiologisches Institut, Im Neuenheimer Feld 326 Universität Heidelberg, D-6900 Heidelberg 1

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POLYMYXIN B BUT NOT POLYMYXIN E MODIFIES CA^{2+}-ACTIVATED K^+ CHANNELS IN MOUSE SKELETAL MUSCLE

R. Weik

The effect of the polycationic peptide-antibiotic polymyxin B was studied using excised inside-out patches from dissociated mouse toe muscles. Polymyxin B, dissolved in an internal solution containing (mM) 155 KCl, 2 $CaCl_2$, 1 $MgCl_2$ and 10 HEPES, produced a block of high conductance (~250 pS) Ca^{2+}-activated K^+ channels, which was more pronounced at more positive potentials. Half-maximal blockage was observed with 0.5µg/ml polymyxin B at E=+30mV. An increase of the internal Ca^{2+}-concentration reduced the block. Polymyxin B did not affect the channel conductance but modified the gating kinetics by decreasing the mean open times and increasing the mean closed times. Without antibiotic, the open times increased with depolarisation, whereas the closed times were voltage-independent. Addition of polymyxin B reversed the voltage-dependency, i.e. now the open times were nearly voltage-independent, whereas the closed times increased with depolarisation. Polymyxin E, another peptide-antibiotic which differs only in one amino acid from polymyxin B, had nearly no effect.
These findings suggest that polymyxin B acts potently on the channel protein of Ca^{2+}-activated K^+ channels and displaces Ca^{2+} ions from their binding sites.

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I. Physiologisches Institut der Universität des Saarlandes, D-6650 Homburg/Saar

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ION CHANNELS IN THE LUMINAL MEMBRANE OF ISOLATED PERFUSED RAT CORTICAL COLLECTING DUCTS (CCD)

E. Schlatter, M. Bleich, and R. Greger

Rat CCDs were perfused in vitro and patch clamp pipettes were inserted into the lumen through the open end of the tubule. Single channel currents were recorded from the luminal membrane of the intact tubule at 37°C. In addition to the previously reported Ca-dependent K^+-channel with a conductance of ~140 pS (Proc.ASN, 383A,1989) we observed another K^+-channel with a conductance of 20-30 pS and a high open probability (P_O) in the cell attached mode. It is highly selective for K^+ over Na^+ and is inhibited by TEA^+, Ba^{++}, quinine, quinidine and verapamil. This K^+-channel is insensitive to large changes of cytosolic Ca^{++} and clamp voltages in the physiological range. These properties make it a likely candidate for the macroscopic luminal K^+-conductance of the CCD responsible for K^+-secretion. This small K^+-channel is only poorly conductive for Rb^+ and NH_4^+ and not at all for Cs^+. It was observed together with the large conductance K^+-channel, which was also colocalized with a Na^+-channel indicating that these three channels reside in the principal cells. In a few membrane patches a highly selective Na^+-channel of ~10 pS was found. This channel could be inhibited by amiloride. It probably reflects the macroscopic Na^+-conductance of the luminal membrane of the CCD. In addition an outwardly rectifying Cl^- selective channel was recorded with a conductance of 30-40 pS. Its P_O is larger at depolarized membrane voltages. The Cl^--channel blocker 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) inhibits the channel. The absence of a colocalization with Na^+ or K^+-channels suggests that this Cl^--channel may be derived from intercalated cells.

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Physiologisches Institut der Universität Freiburg, Hermann-Herder-Straße 7, D-7800 Freiburg

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Apical and basolateral membrane conductances in A6 cells

M. Granitzer, T. Leal, W. Nagel, J. Crabbé.

Confluent monolayers of a renal distal tubule cell line (A6), grown on a permeable support, were mounted in a horizontal Ussing type chamber, short-circuited and impaled with microelectrodes. Specific membrane conductances were calculated from equivalent circuit equations using amiloride (10^{-5}M) during intracellular recording. Transport properties were analyzed under control conditions and during short-term increases in basolateral $[K^+]$ from 2.5 to 20 mmol/l without or in the presence of 2 mmol/l serosal Ba^{++}.

As in most other epithelia, the apical membrane represents the major resistive barrier. Transcellular, apical and basolateral membrane conductances (g_c, g_a and g_i), obtained from 12 acceptable microelectrode studies, averaged 43, 57 and 297 $\mu S/cm^2$, respectively. There is a highly significant positive correlation between short-circuit current (I_{sc}) and g_c, whereas g_i was unrelated to I_{sc}. The I_{sc}, averaging 4.3 $\mu A/cm^2$, was almost completely blocked by amiloride. This was associated with fast hyperpolarization of the intracellular potential (V_{sc}) and increase in fractional apical membrane resistance to almost 100%.

Appreciable K^+-conductance of the basolateral membrane can be derived from the depolarization of V_{sc} after application of high $[K^+]$ or Ba^{++} to the basolateral side. Using the values of V_{sc} during amiloride at normal and high K^+, an apparent transference number of 0.72 can be calculated for K^+ at the basolateral membrane. This value corresponds with the decrease in g_i to about 30% after blockage of the K^+-channels by Ba^{++}. The nature of the remaining conductance is presently unclear. The amiloride sensitive current decreased during high K^+ and Ba^{++}. In part, this is explained by the reduction of the electro-chemical gradient for apical Na^+-uptake due to the depolarization. In addition, g_c decreased to less than 40%, which is considerably smaller than predicted by the GHK constant-field equation. Whether it results from voltage sensitivity of the apical Na^+-permeability, requires further investigation.

Département de Physiologie, Faculté de Médecine, U.C.L., B-1200 Bruxelles, Belgium and Physiologisches Institut, Universität München, Pettenkoferstr.12, 8000 München 2, FRG.

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IN CELLS EXPRESSING RAS ONCOGEN BRADYKININ AND BOMBESIN LEAD TO SUSTAINED OSCILLATIONS OF CELL MEMBRANE POTENTIAL

F. Lang, E. Kahn, F. Friedrich, M. Paulmichl, M. Hammerer, K. Maly, H. Grunicke

The products of ras oncogenes are GTP-binding-proteins which are resistant to inactivation by GTPase activating protein. The functional significance of this property and its significance for malignant transformation remains largely elusive. The present experiments have been performed to test, whether expression of ras oncogen alters electrical properties of 3T3 fibroblasts and their modification by mediators such as bradykinin (BK) or bombesin. Experiments were performed on 3T3 fibroblasts transfected with a transforming Ha-ras MMTV-LTR construct expressing the oncogen upon treatment with dexamethason (+ras). As controls served transfected cells without dexamethason (-ras) and nontransfected cells with dexamethason (oras). All cells were kept in media almost devoid of serum (0.5 %) for 48 - 72 h. In all cells, the cell membrane is hyperpolarized by calcium ionophore A 23187. Patch clamp experiments indeed reveal K^+ channels (30 pS at 0 potential), which are activated by calcium from the intracellular side. According to Fluo3 fluorescence BK leads to transient increase of intracellular calcium. In -ras and oras, BK induces a single, transient hyperpolarization (-ras: from -22 $\pm$ 2 to -48 $\pm$ 3 mV, oras: from -16 $\pm$ 2 to -39 $\pm$ 5 mV). In +ras, BK elicits oscillations of cell membrane potential from -24 $\pm$ 2 up to -59 $\pm$ 3 mV throughout the presence of the hormone. The peak of the oscillations is decreased to -31 $\pm$ 2 mV by increasing extracellular K^+ concentration from 5.4 to 20 mmol/l. The oscillations are partially blocked by 1 μmol/l verapamil and abolished by removal of extracellular calcium. The oscillations are not abolished in the presence of furosemide, amiloride or ouabain or in cells pretreated with pertussis toxin. The oscillations are elicited not only by BK but as well by bombesin. In conclusion, in cells expressing ras oncogen BK and bombesin lead to oscillations of cell membrane potential by calcium sensitive activation of K^+ channels.

Institut für Physiologie der Universität Innsbruck, Fritz-Pregl-Straße 3, A-6010 Innsbruck

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ION CHANNELS IN HUMAN MELANOMA CELLS

I. Böhm and B. Nilius

Ion channels in the membrane of cells from a human melanin producing melanoma cell line (1) (IRG 1) has been investigated with the patch clamp technique. In cell attached patches the most frequently observed channel is a potassium channel with similar properties as a delayed rectifier known e.g. in pineal cells (2) or osteoclasts (3). The channel has a single channel conductance of approximately 10 pS. The permeability ratio between Na and K is about $P_{Na}/P_K \sim 0.03$. The open probability is increased at positive potentials. Whole cell currents and averaged currents show slow activation but no inactivation. The open probability of the channel is down regulated by isoproterenol. Isoproterenol also delays activation. A second type of potassium channels shows rapid inactivation. This channel has a conductance of approximately 12 pS (A-type potassium channel). It is less frequently to observe than the delayed rectifier. Melanocytes also possesses a channel with properties similar to the inward rectifier that is activated at negative potentials. A fourth channel is a non-selective, supposingly Ca-activated cation channel. The channel has a conductance of about 19 pS and cannot discriminate between Na and K. We have not observed voltage-gated Na or Ca channels.

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Julius Bernstein Institute of Physiology, Martin Luther University, Leninallee 6, GDR 4010 Halle (Saale)

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MEFENAMIC ACID AND FLUFENAMIC ACID BLOCK THE NONSELECTIVE CATION CHANNEL IN RAT EXOCRINE PANCREAS

H. Gögelein and D. Dahlem

After stimulation with secretagogues Ca^{2+} dependent nonselective cation channels are activated in the basolateral membrane of rat exocrine pancreatic cells. This channel type was also observed in a number of other secreting epithelia, such as the salivary gland and the distal colon. Recently it was reported that the channel is blocked by 3,5'-dichlorodiphenylamine-2-carboxylic acid (DCDPC, Gögelein and Pfannmüller (1989), Pflügers Arch. 413:287). Now we investigated some drugs which are structurally related to DCDPC. Isolated pancreatic acini or single cells were prepared in the laboratory of Dr. I. Schulz in our institute. Inside-out oriented cell-excised membrane patches were obtained from the basolateral cell membrane. The nonselective cation channel was evoked by exposing the cytosolic side to NaCl-solution containing 1.3 mmol/l Ca^{2+}. The channels appeared mostly in clusters where each channel had a mean open-state probability P_o of about 0.7. Addition of either 10 μmol/l of flufenamic acid or mefenamic acid to the bath decreased P_o to about 50% (n=7 and 5, respectively), whereas 100 μmol/l of these drugs caused complete and reversible inhibition of the channel. The effect of niflumic acid was less pronounced. 10 μmol/l of this drug decreased P_o only slighy, whereas 100 μmol/l decreased P_o from .68±.07 to .21+.08 (n=5). As these drugs are know to exert anti-inflammatory and analgesic effects, we also investigated possible effects of indomethacine, ibuprofen and salicylic acid. However, no pronounced effects were detected with 100 μM of these drugs. It is concluded that the blocking effects of mefenamic and flufenamic acid are related to their specific structure, containing two phenyl rings linked by an amino bridge.

Max-Planck-Institut für Biophysik, Kennedyallee 70
D-6000 Frankfurt/Main 70, F.R.G.

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CELLULAR MECHANISMS INVOLVED IN EPINEPHRINE INDUCED HYPERPOLARIZATION OF MADIN DARBY CANINE KIDNEY (MDCK-) CELLS

F. Friedrich, M. Paulmichl, H. Weiss, S. Waldegger, E. Woell, R. Paulmichl, F. Lang

In MDCK cells epinephrine (EPI) has been shown to hyperpolarize the cell membrane in part by stimulation of phospholipase C, increase of intracellular calcium activity (Cai) and subsequent activation of calcium sensitive K^+ channels. The present study has been performed to further elucidate the intracellular mechanisms involved. To this end, conventional electrophysiology, patch clamp studies and fluorescence measurements (fura 2, BCECF) have been performed. Both, EPI and α_2 agonist BHT 920 lead to sustained increase of Cai, activation of inwardly rectifying K^+ channels (some 80 pS) and hyperpolarization of the cell membrane. In the absence of extracellular calcium EPI leads to transient increase of Cai, activation of the K^+ channels and hyperpolarization. In the absence of extracellular calcium α_2 agonist BHT 920 leads to a transient hyperpolarization of the cell membrane without increasing Cai and appreciable activation of the K^+ channels. Furthermore, in cells pretreated with phorbolester (TPA), EPI leads to a transient hyperpolarization of the cell membrane without increasing Cai. Additional studies have been performed to identify the nature of this transient hyperpolarization: The hyperpolarization is paralleled by a decrease of cell membrane resistance and an increase of the K^+ selectivity of the cell membrane. The hyperpolarization is thus the result of enhanced K^+ conductance. Accordingly, the hyperpolarization is not abolished in the presence of ouabain. In the absence of extracellular chloride and bicarbonate, the EPI induced hyperpolarization is sustained even in cells pretreated with TPA. EPI does not alter intracellular pH significantly. Thus, intracellular alkalinization cannot account for the enhancement of K^+ conductance. In contrast, the EPI induced hyperpolarization is abolished by pretreatment with both, TPA and pertussis toxin. Thus, the TPA insensitive hyperpolarization may be due to G-protein mediated activation of K^+ channels.

Institut für Physiologie der Universität Innsbruck, Fritz-Pregl-Straße 3, A-6010 Innsbruck

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REGULATION AND INTERACTION OF RAT BRAIN POTASSIUM CHANNEL PROTEINS

J.P. Ruppersberg, K.H. Schröter, B. Sakmann, *M. Stocker and *O. Pongs

Rat brain potassium channel (RCK) proteins were expressed in the membrane of *Xenopus* oocytes after injection of the corresponding cRNA into the oocyte or in Hela-cells after transfection with a vector derived from SV40 containing cDNA encoding for RCK proteins. Currents mediated by the potassium channels formed by the RCK proteins were measured with two-microelectrode voltage clamp or cell-attached patch clamp in oocytes and in the whole-cell recording mode of the patch clamp method in the transfected Hela-cells.

With these methods we tested for the effect of c-AMP and phorbolester on potassium currents under various conditions. We found that RCK1, RCK2, RCK3 and RCK4 potassium channels decreased in their activity within minutes in consequence to the dialyzation of the intracellular space by the micropipettes used for the measurements. In contrast to this decrease in RCK1, RCK2 and RCK3 but not in RCK4 the currents could be markedly increased by intracellular application of c-AMP or phorbolesters indicating a stimulation of the channels by phosphorylation. When RCK1 and RCK4 proteins were simultaneously expressed in the same cell heterooligomeric channels called RCK1,4 channels formed which had distinct functional and pharmacological characteristics. The heterooligomeric RCK1,4 channels seemed to be as insensitive to c-AMP dependent phosphorylation as the RCK4 channel.

We assume phosphorylation and changing the subunit structure to be major mechanisms for RCK potassium channel regulation in the mamalian brain.

Abteilung Zellphysiologie des Max-Planck-Instituts für medizinische Forschung, Jahnstr. 29, D-6900 Heidelberg and *Lehrstuhl für Biochemie der Ruhr-Universität Bochum, Universitätsstr. 150, D-4630 Bochum.

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DIFFERENT INTERACTION EFFECTS IN K^+-CHANNELS FOLLOWING CO-EXPRESSION OF WILD TYPE AND SHAKER MUTANT SUBUNITS OF *DROSOPHILA MELANOGASTER*.

R. Wittka & G. Boheim (1); R. Lichtinghagen, M. Stocker & O. Pongs (2)

The shaker locus of Drosophila melanogaster codes for a variety of related K^+-channel subunits, which constitute a subunit family, and of which probably each member by itself forms functional K^+-channels. All *Shaker* proteins share a common core region with 6 putative membrane spanning helices (S1-S6) but have variable NH_2-(A,B,G,D) and COOH-(1,2) termini. There is evidence that all termini face the cytoplasmic membrane side.
Shaker mutants of *Drosophila melanogaster* lack or have abnormal I_A -currents in voltage clamped muscle cells as well as abnormal action potentials with delayed repolarization in nervous tissue. One of the *Shaker* alleles, which eliminates the current completely, is Sh^{KS133} . Another mutant, Sh^{E62}, shows only 28% of wild type current. Gene dosis experiments indicated that Sh^{KS133} belongs to the antimorphic type, and furthermore they showed that the product of Sh^{KS133} significantly interferes with intact K^+-channel subunits, whereas the product of Sh^{E62} does not.
In the genomic sequence of Sh^{KS133} a single base exchange was found, which leads to an amino acid exchange of V to D within the putative extracellular loop between S5 and S6. On the other hand, a defect splicing mechanism in Sh^{E62} causes a translation stop in the class 1 terminus, while class 2 transcripts remain unaffected. By molecular-biological techniques both mutations were introduced into the corresponding cDNA templates. Transcribed cRNA and cRNA-mixtures were then injected into *Xenopus* oocytes. After channel protein expression whole cell I_A-currents were measured. In both cases I_A was completely abolished when only using one of the mutated cRNAs. Injection of 1:1 cRNA-mixtures of either Sh^+/Sh^{KS133} or Sh^+/Sh^{E62} revealed a reduction of the peak current amplitude to 22% of the expected wild type current amplitude (Sh^{KS133}) or no change of that wild type current (Sh^{E62}), respectively. Consequently, Sh^{KS133} produces a defective K^+-channel subunit, which seems to interfere with and to deactivate the normal product, whereas Sh^{E62} does not interact with the intact wild type subunit. This result is confirmed by injection of further cRNA-mixtures (3:1 , 5:1).

Lehrstuhl für Zellphysiologie (1) und Lehrstuhl für Biochemie (2) der Ruhr-Universität Bochum, Postfach 102148, D-4630 Bochum.

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RAT BRAIN AND *DROSOPHILA* POTASSIUM CHANNELS EXPRESSED IN *XENOPUS* OOCYTES ARE MODIFIED BY PROTEIN KINASE C ACTIVATORS.

C. K. Augustine, M. Stocker, O. Pongs, W. Stühmer.

We have examined the effects of various protein kinase C (PKC) activators on the functional properties of some of the K channels that have been cloned from rat brain and the Shaker gene complex of *Drosophila* (EMBO J. 7:1987-1096, 7:2457-2463 1988). PKC activators altered currents produced by the Shaker A K channel (SHA2). The phorbol ester 4βPMA (10 nM) decreased peak SHA2 current by one half and sped up the time constant of inactivation. 10 nM 4αPMA (an inactive phorbol ester) was ineffective. The diacylglycerol analog sn-1,2-diC8 (5-10 μM) mimicked these effects of 4βPMA, while sn-2,3-diC8 (an inactive diacylglycerol) was ineffective (10 μM). The protein kinase inhibitor H-7 (200 μM) increased peak SHA2 current slightly and slowed inactivation kinetics. These agents also reduced the magnitude of non-inactivating currents produced by the rat brain K channels, RCK1 and RCK5. PKC activators did not affect the kinetics or voltage dependence of activation of these currents. These results suggest that the function of several K channels is under the influence of phosphorylation by PKC.

Max-Planck-Institut für biophysikalische Chemie, Abteilung Membranbiophysik, Am Faßberg, D-3400 Göttingen and Ruhr-Universität Bochum, Lehrstuhl für Biochemie, D-4630 Bochum.

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SINGLE CHANNEL RECORDINGS OF ONE TYPE OF NA CHANNEL AND MULTIPLE K CHANNELS IN MYELINATED AXONS

P. Jonas, M. Hermsteiner and W. Vogel

The tibial nerve of the toad *Xenopus* was desheathed and the myelinated fibres were treated for 135 min with 3 mg/ml collagenase in Ringer's and for 35 min with 1 mg/ml protease at 22 °C. Axons with retraction of the myelin sheath were patch-clamped in the nodal and paranodal region with 30-80 MΩ pipettes (Jonas et al. 1989, PNAS 86: 7238-7242). In Ringer's current events are found which reverse sign at the calculated reversal potential for Na^+ ions. They are blocked by tetrodotoxin. The single channel conductance is 11 pS (15 °C). Averaged events show the typical activation and inactivation kinetics of macroscopic Na currents. Three potential-dependent K channels were also identified (I-, F-, and S-channel). The I-channel, which is the most frequent type has a single-channel conductance of 23 pS (inward current, 105 mM K on both sides of the membrane) and activates at potentials between -60 and -30 mV. It deactivates with intermediate kinetics and is blocked by 50-500 nM dendrotoxin. The F-channel has a conductance of 30 pS, activates between -40 and 60 mV, and deactivates with fast kinetics. The former inactivates within tens of seconds, the latter within seconds. The third type, the S-channel has a conductance of 7 pS, and deactivates slowly. All three channels can be blocked by external tetraethylammonium ions. Three components of macroscopic K current have been described recently (Bräu et al. 1990, J. Physiol. 420: 365-385). We suggest that the I-, F- and S-channels form the basis for these current components.
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Physiol. Inst. Aulweg 129, D-6300 Giessen, FRG

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SINGLE POTASSIUM CHANNEL PROPERTIES IN MOUSE CULTURED SCHWANN CELLS.

BY DOROTHE HOPPE, HELMUT KETTENMANN, AND ALEXEJ N. VERKHRATSKY*.

Cultured Schwann cells are characterized by a strong outward rectification of the membrane with a threshold close to the resting membrane potential of about -50 mV. With the patch-clamp technique we identified single channels with a conductance of 10 - 12 pS; the kinetic behavior of these channels can account for the two different types of membrane current components recorded from different cells in the whole cell recording configuration: while some single channels displayed inactivation, other were non-inactivating. Moreover, the time constant of averaged single channel and whole-cell current inactivation were similar and both showed a voltage dependency. These channels are K^+ selective since changes in extracellular $[K^+]$ resulted in changes of the reversal potential as predicted for an exclusively K^+ selective pore. The reversal potentials also predicted an intracellular $[K^+]$ of 60 mM indicating that the K^+ equilibrium potential is slightly negative to the membrane potential. We conclude that cultured Schwann cells express either two types of K^+ channels with similar conductance or a channel which can aquire two functional states and that these channels can account for the membrane currents observed in this cell.

Department of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 364, Heidelberg, West Germany.

**A.A.Bogomoletz Institute of Physiology, Bogomoletz St. 4, Kiev - 24,GSP 252601,Ukraina,U.S.S.R.*

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A RISE IN $[K^+]_o$ SHIFTS THE ACTIVATION CURVE OF THE DELAYED RECTIFIER K^+ CHANNELS IN CULTURED MOUSE SCHWANN CELLS: A POSSIBLE MECHANISM FOR K^+ HOMEOSTASIS.

BY ALEXEJ N. VERKHRATSKY*, D. HOPPE AND HELMUT KETTENMANN.

Cultured mouse Schwann cells are characterized by the expression of an outward rectifying K^+ channel as previously described. In this study we analyzed the effect of the K^+ gradient on channel properties applying the patch-clamp technique in the whole cell and cell attached configuration. In normal $[K^+]_o$ (5.6 mM), channels are activated at potentials more positive than -50 mV thus close to the resting membrane potential. When $[K^+]_o$ was elevated, the threshold of activation shifted to more negative values; in 145 mM $[K^+]_o$ the threshold of activation was -75 - -80 mV mV. Moreover the steepness of the activation curve increased with increasing of extracellular $[K^+]$. The sensitivity of the K^+ channel for $[K^+]_o$ was only apparent at normal intracellular K^+ levels (60 mM); with high intracellular $[K^+]$ (140 mM) channel gating was not affected. This property of the Schwann cell K^+ channel may serve to facilitate the uptake of K^+ in times of activity induced elevations.

Department of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 504, Heidelberg, West Germany.

**A.A.Bogomoletz Institute of Physiology, Bogomoletz St. 4, Kiev - 24,GSP 252601, Ukraina, U.S.S.R.*

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PEPTIDE MEDIATED INACTIVATION OF A POTASSIUM CONDUCTANCE IN LOCUST SKELETAL MUSCLE: INVOLVEMENT OF A SECOND MESSENGER?

K.E. Zittlau and C. Walther

In locust jumping muscle both proctolin (Arg-Tyr-Leu-Pro-Thr) and YGGFMRFamide (Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH_2) lower the resting membrane conductance (by up to -50%) by inactivation of a not voltage dependent, ohmic K^+-conductance (Murck et al., in: *Dynamics and plasticity of neuronal systems*, eds. N. Elsner and W. Singer, p. 433, Stuttgart:Thieme Verlag, 1989). Half-maximal effects are achieved with *ca.* 10^{-11}M and $5*\ 10^{-9}$M, respectively. Intracellular injection under voltage clamp of the known G-protein activator GTPγS renders the response to either peptide irreversible. In order to find out whether the peptides act *via* a secondary messenger and not directly *via* G-protein(s) the effects of various bath-applied drugs were investigated. Neither 8-bromo-cAMP nor 8-bromo-cGMP (10^{-4}M) nor the respective phophodiesterase blockers 3-isobutyl-1-methylxanthine (IBMX, 10^{-4}M) and Na-nitroprusside (10^{-3}M) had an effect on the peptide response or the resting membrane K^+-conductance. 10^{-7}M TPA (12-O-Tetradecanoyl-phorbol-13-acetate), an activator of protein kinase C (PKC), induced a slowly progressing reduction of the resting K^+-conductance while 10^{-7}M 4α-phorbol (12,13-didecanoyl; not supposed to activate protein kinase C) was without effect. The opening probability of a K^+-channel (ca. 100 pS maximum conductance; gating and conductance not markedly voltage sensitive) which is a good candidate for the peptide sensitive conductance was not obviously affected by bath application of either peptide during cell-attached recording. These results point to an involvement of PKC as a mediator for the action of these peptides on the resting K^+-conductance.

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Physiologisches Institut der Universität Marburg, Deutschhausstr.2, D-3550 Marburg

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ACTIVATION OF ATP-SENSITIVE K^+ CHANNELS FROM HUMAN SKELETAL MUSCLE: A NEW METHOD ALLOWS SINGLE CHANNEL RECORDING WITHOUT ENZYME TREATMENT

H.Hatt, C.Franke and S.Quasthoff+

Up to now, recordings with the patch clamp technique from vertebrate skeletal muscle were possible only after enzyme treatment. We report here measurements from membrane blebs formed on skeletal muscle fibres obtained from specimens of routinely performed open muscle biopsies from patients. Only a small amount of material was necessary. The blebs were induced by only mechanical irritation of the fibres without any enzyme treatment. After excision of inside-out pat-ches, single channel activity could be regularly observed in symmetrical solution (in mmol/l: K^+ 140, Ca^{++} 10^{-8}, Cl^- free). The conductance was 20 pS. The channels were reversibly blocked by ATP (> 0.2 mmol/l) or Glibencamide (> 1 µmol/l) applied to the sarcoplasmic side. The block was concentration dependent and the ATP-block could be relieved by addition of EMD 52692 (>1µmol/l), one of the recently characterized K^+ channel openers (Quast and Cook, TIPS 10:431-435, 1989). If K^+ was replaced by Na^+, single channel amplitude decreased, indicating selectivity for K^+ ions of the channel. These data indicate that the recently reported enhancement of membrane K^+ conductance (Quasthoff et al., Pflügers Arch. 414:179, 1989) by EMD 52692 in human skeletal muscle is based on the direct activation of ATP sensitive K^+ channels. Furthermore, membrane blebs are seen to contain channel proteins and the method will allow further studies of normal and diseased membranes of human skeletal muscle.

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Physiologisches Institut der TU München, Biedersteinerstr.29, D-8000 München 40
+Physiologisches Institut der LMU München,

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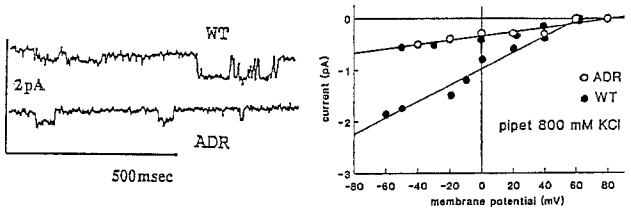
HEREDITARY MYOTONIA IN THE MOUSE INVOLVES QUALITATIVE CHANGES OF SARCOLEMMAL POTASSIUM CHANNELS. A PATCH CLAMP STUDY.

E. Wischmeyer and H. Jockusch

Like in myotonias of man, the hyperexcitability of the muscle of the myotonic ADR mouse is caused primarily by a reduction of chloride conductance to <10% of the wildtype (Mehrke et al., Muscle and Nerve **11**, 440-446, 1988). In the allelic MTO mutant an additional reduction of the potassium conductance by 50% has been demonstrated (Bryant et al., Soc. Neurosci. Abstr. **13**, 1681, 1987).
Using the patch clamp technique, we have recorded single channel currents of enzymatically dissociated toe muscle fibres from normal and myotonic mice. In the cell attached mode we found two types of the inwardly rectifying potassium channels, with current amplitudes of 0.3 and 0.6 pA at resting potential. Whereas both types of channels were present in wildtype fibres, only the 0.3 pA channel was found in ADR muscle. This finding was confirmed on inside out patches and explains the lowered K^+-conductance in myotonic mouse muscle.



A qualitative change of K^+-channels in addition to the lowered chloride conductance points to a generalised membrane defect in myotonic muscles, as has been suspected by several authors (cf. Kuhn & Seiler, Klin. Wochenschr. **48**, 1134-1136, 1970; H. Brinkmeier, Doctoral Thesis, Bielefeld 1988). Supported by DFG, SFB 223.

Lehrstuhl für Entwicklungsbiologie, Universität Bielefeld, Postfach 8640, D-4800 Bielefeld 1, Fed. Rep. Germany

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ANOXIA GENERATES A TIME INDEPENDENT K CURRENT IN ISOLATED CARDIOCYTES OF GUINEA PIG

M. Friedrich, K. Benndorf, G. Bollmann, Hj.Hirche

Ionic currents in single cardiocytes of guinea pig were measured under anoxic conditions ($pO_2 < 0.5$ torr). After variable periods of delay (2-32 min), time independent outward currents developed which reached after 20 to 70 sec a maximum of 3 to 5 nA (0 mV). The current-voltage (IV) relationship of the net current under anoxia was studied between -100 and +90 mV. At physiological and increased (18 mM) extracellular K concentrations it intersected the abscissa at voltages corresponding to the resting potentials of the cells of -88 ± 2 mV and -55 ± 2 mV, respectively. The IV relationships were perfectly linear between -100 mV and +30 mV and negative to the reversal potential much more flat than under control conditions. Within the first minute after the first extra outward current had appeared, reoxygenation led in more than 90% of the cells after three seconds to a complete disappearance of the outward current. Ca currents were not affected at the time of the appearance of extra outward currents.

The results show that anoxia-induced outward current is mainly carried by K ions through K_{ATP} channels which open at ATP concentrations below 1 mM (Noma and Shibasaki, J. Physiol. 363, 463-480 (1985)). At a metabolic blockade these channels not only cause a shortening of the action potential but might also mediate the resting potential. A corresponding decrease of ATP concentration may appear at the time of sufficient glycolytic impairment due to an adenine nucleotide disturbance. The normal Ca channel function is not affected by this degree of ATP depletion.

Institut für Vegetative Physiologie, Universität zu Köln, Robert-Koch-Str. 39, 5000 Köln 41.

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Single channel properties of Opossum Kidney cells (OK).

F.Hollunder-Reese, M.Bleich, M.Mohrmann, R.Greger

OK cells were grown on glass cover slips in DMEM/10%FCS in an atmosphere of 5% CO_2 and 95% air. After two days the cells were subconfluent. A coverslip was transferred into a bath chamber mounted on an invertoscope. Standard patch clamp techniques were used. Most experiments were done in excised inside/out patches.
Two types of K^+-channels (big and intermediate conductance) and nonselective as well as (Cl^-)-channels were found. The big K^+channel was Ca^{2+} dependent and weakly pH dependent. The channel had a mean conductance of 166 ±12 (n=16) pS at clamp voltage Vc=0mV (pipette KCl/bath NaCl). This channel was blocked reversibly by Ba^{++}, $\sim 10^{-5}$ mol/l, n=5. The voltage dependence of this block suggests that Ba^{2+} acts from the cytosolic side. Verapamil $\sim 10^{-5}$ mol/l and quinidine $\sim 10^{-5}$mol/l blocked reversibly. TEA 10^{-2}mol/l blocked only from the outside. The intermediate conductance K^+ channel had a mean conductance of 63 ±13 pS, n=4, (Vc=0mV), and was Ca^{++} independent. Unlike the big K^+ channel, this channel was also observed in cell attached configuration (n=3). In some excised membrane patches both types of K^+-channel coexisted (n=4). One other type of channel (most likely Cl^--channel) had a mean conductance of 77 ±14 pS g(+), and 48 ±9 pS g(-), n=4 and was blocked reversibly by 5-nitro-2-(3-phenyl-propylamino)-benzoate (NPPB) 10^{-5}mol/l. Supported by DFG Gr 480/9 and Mo/398/3-1.
Physiologisches Institut und Kinderklinik der Albert-Ludwigs-Universität, Hermann-Herder-Str.7, D-7800 Freiburg.

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LOCALIZATION, CHARACTERIZATION, AND RECONSTITUTION OF CHLORIDE CHANNELS FROM MAMMALIAN SKELETAL MUSCLE

S. Weber-Schürholz[1], H. Jockusch[1], T. Schürholz[2], M. Akabas[3], D. Landry[3], Q. Al-Awqati[3]

Membrane vesicles were prepared from rabbit skeletal muscle and separated by sucrose gradient centrifugation. Fractions obtained (in the order of increasing density) were outer sarcolemma (SL), T-tubules (TT), sarcoplasmatic reticulum (SR), triads and mitochondria. SL and TT were characterized by high specific binding capacity for ^{3}H-saxitoxin (Na-channel) and ^{3}H-PN 200 (DHP-receptor), respectively. Highest activity in potential sensitive ^{36}Cl-transport and binding of the chloride channel ligand, ^{3}H-indanyloxyacidic acid (IAA 94; Landry, D. et al., Science **224**, 1469, 1989), were found in the SL. We conclude that chloride channels are predominantly localized in the SL and not in the TT as previously proposed (Dulhunty, A., J. Membrane Biol. **45**, 293, 1979).
SL vesicles were solubilized with n-octyl-ß-glucopyranoside and subjected to IAA sepharose affinity chromatography. Bound protein was eluted with 100µM IAA 94 and either analyzed by SDS gel electrophoresis or reconstituted into planar lipid bilayers. The eluate contained a selected set of polypeptides and yielded highly specific chloride channels with four different conductance levels.
These findings may lead to an understanding of myotonia, a hereditary disease affecting chloride conductance.
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[1]Developmental Biology and [2]Physicochemistry Units, University of Bielefeld, 4800 Bielefeld, FRG, [3]College of Physicians and Surgeons, Columbia University, New York, USA.

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DOES IP_4 PLAY A ROLE AS SECOND MESSENGER IN OOCYTES OF XENOPUS LAEVIS?

J.R. Schwarz, C.K. Bauer, A.H. Guse[1], G. Gercken[1], W. Meyerhof[2]

In oocytes previously injected with poly(A)+RNA from cloned anterior pituitary cells (GH_3 cells), receptors of thyrotropin-releasing hormone (TRH) could be expressed. Binding of TRH to these receptors activates a signal transduction pathway in which the formation of inositolphosphates plays an important role (Meyerhof et al. PNAS 1988). Inositol(1,4,5)trisphosphate (IP_3) is believed to directly release Ca^{2+} from an intracellular pool. The function of inositol(1,3,4,5)tetrakisphosphate (IP_4), which is also formed, is still uncertain.

Injection of oocytes with IP_3 elicited Ca^{2+}-dependent chloride currents. These currents could be blocked by co-injection of heparin, which also blocked the TRH-induced current response. Injection of the (2,4,5)IP_3-analogue resulted in pronounced membrane currents, indicating the activation of the IP_3 receptors. Recently we have shown that injection of IP_4 also elicits membrane currents, distinct from those produced by IP_3. This suggests that IP_4 plays a role as second messenger (Mahlmann et al FEBS Lett 1989). In order to exclude the possibility that the "IP_4 effects" were induced by IP_3 formed secondarily from IP_4 by 3-phosphatase activity, we measured the metabolites present following injection of [^{3}H]IP_4. The methods for measuring inositol phosphates were as described in Guse et al. (Biochem J 1989). The main finding was that no [^{3}H](1,4,5)IP_3 could be detected after injection with [^{3}H]IP_4, indicating that significant formation of (1,4,5)IP_3 from IP_4 does not occur. These results support the hypothesis that IP_4 directly functions as a second messenger. Its physiological role, however, remains to be determined.

Physiologisches Institut, Universität Hamburg, Martinistraße 52, D-2000 Hamburg 20, Inst. für Biochemie und Lebensmittelchemie[1], Inst. für Zellbiochemie und klin. Neurobiologie[2], Univ. Hamburg

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EVIDENCE FOR A Cl^- STIMULATED HCO_3^- CHANNEL IN FUSED MDCK CELLS

W. Steigner, H.-J. Westphale, U. Kersting and H. Oberleithner

Madin-Darby-canine-kidney (MDCK) cells in culture resemble properties of intercalated (IC) cells of the cortical collecting duct. The cells transport acid and base equivalents depending on the specific metabolic conditions. Fused MDCK cells can regulate their volume during hypotonic stress by concomitant net efflux of K^+ and HCO_3^- ions. This control mechanism depends on the presence of extracellular HCO_3^- and Cl^- and cannot be inhibited by the stilbene derivate DIDS. Furthermore, measurements of cytoplasmic pH and cell membrane conductance reveal the participation of an electrogenic HCO_3^- transport. Using the patch-clamp technique, we performed single channel measurements in the excised mode (inside/out configuration) with symmetrical HCO_3^- concentrations in the bath medium (24 mM $NaHCO_3$, 110 mM Na-gluconate, 5% CO_2, pH 7.4) and in the pipette solution (24 mM $NaHCO_3$, 220 mM mannitol, 1 mM NaCl, 5% CO_2, pH 7.4). We can disclose a channel with the following characteristics: i) a conductivity of about 17 pS; ii) mean open and closed times of 16 and 5 ms, respectively; iii) an open probability (P_o) of about 0.1 in the physiological range of the membrane potential; P_o increases up to 0.4 at depolarized voltages; iv) a reversal potential near 0 mV; a permeability ratio of HCO_3^- over Cl^- of >2. In presence of a high Cl^- concentration (110 mM) in the bath medium (cytoplasmic face) the channel is activated (P_o doubles compared to controls).

Based on the existence of a voltage and Cl^- sensitive HCO_3^- channel we postulate that an increase of intracellular Cl^- induced by cell depolarization enhances P_o of the HCO_3^- channel. This couples HCO_3^- efflux to Cl^- influx. This mechanism can contribute to cell volume and cell pH regulation.

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Dept. of Physiology, University of Würzburg, Röntgenring 9, 8700 Würzburg, F.R.G.

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DIFFERENT TYPES OF BLOCKERS FOR EPITHELIAL CHLORIDE CHANNELS

M. Tilmann, K. Kunzelmann, R. Greger

The aim of the present study was to examine the potency of various compounds which are known to inhibit Cl^- transport pathways. The blockers were tested in patch clamp experiments on Cl^- channels in excised inside/out membrane patches of cultured HT_{29} colon carcinoma cells and respiratory epithelial cells. The properties of these Cl^- channels have been described in previous reports. They have (i) a conductance of 50-70 pS with (ii) outward rectification and are independent of (iii) cytosolic calcium and (iv) pH. All compounds tested, although of entirely different chemical structure, reduced the open probability (Po) of the examined Cl^- channels reversibly by inducing flickering. The half maximal concentration for inhibition ($Po_{50\%}$) of the different blockers were derived from the dose-response curves. The obtained sequence of the $Po_{50\%}$ values was: 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) $7*10^{-7}$ < amidine[1] $3*10^{-6}$ < indanyloxyacetic acid (IAA)[1] $6*10^{-6}$ < 4,4'-dinitrostilbene-2,2' disulfonic acid (DNDS) 2*10-5 < 4,4'-diisothio cyanatostilbene-2,2'-disulfonic acid (DIDS) 3*10-5 = 4-acetamido-4-isothiocyanato-stilbene-2,2-disulfonic acid (SITS) 3*10-5. The present data do not permit any conclusions as to the mechanism of interaction with the Cl^- channel. Supported by DFG Gr 480/9.

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Physiologisches Institut der Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg

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ANION CURRENT RECORDED FROM THE INNER MITOCHONDRIAL MEMBRANE OF BROWN ADIPOCYTES

T. Klitsch & D. Siemen

Due to the chemiosmotic theory the inner mitochondrial membrane should be impermeable to small inorganic ions. Nevertheless ion fluxes have been described by several groups. Recently Sorgato et al. found an ion channel in rat liver cell mitochondria (Nature 330: 498 - 500, 1987). The authors discussed whether this channel could have the same function as the uncoupling protein of brown adipocytes. This protein uncouples the respiratory chain from ATP-synthesis thus effecting nonshivering thermogenesis. It can be blocked by purine di- and trinucleotides.

We used mitoblasts (swollen mitochondria with removed outer membrane) from brown adipose tissue of 6 - 8 week Sprague-Dawley rats. Mitochondria were isolated according to the method of Cannon and Lindberg (Methods Ezymol. 55: 65 - 78. 1979) by multiple slow (800 x g) and fast (10,000 x g) centrifugation steps. Current recordings were done by means of the patch clamp method (Hamill et al., Nature 391: 85 - 100, 1981) in the "mitoblast-attached" and in the "whole-mitoblast" mode.

Our results show a 110 pS channel with similar kinetics as observed in liver cells. With K-gluconate inside the pipette no anion inward current was observed. We do not see this channel very often in mitoblast-attached patches but we see it regularly in the whole-mitoblast mode. It can be blocked by 20 µM GDP and by 20 µM GMP as well but not by 20 µM cyclic GMP. From this we conclude that the 110 pS channel is an anion channel. It is present in brown fat cells as well as in liver cells. As the uncoupling protein is known not to be blocked by GMP it is most likely that the anion current flowing through the here described 110 pS channel is not identical with the shunt current causing nonshivering thermogenesis.

Physiologisches Institut, Universität Giessen, Aulweg 129, D - 6300 Giessen, FRG

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ZINC SELECTIVELY BLOCKS ONE SUBUNIT COMBINATION OF CLONED AND RECONSTITUTED RAT GABAa-RECEPTORS

A.Draguhn(1),T.A.Verdoorn(1),D.B.Pritchett(2), P.H.Seeburg(2) and B.Sakmann(1)

Cultured human fibroblasts were transiently transfected with cDNA clones encoding rat $\alpha 1$, $\beta 2$ and $\gamma 2$ subunits of GABAa-R (γ-amino-butyric-acid-receptor). GABA-activated currents in cells cotransfected with cDNAs encoding different combinations of GABAa-R subunits were measured with patch clamp whole cell technique (symmetrical 140mM Cl^-).

In cells cotransfected with cDNAs encoding $\alpha 1$- and $\beta 2$- subunits the currents were strongly blocked by micromolar concentrations of Zn++ in a non-competitive manner. At 10μM GABA the current amplitude was reduced by 10 μM Zn++ to 31 ± 15% of control (n=8).

Cotransfection of cells with cDNAs encoding the $\alpha 1$ and $\gamma 2$-subunits or $\alpha 1$-, $\beta 2$- and $\gamma 2$-subunits resulted in a much lower sensitivity to 10μM Zn++ at 10μM GABA: for the $\alpha 1$-,$\gamma 2$-combination the amplitude was 97 ± 12% of control(n=11), for the $\alpha 1$-,$\beta 2$-,$\gamma 2$-combination the current under 10μM Zn++ was 92 ± 14% of control (n=4).

We conclude that the block of GABAa-R by divalent cations depends on the subunit composition of the GABAa-R channel. Receptors composed of $\alpha 1$- and $\beta 2$-subunits are susceptible to block by Zn++, GABAa-R containing a $\gamma 2$-subunit are much less susceptible.

1- MPI fuer medizinische Forschung, Jahnstr.29,D-69 Heidelberg
2- ZMBH,INF 282, D-69 Heidelberg

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BENZODIAZEPINE-PHARMACOLOGY OF RAT GABAa-RECEPTORS RECONSTITUTED FROM CLONED $\alpha 1$ AND $\beta 1$ SUBUNITS

A.Draguhn(1), P.Malherbe(2), H.Möhler(2), and B.Sakmann(1)

Xenopus oocytes were injected with cRNAs encoding $\alpha 1$- and $\beta 1$-subunits of rat GABAa-R(γ-amino-butyric-acid-receptor). After functional expression of GABA-gated Cl^--channels the effect of ligands of the Benzodiazepine-Receptor (BzdR) on current amplitudes was studied.

Benzodiazepine-agonists potentiated the currents in 60% of the 31 cells tested (e.g. 45 ± 21% enhancement by 10μM diazepam, n=8).The reason for the failure of 40% of the oocytes to be potentiated remains unclear.

The competitive BzdR-antagonist Ro 15-1788 surprisingly also enhanced the GABA-activated currents. 10 μM Ro 15-1788 potentiated the response to 2 and 10 μM GABA by 72 ± 12% (n=4). Ro 15-1788 also did not inhibit but further increased the potentiation induced by BzdR-agonists.

The "inverse" BzdR-agonist DMCM also enhanced GABA-activated currents (10μM DMCM induced 51 ± 28% potentation at 1-10μM GABA, n=7).

We conclude that GABAa-R reconstituted from rat $\alpha 1$ and $\beta 1$ subunits contain a BzdR that acts atypically on GABA-induced Cl^--currents.

1-MPI f. med.Forschung,Jahnstr.29,D-69 Heidelberg
2-Research Dpt.,F.Hoffmann-La Roche,Basel,CH

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GLUTAMATE RECEPTORS WITH RAPID OR SLOW TIME COURSES OF RESENSITIZATION

C.Franke, H.Hatt, J.Dudel

Using a liquid filament device for quick solution changes, we have recently shown that glutamate (glu) pulses activate and desensitize quisqualate sensitive excitatory channels completely within 2-5 ms (Dudel et al., Pflügers Arch. 411:291, 1988, Dudel at al., Biophys. J., in press, 1989). We report here the time course of recovery from the desensitized state after removal of glu from the receptor. Pairs of pulses of glu were applied with varying interpulse intervals. The first pulse desensitized the channels completely and the relative amplitude of the response to the second pulse was used to determine resensitization. In crayfish muscle, there is no resensitization up to 1 ms interval. Between 1 and 2 ms intervals, the gradient of recovery is very steep, and at 5 ms interval recovery is complete. The time course of resensitization thus is S-shaped and in its steep section as rapid as desensitization. This time course is not affected by the duration of glu application. A similar type of resensitization behaviour was found also in locust muscle, but another type showed a much slower time course of resensitization. At least 1 s was required for 50 % resensitization and complete recovery was achieved only after 5s (with Ramsey and Usherwood). A wide range of S-shaped resensitization time courses was also observed in embryonic chick spinal cord motoneurons (with Smith and Julie Rosenheimer), activated by quisqualate and kainate, respectively. The highly differentiated de- and resensitization characteristics may serve as effective frequency filters for transmission.

Physiologisches Institut der TU München, Biedersteinerstr.29, 8000 München 40

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DEVELOPMENTAL INCREASE IN THE APV-BLOCKABLE PART OF GLUTAMATE INDUCED WHOLE-CELL CURRENTS IN NEURONES OF RAT PRIMARY VISUAL CORTEX

P. Stern and B. Sakmann

Thin-slices (150-200 μm) of primary visual cortex (area 17) were prepared from Wistar rats in the first three postnatal weeks according to the method of Edwards et al. (Pflüger's Archiv, 1989, 414). Visually identified neurones from layer 4 were patch-clamped in the whole-cell recording mode. Cells were voltage-clamped at a holding potential of -30 mV and voltage-ramps of ±80 mV were applied. Bath-application of 20μM Glutamate resulted in a large increase in the whole-cell currents. In normal Ringer's solution containing 1mM Mg^{2+} a part of this current showed a region of negative slope conductance typical for the NMDA-receptor subpopulation of the glutamate receptors. Addition of 20μM APV blocked this component completely. Under Mg^{2+}-free conditions chord conductances at -80 mV and +50 mV and the slope conductance at 0mV were compared in 20μM glutamate with and without 20μM APV. The percentage of the APV-blockable glutamate-induced currents increased in the first postnatal days from approximately 40% to over 75%. After postnatal day 11 until the end of the third postnatal week this percentage stayed above 75% of the whole current and in some cells exceeded 90%.

In conclusion the percentage of the NMDA-receptor subpopulation of glutamate receptors increases in the neurones of layer 4 of rat visual cortex in the first 12 postnatal days and then stays at this level until the end of the third week.

Abteilung Zellphysiologie des Max-Planck-Instituts für medizinische Forschung
Jahnstraße 29, D - 6900 Heidelberg

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RESPONSES TO EXCITATORY AMINO ACIDS IN NEURONS ACUTELY ISOLATED FROM SPINAL CORD SLICES OF ADULT RATS

F.Zufall, J.Rosenheimer*, C.Franke, D.O.Smith* and H.Hatt

Up to now, desensitization kinetics and pharmacological properties of glutamate receptors of α-motoneurons were studied on embryonic neurons. We report here patch-clamp recordings from aged neurons differentiated in vivo. Spinal cord of adult rats was immersed in ice-cold Hepes buffered saline and cut into 400 µm thick cross sections. Sections were then introduced to a chamber containing bicarbonate-buffered saline (pH 7.4, 37°C) with constant stirring. Following a 30 to 60 min incubation, the medium was replaced with 0.1% trypsin and incubated for an additional 35 min. Single cells were isolated from these slices by mechanical treatment. Cells were allowed to settle in the petri-dish for about 1 h before patch-clamp recordings were made. In whole-cell recordings, large inward currents were elicited in response to pressure applied glutamate, quisqualate, NMDA or kainate with different time courses of desensitization. NMDA-gated currents were rapidly activated and desensitized slowly and incompletely. If Mg^{++} ions were present extracellularly, the channels were blocked by hyperpolarization. Quisqualate-gated currents desensitized to zero current level with a time constant of a few ms. In contrast, no desensitization was observed with the agonist kainate. In outside-out patches, fast application of quisqualate rapidly activated desensitizing channels with characteristics similar to those of embryonic neurons.

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Physiologisches Institut der TU München, Biedersteinerstr. 29, D-8000 München 40
* Department of Physiology, University of Wisconsin, Madison, WI 53706 USA.

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GLUTAMINERGIC RESPONSES IN NEUROPILE GLIAL CELLS AND RETZIUS NEURONES OF THE MEDICINAL LEECH

R. Dörner, K. Ballanyi* and W.-R. Schlue

Glial cells have glutamate receptors similar to those found in neurones (e.g. Sontheimer et al., Glia 1;328, 1988). We have used double-barrelled, ion-sensitive microelectrodes to investigate the effects of glutaminergic agonists on membrane potential (Em) and intracellular ion-activities in neuropile glial cells and in Retzius neurones of isolated leech segmental ganglia. In both types of cells, bath-application of l-glutamate (Glu), kainate (Ka) and quisqualate (Qui) elicited concentration-dependent depolarizations accompanied by increases of the intracellular Na^+ activity (aNai) and by concomitant decreases of the intracellular K^+ activity (aKi). In neuropile glial cells, these alterations of aNai and aKi were preceded by a transient decrease of aNai and an increase of aKi upon administration of Ka and Qui. These initial aNai and aKi transients might be due to glial uptake of neuronal K^+ released during the action of Ka and Qui. As found for Ka, the neuronal and the glial responses to glutaminergic agonists persisted during inhibition of synaptic transmission in solutions containing high Mg^{2+} and low Ca^{2+}. In both types of cells, Retzius neurones and neuropile glial cells, N-methyl-D-aspartate (NMDA) did not affect Em, aNai or aKi.
These results indicate that leech neuropile glial cells have a Ka-prefering non-NMDA glutamate receptor similar to that in the Retzius neurones.

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Inst. f. Zoologie, Lehrstuhl f. Neurobiologie, Heinrich-Heine-Universität, Universitätsstr. 1, D-4000 Düsseldorf, F.R.G.
*Zentr. Physiol. und Pathophysiol., Universität Göttingen, Humboldtallee 23, D-3400 Göttingen, F.R.G.

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PROPERTIES OF A NON-SELECTIVE CATION CHANNEL IN HUMAN VASCULAR ENDOTHELIUM

B. Nilius

In endothelial cells obtained from human umbilical chord, ion channels have been studied by the patch clamp method. Besides an inward rectifying potassium channel and a high conductance Ca-activated potassium channel, a non-selective cation channel has been recorded. This channel is activated by µM concentrations of histamine and nM concentrations of thrombin. Open probability is voltage independent and shows a slow run-down in cell-attached patches. After excision, channel activity runs down to zero open probability in less than 4 min. However, under these conditions the single channel conductance was unchanged and allowed a detailed permeation analysis. The survival time is prolonged in the presence of GTP S. With symmetrical potassium concentrations, the single channel conductance is 28 pS and the reversal potential is 0 mV. With 140 mM Na and 5 mM K in the pipette, the conductance is 26 pS. A reversal potential of -1.5 mV was measured. With 60 mM Ca and 70 mM Na in the pipette, 140 mM K in the bath, the reversal potential was -10.5 mV. The single channel conductance is 5.4 pS measured from inward currents with 110 mM Ca (pipette) and 140 mM K (bath). From the analysis of the reversal potentials, a permeation ratio of K:Na:Ca = 1:0.9:0.2 was calculated. These data refer to the existence of a Ca permeable non-selective cation channel in human endothelial cells that can be activated by agonists as thrombin and histamine. The channel might be a tool to induce a sustained agonist mediated Ca influx.

Julius Bernstein Institute of Physiology, Martin Luther University, Leninallee 6, PSF 302, DDR-4010 Halle (Saale)

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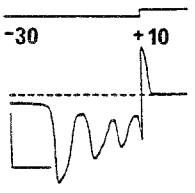
MEMBRANE POTENTIAL AFFECTS THE AGONIST INDUCED $[Ca^{2+}]_i$-MEDIATED Cl⁻-CURRENT IN GUINEA-PIG HEPATOCYTES

B. Kalthof and L. Pott

Stimulation of isolated guinea-pig hepatocytes with the α_1-adrenergic agonist phenylephrine (Phe, 1 µM) causes an increase of the membrane conductance for K^+ and Cl^- (Capiod et al. FEBS Lett **217**: 247, 1987) most likely mediated by a rise in $[Ca^{2+}]_i$ via IP_3-mediated Ca^{2+}-release from internal stores and Ca^{2+}-entry. We have measured the isolated Cl⁻-current using whole-cell patch-clamp in order to obtain information on mechanisms of $[Ca^{2+}]_i$-regulation. Ca^{2+}-dependent K^+-current was blocked by Cs^+ on both sides of the membrane. Upon superfusion with Phe an inward current was recorded at -30 mV holding potential. This current relaxed within several seconds. In some cells the transient increase in Cl⁻-conductance was followed by oscillatory changes of g_{Cl}, which is in line with previous $[Ca^{2+}]_i$-measurements using aequorin (Woods et al. Cell Calcium **8**: 89, 1987) or fura-2 (Kawanishi et al. J. Biol. Chem. **264**: 12859, 1989). The Cl⁻-current could be switched off by voltage steps to positive membrane potentials (Fig. 1). As this effect of depolarization occurred with a delay of several seconds, a direct action of voltage on g_{Cl} seems unlikely. Hyperpolarization of the cell had the opposite effect: it delayed relaxation of the current and, in the presence of Phe, could initiate a second Cl⁻-current. The effect of hyperpolarization was not observed prior to stimulation with Phe. Similar results as with Phe were obtained with extracellular application of ATP (10^{-4} M). Our results support the hypothesis that Ca-entry in hepatocytes is facilitated by hyperpolarization and is reduced or abolished by depolarization.



Effect of depolarization on slow oscillation of Cl⁻-current in the presence of Phe (10^{-6} M). Calibration: 400 pA; 50 s.

Institut für Zellphysiologie, Ruhr-Universität Bochum, Postfach 102148, D-4630 Bochum, FRG // Supported by the DFG (FG KONZELL)

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BIOPHYSICAL MODELS TO DESCRIBE STRUCTURAL DETERMINANTS OF CONDUCTANCE AND SELECTIVITY IN THE NICOTINIC ACETYLCHOLINE RECEPTOR

C.Busch, T. Konno

Xenopus laevis oocytes were used to express injected wild type or mutagenized cDNA of the TORPEDO Acetylcholine receptor (AChR). Three clusters of negatively charged amino acid residues bordering the transmembrane segment M2 of the AChR were identified as determinants of the K+ conductance, selectivity among alkali cations and sensitivity to Mg++ block. Substitution of amino acid residues in these anionic rings resulted in the reconstitution of channels with altered permeability and conductance sequences. We concluded that the charged rings constitute sites of interaction between the channel and the cations. (Imoto et al., Nature, 1888, 335, p.645).
Simulations with barrier models help to understand which equilibrium or kinetic parameters of ion transport could be altered by the mutations. The simulations show that the data are consistent with the assumption of a small negative fixed charge on the extracellular channel mouth and two cation binding sites in the narrow part of the channel, separated by a high energy barrier. Within this model, a mutation may - depending on its location - alter the fixed charge, the binding strength at the sites or the energy barriers of the channel. Most of the mutant data can be described with a model in which only a few parameters, chosen according to the mutant position, are altered.

Max-Planck Institut fuer medizinische Forschung, Jahnstr. 29, D-69oo Heidelberg, FRG

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SINGLE CHANNEL RECORDINGS ON INSECT OLFACTORY RECEPTOR CELLS

H.Hatt, F.Zufall, M.Stengl[+]

The initial steps of the transduction mechanism in olfaction by which odorant binding produces the activation of ion channels are still unknonwn. Recently, a primary long-term culture system of Manduca sexta antennal cells was developed and olfactory neurons were identified with two monoclonal antibodies, one of which recognizes specifically pheromone sensitive neurons (Hishinuma et al., J.Neurosci 8:296-315, 1988).
Using the patch clamp technique, three types of K^+ and at least one type of Na^+ channels could be identified. Na^+ channels recorded in the whole cell mode could be reversibly blocked with 0.1 μM TTX. The predominantly observed K^+ channel type was voltage dependent with a conductance γ of 30 pS. It could be blocked by application of nucleotides (ATP > cGMP > cAMP) to the cytoplasmic side of the membrane. Secondly, a Ca^{++} dependent K^+ channel (γ=66 pS) with low voltage sensitivity was characterized. Third, a transient type of K^+ channel (γ=15 pS) could be activated by depolarizing voltage steps. Cells that had been in culture for at least 18 days could be stimulated with pheromone (bombycal) or female gland extract: An unspecific cation channel is activated and K^+ channel activity was mediated by second messengers. These results indicate that the olfactory neurons differentiate in culture and provide the basis for further studies of pheromone effects.

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Physiologisches Instiut der TU München, Biedersteinerstr.29, 8000 München 40
[+] Univ.of Arizona, ARL Div of Neurobiol., Tucson, AZ 85721, USA

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A23187 AND MELITTIN ACTIVATE A CHLORIDE CONDUCTANCE IN PRIMITIVE RED CELLS OF THE CHICK EMBRYO

Ch. Reinhardt, W. Kaiser and R. Baumann

It has previously been demonstrated that the membrane potential of primitive embryonic red cells is dominated by a proton conductance and the chloride/bicarbonate exchange by band 3 protein in these cells is impaired (Engelke et al. 1988, J. Cell. Physiol. 135,87). In consequence there is a disequilibrium between chloride and proton distribution; the calculated chloride equilibrium potential is around -15 mV whereas E_m is -44 mV at days 4 of development. We have extended our measurements to earlier stages of development, where red cells are in the log growth phase. At resting conditions the E_m values were between -40 to -50 mV and not changed by variation of external sodium, chloride or potassium; stimulation of cells with ATP, db-cAMP, db-cGMP and PMA also did not alter the E_m. However incubation with A23187 or melittin caused a fast, long lasting depolarisation of about 20 to 30 mV, bringing the E_m close to the chloride equilibrium potential.
This depolarisation was not inhibited when cells were treated with DIDS, or when external sodium or calcium were removed, but it was never observed in the absence of external chloride. As the size of the depolarization corresponds to what one observes when cells are treated with the chloride ionophor tributyl-tin we conclude that A23187 and melittin stimulation activates a chloride channel. It may be involved in the volume regulation of proliferating embryonic red cells and can only be activated in the presence of external Cl^-.

Ch. Reinhardt, Physiologisches Institut, Universität Regensburg, 8400 Regensburg, supported by DFG Ba 691/4

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AN ANION CHANNEL FORMING ACTIVITY FROM CLAVIBACTER MICHIGANENSE SUBSP. NEBRASKENSE

T. Schürholz, M. Wilimzig, R. Eichenlaub, E. Neumann

Clavibacter michiganense subsp. nebraskense (Vidaver et al. Int. J. Sys. Bacteriol. 24:482-485, 1974) is a Gram-positive, coryneform bacterium, which is a pathogen of maize. Infection with C. m. subsp. nebraskense causes leaf freckles and wilting (Goss wilt) in the host plant.
Addition of culture medium to the aqueous phase of a planar bilayer chamber, caused formation of ion channels in the lipid membrane. The insertion of the channel component was not voltage dependent. However, the channel conductance showed a two fold voltage dependence. (i) Single channnel conductance increased with voltage; at negative voltage (neg. on the side of insertion) no conductivity could be detected. (ii) The probability of channel opening increased with voltage. The channels were strongly selective for anions, Cl^- > $KSCN^-$ > SO_4^- and unpermeable for gluconate. The incorporated channels were sensitive to protease K.
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Lehrstuhl für Biophysikalische Chemie, Universität Bielefeld, Postfach 8640, D-4800 Bielefeld 1, FRG

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DEVELOPMENT OF A SEROTONIN-INDUCED Cl^- CONDUCTANCE IN IDENTIFIED EMBRYONIC RETZIUS CELLS FROM THE MEDICINAL LEECH

V. LESSMANN, K. GOTTMANN and I.D. DIETZEL

Our aim is to study how neurons differentiate to different subtypes, characterized by diverse receptors and ion channels. Such studies are complicated in many systems by the finding that development of electrical excitability starts very early in embryogenesis, at a time when neuronal subtypes can hardly be identified by morphological criteria.
Here we report a method that allows one to record from identified embryonic Retzius cells in Hirudo Medicinalis. These cells start to take up monoamines as soon as neurites begin to sprout. Following incubation (4 hours) in 300 μM autofluorescent 5,7-dihydroxytryptamine, 50 μM iproniazide, and 30 μM ascorbate added to normal medium (+ 12% FCS) embryonic Retzius cells were visualized in the middle of the forming ganglia. After dissociation in a solution containing 45 mM Mg^{2+}, stained cells could be identified among the largest cells in the culture dish.
Cells identified by this method retained their ability to generate action potentials. Using a single-electrode voltage clamp connected to an electrode allowing internal dialysis, serotonin-(200μM) induced currents were recorded, which reversed at the equilibrium potential for Cl^-. Cl^- currents of more than 10 pA were observed starting with the 10th day of development at 24°C. At this time, voltage-gated ion currents and action potential activity had already developed. Apart from an increase in amplitude serotonin-induced Cl^- currents in embryonic cells were similar to those from freshly cultured adult Retzius cells.

Max-Planck-Institut für Psychiatrie, Abt. Neurophysiologie, Am Klopferspitz 18A, D-8033 Planegg-Martinsried

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KINETIC ANALYSIS OF EXCITATORY POSTSYNAPTIC CURRENTS IN HIPPOCAMPAL GRANULE CELLS

B.U. Keller, Y. Yaari and A. Konnerth

Excitatory synaptic transmission mediated by glutamate receptors was investigated by applying the patch-clamp technique to visually identified neurones in thin *in vitro* hippocampal slices (Edwards et al., Pflügers Archiv, 414, 600-612). Excitatory postsynaptic currents (EPSCs) were evoked in granule cells by field stimulation of the perforant path in the presence of 10μM bicuculline. NMDA mediated EPSCs were isolated and quantified by bath application of 5μM CNQX, which is a specific antagonist for glutamate receptors of the quisqualate/kainate type. Peak NMDA-EPSC amplitudes increased with depolarization, displaying a negative slope conductance at membrane potentials between -70 and -25mV. Perfusing the preparation with Mg^{2+}-free saline eliminated the region of negative slope conductance, in accordance with the physical model of Mg^{2+} blocking NMDA receptor channels. The decays of NMDA-EPSCs were best described by a sum of two exponential functions. Both decay rates depended on membrane voltage, being attenuated by depolarization in an exponential manner (-83 and -121mV / e-fold change, respectively). A significant voltage dependence of these rates persisted even after washout of Mg^{2+} from the bath, indicating that both Mg^{2+}-dependent and Mg^{2+}-independent processes contributed to this voltage sensitivity. On the molecular level, these currents were mediated by single channels with a main conductance level of 50pS and channel properties comparable to NMDA receptor channels in cultured cells. By comparing these results with glutamatergic responses in different neuronal tissue, the functional implications for excitatory synaptic transmission will be discussed.

Arbeitsgruppe Zelluläre Neurophysiologie, Max-Planck-Institut für biophysikalische Chemie, Am Fassberg, 3400 Göttingen, F.R.G.

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GABA-INDUCED CHLORIDE CURRENTS IN 293-CELLS TRANSFECTED WITH SUBUNITS OF THE $GABA_A$-RECEPTOR

K. H. Backus, T. Giller, F. Knoflach, P. Malherbe, P. Pflimlin, G. Trube

The $GABA_A$-benzodiazepine receptor complex is thought to be an assembly of α-, ß- and γ-subunits which exist in a number of isoforms. To investigate electrophysiological differences between various subtypes and subunit combinations, the corresponding cDNAs derived from a rat brain library were subcloned in expression vectors and expressed in the human embryonic kidney cell line 293. Membrane currents were measured using the whole-cell configuration of the patch clamp technique. 1) Cotransfections of ß1 with either α1, α3 or α5 cDNAs lead to receptors sensitive to GABA but insensitive to benzodiazepine receptor agonists. 2) When cells were transfected with the α3ß1γ2 combination the Cl^- current was significantly potentiated by flunitrazepam (10 μM) in 16 out of 30 GABA-sensitive cells (10 μM GABA for 10 s). However, the degree of potentiation varied between 1.2- and 5.6-fold (mean = 2.5 ± 1.4). The inverse agonist ßCCM (1 μM) reduced the GABA-induced current (100 μM GABA) to 0.8-fold (± 0.2) of the control in 8 out of 9 cells. 3) In another triple combination, α1ß1γ2, the GABA-induced current (10 μM GABA) was potentiated 1.3-fold (± 0.2) by flunitrazepam in 6 out of 8 cells. Our results confirm previous findings that the γ-subunit is important for the benzodiazepine sensitivity of the $GABA_A$-receptor Cl^-channel. A variable degree of expression of the γ-subunit might be the reason for the large variations in the amplitude of the flunitrazepam effect.

Pharmaceutical Research Department, F. Hoffmann-La Roche AG, P.O.B., CH-4002 Basel

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DIFFERENTIAL BENZODIAZEPINE PHARMACOLOGY OF RECOMBINANT $GABA_A$ RECEPTORS

G. von Blankenfeld, S. Ymer, D.B. Pritchett, H. Sontheimer, M. Ewert, P.H. Seeburg and H. Kettenmann

Recently the primary sequence of several different GABA receptor subunits was determined by molecular cloning. In this study, we compared GABA activated currents and their modulation by benzodiazepines in cultured human cells transfected with cDNA coding for different $GABA_A$ receptor subunits. Flunitrazepam, a benzodiazepine agonist which potentiates GABA responses in both neurons and astrocytes was only effective in receptor subtypes containing the γ2 subunit (α1ß1γ2 and α5ß1γ2). The ß-carboline DMCM decreased GABA activated currents mediated by GABA receptors composed of α1ß1γ1 and α1ß1γ2 subunits as described for the neuronal $GABA_A$ receptor but increased GABA activated currents via receptors containing the α5 subunit (α5ß1γ1 and α5ß1γ2). A similar result was also observed with astrocytes.
These results suggest that flunitrazepam and DMCM do not act on isosteric sites and that differences in the responsiveness of $GABA_A$ receptors to these compounds are based on different subunit compositions of $GABA_A$ receptors. It further suggests that the previously observed difference in the responsiveness of astrocytes and neurons to the benzodiazepine agonists flunitrazepam and DMCM could be accounted for by differences in their GABA receptor subunit composition.

Neurobiologie der Universität Heidelberg, Im Neuenheimer Feld 345, 6900 Heidelberg.

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SYNAPTIC CURRENTS IN IDENTIFIED HORMONE SECRETING CELLS OF THE RAT PITUITARY.

R. Schneggenburger and A. Konnerth

Hormone secreting cells of the intermediate lobe of the pituitary (IL) are innervated by terminals of neurones originating in the hypothalamus. Whole cell and single channel currents were recorded in visually identified IL cells in thin pituitary slices, by using a modified version of the method described by Edwards et al. (*Pflügers Archiv 414, 1989*). Afferent fiber stimulation induced inhibitory postsynaptic currents (i.p.s.cs) with a latency of 1-2 ms. These i.p.s.cs, recorded in symmetrical Cl^- solution, had a linear I/V relation, reversed around 0 mV and were blocked by bicuculline (K_D = 50 nM), indicating that the activation of $GABA_A$-receptor is mediating the i.p.s.cs. At room temperature and a holding potential of -60 mV the i.p.s.cs had amplitudes of -100 to -500 pA and lasted 100 to 150 ms. They had a fast onset (rise time to half amplitude about 1 ms) and a decay which could be well fitted by the sum of two exponentials, having time constants of ~20 ms and ~50 ms, respectively. GABA-induced currents were enhanced by flunitrazepam (1 µM), while Zn^{2+} and glutamate, known to modulate GABAergic synapses in other systems, did not affect GABA-mediated responses. Addition of 0.5 µM GABA to outside-out patches from IL cells induced single channel currents corresponding to two main conductances of about 14 and 26 pS. The strong synaptic input from hypothalamic neurones to IL cells suggests a powerful central regulation of hormone secretion in the pituitary. The data indicate that this synapse shares some functional similarities with known neuron-neuron GABAergic synapses of the central nervous system.

AG Zelluläre Neurophysiologie, Max-Planck Institut für biophysikalische Chemie, 3400 Göttingen, FRG.

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STABILISATION AND INDUCED REDUCTION OF INTERCELLULAR COMMUNICATION IN ISOLATED PAIRS OF CULTURED MAMMALIAN CELLS

D. Paschke and D.F. Hülser

The alteration of electrical coupling via gap junctions by cAMP, ATP, Ca^{2+}, retinoic acid and by antibodies against liver 26 kD and 21 kD gap junction proteins (connexins - Cx32 and Cx26) was measured with the double whole cell patch clamp method in isolated pairs of BRL (Buffalo Rat Liver) and FL (human amnion) cells. Junctional conductance was monitored throughout the experiment by applying a square pulse of 10 mV at 0.02 Hz to one cell and measuring the resulting current in the other cell. From these records the time course of gap junctional uncoupling was determined during a time span of up to 90 min. Under normal experimental conditions, the cells showed spontaneous uncoupling, where the junctional conductance decreased to about 10% of its initial value after 22 ± 4 min in BRL and after 39 ± 12 min in FL cell pairs.

Filling the patch pipette with buffered saline containing 1 mM db-cAMP and 5 mM ATP results in a stable conductance of about 30% of the initial value after 60 minutes. Addition of 10^{-5}M Ca^{2+} to the pipette solution and 0.5 • 10^{-4}M retinoic acid in the bath medium blocks electrical coupling completely within < 5 min.

With anti-Cx32-antibody in a stabilising pipette solution we achieved 10% coupling in 23 ± 9 min in BRL cell pairs (n=10) while with anti-Cx26-antibody a 10% value was reached after 30 min in two of seven experiments. The results with FL cells point in the same direction.

Biologisches Institut, Abt. Biophysik, Universität Stuttgart, Pfaffenwaldring 57, D-7000 Stuttgart 80, FRG.

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SLOW WHOLE-CELL RECORDING OF MEMBRANE CURRENT AND POTENTIAL DURING REGULATORY VOLUME DECREASE

J.Ubl, H.Murer & H.-A. Kolb

Using the slow whole-cell recording technique, we measured the membrane current and potential, while simultaneously monitoring the size of single Opossum kidney (OK) cells. After a hypotonic shock a transient depolarisation could be measured, which was paralleled by a transient increase of membrane current and of the derived membrane conductance. The maximal cell size was reached within 5-10 minutes. The depolarisation as well as the membrane conductance adopted their maximal values within the same time range. After 20-25 minutes the cells recovered their original cell size. This regulatory volume decrease (RVD) could also be observed in Ca^{2+}-free hypotonic bath solutions. Under this conditions the time course of RVD as well as the change of membrane conductance was slowed down. Addition of 1mM quinine to the isotonic bath solution caused a sustained depolarisation and a decrease of membrane conductance. A hypotonic shock in the presence of 1mM quinine induced a further depolarisation and an increase of membrane conductance. A delayed RVD could be observed. After 40-50 minutes the cells adopted their original size. Addition of 0.1 mM DIDS to the hypotonic bath slowed down the time course of depolarisation and the change of membrane conductance. The relative change of membrane potential and of conductance was significantly reduced compared to control conditions. RVD could be suppressed by DIDS. The data indicate, that a quinine sensitive as well as a quinine insensitive potassium current and a DIDS blockable anion current are involved in RVD of OK-cells.

Universität Konstanz, Fakultät für Biologie, Universitätsstraße 10, D-7750 Konstanz

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KINETIC CHARACTERIZATION OF ENDOGENOUS CELL-TO-CELL CHANNELS BETWEEN PAIRED XENOPUS LAEVIS OOCYTES

J. Ströttchen and G. Boheim

Oocytes from Xenopus laevis frogs were paired after removal of follicel cell and vitelline layers. The dual voltage-clamp technique was used to measure the junctional cell-to-cell conductances. For reference both cells were clamped to the same holding potential. Stepwise changes of the holding potential of only one cell lead to the appearence of an additional current I_j, which corresponds to ion flow between the cells via gap junction channels in the area of membrane contact. The junctional conductance λ_j is defined as the ratio of I_j to the transjunctional voltage (difference of holding potentials) V_j.

After pairing a steady increase of λ_j was observed with a time delay of 4-5 h. 7 h after pairing 11 cell pairs out of 53 had maximum conductances of λ_j = 0.4-2.0 µS, whereas in the other cell pairs the conductance did not exceed a value of λ_j = 0.05 µS. Steady state conductances were symmetrical with respect to both polarities of transjunctional voltage V_j. Maximum λ_j was observed at V_j=0. Increasing V_j lead to a decrease in λ_j, whereby a constant residual conductance of 15-20 % of λ_j remained at high V_j. The time course of cell-to-cell channel inactivation could be described by the sum of two exponential functions. The amplitude of the slow current relaxation exhibited a nonlinear voltage dependence. These properties were independent of the value of the reference holding potential and depended only on V_j.

The complex relaxation amplitude pattern is interpreted in terms of two slow and one fast inactivation processes. At low V_j (20-30 mV) gap junction channels between paired frog oocytes are partially inactivated by a fast and a slow process, which are directly coupled. At higher V_j (40-50 mV) a further slow inactivation of λ_j occurs.

Lehrstuhl für Zellphysiologie, Ruhr-Universität Bochum, Postfach 102148, D-4630 Bochum.

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ELECTRICAL PROPERTIES OF RAT HEPATOCYTES IN PRIMARY CULTURE

F. Wehner, D. Guth and R.K.H. Kinne

In rat hepatocytes grown on gas-permeable membranes (E. Petzinger, et al., In Vitro Cell. Dev. Biol. 24: 491-499,1988) we measured cellular and canalicular potentials and input resistances with double-barelled electrodes. In bicarbonate-containing solutions we found -30.7±1.2 mV (mean ± SEM, n = 37) and -13.9±1.4 mV (n = 22) for cell and canalicular membrane potentials, respectively. Canalicular input resistance was 31.1±4.7 MΩ (n = 22). There was no dependence of these parameters on culture age. Cellular input resistances, however, continuously decreased from 32.3±3.4 MΩ at 2 hours (n = 11) to 8.2±2.1 at 3 days after preparation (n = 6). In ion substitution experiments there were no changes in membrane conductances to K^+, Na^+, and Cl^- that could account for this effect. Cable analysis, however, revealed that the apparent increase in membrane conductance reflects a time-dependent increase in electrical coupling between cells. This coupling was in part sensitive to heptanol (2 mM) but insensitive to octanol (0.5 mM) and 8-Br-cAMP (1 mM). We conclude that for a quantitative analysis of transport mechanisms in cultured cells changes in the degree of cell-cell interaction have to be considered.

Max-Planck-Institut für Systemphysiologie,
Rheinlanddamm 201, 4600 Dortmund 1, FRG

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SINGLE CHANNEL PROPERTIES OF GAP JUNCTION CHANNELS BETWEEN ISOLATED CELL PAIRS

D. Paschke, R. Eckert and D. F. Hülser

Properties of single gap junction channels were investigated in isolated pairs of BRL (Buffalo Rat Liver), FL (human amnion) and PLC (human hepatoma) cells with the double whole cell patch clamp technique.

In BRL and FL cell pairs conductance steps of single channels were observed after spontaneously occuring reductions of gap junctional conductance from more than 20 nS to about 500 pS. PLC cells usually show less initial junctional conductance, single channel events, therefore may be recorded immediately after establishing the double whole cell configuration.

From these records we conclude that the conductance steps during opening or closing of single gap junctional channels are between 25 - 35 pS and 45 - 60 pS in PLC and 45 - 55 pS and 75 - 90 pS for BRL and FL cells, respectively. Larger conductance steps have also been recorded, yet with a very low frequency.

Furthermore, we investigated the influence of retinoic acid and of db-cAMP, both modulators of cell-cell communication, on the properties of single gap junction channels.

Our Experiments indicate that at the single channel level neither retinoic acid nor cAMP significantly influence the conductivity of gap junctions. Preliminary results let us assume that the decrease of cell-cell coupling by retinoic acid may be due to an alteration in the channel kinetics. We can not exclude, however, the possibility of different unit conductances and varying sensitivities to agents such as retinoic acid, cAMP, Ca^{2+} or transjunctional voltage.

Biologisches Institut, Abt. Biophysik, Universität Stuttgart, Pfaffenwaldring 57, D-7000 Stuttgart 80, FRG.

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DESIGN AND OPTIMAL TUNING OF SINGLE AND DOUBLE ELECTRODE VOLTAGE CLAMP SYSTEMS USING METHODS OF OPTIMIZATION BY MODULUS HUGGING

H.-R. Polder and D. Swandulla

Voltage clamp instruments are closed loop control systems using electronic feedback. Modern control theory provides a large variety of solutions for the design and optimal tuning of feedback systems.

Control systems which are composed only of delay elements can be optimized easily by adequate shaping of the "frequency characteristic magnitude": Using controllers with a proportional-integral characteristic (PI-controllers) it is possible to force the modulus of the frequency characteristic *F(jw)*, associated with the transfer function *F(s)* (output to input ratio in the frequency domain) to be as close as possible to one over a wide frequency range ("modulus hugging"). This means that the controlled variable very rapidly reaches the value required by the command variable. The method provides:

1. Control with steady state errors below 0.5%,
2. System stability (no ringing, predictable overshoot),
3. Easy calculation of system parameters and performance (settling time, overshoot, etc.) from the time constants of the control chain according to standard optimization rules ("absolute value optimum" and "symmetrical optimum"),
4. System design using standard graphs and tables,
5. Easy tuning of the controller settings with step commands.

The "absolute value optimum" (AVO) provides the fastest response to a command step with very little overshoot while the "symmetrical optimum" (SO) has the best performance compensating intrinsic disturbance signals.

We have adopted this method for the design and optimal tuning of voltage clamp systems, since their various components (microelectrodes, buffer amplifiers, differential amplifiers etc.) can be described as delay elements (first or second order delays). These systems have time constants in the range of microseconds. In the case of a time-sharing single electrode clamp system, an additional dead-time element caused by the sample and hold amplifiers was considered, which also was approximated by a first order delay with a time constant related to the reciprocal of the switching frequency. These "small" time constants were added to an equivalent time constant T_e (1-100 µs). The cell capacity was considered as an integrating element with a time constant T_m, which is always at least one order of magnitude greater than T_e.

Max-Planck-Institut für Psychiatrie, Abteilung Neurophysiologie,
Am Klopferspitz 18a, 8033 Martinsried

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EFFECTS OF NYSTATIN AND QUINIDINE IN STUDIES ON AMMONIUM ION TRANSPORT ACROSS RUMEN EPITHELIUM.

D. Bödeker, Susanne Hoppe and H. Höller.

Previous studies in nystatin action on biological and artificial membranes indicated that this polyene antibiotic produces small aqueous channels which permit penetration of small monovalent ions. It has been shown that in different epithelia after treatment of the mucosal barrier with such pore-forming antibiotics the remaining electrophysiological characteristics reside mostly in the basolateral membrane.

To study electrophysiological characteristics and the effects of K^+ and NH_4^+ on the basolateral membrane of sheep rumen epithelium we applied 400 U/l nystatin on the mucosal side in Ussing-type chambers. At this concentration nystatin caused a distinct increase of short circuit current (I_{sc}) after application which reached after 60 min a stable plateau 6 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ above that of untreated controls.

The elevated I_{sc} returned to control levels when ouabain was added to the serosal solution, giving evidence that an electrogenic activity of the Na^+/K^+-ATPase at the basolateral membrane was responsible for this increase.

In nystatin treated epithelia addition of K^+ or NH_4^+ resulted in an increase of I_{sc} which was more than threefold higher compared to untreated epithelia. The I_{sc} increase caused by K^+ was more than 3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, and I_{sc} stayed constantly at this level over 20 min while an NH_4^+ induced increase of I_{sc} was transient with an initial step of 1 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. All currents could be blocked rapidly and completely by addition of quinidine (1 mmol/l).

Physiologisches Institut der Tierärztlichen Hochschule Hannover, Bischofsholer Damm 15, D-3000 Hannover 1

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GTP-INDUCED FUSION OF MEMBRANE VESICLES FROM RAT PANCREATIC ENDOPLASMIC RETICULUM, MEASURED WITH FLUORESCENCE DYES AND LIGHT SCATTERING METHODS

W. Hampe, H. Ruf and I. Schulz

Evidence suggests that GTP controls the Ca^{2+}-conveyance between intracellular Ca^{2+} pools. The non-hydrolysable GTP-analog GTPγS does not show these effects (Ghosh et al., Nature 340:236, 1989). Inositol-1,4,5-trisphosphate releases Ca^{2+} from a pool, which is filled with Ca^{2+} via a Ca^{2+}/H^+-countertransporter at the expence of an H^+-gradient, that is established by an H^+-pump using ATP but not GTP as substrate (Thévenod et al., J Membrane Biol 107, 2263-2275, 1989).

We have investigated if GTP induces fusion of vesicles from rat pancreatic endoplasmic reticulum (ER) using light scattering (90°, 640 nm), fluorescence dequenching, and -energy transfer methods. Addition of GTP (10^{-5} mol/l) to ER-vesicles in the presence of the membrane fusogene polyethylene glycol (PEG, 3% w/v) decreased light scattering by 10% and increased fluorescence as measured with the fluorescent membrane probes octadecylrhodamin (R18) and 5-(N-octadecanoyl)-aminofluorescein (F18) by 20% ± 2.5% S.E. Previous addition of ATP (5 mM) increased the GTP-effect to 38.5% ± 3.9% S.E.

GTPγS had no effect on its own and inhibited the GTP induced signals. The protonophore carbonylcyanide-m-chlorophenylhydrazone (CCCP 10^{-5} mol/l) decreased the GTP induced fluorescence increase from 38,5% to 19%, whereas the H^+-ATPase inhibitors 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-Cl 10^{-5} mol/l) and N-ethylmaleimid (NEM 10^{-4} mol/l) abolished the GTP-effect. Omission of Ca^{2+} from the incubation buffer had no effect on the size of the GTP induced signal.

The data indicate that GTP induces fusion of ER-vesicles in which a proton gradient might be involved.

Max-Planck-Institut für Biophysik, Kennedyallee 70,
D-6000 Frankfurt/M. 70

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GTP-INDUCED Ca^{2+} MOVEMENTS IN INOSITOL 1,4,5-TRISPHOSPHATE-SENSITIVE AND -INSENSITIVE INTRACELLULAR Ca^{2+} POOLS FROM RAT PANCREATIC ACINAR CELLS

M. Dehlinger-Kremer, T. Ozawa and I. Schulz

In previous studies we have shown the existence of at least two nonmitochondrial intracellular Ca^{2+} pools: an inositol 1,4,5-trisphosphate (IP_3)-sensitive Ca^{2+} pool (IsCaP) which takes up Ca^{2+} via a Ca^{2+}/H^+ countertransporter at the expense of an H^+ gradient established by an H^+ pump; and an IP_3-insensitive Ca^{2+} pool (IisCaP) which takes up Ca^{2+} via a vanadate-inhibitable Ca^{2+}ATPase (F. Thévenod et al., J Membrane Biol 109: 173, 1989). GTP is proposed to control Ca^{2+} conveyance between intracellular Ca^{2+} pools by forming Ca^{2+} carrying junctions between membranes (Ghosh et al., Nature 340:236, 1989). We have investigated the mechanism for GTP-induced Ca^{2+} uptake and Ca^{2+} release in a fraction from isolated endoplasmic reticulum of rat pancreatic acinar cells, using $^{45}Ca^{2+}$ and a Ca^{2+} macroelectrode.

In the presence of oxalate (10^{-2} mol/l) and the membrane fusogene polyethylene glycol (PEG 3%) GTP (10^{-5} mol/l), but not GTP-γ-S, induces overshooting Ca^{2+} uptake followed by Ca^{2+} release. This Ca^{2+} release is higher in the presence of vanadate (10^{-4} mol/l) indicating that Ca^{2+} reuptake occurs by a vanadate-inhibitable Ca^{2+} pump that compensates in part for Ca^{2+} release. In the presence of the IP_3 analogue inositol 1,4,5-trisphosphorothioate (IPS_3, $3x10^{-5}$ mol/l) or of the Ca^{2+} release agent caffeine ($2x10^{-2}$ mol/l) GTP-induced overshooting Ca^{2+} is abolished or decreased, respectively. With GTP-γ-S the caffeine effect was very small. The data indicate that GTP, but not GTP-γ-S, activates communication between both IsCaP and an IisCaP, the latter showing a caffeine-sensitive Ca^{2+}-induced Ca^{2+} release mechanism.

Max-Planck-Institut für Biophysik, Kennedyallee 70,
D-6000 Frankfurt am Main 70

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DIFFERENT MECHANISMS OF INHIBITION OF SODIUM PUMP IN *XENOPUS LAEVIS* OOCYTES BY DIACYLGLYCEROL ANALOGUES AND PHORBOL ESTER

L. Vasilets*, G.Schmalzing, K.Mädefessel, H. Biel & W.Schwarz

The effects of activation of protein kinase C by phorbol 12-myristate 13-acetate (PMA), 1,2-dioctanoyl-sn-glycerol (diC_8) and 1-oleoyl-2-acetyl-sn-glycerol (OAG) on sodium pump current, membrane capacitance, ouabain binding and ^3H-inulin uptake in Na-loaded *Xenopus* oocytes were studied. Na/K pump current was monitored as difference in steady-state membrane current, produced by changes from 3 mM K-containing to K-free solution in the presence of K-channel blockers. Protein kinase C activators caused a gradual decline in sodium pump current. PMA (50 nM) was the most potent inhibitor, producing a 80% reduction of pump current. A comparable degree of pump inhibition by diacylglycerols required a 1000-fold higher molar concentration. All three compounds decreased the number of ouabain binding sites on the cell surface in proportion to current inhibition. Staurosporine, an inhibitor of protein kinase C, abolished this effect, whereas H7 was ineffective.

Reduction of pump current and ouabain binding sites by PMA and OAG were accompanied by a reduction of total membrane surface, estimated from measurement of membrane capacitance. The PMA-induced increase of ^3H-inulin uptake suggests that the reduction of cell surface area was brought about by endocytosis. In contrast to PMA and OAG, diC_8 did not stimulate endocytosis, as judged from the lack of effect on membrane capacitance and inulin uptake. Independent of the type of kinase C activator, ouabain binding sites lost from the cell surface were recovered after permeabilization of cellular membranes with digitonin plus 0.02% SDS.

The findings suggest that the inhibitory effect of protein kinase C activators on sodium pump current may involve a selective internalization or direct inhibition of surface sodium pumps in addition to a nonspecific removal of pump molecules by endocytosis.

*Permanent address: Inst. of Chem. Phys., Chernogolovka; Noginsky dist.; 142 432 Moscow region (USSR)
MPI für Biophysik, Heinrich-Hoffmann-Str. 7, 6000 Frankfurt

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NH_4^+/NH_3 TRANSPORT PATHWAYS ACROSS THE CELL MEMBRANE OF *XENOPUS LAEVIS* OOCYTES

B. C. Burckhardt, N. Stallmach and E. Frömter

Exposure of cells to 20 mmol/l NH_4Cl has widely been used in studies of cellular acid/base regulation to acidify the cytoplasm (Roos and Boron, Physiol. Rev. 61: 296, 1981). Usually, in such experiments cell pH (pH_i) rises immediately after NH_4Cl exposure and falls only when extracellular NH_4Cl is removed so that NH_3 can leave the cell. When performing such experiments in *Xenopus laevis* oocytes, however, we noticed that after a transient alkalinization pH_i fell already during NH_4Cl exposure within 10 min from 7.50 SD ± 0.12 to 7.01 ± 0.13 (n=13) and remained there within the first minutes after returning to control solutions. Simultaneously, cell membrane potential (V_m) collapsed from -58.9 ± 7.9 to -3.3 ± 5.8 mV, and resistance (R_m) from 3.3 ± 1.2 to 0.5 ± 0.4 MΩ (n=19), but reached control values again when NH_4Cl-free solution was perfused. These data suggested that, in contrast to most other cells, NH_4^+ influx predominated over NH_3 influx, leading to cytoplasmic liberation of H^+ and diffusion of NH_3 into yolk lipids. To identify the presumed NH_4^+ uptake pathways we have used K^+ channel blockers such as Ba^{2+}, quinidine (each 1 mmol/l), tetraethylammonium or Cs^+ (each 20 mmol/l), as well as amiloride, bumetanide (each 1 mmol/l), and ouabain (0.1 mmol/l) to block Na^+/H^+ exchange, $NaKCl_2$ cotransport and $(Na^+ + K^+)$-ATPase. However, none of these drugs prevented or inhibited neither pH_i fall nor V_m and R_m collapse, except ouabain which reduced the fall of V_m by 13.5 ± 8.1% (n=3). In contrast, La^{3+}, diphenylamino-2-carboxylate (DPC), and 4-chloromercuribenzoate (pCMB) (each 1 mmol/l) strongly attenuated the pH_i fall and almost completely eliminated the V_m and R_m response to NH_4Cl exposure. These observations suggest that NH_4^+ enters the oocytes preferentially along non-selective cation channels, presumably the stretch-activated cation channels which had been observed in oocytes previously (Methfessel et al. Pflügers Arch. 407: 577, 1986; Taglietti et al. J. Physiol. 407: 311, 1988).

Zentrum der Physiologie, J. W. Goethe-Universität, Theodor-Stern-Kai 7,
D-6000 Frankfurt/Main 70, FRG

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DEPENDENCY OF Na/K/2Cl-COTRANSPORT ON VOLUME AND INTRACELLULAR MAGNESIUM OF HUMAN RED CELLS

H.Mairbäurl and J.F.Hoffman

The purpose of this study was to analyze the relation of Na/K/2Cl-cotransport (cotransport) with steady-state cell volume (MCV). We measured cotransport in unfractionated and density separated red cells (DSC) of different MCV's from different donors to see if cotransport differences contribute to differences in the distribution of MCV's in the red cell populations. Also the effects of different cellular Mg concentrations and of acutely altered cell volume (using osmotic and isoosmotic techniques) were examined. Cotransport was determined as the bumetanide (10 µM) sensitive ^{22}Na-efflux in the presence of ouabain (50 µM) after adjusting cellular Na (Na_i) and K_i by the nystatin technique to achieve V_{max} for cotransport.

We found a significant inverse correlation between MCV and cotransport in both unfractionated red cells and DSC at V_{max} of cotransport. MCV was correlated directly with red cell 2,3-DPG, whereas total red cell Mg was very similar in various cell fractions indicating that intracellular free Mg (Mg_i) might be lower in red cells with high 2,3-DPG (i.e. high MCV) and vice versa. Altering Mg_i with the ionophore A23187 showed a high Mg_i-sensitivity of cotransport: Depletion of Mg_i inhibited and an elevation of Mg_i activated Na/K/2Cl-cotransport. The K_m for Mg_i corresponds to a medium Mg concentration of about 0.06 mmol/l, set in the presence of A23187. Thus it appears likely that differences in Mg_i among populations of red cells contribute to differences in cotransport activity and MCV.

Measurements of cotransport following acute changes of cell volume showed that cell shrinkage activates, whereas swelling inhibits cotransport. In DSC this response to immediate volume changes was most pronounced in the fraction of cells with the lowest density. When cell volume was changed osmotically, cotransport was higher in the shrunken cells independent of Na_i (5 to 80 mmol/l, reciprocally altered with K_i). The effect of volume persisted, however, when cell volume was altered isoosmotically after appropriate adjustment of Na_i and K_i by the nystatin technique. Further, the response of cotransport to acute volume changes appears to occur independently of Mg_i, since neither decreasing nor increasing Mg_i alters the volume response. Thus the volume sensitivity of cotransport is dependent on changes in the electrochemical driving force, in Mg_i, and on changes in cell volume per se.

Department of Cellular and Molecular Physiology, Yale University Medical School, 333 Cedar Street, New Haven, CT 06511, USA
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EVIDENCE FOR ELECTRONEUTRAL L-ARGININE TRANSPORT IN CULTURED KIDNEY TUBULE CELLS (OK)

J.S. Schwegler[1], E. Schömig[2], A. Heuner[1], S. Silbernagl[1]

Recently, we described an electrogenic transport of L-arginine into the established opossum kidney OK cell line (Pflüger's Arch 415:543-550). In the present study, however, radiotracer uptake experiments show that the major component of saturable ^{3}H-L-arginine uptake in OK cells is independent of the membrane potential.

^{3}H-L-arginine (10 µmol/l) uptake was determined on confluent OK cell monolayers which were grown on plastic dishes (Ø 6 cm) for 7 days. Uptake was saturable and linear with time for at least 60 s. Initial rates of uptake were measured after an incubation period of 20 s. Intracellular breakdown of arginine is not detectable after that incubation period. Cell membrane depolarization by the addition of 3 mmol/l $BaCl_2$ fails to reduce ^{3}H-L-arginine uptake. The complete substitution of extracellular sodium by choline as well as of extracellular chloride by gluconate reduce ^{3}H-L-arginine uptake to 85 ± 3 % and 90 ± 1 % (n = 3) of control, respectively. Extracellular acidification (pH 6.9) and alkalinization (pH 7.9) does not alter ^{3}H-L-arginine uptake significantly (n = 3). The replacement of extracellular sodium by potassium inhibits ^{3}H-L-arginine uptake in a concentration dependent manner. However, only 48 ± 1 % (n = 3) of total ^{3}H-L-arginine uptake are blocked by the complete substitution of sodium by potassium.

Dept. of Physiology[1] and Dept. of Pharmacology[2], University of Würzburg, Röntgenring 9, D-8700 Würzburg, FRG.

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INCREASED Na^+/H^+-ANTIPORT ACTIVITY IN LYMPHOCYTES OF PATIENTS WITH ESSENTIAL HYPERTENSION.

B.O. Göbel, W. Siffert#, A. Butke, G. Hoffmann, M.-K. Meyer zu Brickwedde, H. Vetter, and R. Düsing. MedizinischeUniversitäts-Poliklinik, Wilhelmstr. 35-37, 5300 Bonn 1 und #MPI für Biophysik, Kennedy-Allee 70, 6000 Frankfurt 70, Federal Republic of Germany.

Increased activity of the Na^+/H^+-antiport may be a major abnormality in essential hypertension. We investigated the activity of this transport system in lymphocytes loaded with the fluorescent dye BCECF from 9 patients with essential hypertension [EH; 162±25(SD)/104±11 mm Hg] and 8 normotensive control subjects (C; 116±13/74±10 mm Hg). Cells were acidified by addition of different amounts of Na^+-propionate (10-20 mM). The undissociated acid permeates the cell membrane, thereby decreasing intracellular pH_i to different values below baseline. pH_i slowly recovered to its initial value, a response that was not seen in the presence of ethylisopropylamiloride indicating involvement of Na^+/H^+-exchange. pH_i-recovery (ΔpH_i/min) was plotted against initial pH_i-values (after acidification) and a linear regression analysis yielded different slopes in C (0.21±0.07) and EH (0.70±0.49; $p<0.001$). In contrast, baseline pH_i values were identical in both groups (C: 7.02 ± 0.08 ; EH: 7.02 ± 0.06). Faster pH_i recovery rates in EH versus C suggest either an increased pH_i-sensitivity of the Na^+/H^+-exchanger or an augmented amount of Na^+/H^+-exchangers in lymphocytes of EH. The observation that baseline pH_i was identical in C and EH rules out the possibility that the enhanced activity in EH is caused by "pre-stimulation", e.g. by circulating agents.

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OVEREXPRESSION OF PLATELET NA^+/H^+ EXCHANGE ACTIVITY IN ESSENTIAL HYPERTENSION

W. Siffert, D. Rosskopf and U. Osswald

It was recently reported that platelets of essential hypertensives (EHT) show an increased activity of Na^+/H^+ exchange. The present study aimed at investigating i) whether this increased activity would result in an increased cytosolic pH (pH_i) and ii) whether the increased activity is an epiphenomenon rather than a causative factor in EHT. Platelets were loaded with the fluorescent pH_i dye BCECF and acidified by addition of propionic acid. The recovery of pH_i was recorded and the initial slopes of the fluorescence tracings were used to estimate the activity of Na^+/H^+ exchange. The pH_i of platelets in normotension (NT; 7.14 ± 0.04, n=10) did not differ from that in EHT (7.16 ± 0.04; n=8). In contrast, the initial rate of pH_i recovery from an artificial acid load was three times faster in EHT as compared with NT. Platelets from patients with renal artery stenosis had both a normal pH_i and Na^+/H^+ exchange activity. We conclude that: i) overexpression of Na^+/H^+ exchange activity in EHT does not occur as a result of elevated blood pressure, since this phenomenon is absent in renal artery stenosis. ii) overexpression of Na^+/H^+ exchange activity does not result in an incresed pH_i. iii) The observation that baseline-pH_i was identical in NT and EHT makes it unlikely that the enhanced activity in EHT is caused by "pre-stimulation", e.g. by circulating agonists.

Max-Planck-Institut für Biophysik, Kennedyallee 70
D-6000 Frankfurt/M. 70

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EXPRESSION OF PROBENECID INHIBITABLE P-AMINO-HIPPURATE TRANSPORT IN XENOPUS OOCYTES

J.Steffgen, W.Schwarz and H.Koepsell

Until now information about the sequence and the molecular structure of the probenecid sensitive p-aminohippurate (PAH) exchanger is missing. To test whether expression cloning employing oocytes of Xenopus laevis may be used to clone the PAH transporter, we injected mRNA from kidney (rat and pig) and intestine (rat) into the oocytes. Total RNA was obtained by homogenization of tissue and later phenol extraction and poly(A)$^+$mRNA was separated by oligo (dT) cellulose chromatography. About 50 ng of this mRNA were injected per oocyte (stage V or VI). After 2 or 3 days of incubation at 18°C, uptake of ^{3}H-PAH into single oocytes was measured in oocyte Ringer's solution in the presence or absense of 5 mM probenecid. Already non-injected oocytes showed a PAH uptake from which about 37 % could be blocked by probenecid. The absolut amount of this uptake varied between different animals. No significant increase of PAH uptake could be detected after injection of mRNA from rat intestine. However injection of mRNA from rat (pig) kidney led to a 2.2 (3) fold increase of PAH uptake in comparison to non- or water-injected oocytes which were obtained from the same animal. About 90 % (66 %) of the PAH transport which was increased by mRNA from rat (pig) kidney was inhibited by 5 mM probenecid. Preincubation of the oocytes at about 30°C for 3 h before influx measurements led to a further increase of PAH uptake in the oocytes injected with mRNA from rat kidney by a factor of about 2.6. Also the endogenous PAH transport was stimulated but only about 1.3 fold. The results suggest that probenecid inhibitable PAH transport can be expressed after injection of mRNA from rat and pig kidney.

MPI für Biophysik, Kennedyallee 70, D-6000 Frankfurt 70

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EVIDENCE FOR A DIVALENT CATION SENSITIVE SHORT CIRCUIT CURRENT ACROSS THE ISOLATED RUMEN OF SHEEP

H. Martens, M. Rübbelke and G. Gäbel

The isolated rumen epithelium exhibits in Ussing-chamber studies a short circuit current, I_{sc}, of 0.5-1.5 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. Omitting Mg and Ca on the mucosal side caused a significant increase of the I_{sc} from 1.2±0.2 to 3.8±0.5 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. This enhanced I_{sc} was significantly reduced by subsequent mucosal addition of $MgCl_2$ or $CaCl_2$ (2mM), but not by Mg-EDTA or Ca-EDTA. Further studies showed that other divalent ions (Ba, Sr) reduced the I_{sc} to the same extend. The Ca channel blocker verapamil (0.1 mM) decreased the I_{sc} in the absence of Ca and Mg. The reduction of I_{sc} by divalent ions or verapamil was reversible.

Amiloride (0.1 mM), which inhibits electrogenic Na transport in different tissues, did not change the I_{sc} under these experimental conditions. Ouabain (0.1 mM) in the serosal solution abolished the I_{sc} within 30 - 40 min. Replacement of Na by choline in the mucosal solution reduced or even caused a negative I_{sc}, which was not changed by mucosal addition of Ca.

This divalent cation sensitive I_{sc} of the rumen epithelium shows similarities with a divalent cation sensitive pathway in amphibian epithelia (W. van Driessche et al., Comp. Biochem. Biophys. 90A, 693, 1988). The role of this transport mechanism remains to be elucidated because under physiological conditions the concentrations of Ca and Mg in the ruminal fluid are high enough to block this pathway.

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Institut für Veterinär-Physiologie, Freie Universität Berlin, Koserstr. 20, 1000 Berlin 33

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EXCITATORY AMINO ACID-INDUCED CHANGES IN EXTRACELLULAR CALCIUM CONCENTRATION IN THE RAT HIPPOCAMPUS INDICATE DIFFERENT ACTIVATION OF THE NA^+/CA^{2+} EXCHANGER

J. Arens, J. Stabel and U. Heinemann

All excitatory amino acids produce dose-dependent decreases in $[Ca^{2+}]_o$ and $[Na^+]_o$. However the specific agonists quisqualate, kainate and AMPA induce in addition to the initial decrease a subsequent increase of $[Ca^{2+}]_o$ above baseline which outlasts the application time. NMDA, DL-homocysteic acid and the mixed agonists glutamate and aspartate do not produce an overshooting $[Ca^{2+}]_o$ response. The direction of Ca^{2+}-fluxes across the cell membrane and therefore also the activation of the Na^+/Ca^{2+} exchanger is determined by the electrochemical Na^+-and Ca^{2+}-gradients and the transmembrane potential. To test the hypothesis that activation of the Na^+/Ca^{2+} exchange mechanism is involved in the production of the Ca^{2+}-overshoots we studied the effects of low $[Na^+]_o$, low $[K^+]_o$, Ouabain (5 µM) and lithium (5 mM) on quisqualate-induced $[Ca^{2+}]_o$ changes. All these treatments enhanced the $[Ca^{2+}]_o$ decreases and reduced the overshooting responses in a reversible manner. In some cases lithium could totally block the $[Ca^{2+}]_o$-overshoots which may be explained by a direct blocking effect of lithium on the action of the Na^+/Ca^{2+} exchanger. To clarify further the basic mechanisms which are involved in the generation of quisqualate-induced Ca^{2+}-overshoots we tested the effect of dantrolene, ryanodine and caffeine; drugs which may influence the release of intracellular calcium. Dantrolene (20 µM) and ryanodine (20 µM) had no effect, whereas caffeine (5 mM) reduced the $[Ca^{2+}]_o$ decreases but did not affect the overshoots. The reduction of $[Ca^{2+}]_o$ decreases by caffeine may be explained by a Ca^{2+}-dependent inactivation of Ca^{2+} currents due to an enhanced intracellular $[Ca^{2+}]$.

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Institut für Neurophysiologie, Zentrum für Physiologie und Pathophysiologie, Universität zu Köln, Robert-Koch-Str.39, D-5000 Köln 41

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A PHYSIOLOGICAL ROLE FOR AMINO ACIDS IN THE GLUCOSE TRANSPORT REGULATION OF CARDIAC MYOCYTES

Y. Fischer, J. Thomas, H. Rose & H. Kammermeier

Glucose is one of the major substrates for the energy metabolism of the myocardium. Work (or contraction) and insulin are known to markedly increase glucose transport (GT) across the sarcolemmal membrane of cardiomyocytes, which is the first and probably the rate-limiting step of the glucose metabolism in these cells. Whether other physiological factors might have a similar effect is still unclear. As we previously reported, the GT of isolated, calcium-resistant rat cardiomyocytes was significantly stimulated by partially purified, low molecular weight fractions obtained from a yeast extract (Pflügers Arch., **142**, suppl 1: 50).

We now further purified these fractions by gel filtration chromatography and could separate at least two active components: one major activity was eluted at an apparent molecular weight of ~100 D (peak A) and another less efficient component at ~500-600 D. Using cardiomyocytes (obtained by a modified isolation procedure) that respond very strongly to insulin (10-20 fold stimulation of GT, as compared to control cells), we found that fractions from peak A evoked a 1.5 fold stimulation of 2-deoxy-D-glucose (2-DOG) uptake. An amino acid analysis showed that alanine is, by far, present at the highest concentration in this peak. When tested in the range of concentrations found in peak A, alanine stimulated 2-DOG transport ~1.5 fold, with an EC_{50} of ~150 µM, which corresponds to a physiological plasma concentration. Amoung the other amino acids detected in lower concentrations in peak A, valine had a significant stimulatory effect on GT (also in a physiological range of concentrations, i.e. 30-300 µM).

On the other hand, we found, in a parallel study, that L-cysteine strongly stimulates (2-4 fold) GT in isolated cardiomyocytes; L-cysteine was effective at concentrations as low as 10-100 µM, which, again, corresponds to physiological values measured in human (and rat) plasma.

We conclude (i) that the insulin-like, GT stimulating activity found in the partially purified yeast extract can be, at least in part, ascribed to the monomeric amino acids alanine and valine and (ii) that some amino acids might play a physiological role in the regulation of GT and, consequently, of glucose metabolism, in cardiac myocytes. (supported by the DFG; RO/755 1-1)

Inst. f. Physiologie, Med. Fak. ,RWTH, Pauwelsstr. D-5100 Aachen

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BOTH THIOL AND THIOL OXIDIZING AGENTS ARE POTENT STIMULATORS OF GLUCOSE TRANSPORT IN ISOLATED RAT CARDIOMYOCYTES

Y. Fischer, H. Rose & H. Kammermeier

Thiol/disulfide exchange has often been proposed to be involved in the regulation of glucose metabolism. In particular, thiols/disulfides were reported to be of critical importance for the activity of glucose transport (GT) and its regulation by insulin in different cell types. In order to assess whether this might also apply to cardiac myocytes, we investigated the effects of thiols and sulfhydryl reagents on GT in calcium-resistant rat cardiomyocytes obtained by a new isolation procedure yielding cells that are highly sensitive to insulin (10-20 fold stimulation, as compared to control).

L-cysteine (L-Cys) stimulated GT 2-4 fold, as compared to basal values, with a half-maximal effect at ~30 μM, which, interestingly, is a physiological concentration in human and rat plasma. This stimulatory action was maximal at ~100 μM L-Cys and decreased at higher concentrations. Other thiols including N-acetyl-L-cysteine, reduced gluthatione, dithioerythritol and 2,3-dimercaptopropanol showed a similar biphasic dose-response relationship, suggesting that the effect of L-Cys might be attributed to its sulfhydryl group. As (i) D-Cys was as effective as L-Cys (with exactly the same concentration dependence) and as (ii) the Cys effect could not be inhibited by a high excess of L-serine (that compete with Cys for uptake via the ASC-transport system), it is likely that L-Cys does not need to be transported across the sarcolemmal membrane to induce its effects on GT. On the other hand, the thiol oxidizing agent diamide (3-100 μM) markedly increased GT activity, higher concentrations becoming inhibitory. Furthermore, we found that phenylarsine oxide (PAO), that is known to react with vicinal sulfhydryls and to inhibit insulin action on GT in rat adipocytes, induced the same pattern of stimulation/inhibition as diamide. It is noteworthy that the maximal stimulation (4-7 fold above control) was reached at PAO concentrations (1-3 μM) at which the effect of insulin was only partly antagonized. Thus, it can not be ruled out that PAO acts as a partial agonist of insulin rather than as a mere antagonist.

In conclusion, these results suggest (i) that both thiols and disulfides might play a role in the regulation of GT in cardiomyocytes (possibly in part within the insulin signalling pathway) and (ii) that cysteine might represent a physiologically relevant signal. (supported by the DFG; Ro/755 1-1)

Inst. f. Physiologie, Med. Fak., RWTH, Pauwelsstr. D-5100 Aachen

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INTRACELLULAR Cl^- ACTIVITIES IN NEURONES AND GLIAL CELLS OF THE LEECH CENTRAL NERVOUS SYSTEM AFTER PARTIAL Cl^- SUBSTITUTION

Thomas Munsch and Joachim W. Deitmer

We have measured the intracellular Cl^- activity, aCl_i, in identified neurones and neuropile glial cells of the central nervous system of the leech *Hirudo medicinalis*, using double-barrelled Cl^--sensitive microelectrodes (Corning exchanger 477913). The aCl_i changed considerably in Retzius neurones and neuropile glial cells, when the conventional, high-Cl^- and hypertonic leech saline (219 mosmol/l; 110 mM Cl^--concentration, corresponding to 85 mM Cl^- activity, using an activity coefficient of 0.77) was exchanged by an isotonic saline (186 mosmol/l), in which the Cl^--concentration was reduced to 40 mM (31 mM activity) and substituted by 40 mM DL-malate. This low Cl^- saline appears to match the leech blood closer, since organic anions constitute a large fraction of the anions present in leech blood (A. Wenning, J. exp. Biol. 143:115, 1989). In Retzius neurones aCl_i decreased from 8.5 ± 1.2 mM (± S.D., n=5) in the high-Cl^- leech saline to 4.0 ± 1.7 mM (n=13) in the new, low-Cl^- saline. The Cl^- equilibrium potential E_{Cl} changed from -59 mV to -53 mV, while the membrane potential E_m changed from -43 ± 5 mV to -47 ± 7 mV. In neuropile glial cells aCl_i was 6.3 ±1.6 mM (n=8) in the high-Cl^- saline giving an E_{Cl} of -66 mV at a mean E_m of -68 ± 5 mV; the aCl_i was 2.0 ± 1.1 mM (n=20) in low-Cl^- saline, giving an E_{Cl} of -70 mV at a mean E_m of -72 ± 8.7 mV. In contrast to neurones, Cl^- appeared to be in equilibrium across the glial cell membrane as suggested also by Ballanyi & Schlue, 1990 (J. Physiol., in press). In the presence of CO_2/HCO_3^- buffering (5 % CO_2/24 mM HCO_3^-) the Cl^--sensitive microelectrode reading was 1-5 mV higher in all cells, presumably due to some interference of HCO_3^- to the electrode.

Abteilung für Allgemeine Zoologie, FB Biologie, Universität Kaiserslautern, Postfach 3049, D-6750 Kaiserslautern

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PHOTOMETRIC DETERMINATION OF Na^+/H^+ EXCHANGE IN PLATELETS

D. Rosskopf and W. Siffert

The purpose of the present study was to establish a routine assay for determination of Na^+/H^+ exchange activity in different pathological states. When cells are incubated in a medium consisting of (in mM) Na^+-propionate 140, HEPES 20, glucose 5, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1, pH 6.7, the undissociated propionic acid permeates the cell membrane, acidifies the cytosol and thus activates the Na^+/H^+ exchanger. The internal pH after acidification in this standard medium was 5.7 as estimated from the fluorescence dye BCECF. The continuous uptake of Na^+ then causes osmotic cell swelling. Prewarmed aliquots (37° C) of platelet-rich plasma (PRP; 70 μl) were added to this medium (430 μl). Using a photometer (λ = 680 nm) a rapid decrease in absorbance of such suspensions was observed. The time course of the change in absorbance coresponded to a first order reaction (time constant 22×10^{-3} sec^{-1}). Specific blockers of the exchanger also inhibited the change in aborbance (K_i amiloride, 11μM; K_i ethylisopropylamiloride, 0.1 μM). The change in absorbance depended on the extracellular Na^+ concentration (K_m = 70 mM). The time constant decresed with decreasing Na^+-propionate concentration. Maximum swelling was observed at an external pH of 6.7. A further investigation revealed an incresed activity of Na^+/H^+ exchange in essential hypertensives (time constant 30×10^{-3} sec^{-1}), corresponding to already published data established by more sophisticated and time-consuming methods.

Hence, our method provides a suitable test for routine screening of numerous specimens and for further attempts to clarify the connection between essential hypertension and Na^+/H^+ exchange.

Max-Planck-Institut für Biophysik, Kennedyallee 70
D-6000 Frankfurt/M. 70

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ULEX EUROPAEUS AGGLUTININ I INDUCES A DIDS-SENSITIVE CA INFLUX PATHWAY IN HUMAN ERYTHROCYTES

B. Engelmann, J. Duhm

Of eleven lectins tested, only one, Ulex europaeus agglutinin I (UEA_1) stimulated ^{45}Ca influx into quin-2 loaded erythrocytes (by about twofold) in a dose-dependent manner at subagglutinating concentrations. Na^+ and Rb^+ uptake resistant to ouabain plus furosemide was not altered by UEA_1. The potency of the ABH-blood group specific lectin UEA_1 to stimulate Ca influx decreased with $H=A_2>A_1>B$, e.g., in the same order as the lectins capacity to agglutinate the erythrocytes. UEA_1 is thought to be an α-L fucose specific lectin. Indeed, in the presence of 5mM fucose, the UEA_1 induced component of Ca influx was abolished. Since it is known that most of the protein linked ABH-antigens are attached to bands 3 and 4.5, the effect of inhibitors of the anion exchanger as well as of the glucose and nucleoside transporter was studied. Cytochalasin B and nitrobenzylthioinosine left the Ca influx induced by UEA_1 unaffected, while DIDS caused a dose-dependent inhibition (complete inhibition at 10μM, K_i at about 2μM for A_1 as well as A_2 and H erythrocytes). In addition, also other inhibitors of the anion exchanger such as DNDS (10μM) and dipyridamole (20μM) completely blocked the component of Ca influx elicited by the lectin. The Ca entry blockers verapamil (100μM) and nifedipine (10μM) did not affect the lectin induced Ca influx.

In conclusion, most probably by binding to ABH-blood group antigens, UEA_1 induces a Ca influx pathway in human erythrocytes which is blocked by inhibitors of the anion exchanger.

Physiologisches Institut der Universität München, Pettenkoferstr. 12, 8000 München 2

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CONTRIBUTION OF ELECTROGENIC Na^+-HCO_3^--COTRANSPORT TO GLIAL H^+-BUFFERING IN THE LEECH CENTRAL NERVOUS SYSTEM

Joachim W. Deitmer

The regulation of intra- and extracellular pH in the leech central nervous system occurs via ion transport systems across cell membranes, such as Na^+-H^+ exchange, Cl^--HCO_3^- exchange and/or Na^+-HCO_3^- cotransport. The latter has been shown to operate in glial cells, but not in neurones (Deitmer & Schlue, J. Physiol. 388:261, 1987). It is electrogenic and inwardly directed (Deitmer & Schlue, J. Physiol. 411:179, 1989), thereby accumulating HCO_3^- intracellularly. This leads to a significant increase in the buffering power of glial cytoplasm. I have measured the pH and membrane potential in neuropile glial cells, and the pH in extracellular spaces of the leech c.n.s., using double-barrelled pH-sensitive microelectrodes. Intra- and extracellular pH transients were evoked by addition and removal of weak acids or bases to estimate the apparent buffering power in cells and of the extracellular spaces. The experiments show that the CO_2/HCO_3^--dependent increase in the intracellular buffering power of glial cells is reversed by DIDS (4,4-diisothiocyanostilbene-2,2-disulfphonic acid, 0.3-0.5 mM), which inhibits Na^+-HCO_3^- cotransport. The buffering power of the extracellular spaces, which increased in CO_2/HCO_3^--buffered saline, decreased again after the addition of DIDS. This suggests that the presence of CO_2/HCO_3^-, and hence Na^+-HCO_3^- cotransport across the glial membrane, augments intra- and extracellular apparent buffering power. In contrast the effects of CO_2/HCO_3^- and DIDS on pH_i regulation in neurones are relatively small. The results provide first evidence for the hypothesis that glial cells are directly involved in the regulation of H^+ homeostasis in the nervous system.

Abteilung für Allgemeine Zoologie, FB Biologie, Universität Kaiserslautern, Postfach 3049, D-6750 Kaiserslautern

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Measurement of intracellular free magnesium concentration in skeletal muscle fibers

D. Günzel and S. Galler

Numerous investigations exist on intracellular activities of different anorganic ions, but little is known about intracellular free magnesium concentration ($[Mg^{2+}]_i$) and its regulation. Since Mg^{2+} is able to compete with Ca^{2+} at protein binding sites, $[Mg^{2+}]_i$ might have physiologically important functions in intracellular regulation processes. In the past, determination of $[Mg^{2+}]_i$ was hardly possible because the selectivity of Mg^{2+} sensors was not sufficient to measure $[Mg^{2+}]$ reliably in the intracellular medium with high potassium ion activity. The Mg^{2+} sensor based on the ionophore ETH 5214 (Z. Hu et al. Anal Chem 61:574, 1989) is less sensitive to K^+ interference. We measured with this sensor, using micro glass pipettes with a tip diameter of about 0.5 µm. Mg^{2+} microelectrodes were calibrated in $MgCl_2$ solutions with ionic background similar to that of the intracellular medium.

In fibers of sartorius muscle of the frog *Xenopus leavis*, $[Mg^{2+}]_i$ was determined to be 1.81 ± 0.24 mM (S.E) (n=21) in a Ringer solution containing 0.5 mM $MgCl_2$. As passive distribution of Mg^{2+} across the cell membrane with an electrical potential of -76.8 ± 2.6 mV (S.E.) (n=21) would lead to intracellular Mg^{2+} concentrations in the range of 0.2 M, active extrusion of Mg^{2+} from the muscle cell has to be postulated. When sartorius muscles were incubated for up to ten hours in Ringer solutions ($[MgCl_2]$ = 0.5 mM) in which all Na^+ was replaced by N-methyl-D-glucamine$^+$, $[Mg^{2+}]_i$ increased to 2.62 ± 0.34 mM (n=15). After readdition of Na^+, $[Mg^{2+}]_i$ decreased to 1.72 ± 0.31 mM (n=15). In Na^+-free Ringer solutions containing 5 mM $MgCl_2$, $[Mg^{2+}]_i$ increased to 3.52 ± 0.35 mM (n=15). In this case, readdition of Na^+ lead to $[Mg^{2+}]_i$ of 2.92 ± 0.42 mM (n=15). It can be concluded that a Na^+-dependent Mg^{2+} transport system may partially be responsible for the regulation of intracellular $[Mg^{2+}]$.

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Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 KONSTANZ.

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SODIUM-DEPENDENT AND SODIUM-INDEPENDENT SULFATE TRANSPORTERS FROM RAT INTESTINE ARE EXPRESSED BY mRNA'S OF DIFFERENT SIZE

J.Steffgen, W.Schwarz and H.Koepsell

The oocytes of Xenopus laevis have been used as an expression system for various proteins. We have measured sodium-dependent and sodium-independent (DIDS-sensitive) sulfate uptake in non- or water injected oocytes and in oocytes injected with mRNA from rat intestine. About 50 ng of total mRNA were injected per oocyte (stage V or VI) and 2 or 3 days later uptake of $^{35}SO_4^{2-}$ was measured in oocyte Ringer's solution. For determination of the sodium-dependent component, sodium was replaced by tetramethylammonium. For DIDS-sensitive transport, the fraction of sulfate uptake measured in the presense or absense of sodium which could be inhibited by 50 µM DIDS was determined. In non-injected oocytes about 35 % of sulfate uptake was sodium-dependent, about the same amount of uptake was inhibited by DIDS. Since the absolut amount of sulfate uptake varies beetween different animals, expression experiments were always performed in comparison to oocytes from the same animal. After injection of mRNA from rat intestine total sulfate uptake increased about 2.8 fold in comparison to non- or water-injected oocytes. About half of the increased sulfate uptake in the injected oocytes was sodium dependent and nearly half was DIDS-sensitive. We size-fractionated mRNA on 1 % agarose gel and electroeluted the mRNA. After injection of 10 ng/oocyte of one fraction (1.5-2 kb) the sodium-dependent sulfate uptake increased by about 18 fold. Injection of mRNA of higher molecular weight (3-7 kb) yielded a 8 fold increase of sulfate uptake which was not sodium-dependent but 45 % of this transport could be inhibited by DIDS. The data show, that sodium-dependent and sodium-independent sulfate transport can be expressed by mRNA fractions of different size.

MPI für Biophysik, Kennedyallee 70, D-6000 Frankfurt 70

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ALDOSTERONE REGULATES SODIUM TRANSPORT ACROSS ALVEOLAR EPITHELIUM.

H. Fischer and W. Clauss

Intact lung tissues of the frog *Xenopus laevis* were investigated in Ussing chambers under voltage clamped conditions. Nonstimulated tissues exhibited a distinct Na^+ current from the alveolar to the pleural side which was blockable by low doses of amiloride. The mineralocorticoid aldosterone (1 µmol/l) increased the amiloride-blockable transepithelial Na^+ transport within 4 to 5 hours from 7.7±0.7 µA/cm^2 to 17.9±1.7 µA/cm^2 (means±SE, n=7 and 15). Simultaneously the transepithelial resistance decreased from 754±41 Ω · cm^2 to 646±38 Ω · cm^2 significantly. This stimulatory effect of aldosterone on the transepithelial Na^+ current was totally inhibited when the aldosterone-incubation was carried out together with the antimineralocorticoid spironolactone.

Transepithelial measurements were supplemented by analysis of the fluctuations in the short circuit current. In the presence of amiloride in the alveolar compartment we revealed a Lorentzian noise component in the power density spectra. This enabled us to calculate microscopic kinetic and channel characteristics. The single Na^+ channel current (i_{Na}=0.5 pA) and the blocker kinetics were unaffected by aldosterone-stimulation. The number of apical Na^+ channels, however, increased from 0.085±0.014 µm^{-2} to 0.268±0.054 µm^{-2} in parallel to the transepithelial Na^+ current. This shows that the lung epithelium is a physiological target tissue for aldosterone action.

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Chloride-related current fluctuation in frog skin

W. Nagel and W. Van Driessche

The pathway for conductive transepithelial Cl transport in amphibian skin, which can be activated by serosa positive clamp potentials, has not unequivocally been identified. As possible routes, mitochondria-rich (MR) cells or the tight junctions of the shunt have been proposed. To provide further information on the mode of passage, we have analyzed, on skins of Bufo viridis and Rana esculenta, the current fluctuation of transepithelial current related to this transport of Cl.

Lorentzian components in the power density spectrum (pds) were observed at serosa positive voltage perturbation, i.e. under conditions when Cl conductance (g_{Cl}) is activated. The corner frequency, which ranged between 44 and 100 Hz, was not related to the magnitude of the transepithelial clamp potential. The plateau value S_o, in the order of $60*10^{21}$ Asec/cm^2, increased with the magnitude of I_{Cl}. Substitution of mucosal Cl by NO_3 decreased I_{Cl} and led to disappearance of the Lorentzian in the pds. Inhibitors of g_{Cl} (MK-196, DPC-analogs) had the same effects. After stimulation of g_{Cl} using theophylline or procaine, concomitant increases in I_{Cl} and S_o could be observed. The corner frequency was not systematically altered under any condition.

Assuming that the current fluctuation originates from channels with similar open and closed probablity, we calculate individual channel currents in the order of 30 fA and channels densities between 20 and 300channels/μ^2 macroscopical area. If these channels are localized in the apical membrane of MR-cells, the actual density must be much larger, since MR-cells account for some 1-5 % of the apical surface only. The magnitude of the individual channels current is much lower than reported for membrane channels. We propose that the fluctuation of I_{Cl} originates from specific transport sites in the tight junctions.

Physiologisches Institut, Universität München, Pettenkoferstr.12, D-8000 München 2, FRG and Laboratorium voor Fysiology, KU Leuven, Campus Gasthuisberg, B-3200 Leuven, Belgium.

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LOCALIZATION OF ABSORPTIVE AND SECRETORY ION TRANSPORT IN RAT RECTAL COLON SURFACE CELLS AND CRYPTS BY VOLTAGE SCANNING

A Köckerling, JD Schulzke, D Sorgenfrei and M Fromm

Spatial distribution of conductive ion transport was investigated between surface cells and crypt openings. Totally stripped rat rectal colon was mounted in a modified Ussing-chamber and clamped by an AC current of 30 Hz and ±75 µA/cm². The voltage drop between two positions (nominal distance zero from the epithelial surface and 45 µm apart) was sensed using a piezo-driven stepping electrode (Ling-Gerard type) which was positioned under microscopical control either above surface cells or above crypt openings. From these data and the morphometrically determined area contributions, the conductances per cm² gross tissue area of the two structures were calculated.

- Under control conditions the crypt openings, although covering only 5.5 ± 0.5 % of the gross tissue area in the Ussing chamber, contribute 61 ± 7 % to the total conductance.
- Theophylline (10^{-2} M) increased conductance of the crypt openings from 5.2 ± 0.5 to 8.8 ± 0.9 mS per cm² gross tissue area. Conductance of the surface cells was not significantly changed (n=14, paired t-test).
- After "acute" aldosterone ($3\cdot10^{-9}$ M, in vitro incubation for 6 h), amiloride (10^{-4} M) reduced surface cell conductance from 4.9 ± 0.7 to 2.2 ± 0.3 mS/cm². Conductance of the crypt openings was not significantly changed (n=12).
- After "chronic" aldosteronism (low Na^+, high K^+ diet for 2 weeks), amiloride again reduced surface cell conductance but not crypt conductance (n=13). However, conductances of both, surface cells and crypt openings were increased by a factor of 2 as compared to the conductances under acute aldosterone.

Our data demonstrate that theophylline-induced Cl^- secretion is localized in crypts, but not in surface cells. On the other hand, acute as well as chronic aldosterone induces amiloride-sensitive Na^+ absorption in surface cells, but not in crypts. In addition, chronic aldosterone induces as yet unidentified conductive pathways in both, surface cells and crypts.

Institut für Klinische Physiologie, Klinikum Steglitz, Freie Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45

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DEMONSTRATION OF A VACUOLAR TYPE ATP-DRIVEN H^+ PUMP IN BRUSH-BORDER MEMBRANES OF THE HUMAN PLACENTA

B. Simon, V. Ganapathy, F. H. Leibach, and G. Burckhardt

The brush-border membrane of the placental syncytiotrophoblast forms the first barrier between maternal and fetal circulation systems. To investigate whether besides an Na^+/H^+ exchanger also an ATP-driven H^+ pump is present in the brush-border membrane, vesicles were isolated from normal human term placenta by a Mg^{2+} precipitation technique. The vesicles were solubilized with 1.2% cholate and detergent was removed by overnight dialysis to reorient putative H^+ pumps from the inside to the outside of the membrane. Addition of Mg^{2+} and ATP to these cholate-pretreated placental membranes resulted in a marked intravesicular acidification as visualized by acridine orange proving the presence of an ATP-driven H^+ pump. H^+ uptake was fastest with ATP, much slower with GTP and ITP, and not detectable with UTP and CTP. ADP inhibited uptake with a K_i of 0.07 mM. The divalent cations Mg^{2+} and Mn^{2+} supported best, and Co^{2+} partially, ATP-driven H^+ uptake. ATP-driven H^+ uptake into cholate-pretreated placental vesicles was weakly inhibited by vanadate, azide, and oligomycin. Complete inhibitions, however, were observed with 0.01 mM N-ethylmaleimide (NEM) and 4-chloro-7-nitro-benzoxa-1,3-diazole (NBD-Cl), and 0.2 mM N,N'-dicyclohexylcarbodiimide (DCCD). This inhibitory pattern is typical for "vacuolar" type H^+-ATPases. The stimulation of ATP-driven H^+ uptake by iodide > chloride > nitrate >> sulfate, gluconate suggested that the H^+ pump is electrogenic and requires uptake of permeant anions for charge compensation. Accordingly, ATP-driven H^+ uptake was enhanced by the K^+ ionophor, valinomycin, in the presence of K^+ in the vesicles. In this case, each pumped proton is exchanged with K^+ leaving the vesicles via its ionophor. The data provide the first evidence for the existence in human placental brush-border membranes of an ATP-driven H^+ pump. The high sensitivity of this pump for NEM and NBD-Cl reveals that this pump belongs to the class of "vacuolar" H^+-ATPases.

Max-Planck-Institut für Biophysik, Kennedyallee 70, D-6000 Frankfurt 70, FRG; and Department of Cell and Molecular Biology, School of Medicine, Augusta, GA 30912-2100, USA

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A CALCIUM-SENSITIVE CATION CHANNEL IN FROG COLON

R. Krattenmacher, Rosita Voigt, W. Clauss

Electrogenic ion transport across the colon epithelium of the frog *(Xenopus laevis)* was investigated in an Ussing-chamber under voltage clamp conditions. With NaCl-Ringer on both sides of the tissue, the major part of the short circuit current (I_{sc}) was caused by an electrogenic Na-transport (mucosa to serosa). This transport was insensitive to mucosal amiloride (10^{-4}mmol/l) and could be reversibly increased by removal of mucosal Ca-ions. The amount of this Ca-effect linearly increased with the mucosal pH. Half-maximal inhibition of the Ca-sensitive current was at about 1 µmol/l mucosal Ca. Similar effects as with Ca were obtained with other bivalent cations (Mg, Ba). Noise analysis of the current fluctuations showed that Ca-removal induced a Lorentzian component in the power density spectrum indicating ion channel gating. When either K, Rb, Cs or Li were used as the mucosal main cation, except for Li, Ca-removal from the mucosal solution also resulted in an increase in I_{sc}. The amount of this increase for the different mucosal cations had the sequence K > Na = Rb > Cs > Li. It is concluded that this channel is located in the apical cell membrane and acts as a pathway for Na-absorbing or K-secreting/absorbing processes. Due to the high sensitivity to mucosal Ca, a regulation of the channel conductivity by extracellular Ca is ruled out. We consider that the Ca-sensitivity may indicate an outward rectifying property of this ion channel as described for a similar ion channel in the toad urinary bladder.

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TRANSPORT OF BILE SALTS IN RAT RENAL BASOLATERAL MEMBRANE VESICLES

S. Dietrich and G. Burckhardt

Bile salts, e. g. taurocholate, are actively reabsorbed in the proximal tubules of the rat kidney. They enter the tubule cell across the luminal membrane via a Na^+-dependent transport system. The characteristics of the exit step across the basolateral membrane are unknown. To investigate the bile salt transporter we prepared basolateral membrane vesicles from rat kidney cortex by a Percoll density gradient centrifugation technique. The amount of [^{3}H]-taurocholate associated with the vesicles decreased with decreasing vesicle volume indicating uptake of this bile salt into the vesicle interior rather than mere binding to the membrane. Unlike bile salt transport in the brush-border membrane, [^{3}H]taurocholate uptake into basolateral membrane vesicles was not stimulated by an out>in Na^+ gradient. Unlabeled taurocholate and cholate in the incubation medium inhibited [^{3}H]taurocholate uptake proving the presence of a saturable transporter for conjugated and unconjugated bile salts in the basolateral membrane. Substrates of the transport systems for sulfate, dicarboxylates, and p-aminohippurate (PAH) did not inhibit [^{3}H]-taurocholate uptake when added to the incubation medium. In contrast, unlabeled taurocholate and cholate inhibited strongly the uptake of [^{3}H]PAH and, to a small extent, that of $^{35}SO_4^{2-}$. These data indicate that bile salts are transported by a separate system in the basolateral membrane. In addition they interact with the transporters for PAH and sulfate without being measurably translocated by these systems. [^{3}H]Taurocholate uptake into basolateral membrane vesicles was inhibited by bromosulfophthalein (BSP), the loop diuretics furosemide and bumetanide, by bilirubin, probenecid and 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS). With [^{3}H]cholate comparable results were obtained although the degree of inhibition by organic anions, taurocholate and cholate in the medium was smaller than in experiments with [^{3}H]taurocholate. In conclusion, our data provide evidence for the existence of a separate transport system for bile salts in the basolateral membrane not shared by sulfate, dicarboxylates and PAH.

Max-Planck-Institut für Biophysik, Kennedyallee 70, D-6000 Frankfurt 70, FRG

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EFFECTS OF BARIUM ON POTASSIUM TRANSPORT IN ISOLATED MALPIGHIAN TUBULES OF FORMICA.

A. Leyssens, S.L. Zhang, R. Weltens, P. Steels and E. Van Kerkhove.

In an attempt to elucidate the role of K-channels as a hypothetical pathway for the K-transport through the basolateral membrane of Malpighian tubules of *Formica*, the transepithelial and basolateral (V_{bl}) potential, the specific transepithelial resistance (R_{te}), the relative value of the apical (R_{ap}) over the basolateral (R_{bl}) resistance and the fluid secretion rate were determined in the absence and presence of Ba^{2+} in the bath solution.
V_{bl}, measured in 3 different K-concentrations (5, 51 and 113 mM) , revealed that the basolateral membrane behaved as an almost perfect K-electrode. In the presence of 6 mM Ba^{2+}, this sensitivity was lost (the slope decreased from 49 to 7 mV/ decade). R_{ap}/R_{bl} dropped from 39±9 (n=8) to 1±0 (n=20)while R_{te} increased between 9 and 25%. These results suggest the presence of basolateral K-channels.
Surprisingly, the addition of 6 mM Ba^{2+} resulted in a strong hyperpolarization of the apical membrane potential from -84±2, -51±4 and -41±6 mV to -101±5, -96±6 and -94±6 mV (n=3) in 5, 51 and 113 mM K^+, respectively.
Ba^{2+} had also an effect on the urine formation: secretion rates fell with 87, 89 and 90% in 5, 51 and 113 mM K^+, respectively. We conclude that the net transepithelial K-transport could be inhibited directly by a block of the basolateral K-channels and / or indirectly by a reduced activity of the apical electrogenic cation pump due to an increase of the apical membrane potential.

Laboratory of Physiology, Limburgs Universitair Centrum, Universitaire Campus, 3610 Diepenbeek, Belgium.

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SYNERGISTIC STIMULATION OF ALDOSTERONE AND THYROXINE ON LARGE INTESTINAL SODIUM ABSORPTION IN CHICKEN EMBRYOS

B. Hoffmann, R. Krattenmacher, M. Heinz, B. Habura and W. Clauss

Stimulation of electrogenic Na^+ transport (I_{Na}) across large intestinal epithelium (coprodeum) of 20 days old chicken embryos by thyroxine and aldosterone was studied in Ussing-chambers. Fluctuations in short-circuit current were measured with a low noise voltage-clamp for further noise analysis. With Na-Ringer's solution (supplemented with ß-hydroxy-butyrate, glutamate and mannose) on both sides of the tissue I_{Na} was 4.7 ± 1.3 $\mu A/cm^2$ (mean ± SEM, n=8). Neither thyroxine (10^{-6} mmol/l) nor aldosterone (10^{-7} mmol/l) induced in vitro a significant increase in I_{Na}. Interestingly, when both hormones were given I_{Na} was 11-fold stimulated to 53.1 ± 3.8 $\mu A/cm^2$ within 4-6 hours. To determine the microscopic parameters of this stimulated Na-transport amiloride-induced current fluctuations were analysed. From the plateau values and the corner frequencies of the Lorentzians a Na-channel density (M) of 16 Mio/cm^2 and a single Na-channel current (i_{Na}) of 2.2 pA was calculated. The Michaelis-Menten-constant (K_m) for the amiloride-channel interaction was 0.8 μmol/l. Our results clearly show, that the electrogenic Na-transport system present in large intestine of adult hens already exits in embryonic chicken shortly before hatching. Noise analysis shows that microscopic parameters of apical sodium channels (i_{Na} and M) in this embryonic epithelium are similar to values found in sodium-conserving epithelia of adult animals. Furthermore we demonstrate that at this developmental stage sodium absorption can already be regulated by hormones. Obviously, aldosterone and thyroxine act in a synergistic way.

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The role of mitochondria-rich (MR) cells of frog skin in Na-transport

A. Dörge and W. Nagel

From electrophysiological and flux analyses it was proposed (Ehrenfeld et al. Pfluegers Arch. 414, 59 (1989)) that MR-cells might be substantially involved in amiloride-sensitive Na transport under in vivo-like conditions (low [Na] on the apical side; open circuited). Particularly large was this flux after KCl-adaptation of the frogs.
To test this possibility we determined on KCl-adapted Rana esculenta electrical parameters of principal cells, transepithelial Na net-fluxes and intracellular electrolyte concentrations along with Rb-uptake into principal and MR-cells across the basolateral membrane. Measurements were done under control conditions and after amiloride.
With 2 mM mucosal Na and at open-circuit, the fractional resistance of the apical membrane was from 0.7 to 0.9. After amiloride, it increased virtually to 1.0. Amiloride-induced alterations of transepithelial clamp current at different holding potentials were associated with changes in intracellular potentials of proportionate magnitude. These data indicate that Na passes through principal cells under in vivo-like conditions.
Transepithelial net Na transport, measured from the decrease in [Na] of the apical solution, was observed only at low transepithelial potentials. It was near-completely blocked by amiloride.
The electrolyte composition of principal and MR-cells was essentially unchanged after amiloride. In MR-cells, large variation in [Na] and [Cl] was obtained as usually. Uptake of Rb across the basolateral membrane, which represents a measure of the transport activity of the Na/K-pump, was not different, on the average, in both cells types. It could be reduced by inhibition of net Na transport using amiloride only in principal cells.
Our results indicate that under in vivo-like conditions as well as under "Ussing"-conditions, amiloride-sensitive Na transport occurs mainly, if not exclusively, through the principal cells.

Physiologisches Institut, Universität München, Pettenkoferstr.12, 8000 München 2, FRG

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TRANSPORT OF SHORT-CHAIN FATTY ACIDS ACROSS DIFFERENT SEGMENTS OF THE GUINEA PIG LARGE INTESTINE.

G. Rechkemmer and W. von Engelhardt

Short-chain fatty acids (SCFA: acetate, propionate, butyrate) are produced in the large intestine by anaerobic microbial fermentation of carbohydrates. SCFA are monocarboxylic acids with a pK around 4.8. SCFA constitute the major anions in large intestinal contents. Transport of SCFA across the large intestinal epithelium is not well characterized so far.

Unidirectional isotopic tracer fluxes of ^{14}C-labelled SCFA was measured under short-circuit current conditions across the isolated epithelium of guinea pig caecum, proximal and distal colon. Flux from the mucosal to the serosal side of the preparation (ms-flux) and in the opposite direction (sm-flux) increased linearily with concentration for all three SCFA in all large intestinal segments at concentrations of 1, 10 and 25 mM SCFA.

In the caecum and proximal colon net secretion of SCFA was observed, whereas in the distal colon SCFA were absorbed. Net secretion in the proximal colon decreased with SCFA-chain length, net absorption in the distal colon rose with chain length.

Na transport in the proximal colon was stimulated by SCFA to a small extent. Na transport in the proximal colon is accomplished by Na/H exchange. Na/H exchange can be inhibited by amiloride (0.1 mM). Amiloride significantly increased SCFA secretion in the proximal colon due to inhibition of the ms-flux. In the guinea pig distal colon an ouabain-sensitive K-H-ATPase has been demonstrated. Inhibition of the K-H-ATPase with mucosal ouabain (0.1 mM) abolished SCFA absorption in the distal colon and led to a significant SCFA secretion, similar in magnitude to SCFA secretion observed in the proximal colon.

In conclusion SCFA transport across the large intestinal epithelium appears to be primarily passive but linked to hydrogen ion gradients established by cellular ion transport systems.

Physiologisches Institut der Tierärztlichen Hochschule Hannover, Bischofsholer Damm 15, D-3000 Hannover 1, FRG

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CILIARY EPITHELIAL CELLS IN CULTURE: TRANSEPITHELIAL RESISTANCE AND EFFECTS OF PROTAMINE

O. Strauß and M. Wiederholt

Active ion transport mechanisms in the ciliary epithelium, which is a double cell layer of a pigmented (PE)and a non-pigmented (NPE) epithelium, are believed to contribute to the secretion of aqueous humor into the eye. To investigate the transport properties of the separated epithelia, human NPE cells of a continuous virus transformed cell line (Helbig et al. Invest. Ophtalmol.Vis.Sci 30: 882; 1989)were cultured on porous cellulose filter supports (Millipore HA) and mounted into Ussing-type chambers. 24 hours after seeding a transepithelial resistance of 5 -7 Ωcm^2 was measured. After 3 days the resistance reached its maximum between 15-30Ωcm^2 and decreased after the 8th day. Primary cultures of bovine PE showed a transepithelial resistance of 9-15Ωcm^2 after the fifth day in culture.

Protamine is known to increase transepithelial resistance in leaky epithelia (Fromm et al.,J.Membrane Biol.87:141; 1985). In our studies protamine increased the transepithelial resistance to a mean value of 180% ± 13 (S.D.) in NPE and to 110-120% in PE cells. These effects were reversed by the protamin antangonist heparin. A dose-response relation showed saturation of the resistance at a protamine concentration of 3· 10^{-4}Mol (14,300µg/ml). The protamine effects were maximal at the third to fifth day in culture.

We conclude that resistances in cultured ciliary epithelial cells can be measured comparabel to those obtained in whole ciliary body preparations.

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Institut für klinische Physiologie, Universitätsklinikum Steglitz, Freien Universität Berlin, Hindenburgdamm, D1000 Berlin 45

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COMPARISON OF AMILORIDE AND CDPC EFFECTS ON THE FROG SKIN ELECTROPHYSIOLOGY

D.-G. Margineanu and W. Van Driessche

Recent studies (Helman and Baxendale, FASEB J. 2:A750, 1988; Krattenmacher et al., Pflügers Arch. 412:568, 1988) reported the use of the amiloride analogue 6-chloro-3,5- diaminopyrazine-2-carboxamide (CDPC), as a blocker of sodium channels, suited for inducing Lorentzian noise in epithelia. Here we compare the effects of these two pyrazine derivatives on the short-circuit current (I_{sc}), transepithelial impedance and blocker-induced noise in isolated abdominal skin of *Rana temporaria*.

From the linear dependence of the corner frequency (f_c) of the Lorentzian noise on the amiloride concentration, a mean lifetime of 75 ms and an apparent dissociation constant of 0.8 µmol/l were calculated. The lifetime of CDPC complex with the channels is much shorter (4 ms) and the apparent dissociation constant higher (40 µmol/l), with either sulphate or chloride as major anion. The 50 % inhibition of I_{sc} is produced by about 0.5 µmol/l amiloride and by 150 µmol/l CDPC. In order to reveal the specific effects of the two blockers on the apical and basolateral membranes, we performed impedance measurements in hypotonic sulphate Ringer, in which the Nyquist plots consist in two distinct semicircles. These measurements do not reveal major capacitance changes. But, on the other hand, very significant resistance changes were recorded. The apical resistance is increased by both blockers, it rising to twice the control value in the presence of either 2 µmol/l amiloride or 150 µmol/l CDPC. The basolateral resistance continuously decreases up to highest CDPC concentration used, it reaching 55 % of the control value. At concentrations below 0.1 µmol/l, amiloride also decreases (by about 25%) the basolateral resistance, but above this concentration it continuously increases, reaching twice the control value at 2 µmol/l. The decrease of basolateral resistance might account for the scalloping behaviour of I_{sc}, commonly observed with CDPC, as well as for the increases in I_{sc} above the control values produced by small amiloride concentrations.

Laboratorium voor Fysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium

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EPITHELIAL MONOLAYERS OF DIFFERENTIATED HT-29 CELLS EXHIBIT CRYPT-LIKE CHLORIDE SECRETION

KM Kreusel, M Fromm, U Lempart, D Sorgenfrei and U Hegel

Undifferentiated HT-29 (human colon adenocarcinoma) cells express cAMP-regulated chloride channels. Under glucose-free culture conditions they differentiate. In this study transepithelial transport properties of differentiated HT-29 cells are described.

After prolonged glucose-free culture a clone (HT-29/B6) was selected. The cells were seeded onto filter membranes and the transepithelial resistance (R_t) was monitored daily. R_t exhibited a sigmoidal increase with a plateau value of 421 ± 9 $\Omega \cdot cm^2$ after 7 days.

Light and electron microscopy revealed formation of monolayers consisting of cylindrical cells with tight junctions and a dense apical brush border. Filters were mounted in Ussing chambers and Isc, transepithelial voltage (V_t), and unidirectional Na^+- and Cl^-- fluxes were measured. Control values were V_t -1.1 ± 0.3 mV (mucosa negative), I_{sc} 0.1 ± 0.01, J^{Na}_{NET} -0.3 ± 0.4 (ns), and J^{Cl}_{NET} 0.1 ± 0.2 $\mu mol \cdot h^{-1} cm^{-2}$ (ns).

Serosal forskolin (FSK) 10^{-5}M increased V_t to -17.8 ± 1.5 mV, I_{sc} to 1.9 ± 0.1, J^{Cl}_{NET} to 1.7 ± 0.6 and J^{Na}_{NET} to 0.2 ± 0.4 $\mu mol \cdot h^{-1} cm^{-2}$ (ns). The FSK-induced I_{sc} was decreased to 7% by serosal bumetanide (BUM) 10^{-4}M and reversibly inhibited by the chloride-channel blockers DPC and NPPB with a respective IC_{50} of 10^{-3}M and $3 \cdot 10^{-4}$M.

Serosal dibutyryl-cAMP 10^{-3}M, VIP 10^{-8}M, or prostaglandin E_1 10^{-6}M caused an increase of I_{sc} and V_t which could be reversed by BUM and DPC.

In conclusion, the HT-29/B6 clone shows a cAMP-mediated Cl^- secretion responding to physiological secretagogues. Thus glucose-free culture results in the formation of monolayers possessing attributes of colonic crypts. As the HT-29 cell line is derived, in contrast to other cellular models, from a primary tumor, the clone presented here is most suitable for the transepithelial investigation of intestinal chloride-secretion.

Institut für Klinische Physiologie, Klinikum Steglitz, Freie Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45

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LIPID PEROXIDATION AND DECREASE OF GLUTATHIONE (GSH) IN ACUTE SKIN GRAFTS

A.J.Augustin, *R.Goldstein, E.Purucker and J.Lutz.

Oxygen free radicals are known to be involved in ischemia-reperfusion injury in skin flaps. The commonly used pedicle flap has to be subdivided at least into a lip area and a central zone, which includes the pedicle, because of different oxygen availability. Here, the free flap model,which shows no different areas, was used.
For experiments we used male wistar rats. A 2.5 x 2 cm abdominal wall skin flap was dissected, totally lifted and infolded immediately. The postischemic period lasted 12 hours. Control skin flaps were dissected in the same way. Lipid peroxide level of the skin was determined by modification of the method of Ohkawa et al. (Anal. Biochem. 95, 351, 1979). Glutathione level was determined by the method of Griffith (Anal. Biochem. 106, 207, 1980).
After 12 h skin tissue level of lipid peroxides increased from 185 ± 11 (SEM) nmol/g to 1871 ± 305 nmol/g ($p<0.01$). This elevation of lipid peroxides, indicating oxygen free radical damage, was accompanied by a decrease of tissue glutathione level from 541 ± 30 nmol/g in control animals to 77 ± 17 ($p<0.05$).
These data show free radical formation in the postischemic period, which cannot be compensated by protecting systems like peroxidases (extracellular) or superoxide dismutase and glutathione peroxidase. Glutathione (GSH, reduced form) could have served as electron donor for reduction of hydrogen peroxide:
$2\ GSH + H_2O_2 \text{ -----> } GSSG + 2\ H_2O$.

Physiologisches Institut, Röntgenring 9,
*Dermatologische Klinik, J.-Schneiderstr.2,
D-8700 Würzburg.

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CURRENT FLUCTUATION ANALYSIS OF BASOLATERAL K^+ CHANNELS STIMULATED BY ALDOSTERONE IN ALVEOLAR EPITHELIUM OF *XENOPUS LAEVIS*

B. Illek, H. Fischer, and W. Clauss

Control and aldosterone-stimulated lung tissues were investigated in Ussing chambers under voltage clamp conditions using current fluctuation analysis. In presence of an alveolar-to-pleural K^+ gradient transepithelial K^+ currents were induced by permeabilizing the apical membrane for K^+ with the pore-forming antibiotic amphotericin B (10 μM). This K^+ current (I_K) was blockable by lidocaine and followed Michaelis-Menten kinetics. Halfmaximal lidocaine inhibition concentration was 302±63 μM (mean±SE, n=6). The corner frequencies of the lidocaine-induced Lorentzians increased linearly with blocker concentration.Values for the association and dissociation rate constants were 1.3±0.03 $\mu M^{-1} \cdot s^{-1}$ and 331±15 s^{-1}. The calculated mean single K^+ current was 1.5±0.1 pA and did not change after aldosterone stimulation, corresponding to a single K^+ conductance of 15 pS. After stimulating the tissues for 4 hours with 1μM aldosterone, I_K was significantly increased to 66.9±6.8 $\mu A/cm^2$ (n=9), compared to 44.7±5.3 $\mu A/cm^2$ (n=7) in unstimulated tissues. I_K was in close correlation with the number of K^+ channels (r=0.746, n=10). The regulation of basolateral K^+ channel density permits changes in the rate of transcellular Na^+ transport without modifying intracellular ionic content and volume. This is essential for cell homeostasis in Na^+ transporting epithelia.

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INTRACELLULAR ELEKTRODE MEASUREMENTS IN EPITHELIAL CULTURED CELLS DURING INDEPENDENT CHANGES OF APICAL AND BASOLATERAL FLUIDS

K-P.Thiele, JS.Schwegler, A.Heuner and S.Silbernagl

We recently showed that monolayers of the cultured proximal tubular cell line OK are electrically extremely leaky (Pflügers Arch. 1989, 415:183ff). We now present a method to control both the apical and basolateral fluid of leaky epithelia during measurement of intracellular potential (PD_m). To prove correct separation of both fluid spaces we superfused confluent OK cell monolayers grown on transparent filters with various K^+ concentrations to measure apical (t_K^{ap}), basolateral (t_K^{bl}) and total (t_K^{tot}) K^+ transference numbers. The sum of $t_K^{ap} + t_K^{bl}$ is only 15 ± 5% higher than t_K^{tot} (n=5). To follow a more direct approach for quantifying paracellular K^+ leakage, we superfused the apical side with 5.4 mmol/l K^+, the basolateral side with 20 mmol/l K^+. K^+ concentration on the apical side of a intercellular junction - measured by an extracellular K^+ selective microelectrode - was 5.7 ± 0.1 mmol/l (n=4), indicating that a paracellular K^+ leakage does not disturb measurements under our conditions. The ratio of apical to basolateral K^+ conductance (t_K^{ap}/t_K^{bl}) was 0.45 ± 0.07 (n=5). Therefore, potassium conductance is located to two thirds on the basolateral and to one third on the apical membrane of OK monolayer cells.
We believe that this is a reliable method to study sidedness in leaky epithelia by an electrophysiological approach.

Physiologisches Institut der Universität Würzburg, Röntgenring 9, D-8700 Würzburg

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ERYTHROPOIETIN REGULATION: A 50K PROTEIN WITH AN OXYGEN-DEPENDENT DNA-BINDING PROPERTY

Dittmer, J., and Bauer, C.

HepG2, a hepatoma cell line, produces erythropoietin (EPO) in a physiological manner, i.e. it shows basal and hypoxia-inducable EPO synthesis. In order to look for proteins involved in EPO regulation we analysed nuclear proteins of stimulated and nonstimulated HepG2 for their specific binding to a DNA-fragment of the 5'-flanking region of the EPO gene. We found a 50K protein whose DNA-binding activity could be modulated by metal ions. In presence of Fe^{3+} the DNA was bound to that protein from normoxic but not from hypoxic cells. In contrast, with Zn^{2+} and Co^{2+} no differences in DNA-binding could be seen between proteins from normoxic and hypoxic cells: Zn^{2+} promoted and Co^{2+} suppressed DNA-binding in both cases. The effects of Zn^{2+} and Co^{2+} on DNA-binding correspond to the action of these metal ions on the EPO synthesis in HepG2 cells. The addition of $CoCl_2$ yielded in a 2-3 fold higher EPO synthesis under normoxic conditions whereas in presence of $ZnCl_2$ hypoxia-induced production was lowered down to 60-70% of control value. We suppose that the 50K protein is an iron protein whose DNA-binding activity is regulated directly or indirectly by oxygen.

Physiologisches Institut der Universität Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich

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THE EARLY DECLINE OF ERYTHROPOIETIN PRODUCTION AT CONTINUOUS HYPOXIA IS NOT DUE TO FEEDBACK INHIBITION

K.-U. Eckardt, J. Dittmer, R. Neumann, C. Bauer und A. Kurtz

Serum erythropoietin (EPO) levels in man and rodents are known to increase within 1-2 hours following the onset of normobaric or hypobaric arterial hypoxia, but despite continuous hypoxia decline again prior to an increase in blood oxygen carrying capacity. In order to define the possible mechanisms underlying this phenomeneon, we have investigated (i) how renal EPO mRNA content and EPO production rates underlying the early kinetics of serum EPO levels change under different degrees of normobaric hypoxia and (ii) if a feedback inhibition of either EPO formation or EPO survival in the circulation exists by the hormone itself.

We found that serum immunoreactive EPO in rats peaked after 12 h exposure to 7.5% or 9% oxygen (2949 ± 600 mU/ml and 756 ± 108 mU/ml; mean ± SE) and declined to 29% and 64 % of peak levels, resp., after 36 h of hypoxia. EPO levels in response to 11.5% oxygen showed no consistent change between 12 h and 36 h of hypoxia (122±12 mU/ml and 182±35 mU/ml, resp., mean ± SE). The decline in EPO levels under severe hypoxia (7.5% O_2) was paralleled by a marked reduction in renal EPO mRNA content. Furthermore, the clearance rate of iodinated recombinant human EPO (rhEPO) in normoxic and hypoxic rats was not different, also indicating, that the decline in serum levels is primarily due to diminished hormone production. Considering an EPO half life time of 110 min for endogenous EPO in the rat, the observed reductions in serum EPO after 36 hours corresponded to preceeding declines of calculated EPO production rates from 163 to 62 fold (7.5% O_2) and 36 to 25 fold (9% O_2) basal values. To dermine if EPO exerts direct or indirect feedback effects on its production, rats were injected with 50 IU rh EPO either 12 hours, 6 hours or immediately prior to hypoxia. However, endogenous EPO formation in response to a subsequent hypoxic exposure of 12 hours was unaffected by the exogenous application of EPO.

The results indicate that the early decrease in EPO production at continuous hypoxia is not mediated by a negative feedback control through the effect of EPO on its production sites or target cells. While the reductuion in EPO production rate occurs independent of the amount of EPO produced, the magnitude of the decline appears to be related to the degree of the preceeding stimulation.

Physiologisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich

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RECEPTOR VERSUS ENZYME MEDIATED SPECIFITY OF MINERALOCORTICOID ACTION (Na^+ TRANSPORT) IN RAT RECTAL COLON IN VITRO

M Fromm, JD Schulzke, KM Kreusel, P Clausen, A Krohn, U Lempart, S Lüderitz, U Hegel and K Hierholzer

11-hydroxy-steroid-dehydrogenase (*11-HSD*) catalyses the reactions corticosterone ⟷ 11-dehydro-corticosterone and cortisol ⟷ cortisone. We tested the possibility that local action of this enzyme is responsible for mineralocorticoid (*MCS*) specifity of corticosteroids rather than the classical concept of intracellular steroid receptors.

Rat rectal colon was stripped of the muscularis mucosae, submucosa, and muscularis propria ("total strip") and mounted in Ussing chambers. Short circuit current (Isc) was measured over a time period of 8 hrs. In vitro added aldosterone (*ALDO*, 10^{-8} M) produced an increase of Isc which could be reversed by 10^{-4} M amiloride (=J_{Na}).

- Addition of corticosterone (*B*) in a concentration of 10^{-8} M resulted in no significant increase of J_{Na}.
- Glycyrrhetinic acid (*GLY*, 10^{-6} M), a component of liquorice, alone had no apparent effect on J_{Na}.
- When *GLY* (10^{-6} M) and *B* (10^{-8} M) were added together a definite increase of J_{Na} was observed which was not significantly different from that of *ALDO* (10^{-8} M).
- The metabolite of *B*, 11-dehydro-corticosterone, stimulated J_{Na} at a pharmacologic concentration of 10^{-5} M.
- This effect was abolished in the presence of *GLY* which prevented the formation of *B*.
- Identical results were obtained with cortisol instead of *B*.

We conclude that specifity of *MCS* action in rat rectal colon is controlled by corticosteroid metabolism: *11-HSD*, localized within the target tissue, prevents corticosterone and cortisol from binding to the mineralocorticoid receptor by oxidation of the 11-OH group. It is directly demonstrated in a typical *MCS* target tissue that *GLY* inhibits the action of local *11-HSD*. The distal large intestine takes part in inducing symptoms of hyperaldosteronism at chronic liquorice consumption.

Institut für Klinische Physiologie, Klinikum Steglitz, Freie Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45

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INTERSTITIAL OSMOLARITY AND pH DETERMINE AXIAL DIFFERENTIATION IN RENAL COLLECTING DUCT.

U. Kersting, L. Wojnowski, W. Steigner and H. Oberleithner

The signal (s) inducing cell differentiation in renal collecting duct are still unknown. We used cultured canine kidney (MDCK) cells as a differentiation model for collecting duct epithelium. The cells were grown to confluency and then incubated in different media: pH 6.4 (pH↓); pH 7.7 (pH↑); 600 mosmol/l (osmol↑); 200 mosmol/l (osmol↓). Four days later we tested for the capacity of the apical cell membrane to bind peanut-lectin (PNA). PNA binds in vivo exclusively to the apical membrane of bicarbonate secreting (B-type) intercalated cells. Furthermore, we examined dome formation. These blisters underneath the MDCK epithelium are formed due to Cl^- reabsorption in exchange for HCO_3^-. We measured in rat kidney in vivo pH (ion-selective microelectrodes) and osmolarity in vasa recta of the papilla in antidiuresis and diuresis.

	MDCK cell (MEAN ± SEM)		rat kidney (vasa recta)	
	PNA^+ (%)	domes/cm^2	pH	mosmol/l
osmol↓	97 ± 1	335 ± 21	7.29 ± 0.08	356
pH↑	98 ± 2	256 ± 3	diuresis	
pH↓	74 ± 2	3 ± 0.7	5.7 ± 0.09	1352
osmol↑	5 ± 0.5	0	antidiuresis	

PNA binding capacity of MDCK cells is near zero shortly after cell splitting. The typical PNA binding pattern in a young confluent layer is found in both wild-type and cloned MDCK cells. In alkaline or hypotonic medium PNA binding is enhanced and dome formation is stimulated. PNA positive cells are cuboidal, possess a dark cytoplasm with small vesicles and large intercellular spaces (intercalated cell type). In an acid environment or in hypertonic medium MDCK cells lose their PNA binding capacity and their capability for dome formation. PNA negative cells are flat and possess a light cytoplasm with large vesicles (principal cell type). In rat, high osmolarity is coupled to a high H^+-activity in the interstitial space of the inner medulla. We postulate: After cell splitting MDCK cells differentiate dependent on the metabolic conditions. Osmolarity and pH may force collecting duct cells to differentiate either to principal cells or intercalated cells. B-type intercalated cells (Cl^- reabsorption, HCO_3^- secretion, apical PNA binding) are expressed only in conditions found in renal cortex.

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Dept. of Physiology, Univ. Würzburg, Röntgenring 9, D-8700 Würzburg

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ELECTRICAL ACTIVITY OF MAGNOCELLULAR NEURONES IN THE NUCLEUS PARAVENTRICULARIS IS INCREASED AFTER OSMOTIC CHALLENGE IN THE NEWBORN CHICKEN

R. Großmann, Xu Bin and F. Ellendorff

Neurohypophysial arginine-vasotocin (AVT), an analogue to mammalian arginine-vasopressin, plays a major osmoregulatory role in birds. A close relationship between electrical firing discharge and hormonal activity has been demonstrated in the mammalian hypothalamo-neurohypophysial system. In order to examine whether this feature exists in avian species as well and, if so, is established already early during development single unit recordings from antidromically identified magnocellular neurones in the hypothalamic nucleus paraventricularis (PVN) were performed.

Within 24 hours after hatching chicks were anaesthetized with urethane (3 mg/g, i.p.) and heads fixed in a stereotaxic frame. In each of nine chicks one neurone was studied. Mean spontaneous firing rate, averaged over at least 5 min before any manipulation of plasma osmolality was 0.8 spikes/sec (range 0-3 spikes/sec). In four cells tested with 0.1 ml physiological saline (i.p.) firing frequency was unaffected for up to 30 min. following injection. Injection of 0.1 ml 1.5 M NaCl i.p., an osmotic stimulus which elicited significant amounts of AVT in the newborn chick (Klempt, Ellendorff, Großmann, this meeting), increased mean firing activity of six magnocellular PVN neurones by more than 1 spike/sec during the period 25 to 30 min after injection compared to basic frequency. In one neurone mean firing discharge was increased by more than 4 spikes/sec. Two neurones were unaffected and one neurone inhibited.

Our results show that magnocellular PVN neurones in the newborn chick are osmosensitive and respond to osmotic challenge with increased firing activity, which in turn may lead to secretion of AVT.

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Forschungsbereich Physiologie, Institut für Kleintierzucht FAL, Dörnbergstr.25/27, D - 3100 Celle

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ARGININE-VASOPRESSIN (AVP) ACTS VIA V_1-RECEPTORS ON CYTOSOLIC CALCIUM IN THE ISOLATED PERFUSED RABBIT CORTICAL THICK ASCENDING LIMB (cTAL)

R. Nitschke, U. Fröbe, and R. Greger

Rabbit cTALs were microdissected and perfused in vitro using established methods. The tubules were incubated with fura-2 AM ($2 * 10^{-6}$ mol/l) at room temperature for 45 min and were studied thereafter at 37°C. As a measure of cytosolic calcium the fluorescence emission ratio (R) of 335nm/380nm excitation was monitored at 1 Hz time resolution. Calibration of the cytosolic Ca^{++} activity was carried out at the end of each experiment by exposing the perfused tubule to $0.5 * 10^{-6}$ mol/l digitonin in the presence of defined extracellular calcium activities. In control conditions R was 1,0 ± 0,06 (n = 55) corresponding to a calcium activity of $10-50 * 10^{-9}$ mol/l. Application of AVP (10^{-8} - 10^{-10} mol/l) to the bath perfusate increased R rapidly from 0.93 ± 0,07 to 1,59 ± 0,1 (n = 34). R fell transiently within 7 - 12 min to the control values even in the presence of AVP. Repeated addition of AVP led to reduced calcium responses. The AVP induced transient Ca^{2+} increase was completely dependent on the presence of basolateral calcium, whereas luminal calcium had no influence. The V_2-agonist dDAVP ($10 * 10^{-9}$ mol/l) had no effect on cytosolic calcium (n = 5). The V_1-antagonist (Manning compound, 10^{-8} or 10^{-9} mol/l) blocked the AVP effect completely (n = 5). We conclude that AVP increases cytosolic calcium in rabbit cTAL segments via V_1 - receptors possibly by an initial calcium release from intracellular stores and by calcium influx through the basolateral membrane.

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Physiologisches Institut der Albert-Ludwigs-Universität Freiburg, Hermann-Herder-Straße 7, D-7800 Freiburg

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ANGIOTENSIN II INDUCED RENAL VASOCONSTRICTION IS ESSENTIALLY MEDIATED BY PROTEIN KINASE C ACTIVATION

H. Scholz and A. Kurtz

The aim of this study was to investigate the intracellular pathways along which angiotensin II (ANG II) exerts its constrictor effect on the renal vasculature.

Using the isolated perfused rat kidney model, we examined the role of calmodulin, protein kinase C, and transmembrane calcium influx in ANG II induced renal vasoconstriction. For this purpose, kidneys were isolated from rats and perfused in a recirculating system at constant pressure of 100mmHg using a feedback regulated peristaltic pump. The perfusion medium consisting of a substrate-enriched Krebs-Henseleit buffer with a 10 % fraction of human erythrocytes was dialyzed against a 20 fold volume of saline. Perfusion flow rate was obtained from the pump revolutions and continuously monitored. The tested drugs were infused into the arterial limb of the perfusion circuit.

ANG II (10-100pM) elicited reversible and dose-dependent decrease of perfusion flow rate. Renal vasoconstriction caused by ANG II (100pM) was not altered by the calmodulin antagonists calmidazolium (1μM) and W-7 (10μM). In contrast, staurosporine, a putative C-kinase inhibitor, greatly reduced (10, 30nM) or blunted (100nM) the vasoconstrictory effect of ANG II. Stimulation of C-kinase by phorbol-12-myristate-13-acetate (PMA) (1-100nM) also caused a sustained and dose-dependent decrease of perfusion flow rate. The constrictor effect of PMA was prevented by the preceeding infusion of staurosporine (100nM). As control, the phorbol ester 4α-phorbol 12,13 didecanoate (PDD) (1-100nM) which is known to be inactive in C-kinase stimulation failed to increase renal vascular resistance. Renal vasoconstriction caused by ANG II (100pM) and PMA (100nM) was attenuated by the calcium channel blockers verapamil (5μM) and nifedipine (5μM) and reversibly inhibited by cobaltous chloride (2mM).

Taken together, these findings are compatible with the concept that ANG II induced renal vasoconstriction is essentially mediated by C-kinase activity. Moreover, the activation of protein kinase C seems to require or to induce enhanced transmembrane calcium influx.

Physiologisches Institut der Universität, Winterthurer Strasse 190, CH-8057 Zürich

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EFFECT OF SECOND MESSENGERS ON RENIN SYNTHESIS AND RENIN SECRETION IN PRIMARY CULTURES OF MOUSE RENAL JUXTAGLOMERULAR CELLS

R. della Bruna and A. Kurtz

Utilizing primary cultures of mouse renal juxtraglomerular cells we have examined the effects of the second messengers cAMP, cGMP and protein kinase C on the synthesis and the secretion of renin. Rates of renin secretion were estimated from the cellular release of renin activity, those of synthesis by specific immunoadsorption of protein produced by cells pulsed with ^{35}S-methionine.

An increase of cAMP induced by forskolin (1μM) or isoproterenol (10μM) stimulated both the synthesis and the secretion of renin. Stimulation of secretion by cAMP occurred within minutes, stimulation of synthesis was observable with a delay of around eight hours.

An increase of cGMP by 8-bromo-cGMP (100μM) inhibited only basal renin secretion and was without effect on synthesis.

Activation of protein kinase C by the phorbol ester PMA (10nM) had no significant effect on basal and stimulated secretion nor on basal synthesis but blunted the increase of renin synthesis induced by cAMP.

Our findings suggest that cAMP is an important regulator of both synthesis and secretion of renin in renal juxtaglomerular cells. The inhibitory effect of C-kinase activation on cAMP-induced stimulation of renin synthesis could provide a novel explanation for the inhibitory effect of angiotensin II on renin synthesis in vivo.

Physiologisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich

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CONTROL OF PRESSURE NATRIURESIS IN CONSCIOUS DOGS: ROLE OF THE SYMPATHETIC NERVOUS SYSTEM

H. Ehmke, P.B. Persson, M. Seyfarth and H.R. Kirchheim

The relationship between renal artery pressure (RAP) and urinary sodium output ($U_{Na}V$) was investigated during (1) control conditions, (2) common carotid occlusion (CCO), (3) CCO with intrarenal prazosin infusion, and (4) low-dose intrarenal methoxamine infusion. Pressure natriuresis curves (PNC) were determined in 12 conscious dogs on a normal-salt diet. RAP was reduced in 10 mmHg steps by inflation of a cuff placed around the renal artery. (1) Under control conditions, a reduction in RAP resulted in a strong decrease in urine output and $U_{Na}V$. In all dogs, the PNC was found to be closely related to the individual resting blood pressure; urine flow rate reached zero 30-40 mmHg below resting blood pressure. (2) A baroreflex activation of the sympathetic nervous system by CCO shifted the PNC by 11.1 ± 3.1 mmHg ($P < 0.01$; $n=8$) to the right. The sensitivity of pressure natriuresis was not affected by CCO. (3) The shift was blocked, when the selective alpha$_1$-adrenoceptor antagonist prazosin was infused intrarenally during CCO ($n=9$). Without CCO, prazosin did not alter $U_{Na}V$ at the control RAP. (4) Similar to CCO, intrarenal infusion of the selective alpha$_1$-adrenoceptor agonist methoxamine shifted the PNC by 19.3 ± 7.7 mmHg to the right without altering the slope of the PNC ($n=3$). Neither control renal blood flow nor glomerular filtration rate were changed by the methoxamine infusion. These results indicate that the sympathetic nervous system regulates $U_{Na}V$ by shifting the PNC through intrarenal alpha$_1$-adrenoceptors without altering its slope.

I. Physiologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg

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INTERACTION OF ANGIOTENSIN II AND ADENOSINE IN RENAL MICROCIRCULATION

M.S. Dietrich, N. Parekh and M. Steinhausen
with the technical assistance of R. Dussel

It is still a subject of controversy to what extent an inhibition of angiotensin II (A II) can block the renal vasoconstriction due to adenosine. The purpose of the present investigation was to analyse the interaction of these two vasoconstrictory substances by means of in vivo microscopy in the split hydronephrotic rat kidney (cf. for the technique: Kidney Int 35: 1151-1160, 1989). In agreement with Spielmann and Osswald (Am J Physiol 237: F 463-467, 1979), we found in a first series of experiments that local application of the A II-antagonist saralasin (10^{-6}M) abolished the vasoconstriction and the reduction of glomerular blood flow induced by the A1-adenosine receptor agonist N6-cyclohexyladenosine (CHA, local concentration 10^{-7}M). Without saralasin, CHA reduced glomerular blood flow and decreased vessel diameters as previously reported (Holz and Steinhausen, Renal Physiol 10: 272-282, 1987). In a second series of experiments, we found that the blockade of CHA by the selective A1-adenosine receptor antagonist 1,3-dipropyl-8-cyclopentyl-xanthine (DPCPX, 10^{-8}M) did not abolish the vasoconstrictory action of A II (10^{-8}M). In separate series, we confirmed the inhibitory action of DPCPX on the adenosine-induced vasoconstriction and the stability of our preparation.
We suppose that adenosine needs a functioning A-II-receptor system for its vasoconstrictory action, whereas A-II can induce a non-adenosine-dependent vasoconstriction.

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I. Physiologisches Institut der Universität
Im Neuenheimer Feld 326
D-6900 Heidelberg

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Electrophysiological andpharmacological differences between mesenchymal cells of the renal cortex

C. P. Bührle and R. Nobiling, I. Physiologisches Institut, Universität Heidelberg, INF 326, 69 Heidelberg, Germany (West)

Little is known about the comparative electrical membrane properties of mesenchymal cells within the kidney cortex, owing to difficulties of their microtopographical localization. The microvessels and glomeruli embedded in a vast mass of tubular epithelia (TBs). Three types of preparations have been developed to overcome this difficulty: the mouse hydronephrotic mouse kidney, the mouse kidney slice, and cultured renal cortical cells. We investigated, whether vascular smooth muscle cells (VSMCs), juxtaglomerular epithelioid cells (JGECs), mesangial cells (MCs) and tubular epithelia differ sufficiently in their electrical membrane properties or in their responses to vasoactive agents, as to permit an electrophysiological discrimination when visual identification is impossible. Within the experimental scatter, membrane potential and cellular input resistance do not permit to discriminate between JGECs, VSMCs and MCs in all preparations. Only tubular epithelia are easily discernible owing to their high membrane potential and low input resistance. In all preparations, JGECs, VSMCs and MCs reacted with depolarizations in response to the vasoactive peptides AVP and ANG II. The only difference consists in the absence of alpha-receptors on MCs as opposed to VSMCs and JGECs. Cells from TBs did not react to the application of any vasoactive substance.

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INTERACTION OF STEROID HORMONES WITH THE CONTRALUMINAL ANION TRANSPORTERS IN THE PROXIMAL RENAL TUBULE

K. J. Ullrich, G. Rumrich, S. Klöss and F. Papavassiliou

The prevailing hypothesis is that steroid hormones cross the cell membrane by simple diffusion. Our specificity scheme of the contraluminal anion transporters (Am J Physiol 254:F453, 1988) would predict that steroid hormones are also transported by the contraluminal para-aminohippurate (PAH) transporter. To test this we applied the stop flow microperfusion technique of the peritubular capillaries and studied the interaction of steroid hormones with the contraluminal uptake of ^{3}H-PAH and ^{35}S-sulfate: Pregnenolone (5-pregnen-3ß-ol-20-one) and progesterone (4-pregnene-3,20-dione) do not interact with the PAH transporter. If, however, progesterone has one additional OH-group in position 6, 17α or 21, or two additional OH-groups either in position 11 and 21 (corticosterone), or in position 17α and 21 (11-deoxycortisol), the compounds exert a high to moderate inhibitory potency against contraluminal PAH-transport (app. $K_{i,PAH}$ 0.13-0.38 mmol/l). If the pregnenolone or progesterone molecule has 3 additional OH-groups to yield cortisol or urocortisol interaction with the PAH transporter decreases. If cortisone or cortisol has an additional OH-group in position 6 interaction with the PAH transporter vanishes. 21-sulfatation or acetylation of corticosterone does not change its $K_{i,PAH}$ ($\approx$ 0.2 mmol/l). Sulfatation, however, exerts additional interaction with the sulfate transporter (app. $K_{i,SO_4^{2-}}$ 3.2 mmol/l). 1s influx of ^{3}H-cortisol was inhibited by 29% with 10 mmol/l probenecid when the starting concentration of cortisol was 0.1 mmol/l, and by 18% when it was 1 mmol/l. The data show that steroid hormones are indeed transported by the PAH transporter, probably in addition to diffussion through the lipid bilayer.

Max-Planck-Institut für Biophysik, Kennedyallee 70,
D-6000 Frankfurt/M. 70

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WHOLE CELL RECORDING OF SODIUM-COUPLED ALANINE TRANSPORT IN SINGLE PROXIMAL TUBULE CELLS

J. Hoyer and H. Gögelein

Single cells from proximal convoluted tubules (PCT) were isolated from cortical slices of New Zealand White rabbits (800-1200g). Alanine driven sodium current was measured with the tight-seal whole-cell recording method. Addition of L-alanine to the extracellular side induced an inward-directed sodium current and a cell depolarization. The cotransport current was sodium- and voltage-dependent, stereospecific, and independent on extracellular pH (pH 6.4 to 8.4). It followed simple Michaelis-Menten kinetics with an apparent K_m for alanine of 6.6 mmol/l and an I_{max} of 0.98 pA/pF at -60 mV (bath solution (in mmol/l): 140 Na-cyclamate, 1.3 Ca^{2+}, 0 - 40 alanine; pipette: 140 Tris-cyclamate, 10^{-7} Ca^{2+}, 0 alanine). Transport rate at physiological alanine concentration was in the same order of magnitude as the estimated cotransport current required for 90% alanine reabsorption in PCT. A coupling stoichiometry of 1 Na^+ and 1 alanine was estimated by Hill plots of cotransport current data. Apparent K_m for Na^+ and apparent I_{max} were potential dependent, whereas apparent K_m for alanine was potential independent in absence or presence of a large inward-directed Na^+-gradient. Apparent K_m for alanine increased when inward-directed sodium gradient was decreased. From these kinetic data and from additional theoretical treatment a cotransport model with a simultaneous transport mechanism, a potential dependent binding or unbinding of sodium, and a negatively charged empty carrier is derived.

Max-Planck-Institut für Biophysik, Kennedyallee 70,
D-6000 Frankfurt/Main, F.R.G.

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CHARACTERIZATION OF THE PAH-TRANSPORT SYSTEM IN BOVINE RENAL BASOLATERAL MEMBRANE VESICLES

C. Schmitt and G. Burckhardt

Amphiphilic anions, e. g. p-aminohippurate (PAH), are excreted in proximal tubules of mammalian kidneys. To characterize the transporter responsible for uptake of PAH from blood into the cell, basolateral membrane vesicles were prepared from bovine kidney cortex using a Percoll density gradient. [^{3}H]PAH uptake was enhanced by preloading the vesicles with unlabeled PAH and 2-oxoglutarate proving the presence of a transporter that exchanges external [^{3}H]PAH with intravesicular PAH or 2-oxoglutarate. PAH uptake was independent of gluconate, sulfate, chloride and bromide in the medium (75-150 mM), and was not influenced by changes in membrane potential suggesting electroneutrality even when monovalent PAH is exchanged for divalent 2-oxoglutarate. An exchange of PAH for 2-oxoglutarate plus H^+ seems not to occur since PAH/2-oxoglutarate exchange was not influenced by pH. Na^+ stimulated [^{3}H]PAH uptake in the presence, but not in the absence, of 2-oxoglutarate in the incubation medium. Thus, similar to the rat kidney, the PAH transporter itself is Na^+-independent, but can be driven by 2-oxoglutarate which is intravesicularly accumulated by the Na^+-coupled dicarboxylate transporter. PAH uptake was stimulated by a pH difference ($pH_{out} < pH_{in}$) suggesting PAH/OH^- exchange as a possible mode of the transporter. PAH/OH^- and PAH/PAH exchange were inhibited to the same degree by unlabeled PAH, probenecid, and 2-oxoglutarate in the medium proving that the same transporter was operative under both experimental conditions. Benzylpenicillin inhibited [^{3}H]PAH uptake with an apparent K_i of 1.5 mM. Cephalexin inhibited also, but with a weaker potency (app. $K_i > 5$ mM). In conclusion, our data reveal the presence of a Na^+-independent PAH/anion exchanger in bovine renal basolateral membrane vesicles with characteristics described earlier for the respective system in vesicles from the rat kidney. Due to the greater abundance, basolateral membrane vesicles prepared from bovine kidney provide a rich source for future isolation of the PAH transporter protein.

Max-Planck-Institut für Biophysik, Kennedyallee 70, D-6000 Frankfurt 70, FRG

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MERCURY IONS DISSIPATE THE TRANSEPITHELIAL POTENTIAL DIFFERENCE IN DISTAL CONVOLUTED TUBULES OF THE RAT KIDNEY.

A. Jungwirth, M. Ritter, F. Lang

The kidney is the main target organ for mercury toxicity. Acute administration of mercury ions is followed by marked alterations of renal electrolyte excretion. The present study has been designed to elucidate the acute effects of mercury ions on electrogenic transport systems in distal convoluted tubules. To this end, male Munich Wistar rats have been prepared for micropuncture in the usual way. Distal tubules have been identified by injection of lissamine green and perfused utilizing a microperfusion pipette allowing multiple fluid exchange. Transepithelial potential difference (Vte) has been recorded continuously, utilizing a conventional microelectrode. During perfusion with a solution composed of (in mmol/l) 141 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.2 $CaCl_2$, 0.8 Na_2HPO_4, 0.2 NaH_2PO_4, 5.0 $NaHCO_3$ Vte amounts to -19 $\pm$ 3 mV. Increase of luminal potassium concentration to 20 mmol/l hyperpolarizes the epithelium by -5.4 $\pm$ 0.7 mV. Amiloride (5 μmol/l) depolarizes the epithelium to -3.1 $\pm$ 1.2 mV. Mercury ions (10 μmol/l) lead to a gradual depolarization of the epithelium approaching -9.3 $\pm$ 2.7 mV within 8 minutes. For significant depolarization, 0.1 μmol/l mercury ions are required, half maximal depolarization is observed at approx. 3 μmol/l. A linear correlation occurs between Vte before application of mercury ions and the depolarization caused by mercury ions. Further analysis reveals that the depolarization is mainly due to a decline of the amiloride sensitive portion of Vte, whereas the potassium sensitive portion of Vte is not significantly altered by mercury ions.
In conclusion, mercury ions depolarize the distal transepithelial potential difference, an effect possibly contributing to the altered renal electrolyte excretion following acute administration of mercury ions. The depolarization is at least in part due to an inhibition of the amiloride sensitive sodium channels at the luminal cell membrane of principal cells.

Institut für Physiologie der Universität Innsbruck, Fritz-Pregl-Straße 3, A-6010 Innsbruck

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INTRACELLULAR ACCUMULATION AND TRANSEPITHELIAL REABSORPTION OF HCO_3^- INDUCED BY HYPERTONIC STRESS IN CULTURED KIDNEY (MDCK) CELLS.

L. Wojnowski, U. Kersting, H.-J. Westphale, and H. Oberleithner

MDCK cells resemble properties of renal intercalated cells. They release $KHCO_3$ when exposed to hypotonic stress. Now we report on mechanisms of volume regulation activated by hypertonic conditions. By means of pH-sensitive microelectrodes we studied pH in domes formed in monolayers due to transepithelial salt transport and measured cytoplasmic pH in fused MDCK cells.
Application of hypertonic Ringer solution (+300 mOsm mannitol or NaCl) induces a transient alkalinization (ΔpH: 0.17 $\pm$ 0.02, n=11) of the dome-fluid (steady-state control pH = 7.32 $\pm$ 0.02). This indicates a transient HCO_3^- accumulation in the basolateral dome fluid. It cannot be explained by shrinkage of the dome-volume evaluated optically. Pretreatment with the carbonic anhydrase inhibitor acetazolamide (1 mM) inhibits the rate of dome-alkalinization by 37%. Hypertonic stress applied to fused MDCK cells induces a transient alkalinization of the cytoplasm (ΔpH: 0.19 $\pm$ 0.02, n=7) starting from a steady-state intracellular pH of 7.36 $\pm$ 0.01.
We conclude that (i) MDCK cells accumulate HCO_3^- in response to acute hypertonic stress; (ii) the increase of intracellular HCO_3^- concentration is mediated by carbonic anhydrase. This enzyme catalyzes cytoplasmic HCO_3^- production and is responsible for its accumulation; (iii) hyperosmotic conditions that resemble antidiuresis in kidney could increase at least transiently transepithelial reabsorption of HCO_3^- in renal collecting duct.
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REGULATION OF SORBITOL RELEASE FROM RAT INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS MAY INVOLVE CYTOSOLIC CALCIUM

C. Bevan, C. Theiss, and R.K.H. Kinne

Hypoosmotic stress leads to a rapid loss of the organic osmolyte sorbitol from IMCD cells in vitro. In order to investigate the role of various intracellular mediators on this phenomenon, IMCD cells were isolated in 600 mosm/l buffer and then resuspended in either 300 or 600 mosm/l buffer (osmolarity adjusted with NaCl) for 20 min. The intra- and extracellular sorbitol content was measured enzymatically. Under control conditions at 600 mosm, addition of the Ca^{2+} ionophore A23187 (10 uM) in the presence but not in the absence of calcium increased sorbitol release from 20 + 4% of the total sorbitol content to 32 + 3% (mean + S.D., n = 4; P<0.01). Under hypoosmotic conditions at 300 mosm, sorbitol release was significantly reduced from 51 + 2% to 29 + 4% in Ca^{2+}-free (1 mM EGTA) buffer, and to 30 + 4% by 20 uM trifluoroperazine, a calmodulin inhibitor (mean + S.D., n = 4; P<0.01). Inhibitors of calcium influx such as verapamil (0.1 mM) had no effect. In addition, the change in sorbitol permeability does not seem to involve protein kinase C since neither 1 uM phorbol 12-myristate 13-acetate nor 1 mM neomycin had any significant effect on sorbitol release. These results suggest that during osmoregulation, intracellular calcium may regulate sorbitol permeability of IMCD plasma membranes by a calmodulin-dependent mechanism.

Max-Planck-Institut für Systemphysiologie, Rheinlanddamm 201, 4600 Dortmund 1, FRG

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Parathyroid hormone increases thiol-protease activity by activation of protein kinase C in cultured kidney tubule cells (OK)

Liliana Denek[1], Johann S. Schwegler[2], Roland Schäfer[1], Albert J. Augustin[2] and August Heidland[1]

Intracellular proteases were measured in cultured opossum kidney cells during chronic (24h) exposure to parathyroid hormone (PTH) by assaying the rate of breakdown of azocaseine at pH 5.4. PTH increases the total proteolytic activity from a control of 6.4 ± 0.1 U/mg protein/45min in a saturable, dose-dependent manner to a maximum of 14.2 U/mg/45min (n=4). Saturation occurs at as low a PTH concentration as 10^{-12} mol/l. E64 (1 mmol/l), an unspecific inhibitor of thiol proteases, markedly reduces this PTH mediated increase (7.3 ± 0.3 U/mg/45min vs. control 6.5 ± 0.1 U/mg/45min at 10^{-11} mol/l PTH). Application of the phorbol ester TPA (10^{-10} mol/l, 24h) mimicks the effect of PTH on total protease activity (12.2 ± 0.3 U/mg/45min vs. control 6.4 ± 0.1 U/mg/45min; n=4). This TPA-induced increase is also blocked by 1 mmol/l E64. Staurosporin (10^{-7} mol/l), a potent inhibitor of protein kinase C, is equally effective in blocking the TPA- and PTH-induced protease activity increase (TPA + staurosporin: 4.9 ± 0.1 U/mg/45min vs. control 6.2 ± 0.0 U/mg/45min; PTH + staurosporin: 5.2 ± 0.2 U/mg/45min vs. control 6.2 ± 0.0 U/mg/45min; n=4). Staurosporin on its own has no significant effect on protease activity. Both the calcium ionophore A23187 ($5 \cdot 10^{-7}$ mol/l, 24h) and dibutyrylic cAMP (10^{-4} mol/l, 24h) significantly reduce azocaseine breakdown ($p<0.05$).
These data are highly suggestive of the notion that PTH increases the intracellular protease activity by an activation of the protein kinase C.

[1]Dept. of Nephrology, Josef-Schneider-Str.2, and [2]Dept. of Physiology, Röntgenring 9, University of Würzburg, 8700 Würzburg, FRG

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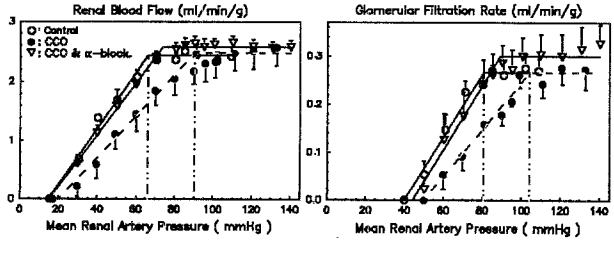
SYMPATHETIC MODULATION OF RENAL AUTOREGULATION

P.B. Persson, H. Ehmke, B. Nafz, A. Just and H.R. Kirchheim

The sympathetic nervous system may exert an important influence on renal autoregulation (AR). This study was designed to investigate the mechanisms by which a moderate sympathetic stimulus influences AR of renal blood flow (RBF) and glomerular filtration rate (GFR) in 39 experiments in 7 conscious dogs. Common carotid occlusion (CCO) increases renal nerve activity by some 60%, and impaired AR by increasing the lower autoregulatory limit of RBF and GFR by 21-30 mmHg (fig. 1). The impaired AR can be of significant clinical importance in several pathophysiological states characterized by a high sympathetic tone along with a normal to low arterial pressure. Two common examples for a such combination are congestive heart failure and cardiovascular shock.
The impairment of AR was reversed by an intrarenal infusion of an α_1 antagonist (Prazosin, fig. 1). An intrarenal infusion of the α_1-adrenoceptor agonist methoxamine induced a similar effect as CCO. In another group it was shown, that a combination of CCO with an intrarenal AII-blockade (saralasin) did not significantly alter the response to CCO.
These data demonstrate an α_1-adrenergic resetting of renal autoregulation. An augmented A II-formation does not play a major role in mediating this effect.



I. Physiologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg

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PROGESTERONE REDUCES THE POTASSIUM CONDUCTANCE IN MADIN DARBY CANINE KIDNEY (MDCK-) CELLS

M. Steidl, G. Pinggera, M. Paulmichl, F. Lang

Progesterone leads to natriuresis, an effect largely attributed to displacement of aldosterone from its receptor. The present study has been performed to elucidate the interaction of aldosterone and progesterone in MDCK cells. Surprisingly, the experiments revealed an acute effect of progesterone on the potential difference across the cell membrane (PD) in the absence of aldosterone, which was then analysed further: 0.1, 1 and 10 μmol/l progesterone depolarize the cell membrane of MDCK cells (from -51.8 ± 1.4 mV) by 1.3 ± 0.5, 4.1 ± 0.7 and 12.3 ± 1.5 mV, resp. Acute application of other steroids, such as aldosterone, hydrocortisone, hydroxyprogesterone, estradiol and dihydroxy-testosterone (each up to 10 μmol/l) do not significantly alter PD. 1, 5 and 10 μmol/l progesterone decrease the potassium selectivity of the cell membrane from 0.43 ± 0.03 to 0.29 ± 0.01, 0.20 ± 0.02 and 0.18 ± 0.01, resp. Since at the same time progesterone increases the resistance of the cell membrane, the decrease of potassium selectivity reflects a decrease of the potassium conductance of the cell membrane. Barium (10 mmol/l) depolarizes the cell membrane to -27.1 ± 1.5 mV and increase of extracellular K^+ concentration to 40 mmol/l depolarizes the cell membrane to -24.1 ± 0.7 mV. In the presence of either barium or 40 mmol/l K^+ the effect of progesterone (5 μmol/l) is almost abolished (1.2 ± 0.4 and 1.6 ± 0.3 mV, resp.). Removal of extracellular chloride or bicarbonate hyperpolarize the cell membrane (by -6.6 ± 1.9 and -10.8 ± 2.1 mV, resp.) but do not abolish the depolarizing effect of 5 μmol/l progesterone (13.9 ± 1.9 and 12.9 ± 1.8 mV, resp.). 10 μmol/l amiloride has no significant effect on the cell membrane potential, whereas 1 mmol/l amiloride depolarizes the cell membrane by 5.1 ± 0.6 mV. Neither concentration of amiloride interferes significantly with the depolarizing effect of 5 μmol/l progesterone (10.8 ± 0.8 and 9.5 ± 0.7 mV, resp.). In conclusion, acute administration of progesterone depolarizes MDCK cells by decreasing the potassium conductance of the cell membrane.

Institut für Physiologie der Universität Innsbruck, Fritz-Pregl-Straße 3, A-6010 Innsbruck

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RECEPTOR-MEDIATED INHIBITION OF VASOPRESSIN AND ALDOSTERONE RELEASE BY ANF IN CONSCIOUS RABBITS

R. Gerstberger, H. Schütz and E. Simon

Atrial natriuretic factor (ANF) antagonizes systemic and central effects of the osmoregulatory hormones vasopressin (ADH), angiotensin II (ANG-II) and aldosterone (ALDO) by (1) lowering their circulating levels and (2) antagonistic actions at the same target organ. To study receptor-mediated alterations in circulating ADH and ALDO, ANF was infused i.v. at 15 pmoles/min/kg b.w. (15 min) resulting in increased plasma levels of 270 ± 55 compared to 63 ± 14 pg/ml at slightly diminished arterial blood pressure (-8 ± 3 mm Hg). Plasma osmolality, oncotic pressure, sodium concentration and the hematocrit remained unchanged. In animals either slightly dehydrated or pretreated with 10 pmoles/min/kg b.w. ANG-II, both ADH and ALDO secretion were suppressed by 20-50 % at unchanged corticosterone levels. Receptor-autoradiography using 125I-labelled ANF revealed high affinity ANF-specific binding sites in the zona glomerulosa of the rabbits' adrenal and renal structures also endowed with receptors for ANG-II. In the hypothalamo-neurohypophyseal axis, the choroid plexi, the havily vascularized periventricular region of the IIIrd ventricle, the median eminence and the neural lobe - all strutures accessible to blood-borne ANF - were densely labelled by both radioiodinated ANF and brain natriuretic factor (BNF). Displacement studies support the specificity of the binding sites for ANF/BNF. Endogenous ANF may therefore be of regulatory importance in controlling the release of ADH and ALDO as well as inhibiting angiotensinergic actions.

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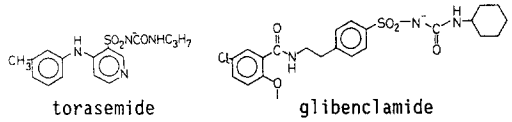
STRUCTURE-ACTIVITY RELATION OF SULFONYLUREA COMPOUNDS (SUC) ON NaCl-TRANSPORT IN ISOLATED PERFUSED RABBIT CORTICAL THICK ASCENDING LIMBS OF HENLE'S LOOP (cTAL)

E. Lohrmann, R. Nitschke, E. Schlatter, B. Masereel, J. Delarge, H.J. Lang, H.C. Englert, R. Greger

Antidiabetic SUCs inhibit ATP-dependent K^+-channels in pancreatic ß-cells; diuretic SUCs inhibit the $Na^+2Cl^-K^+$-cotransporter and Cl^--channels in the cTAL. This study examines the structure-activity relation of SUCs on equivalent short circuit current (I_{SC}) in cTAL segments. Diuretic SUCs were derivatives of torasemide (TOR), with the meta-toluol- (R_1) and the iso-propyl-group (R_2) replaced by cyclo-alkyl residues (6-8). Half maximal inhibition of I_{SC} after luminal ($IC_{50,l}$, µmol/l) and after basolateral addition ($IC_{50,bl}$), and lipid solubility as log of octanol/water-distribution (P) were determined.



torasemide glibenclamide

R_1/R_2	TOR	6/6	6/7	6/8	7/6	8/6	8/7	8/8
$IC_{50,bl}$	37	>100	>100	85	>100	80	20	70
$IC_{50,l}$	.24	30	9.6	14	3.5	.40	2.0	.10
P	.45	1.3	1.7	2.1	1.7	2.1	2.4	2.7

The introduction of cyclo-alkyl residues increases lipid solubility and preserves the affinity to the $Na^+2Cl^-K^+$-cotransporter (e.g. 8/8). Antidiabetic SUCs (glibenclamide, glipizide, gliquidone, glibornuride, glisoxepide, tolbutamide) had no effect from the lumen and basolateral side except for glibenclamide which inhibited I_{SC} with an $IC_{50,bl}$ of 80 µmol/l. We conclude that antidiabetic SUCs do not inhibit the luminal cotransporter and K^+-channel nor the basolateral Cl^--channel in cTAL. Supported by DFG Gr 480/9

Physiologisches Institut der Albert-Ludwigs-Universität, Hermann-Herder-Straße 7, D 7800 Freiburg, FRG

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ROLE OF LIPOPHILITY IN THE RENAL EXCRETION OF MIDDLE-WEIGHT PROTEINS IN THE RAT

D. Caliebe, M. Mályusz, P. Wrigge and G. Gronow

In order to clarify the role of lipophility in the tubular reabsorption of partially filtered middle-weight proteins, the excretion rate of enzymes of similar size (46 - 57 kDa) but of different isoelectric points (IP) and lipophility was studied in the rat 1.) in vivo after a bolus injection, 2.) in vitro on isolated rat kidney tubules and renal cortical slices. The following 125I-labelled enzymes were used: rat pancreatic lipase (RL, 48 kDa, IP: 7.0 ≥ 6.5 ≥ 6.3), human pancreatic lipase (HL, 46 kDa, IP: 5.8 ≥ 5.85), rat pancreatic amylase (RPA, 54 kDa, IP: 8.5 ≥ 8.3), rat salivary amylase (RSA, 57 kDa, IP: 5.0 ≥ 4.7 ≥ 4.5). Ad 1.): The injected activity was excreted according to the following pattern (the best filtered protein showing the smallest degree of excretion and vice versa):

	half life ($t_{½}$, min)	sieving coeff.	excreted ^{125}I-act. % of load	% protein-bound hereof
HL	17.5	0.129	6.8	20.9
RL	17.1	0.126	13.0	16.8
RPA	20.0	0.118	15.9	60.2
RSA	65.0	0.028	16.2	72.9

ad 2.) In vitro experiments carried out on renal cortical slices and on isolated kidney tubules show a.) faster reabsorption of 125I-lipases (HL ≥ RL) than of -amylases (RPA ≥ RSA), b.) faster release of 125I-di-iodo-tyrosin from lipases than from amylases (RPA ≥ RSA). - Extraction of highly purified preparations with neutral paraffin-oil resulted in the decrease of activity by: HL = 99 % = RL = 98 %; RPA = 75 %; RSA = 0 - 0.1 %. We conclude that 1.) reabsorption of middle-weight proteins does not occur strictly according to their IP; 2.) lipophility of filtered proteins seems to be of importance for their tubular reabsorption.

Physiologisches Institut der Universität Kiel, Olshausenstraße 40, D-2300 Kiel 1

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Kinetics of intracellular Ca⁺⁺-changes in single glomerular mesangial cells

R. Nobiling and C. P. Bührle, I. Physiologisches Institut der Universität Heidelberg, INF 326, 69 Heidelberg

Measurements of intracellular free calcium in individual glomeruluar mesangial cells (MCs) were done using a microscope fluorimeter with dual wavelength UV-excitation, the Ca^{++}-sensitive dye fura-2, and extremely sensitive, photon counting-based light detection. Fast (up to 200 x s^{-1}) filter changes allow quasi-continuous measurements at different wavelengths (Nobiling and Bührle, J. Micr. Nov 1989:156;149-161).

Cultured MCs are frequently used as models for the closely related vascular smooth muscle cells of the kidney vessels. The important role of Ca^{++} for cellular stimulation, e.g. in renin secretion and contraction, is still widely accepted. Many details, however, are still unclear and subject to extensive work. We report here certain chararacteristics of the Ca^{++} transients that occur in MCs after the application of the vasoactive peptides AVP and ANG II: Generally, a delayed cellular reaction with respect to the onset of the Ca^{++} increase is observed. This delay is not only dependent on the concentration of the agonists, but also on the temperature of the superfusion medium. An analysis of the kinetics demonstrates, that, obviously, two processes are involved in the generation of these transients. The identification and correlation to cellular processes such as transmembrane currents and or Ca^{++} liberation from intracellular stores may contribute to a better understanding of the processing of stimuli in these cells.

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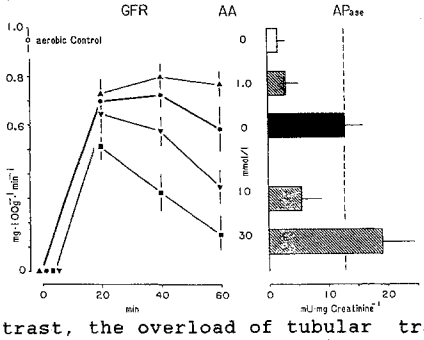
DOSE-DEPENDENT AMINO ACID EFFECTS ON POSTISCHEMIC RENAL FUNCTION IN THE RAT

Klause,N., M.Mályusz, H.Zinnert, and G. Gronow

High levels of plasma amino acids (AA)increase renal blood flow and glomerular filtration rate (GFR) in the intact kidney. We studied effects of infusion of variable concentrations of an isomolar AA mixture of proline, glycine, and aspartic acid on postischemic renal function in rats. GFR (creatinine clearance) and urinary losses of lysosomal acid phosphatase (APase) were measured after 30 min renal ischemia in situ. Without AA-infusion, postischemic APase loss increased markedly, whereas GFR declined by about 40% (see figure). Infusion of AA at ≤ 10 mM (maximal plasma) level had a cytoprotective effect (loss of lysosomal APase declined significantly). In contrast, the overload of tubular transport systems by 30 mM AA caused a marked increase in APase release. At 1 mM AA infusion, vasodilation induced a stabilization of postischemic GFR. At ≤ 10 mM AA infusion, however, GFR fell in parallel to a decrease in mean arterial blood pressure. It is concluded that AA in a physiological range of plasma concentration (≤1mM) may support postischemic renal function.



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RENAL PRODUCTION OF ERYTHROPOIETIN
- STUDIES IN THE ISOLATED PERFUSED RAT KIDNEY -

H. Pagel, W. Jelkmann and Ch. Weiss

The dependency of the production of the renal glycoprotein hormone erythropoietin (Epo) on the renal O_2 supply was studied in the isolated perfused rat kidney (IPRK). The kidneys were perfused at constant perfusion pressure (100 mmHg) in a recirculation system with a substrate enriched Krebs-Henseleit solution containing 60 g/l bovine serum albumin and freshly drawn human erythrocytes.
When the kidneys were perfused at an arterial pO_2 of 720 or 150 mmHg (hematocrit 5 %), Epo production measured by RIA was very low (0.1-0.2 U/g kidney after 3 h of perfusion). At a pO_2 of 20 mmHg, Epo production increased significantly to 0.9 U/g kidney. However, the production of Epo was little affected by changes in the hematocrit - i.e. the O_2 carrying capacity of the perfusion medium - in a range between 40 and 0 %.
These results indicate that the production of Epo in the IPRK is mainly under the control of the arterial pO_2.

Institut für Physiologie, Medizinische Universität zu Lübeck, Ratzeburger Allee 160, D-2400 Lübeck 1

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GSH AND GSSG CHANGES IN KIDNEY, LIVER AND GUT AFTER AORTIC OCCLUSION AND REPERFUSION

E.Purucker, A.J.Augustin and J.Lutz

Reduced glutathione (GSH) as specific substrate for GSH-peroxidase plays a critical role in detoxification of hydrogen peroxide and other reactive oxygen species. The relationship betweeen GSH and its oxidized disulfide form (GSSG) is thought to have an important effect on oxidation-reduction state of protein-thiols, resulting in enhanced or diminished activity of, e.g., catalytic enzymes.
In male wistar rats we investigated the tissue concentration of GSH and GSSG in controls, after 45 min of subdiaphragmatic aortic occlusion, and after reperfusion of 45 min. GSH and GSSG were determined by a modification of the method of Griffith (Anal. Biochem. 106 207, 1980). Tissue was homogenized in 5% 5-sulphosalicylic acid immediately after dissection to prevent GSH-loss and conversion of GSH to GSSG.
GSH in controls amounted to ($x \pm SEM$ in $\mu M/g$ wet weight): kidney 2.08 ± 0.11, liver 6.18 ± 0.61, gut 2.23 + 0.07. After 45 min of occlusion the values decreased by 29.7%, 21.8% and 17.1% resp. ($p < 0.05$). 45 min after reperfusion the concentrations no longer differed significantly from controls.
GSSG levels ($x \pm SEM$ in nM/g ww) in controls were: kidney 35.9 ± 4.4, liver 195.0 ± 18.6, and gut 9.2 ± .4. After the occlusion period the values decreased by 51.5 % in the kidney and 38.1 % in the liver, they rose by 114.1 % in the gut ($p < 0.05$). After reperfusion in the kidney a slight increase by 23.7% of control occurred, but without statistical significance; in liver the decrease continued by 60.6% of control, whereas in the gut a large increase by 279.3% of control took place (both values significant, $p < 0.05$).
Using the GSSG/GSH ratio as a measure of recovery from occlusion - reperfusion stress, we interpret a decrease in this ratio, as in the liver, as a good response, a nonsignificant increase, as in the kidney, as a fair response, and an increase, as in the gut, as a progressive injury during reperfusion.

Physiologisches Institut, Röntgenring 9, D-8700 Würzburg.

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THE AVIAN SALT GLAND: A TARGET FOR THE ATRIAL NATRIURETIC FACTOR (ANF)

H. Schütz, R. Gerstberger and E. Simon

Functional ANF systems are involved in body fluid homeostasis of vertebrates regulating electrolyte and water transport. In addition to the kidneys as osmoregulatory organs, marine birds possess supraorbital salt glands to excrete a strongly hypertonic salt solution (NaCl). To elucidate the role of chicken ANF (cANF) in the control of salt gland function, conscious salt-acclimated Pekin ducks received 15 pmoles/min/kg b.w. cANF for 10 min at two states of salt gland activity. (1) During steady-state diuresis and salt gland activity induced by systemic infusion of 1.1 ml/min isotonic Krebs-Ringer solution, cANF applied i.v. enhanced secretion rate from 0.21 to 0.29 ml/min and osmolality of secretion from 870 to 910 mOsm/kg. At threshold conditions of salt gland activity, cANF infused i.c. stimulated secretion rate from 0.07 to 0.15 ml/min at elevated osmolality of 760 compared to 480 mOsm/kg. Employing receptor autoradiography with 125I-BH labelled cANF as ligand, specific binding sites could be demonstrated throughout the salt gland tissue of both fresh-water (FD) and salt-acclimated (SD) animals. Scatchard analysis using an enriched membrane fraction revealed high affinity (KD = 0.9 nM) binding sites of 270 fmoles/mg protein density by radioreceptor assay. Displacement studies with unlabelled chicken and human ANF showed comparable KI values for both peptides in FD as well as SD, suggestive of the molecules' ring structure to be recognized by the receptor. Our results indicate an important role for ANF in salt and water homeostasis of birds.

MPI für physiol. & klin. Forschung, W.G. Kerckhoff Institut, Parkstraße 1, D-6350 Bad Nauheim

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INTERSTITIAL VOLUME PERCEPTION CONTRIBUTES TO EFFERENT CONTROL OF RENAL FUNCTION IN BIRDS

R. Keil, R. Gerstberger and E. Simon

The role of the interstitial fluid volume (ISFV) in body fluid homeostasis is heavily debated. Using the salt-acclimated Pekin duck as a model, we attempted to elucidate the influence of ISFV on afferent and efferent control of renal function including the contribution of the osmoregulatory hormones Arg^8-Vasotocin (AVT) and Val^5-Angiotensin (AII). During steady-state diuresis driven by systemic infusion of 1.1 ml/min of isotonic Krebs-Ringer solution (KR), dextran-70 (5 %) was added for 30 min inducing directed fluid shifts between the two extracellular fluid compartments. Hematocrit decreased from 36.4 to 32.3 % with concomitant rise in plasma colloidosmotic pressure from 9.5 to 12.9 mmHg at constant plasma osmolality and electrolyte concentrations. Depletion of ISFV caused a marked antidiuresis to < 50 % of control values accompanied by reduced osmolal excretion. Effective renal plasma flow dropped from 27.3 to 21.7 ml/min/kg and glomerular filtration rate decreased from 3.20 to 2.36 ml/min/kg indicating a fall in filtration fraction. The calculated fractional water excretion was markedly inhibited suggestive of tubular contributions to the antidiuresis. Plasma levels of both AVT and AII remained unchanged excluding their involvment in the observed reactions. Cardiovascular side-effects of the dextran application can be ruled out due to the constancy of mean arterial pressure and central venous pressure. Thus, our results suggest a contribution of the ISFV to renal function in the salt-acclimated Pekin duck.

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INFLUENCE OF MAXIMAL AND SUBMAXIMAL EXERCISE UNDER NORMOXIC AND HYPOXIC CONDITIONS ON PLASMA ATRIAL NATRIURETIC PEPTIDE AND ALDOSTERONE LEVEL.

W. Schmidt, C. Kröger, E.G. Brabant, A. Hilgendorf, and S. Strauch

The present study was designed to investigate the influence of exercise intensity and duration as well as of inspiratory oxygen content on plasma atrial natriuretic peptide (ANP) concentration and to compare with the behavior of plasma aldosterone. Nine untrained male subjects performed a vita maxima test (ME) on a bicycle ergometer and a 60 min lasting submaximal test with 60% of maximal performance (SE) under normoxia (N) and normobaric hypoxia (H), (PO2 92 mmHg). Five subjects were exposed to resting hypoxia for 90 min. [ANP] was mostly affected by exercise intensity (5 min after ME-N: +298.1 ± 117.4%) and less by exercise duration (at the end of SE-N: +229.5 ± 88.1%). Hypoxia has no effect at rest and reduces the exercise response (ME-H: +184.3 ± 76.8%, SE-H: +172.4 ± 41.5%). In contrast to ANP, Aldo response was more affected by duration at submaximal level (+290.1 ± 89.9%) than by short maximal exercise (+235.7 ± 62.7%). Hypoxia exposition rapidly decreased [Aldo] (-28.5 ± 7.4% after 30 min., $2p < 0.01$), but did not influence the exercise effects (ME-H: +206.2 ± 69.7%, SE-H: +321.6 ± 126.3%). [ANP] increase was faster than [Aldo] during the maximal tests and not different during submaximal exercise. Changes in plasma volume, sodium, and osmolality were most pronounced during maximal exercise (for ME-H: PV -13.1 ± 3.6%, sodium +5.9 ± 2.7 mmol/l, Osm +18.4 ± 6.5 mosmol/kg). Regression analysis yields higher correlations between changes in [ANP] and osmolality (ME-N: r=0.64) than for changes in sodium (r=0.51), heart rate (r=0.41), blood pressure (r=0.56), and changes in plasma volume (r=-0.51). It is concluded that besides other mechanisms increased osmolality might be involved in the exercise dependent increase of plasma [ANP].

Abt. Sport- u. Arbeitsphysiologie, Med. Hochschule Hannover, Konstanty-Gutschow-Str. 8, D-3000 Hannover 61

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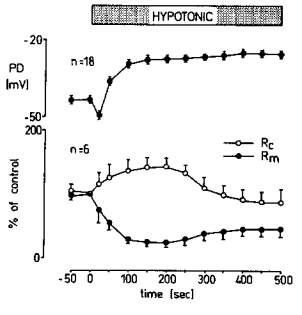
FURTHER STUDIES ON REGULATORY VOLUME DECREASE (RVD) IN MADIN DARBY CANINE KIDNEY CELLS.

M. Ritter, M. Paulmichl, F. Lang

As shown previously, Madin Darby Canine Kidney (MDCK-) cells are able to regulate their cell volume in hypotonic extracellular fluid. If exposed to hypotonic media (removal of 80 mmol/l mannitol), they initially swell but then gradually shrink close to their volume in isotonic extracellular fluid. In the present study cellular cable analysis has been applied to determine the potential difference across the cell membrane (PD), the cell membrane resistance (Rm) and the intercellular coupling resistance (Rc) during RVD. Furthermore, fluorescence measurements have been performed to determine intracellular calcium (Cai) and hydrogen ion (pHi) concentration. Exposure of MDCK cells to hypotonic perfusate leads to a transient hyperpolarization of the cell membrane, followed by a sustained depolarization, to a transient increase of Rc and a sustained decrease of Rm. The decrease of Rm probably reflects activation of an anion channel, since it is paralleled by a decrease of potassium selectivity of the cell membrane (from 0.55 ± 0.07 to 0.09 ± 0.01) and an increase of chloride selectivity (from virtually zero to 0.34 ± 0.02) of the cell membrane. Cai approaches 67 ± 8 nmol/l and intracellular pH 7.23 ± 0.04 in cells exposed to isotonic extracellular fluid (80 mmol/l mannitol). Reduction of bath osmolarity by removal of mannitol increases slightly but significantly intracellular calcium activity (74 ± 7 nmol/l) and leads to a significant acidification (pH 7.05 ± 0.04) of the cellular fluid. In conclusion, RVD in MDCK cells is at least in part accomplished by the activation of an anion channel, and is paralleled by intracellular acidosis. RVD in MDCK cells does apparently not require a substantial increase of Cai.



Institut für Physiologie der Universität Innsbruck, Fritz-Pregl-Straße 3, A-6010 Innsbruck

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UPTAKE OF $SO_4$35 IN RAT PAPILLARY COLLECTING DUCTS (PCD) STUDIED IN THE ISOLATED PAPILLA *

A. Frick (with the techn. assist. of M. Speier and M. Däs)

Microperfusion studies were performed with PCD in situ in the isolated papilla of the rat kidney to study the uptake of $SO_4$35 in this final segment of the nephron.
Male Wistar rats (about 250g b.w.) were anesthetized with Inactin intraperitoneally and the kidneys removed by an abdominal approach. The papilla was prepared according to the method of Morgan et al. (Am. J. Physiol. 214, 574-581, 1968) as modified by Häberle et al. (Renal Physiology 9, 54, 1986). The exercised papilla was placed in a special chamber containing a bathing medium: Urea 700 mM, NaCl 150 mM, $NaHCO_3$ 25 mM, sodium acetate 20 mM, Glucose 11 mM, KCl 10 mM, NaH_2PO_4 2.4 mM, $MgSO_4$ 2.4 mM, $CaCl_2$ 2 mM (Merck). The ducts were microperfused at 30 nl/min with the following solution: Urea 700 mM, NaCl 150 mM, $NaHCO_3$ 25 mM, KCl 10 mM, K_2HPO_4 5 mM, $MgSO_4$ 2.4 mM, $CaCl_2$ 2 mM and containing Inulin-H3 and $SO_4$35 (Amersham Buchler). The bathing medium was also used to perfuse the vasa recta. ADH (10^{-8} M; Sigma) was added to the bathing medium in some experiments.
Only results with Inulin-H3 ratios - collected fluid (CF)/perfused fluid (PF) - of 0.95 to 1.0 were accepted. Although the results obtained are from different lengths of perfused segments of PCD (from 0.2 to 1.5 mm) the pooled results are reported here. Under these conditions the (CF/PF)$SO_4$35 ratios were 0.87 ± 0.07 (57) (mean ± SD, n), a value significantly less than 1. Addition of ADH did not change these $SO_4$35 ratios (n=48) significantly.
It is concluded that $SO_4$35 disappears from the perfusion fluid. This may represent either binding to the apical membrane or transport across this membrane into the cellular fluid. Further experiments are necessary to elucidate the transport of this divalent anion in these cells.
Deutsche Forschungsgemeinschaft Fr 239/9-3.
* These experiments were performed in the laboratory of Dr. D.A. Häberle.
Physiologisches Institut der Universität München, Pettenkoferstraße 12,D-8000 München 2.

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MERCAPTURIC ACID FORMATION IN CULTURED OPOSSUM KIDNEY CELLS (OK)

N. Golenhofen, A. Heuner, S. Mildenberger, J. S. Schwegler, S. Silbernagl

The kidney is known to participate in mercapturic acid formation. We investigated the last step of this formation, the N-acetylation of cysteine S-conjugates, in the established OK kidney cell line, which shows characteristics of the proximal tubule. We used S-benzyl-L-cysteine (BC) as model sustance for such a cysteine S-conjugate. Methods: OK cells were grown on plastic petri dishes of 3.5 cm diameter.The intracellular concentration of BC was measured in confluent monolayers incubated with medium containing 1 mmol/l BC, in some experiments 1 mmol/l BC and 30 mmol/l L-phenylalanin. The concentration of the mercapturate N-acetyl-S-benzyl-L-cysteine (AcBC) in the medium, i.e. in the extracellular space, was measured during incubation with various BC-concentrations (0.75mM, 1.0mM, 1.25mM). BC and AcBC were quantified by reversed phase HPLC. Results: BC accumulated in the intracellular compartment to twenty times more than in the extracellular one, this accumulation being significantly reduced by the addition of 30 mmol/l L-phenylalanin. After uptake into the cells BC was acetylated and the acetylated product occured at a rate of 20 nmol/h/dish in the extracellular space. This rate was independent from the extracellular BC-concentration. Conclusions: OK cells reabsorbed BC via the neutral amino acid carrier. In the intracellular space BC is transformed to AcBC which is secreted into the extracellular compartment. Acetylation and/or secretion proceed to a maximum rate of 20nmol/h/dish.

Physiologisches Institut der Universität Würzburg, Röntgenring 9, 8700 Würzburg

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EFFECTS OF ALTERATION OF MIXED VENOUS pO_2 ON THE BLOOD VOLUME REGULATION

B. Flemming, D. Roloff, T. Wronski, C. Bauer, and W. Jelkmann

An extracorporal veno-venous bypass applying an oxygenator to alter mixed venous pO_2 (pO_{2mv}) nearly about 1.3 kPa was used in experiments on anaesthetized cats. Comparing the alterations of pO_{2mv} in cats in osmotic diuresis the higher level of pO_{2mv} was connected with an increase of the renal sodium excretion (60%) (B. Flemming et al., Biomed. Biochim. Acta 44:1687, 1985) if the tip of the reperfusion catheter was located in the inferior vena cava. The same effect was found in rats after administration of plasma or its different fractions (low molecular weight fraction (MW<700), lipid soluble extract) from cats with high pO_{2mv} (D. Roloff et al.,Physiol.bohemoslov. 37:83, 1988). The renal sodium retention of cats with low pO_{2mv} in chloralose-urethan anaesthesia was coupled with a significant increase of erythropoeitin blood level (25 mU/ml after 2 h) without significant changes of clearances of PAH and Inulin.
Our results seem to suggest a possible role of decreased mixed venous pO_2 on renal sodium retention and the release of erythropoietin in the blood volume regulation, i.e. in anemia, heart failure, heavy exercise and so on.

Institute of Physiology, Humboldt-University Berlin Hessische Strasse 3/4, GDR 1040 Berlin

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ROLE OF AN INCREASE IN K^+-PERMEABILITY (P_K) OF THE PANCREATIC B-CELL MEMBRANE FOR THE INHIBITION OF INSULIN RELEASE BY ADRENALINE AND GALANIN

G. Drews, A. Debuyser, J.C. Henquin

The mechanisms by which adrenaline and the neuropeptide galanin affect pancreatic B-cell function were studied with mouse islets. In B-cells stimulated by 15 mM glucose, adrenaline (100 nM) and galanin (50 nM) caused a transient marked hyperpolarization followed by a sustained partial inhibition of electrical activity. These changes were accompanied by a biphasic decrease in $^{86}Rb^+$ efflux from islet cells and nearly complete (90 %) inhibition of insulin release. Diazoxide (10-15 µM), a selective activator of ATP-sensitive K^+ channels largely mimicked these effects. When P_K was markedly increased by 100 µM diazoxide and the hyperpolarization reversed by high K^+, adrenaline and galanin were without effect on the membrane potential and $^{86}Rb^+$ efflux, but still partially inhibited insulin release. Tolbutamide (which blocks K-ATP channels) or arginine (which depolarizes because of its transport in a positively charged form) largely prevented the ability of adrenaline and galanin to affect the membrane potential of B-cells. They also decreased the inhibitory potency of adrenaline and galanin on insulin release. In conclusion, inhibition of insulin release by adrenaline and galanin involves at least two mechanisms: a partial repolarization of the B-cell membrane through an increase in P_K, and a mechanism independent of changes in membrane potential.

I. Physiol. Inst. der Universität d. Saarlandes, Homburg, FRG; Unité de Diabétologie et Nutrition, University de Louvain, Brussels, Belgium

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CALCIUM CURRENTS IN SECRETORY CELLS OF RAT ANTERIOR PITUITARY THIN SLICES.

M.B. Jackson, S.A. DeRiemer and A. Konnerth

Thin slices prepared from the pituitaries of 15 to 25 day-old rats were studied with the patch clamp technique under Nomarski optics to allow visual identification of cells (Edwards et al, *Pflügers Archiv 414, 1989*). In the presence of tetrodotoxin to block Na currents, and with intracellular Cs and TEA to block K^+ currents, depolarizing voltage steps from negative holding potentials activated Ca^{2+} currents in all cells tested. In recordings made from the most frequently observed (80-90%), "small" cell type (diameter 6 to 9 µm), a high voltage-activated, non-inactivating Ca^{2+} current was observed, which was partially blocked by either nimodipine or omega-conotoxin, and enhanced by BayK 8644. A complete block was observed following application of 20 µM Cd^{2+}. In contrast, 100 µM Ni^{2+} was ineffective. The "large" secretory cells of the anterior pituitary (diameter 9 to 15 µm) had Ca^{2+} currents with an inactivating and a non-inactivating component. The transient component could only be activated from holding potentials equal to or more negative than -80 mV. The non-inactivating Ca^{2+} current resembled the current observed in the small cell type in terms of its activation properties. This study shows that cells in the intact pituitary can be distinguished on the basis of both size and membrane characteristics. By combining this approach with immunocytochemical techniques, we hope to elucidate and distinguish different membrane mechanisms in the regulation of secretion of different pituitary hormones.

AG Zelluläre Neurophysiologie, Max-Planck Institut für biophysikalische Chemie, 3400 Göttingen, FRG.

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SINGLE CHANNEL RECORDINGS OF VOLTAGE-DEPENDENT Ca^{2+} CHANNELS IN FUSED ENDOPLASMIC RETICULUM VESICLES FROM RAT EXOCRINE PANCREAS.

A. Schmid, I. Schulz and H. Gögelein

The endoplasmic reticulum (ER) is known to play a central role in regulation of cytoplasmic free Ca^{2+}-concentration. With the dehydration/rehydration method we fused isolated ER vesicles from rat exocrine pancreas and investigated the fused vesicles by means of the patch-clamp technique. With K^+-solution (75 mmol/l KOH, 280 mmol/l HEPES, 10 µmol/l $CaCl_2$) in the pipette and Ba^{2+}-solution (50 mmol/l $Ba(OH)_2$, 280 mmol/l HEPES) in the bath single channels with a mean conductance of 47±4 pS (n=13) were recorded. The channel activity was markedly voltage regulated. At positive clamp potentials (0 to +30 mV, sign referred to the bath) the channel was most of the time in its open state, whereas, small negative voltages (-10 mV) caused channel inactivation. The extrapolated reversal potential is more negative than -30 mV indicating a $P_{Ba^{2+}} : P_{K^+}$ ratio of at least 5 : 1. Replacement of Ba^{2+} by Ca^{2+} demonstrated that the channel is also permeable to Ca^{2+}. Caffeine (10 mmol/l) in the bath or in the pipette activated the channel in part of the experiments. Ruthenium red (10 µmol/l) in the pipette caused complete channel inhibition. Ryanodine (10 - 200 µmol/l) and nifedipine (10 - 100 µmol/l) on either side of the membrane patch had no effect on channel activity. The channel was not dependent on the free Ca^{2+} concentration ($<10^{-9}$ to 10^{-3} mol/l) on both sides. We conclude that the voltage-dependent Ca^{2+} channel mediates Ca^{2+} release from a caffeine and ruthenium red sensitive but IP_3-insensitive intracellular Ca^{2+} pool.

Max-Planck-Institut für Biophysik, Kennedyallee 70
D-6000 Frankfurt/Main 70, F.R.G.

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CHOLECYSTOKININ (CCK) ACTIVATES DIFFERENT GTP-BINDING PROTEINS (G-PROTEINS) IN RAT PANCREATIC ACINAR CELLS

S. Schnefel, A. Pröfrock, K.-D. Hinsch* and I. Schulz

CCK activates phospholipase C (PLC) via 40 kDa G-protein(s) (S. Schnefel et al., FEBS Lett 230:125, 1988). Following ADP-ribosylation of pancreatic plasma membranes with cholera toxin (CT) or with pertussis toxin (PT), three 40/41 kDa proteins - pI-values of 5.30, 5.60 and 5.75 - were detected by two-dimensional gel electrophoresis. In addition, CT ADP-ribosylated five 45 and five 48 kDa proteins with pI-values of 5.60, 5.80, 5.95, 6.10, 6.30 and 5.35, 5.50, 5.65, 5.70 and 5.80, respectively, presumably corresponding to the low and high molecular forms of $G_s\alpha$ subunits of the adenyl cyclase. CCK enhanced CT-induced ADP-ribosylation of one 45 and of all 48 kDa proteins and decreased CT- and PT-induced ADP-ribosylation of all 40/41 kDa proteins. Incorporation of the photoaffinity analogue [α-^{32P}]GTP-γ-azidoanilide into the three 40/41 kDa proteins and into one 48 kDa protein was stimulated by CCK. Using affinity purified antipeptide antibodies raised against specific sequences of aminoacids in α-subunits of G_{i1}, G_{i2} and G_{i3} we identified the three 40/41 kDa proteins as α_{i1}, α_{i2} and α_{i3}. The data indicate that CCK-receptors functionally interact with six G_s and with three G_i-proteins: G_{i1}, G_{i2} and G_{i3}. We assume that one, two or all of the three G_i-proteins are involved in regulation of phospholipase C activity in pancreatic acinar cells.

Max-Planck-Institut für Biophysik, Kennedyallee 70, D-6000 Frankfurt am Main 70;
*Institut für Pharmakologie der Freien Universität Berlin, Thielallee 69/73, D-1000 Berlin 33

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MEASUREMENT OF MAGNETIC RELAXATION ABOVE THE LIVER BY MEANS OF NEEDLE-SHAPED GAMMA Fe_2O_3 PARTICLES

J. Lutz, A.J. Augustin and J. Milz

The alignment of magnetic particles, phagocytosed in the liver of laboratory animals can be sensed magnetometrically after application of a strong external magnetic field [Gehr et al., Nature 302, 336 (1983), Weinstock et al., Cells of the Hepatic Sinoid 1, Acad. Press NY p. 51 (1986)]. By this method the capacity of the macrophage system under different loads can be examined. In contrast to formerly used Fe_3O_4 (magnetite) particles or Fe_2O_3 particles, gained by combustion of iron pentacarbonyl, we used a suspension (50 mg/ml) of needle-shaped Fe_2O_3 particles of ca. 0.3 um length with a diameter of less than 0.05 um, as used for production of tape material (BASF, Ludwigshafen, FRG).

Male Wistar rats were injected iv. with 2.5 to 5 mg/kg bwt. of the iron oxide and were magnetized after different time intervals in a magnetic field of 0.26 Tesla (2600 Gauss) for 30 s. Immediately afterwards the animals were put into a magnetically shielded chamber. The depilated skin area above the right lower rib cage corner was held in close contact to a double FOERSTER probe (Magnetoscop 1.068, Inst. Dr. Foerster, Reutlingen, FRG) in a gradiometer mode of field detection. From the curves of declining magnetism the relaxation constant, k, was calculated according to: $k = (\ln c_1 - \ln c_2) / (t_2 - t_1)$. k and the correlation coefficient, r, were determined from curves fitted by the method of least-squares to 12 values obtained between 0.3 and 10 min after the end of magnetization. 95% of the curves gave a correlation coefficient $r > .940$.
In controls, k yielded a mean of .055 ± .003 (± SEM), gained from 42 relaxation curves. 6 - 12 hours after an iv. injection of 4 g lipid emulsion/kg bwt., k decreased to .035 ± .003 ($p<0.001$). After administration of 4 g perfluorochemicals (PFC, Fluosol DA), k was depressed to .018 ± .003 ($p<0.001$). k regained 80 % of its initial value within 1 day in the case of lipid emulsion administration, but not before 30 days in the case of PFC.

By these experiments it could be shown that needle-shaped magnetic particles rapidly change their alignment in vivo and that this change is markedly slowed by substances that are taken up by sessile macrophages.

Physiologisches Institut der Universität, Röntgenring 9, D-87 Würzburg
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pH_i-RECOVERY AND H^+-EXTRUSION IN ISOLATED RABBIT PARIETAL CELLS IN THE ABSENCE AND PRESENCE OF CO_2/HCO_3^-: Na^+/H^+ EXCHANGE, Na^+/HCO_3^- COTRANSPORT, OR BOTH?

G. Lamprecht und U. Seidler

Recovery of pH_i after an intracellular acid load requires active H^+- extrusion mechanisms. In some cell types, the predominant acid extrusion mechanisms in the absence of CO_2/HCO_3^- is the Na^+/H^+ exchanger, whereas in the presence of CO_2/HCO_3^-, a Na^+/HCO_3^- cotransporter becomes the predominant acid extruder. Controversy exists as to the situation in the parietal cell. To clarify the issue, we measured intracellular buffering capacity (β_i), pH_i-recovery (dpH_i/dt) and H^+-extrusion rates ($dpH_i/dt \times \beta_i$) in isolated rabbit parietal cells in the presence and absence of HCO_3 (required for Na^+/HCO_3^- cotransport), of 1 mM amiloride (Na^+/H^+ exchange inhibitor), and of external Na^+ (Na^+_o) (required for Na^+/H^+ exchange and Na/HCO_3^- cotransport). All experiments were performed at an extracellular pH (pH_o) of 7.4 in Cl^--free buffer (to suppress Cl^-/HCO_3^- exchange activity). pH_i was measured fluorometrically after loading isolated Cl^--depleted rabbit parietal cells with the pH-sensitive dye BCECF. β_i was determined over the pH_i range from 6.4 - 7.4 in the absence and presence of 20% CO_2/96 mM HCO_3^-. Results: β_i in the absence of CO_2/HCO_3^- decreased from 54±6 mM/pH-unit at pH_i 6.4 to 21±4 mM/pH-unit at pH_i 7.4. In CO_2/HCO_3^-, β_i increased from 72±6 mM/pH-unit at pH_i 6.4 to 229±4 at pH_i 7.4. Due to this difference in β_i, a higher intracellular acid load was required to acidify the cytoplasm to the same pH_i (6.7) in the presence than the absence of CO2/HCO_3^-. The initial H^+-efflux rate in the absence of CO_2/HCO_3^- was 9.04±1.3 mM/min and dropped very rapidly ($t_{1/2}$=1.5 min) as pH_i approached steady-state levels (7.43). In CO_2/HCO_3^-, the initial H^+-efflux rate was the same (8.2±0.9 mM/min), but the efflux rate remained high for a much longer time ($t_{1/2}$=4.5 min). The dependency of H^+-efflux rate on pH_i showed a strong inverse correlation (demonstrating the strong dependency of the Na^+/H^+ exchange rate on the pH_i), but was identical with or without CO_2/HCO_3^-, arguing against an additional acid extrusion mechanism in the presence of CO_2/HCO_3^-. In the presence of 1 mM amiloride, maximal H^+-efflux rates were inhibited to the same degree (78±4 and 77±7) in the absence and presence of CO_2/HCO_3^-; efflux was abolished in the absence of Na^+_o with or without CO_2/HCO_3^-. Summary and conclusions: During recovery from an identically low pH_i, the parietal cells had to extrude a higher acid load in the presence than in the absence of CO_2/HCO_3^-, resulting in higher overall H^+ -extrusion rates during pH_i-recovery. For a given pH_i the H^+-efflux rates were identical with and without CO_2/HCO_3^-, arguing against a role of the Na^+/HCO_3^- cotransporter in acid extrusion from isolated rabbit parietal cells. The only identifiable acid extruder in this experimental setting was the Na^+/H^+ exchanger.

II. Medizinische Klinik der TU München, Ismaninger Str. 22, 8 München 80

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STEADY-STATE pH (pH_i) IN GASTRIC PARIETAL AND SURFACE, BUT NOT CHIEF CELLS, IS MORE ACIDIC IN THE PRESENCE THAN THE ABSENCE OF HCO_3^-: IS THIS DUE TO Cl^-/HCO_3^- EXCHANGE, Cl-INDEPENDENT HCO_3^- TRANSPORT, OR BOTH?

U. Seidler und W. Silen

Cellular pH-regulation systems have usually been studied after the application of acute intracellular acid and base loads. What actually determines the steady-state pH_i has remained largely speculative. We have found that isolated and highly purified rabbit parietal and surface cells, but not chief cells, have a consistently more acidic steady-state pH_i in the presence than in the absence of CO_2/HCO_3^- at the same external pH (pH_o) of 7.4 This effect could be due to HCO_3^--efflux in exchange for Cl^- or electrogenic HCO_3^- efflux, possibly coupled to Na^+. Other known HCO_3^--dependent ion transport mechanisms alkalinize the cytosol and therefore cannot explain the observed effect. Methods: To clarify this issue, pH_i was measured fluorometrically in highly purified rabbit parietal, chief and surface cells after loading with the pH-sensitive dye BCECF and incubation for 30 min, 1, 2, and 3 h in buffer that contained either Cl^- and HCO_3^-, only Cl^- or HCO_3^-, or neither Cl^- nor HCO_3^-, for each of the four conditions at buffer pH-values for the pH-range from 6.2 to 7.8. Results: All three cell types in- and decreased steady-state pH_i with in- and decreasing pH_o, but the difference between pH_i and pH_o also increased both a high and low pH_o, demonstrating a remarkable capability of the cells to maintain a pH-gradient for very long time periods. In chief cells, steady-state pH_i was not significantly different in the presence or absence of either HCO_3^- or Cl^- for any pH_o tested. In surface cells, the presence of CO_2/HCO_3^- resulted in a more acidic steady-state pH_i (at equivalent pH_o), which was not influenced by the absence or presence of Cl^-. In parietal cells, the presence of CO_2/HCO_3^- resulted in a more acidic pH_i both in the presence and absence of Cl^-, but more so in its presence.
Conclusions: 1.) All three gastric cells types were able to maintain a considerable pH-gradient between cell interior and surrounding medium. 2.) Maintenance of chief cell pH_i does not involve Cl^- and HCO_3^--dependent ion transporters, 3.) in surface cells, Cl^--independent HCO_3^- efflux acidifies pH_i under steady-state conditions, and 4.) in parietal cells, the more acidic steady-state pH_i in the presence of CO_2/HCO_3^- is predominantly caused by Cl^- -independent HCO_3^- efflux, and partly by Cl^-/HCO_3^- exchange.

II. Medizinische Klinik der TU München, Ismaninger Str. 22, 8 München 80

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EFFECTS OF ENTERAL AND PARENTERAL APPLICATIONS OF DEXTROSE SOLUTIONS ON THE MOTILITY OF THE GUT IN CONSCIOUS RATS

O. Aziz, W. Reschke and G. Fischer

Chronic catheters were implanted in the antrum, duodenum, jejunum, V. cava, A. carotis, A. mesenterica of rats. Solutions of 5,2 % (isotonic) or 30 % (as used for total parenteral nutrition) of dextrose were infused at a rate of 100-500 µl/min for 2,5 - 10 min in the various sites and manometric measurements of motility performed in one or more sites in the gut. The aim was to study the ability of the nutrient a) to increase motility regardless of luminal load, b) to disperse phase III of the migrating motor complex (MMC), c) to induce retrograde inhibition of motility (brake effect).

Results: Both enteral and parenteral 5 % dextrose increases motility in a variable manner compared to isotonic NaCl. Intracarotid infusions to the CNS do not enhance the effect. Infusions 3 - 6 cm distal to Oddi's sphincter inhibit motility in the prox. duodenum. 30 % dextrose into the antrum changes phase I immediately to phase II-like activity in the duodenum and proximal jejunum and disrupts the MMC. Into the A. mesenterica sup., an enhancement of the next 2 phases III is observed, with subsequent abolishment of MMCs, particularly in the distal jejunum. It is concluded that dextrose can affect motility from the blood side.

Angewandte Physiologie im Institut für Normale und Pathologische Physiologie der Philipps-Universität, Karl-von Frisch-Straße/Lahnberge, 3550 Marburg/Lahn

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INTESTINAL AND HEPATIC LIPID PEROXIDATION AFTER AORTIC OCCLUSION AND REPERFUSION

A.J.Augustin, E.Purucker, J.S.Schwegler and J.Lutz

Ischemia induced oxygen free radical damage can be initiated in different ways, e.g. the xanthine oxidase mechanism or activated neutrophils. The initiating system, coming to the fore, seems to be dependent on the respective ischemic tissue. This reveals to the different ischemic tolerance of the organs.

Experiments were done with 12 male wistar rats by reversibly occluding the aorta subdiaphragmatically for 45 min. In one group the aorta was reopened for 45 min (reperfusion time); a second group without reflow served as controls. The tissue level of lipid peroxides (LPO) was determined by a modification of the method of Ohkawa et al., Anal. Biochem. 95, 351 (1979).

The intestinal tissue-level increased during the ischemic period from 68 ± 29 nmol/g (basic LPO - content ± SEM) to 641 ± 40 ($p<0.001$), whereas the liver LPO-level remained nearly constant (137 ± 17 vs. 125 ± 13). In the reperfusion period the intestinal lipid peroxidation went up to 1029 ± 155. LPO-content of the liver increased to 480 ± 59 ($p<0.05$) after this time.

These data indicate intestinal oxygen free radical damage already in the ischemic period, whereas hepatic tissue sustains damage only during reperfusion. The higher ischemic tolerance of the liver is explicable by the higher oxygen extraction ratio (Lutz et al., Pflüg. Arch. 360, 7, 1975) and - concerning free radical damage - by the different sources of the initiating radicals in intestine and liver. Neutrophils should play a major role in the intestine, whereas liver tissue can be primarly altered by the xanthine oxidase mechanism.

Physiologisches Institut der Universität, Röntgenring 9, D8700 Würzburg

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DIURESIS IN FORMICA POLYCTENA AND ACHETA DOMESTICA: EFFECT OF CRUDE EXTRACTS AND PARTIAL PURIFIED FACTORS.

N. De Decker(1), T. Hayes(2), G. Coast(3), P.Steels(1) and E. Van Kerkhove(1).

When studying the Malpighian tubules of *Formica* throughout the year, ants were found with inactive tubules and others with tubules secreting at various rates (8-800 pl/min.). This could be due to the presence of different levels of a hormonally regulated active state. Crude head extracts were made and tested in 2 diuretic bioassays, which measure the volume of the secreted droplet of the Malpighian tubule, in a given time interval.In a slow *Formica* assay (Van Kerkhove et all,1989), the crude extract gave 2 successive stimulations of the fluid secretion, one when the extract was added in the bath solution and a second when the extract was washed out. In a quick *Acheta* assay (Coast,1988), a single diuretic effect of the crude extract was observed. Purification of the crude extract was performed by means of RP-HPLC. It resolves the activity into 2 major fractions, which were active in the *Acheta* bioassay. On a Bio-rad Hi-pore semi-prep. column, their retention times were 29-34 min. and 34-39 min.The average effect of the stimulation increased fluid secretion with 65 % for the 29-34 fraction and with 45 % for the 34-39 fraction. Further purification of these active fractions is accomphished by additional RP-HPLC.

These studies provide evidence for the presence of diuretic factors in *Formica* and a sensitive and quick bioassay for these factors.

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(1) Limburgs Universitair Centrum, Dept. WNIFysiologie, Universitaire Campus, B-3610 Diepenbeek, (2) Dept. of Entomology, Texas A & M University, College Station, TX 77843, USA, (3) Dept. of Biology, Birkbeck College, London, WC1 7HX, U.K.

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GTP-β-S INDUCES CALCIUM OSCILLATIONS BUT NOT EXOCYTOSIS IN MAST CELLS.

F. von zur Mühlen, F. Eckstein*, R. Penner & E. Neher.

Several G proteins are involved in stimulus-secretion coupling in rat mast cells. GTP-γ-S has been found to induce a transient rise in intracellular calcium as well as mast cell degranulation. In an attempt to further differentiate the role of various G proteins in this response, the thiosylated GTP-analogues GTP-α-S and GTP-β-S (R- and S-isomers) were introduced into rat peritoneal mast cells via a patch pipette. Degranulation and intracellular calcium were monitored by cell capacitance and Fura-2 measurements.

R-GTP-α-S had effects similar to, but somewhat weaker than, GTP-γ-S (the S-isomer was largely ineffective). GTP-β-S (the R-isomer more so than the S-form) was found to induce repetitive large calcium spikes which were not regularily accompanied by degranulation. These calcium oscillations appeared with an average latency of 230 s and continued for up to 15 min before damping out. They were thus distinct from the rapid and short calcium changes mediated by GTP-γ-S and R-GTP-α-S.

The oscillations were independent of extracellular calcium. They were abolished by high concentrations of IP_3 (10 µM) as well as heparin (500 µg/ml), and GDP-β-S (300 µM), implicating G protein-mediated PI turnover in their generation. Their frequency could be modulated by changing the intracellular ATP concentration.

These results provide further evidence that calcium transients are not necessarily linked to exocytosis. They suggest that GTP-β-S selectively activates a G protein related to intracellular calcium signalling. The system could moreover provide a valuable model for studying the generation of calcium oscillations.

Max-Planck-Institutes for Biophysical Chemistry and *Experimental Medicine, D-3400 Göttingen, FRG

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EPITHELIAL ION TRANSPORT IN THE SHORT BOWEL SYNDROME

JD Schulzke[1], M Fromm[2], U Hegel[2], CJ Bentzel[3], EO Riecken[1]

Epithelial ion transport and morphology in the short bowel syndrome was characterized in vitro using rat ileum 2 months after 70% proximal small intestinal resection.

Subepithelial (R^{SUB}) and epithelial resistance (R^E) was distinguished by impedance analysis. R^E decreased from 27 ± 1 (n=16) in control to 21 ± 1 Ω·cm² (n=19; P<0.001) in the short bowel. Due to variable R^{SUB} at different stages of intestinal adaptation, comparison of active transport rates requires correction for the ratio $(R^E+R^{SUB})/R^E$ which was 1.6 ± 0.1 in control and 2.2 ± 0.2 (P<0.01) in short bowel.

Na- and Cl-fluxes were measured in HCO_3-containing medium. Fluxes were corrected for bath and subepithelial resistance. In control ileum, net Na- and Cl-absorption were of same magnitude compatible with electroneutral NaCl-absorption. Isc was due to the residual flux and assigned to HCO_3-secretion. Neither NaCl-absorption nor HCO_3-secretion was significantly changed in the short bowel. However, Na/glucose-cotransport (measured as the glucose-induced change in Isc) increased in V_{max} from 2.0 ± 0.3 in control to 5.0 ± 1.0 μeq·h⁻¹cm⁻² (P<0.01) in short bowel, while K_m remained unchanged.

Freeze fracture EM of tight junction structure showed no change in strand number, but a slight increase in tight junction depth in short bowel. This, however, can not explain the decrease in epithelial resistance and may be due to the higher mitotic index in short bowel. In contrast, microdissection morphometry showed an increase in villus height from 121 ± 5 μm (n=7) to 156 ± 8 μm (n=6; P<0.05) in short bowel.

We conclude that the mucosa in the short bowel syndrome is characterized by decreased epithelial resistance per cm² of gross area due to mucosal surface amplification. HCO_3-secretion and electroneutral NaCl-absorption is unchanged, while glucose-coupled Na-absorption increases to 250% in the short bowel syndrome. This can be considered as an adaptive response to the reduced absorptive area of the remaining intestine.

Depts. [1]Gastroenterology and [2]Clinical Physiology, Klinikum Steglitz, FU Berlin, FRG; [3]Dept. Medicine, East Carolina University, Greenville, NC, USA.

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NONSELECTIVE CATION-CHANNELS IN THE BASOLATERAL MEMBRANE OF CRYPT CELLS FROM RAT DISTAL COLON

C. Siemer and H. Gögelein

Intact crypts from rat distal colon were isolated and investigated with the patch-clamp technique. The isolated distal colon was inverted and filled with a Ca^{2+}-free NaCl-solution (in mM: 127 NaCl, 5 KCl, 1 $MgCl_2$, 5 glucose, 5 Na-pyruvate, 10 HEPES, 5 EDTA, 1% albumin, pH 7.4). After incubation in Ca^{2+}-free NaCl-solution at 37° C for 15 minutes , entire crypts as well as single cells were obtained by rapid shaking. Then the crypts were twice centrifuged at 500 g for 1 minute and resuspended in a NaCl-solution containing 1.25 mM $CaCl_2$ (in mM: 127 NaCl, 5 KCl, 1 $MgCl_2$, 1.25 $CaCl_2$, 5 glucose, 5 Na-pyruvate, 10 HEPES, 1% albumin, pH 7.4). The isolated crypts were stored on ice until use.

We investigated single channel currents in the basolateral membrane of cells from the bottom of the crypt. All experiments were performed at 35±1° C. In cell attached and cell excised patches nonselective cation-channels with a conductance of 37.4±0.6 pS (n=87) were found, which did not discriminate between potassium and sodium ions and which were impermeable to chloride ions. In some cell attached experiments the channel could be evoked by carbachol (100 μM, n=6) or by the calcium-ionophore A23187 (1 μM, n=2) added to the bath-medium. The channel was inhibited, when free Ca^{2+} was decreased at the cytosolic side (n=5). 3',5-dichlorodiphenylamine-2-carboxylic acid (DCDPC, 50 μM) and mefenamic acid (100 μM) added to the cytosolic side in cell excised experiments inhibited the channel completely and reversibly. The channel shows similar properties as the nonselective cation-channel in rat pancreatic acinar cells. Thus, it is likely that also in cells from rat distal colon nonselective cation-channels in the basolateral membrane are involved in the mechanism of salt secretion.

Max-Planck-Institut für Biophysik, Kennedyallee 70, D-6000 Frankfurt/Main 70, F.R.G.

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THE INFLUENCE OF Na ON THE TRANSPORT OF BENZOIC ACID ACROSS THE ISOLATED RUMEN EPITHELIUM OF SHEEP

G. Gäbel, P. Rothenpieler, E. Smith and H. Martens

Previous investigations (Gäbel et al., Pflügers Arch. 411, R107) have shown that the Na transport across the rumen epithelium can be stimulated by weak acids like short chain fatty acids (SCFA). The stimulation was mainly due to activation of a Na^+/H^+ exchange on the mucosal side of the tissue.

It was the aim of the present study to elucidate interactions in the opposite direction, i.e. whether Na may influence the permeation of weak acids. For that purpose unidirectional flux rates of ^{22}Na and ^{14}C-benzoic acid were measured under short circuit conditions across isolated, stripped rumen mucosa of sheep in Ussing-chamber experiments. ^{14}C-benzoic acid was chosen as a representative for weak acids instead of labelled SCFA since it is metabolized to a much smaller extent in the tissue.

In a HCO_3 buffered solution the mucosal to serosal flux of benzoic acid ($J_{ms}^{Benz\cdot}$) was significantly larger than the corresponding serosal to mucosal flux ($J_{sm}^{Benz\cdot}$) Replacement of Na by choline on the mucosal side or on both sides of the tissue decreased $J_{ms}^{Benz\cdot}$ leading to a reduction of $J_{net}^{Benz\cdot}$. Serosal addition of 0.1 mM ouabain diminished $J_{ms}^{Benz\cdot}$ and $J_{net}^{Benz\cdot}$ only in Na containing solutions.

Elevation of the pCO_2 from 5 kPa to 19 kPa ([HCO_3] constant; pH decrease: 7.3 to 6.7) on both sides of the tissue led to similar relative increases of the Na and benzoic acid fluxes: J_{ms}^{Na} increased by 70%, J_{net}^{Na} by 108%, $J_{ms}^{Benz\cdot}$ by 68% and $J_{net}^{Benz\cdot}$ by 108%. The pCO_2 induced increase of the unidirectional and net fluxes of both Na and benzoic acid could almost be abolished by mucosal addition of 1 mM amiloride. Elevation of the pCO_2 only on the mucosal side increased the amiloride sensitive Na and benzoic acid flux to a similar extent as the elevation of the pCO_2 on both sides of the tissue.

Our results suggest that the transport of weak acids like benzoic acid across the rumen epithelium may partly depend on the activity of the Na^+/H^+ exchange. The extrusion of H^+ by the exchange may lead to protonation of dissociated acid anions thus facilitating the uptake of undissociated acids. On the other hand the elevation of the intracellular pH by the Na^+/H^+-exchange leads to an increase of the transmembranal gradient of the undissociated acid.

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Institut für Veterinärphysiologie, FU Berlin, Koserstr. 20, D-1000 Berlin 33.

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THE INVOLVEMENT OF CALCIUM AND OXYGEN DERIVED FREE RADICALS IN ETHANOL-INDUCED RAT'S GASTRIC MUCOSA INJURY

K.R. Lutnicki, J.T. Wróbel, A. Ledwożyw, E.Pietraś

Free radicals play major role in producing the microvascular and parenchymal damage of gastric mucosa. Ca^{++} are the most common and universal factors in cells function regulation. The effect of verapamil /as a potent calcium antagonist/ and extracellular Ca^{++} on the free radicals generation, and on the activity of antioxidant enzymes in rat stomach mucosa after ethanol injury was assesed. The experiments were carried out on 100 albino rats, divided as follows: A-rats administrated with saline, B-supported with ethanol, C-verapamil+ethanol treated group, D-calcium gluconate plus ethanol, E-verapamil+ethanol+calcium gluconate treated group. For each group the ulcer index, the amount of peroxidation products/MDA, conjugated diens, hydroperoxides/ and antioxidant enzymes activity were estimated./SOD, peroxidase, glutationyl peroxidase, glutationyl reductase/.

50% ethanol given oraly causes severe injury in stomach mucosa. The enhanced levels of above mentioned peroxidation products and decreased activity of enzymes was observed. Verapamil given i.p. beforehand ethanol enhances mucosa injury and causes increase in activity of antioxidant enzymes, except catalase. The lower levels of peroxide products was observed. Calcium given oraly beforehand ethanol has no significant influence on the enzymes, but has the protective effect on stomach mucosa, decreasing the level of peroxide products. This finding do suggest, that free radicals play the main role in the induction of gastric mucosa injury. The alternations in Ca^{++} influx into gastric cells have the secondary protective effect, involved with its regulatory function.

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PRIMITIVE AND DEFINITIVE ERYTHROPOIESIS OF 4-8 DAYS OLD NORMOXIC, HYPOXIC AND HYPEROXIC CHICK EMBRYOS

S. Sender and R. Baumann

In avian embryos the switch from embryonic to adult hemoglobin production is cell lineage specific and caused by the substitution of primitive red cells with embryonic hemoglobin by definitive red cells with adult hemoglobin. The mechanisms controlling the timing of the switch are not understood. We have previously observed that incubation of chick embryos at reduced PO_2 causes premature appearance of adult hemoglobin. Using specific antibodies against primitive and definitive red cells we have investigated the time course of the switch and assessed the contribution of the yolc sac and intraembryonic hemopoiesis to the production of the first population of definitive red cells. The experiments were carried out with embryos incubated for 4 to 8 days in air, 13.5% O_2 or 100% O_2. The results show that the rate of early embryonic erythropoiesis is independent of ambient PO_2. The maximum size of the first red cell population produced in the yolc sac is about 170 million cells. Depending on the incubation PO_2 this population consists entirely of primitive red cells (100%oxygen) or contains up to 50% definitive red cells (13.5%oxygen). Circulating definitive red cells are already found at day 4.5 to 5 in hypoxia but only at day 7 in hyperoxia. The development of intraembryonic erythropoietic sites is unaffected by PO_2; the sites produce only definitive red cells and cannot be labelled for adult hemoglobin prior to day 6. Thus in hypoxia and normoxia the first definitive red cells arise in the yolc sac, whereas in hyperoxia definitive red cells appear only when intraembryonic erythropoiesis has started.

Zentrum Physiologie, Medizinische Hochschule Hannover, D-3000 Hannover 61. supported by DFG Ba 691/3

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OXYGEN TRANSPORT PROPERTIES OF BLOOD AND HEMOGLOBIN SOLUTIONS IN *LAMA* SPECIES.

Klaus D. Jürgens and T. Kleinschmidt

The four South American *lama* species vicuña (*Lama vicugna*), guanaco (*Lama guanacoë*), llama (*Lama glama*) and alpaca (*Lama pacos*) are adapted to permanent life at high altitude. We studied functional and structural properties of blood and hemoglobins of these species with respect to interspecific differences. No significant differences were found in hematocrit, red cell count, blood hemoglobin concentration, Bohr effect, and intracellular concentration of 2,3 DPG, whereas the blood half saturation pressure was remarkably lower in the vicuña than in the other camelids. The P_{50} value of around 17 Torr, which was found in this species, is the lowest so far reported for a mammal. The influence of the allosteric cofactors 2,3 DPG and chloride on the hemoglobin oxygen affinity of *lama* species was checked in hemoglobin solutions. The intrinsic P_{50}, which was found to be nearly identical in all four species, was increased considerably less by both cofactors in vicuña hemoglobin than in the other three camelid hemoglobins.

To reveal structural reasons for different cofactor action in different *lama* species, the amino acid sequences of their hemoglobins have been determined. Guanaco and alpaca hemoglobins were found to have an identical sequence, in llama hemoglobin α122 is Asp instead of His. Two other substitutions are seen in vicuña hemoglobin, α10 Ile —> Val and α130 Ala —> Thr. The latter is in the vicinity of a chloride binding site and likely to be responsible for the weak binding of this cofactor. The reason for the weaker action of 2,3 DPG is not obvious from these results.

Zentrum Physiologie, Medizin. Hochschule Hannover, Konstanty-Gutschow-Str. 8, 3000 Hannover 61.
Abt. Proteinchemie, MPI für Biochemie, Martinsried.

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THE HALDANE-EFFECT IN RABBIT BLOOD DURING RESPIRATORY AND METABOLIC ACID-BASE DISTURBANCES

F. Werkmeister, H. Kiwull-Schöne and P. Kiwull

Detailed data concerning the HALDANE effect (HE) in a wide range of respiratory and metabolic acid-base conditions are available for human blood. Within the physiological range of acid-base disturbances, the HE, e.g. the difference in pH (ΔpH) of oxygenated and deoxygenated hemoglobin (Hb) was shown by (1) to be linearily related to the logarithm of the bicarbonate concentration (log HCO_3^-). Since there are differences in the binding affinities of Hb in different species, the question arises, whether the empirical relationship derived for humans (1) can also be used to estimate the HE in rabbits, as common laboratory animals.
Therefore, arterial blood was taken from anaesthetized rabbits, and samples were treated by different concentrations of lactic acid or $NaHCO_3$, in order to achieve different metabolic acid-base conditions in the pH-range between 7.1 and 7.5. Subsequently, the samples were equilibrated in random sequence by 4% and 8% CO_2 in oxygen or nitrogen. For regression analysis, 80 pairs of ΔpH (y) and log HCO_3^- (x) were calculated from 640 measured pH-values. Considering the mean Hb-concentration ($\pm$SEM) of 11.4 $\pm$0.35 g/dl, the following linear relationship (r = 0.96)

resulted for rabbit blood $y = (2.0 - \log x)\ Hb/200$
compared to humans (1) $y = (1.9 - \log x)\ Hb/225$

and to dogs, calculated from (2) $y = (1.8 - \log x)\ Hb/150$

By using the formula for human blood (1), the HE would have been underestimated by about 20% in rabbits and by about 50% in dogs. This is of special importance during hypoxia, when determining the PCO_2 by the equilibration (Astrup) method. If the appropriate HE for the species is not considered, the resulting PCO_2 may be erroneous by up to several 100 Pa.

(1) v. Mengden et al., (1969) Respir. Physiol. 6, 151-159
(2) Reeves et al., (1982) J. Appl. Physiol. 53, 87-95

Institut für Physiologie der Ruhr-Universität, D-4630 Bochum

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TRANSCUTANEOUS TRANSPORT OF LMW-HEPARIN

J. Vahle, M. Ott and R.E. Zimmermann

The question as to whether heparin is able to permeate human skin arouses controversy among physiological and dermatological experts.
Some scientists claim that heparin enhances the resorption of subcutaneous hematoma, improve endothelial proliferation by including capillary growth and, therefore, enhancing blood supply to tissues and organs controversial pont in current research isthe antiphlogistic effect of heparin on cutaneous inflammation.
To investigate the later effects we used a low molecular weight heparin (average molecular weight 3200 dalton) supplied as an ointment with 30 000 units/100 g and were able to show at first that heparin permeates human skin in vitro as well as in vivo. Applying heparin topically in vivo we found an increase of systemic anti-factor IIa-activity in the test persons plasma.
In a second experiment we produced hematoma by injecting blood subcutaneously in order to test the effects of heparin on the resorption of subcutaneous hematoma by measuring the skin colour with a specific reflexion photometer.
Finally we induced erythema by applying UV-A radiation and compared the visible changes of inflammation between the placebo and heparin treated groups.
The results of our experimental studies led us to the conclusion that topically applied heparin has significant and evident local effects although its systemic potency turned out to be poor.

Physiologisches Institut II der Wetfälischen Wilhelms-Universität Münster, Robert-Koch-Str. 28, D-4400 Münster

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A COMPARISON OF DIFFERENT FIBRINOLYTIC AGENTS BY A FLUORESCENCE MEASUREMENT METHOD (IN VITRO)

J.G. Elsing and R.E. Zimmermann

In order to measure fibrinolytic activities the most common methods are the fibrin plate assay (Astrup and Mullertz, 1952), the standard hanging clot method (Von Kaulla and Taylor, 1961) and the lysis of radioactive labelled clots. We tried to develop a new method because the former techniques have some disadvantages. Human fibrinogen was labelled with fluoresceinisothiocyanate (FITC), purified on Sepharose 4 B-columns, dialysed, lyophilised and stored at - 20°C.
FITC-labelled clots were preformed in a flexible tube by adding thrombin to the fibrinogen which led to clots with defined size. The lysis of the labelled clots was continuosly recorded at 522 nm after exitation of the supernatant at 498 nm.
The obtained results showed a variation coefficient (VD) of 4.3-5.2 % determined with 30 and 50 U streptokinase per 2 ml plasminogen phosphate-buffer after 3 to 4 hours of lysis.
According to the producers specification of lytic activity or the molar basis equivalent the fibrinolytic agents (streptokinase, APSAC, t-pa, urokinase) were compared with one another.The lytical activity of plasmin was also determined. At low concentrations Streptokinase and APSAC seemed to be more efficient than urokinase and t-pa, but at higher concentrations of the lytic agents they presented similar activities.
Fibrinspecifity was measured after clot incubation with the fibrinolytic agent, washing and measuring the residual lytic activity of the clot. t-pa presented the highest fibrinspecifity of all agents investigated.

Physiologisches Institut II der Westfälischen Wilhelms-Universität Münster, Robert-Koch-Str. 28, D-4400 Münster

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REGULATION OF CARBONIC ANHYDRASE EXPRESSION IN EMBRYONIC RED CELLS

D. Million, P. Zillner and R. Baumann

During chick embryonic development carbonic anhydrase (C.A.) expression of the erythrocytes is kept at a very low level until the last week of incubation. We have obtained evidence that hypoxia is the physiological stimulus for the rapid onset of C.A. synthesis before hatching (Baumann et al. Dev. Biol. 116, 548 (1986)). Looking for putative hormonal signals we have carried out in vitro incubations of embryonic red cells and tested prostaglandins, Calcitriol, Thyroxin, corticosteroids, catecholamines, c-AMP, c-GMP, A23187, TPA, choleratoxin, FCS, chick serum and human plasma with no effect.- However incubation with plasma obtained from embryos at least 7 days old or adult chick plasma significantly increased the C.A. activity. After a lag phase of 4 h, C.A. activity increased to 5018 $\pm$ 1300 U/gHb at 6h compared to 2386 $\pm$ 818 U/gHb in the controls. This increase was not observed when the incubation was carried out in the additional presence of Actinomycin D, Cycloheximid, ALF_4^- or pertussis toxin, or when plasma was heat inactivated. We conclude that embryonic plasma contains a heat labile factor which stimulates C.A. synthesis by activating transcription. In vivo its action is suppressed during most of development by an inhibitor continually supplied and rapidly inactivated (otherwise embryonic plasma would not cause an increase of C.A. activity in vitro). Its production may be controlled by the blood PO_2 and the inhibitory action may in part rely on activation of G-proteins. The fall of the blood PO_2 before hatching reliefs inhibition and hence activates C.A. synthesis.

D. Million, Physiologisches Institut, Universität Regensburg, 8400 Regensburg

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INHIBITORY EFFECT OF ADENOSINE (ADO) ON OXYGEN RADICAL PRODUCTION (ORP) AND INTRACELLULAR CALCIUM IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES (PMNL)

M. Thiel, H. Bardenheuer, K. Peter

Introduction: The release of oxygen radicals from activated PMNL can play a major role in the pathogenesis of ARDS, septic multiple organ failure and ischemia-reperfusion injuries. ADO, which has been reported to be enhanced during sepsis and ischemia, is a well known inhibitor of various cell-systems most likely by interfering with intracellular calcium (Ca_i). Therefore, this study investigates the effect of ADO on the ORP and on Ca_i in human PMNL. **Material and Methods:** PMNL of healthy volunteers were activated by Ca-dependent (FMLP, ZAS, (1)) and Ca-independent (PMA(2)) stimuli. The maximal ORP was determined by luminol-enhanced peakchemiluminescence (PCL) and Ca_i was measured by quin-2 flourescence. **Results:** **Tab.1:** ADO dose-dependently inhibited the ORP during Ca-dependent (1), but not with Ca-independent (2) stimulation. **Tab.2:** The ADO-receptor specific antagonist PT totally reversed this inhibition. **Tab.3:** ADO reduces the FMLP induced increase of Ca_i. In contrast, Ca_i is elevated, when ADO is antagonized by PT or metabolized by ADA. **Conclusions:** The inhibition of ORP by ADO is receptor-mediated and restricted to Ca-dependent stimuli. ADO's action is most likely mediated by a direct reduction of Ca_i. Thus, ADO might be part of a negative feedback (PMNL-activation -> ORP -> cell injury -> ADO -> PMNL-inhibition) to inhibit inflammatory processes.

Tab.1: Effect of ADO on PCL (cpmx10^3) of activated PMNL

		ADO	(x	10^{-6}	M)
Basal	0	1	10	100	1000
0.28 FMLP(1)	6.3	5.1	4.7*	3.3*	2.5*
0.17 ZAS(1)	0.48	0.38	0.30*	0.26**	0.18**
0.86 PMA(2)	96	121	97	101	105

mean, n=8, *p<0.05; **p<0.001, vs. stimulation without ADO, paired t-test

Tab.2: Effect of 8-phenyltheophylline (PT) on ADO mediated inhibition of FMLP stimulated PCL (cpmx10^3)

Control	ADO($1x10^{-6}$M)	ADO/PT	PT($5x10^{-5}$M)
21.2	15.5*	19.9+	22.9

mean, n=6, *p<0.01 vs. control +p<0.01 vs. ADO

Tab.3: Effects of ADO , PT and ADA (ADO-deaminase) on Ca_i ($1x10^{-9}$M) of FMLP stimulated PMNL

	(n)	Basal	FMLP-stimulation 3'	10'
Control	(6)	124	272*	186*
ADO($1x10^{-4}$M)		104	195*	121*
Control	(3)	107	248	164
ADO($1x10^{-6}$M)		107	215	144
ADO/PT		104	248	185
PT($5x10^{-5}$M)		131*	284*	200*
Control	(6)	129*	294*	187
ADA(2U/ml)		178*	328*	284*

mean, *p<0.05 vs. control

Institute of Anesthesiology, University of Munich, 8000 München 70, FRG

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CYTOCHROME P_{450} IN THE CONTROL OF THE PRODUCTION OF ERYTHROPOIETIN IN HEPATOMA CELL CULTURES

*W. Jelkmann, +J. Fandrey and ++C.P. Siegers

The mechanism by which hypoxia stimulates the production of erythropoietin (Epo) in the kidneys and the liver is still poorly understood. Recently Goldberg et al. (Science 242: 1412, 1988) have reported that hypoxia triggers Epo gene expression in hepatoma cells of the line Hep3B and that this gene expression requires the formation of a heme protein. To further elucidate the biochemical nature of the O_2 sensitive hemoprotein controlling the synthesis of Epo, we have investigated the effects of agents interfering with microsomal mixed-functional oxidases (cytochrome P_{450} and its reductase) on the production of Epo in hepatoma cell cultures of the line HepG2.

Radioimmunological measurements showed that the production of Epo increased when agents known to induce P_{450} microsomal hemoproteins, in particular cytochrome P_{450} reductase, were added to the cultures, namely phenobarbital, 3-methylcholanthrene, cobaltous chloride and thyroid hormones. On the other hand, the production of Epo was suppressed in the presence of compounds that inhibit microsomal mixed-functional oxidases. Diethyldithiocarbamate and cysteamine chloride were found to act this way.

Based on these findings it is proposed that O_2 sensitive hemoproteins of the microsomal mixed-functional oxidases are critically involved in the control of the synthesis of Epo.

*Physiologisches Institut der Universität Bonn, Nußallee 11, D-5300 Bonn 1, und Institute für +Physiologie und ++Toxikologie, Medizinische Universität, D-2400 Lübeck 1

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METABOLIC EFFECTS ON SARCOPLASMIC RETICULUM AND MYOSIN EXPRESSION IN THE HEART OF DIABETIC AND FASTED RATS

H. Rupp, V. Elimban*, N.S. Dhalla*

In the diabetic heart, the molecular structure of the myocyte is markedly changed involving sarcoplasmic reticulum (SR) and myosin. In order to define the signals involved in the subcellular changes, the effect of the physiological load of intermittent fasting (1 d fasting, 1 d ad libitum feeding; 6 wk total) was studied. During fasting plasma insulin was reduced to 9 uU/ml vs. 17 uU/ml of controls (C) and 4 uU/ml of diabetic rats (65 mg/kg streptozotocin). Intermittent fasting induced changes in Ca^{2+}-stimulated ATPase of SR (95 vs. 135 nMol P/mg/min of C) and in myosin V1 (38% vs. 53% of C) which were equidirectional to diabetic rats (75 nMol P/mg/min; 15% V1). Treatment of intermittently fasted rats with sucrose (0.8% in drinking water) prevented the changes in SR and myosin. Plasma glucose levels were reduced by sucrose feeding, particularly after refeeding, indicating an enhanced peripheral glucose utilization. Thyroid hormones and growth characteristics were unaffected by the sucrose treatment which increased daily calorie intake by only 1%. It is concluded that also under physiological conditions shifts in fuel metabolism can affect the subcellular structure of the myocyte and that the administration of sucrose in a low dose provides an efficient means for restructuring of the myocyte.

Physiological Institute II, University of Tübingen, Gmelinstr. 5, D-7400 Tübingen, and *St. Boniface General Hospital Research Centre, Winnipeg, Canada

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CHANGE OF MYOSIN ISOENZYME EXPRESSION IN THE VENTRICLE OF COLD ADAPTED RATS AND HIBERNATING EUROPEAN HAMSTERS

I. Morano, **B. Agostini, *M. Mühleisen, and **W.F.H.M. Mommaerts

Adult Sprague Dawley-rats kept for 6 weeks at +4°C for cold adaptation were killed at the age of 20 weeks. Adult European Hamsters hibernating (HH) in a cold and dark chamber at ±4°C starting in November were killed in January. Summer active hamsters (SH) were killed in July.
Left ventricular myosin isoenzymes (MI) of rats and hamsters were studied by the pyrophosphate gelelectrophoresis technique displaying three components with increasing mobility V3, V2, and V1 identically in both species investigated. Values are given as means. SD were <10%.
Summer active hamsters (SH) expressed mainly V3, while during hibernation (HH) the V1-form dominated: MI patterns (%V1/%V3) were 15/68 and 70/10 in SH and HH respectively. Ca2+-dependent ATPase activity of ventricular myofibrils (MF) was higher in HH than in SH: at maximal Ca^{2+}-activation (10µM Ca^{2+}) MF ATPase-activity (nmol P_i/mg/min) was 86,4 in HH and 26,6 in SH.
MI patterns of control rats kept at 25°C were 65/15 and changed in favour of the V1-form to 85/5 in the cold adapted rats. At maximal Ca^{2+}-activation MF ATPase activity was 95 and 80 for control and cold-adapted rats respectively.
The shift of myosin isoenzyme expression to the highly active V1-form during hibernation and coldexposure could be related to an enhanced activity of the thyroid gland.

II. Physiologisches Institut, Im Neuenheimer Feld 326, *I. Biochemisches Institut, Im Neuenheimer Feld 346, and **Max-Planck-Institut für Medizinische Forschung, Jahnstraße 29, D-6900 Heidelberg

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DUAL EFFECTS OF INTRACELLULARLY APPLIED cGMP ON L-TYPE CALCIUM CURRENT IN ISOLATED GUINEA PIG VENTRICULAR MYOCYTES

K. Ono and W. Trautwein

The effect of cGMP on Ca current (I_{Ca}) was investigated in isolated guinea-pig ventricular cells using whole-cell patch clamp method combined with intracellular perfusion technique. When I_{Ca} was increased by isoprenaline (0.1 µM) or by intracellular perfusion with cAMP (50-100 µM), cGMP (1-10 µM) induced an additional increase of I_{Ca}. This effect was reversible and could be repeated in the same cell. However, cGMP (1-10 µM) had no effect when I_{Ca} was maximally stimulated by the non-hydrolyzable 4-chloro-phenyl-thio cAMP (5-100 µM). On the contrary, in the presence of nonselective phosphodiesterase inhibitor, IBMX (40 µM), I_{Ca} stimulated by isoprenaline (0.1 µM) was reduced by cGMP (10 µM). This effect was partially reversible. Furthermore, high concentration of cGMP (100 µM) also inhibited I_{Ca} which was elevated by intracellular perfusion with cAMP (50 µM). 5'-GMP, the metabolite of cGMP, had no effect on I_{Ca}. 8-bromo cGMP, a potent activator of cGMP dependent protein kinase, strongly reduced I_{Ca} which was stimulated by isoprenaline (0.1 µM) or cAMP (50 µM). To test whether cGMP dependent protein kinase affects I_{Ca}, we perfused the pipettes with the active fragment of cGMP dependent protein kinase (67 kDa). In some experiments, I_{Ca} was reduced when cG kinase (0.3-1 µM) was intracellularly applied. It is concluded that cGMP regulates I_{Ca} in two ways. Firstly, cGMP increases I_{Ca} by inhibiting hydrolysis of cAMP. Secondly, cGMP inhibits I_{Ca}, probably due to an activation of active cGMP dependent protein kinase.

II. Physiologisches Institut der Universität des Saarlandes, D-6650 Homburg/Saar

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SARCOMERE SHORTENING AND CALCIUM CURRENT IN ISOLATED CARDIAC MYOCYTES

M.Wussling, W.Schenk and W.Boldt

The relative contribution of the calcium influx to the myofilament activation was shown to increase with the post rest recovery in several heart tissues in the manner of a positive staircase (D.M. Bers, Am J Physiol 248:H 366, 1985). There was no close relationship between calcium influx and tension staircases. In the present study the attention is focused to the post rest recovery of the slow inward current and the sarcomere shortening in enzymatically isolated heart cells of rat and guinea pig. Measurements were performed by means of the whole cell clamp technique (C.P. Hamill, A. Marty, E. Neher, B. Sakman and F.J. Sigworth, Pflügers Arch 391:85, 1981) and laser diffractometry (M. Wussling, W. Schenk and B. Nilius, J Mol Cell Cardiol 19:897, 1987).

When regularly paced after a rest period of at least three minutes we observed in rat cardiac myocytes a negative staircase of the peak slow inward current (depolarization steps from -45 to +10 mV, step duration 300 ms) as well as of the peak sarcomere shortening. The magnitude of each staircase was dependent on the stimulation rate. Guinea pig cardiac myocytes showed a negative staircase of the slow inward current, too, but a positive one of the sarcomere shortening. In rat cardiac myocytes the slow inward current was small compared to guinea pig heart cells whereas the extent of the steady state sarcomere shortening amounted to about 200 nm at a stimulus frequency of 60/min in all cells investigated.

J.-Bernstein-Institut für Physiologie der Martin-Luther-Universität Halle-Wittenberg, PSF 302, DDR 4020 Halle/Saale

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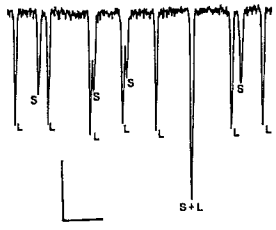
TWO COMPONENTS OF Ca^{2+}-RELEASE FROM SARCOPLASMIC RETICULUM IN GUINEA-PIG ATRIAL MYOCYTES

P. Lipp and L. Pott

An intracellular Ca^{2+}-transient due to Ca^{2+}-release from the sarcoplasmic reticulum causes a transient inward current (I_{ti}) at negative membrane potentials which is carried by electrogenic Na^+-Ca^{2+} exchange (Lipp & Pott J. Physiol. 397: 601, 1988). I_{ti}-measurements were used to study properties of spontaneous and Ca^{2+}-induced Ca^{2+}-release (CICR) from the SR of myocytes dialyszed via patch-clamp pipettes with solutions containing (mM) Cs_3Citrate (0-55), NaCl (10), CsCl (10), MgATP (5), CsAspartate (120-0), Hepes (10, pH 7.4), EGTA 50-250 μM. In the majority of myocytes both spontaneous I_{ti} (at $EGTA_i \leq 100$ μM) and I_{ti} evoked by L-type I_{Ca}, the latter representing CICR, displayed two caffeine-sensitive components (S and L) which could be identified by their different amplitudes or time-integrals respectively. S and L could occur independently or simultaneously, resulting in I_{ti}-amplitudes corresponding to S+L (Fig. 1). If spontaneous Ca^{2+}-release was supressed ($EGTA_i \geq 200$ μM) in a fraction of about 40 % of the myocytes the occurence of L was dependent on the presence of S. During trains of repetitive depolarizations S and L displayed different kinetics of 'loading'. We suggest S and L to represent two different functional compartments of the SR which might reflect the previously described morphological compartmentization. As in more than 150 cells studied under this aspect, we never detected more than two components of the release signal, local fluctuations of $[Ca^{2+}]_i$ as a reason for the above observations can be excluded.



Spontaneous I_{ti}'s recorded at -50 mV holding potential. Three classes of amplitudes (S, L and S+L) can be identified. Calibration: 10 pA, 5s.

Institut für Zellphysiologie, Ruhr-Universität Bochum, Postfach 102148, D-4630 Bochum, FRG // Supported by the DFG (FG KONZELL)

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LATE CONTRACTION IN VENTRICULAR MYOCYTES ACTIVATED BY THE NA-CA EXCHANGE

K. Schüttler and G. Szymanski

When single ventricular myocytes of guinea-pigs were incubated for 90 min in 10 uM ryanodine a late contraction (measured by an optical technique) was obtained. This late contraction was found to be increased when the transmembrane Na gradient was reduced either by reducing $/Na/_o$ or by experimental manoeuvres expected to increase $/Na/_i$. The late contraction increased with membrane potential during test pulses ("whole-cell clamp technique") over the range -30 to +70 mV. Late contraction continued to develop over the whole duration of test pulse or of action potential. In another approach a late contraction could be kinetically isolated: When a train of normal contractions (.2 Hz) was interrupted by a period of rest of 10 to 15 min the first post-rest contraction was found to be a late contraction. This so-called rested-state contraction was found to be increased as well when the transmembrane Na gradient was moderately reduced. The evidence suggests that the late contraction isolated either pharmacologically or kinetically is substantially activated by Ca entering the cell via the sarcolemmal Na-Ca exchange.

In dependence on the stimulation pattern the following properties of the ryanodine-isolated component were found in guinea-pig papillary muscles: (1) no postextrasystolic potentiation, (2) faster restitution, (3) nearly complete decrease of developed tension of the first beat when the stimulus interval was abruptly shortened, (4) positive (monotonous) staircase after short periods of rest. These properties were similar to those of tissues the contraction of which is dominated by a sarcolemmal Ca source. In addition to, they are in agreement with the hypothesis on a Na-Ca exchange activated late contraction.

Pharmakologisches Institut der Universität-Gesamthochschule Essen, Hufelandstr. 55, D-4300 Essen 1

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ELECTRONPROBE-MICROANALYSIS OF CARDIAC MYOCYTES: PAIRED VOLTAGE-CLAMP PULSES POTENTIATE CONTRACTILITY AND INCREASE TOTAL MYOPLASMIC CALCIUM-CONCENTRATION TO MILLIMOLAR VALUES

M.F. Wendt-Gallitelli and G. Isenberg

Electronprobe-microanalysis (EPMA) of multicellular ventricular trabeculae has shown [1] that noradrenaline or ouabain, potentiating isometric force without increasing the diastolic tension, increase diastolic total $[Ca]_m$ up to 5 mmol/kg d.w.. Since total $[Ca]_m$ may have analyzed in superficial cells (partially intoxicated?) but tension originated from all cells, the influence of potentiation on total $[Ca]_m$ was re-investigated in single guinea-pig ventricular myocytes (37 °C, 2 mM $[Ca^{2+}]_o$).

28 cells were potentiated by paired pulses (1 Hz) and shock-frozen for EPMA. Voltage-clamp set the diastolic potential to -80 mV. The first pulse induced Ca-influx through Ca-channels (180 ms to 0 mV). The second pulse activated Ca-influx through the Na,Ca-exchanger (160 ms to +50mV). The change from single to paired pulses increased the extent of shortening three-fold but let the diastolic re-lengthening almost unchanged (sarcomere length 1.81 instead 1.85 μm).

EPMA measured during diastole a total $[Ca]_m$ of 2.3 ± 0.4 mmol/kg d.w. (mean ± S.E., n=52) which corresponds to 0.8 mM. 30-60 ms after start of the first pulse, total [Ca] was 5.6 ± 0.4 mmol/kg d.w. (1.9 mM, n=62), 30-60 ms later it was 3.0 ± 0.5 mmol/kg d.w.(1mM, n=33). The time course of total $[Ca]_m$ corresponds the one of free $[Ca^{2+}]$ measured with fluorescent probes. But, total [Ca] (bound and free) exceeds free $[Ca^{2+}]$ by a factor >1000. Our results suggest that potentiation of contraction goes along with excessive binding of Ca to myoplasmic constituents. These Ca-buffers may have a capacitance 10 times higher than originally thought [2].

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Institut für Physiologie II, Gmelinstr. 5, 7400 Tübingen und Zentrum für Physiologie, Robert-Koch-Str. 39, 5000 Köln.

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EFFECTS OF APP 201-533, A Ca SENSITIZING AGENT, ON Ca ACTIVATION OF HUMAN LEFT ATRIAL SKINNED FIBRES FROM CONTROL AND IDIOPATHIC CARDIOMYOPATHIC HEARTS

J.W. Herzig#, H. Depersin#, G. Grupp* and I. Grupp*

Human left atrial trabeculae were dissected from a non diseased heart (Control) and from an explant heart obtained from a 54 years old woman suffering from idiopathic cardiomyopathy (ICM) and undergoing cardiac transplantation. Using Triton X100, skinned fibres were prepared and isometrically activated in EGTA buffered Ca solutions in presence of MgATP and an ATP reconstituting system (Pyruvate Kinase and PEP). Ca activation resulted in sigmoidal force-pCa relationships, EC50 for Ca activation being consistently lower in ICM (~1500 nM) than in control preparations (~2200 nM), i. e. cardiomyopathic fibres were more sensitive to Ca than control fibres. In ICM and control skinned fibres, application of the positive inotropic agent APP 201-533 (3-Amino-6-methyl-5-phenyl-2(1H)-pyridinone) led to a concentration dependent leftward shift of the Ca activation curves by up to ~800 nM, which again resulted in higher Ca sensitivity in ICM than in control skinned fibres. Two principally alternative interpretations appear obvious:

-Either Ca sensitization is a pathophysiological process resulting in pump dysfunction, a situation which would be worsened by a Ca sensitizing agent

-or Ca sensitization is a physiological compensatory process elicited by and counterbalancing pump dysfunction, a situation which should be supported and enforced by a Ca sensitizing agent.

Pharmaceuticals Division, Department Research CVS Ciba-Geigy Ltd, Basle, Switzerland
* Department of Physiology, University of Cincinnati, Ohio, USA

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CONTRACTION AND ACTIVE NA/K TRANSPORT OF SHEEP CARDIAC PURKINJE FIBRES AS AFFECTED BY 22, 23-DIHYDROBUFALIN, A NEW CARDIOACTIVE STEROID

H.G. Glitsch, H. Pusch and Ch. Zylka

Werner et al. (Angew. Chem. 28, 1359-61, 1989) have recently described a general synthetic pathway to bufenolides where the lactone moiety carries a residue to which various reactive or activatable groups suitable for affinity labelling could be attached. By means of these compounds the binding site of the Na/K-ATPase for the lactone grouping of cardioactive steroids could be identfied. An essential prerequisite is, of course, that the bufenolides share the fundamental pharmacological characteristics of known cardioactive steroids. We checked whether one of these compounds, 22, 23-dihydrobufalin (DHB), exerts a positive inotropic effect and an inhibition of the Na/K pump in sheep cardiac Purkinje fibres. Under the experimental conditions to be described DHB evokes a concentration dependent, reversible positive inotropic effect similar to that caused by ouabain. Furthermore, DHB inhibits reversibly the Na/K pump studied by means of Na sensitive microelectrodes in voltage clamped Purkinje fibres. Assuming a single type of DHB receptor a K'_D value of $(9 \pm 0{,}73)\cdot10^{-7}$ M (n = 10) for the DHB-Na/K pump interaction can be derived from measurements in Tyrode solution containing 5.4 mM KCl. The value is comparable to that found for ouabain ($(9.2 \pm 7.7)\cdot10^{-7}$ M; n = 9) but larger than the K'_D value calculated for bufalin ($(1.2 \pm 1.7)\cdot10^{-7}$ M; n = 8). We conclude that DHB has at least two important characteristics in common with cardioactive steroids. It causes a positive inotropic effect and an inhibition of the Na/K pump.

Institut für Zellphysiologie, Ruhr-Universität Bochum, Postfach 102148, 4630 Bochum, F.R.G.
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DOES A LONGTERM BLOCKADE OF THE α- OR β-ADRENERGIC RECEPTORS HAVE INFLUENCE ON THE DEGREE OF LEFT VENTRICULAR HYPERTROPHY, CONFIGURATION AND PUMPING PERFORMANCE?

M. Brändle and R. Jacob

As recent investigations (Laks & Moraday 76; Östman-Smith 79, 81) have revealed, the catecholamines and their receptors play a central role as a mediator of cardiac growth in the adaptation of the heart to increased load. Furthermore, it is known that in terminal cardiac insufficiency the number of adrenergic receptors decreases in relation to the severity of the disease (Ayobe & Tarazi 83).
It was the aim of the present study also in view of the frequent application of sympathicolytica in the clinic to investigate the effect of the removal of adrenergic stimuli on ventricular configuration under unchanged loading conditions. It would be conceivable that the ventricular radius-wall thickness ratio could be enhanced by inhibition of a catecholamine induced stimulation of protein synthesis. Thus the pumping function of the heart could be affected even on the basis of geometric factors alone.
The investigations were made in Wistar rats with experimental supravalvular aortic stenosis (AS). The animals were divided into four groups: a) sham-operated (SO); b) AS; c) AS + Atenolol 50 mg/kg b.w. daily; d) AS + Terazosin 3 mg/kg b.w. daily. - All measurements were performed after an observation period of one year. In all AS groups the survival rate was 70%. The degree of hypertrophy of the AS animals was about 50% as compared to the SO group. No significant differences were found within the AS groups. Likewise, the end-diastolic P-V relations, as well as the calculated myocardial elastic constants, were identical in all AS animals. The assessment of cardiac performance showed no significant differences, except when compared to the SO group.
Conclusions: A change in the ventricular configuration towards structural dilatation due to discontinuation of α- or β-adrenergic stimuli can, with certainty, be excluded for the chosen experimental conditions.

Physiologisches Institut II, Universität Tübingen, Gmelinstr. 5, D-7400 Tübingen

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EFFECT OF REPERFUSION-PRESSURE ON POST-ISCHEMIC RECOVERY OF NORMAL AND HYPERTROPHIED RAT HEARTS

T.Stein,W.Isselhard,J.Sturz,T.Minor,P.Wingenfeld

Recovery (Rc) from global ischemia was studied in normal and hypertension-adapted (14 d suprarenal aorta-constriction) hypertrophied hearts (NH, HH) perfused (Langendorff-technique) with Krebs-Henseleit-solution (KHS, 35°C, 30 min), and cardioplegic St. Thomas' Hospital-solution (25°C, 5 min, 65 mm Hg), kept ischemic for 40 min at 25°C, and reperfused with KHS (35°C, 45 min). During KHS-perfusion, perfusion pressure (PP) was either 75 or 130 mm Hg. Reperfusion (Rp) was either abrupt (A, PP within 1 min) or gentle (G, PP within 30 min).
LVSP and ATP tissue levels as selected parameters of functional and metabolic Rc at 45 min Rp were:

Hearts			NH		HH	
RP	Mode		A	G	A	G
PP 75 mm Hg		LVSP %Rc	75 * 92		83	89
		mm Hg	91 * 112		99	124
		ATP µmol/g	3.53 * 4.20		3.65 * 4.41	
PP 130 mm Hg		LVSP %Rc	63 * 75		71 * 87	
		mm Hg	72	81	89	108
		ATP µmol/g	2.79	3.20	2.91	3.32

%Rc: Pre-arrest value = 100% *p 0.05 or smaller

Conclusions: Rp with the lower PP resulted in a better post-ischemic Rc of funtion and metabolic status. Gentle Rp increased the extent of metabolic Rc at all conditions and of functional Rc except for HH at lower PP. Except of HH at lower PP, the gain in Rc by gentle Rp was more pronounced at the lower PP. HH tolerated the higher PP better.

Institut für Experimentelle Medizin, Robert-Koch-Straße 10, D 5000 Köln 41

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CONTRACTILE BLOCKADE PREVENTS HYPERCONTRACTURE IN ANOXIC-REOXYGENATED CARDIOMYOCYTES

B. Siegmund, T. Klietz, P. Schwartz, H.M. Piper

Reoxygenation after 120 min substrate-free anoxia causes sudden hypercontracture in isolated rat cardiomyocytes. Reoxygenated hypercontracted cardiomyocytes maintain their sarcolemmal integrity (no enzyme release) and re-establish a nearly normal phosphorylation potential within 15 min (Siegmund et al., Am J. Physiol., in press). In the same model it was now investigated whether a temporary contractile blockade by 20 mM 2,3-butanedione monoxime (BDM) can prevent reoxygenation-induced hypercontracture. When BDM was present during 120 min anoxia and removed immediately before reoxygenation, it had no effect, but when it was present during 120 min anoxia and the subsequent 15 min reoxygenation, hypercontracture was prevented in 85 % of the cells. The anoxic changes of high-energy phosphate contents, the phosphorylation potential and the ultrastructure remained unaffected by the presence of BDM. When BDM was applied anoxically immediately prior to reoxygenation, it also prevented hypercontracture. When it was washed out after the first 15 min of reoxygenation, contracture still remained absent but the cells could be electrically stimulated to contract. Conclusions: The results demonstrate that a temporary contractile blockade (15 min) at the onset of reoxygenation prevents hypercontracture in anoxic-reoxygenated cardiomyocytes. This result, the energetic recovery and sarcolemmal integrity of cardiomyocytes in anoxia-reoxygenation, demonstrate that reoxygenation-induced hypercontracture is not based on an already irreversible cell damage. (Supported by the DFG).

Physiologie I, Universität Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf.

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XAMOTEROL RECRUITS AN INOTROPIC RESERVE IN REPERFUSED MYOCARDIUM WITHOUT DETRIMENTAL EFFECTS ON ITS SUBSEQUENT RECOVERY.

Christa Linder, Stefan Schäfer, and Gerd Heusch

The β_1-adrenoceptor partial agonist xamoterol (X) is characterized by the combination of its negative chronotropic, anti-ischemic effect during sympathetic activation and its positive inotropic effect at a low resting cardiac sympathetic tone. X may therefore offer a new therapeutic approach in patients with exercise-induced myocardial ischemia and subsequent post-ischemic dysfunction. In 8 anesthetized dogs, we tested the effect of X on regional myocardial function (sonomicrometry) during 8 h reperfusion (R) after a 15 min LCX-occlusion. During occlusion, mean systolic thickening velocity (V) decreased from 9.31±1.91 (SD) mm/s to -1.35±2.72 mm/s. X (100 µg/kg i.v., infused at 10 min R) increased V from 1.47±2.34 mm/s (10 min R) to 7.13±3.55 mm/s (30 min R, $p<0.05$), whereas in a placebo-group (P, n=8) V remained unchanged (3.14±3.30 mm/s at 10 min R vs. 2.96±3.74 mm/s at 30 min R). At 8 h R, V was not different in both groups (X: 7.97±4.23 mm/s vs. P:6.87±4.00 mm/s). Histological examination revealed no difference in the extent of necrosis between the two groups.
Conclusion: Xamoterol recruits an inotropic reserve in reperfused myocardium and this recruitment does not compromise functional recovery and structural integrity of the reperfused myocardium.

Abteilung für Pathophysiologie, Universitätsklinikum Essen, Hufelandstr. 55, 4300 Essen

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MYOCARDIAL RECOVERY AFTER LOW-FLOW ISCHEMIA: PHARMACOLOGICAL INTERVENTIONS AND EFFECTS OF GRANULOCYTES

B.F. Becker, N. Reinholz, P. Raschke, B. Leipert and E. Gerlach

The attenuated recovery of function in ischemic myocardium subsequent to a restoration of flow has been ascribed to both the coronary no-reflow phenomenon and to myocardial stunning. Oxygen radicals and oxidants, formed especially during reflow by polymorphonuclear granulocytes (PMN), are deemed to be a cause. To better differentiate between damage resulting directly from hypoxia and that suffered upon reflow, isolated guinea pig hearts were subjected to low-flow ischemia (1 ml/min, 37°C, 30min) and reperfusion in the absence and presence of a) various radical scavengers and antioxidants, and b) autologous PMN (infusion rate: 10^6 cells/min). Myocardial function was assessed from the performance of pressure-volume work prior to and after ischemia.
Results: 1) External heart work recovered to about 35% of the pre-ischemic value (control). Supplementation of the Krebs-Henseleit perfusate with superoxide dismutase + catalase, uric acid, captopril or allopurinol enhanced recovery to between 51% and 62%. However, the non-scavenger substances ramiprilat, bradykinin and iloprost were similarly effective. 2) For all conditions functional recovery was directly, albeit rather loosely, related to the myocardial ATP content (r=0,62) and to the total loss of purine compounds during the low-flow phase (0.3-0.5 µmol/g, r=0.52), but not to purine loss during reperfusion (0.11-0.16 µmol/g), lactate release or post-ischemic coronary flow. 3) Perfusion with unstimulated PMN, either before, during or after the low-flow phase, did not noticeably alter myocardial function or coronary flow.
Conclusions: The level of adenine nucleotides remaining in the myocardium after a period of low-flow ischemia is a major determinant of functional recovery. As evidenced by the purine losses, the ischemic and not the reflow phase is decisive for this aspect. The large scatter of performance for hearts with similar ATP values suggests, however, that in some cases work performance is under additional restraints (stunned myocardium). The beneficial effects of the various additives tested probably arise from influences on this phenomenon, though the modes of action are obviously multiple. As to be expected, passage of unstimulated PMN through the coronary system does not lead to acute vascular or heart muscle dysfunction. Ischemia of a reversible degree surprisingly will not alter this behaviour.

Physiologisches Institut der Universität München, Pettenkoferstr. 12, 8000 München 2

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LEFT VENTRICULAR ASYNCHRONY IS AN INDICATOR OF REGIONAL MYOCARDIAL DYSFUNCTION.

Stefan Schäfer and Gerd Heusch

There is a marked heterogeneity of myocardial wall thickening within the left ventricle and between different individuals. It is therefore difficult to detect regional myocardial dysfunction from absolute values of systolic wall thickening (WT). We now tested whether the extent of left ventricular asynchrony can be used to quantify the severity of regional myocardial dysfunction. In 6 open-chest dogs, regional myocardial wall thickness (sonomicrometry) was measured under control conditions (C), at three degrees of ischemic dysfunction produced by at least 4 min steady state stenoses on the left circumflex coronary artery (I_1-I_3) and after release of a 15 min LCX-occlusion, when two matched degrees of reperfusion dysfunction (R_1-R_2) were present. Two indexes of left ventricular asynchrony were calculated: (1) post-ejection thickening (PET) and (2) the phase difference of the first Fourier harmonic of posterior versus anterior wall motion (PD). WT was decreased from 15.3±3.1 (SD) % (C) to 9.7±1.4 % (I_1), 4.2±1.6 % (I_2), and -3.7±3.1 % (I_3). Conversely, PET increased from 0.02±0.4 mm (C) to 0.15±0.22 mm (I_1), 0.19±0.15 mm (I_2), and 0.50±0.26 mm (I_3). PD increased from 9±28 degrees (C) to 22±19 degrees (I_1), 54±18 degrees (I_2), and 107±21 degrees (I_3). During reperfusion, PET and PD recovered to 0.34±0.19 mm and 36±24 degrees (R_1) and 0.25±0.31 mm and 29±8 degrees (R_2). There were inverse linear relationships between WT and PET ($r=-0.82$, $p<0.001$) and between WT and PD ($r=-0.86$, $p<0.001$). Inotropic stimulation by postextrasystolic potentiation or norepinephrine (0.5-1.0 µg/kg*min i.v.) increased posterior and anterior WT but did not alter the extent of left ventricular asynchrony. Thus, the severity of regional myocardial dysfunction at a given inotropic state can be determined by analysis of left ventricular asynchrony using PET or PD.

Abteilung für Pathophysiologie, Universitätsklinikum Essen, Hufelandstr. 55, 4300 Essen

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QUANTITATIVE ANALYSIS OF CARDIAC DYNAMICS BASED ON FRANK's DIAGRAM AND COMPUTER SIMULATION. SIGNIFICANCE OF MATHEMATICAL MODELS

B. Dierberger, R.W. Gülch and R. Jacob

A methodological approach for analysing the determinants of stroke volume under the conditions of altered ventricular geometrical configuration is presented and applied to left ventricular dynamics of rats with experimental aortic stenosis and spontaneous hypertension. The analysis is based on Frank's pressure-volume (P-V) diagram and on model calculations. The relationship describing stroke volume as a function of end-diastolic inner radius or inner volume, respectively, is calculated after transforming P-V relations into stress-length (circumference) relations. Changes of this relation permit a quantitative evaluation of the significance of the following factors for stroke volume: Ventricular inner dimensions, wall thickness, "myocardial contractility" and distensibility, preload (end-diastolic wall stress) and systolic pressure load (end-systolic pressure).
An ascending branch of the relationship between stroke volume and end-diastolic ventricular size can be demonstrated using the model of a thickwalled sphere as well as some more sophisticated models such an ellipsoid of revolution with uniform or non-uniform wall thickness. The calculated value of stroke volume is only slightly affected by the choice of the model used up to a three-fold increase in inner volume. - It is shown in the present examples that, as a rule, myocardial alterations (reduced contractility and distensibility) are the decisive factors for impaired cardiac pumping function. - The methodological approach presented is certainly an indispensible completion of Frank's P-V diagram. Presupposing exact measurements this concept can also be applied to clinical cases. Current investigations should clarify whether further improvement of analysis of ventricular function is possible based on the finite element theory.

Physiologisches Institut II, Universität Tübingen, Gmelinstr. 5, D-7400 Tübingen

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VENTRICULAR MYOCYTES: INACTIVATION CURVES OF FORCE AND CA-CURRENT DISSOCIATE AT NEGATIVE POTENTIALS

G. Isenberg, M. Leverkus, T. Mitsuiye

Isometric contractions were measured in single myocytes isolated from guinea-pig ventricles. By means of poly-L-lysine, one cell was attached to the beveled ends of a pair of thin glass rods. Contraction displaced the rods by 1-3 μm, with the calibrated compliance (0.72 m/N) the displacement could be transformed into a force signal.

By means of the whole cell patch-clamp technique, the cells were depolarized at 1 Hz with 160 ms long clamp-pulses from -45 mV to +5 mV. At 35°C and 1.8 mM $[Ca]_o$, force peaked within 96 ± 20 ms to 3.2 ± 1.6 mN/mm^2 (mean ± S.D., n=9). More positive holding potentials decreased peak force and peak calcium current in parallel, the inactivation curves of current and force had similar slopes (-9 mV) and 50% inactivation potentials (-20 mV).

Holding potentials more negative than -45 mV potentiated peak force but they did not significantly change the calcium current. Contraction was maximal at a holding potential of -80 mV; pulses from -80 to +5 mV evoked force twitches 6.4 ± 0.5 times larger than those from -45 to +5 mV. Between -80 and -45 mV, more positive holding potentials reduced twitch force along an S-shaped inactivation curve that had a slope of -20 mV and a midpoint at -57 mV. For testing the possibility, inactivation of contraction being related to inactivation of the fast sodium current, the experiments were repeated in the presence of 30 μM TTX. TTX did not significantly change the inactivation curve of force, the slope (-18 mV) and potential of half maximal inactivation (-60 mV) were similar in the presence and absence of TTX.

We discuss the results to suggest that activator-Ca derives not exclusively from Ca-induced SR-Ca-release. It may be provided also by depolarization-induced SR-Ca-release, the contribution of which decreasing with less negative holding potentials.

[1] Shepherd,N, Vornanen,M, Isenberg, G, Am J Physiol 258, in press

Zentrum für Physiologie, Robert-Koch-Str. 39, 5000 Köln.

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Mg^{2+} - Ca^{2+} - INTERACTION IN CARDIAC MUSCLE

U. Pohl, S.Y. Wang, R. Meyer, H.G. Haas

Membrane currents of isolated ventricular myocytes of the guinea-pig were investigated by patch clamp in the whole cell recording mode. External Mg^{2+}-concentration was varied and its effect on membrane currents was tested. Emphasis was placed on activation and inactivation of current flow through Ca^{2+} channels of the L-type. Using Tyrode solution with normal concentrations of Na^+, K^+, and Ca^{2+}, an increase in $[Mg^{2+}]_o$ from 1 to 2.5; 5; 10 or 20 mM caused a shift of the steady activation curve for I_{Ca} by 4; 8; 12 or 26 mV, respectively, to more positive potentials. Shape and steepness of the activation curve were almost unaffected by Mg^{2+}. The effect of Mg^{2+} on activation gating was found to be superimposable with the effect of other agents. When external Na^+ was replaced by choline and Mg^{2+} was increased subsequently, either change was followed by a shift in activation gating to more positive potentials and the effect of increased $[Mg^{2+}]_o$ was simply added to that of choline. The effects of Mg^{2+} on activation of Ca^{2+} channels may be interpreted as due to a decrease of membrane surface potential by binding of Mg ions to fixed negative surface charges.

Unlike activation, influence of $[Mg^{2+}]_o$ on steady inactivation of I_{Ca} was less pronounced and not clear. In some experiments increasing $[Mg^{2+}]_o$ from 1 to 10 mM had almost no effect on the availiability of I_{Ca} while in others a shift of the steady inactivation curve to more positive potentials was observed. The shift of the inactivation curve was always smaller than the shift of the activation curve obtained from the same cell. The apparent discrepancy between the effects of $[Mg^{2+}]_o$ on I_{Ca} activation and inactivation could be explained in several ways: i) inactivation gates may be located deeper in the membrane so that they are less affected by changes in the surface potential or ii) I_{Ca} inactivation is a $[Ca^{2+}]_i$-dependent process rather than a voltage dependent process.

In a Ca^{2+}-free, Mg^{2+}-free solution with normal $[Na^+]_o$ and 2 mM EGTA a long lasting inward current developed which is thought to be carried by a flow of Na ions through the L-type channels. This current was reversibly blocked by addition of Mg^{2+}.

Physiologisches Inst. der Universität Bonn, Wilhelmstr.31, D-5300 Bonn 1

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PROTEOLYTIC PROCESSING OF ATRIAL NATRIURETIC PEPTIDE (ANP) FROM PROHORMONE: A LANGENDORFF HEART STUDY.

M.Ph. Christmann, Th. Dörner, D. Hock, R. Hertel, M. Gagelmann and W.G. Forssmann

ANP is stored as a prohormone (126 amino acid residues) in specific granules of atrial myoendocrine cells. The circulating ANP comprises 28 residues of the C-terminus of the prohormone and is involved in the regulation of blood pressure and electrolyte balance. However, the proteolytic process for release of ANP from the prohormone is still a matter of debate. In order to determine whether processing is cosecretorial or occurs after release from the myoendocrine cells isolated rat hearts were perfused (Langendorff technique) with N-terminal extended forms of ANP (porcine ANP-39-126 and human ANP-95-126). The cleavage products were separated by HPLC and analyzed by amino acid sequencing. The amino acid sequences of porcine, human and rat ANP are identical except for Met in position 110 which is replaced by Ile in case of rat ANP, enabling discrimination between exogenously administered ANP analogs and endogenous peptide. During 30 min recirculation, either porcine ANP-39-126 or human ANP-95-126 were added (100 µg/30 ml). For control peptides (10 µg) were incubated in perfusate alone and Krebs-Henseleit buffer. The peaks separated by RP-HPLC were collected, pooled and rechromatographed for sequencing. Our experiments demonstrate that during perfusion porcine ANP-39-126 and human ANP-95-126 are processed to the circulating bioactive ANP-99-126 (α-ANP). A similiar result is obtained during control incubation, while no processing is detected with buffer alone. The analysis by amino acid sequencing for the first time, demonstrates that i.) prohormone may be proteolytically cleaved between Arg^{98} and Ser^{99} following release from the myoendocrine cells and the processing ii.) is achieved by a membrane bound enzyme which is eluted during coronary perfusion.

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Institut für Anatomie & Zellbiologie III, Universität Heidelberg, INF 307, 6900 Heidelberg, FRG

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ATPase ACTIVITY OF PIG HEART SKINNED FIBERS ; EFFECTS OF SKINNING PROCEDURE AND APP 201-533, A Ca-SENSITIZING AGENT

W.J. Leijendekker and J.W. Herzig

The effect of varied skinning procedures with Triton X-100 (T) on ATPase activity and isometric force of isolated pig heart fiber bundles has been examined. Moreover, the effects of APP 201-533, a Ca-sensitizing agent, on these preparations were studied. Thin (0.3-0.5 mm) right ventricular fiber bundles were exposed at 4°C to T for varied times, with ATP (5 mM) and an ATP reconstituting system of PEP (10 mM) and pyruvate kinase present, or without ATP. The ATPase activity of the skinned fiber bundles was measured using an NADH fluorescence coupled assay. The ATPase activities at pCa 4.3 (Ca-activated) and pCa > 8 (basal activity) in presence of 10 mM EGTA were measured. The ratio of Ca-activated to basal ATPase activity increased from 3 (0.1 % T, 30 min) to 7 and 10, respectively (1 % T, 3 hrs and 20 hrs, n > 3), independent of the presence of ATP. More extensive treatment with T decreased the ratio of Ca-activated ATPase activity to force by 35-40 % (0.1 % T for 30 min compared to 1 % T for 3 hrs). The ratio Ca-activated ATPase to force of fibers extracted in presence of ATP was 30-50 % lower than in fibers extracted without ATP, and seemed independent of T treatment. These results suggest that prolonged T treatment may extract "contaminant" Ca-activated ATPases; alternatively, the turnover rate of actomyosin ATPase could be dependent on the time of exposure of the fibers to T and to ATP in the extraction solution.

Furthermore, in fibers extracted with 1 % T for 4 hrs without ATP, 500 uM APP 201-533 increased force by 70-100 % (at pCa ~6), the ratio of ATPase to force being unaffected by APP 201-533.

Pharmaceuticals Division, Department Research CVS, Ciba-Geigy Ltd, Basle, Switzerland

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EFFICIENCY OF THE HEART UNDER ADRENALINE

G. Kissling

The investigations were performed on a modified heart-lung preparation of the rat. After opening the chest under urethane anaesthesia (1.2g/kg b.w.) the aortic root as well as both caval veins were ligated and the aortic root connected to the vena cava inferior via a shunt with a Starling resistance. Via this resistance left ventricular afterload could be adjusted arbitrarily. The following haemodynamic parameters were measured: right and left ventricular pressure, central aortic pressure, pulmonary flow and the flow in the shunt circuit. The oxygen consumption of the preparation was determined by means of the difference in oxygen concentration between the inspired and expired air and the respiratory volume per minute. The efficiency was calculated from the external work and the oxygen consumption under control conditions as well as under adrenaline (bolus: 60µg/kg, continuous infusion: 10µg/kg·min).

Our investigations have confirmed that the efficiency of the heart depends on the external mechanical conditions: compared to high pressure loads, at low pressure loading a certain amount of work is performed with a significantly increased efficiency. Under the chosen experimental conditions, a given work is performed under adrenaline with a reduced pressure development and a higher volume shift compared to the control conditions. Simultaneously, the efficiency increases significantly. However, the improvement in efficiency under adrenaline cannot be attributed to the more favourable mechanical conditions alone. Even in cases where a given work is performed under identical pressure and volume loading, adrenaline significantly improves efficiency.

Physiologisches Institut II, Universität Tübingen, Gmelinstr. 5, D-7400 Tübingen

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CA-SYNERGISTIC AND CA-ANTAGONISTIC EFFECTS ON NET ^{45}CA UPTAKE INTO NEONATAL RAT MYOCYTES - A MODEL TO STUDY MODULATION OF CA INFLUX IN CELL CULTURES.

H.Michel, A.Goetz, M.Frey a. G.Fleckenstein-Grün
Study Group for Calcium Antagonism, Physiological Institute, University of 7800 Freiburg i.Br., FRG

Transmembrane calcium (Ca) influx triggers cascades of enzymatic reactions in a variety of cell types in both, physiological and pathophysiological processes. Consequently, modulation of transmembrane Ca entry by Ca promoters or Ca-antagonistic inhibitors is effectively used for pharmacological interventions. In order to investigate various mechanisms of influencing transmembrane Ca influx on a cellular level, net ^{45}Ca uptake was measured on monolayers of neonatal rat myocytes, cultured by standard methods. ^{45}Ca (0.02 µCi/l) was added for 5 min. Subsequently, the monolayers were washed with ice-cold, Ca-free, EGTA (2.5 mmol/l)-containing solution, detached and lysed. From the cell lysate samples were taken for fluorescent protein determination (excitation 286 nm, emission 338 nm) and for measurement of ^{45}Ca activity by liquid scintillation. Under control conditions (37°C, pH 7.4, Ca_o:1 mmol/l) net ^{45}Ca uptake was 131.18 ± 10.13 ng Ca/mg protein. Additional Ca uptake above control values - amounting to 35%, 34%, or 74% respectively - was evoked by three different mechanisms: (1) Depolarization (n=10) with K_o=65 mmol/l; (2) ß-receptor stimulation (n=10) with $1x10^{-6}$ mol/l isoproterenol or (3) prolongation of the opening state of L-type Ca channels (n=4) with $5x10^{-8}$ mol/l Bay K 8644. Conversely, highly specific Ca antagonists ($1x10^{-6}$ mol/l) reduced transmembrane Ca entry below normal: Verapamil by 43.2 ± 5.6 % (n=6), diltiazem by 26.3 ± 4.5% (n=3) and nifedipine by 20.5 ± 3.4% (n=5). The data suggest, that determination of net ^{45}Ca uptake into neonatal rat myocytes offers an adequate model for studies on modulation of transmembrane Ca influx in cell cultures.

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HEMODYNAMIC EFFECTS OF A BRADYCARDIC AGENT ON THE CANINE HEART

Jochen D. Schipke*, Yasuhiko Harasawa, Seiryo Sugiura, Joe Alexander Jr., and Daniel Burkhoff

Bradycardic agents are supposed to improve the oxygen demand/supply ratio during ischemia both by reducing heart rate (HR) and by increasing diastolic coronary flow. We tested whether the benzazepinon UL-FS49 acts exclusively on sinus node cells or additionally affects myocardial contractile state. To avoid interactions with the peripheral system, we performed experiments on isolated, blood-perfused canine hearts and determined HR (1/min), duration of systole and diastole (Tsys, Tdia, ms), ventricular contractile state (peak isovolumic systolic pressure: LVISP, mmHg), myocardial oxygen consumption (MVO_2, ml/min·100g), and cardiac output (CO, l/min) during control and after injection of UL-FS49 (1 mg/kg i.c.).

	HR	Tsys	Tdia	LVISP	MVO_2	CO
control	104±7	280±16	324±51	72±6	6.9±.5	1.1±.1
UL-FS49	93±7*	313±19*	427±44*	72±6	6.0±.4*	1.2±.1

*$p < 0.05$ control vs. UL-FS49; data are mean ± SEM

We conclude that UL-FS49 decreases MVO_2 by bradycardia but not by reduced contractility. Hence, the agent does not further depress ischemic ventricular function. Myocardial blood flow will, additionally, be improved via prolonged diastole. Moreover, peripheral blood flow is maintained at the dose used. Thus, usage of bradycardic agents could become an alternative strategy in treating ischemic myocardial disease.

Dept. of Biomedical Engineering, The Johns Hopkins Univ., Baltimore, USA; *Inst. für Experimentelle Chirurgie, Univ. Düsseldorf

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NOVEL CARDIOTONIC DRUGS - A COMPARATIVE STUDY ON THEIR Ca^{2+}-SENSITIZING EFFECT ON THE CONTRACTILE PROTEINS OF GUINEA-PIG PAPILLARY MUSCLE.

L. Kling, B. Müller-Beckmann, M. Schroth, G. Sponer, E. Böhm, K. Strein

A novel approach for the positive inotropic therapy of heart failure may be to alter the response of the myofilaments to Ca^{2+} and by so doing to achieve an increased contractile state with little change in the free Ca^{2+} transient during the beat (SOLARO, NSAP 340, Suppl. II, R89, 1989). Ca^{2+}-responsiveness of the contractile proteins can be investigated by studying the relationship between force and Ca^{2+} concentration in skinned fibres (RÜEGG and MORANO, J. Cardiovasc. Pharmacol. 14, Suppl. 3, S20, 1989). We now investigated the influence of the PDE III-inhibiting positive inotropic drugs adibendan (A), MCI-154 (M), pimobendane (P), and the active metabolite of P, UD-CG 212 (U), on the Ca^{2+}-responsiveness of skinned fibres of guinea-pig papillary muscle.

Guinea-pig papillary muscles were chemically skinned using 50 % glycerol and 1 % triton X100. Subsequently Ca^{2+}-concentration response curves (1.6 - 4.5 µM) were performed in the absence and presence of various concentrations of the test compounds and the EC_{50} % were calculated. The Ca^{2+}-responsiveness of the skinned fibres in the presence of the drugs was calculated as the shift of the EC_{50} % expressed as its negative decadic logarithm of the molar concentration (pCa_{50}). Values higher than -0.09 (result obtained in control experiments) represent a Ca^{2+}-sensitization, those smaller than -0.09 characterize a Ca^{2+}-desensitization of the contractile proteins.

Results (means, n = 4-6):

drugs	pCa_{50} shift in the presence of ... µM of the test compounds		
	10	100	500
A	-0.04 a	-0.03 a	+0.06 a
M	-0.04 a	-0.06	+0.04 a
P	-0.04	+0.12 a	n.d.
U	-0.06	-0.25 b	-0.70 b

a = $p<0.05$ for Ca^{2+}-sensitization
b = $p<0.05$ for Ca^{2+}-desensitization
n.d. = not determined due to limited solubility of the compound

An increase of the Ca^{2+}-responsiveness was observed in the rank order P>A>M at concentrations higher than 10 µM. However, U decreased the Ca^{2+}-responsiveness of the cardiac myofibrils at concentrations higher than 10 µM. The relative contribution of the Ca^{2+}-sensitization to the overall inotropic activity of A, M and P in vivo remains to be determined since the PDE III inhibiting action of these drugs also induce an increase in myocardial contractility. Nevertheless, our results suggest that Ca^{2+}-sensitization does not contribute to the inotropic effect of U in vivo, in particular in guinea pigs.

Dept. of Cardiovascular Pharmacology, Boehringer Mannheim GmbH, D-6800 Mannheim 31, FRG
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EFFECTS OF POSITIVE AND NEGATIVE INOTROPIC AGENTS ON CORONARY PERFUSION PRESSURE INDUCED INCREASE OF LEFT VENTRICULAR PERFORMANCE

A. Dorszewski and G. Arnold

Previous studies have shown that an increase in coronary perfusion pressure (CPP) with aortic pressure (PAO) kept constant resulted in a rise of left ventricular performance, while left ventricular end-diastolic pressure (LVEDP) decreased, thus demonstrating the garden-hose-effect (GHE).
In isolated, saline perfused guinea pig hearts we studied the coronary flow induced changes in CPP in a range between 30 and 70 mmHg during norepinephrine (N) and verapamil (V) infusions at a constant heart rate. To adjust drug concentration equivalently to coronary flow, the latter could be varied independently. We measured left ventricular pressure (LVP), LVEDP and myocardial contractility (dP/dt_{max}) during control (C) and infusion of the drugs. The differences of the left ventricular parameters were calculated between the data shown for example at a CPP of 40 and 60 mmHg:

	NOREPINEPHRINE				VERAPAMIL		
	ΔLVP mmHg	ΔLVEDP mmHg	$\Delta dP/dt_{max}$ mmHg/s		ΔLVP mmHg	ΔLVEDP mmHg	$\Delta dP/dt_{max}$ mmHg/s
C	5,0 ± 0,9^{+}	2,6 ± 0,5^{+}	117 ± 10,5^{+}	C	4,3 ± 0,6*	2,1 ± 0,6*	107 ± 15,9*
N	2,5 ± 0,8	1,7 ± 0,6	66 ± 16,1	V	3,9 ± 0,8	2,2 ± 0,4	90 ± 14,3

(n = 15; data are mean ± SEM; + p < 0,05; C vs. N; * p > 0,1; C vs. V)

During N we observed a decrease of GHE, but no significant change under V. Corresponding data were obtained at different CPP. We conclude that the decrease of GHE under N is the result of a higher performance level of the heart. The influence of V might be explained by an inhibition of excitation-contraction-coupling resulting in no increase of GHE.

Inst. für Exp. Chirurgie, Universität Düsseldorf
Moorenstr.5, 4000 Düsseldorf

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CALCIUM CURRENTS IN ISOLATED RAT ATRIAL MYOCYTES.

G.K. Pike , D.J. Bryant, M.L. Roberts and R. H.Fink.

Atrial calcium currents (I_{Ca}) have been studied in many species including guinea-pig, dog, rabbit, and human, and notable interspecies differences have been found. Surprisingly, little work has been done on rat atrial myocytes and therefore this study was aimed at quantifying I_{Ca} in these cells. Atrial myocytes were isolated using a technique similar to that described by Isenberg and Klockner (Pflügers Arch. 395, 6-18, 1982) and kept in a modified 'KB' medium for up to 3 days. Cells were suspended in Tyrode solution (in mM: NaCl 150, KCl 5.4, Glucose 10, HEPES 5, $MgCl_2$ 1.2, $CaCl_2$ 3.6, TTX 0.02mM, pH 7.4). The electrode solution contained (in mM: KCl 140, $MgCl_2$ 3.5, $CaCl_2$ 2, EGTA 11, Na_2ATP 0 or 5, HEPES 10 pH 7.1). Whole cell recordings (WCR) were obtained with cells held at -80mv. At 2-5 mins after WCR, $I_{Ca}max$ was 26.03 ± 9.28 pA/pF (n=9,SE), activated at about -35 mv, and peaked at -20 mv. Presence or absence of ATP in the patch electrode did not appear to influence I_{Ca}. Rundown of I_{Ca} varied from cell to cell, being reduced in magnitude by 50% often after 5-10 mins, but in some cells was unchanged after 30-40 mins. The time to peak current varied between 4 and 20 milliseconds. Interestingly, I_{Ca} in Na^+ and K^+ free solutions (TEA and Cs substituted respectively) was only 6.31 ± 0.74 pA/pF (n=16,SE), with a shift in activation voltage to -15 mv, reaching peak values at 10 mv. Again, the presence or absence of ATP with or without 0.1mM Cyanide did not influence I_{Ca}. Rundown occurred at a similar rate compared with cells in normal solutions. Furthermore, there was no correlation between tip size (or series resistance) and the rate of rundown. Supported by NHF (Aust.) and CSIRO/URG to RHF and MLR.

Department of Physiology, University of Adelaide, Adelaide, Australia, SA 5000.

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METABOLIC EFFECTS ON SARCOPLASMIC RETICULUM AND MYOSIN EXPRESSION IN CARDIAC HYPERTROPHY

H. Rupp, R. Jacob, V. Elimban*, N.S. Dhalla*

Pressure overload of rat heart leads to an increase in the proportion of myosin V3 and a reduced Ca^{2+}-stimulated ATPase activity of sarcoplasmic reticulum (SR). Because a longstanding overload is likely to result in cardiac failure, it was attempted to prevent the subcellular changes by interventions which preferably do not provide an additional load for the heart. Administration of sucrose in the drinking water in a low dose (0.8%) proved to be a most efficient means. The sucrose intake corresponded to approx. 1% of the daily consumed calories and did neither affect ventricular weight nor other growth characteristics. In rats with abdominal aortic stenosis (AS), the sucrose treatment prevented the reduction in V1 (54% control, 36% AS, 54% AS + sucrose) and the reduction in SR ATPase activity given in nmol P/mg/min (145 control, 89 AS, 131 AS + sucrose). The proportion of V1 can also be increased by a fat-rich diet (20% mackerel oil). In the case of SHR, V1 increased from 21% to 30% and the rate of Ca^{2+}-uptake of SR increased from 55 to 105 nmol Ca^{2+}/mg/min. This treatment which was associated with an increased daily calorie intake led, however, to increased growth parameters. Taken together, the data show that a restructuring of the myocyte from pressure loaded ventricles can be achieved by interventions which most probably act via an altered fuel metabolism of the heart.

Physiological Institute II, University of Tübingen, Gmelinstr. 5, D-7400 Tübingen, and *St. Boniface General Hospital Research Centre, Winnipeg, Canada

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MATHEMATICAL ANALYSIS OF THE RELAXATION CHARACTERISTICS OF PAPILLARY MUSCLE UNDER THE INFLUENCE OF ISOPRENALINE AND PHENYLEPHRINE IN SWIM-TRAINED RATS

Ch. Ross and R. Jacob

A mathematical analysis is presented permitting quantiative description of myocardial muscle relaxation using only one function with the form:

$\sigma = \sigma_{max} e^{-\gamma\tau\lambda}$

σ_{max} = Max. stress developed during an isometric contraction
γ and λ = Constants
τ = Time elapsed after σ_{max} was reached.

A double logarithmic plot of this function yields a straight line of the form $y = mx + c$ where the gradient $m = \lambda$ and the y-intercept c can be used to evaluate the second constant γ. To test the sensitivity of this mathematic tool, the effect of a swim-training programme, α-receptor stimulation with phenylephrine and β-receptor stimulation with isorpenaline was investigated.
The relaxation characteristics of muscles from swim-trained rats are similar to those of muscles under the influence of isoprenaline. This is clearly visible in the rectified plot which showed a parallel shift to the left, representing a speeding up of muscle relaxation. Phenylephrine, on the other hand, showed an increase in gradient in the rectified plot which represents an initial increase in the velocity of relaxation with a subsequent decrease.
The results reveal that such a rectification is very sensitive to variations in the form of the mechanogram and makes small differences in the muscle relaxation characteristics very conspicuous. Furthermore, it permits description of relaxation simply with only the two constants λ and γ.

Physiologisches Institut II, Universität Tübingen, Gmelinstr. 5, D-7400 Tübingen

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DOUBLE-LOOP SERVO-SYSTEM FOR PRESSURE-CONSTANT PERFUSION OF THE CORONARY CIRCULATION

A. Skyschally, R. Schulz, C. Linder, G. Heusch

Coronary autoregulation or vasoactive substances can cause transient changes in vascular resistance. The quantitative evaluation of these effects requires the constancy and reproducibility of one major determinant of coronary blood flow, i.e. coronary perfusion pressure. A constant perfusion pressure also avoids a pressure dependent transmural blood flow redistribution. Due to the transient nature of changes in coronary vasomotor tone, such a perfusion system must have a fast regulatory response. A roller-pump, combined with an air chamber of 40 ml, provides blood at a pressure of up to 700 mmHg and a maximal flow of 350 ml/min. The pressure in the air chamber is measured and the pump speed is controlled by the first servo-loop to maintain this pressure. To reduce the high pressure of the windkessel to the selected coronary perfusion pressure, a variable flow-resistance, formed by a clamped silicone tubing, is inserted into the output line of the system. A fast servomotor performs the clamping and is controlled by an electronic regulator using a feedback-loop to the pressure signal measured at the tip of the perfusion cannula by a B&H 4-327-I transducer. The dead volume of the whole system is about 60 ml. The dynamic performance was tested in vitro and in acute experiments. Pressure steps with a simulated load are fully regulated within 300 ms and the overshoot is less than 10 mmHg. In vivo, pressure is stabilized within less than two cardiac cycles during postocclusive reactive hyperemia with a peak flow of about 300 ml/min. During five hours of perfusion in vitro and in vivo hemolysis was less than 5.1%. Conclusion : This perfusion system is suitable for experiments with constant perfusion pressure and permits the investigation of transient changes in coronary vasomotion. It combines fast regulatory performance with a low dead volume.

Abt. f. Pathophysiologie, Universitätsklinikum Essen, Hufelandstraße 55, D-4300 Essen

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HEMODYNAMIC DEPENDENCY OF MYOCARDIAL OXYGEN CONSUMPTION INDICES

J.D. Schipke*, D. Burkhoff, and J. Alexander Jr.

Myocardial oxygen consumption (MVO_2) is difficult to measure *in situ*. Accordingly, multiple indices exist to predict MVO_2 from more easily measurable physiological signals. We tested the dependency of Bretschneider's total energy requirement (Et), Rooke and Feigl's pressure work index (PWI), and Suga's pressure-volume area (PVA) on afterloading conditions and contractile state. MVO_2 was measured according to Fick's principle in 7 isolated, canine hearts at 5 end-diastolic volumes at each of 4 settings of afterload resistance: 1.5, 3.0 and 6.0 mmHg·s/ml, and the heart contracting isovolumically. The measured values of MVO_2 (M_{MVO_2}) were compared to values predicted (P_{MVO_2}) by the three indices. There was no consistent influence of afterloaded resistance on the M_{MVO_2}-P_{MVO_2} relations. In 5 of the hearts we also tested the indices when contractility and heart rate were varied simultaneously. In this case, there was a larger influence on the M_{MVO_2}-P_{MVO_2} relation for the three indices. While there was always a high degree of correlation between M_{MVO_2} and P_{MVO_2} for each of the parameters under the conditions tested, there was significant variability in the regression coefficients from one heart to another. As a result, linear regression applied to the data pooled from all hearts and all conditions provided regression coefficients that were different than those obtained in the individual hearts. We conclude that there is a minor influence of afterload and a larger influence of contractile state on the three indices of MVO_2 which we tested.

Dept. of Biomedical Engineering, The Johns Hopkins Univ., Baltimore, USA; *Inst. für Experimentelle Chirurgie Univ. Düsseldorf

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CALIBRATION METHODS FOR INTRACELLULAR ION MEASUREMENTS WITH FLUORESCENT DYES

F. Gollnick, R. Meyer, S.Y. Wang

The new generation of dual wavelenght fluorescent dyes for ion measurement and localization allows absolute quantitation. By microspectrofluorimetry and/or by image analysis intracellular ion-concentrations can be measured in single cells. To make use of these facilities reliable calibration procedures are necessary. Different in vivo or in vitro calibration procedures have been developed in the last years. We have compared some of these with respect to their applicability to two cell types: 1. isolated heart muscle cells, 2. fresh water amebas (Amoeba proteus). Three in vitro calibration procedures and one in vivo procedure were tested. Two of these work in vitro with pure ionic mixtures and can be carried out easily. The composed solutions resemble the ionic and osmotic composition of cytoplasm. Solutions were measured as thin layer (1 mm thick) in a special chamber or in microcapillaries with rectangular profile of 50 μm height (viewing pathlength) and 500 μm width. The third in vitro method was to bring a cold methanol extracted cell model in a small droplet (cell volume) of calibration solution. This led to lens effects and drying artifacts. In vivo calibration was carried out using the R_{min}/R_{max}-technique (G. Grynkiewicz et al., J Biol Chem 260:3440, 1985). R_{min} was evaluated in fura-2 loaded heart cells by internal perfusion from a patch clamp pipette with 10 mM EGTA (less than 1 nM Ca^{2+}). R_{max} (1 mM Ca^{2+}) was measured by permeabilisation of the cells with digitonin. All tested methods reveal potential sources of artifacts, e.g. ratios (340/380 nm) of R_{max} varied between 3.7 ± 0.7 (in vivo calibration), 6.5 ± 0.7 (calibration with capillaries), and 11.8 ± 1.5 (calibration in thin layers); R_{min} was always around 0.5 independent of the calibration method (±S.D.). K_d as calculated from measurements with capillaries was 460 ± 79 nM. The great variety of R_{max}-values which are relevant for the determination of K_d can be explained by the small signal to noise ratio at excitation with 380 nm. This leads to artificially elevated ratios in the case of 1 mm-layers. At in vivo calibrations loss of dye due to digitonin treatment may suppress the ratio before the real maximum is reached. The biggest quantitation errors occur at high Ca^{2+}-levels beyond the physiological range. Thus absolute quantitation seems to be useful although intracellular K_d-values are still uncertain.

Physiologisches Institut, Universität Bonn, Wilhelmstr. 31, D-5300 Bonn 1

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A NEW THEORY OF THE MONOPHASIC ACTION POTENTIAL

Kh. Peter, H. Antoni

In the past the Monophasic Action Potential (MAP), derived by means of extracellular suction or pressure electrodes, was generally explained in terms of the "injury theory". This postulates that the cells, "injured" by suction or pressure, are depolarized and serve as a reference electrode.

We offer a new explanation ("coupling theory"), which postulates an increase in conductance of the membranes under suction or pressure. Thus, the extracellular MAP electrode becomes able to register the intracellular potential which is clamped to the neighbouring cell potentials by the low resistance of the gap junctions. This means that the MAP consists of two signals: the local extracellular potential and the intracellular potential, diminished in amplitude, due to the resistance of the gap junctions and the membranes under suction or pressure.

The following findings support the "coupling theory": (1) Within the MAP signal the local extracellular potential can be identified. (2) Inside the cells under suction or pressure action potentials can be recorded by means of microelectrodes. (3) The typical potential distribution of the MAP with 1/3 positive and 2/3 negative can be explained. (4) Deformations of the MAP-plateau are the results of excitatory phenomena occurring at the membranes under suction or pressure. (5) The time course of the decrease in MAP-amplitude corresponds to the electrical decoupling of the gap junctions (healing over).

The "coupling theory" allows a stringent interpretation of the MAP and offers a more precise evaluation of the intracellular action potential.

Physiologisches Institut der Universität Freiburg i. Br.

Hermann Herder Str. 7, D-7800 Freiburg i. Br.

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CARDIAC SAH-HYDROLASE ACTIVITY OF DIFFERENT MAMMALIAN SPECIES. R. Franken-Weyers, M.M. Borst, A. Deussen, J. Schrader.

In the dog heart in situ measurement of the accumulation of S-adenosylhomocysteine (SAH) can serve as a sensitive index of regional free adenosine (Deussen et al., Circ. Res. 63: 250-261; 1988). This method is based on the enzymatic properties of SAH-hydrolase, a cytosolic enzyme homogenously distributed in canine myocardium. In order to investigate whether the "SAH-method" of measuring adenosine is limited to the dog heart, the activity of the SAH-hydrolase was determined in hearts of various species (dog, rat, guinea pig, sheep, rabbit, bovine) including human tissue samples taken from explanted hearts of patients suffering from dilative cardiomyopathy. Cytosolic extracts were prepared from tissue of the left and right ventricular wall, the septum and the atria. Homogenates were assayed for SAH-hydrolase activity in direction of SAH-synthesis using HPLC-techniques.
In each species analyzed a substantial SAH-hydrolase activity was found: bovine myocardium exhibited the highest activity (4.54 $\pm$ 0.16 nmol/min/mg protein) followed by guinea pig (2.27 $\pm$ 0.40), sheep (1.77 $\pm$ 0.10), pig (0.96 $\pm$ 0.14), rat (0.94 $\pm$ 0.06), rabbit (0.90 $\pm$ 0.04) and dog (0.88 $\pm$ 0.18). Human SAH-hydrolase activity amounted to 0.78 $\pm$ 0.09 nmol/min/mg protein. Enzym activity in most species was homogenously distributed over the different regions of the heart. Only the atria of pig and dog exhibited activities 70 % above those of the other myocardial regions. In wistar rats aged 1 month, 4 months and 2 years mean hydrolase activity was 0.87, 0.90 and 1.07 nmol/min/mg protein, respectively.
Conclusions: The cardiac activity and distribution of SAH-hydrolase in various species including man is adequate to assess the regional adenosine metabolism by means of the "SAH-method".

Zentrum für Physiologie, Heinrich-Heine-Universität Düsseldorf, Moorenstr.5, 4000 Düsseldorf 1

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ENERGETIC ASPECTS OF ISOLATED CARDIOMYOCYTES

H. Rose, S. Pöpping, H. Kammermeier

A new technique was developed to measure simultanously oxygen consumption and shortening of isolated ventricular myocytes of rat hearts during stimulation. The length-time intergral (I_t) was taken as a measure of the "work" performed by the contracting cells and correlated to the oxygen consumption per beat (V_bO_2). Stepwise inhibition of the actin-myosin interaction by addition of 2,3-butanedione monoxime (BDM) was characterized by a linear relationship between I_t and V_bO_2. Extrapolation to the point of complete inhibition of the actin- myosin ATP-ase led to an oxygen consumption due only to the cycling of ions (Ca^{++},Na^+,K^+), related to contraction.

Basal oxygen consumption was 215 $\pm$ 14 nl/mg_{Pr}* min and V_bO_2 amounted to 0.722 nl/mg_{Pr}* beat (with 0.5 mM $[Ca^{++}]_e$), 21 % of which was used for Ioncycling. This value increased to about 40 % of V_bO_2 of the unloaded cells when $[Ca^{++}]_e$ was 1.8 mM. The influence of inotropic drugs on calcium cycling should be detectable with this new method.

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Institute of Physiology, Med. Fac. RWTH Aachen, Pauwelsstr. D-5100 Aachen, FRG

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COMPUTER ASSISTED MEASUREMENT OF CONTRACTIONS AND OXYGEN CONSUMPTION OF ISOLATED CARDIAC MYOCYTES

H. Rose, K. H. Strotmann & H. Kammermeier

The course of contraction of isolated cardiomyocytes is difficult to measure mechanically and most optical methods have a poor time resolution. Thus we developed a technique with a resolution of less than 1 ms. The myocytes (some of which stick to a cover slip) were stimulated electrically by biphasic pulses in a stimulation chamber, observed through a microscope and CCD camera, and the images were recorded and digitized. 36 frames illuminated by a stroboscope were taken at increasing time intervals between stimuli and snap. The difference between these frames and a reference frame of the cells in the relaxed state gave the information to quantify the contractions. This system was coupled with a pO_2-electrode, thus oxygen consumption and contraction could be measured simultaneously.

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Institute of Physiology, Med. Fac. RWTH Aachen, Pauwelsstr. D-5100 AACHEN

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EVALUATION OF INOTROPIC REACTIONS OF ISOLATED CARDIAC MYOCYTES

H. Rose, B. Lukascek & H. Kammermeier

Isolated Myocytes of adult rats were fixed on petridishes and stimulated by alternating series of biphasic electrical pulses (1 Hz, 30 s) and quiescence (30 s). Inverted microscope, CCD camera and dimension analyser enabled us to measure the amplitude (ΔL) shortening- and relaxation-velocity (+/-dL/dt) both, from (i) the first contraction of a serie and (ii) for the steady state contractions. The "Treppe" phenomenon could thus be quantified.

From these data the contractionrate r+ ((dL/dt_{max})/L) and relaxationrate r- ((dL/dt_{min})/L) were calculated. Reduction of temperature (from 37 to 21°C) led to a strong positive inotropic response, which was accompanied by a reduction in r+ and r- (E_A= 48 kJ/mol). 2,3 butanedionemonoxime (BDM) significantly reduced ΔL, dL/dt, while r+ and r- remained unchanged. Reduction of temperature as well as 10 mM caffeine reduced the BDM efficiency. Caffeine alone abolished the negativ treppe and reduced r-. Oscillations occurred during the relaxation, they were enhanced if Na+ was replaced by Li^+. With 50 % Na+ exchange r- was reduced by 65 % and time of relaxation was extremely prolonged. With the setup the role of SR and Na/Ca-exchanger can be assessed.

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Institute of Physiology, Med. Fac. RWTH Aachen, Pauwelsstr. D-5100 AACHEN

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DETERMINATION OF EFFICACY OF SOME VASOACTIVE AGENTS ON TRANSMURAL DISTRIBUTION OF CORONARY FLOW IN ISOLATED RABBIT HEARTS BY MODIFIED THERMODILUTION TECHNIQUE

T. Karnath, K. Güttler, W. Klaus

Clinical finding that the subendocardial layer of the heart is more susceptible to ischemic damage than the other ventricular regions has focussed the experimental research on the analysis of transmural distribution of myocardial blood flow. In order to more simplify the assessment of flow distribution we have modified the conventional thermic methods. Bolus injection of cold Tyrode solution (200 µl) into the aorta of thermic isolated Langendorff-perfused rabbit hearts induced typical epicardial (EPI) resp. endocardial (ENDO) time courses of temperature, which were recorded by two NTC-thermistors (diameter:0.5 mm) installed at the top of every branch of a special constructed forceps (length: 15 mm), whereby temperature measurements of corresponding epicardial and endocardial areas of the left ventricular wall were enabled. The study was undertaken to evaluate this indirect method of analysing transient temperature courses as function of transmural flow distribution and to elucidate if adenosine and diltiazem increase blood flow proportionately in subepicardium and subendocardium of nonischemic regions. Vasoconstriction produced by ornipressin (0.02 IE) showed a reduction of the minimal temperature value (T_{min}: - 28 ± 8 % EPI; - 11 ± 6 % ENDO) in comparison to control; that means that the subepicardial flow is more reduced than the subendocardial flow. Vasodilatation caused by diltiazem (1 µM) induced proportionate distribution of coronary flow to subepicardial and subendocardial layers (T_{min}: + 39 ± 3 % EPI; + 39 ± 1 % ENDO). In contrast, adenosine (0.2 µM) produced a greater increase in subepicardial flow (T_{min}: + 42 ± 6 % EPI) than in subendocardial flow (T_{min}: + 25 ± 6 % ENDO). These results suggest that the developed method is appropriate to determine the transmural distribution of coronary flow in isolated hearts.

Institut für Pharmakologie der Universität zu Köln, Gleuelerstr. 24, D-5000 Köln 41

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RHYTHM PHENOMENA OF NEONATAL HEART CELL CULTURES DUE TO EXTERNAL STIMULATION

K. Haverkampf, M. Guhlmann, H. Antoni

Action potentials and coupling phenomena derived from whole cell patch clamp recordings in cell cultures of neonatal rat heart were evaluated and the development of spontaeous beating rhythms was analyzed.
Morphologically, the cells in culture are first single and uncoupled, then they grow to pairs, afterwards to clusters of many cells up to a monolayer covering the whole culture dish. The electrical and mechanical activity of the culture varies during growth due to the varying interaction between the cells. This leads to more or less complicated patterns of spontaneous beating, which can be classified into distinct groups.
To investigate the cell layer under controlled conditions, we stimulated the whole culture via field application in the frequency range between 2 and 20 Hz. By this means we were able to compare the behaviour of our strongly coupled cell monolayer of simple geometry with the response of a single cell and of the much more complicated structure of the whole heart.
The cell layer shows a similar behaviour as a single cell in that entrainment regions can be found as well as irregular responses which are chaotic in nature. A chaotic behaviour is also observed in the sponteous bursting mode of the culture. From this it can be concluded that during this bursting mode the sodium system must be often inactivated by partial voltage clamping due to strong coupling between cells in the culture.

Physiologisches Institut, Universtät Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg i. Br.

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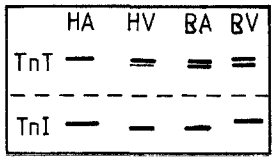
TROPONIN-POLYMORPHISM AND CALCIUM SENSITIVITY IN HUMAN AND BOVINE MYOCARDIUM

S. Schmidt-Ott, U. Heuschen*, G. Mall*, G. Pfitzer

We report differences in the troponin-subunit isoform pattern of human and bovine atrial and ventricular muscle, and this diversity may - at least partly - account for the different calcium sensitivities of skinned fibre preparations (neg. log. of Ca^{2+} required for 50% activation of force (pCa) at pH 6.7 an 10 mM MgATP):

	bovine		human	
	atrium	*ventricle*	*atrium*	*ventricle*
pCa_{50}	5,44	5,64	5,07	5,27

Myofibrillar cardiac proteins were separated by SDS-PAGE, TnT-isoforms were identified by immunoblotting and TnI-isoforms by phosphorylation of TnI with A-Kinase, followed by autoradiography. In bovine ventricle and atria 2 isoforms with similar distribution pattern were found (cf Tobakman and Lee, J Biol Chem 262:4059, 1987). Whereas in human ventricles (3 hrs post mortem) 2 isoforms of TnT could be detected, atria contained only one band. However, proteolytic degradation cannot be excluded. Note (figure), that compared with the TnI bands of human ventricle and bovine atrium, the bands of human atrium and bovine ventricle seem to migrate more slowly on 10-14% SDS-gradient gels:



migration pattern of troponin I (TnI) and troponin T (TnT) isoforms in human atrium and ventricle (HA, HV) and bovine atrium and ventricle (BA,BV), purified bovine troponin was used as standard (not shown).

II. Physiologisches Institut und *Pathologisches Institut der Universität Heidelberg, INF 326 und 220, 6900 Heidelberg

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PHOSPHORYLATION AND THIOPHOSPHORYLATION OF MYOSIN LIGHT CHAINS: DIFFERENT EFFECTS ON MECHANICAL PROPERTIES OF CHEMICALLY SKINNED PIG VENTRICULAR FIBERS

J. Rösch+, I. Morano+, A. Arner *, J.C. Rüegg+

V_{max} (maximal, unloaded shortening velocity) of skinned ventricular fibers of the pig was determined by the slack-test method of EDMAN 1978 and the force-velocity relation by the isotonic quick release method (JEWELL and WILKIE 1958). V_{max} was 1.53 muscle length s^{-1} (MLs^{-1}) and 1.94 MLs^{-1} using the force-velocity relation (curve fitting to the HILL 1938 equation) and the slack-test, respectively. The influence of myosin light chain phosphorylation (incubation with myosin light chain kinase = MLCK, calmodulin and ATP) and thiophosphorylation (incubation with MLCK, calmodulin and ATPγS) on Ca^{2+} sensitivity and V_{max} of chemically skinned pig ventricular fibers has been studied.
Phosphorylation increased the Ca^{2+} sensitivity of skinned fibers but had no influence on V_{max}. Thiophosphorylation decreased V_{max} but had no influence on Ca^{2+} sensitivity.
Chemical modification of the myosin light chains of the skinned pig fibers has been studied using (γ-^{32}P)ATP or (γ-$P^{35}S$)ATP (250µCi each) and autoradiography. Incubation of skinned fibers with labeled ATP led to a phosphate incorporation into the 18 kDa myosin light chain (P-light chain or regulatory light chain) while incubation with labeled ATPγS led to an unexpected incorporation of thiophosphate into the 28 kDa myosin light chain (alkali light chain) and tropomyosin.
We suggest that the different phosphorylation profiles of the myosin light chains after phosphorylation or thiophosphorylation are responsible for the different mechanical behaviours of phosphorylated and thiophosphorylated skinned ventricular fibers.

+II. Physiologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg, *Institut für Physiologie und Biophysik der Universität Lund, Schweden

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THE EFFECT OF THE α_2-ADRENOCEPTOR ANTAGONIST BDF 8933 ON POSTSTENOTIC CORONARY RESISTANCE AND MYOCARDIAL FUNCTION.

K. Kröger, J. D. Schipke, V. Thämer.

Distal a severe stenosis sympathetic activation can induce ischemic myocardial dysfunction due to an adrenergic coronary constriction. We tested the effect of the new α_2-adrenoceptor-antagonist BDF 8933, that is ought to have a higher affinity to post- than to presynaptic α_2-adrenoceptors, on this mechanism. In anesthetized mongrel dogs sympathetic activation was performed by electrical stimulation of the left n. cervicalis ventrolateralis before and after production of a severe stenosis on the ramus circumflexus of the left coronary artery and after injection of 150 ug/kg of BDF 8933. In one group (n = 7) enddiastolic coronary resistance and in an other group (n = 6) systolic myocardial wall thickening (sonomicrometry) was calculated.

	without stenosis		stenosis		stenosis+BDF 8933	
	control	SNS	control	SNS	control	SNS
R	1.4±0.7	1.1±0.5*	1.2±0.7	1.4±1.1	1.3±1.2	1.0±0.9
WTp	12.7±3.6	21.9±8.4*	5.4±4.0	2.1±5.1*	4.2±4.2	5.6±3.6
WTc	15.3±3.9	19.2±6.9*	19.6±6.7	22.8±6.7*	15.9±6.0	25.4±7.3*
LVP	131±17	161±21*	124±18	156±21*	142±27$	177±26*

* = p<0.05 SNS vs control, $ = p<0.05 vs controls before BDF 8933, R = coronary resistance (mmHg*min*100g/ml); WT% = systolic wall thickening (%) in poststenotic (p) and control area (c); LVP = leftventricular peak systolic pressure in both groups, SNS = sympathetic nerve stimulation.

Conclusion: The new α_2-adrenoceptor-antagonist BDF 8933 prevents sympathetic-induced myocardial dysfunction in poststenotic myocardium and increases peak left ventricular pressure.

Abt. Herz- und Kreislaufphysiologie der Universität Düsseldorf, Moorenstraße 5, 4000 Düsseldorf.

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MEAN SYSTOLIC WALL THICKENING DESCRIBES THE POSTOCCLUSIVE MYOCARDIAL FUNCTION ONLY INCOMPLETELY

Thomas Ehring[‡], Jochen D. Schipke[‡] and Volker Thämer.

Mean systolic wall thickening (WT) is often used for the description of myocardial function. However, WT does not account for early systolic wall thinning and postsystolic wall thickening, which can both be observed in ischemic and reperfused myocardium.
In 10 open-chest dogs the myocardial function of the posterior wall was investigated after 14 min occlusion of one or two side branches of the left circumflex coronary artery. In the ischemic myocardium the function was assessed using sonomicrometry. Mean systolic wall thickening expressed as a percentage of end-diastolic wall thickness was calculated, as well as maximal systolic wall thickening (XT) and maximal wall thickening during the contraction cycle (GT) expressed as a percentage of the minimal systolic wall thickness. Results:

	WT	XT	GT
Control	13.1 ± 2.3	16.5 ± 2.4	16.9 ± 2.4
Occlusion:	-8.1 ± 1.7*#a	3.4 ± 1.0*b	14.1 ± 3.2

(Mean ± SEM; p < 0.05: * WT,XT vs. GT; # WT vs. XT; a,b Occlusion vs. Control)
The data demonstrate that GT, in contrast to WT, is only slightly reduced after 14 min coronary occlusion. The value of XT shows that the postocclusive region participates in shifting the intraventricular volume during systole.

Physiologisches Institut, Abt. Herz- und Kreislaufphysiologie und [‡]Inst. für Experimentelle Chirurgie der Universität Düsseldorf, Moorenstr. 5, 4000 Düsseldorf 1

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DEPENDENCY OF POSTSYSTOLIC MYOCARDIAL WALL THICKENING ON HEART RATE

Thomas Ehring[‡], Jochen D. Schipke[‡] and Volker Thämer.

The extent of postsystolic myocardial wall thickening (WT_p [%]) has been described as a marker of myocardial dysfunction during ischemia and reperfusion. However, the dependency of postsystolic myocardial wall thickening on heart rate (HR [1/min]) is unknown. To investigate this dependency experiments were performed in 6 open-chest dogs. One or two side branches of the left circumflex coronary artery were occluded for 15 min to induce reperfusion dysfunction in a posterior myocardial area. 30 min after onset of reperfusion, global ventricular function was evaluated by measuring peak left ventricular pressure (LVP [mmHg]). Both regional myocardial function in the reperfused (WT_i [%]) and in an anterior control area (WT_c [%]) was assessed using sonomicrometry. HR was varied by left atrial pacing. Results:

HR	LVP	WT_c	WT_i	WT_p
143±9	98±18	18.5±6.4	6.2±4.7	0.35±0.16
179±5*	98±20	14.0±7.3*	6.2±3.4	0.19±0.08*

(*p < 0.05; Mean ± SD)
These data demonstrate that global ventricular function was unaffected by changes in HR. In contrast to a significant decrease in WT_c posterior myocardial function was unchanged. This might in part be due to the recruitment of an increasing portion of WT_p into systole with increasing HR. We conclude that the description of myocardial dysfunction with the help of WT_p is limited if HR changes.

Physiologisches Institut, Abt. Herz- und Kreislaufphysiologie und [‡]Inst. für Experimentelle Chirurgie der Universität Düsseldorf, Moorenstr. 5, 4000 Düsseldorf 1

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AMELIORATION OF OXYGEN SUPPLY IN ISCHEMIC MYOCARDIUM BY A SIMULTANEOUS ENHANCEMENT OF CORONARY BLOOD FLOW AND A REDUCTION OF MYOCARDIAL FUNCTION

Thomas Ehring[‡] and Volker Thämer.

In the presence of a severe coronary stenosis oxygen supply in the poststenotic myocardium is reduced. In this situation, a substance which can both increase coronary blood flow and decrease myocardial performance could have a beneficial effect on the poststenotic myocardium. The structurally novel benzopyran K^+-agonist BRL 34915 relaxes vascular smooth muscle cells. Additionally, cardio-depressant properties have been reported about this drug.
We therefore investigated in 6 open-chest dogs, if cumulative intracoronary doses (1 µg, 4 µg, 14 µg) of BRL 34915 could enhance coronary blood flow and simultaneously reduce myocardial function in poststenotic myocardium, thereby increasing oxygen supply and lowering oxygen demand.
This substance increased mean left circumflex coronary blood flow [ml/min*100g] dose-dependently from 59 ± 12 (mean ± SEM) (no BRL) to 227 ± 44 (14 µg BRL) (p < 0.05) in intact coronary arteries and from 36 ± 7 to 74 ± 13 (p < 0.05) distal to a severe stenosis, respectively. In contrast, posterior systolic wall thickening [%] (Sonomicrometry) was significantly decreased only by a cumulative dose of 14 µg BRL from 9.7 ± 1.8 (no BRL) to 7.8 ± 2.1 (14 µg BRL) (p < 0.05) in the presence of intact coronary arteries and from 8.7 ± 2.0 (no BRL) to 4.1 ± 1.4 (14 µg BRL) (p < 0.05) in the ischemic myocardium.
These results demonstrate, that BRL 34915 can simultaneously enhance coronary blood flow in the poststenotic myocardium and decrease myocardial function, potentially narrowing the gap between oxygen supply and demand.

Physiologisches Institut, Abt. Herz- und Kreislaufphysiol. und [‡]Inst. für Exper. Chirurgie der Universität Düsseldorf, Moorenstr. 5, 4000 Düsseldorf 1

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PROBLEMS IN TIMING OF END-SYSTOLE FOR THE CALCULATION OF REGIONAL MYOCARDIAL FUNCTION DURING VENTRICULAR ASYNCHRONY.

R. Schulz, B.D. Guth and G. Heusch

The analysis of regional myocardial function is often based on a comparison of myocardial wall thickness at end-diastole to that at end-systole (%WTh). Consequently, the accurate calculation of regional myocardial function depends on the exact definition of the end of systole. The maximum negative value of the first derivative of left ventricular pressure (-dP/dt) has been shown to be a good estimate of the end of systole under varying pre- and afterload conditions. During asynchronous ventricular contraction, as occurring during regional myocardial ischemia or regional inotropic stimulation, the relaxation of the ventricle is impaired, resulting in a decrease and a shape change of -dP/dt. This decrease and shape change of -dP/dt, however, may lead to an inaccurate definition of the end-systole and thereby cause errors in the calculation of %WTh. In 7 anesthetized (Isoflurane) swine the left anterior descending coronary artery was cannulated and perfused at constant flow rates. Dobutamine (D) was infused regionally and the inotropic stimulation repeated during coronary hypoperfusion (HP). %WTh (sonomicrometry) was calculated using either -dP/dt or the closure of the aortic valve (CAO) for defining the end-systole. The aortic flow was measured via an electromagnetic flow probe around the ascending aorta and CAO was determined by the stoppage of forward flow. A close correlation between the end-systole defined by the CAO or -dP/dt existed during control conditions, dobutamine stimulation and hypoperfusion ($r=.86$, $p<.01$; $r=.94$, $p<.001$; $r=.96$, $p<.001$). During HP+D, however, there was no correlation between the end-systole defined by -dP/dt or the CAO ($r=.22$, NS). %WTh calculated using -dP/dt for defining the end-systole was underestimated when compared to %WTh calculated by use of the CAO (2.9 ± 6.8 vs 7.3 ± 7.8, $p<.05$). Thus, during ventricular asynchrony the use of -dP/dt for defining the end-systole can lead to inaccurate calculation of regional myocardial function.

Abt. für Pathophysiologie, Univ. Essen, Hufelandstr. 55, 4300 Essen, FRG.

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CORRELATION BETWEEN LEFT VENTRICULAR ATP CONCENTRATION AND MORPHOMETRICALLY DETERMINED MITOCHONDRIAL SWELLING DURING GLOBAL ISCHEMIA*)

Ph.A. Schnabel[1], A.Schmiedl[1], M.M.Gebhard[1], J.Richter[2], and H.J.Bretschneider[1]

The effects of different methods of cardiac arrest on left ventricular ATP and mitochondrial swelling were evaluted.

Canine hearts were arrested ischemically (iCA, n=6) or cardioplegically by elevation of Mg^{++} and K^{+} plus procaine (St. Thomas, n=6), by reduction of Ca^{++} and Na^{+} to cytoplasmatic values plus histidine-buffering (HTK, n=6) and with HTK+4mM Tryptophan (n=5) or HTK+50μM Ca^{++} (n=3). Isolated left ventricles were incubated at 25°C in a physiological electrolyte solution (iCA) or in the corresponding cardioplegic solution. Samples were taken at the onset and during ischemia. ATP was measured enzymatically. Mitochondrial swelling was determined by the surface to volume ratio of mitochondria ($S_V ratio_{Mi}$) in 50 test fields per sample and 3 samples per animal and time point. Additionally intact HTK-arrested hearts left in situ were analyzed after 300 min ischemia (n=3).

At the onset of ischemia ATP is about 6 $\mu mol/g_{ww}$ in all groups (n.s.), whereas $S_V ratio_{Mi}$ shows significant differences between the groups ($p<0.01$). During ischemia between ATP concentrations of 5.4 and 1.7 $\mu mol/g_{ww}$ $S_V ratio_{Mi}$ decreases corresponding to the ATP decline in all groups, for 99 analyses the correlation coefficient between ATP and $S_V ratio_{Mi}$ is $r=0.76$ ($p<0.001$).

Thus, the degree of mitochondrial swelling at the onset of ischemia depends on the method of cardiac arrest but during ischemia - below an ATP level of about 5.5 $\mu mol/g_{ww}$ - correlates with ATP independent of the experimental procedure.

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[1]Zentrum Physiologie and Pathophysiologie, [2]Zentrum Anatomie der Universität Göttingen, Humboldtallee 23, 3400 Göttingen

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MINIMAL α_1- AND α_2- ADRENERGIC CORONARY VASOCONSTRICTION IN ANESTHETIZED SWINE.

B. D. Guth, R. Schulz, R. J. Oudiz and G. Heusch

α_2-adrenergic coronary vasoconstriction contributes to the initiation of poststenotic myocardial ischemia in anesthetized and conscious dogs. However, during acute coronary events the porcine coronary circulation may be a more suitable model for human coronary artery disease. Seven anesthetized swine were therefore studied to determine the responsiveness of the porcine coronary circulation to α-adrenoceptor activation using either the selective α_1- agonist methoxamine or the selective α_2- agonist BHT 933. The swine were anesthetized with either isoflurane (6) or morphine/chloralose (1) and studied following bilateral cervical vagotomy and beta-adrenergic blockade with propranolol (2 mg/kg). The left anterior descending coronary artery (LAD) was cannulated and perfused by means of a pump at a constant flow rate of 19±10 ml/min throughout. Graded dosages of the two α-adrenergic agonists (4.1 - 43.2 µg/kg/min) were administered while coronary arterial pressure was measured through a side arm of the cannula. Left ventricular pressure (micromanometer), dP/dt, systemic arterial pressure, and regional myocardial wall thickening (sonomicrometry) were continously recorded. During methoxamine infusion, none of the seven swine exhibited any changes in coronary pressure prior to changes in systemic arterial pressure. Only four of seven swine showed increases in coronary arterial pressure independent from increases in systemic pressure during the infusion of BHT 933; the increases in coronary arterial pressure were 15, 15, 20 and 35%. No changes in regional myocardial function occurred during the infusion of the α_1- or α_2- agonists. These results indicate that swine have relatively little response to α_2-adrenoceptor activation and that no α_1-adrenergic vasoconstriction is apparent. Thus, swine may not be a suitable model for the study of α-adrenergic vasoconstrictive mechanisms, which have been demonstrated in human coronary artery disease.

Abt. für Pathophysiologie, Univ. Essen, Hufelandstr. 55, 4300 Essen, FRG.

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INFLUENCE OF THE METHOD OF CARDIAC ARREST AND OF THE ATP-CONCENTRATION ON THE CONTRACTION STATE OF WORKING MYOCARDIUM DURING GLOBAL ISCHEMIA*)

A.Schmiedl[1], Ph.Schnabel[1], M.M.Gebhard[1], J.Richter[2] and H.J.Bretschneider[1]

The aim of this study was to evaluate whether the contraction state of sarcomeres during ischemia is influenced by the decline of ATP or - independent of the ATP-content - by the method of cardiac arrest.

Left ventricular samples were taken from canine hearts at the onset of global ischemia immediately after aortic cross clamping (group 1,n=6), after coronary perfusion with St. Thomas- (group 2,n=6) or HTK-solution (group 3,n=6) and during ischemia (25°C) at defined times. Biochemical analysis ensued enzymatically. The contraction state of sarcomeres was evaluated according to a score in 30 fields per sample and 3 samples per animal and time point.

At the onset of ischemia all groups show almost identical ATP values, but significant differences in the percentage of relaxed sarcomeres (group 1: 5.7%; group 2: 0.8%; group 3: 30.7%). During ischemia between 4 $\mu mol/g_{ww}$ ATP - the practical limit of resuscitability - and 2 $\mu mol/g_{ww}$ ATP - the theoretical limit of resuscitability - sarcomeres show an increase of relaxation up to maximal values at different time points depending on the method applied: in group 1 to 82.0% at 2.8 $\mu mol/g$ ATP after 90 min; in group 2 to 60.4% at 3.3 $\mu mol/g$ ATP after 180 min; in group 3 to 98.8% at 3.8 $\mu mol/g$ ATP after 240 min. Beyond these times the percentage of contracted sarcomeres increases in all groups.

Thus, the development of relaxation and contraction during global ischemia predominantly depends on the method of cardiac arrest, whereby the composition of the corresponding solution (group 1: physiological electrolyte content; group 2: slight reduction of Ca^{++}, elevation of Mg^{++} and K^{+} plus procaine; group 3: reduction of Na^{+} and Ca^{++} to cytoplasmatic values plus histidine) plays a major role for the percentage of relaxed sarcomeres. Nevertheless, the times of maximal relaxation and the following onset of contraction seem to be influenced by the ATP concentration.

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[1]Zentrum Physiologie, [2]Zentrum Anatomie der Universität Göttingen, Humboldtallee 23, D-3400 Göttingen

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GLYCOLYTIC ATP PRODUCTION AS DETERMINANT OF ENDOTHELIAL MACROMOLECULE PERMEABILITY

H. Watanabe, R. Spahr, H.M. Piper

Macromolecule permeability increases in ischemic myocardium. The dependence of endothelial permeability on the energetic state was investigated in the model of rat coronary microvascular endothelial cells (CMEC) and porcine aortic macrovascular endothelial cells (AMEC). Confluent monolayers of CMEC and AMEC on filter membranes were incubated with 10 % fetal calf serum (FCS) in modified Tyrode solution (pH 7.4; 37 °C). Macromolecule permeability was determined with the aid of FITC labelled albumin. Under control conditions, the permeability was 11.3 x 10^{-6} cm/sec with CMEC and 5.6 with AMEC. In both endothelial cell preparations, permeability remained unaltered, when incubated for 1 h with 5 mM KCN. When 10 mM deoxyglucose was present together with KCN, macromolecule permeability increased in CMEC by 41 %, and in AMEC by 39 %. After 1 h with KCN and deoxyglucose, the increased permeability could be completely reversed in both cell preparations by an 1 hour-return to culture medium (medium 199 with 10 % FCS).

Conclusions: Macromolecule permeability of microvascular and macrovascular endothelial cells is energy dependent. Glycolytic energy production is sufficient to maintain a normal permeability. Permeability changes caused by energy depletion do not indicate irreversible endothelial injury.

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Physiologie I, Universität Düsseldorf, Moorenstr. 5 D-4000 Düsseldorf

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TRANSCAPILLARY GRADIENTS OF NORADRENALINE (NA) AND URIC ACID (UA): PROPERTIES OF THE ENDOTHELIAL CELL

O.Obst and H.Kammermeier

Interstitial (is) and venous (ven) concentrations of NA and UA and rates of uptake (NA) and release (UA) were estimated at equilibrium in saline perfused rat hearts.

1) Perfusion with 10^{-8},10^{-7},and 10^{-6}M NA lead to a transcapillary gradient of NA of 246, 163, and 124% (ven/is), and to a NA-removal of 19, 136, and 928 pmol*min^{-1}*g^{-1}. Corresponding to the transcapillary gradient the is-dose-response-relationsships are shifted to the left as compared with the vascular curves. Blockade of the catecholamine-uptake with 1µM desipramine (DMI) and 100µM o-methylisoprenaline (OMI) abolished the transcapillary gradient as well as the removal. Calculation of the permeability*surface(P*S)-product showed a decrease in the apparent NA-permeability with OMI but not with DMI, suggesting an OMI-sensitive, endothelial NA-uptake-mechanism.

2) The release of UA amounted to 2.8 nmol*min^{-1}*g^{-1} at control with a transcapillary gradient of 330% (is/ven). Administration of 10^{-8},10^{-7}, and 10^{-6}M NA increased the UA-release (to 3.0, 4.8, and 6.3nmol *min^{-1}*g^{-1}) as well as the transcapillary gradient (to 360, 424, and 443%). Calculation of the P*S-product showed a decreasing apparent UA-permeability with increasing NA-concentrations, suggesting a shift from the venous to the interstitial UA-release.

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Abt. Physiologie der RWTH Aachen, Klinikum Aachen, Pauwelsstr., D-5100 Aachen

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CARDIAC ENDOTHELIAL FUNCTION INVESTIGATED BY EXPERIMENTAL ALTERATION BY GASPERFUSION (GP)

H. Mertens, O. Obst, H. Kammermeier

Isolated rat hearts were perfused under constant pressure with saline solution and to manipulate the endothelial function with carbogengas for 20 min.

The following consequences of the GP could be observed: 1) Purine nucleoside phosphorylase, an endothelial cytosolic marker enzyme, could be detected in the interstitial transudate (IT): 3.3 ± 2.1 mU/g and in the venous effluent (VE): 5.0 ± 2.6 mU/g with a concentration gradient IT/VE greater than 100:1 (total content: 180 mU/g_{heart}). 2) The production rate of the IT was increased upto a factor of 5. 3) The coronary response to 2 min anoxia was inverted from an increase before GP to a decrease. 4) A substantial alteration of the myocytes could be largely excluded because: a) during the first 16 min after GP the LDH release in the IT amounted to less than 1 % of the total content (210 U/g_{heart}), b) the VO_2 was unchanged, c) the pressure-rate product was reduced by 20 %, d) the coronary flow reached a stable level of about 2/3 of controls, e) the myogenic autoregulation was maintained. 5) The rate of uric acid (UA) release was unchanged before and after GP. 6) The transcapillary UA gradient during noradrenaline (NA) administration (10^{-7} , 10^{-6} M) significantly decreased after GP. 7) The transcapillary permeability of NA increased after GP.

The points 1) and 2) are interpreted in terms of an endothelial lesion paralleled by a partial (1)) abolition (2),3),6),7)) of the barrier function.

Inst.f.Physiologie,Med.Fak.RWTH, Pauwelsstr.,D-5100 Aachen

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CALCIUM-DEPENDENCY OF EDRF/NO SYNTHESIS IN ENDOTHELIAL CYTOSOL IS MODULATED BY CALMODULIN

A. Mülsch and R. Busse

A transmembrane calcium influx is involved in the agonist-induced release of endothelium-derived relaxant factor/nitric oxide (EDRF/NO) from endothelial cells (A. Lückhoff et al., Br J Pharmacol 95: 189, 1988). Recently we have demonstrated that NADPH-dependent EDRF/NO synthesis from L-arginine in isolated endothelial cytosol is increased by free calcium ions (EC_{50} 0.3 µM; A. Mülsch et al., Naunyn-Schmiedeberg's Arch Pharmacol, in press). We now studied whether calmodulin, which is known to mediate calcium sensitivity to a variety of target enzymes, could mediate the calcium sensitivity of EDRF/NO synthesis in isolated endothelial cytosol. EDRF/NO was quantified by activation of a purified soluble guanylate cyclase coincubated with cytosol from freshly harvested pocine aortic endothelial cells. With 0.1 mg cytosolic protein/ml the calcium-dependent, but not the calcium-independent EDRF /NO synthesis was potently inhibited by the calmodulin antagonists melittin, mastoparan, and calcineurin (IC_{50} 1 µM, 1 µM and 1 unit/50 µl, respectively), but not by fendiline, trifluoperazine or calmidazolium (up to 10 µM). The inhibitory potency was increased by preincubation of inhibitors with cytosol (10 min, 0°C). Inhibition was overcome by exogenously added porcine brain calmodulin, or by addition of heat-denatured endothelial cytosol, which contained about 0.3 µM calmodulin, as measured by radioimmunoassay. We conclude that calcium-calmodulin is tightly associated with the enzyme(s) catalyzing EDRF/NO synthesis, thereby providing the calcium sensitivity of endothelial EDRF/NO synthesis.

Institute of Applied Physiology, University Freiburg, Hermann-Herderstr. 7, D-7800 Freiburg, FRG

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EDRF AUGMENTS CORONARY CONDUCTIVITY THROUGH ATTENUATION OF MYOGENIC AUTOREGULATION.

U. Pohl, D. Lamontagne, E. Bassenge, R. Busse

The myogenic vascular response to increases in transmural pressure represents a mechanism which potentially reduces the coronary reserve. Since EDRF is released in increased amounts in response to elevated shear stress, it was tested whether EDRF interferes with the magnitude of myogenic responses of the coronary vascular bed to increases in perfusion pressure. Isolated rabbit hearts (n=14; Langendorff technique) were perfused under constant pressure conditions with a modified Krebs-Henseleit solution. The resulting flow was measured by means of an electromagnetic flow probe. Flow responses to changes in perfusion pressure were investigated before and after pretreatment of the coronary circulation with the stereospecific inhibitor of EDRF-synthesis N^G-nitro-L-arginine (L-NNA; 30 µM). A sudden increase in the perfusion pressure from 70 to 120 mm Hg induced an initial increase in flow by 108 ±13% (corresponding to a decrease in vascular resistance(VR) by 22 ±4%). This was followed within 20 seconds by a renewed increase in VR by 12 ±4%. After L-NNA, this increase in VR was significantly higher (55 ±15%; $p<0.02$). During a stepwise (15 mm Hg) increase in perfusion pressure from 45 to 120 mm Hg the flow increased from 24 ±2 to 55 ±3 ml/min (+136 ±15%; n=7). After L-NNA the flow increased from 14 ±3 ml/min to only 22 ±5 ml/min (+57 ±17%; $p<0.05$). In contrast, the D-stereoisomer D-NNA did not significantly affect the vascular responses to changes in perfusion pressure. It is concluded that EDRF significantly attenuates myogenic responses to increases in coronary perfusion pressure which would otherwise tend to reduce the coronary conductivity. This effect may be mediated by an enhanced release of EDRF due to changes in shear stress at the endothelial surface.

Institut für Angewandte Physiologie der Universität Freiburg, Hermann-Herder-str. 7, 7800 Freiburg, FRG

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INHIBITORY EFFECTS OF EDRF-MEDIATED FLOW-INDUCED DILATION ON MYOGENIC VASOCONSTRICTION.

U. Pohl, K. Herlan, A. Huang and E. Bassenge.

Both, the pressure-induced myogenic vasoconstriction and the endothelium-mediated flow-dependent dilation represent positive feedback mechanisms which, if unopposed, could lead to an unstability in the circulatory system. It was tested whether the flow-dependent dilation (FDD) could counteract the myogenic constrictor response (MR) when pressure and flow increased simultaneously. Small rabbit mesenteric arteries (diameter 224 ±4 µm, mean ±SEM; total n=178) pump-perfused in situ with Tyrode's solution at constant flow and superfused with norepinephrine (0.2 µM) were investigated. When flow was rapidly increased to 2.2 ±0.1 times control, the input perfusion pressure rose by 155 ±11%. Vascular diameters, measured by a video dimension analyzer, first increased passively by 9 ±1%, and then decreased by 10 ±1% (MR). This was followed by a 17 ±2% increase in diameter (FDD). Side branches of the same size which were exposed to the same increase in pressure but had been ligated distal to the site of observation did not dilate after the MR. FDD was 70 % greater when the perfusate viscosity was increased with dextran 60 (6% solution) indicating that the augmentation of shear stress acted as the stimulus. In presence of either EDRF-inhibitor, hemoglobin (10 µM) or N^G-nitro-l-arginine (0.3 mM), FDD was abolished, indicating that the dilator response was mediated by EDRF. Preincubation with neuraminidase (0.2 U/ml, 20-40 min), which removes part of the membrane glycocalyx, also abolished the FDD, but did not affect the dilator response to the EDRF-stimulus acetylcholine (1µM). It is concluded that the MR in small mesenteric arteries can be antagonized by EDRF (FDD), which is released when increased shear stress acts upon the glycocalyx. Especially under conditions of simultaneous increases in pressure and flow (e.g. active hyperemia), FDD may be an important mechanism in opposing the MR, which would otherwise tend to reduce vascular conductivity.

Institut für Angewandte Physiologie der Universität Freiburg, Hermann-Herder-Str.7, D-7800 Freiburg, FRG

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ENHANCEMENT BY L-ARGININE OF ACETYLCHOLINE-INDUCED RELAXATION IN ISOLATED RAT BASILAR ARTERY

J.R.L. Mackert, L. Schilling, A.A. Parsons*, and M. Wahl
*Royal Society Exchange Fellow

The endothelium can affect vascular tone by releasing vasoactive compounds, one of these termed "endothelium-derived relaxing factor" (EDRF). Recently, NO, probably derived from L-arginine has been shown to account for at least a part of the EDRF-action, which can be elicited by acetylcholine (ACh) and which can be blocked by Methylene blue (MB) or Oxy-haemoglobin (Oxy-Hb). Therefore, we studied the effects of L-arginine on precontracted rat basilar artery in-vitro and on ACh-induced EDRF-dependent relaxation in the presence of MB or Oxy-Hb.

Basilar arteries removed from normotensive rats were cut in ring segments and set up for measurement of isometric force in a modified Krebs-Högestätt solution. Bath temperature was 37°C and pH was kept constant by continuously bubbling with a mixture of O_2/CO_2. Resting tension was set at 2mN. The segments were precontracted with 5-HT (1 µM). On sustained contraction cumulative concentration-effect curves to ACh and L-arginine were formed first in the absence and then in the presence of MB or Oxy-Hb (1 and 10 µM each).

On sustained tension to 5-HT both ACh and L-arginine produced concentration-related relaxation with pD_2 values ($-\log_{10} EC_{50}$) of 6.5 ± 0.1 and 5.5 ± 0.3 and maximum effects of 48% and 22.1% of the 5-HT spasm, respectively. When applied together, ACh and L-arginine induced a slightly bigger relaxation than ACh alone. MB and Oxy-Hb induced a variable increase of resting tone. Only the Oxy-Hb-induced contraction could be reversed by ACh and L-arginine when applied together. MB and Oxy-Hb also inhibited the ACh-induced relaxation of 5-HT spasm. Additional application of L-arginine (100µM) could reverse the inhibition of ACh-relaxation elicited by Oxy-Hb, but not that induced by MB.

The effects of MB and Oxy-Hb on resting tension might indicate a basal release of EDRF under our experimental conditions. The ACh-induced EDRF-dependent relaxation can obviously be enhanced by application of L-arginine, the suggested precursor for NO-production. An increased production of NO might also explain the reversal of Oxy-Hb induced inhibition of ACh-relaxation.

Physiologisches Institut der Universität München, Pettenkoferstraße 12, 8000 München 2

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DETERMINANTS OF S-ADENOSYLHOMOCYSTEINE (SAH) ACCUMULATION IN HEART AND BRAIN.

A. Deussen, R. Franken-Weyers, J. Schrader

In the presence of homocysteine rate of SAH accumulation permits to study regional changes of free adenosine in the heart (Deussen et al., Circ. Res 63; 240-249, 1988). In the present study we wished to compare the single determinants (substrate concentration, enzyme distribution) of this reaction (adenosine + homocysteine ⟶ SAH + H_2O) in heart and brain.

In a first set of experiments blood pressure of anesthetized rats was systematically varied between 150 and 30 mmHg while homocysteine (10 µmol/kg/min, i.v.) was applied over 30 min. This enhanced the plasma concentration of homocysteine 60 fold to 389 µM. Homocysteine concentrations in heart and brain were 147 and 35 µM, respectively. Above 60 mmHg SAH remained constant (1.8 nmol/g) but increased 3- (brain) and 30 fold (heart) below 60 mmHg. Total tissue adenosine did not change significantly over the entire blood pressure range.

In a second experimental series adenosine formation was maximally stimulated by systemic ischemia elicited by dissection of the abdominal aorta. In both organs under study this enhanced adenosine levels 30-40 fold above control within 3 min. While cardiac SAH linearly increased 30 fold within 5 min, brain SAH was elevated only 4 fold.

Determination of SAH-hydrolase activity revealed mean activities of 550 and 390 nmol/min/g in heart and brain. Enzymatic activity was homogenously distributed between different cardiac regions, whilst it differed considerably between distinct brain regions (cerebellum 810, striatum 147 nmol/min/g).

Conclusions: 1) Adenosine is unlikely to be the mediator of flow autoregulation in heart and brain. 2) In the brain, homocysteine availability is limited presumably due to the blood brain barrier. 3) The heterogeneity of SAH-hydrolase activity in brain precludes the quantification of local changes in free cytosolic adenosine by the "SAH-method".

Zentrum für Physiologie, Heinrich-Heine-Universität Düsseldorf, Moorenstr. 5, 4000 Düsseldorf 1

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THE cAMP-DEPENDENT PHOSPHORYLATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) PREVENTS PROTEOLYSIS BY ENDOPROTEASE-24.11 BUT HAS NO EFFECT ON THE HALF-LIFE OF ANP IN THE BLOOD.

Th. Dörner, M. Gagelmann, D. Hock, M.Ph. Christmann and W.G. Forssmann

Exogenously administered ANP in the circulation exhibits a short half-life time of 1 to 2 min. In human coronary sinus blood proteolysis of ANP within the disulfide-linked loop between Cys-105 and Phe-105 amounts to 20-30% (T.G. Yandle et al. BBRC 146:832, 1987) indicating participation of the endoprotease-24.11. This hypothesis was supported by studies showing increased levels of exogenous ANP following ANP infusion in the presence of endoprotease-24.11 inhibitor (G.M. Olins et al. Mol Cell Endocrinol 61:201, 1989).

We found that cAMP-dependent phosphorylation of ANP at the serine residue in position 104 is associated with decreased vasorelaxant potency and, furthermore, that phosphorylation prevents proteolysis of ANP by the endoprotease-24.11. (The protease represents the major ANP-degrading proteolytic activity in the kidney with the highest concentration in the proximal tubulus.) In order to determine whether the endoprotease-24.11 contributes to the ANP clearance ^{32}P-labelled ANP together with unlabelled ANP (27-32µg ANP, spec. act. 3µCi) were injected into anesthetized rats (phentobarbital i.p. 60mg/kg) and blood samples were removed. The determination of ANP in the plasma was achieved by measuring of the radioactivity following separation by RP-HPLC. (^{125}I-labelled ANP was used for the controls with unphosphorylated ANP.) We found similar clearance rates for phosphorylated and unphosphorylated ANP. Approximately 50% of the peptides were removed from the plasma within 1 to 1.5 min.

The results indicate that the endoprotease-24.11 plays no major role in the clearance of ANP from the blood. However, because of high endoprotease-24.11 concentration in the kidney the protease may be involved in the regulation of specific ANP functions in this organ.

Institut für Anatomie und Zellbiologie III, Universität Heidelberg, INF 307, 6900 Heidelberg, FRG.

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EXCESSIVE MURAL CALCIUM UPTAKE IN DIFFERENT TYPES OF HUMAN ARTERIOSCLEROTIC VESSELS.

F.Thimm, M.Frey, G.Fleckenstein-Grün,a.A.Fleckenstein. Study Group for Calcium Antagonism, Physiol. Inst., Univ.Freiburg, Hermann-Herder-Str.7, D-7800 Freiburg,

Chemical analysis of stenosing coronary plaques which had caused large coronary infarction and death revealed tremendous accumulation of calcium (86-fold above normal) whereas free and total cholesterol rose by only 1.8 and 1.7 times resp.As already shown in previous animal experiments (1-3) Ca overload has to be considered a pathogenic factor in the development of arteriosclerosis. Thus in human coronary walls, the severity of plaque formation (Grade I-III according to WHO classification) positively correlates with the degree of Ca accumulation. In contrast, there is no or even an inverse correlation between coronary plaque development and the free or total mural cholesterol contents. Aortic plaques (Grade II and III) contain more cholesterol than coronary plaques of Grade II or III do. However again, also in these aortic lesions, tremendous Ca overload that positively correlates with the degree of arteriosclerosis is the most spectacular phenomenon. Sclerotic wall tissue of human femoral arteries is also characterized by an 80-90-fold increase in Ca above that of healthy young individuals. Here too, the augmentation of free and total cholesterol is modest. In arteriosclerotic dorsal foot arteries, excessive mural calcium overload takes place without any significant change in the cholesterol fractions.

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FLUORESCENT STAINING OF THE TOTAL AND PERFUSED CAPILLARY NETWORK IN THE BRAIN

H.Theilen, U.Göbel and W.Kuschinsky

Previous studies have described a large fraction (40-50%) of nonperfused capillaries in the rat brain (e.g. H.R. Weiss et al., Circ Res 51:494-503,1982; H.R. Weiss, Microvasc Res 36:172-180,1988). These authors used FITC-dextran as an intravascular marker to measure the density of perfused capillaries. The density of morphologically existing capillaries was determined by the alkaline phosphatase (AP-) method. Both methods as used by Weiss et al. can be criticized: air drying of the cryosections may underestimate the intravascular marker; the light microscopical AP-method may not be sensitive enough to stain all morphologically existing capillaries in the brain.

Therefore we have developed a fluorescent double staining technique to quantify the density of perfused and morphologically existing capillaries in rat brain. First the perfused capillaries were visualized by intravascular Evans blue, which was allowed to circulate for at least 10 seconds. Then the morphologically existing capillaries were stained by using a newly developed immunohistochemical fluorescent method. The capillary wall constituent fibronectin was visualized by a primary antibody directed against fibronectin and a second FITC-coupled antibody (indirect immunofluorescence). The existing capillaries were relocated in the same measuring field of the same brain section. This kind of double staining resulted in identical capillary counts (perfused-existing) in 97%. In contrast, the AP-technique yielded capillary counts that were consistently 30% lower.

Conclusions: 1)Fluorescence methods show a perfusion of virtually all capillaries in the brain of awake normocapnic rats; 2)The complete capillary perfusion found already 10 seconds after injection of the intravascular marker gives no evidence for a capillary reserve in the brain.

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Physiologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg.

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CEREBRAL AND CARDIAC RESPONSES TO UNILATERAL STIMULATION OF CAROTID SINUS BARORECEPTORS

Th. Elbert, M. Tafil-Klawe*, H. Rau, W. Lutzenberger

The present study was designed to investigate effects of unilateral baroreceptor modulation on higher brain structures by measuring event-related potentials in response to activation and to inactivation of baroreceptors located in either the right or the left carotid sinus.

Ten healthy right-handed men participated in the experiment (mean 33 years). Baroreceptor activation (BA) was evoked by applying a negative pressure for intervals of 6s each, either to the left or to the right sinus region, using two separate small chambers. Baroreceptor inactivation (BI) was achieved by applying a positive pressure to one side at a time. Pressure changes amounted to -28.6 ± 0.4 mmHg during BA and to $+19.2 \pm 0.2$ mmHg for BI. In response to BA heart rate dropped significantly, validating that baroreceptors indeed were activated. Correspondingly heart rate increased when a positive pressure was applied to one of the two carotid sinus regions. Nine of the ten subjects responded with the more pronounced **chronotropic** effects to pressure manipulations over the **right** than for those over the left carotid sinus. Compared to BI, the slow negative brain potential was significantly reduced during baroreceptor stimulation, repilcating earlier reports (Elbert et al., 1988). Over parietal cortex, the **reduction in cortical negativity under BA was more pronounced ipsilateral to the site of stimulation** than contralateral. The reverse effect was observed for BI.

Using the same stimulation devices, Tafil-Klawe et al. 1990 demonstrated that the baroreceptors on the **left** have relatively larger **inotropic** effects. Results are in line with the morphological and functional asymmetry of cardiac innervation. The carotid sinus nerves project bilaterally to the dorsomedial part of the solitary nucleus. Thereby uncrossed pathways seem to be of primary importance. Furthermore, the observed effects suggests a considerable impact of baroreceptors on higher (cortical) centers.

Dept. of Clin. & Physiol. Psychology, University, 74 Tübingen, Germany
*Dept. of Physiology, Medical Academy, Warsaw, Poland

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PECULIARITIES OF VITAMIN D3-INDUCED EXCESSIVE CALCIUM ACCUMULATION IN DIFFERENT RAT ARTERIES.

M.Frey, F.Thimm, G.Fleckenstein-Grün, A.Fleckenstein.
Study Group for Calcium Antagonism, Physiol. Inst., Univ.Freiburg, Hermann-Herder-Str.7, D-7800 Freiburg.

Vitamin D3-intoxication (one single dose of 300.000 I.U./kg i.m.) leads to severe calcific sclerosis in coronary, mesenteric, and renal arteries of rats as well as in the aorta within 4 days (1-2).This type of arteriosclerosis is characterized by necrotization of Ca-overloaded smooth muscle cells and by incrustation of the elastic fibres with Ca salts. Interestingly, not all arteries respond in this way to vitamin D3 overdoses. In fact, the brain arteries (A.basilaris, A.cerebri media) exhibit a totally different behaviour, since (as shown in the Table) they proved to be rather resistant to vitamin D3-induced Ca accumulation. The preferential binding sites for Ca in the arterial walls are generally represented by the vascular smooth muscle cells and the elastic fibres. However, the cerebral vessels, particularly the basilar arteries, are scarcely provided with smooth muscle cells and elastic elements. Thus they are probably lacking a pronounced Ca-binding capacity.

Mural Ca content mmol/kg dry tissue weight		
	Controls	Vitamin D3
Coronary art.	11.2 ± 0.64(n=30)	307.6 ± 23.8(n=94)
Basilar art.	10.3 ± 1.0 (n=21)	12.0 ± 1.4 (n=15)

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EFFECT OF ENHANCED OXYGEN DELIVERY ON PLASMA ADENOSINE [ADO] IN PATIENTS WITH SEPSIS

H. BARDENHEUER, H. FORST, M. HALLER, K.PETER

INTRODUCTION: IMPAIRED TISSUE OXYGENATION CAN HAVE DELETERIOUS EFFECTS ON THE OUTCOME OF CRITICALLY ILL PATIENTS. SINCE ADO IS A SENSITIVE MARKER OF TISSUE ISCHEMIA IN MAN, THIS STUDY INVESTIGATES THE INFLUENCE OF AN INCREASE IN OXYGEN DELIVERY [DO2] FOLLOWING BLOOD TRANSFUSION [BT] ON PLASMA ADO AND LACTATE IN PATIENTS WITH SEVERE SEPSIS.
METHODS: IN 10 PATIENTS (AGE 46 +/- 20) WITH HCT < 30% MEAN ARTERIAL BLOOD PRESSURE [MAP], HEART RATE [HR], CARDIAC INDEX [CI], AND PULMONARY CAPILLARY WEDGE PRESSURE [PCWP] WERE DETERMINED BEFORE [C], 10 AND 60 MIN, AND 24 HOURS AFTER BT. ALSO DO2 AND OXYGEN UPTAKE (CALORIMETR.) [VO2] WERE DETERMINED AND PLASMA ADO, PH AND LACTATE [LAC] ANALYZED.
RESULTS: TABLE 1: (MEAN VALUES; N=10; * P < 0.05)

		TIME AFTER BT		
	C	10'	60'	24 H
MAP (MM HG)	77	99*	93*	81
HR (1/MIN)	92	88	90	88
CI (L/MIN/M2)	4.7	4.9	5.0	5.1
PCWP (MM HG)	10	14*	11	12
HCT (%)	27	39*	38*	37*
DO2 (ML/MIN/KG)	13	18*	19*	19*
VO2 (ML/MIN/KG)	3.3	3.5	3.6*	3.5
ADO (NMOL/L)	617	169*	310*	216*
LAC (MMOL/L)	1.07	0.90	0.82	0.98
PH	7.42	7.40	7.41	7.40

CONCLUSIONS: 1.) BT ELEVATES MAP, PCWP, AND DO2. 2.) THE ENHANCED VO2 [VO2= 2.5 + 0.06 X DO2, R= 0.54) IS PARALLELED BY A DECREASE IN ADO AND LAC. 3.) BEFORE BT ADO IS 4-5 TIMES HIGHER THAN IN NON-SEPTIC PATIENTS. ADO BEST REFLECTS THE IMPROVEMENT IN DO2.

INSTITUT FÜR ANAESTHESIOLOGIE, UNIVERSITÄT MÜNCHEN, KLINIKUM GROßHADERN, D-8000 MÜNCHEN 70

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EFFECT OF ACUTE INFRARENAL AORTIC CONSTRICTION ON SYSTEMIC ADENOSINE [ADO] IN MAN

H. BARDENHEUER, C. SANTJOHANSER, L. LAUTERJUNG, K. PETER

INTRODUCTION: THE PRESENT STUDY INVESTIGATES THE CHANGES OF HEMODYNAMIC AND METABOLIC PARAMETERS AFTER ACUTE ENHANCEMENT OF AFTERLOAD IN PATIENTS UNDERGOING ABDOMINAL AORTIC ANEURYSMECTOMY.
METHODS: IN 18 PATIENTS (66 +/- 11 YEARS (SD)) ADO, HYPOXANTHINE (HYPO) AND LACTATE (LAC) WERE ANALYZED IN ARTERIAL (A) AND PULMONARY-ARTERIAL (PA) BLOOD BEFORE (C), DURING CLAMPING, AND DECLAMPING. BLOOD PRESSURE (SBP), PULMONARY WEDGE PRESSURE (PCWP), CARDIAC OUTPUT (CO), SYSTEMIC (SVR) AND PULMONARY VASCULAR RESISTANCE (PVR) WERE DETERMINED.
RESULTS: TABLE 1: (MEAN VALUES, N=18; * P < .05)

		CLAMPING		DECLAMPING	
	C	5'	30'	5'	20'
SBP (MM HG)	113	125*	128*	129	114
CO (L/MIN)	7.4	6.8*	5.3*	6.7	7.2
STROKE WORK (NM)	1.4	1.8*	1.6	1.8	1.6
SVR (DYN*S/CM5)	1088	1100	1670*	1358	1131
PVR (DYN*S/CM5)	159	181	204	226*	203
PCWP(MM HG)	17	17	18	23*	15
ADOA (NM)	185	494*	367	392	230
ADOPA (NM)	172	388	339	391	347
HYPOA (UM)	0.95	1.00	0.98	2.26*	1.71*
HYPOPA (UM)	1.01	0.88	1.36	2.02*	2.09*
LACA (MM)	0.58	0.58	0.66	1.24*	0.99*
LACPA (MM)	0.56	0.54	0.66	1.16*	0.96*
PHA	7.38	7.38	7.38	7.31*	7.35

SUMMARY: 1.) IN PATIENTS ADO, BUT NOT LAC PARALLELS THE CHANGES IN SBP, CO, AND STROKE WORK DURING ACUTE AORTIC CONSTRICTION. 2.) DURING REPERFUSION ADO, HYPO, LAC and H+ ARE WASHED OUT into the venous blood INDICATING A SIGNIFICANT IMPAIRMENT IN TISSUE OXYGENATION DURING CLAMPING.

INSTITUT FÜR ANAESTHESIOLOGIE, UNIVERSITÄT MÜNCHEN, KLINIKUM GROßHADERN, 8000 MÜNCHEN 70

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FLOW-INDUCED CONTROL OF SMOOTH MUSCLE TONE AT MICROVASCULAR LEVEL

V. Smieško, D.J. Lang and P.C. Johnson

Flow-induced, endothelium-mediated dilation has been demonstrated mainly in large conduit arteries. To determine whether the dilation exists at microvascular level we studied the effect of an increase in blood flow velocity on diameter of arcading arteriole /AA/, connecting two adjacent triangular vascular sectors in the rat mesentery. The velocity was increased by occlusion of the feed artery to one of these two vascular sectors so that during occlusion the affected sector was supplied by collateral flow through the AA. The diameter of the AA and red cell velocity through it were monitored with a dual slit video microscope system / P.C. Johnson and M. Intaglietta, Am J Physiol 231:1686, 1976 /.

The occlusion of feed artery increased red cell velocity in the AA from 9.9 ± 1.1 to 66.2 ± 3.5 mm/s /±SEM/. After a delay of 7.7 ± 0.6 s the AA dilated by 68.5 ± 3.5 % of the initial diameter /68.1 ± 2.4 µm/. The dilation : /a/ was equal to the maximal dilation attained with topical application of papaverine, /b/ was sustained for the duration of increased velocity, /c/ was smaller during partial occlusion than during total occlusion, /d/ was absent in adjacent non-arcading arteriole without velocity increase. The dilation enlarged collateral volume flow to ischemic vascular sector by additional 190 % as compared to the increased flow immediately after occlusion. These observations indicate that a potent flow-dependent dilator mechanism is present in arterioles under 100 microns diameter.

Institute of Normal and Pathological Physiology , Slovak Academy of Sciences , Sienkiewiczova 1 , 813 71 Bratislava , Czechoslovakia

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CEREBRAL BLOOD FLOW, GLUCOSE USE AND CEREBROSPINAL FLUID ELECTROLYTES DURING CHRONIC METABOLIC ALKALOSIS

H. Schröck and W. Kuschinsky

Chronic metabolic alkalosis was induced in rats by keeping them on a low K^+ diet for either 15 or 27 days with 0.2 M $NaHCO_3$ as drinking fluid. Local cerebral blood flow (LCBF) and local cerebral glucose utilization (LCGU) were measured in 31 different structures of the brain by means of the (^{14}C)-iodoantipyrine and (^{14}C)2-deoxyglucose method. The treatment induced moderate (15 days, BE=16 mM) to severe (27 days, BE=25 mM) hypochloremic metabolic alkalosis in addition to severe K^+ depletion. No change was detectable in average LCGU and LCBF during moderate metabolic alkalosis when compared to controls, despite a significant decrease in CSF K^+ and H^+ concentrations. In a previous study (Schröck and Kuschinsky, Am. J. Physiol. 254, H250-H257,1988) a comparable K^+ depletion but without hypochloremic alkalosis had been induced which had resulted in a decrease in average LCBF of 19 to 25%; this had been attributed to the decreased CSF K^+ and H^+ concentrations. The present study shows that moderate metabolic alkalosis including K^+ depletion prevents such a reduction in LCBF. During severe metabolic alkalosis LCBF was decreased by 19% and average LCGU was decreased by 24% when compared to controls. The decrease in average LCBF during severe metabolic alkalosis can be attributed to a decreased cerebral metabolism and a decreased CSF H^+ concentration.

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Physiologisches Institut I der Universität
Im Neuenheimer Feld 326, 69 Heidelberg

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PRES: A TECHNIQUE FOR THE CONTROLLED STIMULATION OF THE CAROTID BARORECEPTORS IN MAN

Rau, H., Elbert, T., Geiger, B., & Lutzenberger, W.

The cervical neck suction has proven to be a valid method for the stimulation and inhibition of carotid baroreceptors. When employing this technique, control conditions seem to be difficult to arrange. Without an appropriate control procedure, it is difficult to be confident that an observed bahevioral effect is due only to the pressure differential created across the sinus wall and not simple distraction from the suction associated sensation. The present method was developed to allow a psychologically equivalent 'placebo'condition.
Because the carotid stretch receptors are not only sensitive to static levels of pressure, but also to rate of change, it is possible to manipulate receptor firing rate through changes in pulse amplitude. The device constructed relies on the application of short changes in cuff pressure tied to different phases within the heart cycle (PRES: phase related external suction). A brief external suction during the systole has stronger stimulatory effects on baroreceptors than the application of the same pressure pulse during the diastole. This is verified by the evoked HR deceleration. In order to allow an ongoing period of stimulation, a train of alternating negative/positive pressure pulses is applied. In the stimulation condition the ECG R-wave triggeres a negative pulse which is followed by a positive one during the diastole. In the control condition this relationship is reversed.
Systematic differential effects between the two conditions on cardiovascular measures (particularly heart rate) have been found in all of the 26 subjects investigated. As revealed by questionnaires and interviews, subjects did not become aware of the pphase relationship between pressure changes with their cardioac cycle and were unable to distinguish intervals during which the baroreceptors were stimulated from control times.

Eberhard-Karls Universität Tübingen, Klinische & Physiologische Psychologie; Gartenstr. 29, D 7400 Tübingen

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DETERMINATION OF STROKE VOLUME BY IMPEDANCE CARDIOGRAPHY: VARIABILITY, REPRODUCIBILITY AND VALIDITY

G. Koch, H.E. Koralewski and F.H. Perschel

Non-invasive assessment of stroke volume (SV) is mainly limited to 2 methods: impedance cardiography (ICG) and calculation from echocardiographic measurements of the systolic and diastolic changes of left ventricular dimensions. Impedance techniques are particularly attractive since they allow for beat-to-beat evaluation as well as for measurement of steady state SV. The purpose of the present study was to provide data on the variability of measurements of some key hemodynamic data including SV at several days' intervals as they are often needed to be done in pharmacological intervention trials. Moreover, data on the reproducibility and the validity of the method as evaluated from the changes when raising from the supine to the seated posture are provided. Variability: 12 healthy male volunteers aged 22 to 29 ys were studied 3 times at 1-week intervals under rigorously standardised steady state conditions in the supine posture; all parameters were recorded on-line and computed using an OS 9 system. SV was averaged over 20-second intervals. The values used are the means obtained during 10 such intervals; they thus correspond to a mean value as measured over 200-second periods. Mean values ± SD for SV were 99 ± 26, 109 ± 21 and 98 ± 16 ml without any statistically significant difference. The corresponding values obtained for cardiac output and total systemic vascular resistance were 6.3 ± 1.3, 6.9 ± 1.4, 6.1 ± 0.7 l/min and 16.2 ± 4.7, 14.4 ± 4.1 and 15.6 ± 2.5 units respectively, again without statistically significant differences. Reproducibility was tested by considering the values obtained at two consecutive 100 msec periods as a duplicate determination. Regarding SV, the mean difference was 5 % of the mean value (96 ml, n = 104), the corresponding coefficient of variation 2.7 %. Validity: In 5 volunteers the hemodynamic parameters including SV were measured twice at an interval of approximately 5 min in the supine and the seated posture, i.e. in the course of a manoeuvre known to affect SV in a predictable way. Sitting up resulted in a mean reduction of 14.6 and 13.4 % of the initial (supine) SV without a statistically significant difference. In previous studies it has been shown that the orthostatic changes of SV measured by ICG compare favorably with those obtained by the Fick method.

It is concluded that ICG can be used for determination of SV changes as a substitute for invasive methods provided adequate standardisation is achieved. This is at least valid under resting conditions.

Physiologisches Institut, Freie Universität Berlin, Arnimallee 22, 1000 Berlin 33

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EARLY POSTOPERATIVE CHANGES OF TISSUE PO_2 IN THE LOWER LEG FOLLOWING VASCULAR SURGERY.

K. Wagner, U. Krüger, M. Albrecht, G. Hohlbach, Ch. Weiss

Only few data on muscle pO_2 in patients after vascular surgery for peripheral arterial disease (pad) are available. We undertook an on-going study measuring tissue-pO_2 in the m. tibialis anterior or m. gastrocnemius before, during and after (day 2-4 and day 10-14) vascular reconstruction. 11 patients so far have been studied (age range 47-71 years), 7 patients were in stage 2b of pad, 3 in stage 3 and 1 patient in stage 4 (according to the Fontaine-classification). Tissue-pO_2 was determined with a pO_2-histograph (Ch. Weiss, W. Fleckenstein, in: Grote, Thews (ed): Funktionsanalyse biologischer Systeme 15:155, 1986). The following blood parameters and parameters of cardiovascular and pulmonary functions were monitored: mean arterial pressure, central venous pressure, heart rate, arterial and venous blood gases, hemoglobin and hematocrit, serum Na^+ and K^+, body core temperature and tissue temperature.
Mean preoperative tissue-pO_2 values laid at 22.7 ± 4.4 mm Hg (see table below; physiological value in the m. tibialis ant.: 27.2 ± 4.4 mm Hg (Ehrly et al, Klin Wschr 53:687, 1975)). The intraoperative measurements taken 10 - 30 minutes after reestablished blood flow showed that mean tissue-pO_2 rose to 29.3 ± 4.7 mm hg with considerably more pO_2-values lying in the range of 40 - 80 mm Hg. On the 2nd - 4th postoperative day pO_2-measurements revealed unexpectedly low mean values at 19.2 ± 6.5 mm Hg while none of the patients had a vessel occlusion. The histogram showed an increase of the relative frequency of very low pO_2 values in the range of 0 - 10 mm Hg (left shift). However, mean tissue-pO_2 values measured on the 10th-14th postoperative day amounted to 26.5 ± 1.1 mm Hg, close to the physiological value. Since there was neither an indication of a postoperative vessel occlusion nor a correlation of the level of the muscle tissue-pO_2 with any of the other monitored parameters our finding of a transient decrease of postoperative tissue-pO_2 on day 2-4 is assumably the result of a reperfusion damage, the cause of which is presently being studied by us.

Table tissue-pO_2 (mm Hg)

preop	intraop	postop 2-4d	postop 10-14d
22,7±4,4	29.3±4.6	19.2±6.5	26.5±1.1

Institut für Physiologie, Med. Universität Lübeck, Ratzeburger Allee 160, 2400 Lübeck

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RED CELL POTASSIUM CONCENTRATION AND RED CELL VOLUME DURING EXHAUSTIVE EXERCISE WITH A SMALL MUSCLE GROUP

N. Maassen, M. Foerster,

Red cell volume during exercise is reported to be constant. This is only possible if all factors influencing the volume (mainly pH and tissue osmolality) are counterbalancing each other. Some authors discuss a K^+ uptake as a volume regulating factor.
Methods: 7 male volunteers performed handgrip exercise until exhaustion. Skin blood flow was reduced by cooling. Blood was drawn from a cubital vein. [HB], HCT, [Prot], Osmolality, pH_{pl}, $[K^+]_{pl}$, and $[K^+]$ in hemolyzed blood were measured. $[K^+]_{ery}$ was calculated and related to the water phase. Results: During exercise the following changes were measured: [PROT] from 7.2 +/- 0.3 to 7.7 +/- 0.4 g/dl; [HB]: 15.2 +/- 0.6 to 16.2 +/- 0.7 g/dl; MCHC: 34.5 +/- 0.6 to 35.4 +/- 1.0 g/dl; $[K^+]_{pl}$: 4.2 +/- 0.15 to 7.2 +/- 0.52 mmol/kg H_2O; $[K^+]_{ery}$: 134.7+/- 9.0 to 138.6 +/- 4.0 mmol/kg H_2O; pH_{pl}: 7.352 +/- 0.026 to 7.175 +/- 0.046; OSM: 293.8 +/- 4.2 to 319.3 +/- 8.7 mosmol/kg H_2O. All changes were significant except $[K+]_{ery}$.
Discussion: The decrease in pH would cause an increase in red cell volume but that is over compensated by the high tissue osmolality which leads to a shrinking of the red cells and thus to an increase of MCHC. $[K+]_{ery}$ is influenced by water shifts only but is no volume regulating factor in the red cell under these conditions.

Department of Sports- and Exercise Physiologie, Medical School, D-3000 Hannover 61, F.R.G.

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BLOOD FLOW RESPONSES OF THE INTERNAL MAXILLARY ARTERY AND OF ITS VASCULAR COMPARTMENTS - AVA AND CAPILLARIES - TO INCREASES IN SYSTEMIC ARTERIAL BLOOD PRESSURE.

J. Mühling, F. Bari and K. Pleschka

To evaluate the role of baroreceptor reflexes in blood flow control of the facial and nasal vasculature, total blood flow and perfusion pressure (PP) of the internal maxillary artery (IMA-FLOW) were recorded bilaterally at rest and during baroreceptor stimulation (ST) in 10 anesthetized, paralyzed and artificially ventilated dogs. ST was achieved by increasing spontaneously established blood pressure (BP) in the aortic arch with an inflatable balloon, positioned in the descending aorta. Mean increases in regional BP were 35.4 (SE 1.94) mmHg (ST_1); 53.1 (SE 1.27) mmHg (ST_2), and 71.7 (SE 2.53) mmHg (ST_3). Distribution of IMA-FLOW to precapillaries (CAP-FLOW) and arteriovenous anastomoses (AVA-FLOW) was determined unilaterally by the tracer microspheres technique (Hashimoto et al., Pflügers Arch. 410:589, 1987) during the three periods of ST and the corresponding resting conditions. Further variables measured or determined were cardiac output (CO) of the right heart, heart rate (HR), and peripheral resistance of the IMA (R-IMA). The results are listed in the table as percentage changes during ST. (* indicates significance at $P < 0.05$)

	ST_1	ST_2 ipsilateral	ST_3	ST_1	ST_2 contralateral	ST_3
PP :	+23.9*	+51.5*	+68.0*	+36.5*	+50.8*	+70.0*
R-IMA :	−11.0	−10.5*	− 2.5	− 2.4	−16.7*	− 8.8
IMA-FLOW :	+36.2*	+74.2*	+76.0*	+31.1*	+82.2*	+75.0*
AVA-FLOW :	+37.8*	+84.7*	+106.1*			
CAP-FLOW :	+33.2	+61.1*	+48.1			
HR :		−43.4*	−41.9*	−37.5*		
CO :		−24.0*	−19.2*	−28.1*		

The results demonstrate that ST_{1-3} caused significant increases in IMA-FLOW concomitant with significant stimulus strength dependent increases in PP. The increase in IMA-FLOW was mainly due to marked increases in AVA-FLOW rather than to increases in CAP-FLOW. Because of the less pronounced decreases in R-IMA it appears that the IMA-FLOW responses were passively induced. On the other hand the preferential increase of AVA-FLOW during all periods of ST_{1-3} could also be reflexive to baroreceptor stimulation. Unimpairment of baroreceptor reflex was indicated by decreases in heart rate in association with decreases in cardiac output during ST_{1-3}.

MPI für Physiol. & Klin. Forschung, W. G. Kerckhoff-Institut, D-6350 Bad Nauheim

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CARDIOVASCULAR EFFECT OF DIPA-DCA COMPARED TO CLONIDIN IN ANAESTHETIZED GUINEA-PIGS

H.D.Schmidt, H. Groß, and W. Loock

In previous studies in cats DIPA-DCA proved to have a strong sympathicolytic effect (Schmidt et al Pflügers Arch. 406, R84, R85, 1986) which is presumably due to activity on the ganglionic level. In the present study DIPA-DCA is compared to the centrally-acting sympathicolytic agent clonidin. In 32 anaesthetized guinea-pigs, increasing doses of DIPA/DCA, up to 5mg/kg, progressively decreased aortic pressure (AP -33%), heart rate (HR -10%), cardiac output (CO -12%), and total peripheral resistance (TPR -25%). Similar effects were seen with 1 µg/kg clonidin: AP -39%, HR -10%, CO -9%, and TPR -38%. When clonidin was injected after 5mg/kg DIPA/DCA, AP increased by +23%. This proves that DIPA-DCA had completely blocked the sympathetic system, revealing the effect of clonidin on peripheral α_1-receptors. When DIPA/DCA was given after premedication with clonidin AP no longer decreased, but increased considerably (+8% at 5mg/kg and +16% at 20 mg/kg). TPR increased (+19%) while CO remained unchanged and HR decreased slightly. This effect on peripheral vessels cannot be prevented by praxocin and is also present when the ganglion blocking agent, hexamethonium, is used.
Conclusion: The maximal cardiovascular effect of DIPA-DCA is qualitatively and quantitatively similar to clonidin. It also shows a stimulatory effect on the peripheral vasculature which is not, however, mediated via α_1-receptors. DIPA-DCA seems as suitable as clonidin as a non-ß-receptor blocking sympathetic agent.

Physiologisches Institut der Freien Universität Berlin, Arnimallee 22, 1000 Berlin 33, FRG

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A Hall-effect sensor for recording pulse waves and diameter changes in cutaneous veins

C. Stick, U. Diefenbacher, D. Gronewold, M. Tobinsky, A. Walther-Behrends and E. Witzleb

When recording venous pulse waves to determine the pulse wave velocity special problems arise from the low blood pressure in these vessels. Accordingly, most of the numerous pulse transducers designed for recording arterial pulses are not suited for veins. A pulse transducer is presented, which is custom-made for application in the veins. The transducer is based on the Hall-effect. A small magnet is fixed to the skin over a cutaneous vein. The movements of this magnet as transmitted from the vessel wall are detected as changes in the magnetic field by a Hall-sensor. This sensor - an integrated circuit (IC) - is mounted on a holder and positioned over the magnet. The changes in the magnetic field are transformed into a voltage signal, which is fed to the analogue-digital-converter of a personal computer. The output voltage of the IC depends in an exponential manner on the distance between the south pole of the magnet and the IC. Allowing for the respective calibration curve the varying distance between magnet and IC can be calculated from the output voltage of the IC. Thus, the transducer cannot only record pulse waves, but also diameter changes in veins. Using two of the described pulse transducers, which were placed over a vein on the dorsum of the hand and over the cephalic vein respectively, the wave velocity of artificially induced pulse waves could be measured. First results show this variable to be closely related to changes in venous blood flow velocity and to changes in venous diameter.

Institut für angewandte Physiologie und medizinische Klimatologie der Christian-Albrechts-Universität, Hermann-Rodeald-Str. 5, D-2300 Kiel

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COMPARISON OF THE EFFECTS OF COOLING THE THORACIC SKIN ON CARDIOVASCULAR PARAMETERS IN BROODY AND NON-BROODY BANTAM HENS

M. Brummermann and R. E. Reinertsen

During the breeding season the ventral skin of many birds undergoes defeathering and hypervascularisation forming the brood patch.

Stepwise cooling of the brood patch of 14 broody and a corresponding shaved skin area of 13 non-broody Bantam hens with stimulation temperatures (T_s) from 35 to 15 °C resulted in a significantly lesser decrease of breast skin temperature in the broody than in the non-broody hens. Deep body temperature was lowered significantly only in broody hens.

Oxygen uptake was inversely correlated to T_s in both groups, the effect being up to 2.5 times as high in broody than in non-broody hens.

Cardiac output which was 674±134 in 8 broody and 360±200 ml/min·kg in 8 non-broody hens increased with decreasing T_s with a steeper slope in the broody than in the non-broody hens, mainly due to a tachycardia which was much more pronounced in the brooding hens, and partly to a slight increase in stroke volume (SV) in both groups, the broody hens having a 45% higher SV already at resting conditions. Since blood pressure was rather stable and equal in both groups, this accounts for an initially lower total peripheral resistance (TPR) and a greater decrease of TPR during cold stimulation in broody than in non-broody hens.

Thus in incubating hens the heat transfer to cold eggs seems to be achieved by two factors, a permanent enlargement of the vascular bed and an additional vasodilation in reaction to cold stimulations of the brood patch, both increasing the thermal conductance.

Department of Zoology, University of Trondheim, N-7055 Trondheim, Norway

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DISSOCIATION BETWEEN MYOCARDIAL CAPILLARY AND FIBER DENSITY IN RATS WITH IN VIVO AGED RED CELLS

H. Rogausch and W.M. Rohn

In rats, in vivo aging of the erythrocytes increases the cardiac intercapillary distances (H.Rogausch, Int. J. Microcirc.: clin. exptl., in press). In the following experiments we tested whether the increased distances were associated with a greater cross sectional area of individual myocardial fibers. After 5 weeks of erythrocyte aging by the method of Ganzoni (J. clin. Invest. 50: 1373, 1971), the plasma of the animals was stained with FITC-globulin and the hearts were rapidly frozen according to the method of Vetterlein (Am. J. Physiol. 242: H133, 1982). In alternating cryostate sections at the greatest circumference of the hearts, the fibers were visualized by v. Gieson's stain and the capillaries by fluorescence microscopy. Mean intercapillary distance increased by 14% ($p<0.009$), mainly due to a pronounced increase of the larger distances. The mean cross sectional area of myocardial fibers, however, was not significantly altered (246±86µm² in controls and 269±97µm² in animals with aged erythrocytes, $p>0.05$). Therefore heart hypertrophy was not the cause of the increased intercapillary distance. In HE-stained sections no signs of myocardial cell damage were seen. The results suggest that the decrease in the capillary density is caused by a reduction of microcirculatory flow without visible myocardial cell damage.

Dept. Physiol., Deutschhausstr.2, 3550 Marburg, FRG

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CHARACTERIZATION OF A LOW AFFINITY COMPONENT FOR OXYGEN IN THE RAT CATORID BODY IN VITRO

H. Acker, M. Delpiano, E. Dufau, J. Hentschel

It has been shown that the rat carotid body superfused in vitro with low PO_2 (20 Torr) exhibited an optical absorbance spectrum which resembles the reduced spectrum of the NADPH oxidase in neutrophiles. Diphenyl iodonium (DPI) as a specific inhibitor of the oxidase attenuated the reduced absorbance spectrum in the carotid body (Acker et al., FEBS Lett. 256, 75-78, 1989). For further characterization microscope fluorescence measurements of FAD (460 nm/523 nm) and NAD(P)H (366 nm/465 nm) have been carried out in the rat carotid body in vitro. Lowering the PO_2 gradually in the superfusion medium from 340 Torr to 20 Torr was followed by a gradual decrease of the FAD fluorescence of about 15% indicating a reduction of FAD (n=6). The gradual PO_2 decrease in the superfusion medium was also followed by a gradual increase of the NAD(P)H fluorescence of about 15% (n=6). The apparent $KmPO_2$ for FAD as well as for NAD(P)H was 62 Torr indicating a close functional relationship between both components. DPI (10 µM) inhibited the hypoxia-induced NAD(P)H as well as FAD changes. Concomitantly the hypoxic nervous excitation of the rat carotid body could be depressed by the same dose of DPI. Superfusion of the rat carotid body with low sodium (20 mM) and amiloride (1 mM) attenuated the hypoxia-induced light transmission changes especially at 460 nm, the FAD absorbance maximum (n=10). These data suggest a membrane localization of the NAD(P)H oxidase in the carotid body, which has a low affinity for oxygen and might participate with O_2 radical generation as an O_2 sensor protein in the chemoreceptive process.

Max-Planck-Institut für Systemphysiologie, Rheinlanddamm 201, 4600 Dortmund 1, FRG

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INVESTIGATION OF OXYGEN TRANSPORT TO TISSUES DURING REST AND ERGOMETER WORK BY USING OXYGEN ISOTOPES

H. Heller and K.-D. Schuster

Due to the different molecular weights of the isotopic molecules $^{16}O^{18}O$ and $^{16}O_2$, a fractionation effect occurs between them when passing through the pathways of oxygen transport and metabolism. Several constituent processes of these pathways such as O_2 diffusion and O_2 utilization are known to have high fractionations (3 and 1.3 % respectively). In recent experiments performed on healthy humans at rest, the uptake rate of $^{16}O_2$ was found to be 0.9 % above that of the heavier molecule (Schuster and Pflug, Oxygen Transport to Tissue, XI, Adv. Exp. Med. Biol. (1989)). We investigated the question as to whether diffusion becomes a limiting process of oxygen transport during ergometer work, thus causing an increase of overall fractionation effect. Experiments were carried out on 6 healthy humans at rest and during ergometer work for 10 minutes and varying loadings of 50, 100, 150, 200 and 250 W. Samples from inspiratory and expiratory gas were analysed by mass spectrometry. At rest uptake, subsequent transport and utilization of oxygen occurred with a 0.72 % ± 0.08 % higher rate for $^{16}O_2$ than for $^{16}O^{18}O$. During ergometer work this value steadily decreased to 0.31 % ± 0.04 % at 250 W. These results suggest: (1) At rest the overall fractionation effect of respiration between $^{16}O^{18}O$ and $^{16}O_2$ is below the value of 1.3 % which is in line with a limitation of oxygen uptake mainly due to metabolism, (2) the decrease of overall fractionation effect during ergometer work indicates that oxygen transport is not limited by diffusion up to work loads of 250 W, otherwise an increase of fractionation effect should have occurred.

Physiologisches Institut I der Universität Bonn, Nussallee 11, D-5300 Bonn 1

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DISPERSION OF INSPIRED AEROSOL BOLI AND DETERMINATION OF EFFECTIVE AIRWAY DIAMETERS BY A NEW AEROSOL METHOD IN HEALTHY SMOKERS BEFORE AND AFTER BRONCHODILATION.

R. Siekmeier+, H. Kronenberger*, H. Lintl+, Ch.F. Schiller-Scotland+, J. Gebhart+, J. Heyder++, J. Meier-Sydow*, W. Stahlhofen+.

Dispersion and half-width of aerosol boli can be determined by an aerosol method during in- and exspiration. The dispersion of the inhaled aerosol boli with a half-width of 70 ml is a parameter for convective gas mixing during respiration. Another method recently described by J. HEYDER (J. Aer. Med.;1989; 2;89-97) allows the noninvasive determination of effective airway diameters (EAD) as a function of volumetric lung depth (LD). This method depends on settling velocity and deposition rate of an inert aerosol in the respiration tract during a defined time of breath-holding. Methods: Aerosol dispersion and EAD were determined in a group of 18 healthy smokers (age: 25.1 yrs.; pack yrs.: 11.8) with normal lung function tests. EAD were assessed in LD between 150 and 800ml. Every subject was measured twice within one week before and after bronchodilation by a β_2-agonist (Formoterol) and used as its own standard.
Results: Relative changes (increase) of EAD by Formoterol [% baseline] are shown in table.

LD [ml]	150	200	250	300	400	500	600	700	800
EAD [%]	37.1	28.9	23.9	18.3	12.5	10.0	7.7	6.3	6.6

Discussion and Conclusions: As a function of LD bolus half-width increase whereas EAD decrease. Inhalation of the β_2-agonist causes significant increases of EAD in LD between 150 and 300 ml ($p<0.05$, WILCOXON). The relative changes of EAD decrease with increasing LD, whereas no significant changes of bolus half-width were found. We suggest that in healthy subjects convective gas transport is not influenced by bronchodilation.

+) Ges. f. Strahlen- und Umweltforschung, Frankfurt/M.
*) University Hospital, Dep. of Intern. Med., Div. of Pneumology, Frankfurt/M.
++) Ges. f. Strahlen- und Umweltforschung, Projekt Inhalation, München, FRG

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A NEW METHOD FOR CONTINUOUS REGISTRATION OF O_2-SATURATION AND PO_2 OF FLOWING SHEARED BLOOD.

K.Mottaghy, C.Geisen, W.Richter and J.Beckman

Standard-O_2-dissociation curves (ODC) are measured in non-flowing blood under equilibrium conditions, neglecting rheological aspects. The recently introduced rheo-oxymetry in contrast, allowed the evaluation of the oxygen uptake or delivery in flowing blood by continuous Po_2 registration. During these measurements, blood is sheared in a Couette-flow-system while being exposed to the oxygen phase across a membrane for defined contact times. An additional simultaneous saturation measurement would allow a complete analysis of the gas exchange properties of sheared blood. In a new approach, this continous O_2-saturation measurement was achieved using a new wavelength scanning oximeter. This device was originally developed for measurements of cutaneous haemoglobin spectra, according to Lübbers et al.. We adapted it's photometric sensor to a thin layer cuvette connected to the vent of the Couette rheo-oxymeter. The blood's reflection spectrum is continuously scanned in a wavelength range from 500 to 600 nm. The momentary saturation is calculated on the basis of a non-linear two-component analysis enabling rapid measurements. This, in combination with a simultaneous Po_2 measurement now allows the measurement of a 'dynamic' ODC of blood under shear conditions. Haemorheological effects on the oxygen uptake have been proven under several circumstances. Artificial rigidification of RBC's e. g. leads to a slower O_2-saturation (shear rate 370 s^{-1}). It has been observed, that impaired RBC-deformability reduces the oxygen uptake even when the standard-ODC is left-shifted.

Institut für Physiologie, Klinikum der RWTH, Pauwelsstr. D-5100 Aachen

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PATHOPHYSIOLOGICAL MECHANISMS OF LIPOPOLYSACCHARID INDUCED RESPIRATORY AND CARDIO-VASCULAR CHANGES

W. Marek, and W.T. Ulmer

Lipopolysaccharid (LPS) induced biphasic changes in airway resistance, pulmonary artery pressure and vascular permeability represent an accepted model of adult respiratory distress syndrome (ARDS).
Methods: Besides direct vagal reflex mechanisms, we studied the contribution of mediators like histamine, platelet activating factor (PAF), and leucotrienes by application of their specific antagonist before i.v. infusion of 0,2 ug/kg LPS from Escherichia coli endotoxin in anaesthetized sheep.

Results: LPS infusion results in an increase in dynamic elastance (Edyn) as a representative of airway resistance from 2.0 ± 0.9 to 7.5 ± 3.6 mmHg/100ml VT after 50 min, along with an increase of systolic pulmonary artery pressure from 15 ± 2.8 to 34 ± 10 mmHg with a maximum after 30 min. Both result in a severe arterial hypoxaemia. The late phase of response after 2-5 hours was characterized by a severe decrease in the number of leucocytes in arterial blood from 4.100 ± 860 to 850 ± 240 cells/ml and the developement of lung oedema along with mild respiratory and cardiovascular changes.
Inspite of allergen induced airway obstruction, bilateral vagotomy only slightly diminished the respiratory and cardio-vascular responses to LPS. While histamine H1-receptor antagonists hardly showed any changes of the responses to LPS infusion, between 30-50 % of the responses were diminished after pretreatment with the PAF-antagonist WEB-2086 or the lipoxygenase inhibitor HWA-214.

Conclusion: Platelet activating factor and lipoxygenase products contribute to the LPS-induced respiratory and cardio-vascular changes, while vagal reflex-mechanisms and histamine are of minor importance.

Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin an der Ruhr-Universität Bochum, Gilsingstr. 14, 4630 Bochum 1, FRG.

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PERIPHERAL CHEMORECEPTOR RESTING DRIVE IN NORMOTENSIVE AND HYPERTENSIVE SLEEP APNEA PATIENTS

M.Tafil-KLawe*, A.E.Thiele, F.Raschke, J.H.Peter, G. Hildebrandt

Augmented carotid chemoreceptor resting drive has been suggested to be involved in the pathophysiology of human essential hypertension (1,2,3). Relationship between arterial hypertension and sleep apnea syndrome was frequently reported (4). Do arterial chemoreceptors play a role in the pathophysiology of sleep apnea syndrome (5)?
The ventilatory response to inactivation of carotid chemoreceptors (CCh) was studied in 15 normotensive patients with sleep apnea syndrome (SAS), in 13 hypertensive (H-SAS) and in 30 age-matched controls (C). The inactivation of CCh was due to pure oxygen breathing for 1 min. A significant decrease in ventilation was obsereved in all groups of subjects:

C	16.3 ± 2.5%
SAS	15.9 ± 2.1%
H-SAS	27.47 ± 3.9% (SEM).

The reduction of ventilation was significantly higher in H-SAS patients as compared to SAS and C subjects $p<(0.001)$.
Our results suggest that H-SAS patients have an augmented resting CCh drive whereas the SAS patients did not differ from the C subjects. The increase in tonic CCh activity can probably contribute in causing arterial hypertension in H-SAS patients.

1. Przybylski 1981, Med.Hypoth.; 2. Trzebski et al. 1982, Cardiovasc.Res.; 3. Tafil-Klawe eet al. 1985, Acta physiol.Pol.; 4. Gislason 1987, Acta Med.Sc.; 5. Przybylski et al. 1986, Med.Hypoth.

Institut für Arbeitsphysiologie und Rehabilitationsforschung, Robert-Koch Str.7A, 3550 Marburg; Zentrum für Innere Medizin, Marburg; *Department of Physiology, Medical Academy, Warsaw.

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INFLUENCE OF CHANGES IN LUNG VOLUME ON AIRWAY DIAMETERS IN HEALTHY SUBJECTS: ASSESSMENT WITH A NEW AEROSOL RECOVERY METHOD.

H. Kronenberger*, J. Gebhart+, T. Kullmer*, H. Lintl+, Ch.F. Schiller-Scotland+, R. Siekmeier+, J. Heyder++, J. Meier-Sydow*, W.Stahlhofen+.

Our group has developed a new method which enables us to determine non-invasively effective (mean) airway diameters (EAD [mm]) as a function of volumetric lung depth (LD [ml]). The method is based on the inhalation and measurement of the recovery of a monodisperse aerosol during a single breath (J. HEYDER: J.Aersol.Med. 89;2:89-97). It is well known that airway resistance and thus airway calibre is influenced by lung volume. In this study we evaluate the effect of changes in lung volume on EAD. Methods: 13 healthy smokers (age: 24.9 yrs.; pack yrs.: 10.0) with normal lung function tests were subjected to successive EAD-measurements in LD 100-800 ml. Routine procedure (RP) starts at 1 l below FRC with inhalation of 2 l of aerosol followed by different respiratory pauses so that the EAD are determined at a lung volume of 1 l above FRC. In the altered procedure (AP) the inhalation starts at FRC, so that the lung volume for the measurement is raised to 2 liters above FRC (1 l above RP-level). Results of EAD for RP (EAD_{RP}) and AP (EAD_{AP}) are shown in the table (@: Statistics $p<0.05$ [WILCOXON]).

LD	100@	150@	200@	250@	300@	400@	500@	600	700	800
EAD_{RP}	2.29	1.31	0.87	0.71	0.63	0.52	0.45	0.41	0.37	035
EAD_{AP}	4.18	1.94	1.19	0.91	0.75	0.58	0.49	0.44	0.40	037

Discussion and Conclusions: As expected the elevation of lung volume by 1 l causes a significant increase of EAD in LD between 100 and 500 ml. In more peripheral air spaces no change can be observed. These marked changes of EAD have the same extent as those described in healthy subjects after inhalation of bronchodilatory pharmaca (H. KRONENBERGER ET AL: Eur. Resp. J. 89;2:392s). Thus, these data confirm the value of the new aerosol method for the evaluation of airway calibres.

*) University Hospital, Dep. of Intern. Med., Div. of Pneumology, Frankfurt/M.,
+) Ges. f. Strahlen- und Umweltforschung, Frankfurt/M.,
++) Ges. f. Strahlen- und Umweltforschung, Projekt Inhalation, München, FRG

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RESPIRATORY EFFECTS OF AMINOPHYLLINE IN ADULT RABBITS

G. Kozianka-Burghof, H. Kiwull-Schöne and P. Kiwull

Since aminophylline* (A), the complex of theophylline and the solvent ethylenediamine (EDA), is suitable for the treatment of respiratory failure after birth, it often has been studied in newborn rabbits. For adult rabbits, only few data are available about respiratory drug and/or solvent effects at central and effector level.

Therefore, anaesthetized adult rabbits, spontaneously breathing O_2-enriched air, were studied before and during intravenous infusion either of A (loading dose: 80mg/kg for 10 min, maintenance dose: 10mg/kg/h for about 90 min) or only of EDA in equivalent molar amounts. Tidal volume and inspiratory/expiratory durations (V_T, T_I, T_E), transpulmonary pressure (P_{TP}), phrenic nerve activity (PNA) and end-tidal PCO_2 were continuously determined, as well as from blood samples, the arterial PCO_2, pH, standard HCO_3^- and theophylline concentration.

After the initial loading infusion of either A or EDA, V_T, P_{TP} and PNA invariably decreased by 10-20%. During the maintenance infusion, V_T returned to control, and P_{TP} rose beyond in both cases, although PNA rose only in case of A. From the onset of infusion, A led to a pronounced (70%) and EDA to a smaller (20%) rise in respiratory rate. Therefore, ventilation ($\dot{V}$) was enhanced immediately and then maintained at a high level by A, but EDA caused even an initial depression, followed by a delayed small and transient rise. Both response-types of $\dot{V}$ were accompanied by about the same decrease in arterial PCO_2 by 0.8-1.0 kPa.

We conclude that EDA in aminophylline partly inhibits the respiratory drive elicited by theophylline at central level, but at the same time enhances the effectivity of respiratory control for the gas-exchange at effector level.

* Euphyllin®

Institut für Physiologie der Ruhr-Universität, D-4630 Bochum

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CIRCADIAN VARIATION OF NASAL AND ORAL RESISTANCE

A.E.Thiele, F.Raschke and G.Hildebrandt

Nasal resistance accounts for about half of the total airway resistance, but it has gained only little interest as compared to tracheal and bronchial respiratory resistance. Because nasal resistance is known to be highly variable both within and among subjects, we studied the 24-h variations of nasal resistance and airflow laterality (nasal cycle) under strictly standardized metabolic, climatic, positional and social conditions.

Methods: In 10 healthy male subjects oral and nasal resistance and nasal laterality were measured every 2h by means of the forced oscillation method over 24h. The experiment was carried out in a climatic chamber keeping the subjects under bedrest and low-protein isocaloric diet. Heart rate (HR) and rectal temperature were measured continuously. Self rated alertness (Thayer list) was assessed at each 2h run.

Results: Nasal and oral resistance measurements showed pronounced 24h variations with a maximum of the oral resistance at 5.00h. The maximum of the nasal resistance was preceded for about 4h. Nasal laterality showed a cycle length of 4h on average. HR and rectal temperature showed the well known circadian rhythm with a minimum at 5h.

Discussion: The phase lag between diurnal variations of nasal and oral resistance is indicative for different mechanisms of their regulation. Whereas the neural part of nocturnal increase of oral resistance is known to be mediated by the parasympathetic, the nocturnal increase of nasal resistance may be induced by a reduction in tone of the adrenergic system. Additionally there is evidence for a reciprocal control of vasomotoric activity in the capacitance vessels of the turbinates originating in the lower brain stem (Bamford & Eccles, Pflügers Arch.,394:139,1982). Our results have some implications in the pathogenesis of nocturnal breathing disorders.

Institut für Arbeitsphysiologie und Rehabilitationsforschung der Universität Marburg, Robert-Koch Str.7A, D-3550 Marburg

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CHEMICALLY EVOKED SIGHS IN THE ANAESTHETIZED CAT AND IN THE SLEEPING INFANT AFTER LOSS OF CENTRAL CHEMOSENSITIVITY

M.E. Schlaefke, H. Gnuschke, T. Schaefer, D. Schaefer, and C. Schaefer

Sighs/h were evaluated as a chemically evoked respiratory 'arousal', and studied in aneasthetized cats before and after elimination of central chemosensitivity by local superfusion of glycine (2%) on the ventral medullary surface, and also in infants who did not respond to CO_2 (Ondine's Curse Syndrome, OCS). In the cat spontaneous sighs (1,2/h) vanished with glycine, blocking central chemosensitivity. In the control an increase of $FICO_2$ to 7 % in O_2 was accompanied by an increase of sighs to 22/h. During glycine-blockade sighs/h increased from 0 to 1,2 only when $FICO_2$ was increased now. Hypoxia (FIO_2 10 %) during glycine-blockade caused an increase of sighs to 133/h. In the spontaneously sleeping infant suffering from OCS PCO_2 rises to 75 mmHg and more during NREM-sleep and drops down to 55 mmHG during REM-sleep. We find 140 sighs/h linked to REM-sleep phases. Corresponding to the findings in the cat we conclude that sighs in OCS during sleep are due to peripheral chemoreceptor activity, and further that the threshold is high during NREM-sleep and lowered during REM-sleep. The life threatening aggravation of respiratory acidosis during NREM-sleep in infants with OCS may be explained by the threshold elevation of peripheral chemoreceptors and must not necessarily be the result of sleep dependent depression of respiratory sensitivity to CO_2 except the part of peripheral chemoreceptors.

Abteilung für angewandte Physiologie, Ruhr - Universität, D-4630 Bochum 1.

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FADE AT THE NEUROMUSCULAR JUNCTION IS CAUSED BY POSTSYNAPTIC EFFECTS

Ronald J. Bradley, Raimund Sterz and Klaus Peper

Fade or run-down at the mammalian neuromuscular junction (nmj) can be observed in the presence of 1 µM d-tubocurarine (d-TC) or in myasthenic patients: in a nerve train stimulation the 4th pulse elicit an amplitude in compound action potential (CAP) of, e.g. only 25% of that of the first pulse. It was assumed that positive feedback via postulated presynaptic AChR is necessary to counteract a natural fade in ACh-release. If the presynaptic AChRs are blocked by d-TC, a decreasing amount of ACh is released in each stimulation. We tried here to bypass the presynaptic part of nmj by applying constant doses of 4000 pCoulomb ACh ionophoretically very close to a voltage-clamped endplate. Under this condition, the fade ratio 4th/1d (endplate current, epc) was 0.93 (baseline-shift was corrected). If, however, 1 µM d-TC was added, the fade ratio became 0.60 (i.e., fade occurs). With much smaller ACh-pulses of 66 pCoulomb the ratio remained at 0.98 (i.e., no fade). It was further observed that small doses of α-Toxin indeed cause severe fade, although it is generally assumed that this toxin does not bind to presynaptic structures. We therefore propose here that the phenomenon of fade can be explained, partly or completely, by postsynaptic effects. Since fade occurred only under high saturating concentrations of ACh (as is the case for ACh released by the nerve), we propose that open-channel blocking or some other allosteric effect of d-TC on the post-synaptic AChR is the real cause of fade.

School of Medicine, University of Alabama, Birmingham, USA and Physiologisches Institut, Universität des Saarlandes, D-6650 Homburg/Saar.

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VOLTAGE DEPENDENT CONTRACTILE ACTIVATION AND INTRAMEMBRANE CHARGE MOVEMENTS IN MYOTOME CELLS OF THE LANCELET (BRANCHIOSTOMA LANCEOLATUM).

By R. Benterbusch and W. Melzer

According to a recent hypothesis Ca release from the sarcoplasmic reticulum became physically linked during evolution of vertebrate skeletal muscle to a modified Ca channel (the "dihydropyridine receptor") resulting in a more efficient control of this process by the membrane potential.
By studying the protochordate Branchiostoma we expect gaining information about the voltage-controlled Ca release in an early evolutionary state.
We performed whole-membrane voltage clamp on resealed fractions of myotome cells and studied contractile activation as a function of voltage. Contractions persisted after eliminating the Ca currents indicating a current-independent but voltage-controlled mechanism.
The voltage dependence of fractional isotonic shortening S/Smax could be fitted by a Boltzmann distribution of the form $S/Smax = 1/(1+\exp(V_{0.5}-V)/k)$ with $V_{0.5}=-6mV$ and $k=8mV$.
Searching for a voltage sensor signal we eliminated virtually all transmembrane ionic currents. A short transient current with the characteristics of an intramembrane charge movement persisted. Fitting the charge-voltage relation by a Boltzmann distribution we obtained a maximal charge density of 11pC per pF linear capacitance, a half maximal charge displacement at $V_{0.5}=-10mV$ and a value for k of 10mV, corresponding to the movement of 2.5 electronic charges per voltage sensor unit across the membrane.
With its voltage dependence and kinetics this charge movement signal could be involved in the control of intracellular Ca release as well as in the gating of the Ca current present in Branchiostoma myotome cells.

Lehrstuhl für Zellphysiologie, Ruhr-Universität Bochum, Universitätsstr. 150, Gebäude ND-4, D-4630 Bochum 1

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ACTIN-BINDING KINETICS OF WEAK-BINDING CROSSBRIDGES IN SKINNED SINGLE SKELETAL MUSCLE FIBERS: EFFECT OF CA^{++} ON ATTACHMENT AND DETACHMENT MEASURED BY MECHANICAL AND X-RAY DIFFRACTION EXPERIMENTS. Th. Kraft, L.C. Yu, B. Brenner

If calcium-regulation in muscle acts through steric blocking of the attachment of weak-binding crossbridges (CB), an almost all-or-none effect of calcium on attachment of these CB and also on their actin-affinity is expected. Biochemical findings show that calcium increases the actin-affinity of these CB only 2 to 5fold. Studying the calcium effect of weak-binding CB in the presence of MgATP is difficult because these CB always go through the complete CB-cycle, including weak- and strong-binding states. For this reason in our experiments we used the non-hydrolysable analogue $MgATP_{\gamma}S$ instead of MgATP. It will be shown that, different from other ATP-analogues, CB in the presence of $MgATP_{\gamma}S$ are of the weak-binding type. Mechanical measurements of fiber stiffness and the equatorial reflections [1,0] and [1,1] in X-ray diffraction patterns revealed that calcium has three main effects on CB in the presence of $MgATP_{\gamma}S$:
-At full saturation with $ATP_{\gamma}S$ (in the presence of calcium at 1°C at least 10 mM $MgATP_{\gamma}S$ are required) calcium increases the actin-affinity of weak-binding CB by only 2 to 10 fold, with truly complete saturation there might be no effect at all.
-While the fraction of attached CB is only slightly affected, the kinetics of reversible actin-attachment are very sensitive to calcium. In the presence of calcium the rate constant for CB-detachment, k^-, is reduced almost 100 fold, as revealed by stiffness-speed relations. Based on the small effect on actin-affinity, k^+ apparently is much reduced as well.
-We also found a profound calcium-effect on binding of $MgATP_{\gamma}S$ to the CB. In the presence of calcium at low temperature about 1000 fold higher $MgATP_{\gamma}S$-concentrations are required for full saturation. The large effect of calcium on stiffness and on equatorial diffraction patterns at high temperature were found to be due to incomplete saturation of CB with $MgATP_{\gamma}S$.
The small calcium-effect on the actin-affinity of weak-binding CB is inconsistent with an all-or-none effect on actin-affinity required for regulation of CB-activation via steric-blocking of attachment. More likely, there is great evidence that regulation of the CB-cycle occurs at a step subsequent to attachment.

Abt. für Allg.Physiologie, Universität Ulm, Albert Einstein Allee 11, 7900 Ulm

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REPETITIVE LENGTH CHANGES INCREASE THE MgATPase ACTIVITY OF SKINNED LIMULUS FIBRES

H.J. Kuhn and K. Blankenbach

Chemically skinned fibre bundles from the telson levator muscle of the horse shoe crab, Limulus, were incubated in solutions containing (in mM): ATP 5, EGTA 20, PEP 15, imidazole 40, NADH 0.3; pyruvate kinase 100 U/ml, lactate dehydrogenase 100 U/ml; pCa = 5, pMg 2.7, pH 6.7, 20 °C. The ATPase activity was determined photometrically using a coupled enzymatic system (PEP, NADH, pyruvate kinase, lactat dehydrogenase). Rectangular length changes (L = 1% Lo, ramp duration 2.5 - 10 ms, repetition frequency 12.5 - 200 Hz) were applied for 4 - 6 minutes.

The extra ATPase activity induced by the repetitive length changes was up to 50% the isometric value. Up to 50 Hz, the extra ATPase activity increased in direct proportion with the repetition frequency. During the period of applied length changes the mean fibre tension at rest length was lower than in the isometric state.

We think that the extra ATPase activity was induced during the release phases of the length changes. If so, during each release phase ATPase activity would be increased by 2 - 3% of its value in the isometric state although such a release phase does abolish tension completely.

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Abteilung für Allgemeine Physiologie der Universität Ulm, Albert-Einstein-Allee 11, D-7900 Ulm, FRG

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CONTRACTILE PROPERTIES AND FIBRE TYPE COMPOSITION OF SLOW AND FAST TWITCH MUSCLES OF THE WALTZING MOUSE

G. Asmussen, I. Kunze and K.-S. Pieper

Due to a genetically determined degeneration of the neuroepithelium of the vestibular apparatus waltzing mice (WM) show a motor hyperactivity characterized by stereotypically performed rotating movements. The influence of such a hypermobility on the contractile, histochemical and some biochemical properties of the slow-twitch soleus (SOL) and the fast-twitch extensor digitorum longus (EDL) muscles of adult (3 month old) WM was investigated. SOL and EDL of age matched mice with normal motor activity (CM) served as controls. In comparison to controls the time parameters of the single twitch and of the tetanic tension development of the SOL as well as the EDL of WM are prolonged. The SOL of WM develops a higher maximum tetanic force per unit cross-sectional area. The EDL of WM shows a smaller posttetanic potentiation (10%) than that of CM (44%); also the cooling potentiation of the EDL is smaller in WM (50%) than in CM (65%). The SOL of WM contains more type I fibres (84%) than that of CM (65%) and the activities of the lactate dehydrogenase, malate dehydrogenase and phosphoglycerate kinase are significantly lower in the SOL of WM. There are no striking differences in fibre type composition and enzyme activities measured between EDL of WM and CM. The results are discussed in comparison with other models of muscular hyperactivity.

Carl-Ludwig-Institut für Physiologie der Karl-Marx-Universität Leipzig, Liebigstr. 27. DDR-7010 Leipzig

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THE SITE OF CAFFEINE ACTION IN SLOW MUSCLE FIBRES OF THE FROG (*RANA ESCULENTA*)

J. Steinmetz and H. Schmidt

It is known that caffeine elicits contractures in frog twitch and slow muscle fibres(Axelsson & Thesleff, Acta Physiol.Scand.44:55,1958; Lüttgau & Oetliker,J.Physiol.Lond.194:51, 1968; Nasledov et al.,Experientia 28:1305, 1972). In the present experiments caffeine was applied extra- and intracellularly in frog slow fibres. When added to the bath solution the threshold caffeine concentration was near 2 mmol/l; maximum tension was obtained with 12-20 mmol/l. The dose-response (DR) curve was shifted towards lower concentrations after reducing the Ca^{++}-concentration of the medium. Long-lasting Ca^{++}-deprivation did not affect the contracture elicited with high caffeine concentrations, but reduced the effect of submaximal caffeine concentrations. Increasing the Ca^{++}-concentration had opposite effects. In contrast to K-contractures there was only a very small shift of the caffeine DR-curve when Ni^{++} replaced Ca^{++}.- Caffeine was also applied locally by pressure ejection from a micropipette positioned near the outer surface of a slow fibre. Localized contractures were obtained upon application of 47-138 pl caffeine solution (40 mmol/l; four fibres). After impalement of the fibres similar caffeine doses were never observed to elicit a mechanical response. It is concluded from these results that caffeine releases Ca^{++} from an intracellular store by acting on an external membrane site different from that which is involved in Ca^{++}-release following depolarization of the membrane with a K^{+}-rich solution.

I.Physiologisches Institut der Universität des Saarlandes, D-6650 Homburg/Saar, FRG

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DIVALENT CATIONS AND ACTIVATION OF CONTRACTILE FORCE IN SLOW MUSCLE FIBRES OF THE FROG

P. Krippeit-Drews and H. Schmidt

Isometric contracture tension was recorded from single slow fibres of *Rana temporaria* and *esculenta*. Omission of Ca^{++} from the medium had time dependent effects on the amplitude of K^{+}-contractures elicited by application of K^{+}-rich solutions. After short exposure to Ca^{++}-free (0 Ca^{++}) Ringer the threshold K^{+}-concentration was reduced, but the dose-response (DR-) curve was flattened, and maximum force was reduced by about 5-10 %. Replacement of Ca^{++} by 1.8 mmol/l Ni^{++} or Co^{++} shifted the DR-curve clearly towards higher K^{+}-concentrations. Substitution of Mg^{++} for Ca^{++} slightly increased the K^{+}-threshold, but the DR-curve was much less steep than those for Ca^{++}, Ni^{++} and Co^{++}. Long-term exposure (40-60 min) to 0 Ca^{++}-Ringer completely abolished the response to high K^{+}-Ringer. Contractile force could not only be restored by readmission of Ca^{++} but also by application of 1.8 mmol/l Ni^{++};Co^{++},Mn^{++} and Mg^{++} were less effective. In contrast to K^{+}-contractures the response to high caffeine concentrations (12-20 mmol/l) was very little affected, even after several hours of exposure to 0 Ca^{++}-Ringer. The following conclusions can be drawn from these results: 1. Excitation-contraction coupling in frog slow fibres is supported by divalent cations bound to an extracellular site; their order of efficiency is Ca^{++} = Ni^{++} > Co^{++} > Mn^{++} >> Mg^{++}. 2. Block of excitation-contraction coupling in 0 Ca^{++}-Ringer is not due to exhaustion of intracellular Ca^{++}.

I. Physiologisches Institut, Universität des Saarlandes, D-6650 Homburg/Saar, FRG

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SIZE AND HISTOCHEMICAL PROFILE OF RAT MUSCLE FIBERS AFTER DEAFFERENTATION AND IN COMBINATION WITH PERIPHERAL NERVE CRUSH

U. Hübschen and A.C. Nacimiento

Whereas much information has accumulated about changes in motor units after peripheral nerve lesion much less is known about the role of afferents during posttraumatic regeneration.To approach this question we first performed a unilateral rhizotomy of dorsal roots L2-L5 in rats and tested the influence of deafferentation.Then we added an ipsilateral peripheral crush of the common peroneal nerve.1,2,3,4 and 6 weeks p.o.we measured size and and made enzymhistochemical typing of single muscle fibers (slow twich oxidative,SO;fast twich oxidative glycolytic,FOG;fast twich glycolytic,FG) in tibial anterior or extensor digitorum longus muscle.
Deafferentation produces an ipsilateral decrease of SO-and FOG-fibers'size at 2 weeks,a temporar recovery at 4 weeks and a decrease again at 6 weeks.FG-fibers were larger than normal after 6 weeks.Contralateral fibers of all types behaved like ipsilateral SO and FOG-types.The relative amounts of FG-fibers increased ipsilateral in the first 4 weeks and decreased later,the contralateral SO-fibers'amount increased steadily in the whole observation time. The combined lesion produced ipsilateral a reduced FG-fiber size up to 4 weeks.FOG-fibers'size was first reduced but recovered at 4 weeks. The relative amount of FOG-fibers was higher than normal in the first 3 weeks,FG-fibers'amount was subnormal in the 2nd week.Possible mechanisms causing these changes will be discussed.

Neurochirurgisches Forschungslaboratorium der Universitätskliniken, D-6650 Homburg/Saar

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GABA and inhibitory nerve stimulation produce different effects on intracellular pH in crustacean muscle fibers

D.Günzel, S.Galler and W.Rathmayer

Most arthropod skeletal muscle fibers receive, in addition to their excitatory innervation, input from inhibitory motoneurons. The transmitter of these inhibitory neurons was shown to be γ-aminobutyric acid (GABA), which opens Cl^- channels. Inhibitory activity is able to release catch-like contraction, which is a prominent feature of many arthropod muscles. K. Kaila and J. Voipio (Nature 330:163, 1987) showed for the opener muscle in crayfish that GABA, in addition to increasing Cl^- permeability, also increased HCO_3^- conductance, which resulted in intracellular acidification of the muscle fibers.

To determine whether activity of inhibitory neurons produces a similar change in intracellular pH (pH_i) of crustacean muscle fibers, pH_i was continously monitored in fibers of walking leg muscles of the crab *Eriphia spinifrons* and the crayfish *Pacifastacus leniusculus* during repetitive stimulation of specific or common inhibitory motoneurons with 40/s for 2 to 5 min. Muscles were bathed in salines containing CO_2 (1%) / HCO_3^- (10mM). pH_i was measured with microelectrodes filled with a sensor based on the neutral ionophore ETH 1907 (time constant smaller than 0.6 s). In no preparation a change in pH_i was observed upon stimulation of the inhibitors (8 preparations, 25 fibers). On the other hand, in all muscle fibers investigated (including fibers which lack inhibitory innervation), 10^{-5} M GABA produced a fall in pH_i by 0.17 ± 0.07 (S.D.) pH units (n=12). We conclude that HCO_3^- conductance can not be activated through synaptic GABA-receptors. Therefore, release of catch-like contraction is evidently not caused by intracellular acidification of muscle fibers.

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Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz

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INFLUENCE OF FIBRE TYPE COMPOSITION OF SKELETAL MUSCLES ON TEMPERATURE DEPENDENCE AND ACTIVATION ENERGIES OF CONTRACTION PARAMETERS

U. Gaunitz and G. Asmussen

The temperature dependence of contractile parameters of slow twitch soleus (SOL) and fast twitch extensor digitorum longus (EDL) muscles were measured in mice, rats and guinea pigs in a range from 15° to 37°C. The logarithm of time parameters recorded were plotted versus the reciprocal temperature (Arrhenius plot). The slope of the relation is a formal measure of the energy of activation of the basic processes. Straight lines (constant activation energies) are not achieved in all cases. The best mathematical approximation is obtained by a polynom of second order (3 coefficients) that means linear changing activation energies. The activation energies of the contraction process of EDL and SOL are comparable. Considerable differences were found in the temperature dependence of the relaxation process of SOL of different species. In the sequence mouse-rat-guinea pig the activation energies increased. In the same sequence the proportion of slow twitch fibres in SOL increase. The SOL of guinea pig is composed by slow twitch fibres only but the SOL of mice and rats contains a remarkable amount of fast twitch fibres (II A). It is suggested that the strong temperature dependence of the twitch relaxation is caused by the amount of slow twitch fibres in the muscles. The more complicate kinetic mechanism cannot be explained by a single-step-reaction. The interpretations are supported by previous data of single fibres from a mouse foot muscle (J. Lännergren and H. Westerblad, J. Physiol. 390: 285, 1987).

Carl-Ludwig-Institut für Physiologie der Karl-Marx-Universität Leipzig, Liebigstraße 27, DDR-7010 Leipzig

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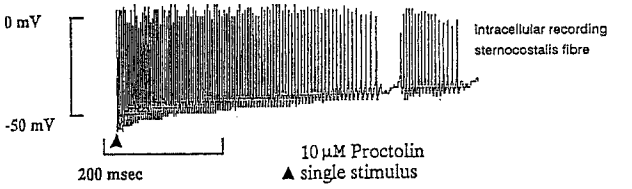
THE PENTAPEPTIDE PROCTOLIN INDUCES ELECTRICAL MYOTONIA IN MAMMALIAN MUSCLE

E. Wischmeyer, D. Költgen & H. Jockusch

In mammalian muscle, mutations as well as a number of agents that interfere with Cl^--conductance lead to a hyperexcitability of the plasma membrane and thereby to the symptoms of myotonia i.e. unscheduled runs of action potentials and aftercontractions. Proctolin (R-Y-L-P-T) has recently been reported to induce aftercontractions in insect muscle (Herrmann, C. E. & Schmitz, J. in: N. Elsner & W. Singer (eds.): Dynamics and Plasticity in Neuronal Systems, Thieme, Stuttgart, New York, p 129, 1989). Therefore we tested the effect of proctolin on mammalian muscle, both by intracellular recording and by contraction measurements. Whereas 10 µM proctolin after 30 minutes caused mouse sternocostalis muscle to produce 'myotonic runs' and visible contractions of the stimulated fibers, no aftercontractions were measured in whole EDL muscles.



The same phenomenon had been observed after treatment of mouse muscles with 4 ß-phorbolesters and other activators of protein kinase C (H. Brinkmeier & H. Jockusch, Bioch. Biophys. Res. Comm. 148, 1383-1389, 1987). The discrepancy between electrical and mechanical myotonia has not yet been resolved. As activators of protein kinase C reduce Cl^--conductance, proctolin may act likewise on ion channels via a second messenger mechanism, but it remains to be resolved which channels are affected (Walther, C. & Zittlau, K. E., J. of Physiol. 410, 32 P, 1989).

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Entwicklungsbiologie, W7, Universität Bielefeld, Postfach 8640, 4800 Bielefeld, Fed. Rep. Germany

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ADULT MOUSE INTEROSSEOUS MUSCLE HAS ION CHANNELS THAT ARE ACTIVE AT THE RESTING POTENTIAL

H. Brinkmeier, E. Zachar and R. Rüdel

We investigated the single channel activity in patches of sarcolemmal vesicles obtained from intact fibers by treatment with 140 mM KCl, and also in membrane patches of collagenase-treated fibers voltage-clamped in the cell-attached and inside-out configurations. The 3 methods yielded corresponding results. Under physiological ion conditions, we regularly observed several Ca^{2+}-dependent K channels and several delayed rectifier K channels in a patch. The Ca^{2+}-dependent K channels (145 pS) opened only at voltages positive to +40 mV, the delayed rectifier channels (17 pS) were activated by voltage steps from -60 to -20 or 0 mV. The inactivation of the delayed rectifier was never complete as the channels kept opening and closing at constant voltages between -50 and +20 mV.

In about 1 out of 5 patches, we recorded channel activity (see Fig. 1) near the resting potential (from -110 to -70 mV) with a linear current-voltage relationship yielding a conductance of about 10 pS. We have not yet established whether these channels conduct Cl or Na ions.

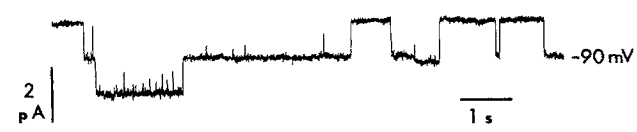

Fig. 1. Channel activity observed in an inside-out patch with 140 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ in the pipette and 140 mM KCl, 1 mM EGTA, 1 mM $MgCl_2$ in the bath. Both solutions were buffered with 1 mM Tris-Cl to pH 7.4.

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Abteilung für Allgemeine Physiologie der Universität Ulm, Albert-Einstein-Allee 11, D-7900 Ulm, FRG

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CHANGE IN THE CALCIUM TRANSPORT ATPase OF SKELETAL MUSCLE SARCOPLASMIC RETICULUM DURING HIBERNATION

B.Agostini,B.Soltau,L. De Martino and W. Hasselbach

Adult European hamsters (Cricetus cricetus L.) hibernating in a cold chamber at + 2°C, relative humidity 90 + 5%, starting in November were killed between December 10th and February 10th. Summer active animals, kept under standard laboratory conditions, were killed between June 10th and August 10th. Ca^{2+}-uptake and release of sarcoplasmic reticulum (SR) were studied with crude homogenates of mixed skeletal muscles in the presence of 5mM NaN_3 to inhibit mitochondrial activity. Ca^{2+}-release was studied by addition of 0.2M KCl - 0.01M caffeine to suspensions of Ca^{2+}-loaded SR vesicles. Muscle fibre types were identified and measured on cryostate sections stained by the usual methods.
Ca^{2+}-uptake rate and Ca^{2+}-accumulation of SR are increased by 50% and respectively 20% in the hibernating hamsters,as compared with the summer active animals. The study of Ca^{2+}-release by 0.2M KCl - 0.01M caffeine did not show any difference between the two groups of animals.Kinetic experiments on the dependence on free Ca^{2+} concentration of Ca^{2+}-uptake rate confirmed that hibernation alters the Ca^{2+}-transport ATPase of skeletal muscle SR in the sense of that of fast-twitch-muscle. By contrast, morphometric cytochemistry shows that the atrophy by 20% of various muscles of hibernating animals reaches values up to 40% in the fast-twitch-type II fibres, which are known to contain a well developed SR.
The results suggest the occurence during hibernation of some modulation in the properties of Ca^{2+}-transport ATPase of SR rather than an increase in the amount of the enzyme within the membrane.

Max-Planck-Institut für Medizinische Forschung, Jahnstraße 29, D-6900 Heidelberg

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EFFECTS OF MYOSIN LIGHT CHAIN PHOSPHORYLATION ON ISOMETRIC FORCE AND CROSS-BRIDGE TURNOVER KINETICS

B. Brenner, I. Morano*

Isometric tension and the rate constant of force redevelopment after a short period of shortening and restretch to the initial sarcomere length (k_{redev}) increase upon Ca^{2+} (Brenner, PNAS 85, 1988) and myosin P-light chain phosphorylation (MPLC-p) (METZGER et al. J.Gen.Physiol. 93, 1989). Here we studied the effect of incubation of Ca^{2+}-activated skinned psoas fibers with myosin light chain kinase (MLCK) on the time-course of k_{redev} and MPLC-p. Fibers were incubated at 15°C with contraction solution containing 1µM calmodulin and 100nM MLCK. Single fibers were analyzed by a Micro-2D-PAGE-technique after TCA-denaturation. Values are means±SEM.
Incubation with MLCK at pCa 5.6 (15% activation) increased tension from 15.1±5mN to 30.5±11mN, k_{redev} from 1.6±0.2s^{-1} to 2.8±0.1s^{-1} (n=5) while MPLC-p raised from 10% to 25%. The half-time for the increase in k_{redev} and MPLC phosphorylation ranged between 30s and 60s. Effects of MPLC-p on k_{redev} was lower at higher Ca^{2+} concentrations: around 90% of full Ca^{2+}-activation, tension increased from 93.4±5.3mN to 99.2±4.1mN while k_{redev} increased from 4.9±0.6s^{-1} to 6.4±0.5s^{-1} (n=3). Our observations suggest an increase in cross-bridge turnover kinetics (specifically f_{app}) with MPLC-p thus favouring accumulation of force generating cross-bridges. The half-time in the order of 30-60s shows that MPLC-p has the potential of representing an important mechanism for short-term modulation of contractile function in striated muscle.

Abteilung für Allgemeine Physiologie, Universität Ulm, Oberer Eselsberg, 7900 Ulm, and *II. Physiologisches Institut, Universität Heidelberg, INF 326, 6900 Heidelberg

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INCREASED EXTRACELLULAR K^+-CONCENTRATION OR TETANIC STIMULATION REDUCE THE CONDUCTION VELOCITY OF ISOLATED FAST AND SLOW TWITCH RAT MUSCLES.

F. Kössler, F. Lange, and G. Küchler

Extracellular K^+ (K^+_e) accumulation during muscle activity has been suggested as a factor of changes in the conduction velocity (c.v.) of action potentials and of fatigue (C. Juel, Pflügers Arch. 406: 458, 1986). A study has been made to compare the effects of variation in K^+_e with the effect of tetanic stimulation on c.v. The c.v. was measured by stimulation (single impulse, 0.02 Hz) of isolated fast twitch m. extensor digitorum longus (EDL) and of slow twitch m. soleus (SOL) held in bath solution (22 °C) containing 0, 5, and 10 mmol/l K^+. In Tyrode solution (5 mmol/l K^+) the c.v. of the muscles was1.14 ±0.27 m/s (8 EDL) and 0.97 ±0.18 m/s (10 SOL). In solution without K^+ the c.v. increased by about 30 % in SOL but 60 % in EDL muscles; in solution with 10 mmol/l K^+ the c.v. dropped by about 15 % (SOL) and 30 % (EDL); a rise to 15 mmol/l K^+ did not increase this effect markedly. The changes were completely (SOL) or partly (EDL) reversible in Tyrode solution within 30 minutes.
Immediately after tetanic stimulation in Tyrode solution (3x20 s, 50 Hz) the maximum developed tension was decreased to 40 % in SOL but less than 5 % in EDL muscles; the c.v. dropped to 80 % (SOL) and 60 % (EDL).
The significantly stronger influence of the K^+_e on EDL muscles parallels their higher fatiguability. It seems that K^+_e-accumulation during muscle activity acts as one factor causing fatigue.

Zentralinstitut für Arbeitsmedizin der DDR, DB Arbeitsphysiologie
Nöldnerstraße 40-42, DDR-1134 Berlin

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A PEPTIDE MIMETIC OF THE CARDIAC TROPONIN-I SEQUENCE 137-148 INHIBITS CONTRACTION OF SKINNED CARDIAC AND SKELETAL MUSCLE

J.C. Rüegg[1], C. Zeugner[1], J. Van Eyk[2] und R.S. Hodges[2]

In calcium activation of muscle contraction the interaction of troponin-C and troponin-I is increased. In vitro this interaction can be competitively inhibited by a peptide mimetic of the TnI-recognition site for troponin-C (Rüegg et al., Pflügers Arch. 414:430, 1989). Its cardiac analogue has the sequence TnI 137-148 Gly-Lys-Phe-Lys-Arg-Pro-Thr-Leu-Arg-Arg-Val-Arg and inhibits partly (22±2.6% at 50 µM) the isometric contraction of skinned pig ventricle muscle but not that of skinned rabbit psoas muscle fibres under conditions of maximal Ca^{2+}-activation (pCa 4.3). In both preparations, the calcium/force relationship is shifted towards higher Ca^{2+}-concentrations by 0.3 pCa-units by the peptides. At submaximal Ca^{2+}-activation, contraction is also inhibited by the pro-143-analogue peptide, whereas the gly-analogue activates as a Ca-sensitizer, see Table:

Peptide (50 µM)	TnI (137-148)	Pro-143	Gly-143
Cardiac fibres	37.3±2.8(5)	30.7± 6.4(4)	201.1±15.7(6)
Skeletal fibres	12.3±3.3(6)	18.18±7.0(3)	72.4± 12.9(3)

Figures are means ±SE of fractional force (in% of control) at pCa 5.2, pH 6.7, 10 mM ATP, 12 mM $MgCl_2$, 5 mM CaEGTA, 30 mM imidazole, 10 mM creatine phosphate, 5 mM P_i, I=0.08 of triton-skinned fibre bundles.

[1]II. Physiologisches Institut, Univ. Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg
[2]Dept. Biochemistry, Univ. of Alberta, 474 Medical Sciences Building, Edmonton, Canada T6G, 2H7

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ACETYLCHOLINE-INDUCED CURRENTS IN DENERVATED MOUSE SOLEUS: EFFECTS OF ANTAGONISTS

H. Lorkovic

ACh-induced currents were measured in partially depolarized mouse soleus muscles denervated for 3-6 days by using a point voltage clamp. When 0.25 µM d-tubocurarine (d-Tc) were used, the weak currents provoked by 0.1 µM ACh at a holding potential -20 mV were barely affected, while the large currents provoked by 2-5 µM ACh were decreased by more than 50%. By contrast, weak and strong ACh-induced currents were proportionally diminished when, under similar conditions, 20-100 µM ipratropium was used. Currents were proportionally diminished by d-Tc when the holding potential was set at +15 mV, a level corresponding to the reversal potential of the current provoked by low ACh concentrations. In non-denervated flexor digitorum brevis muscles, d-Tc had the same relative effect at low and at high ACh concentrations independent of the holding potential. The reversal potential for the ACh-induced currents was about +14 mV for low [ACh] and decreased to about +3 mV with 4 µM ACh in denervated soleus muscles. It was concluded that denervated soleus muscles, in contrast to endplate regions of non-denervated mouse muscles, contain a low proportion of highly ACh-sensitive, weakly d-Tc sensitive, predominantly Na^+-permeable ACh receptors. These receptors are presumably responsible for the non-fading ACh-induced currents described before for the denervated mouse soleus muscle.

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Abteilung für Allgemeine Physiologie der Universität Ulm, Albert-Einstein-Allee 11, D-7900 Ulm, FRG

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Depression of neuromuscular transmission by acetazolamide in the rat diaphragm

N. Clemens and P. Scheid

We have earlier reported (Pflügers Arch. **406**, 1986, R 29) a reduction in the force of isometric contraction of the rat diaphragm after exposure to the carbonic anhydrase (CA) inhibitors acetazolamide and ethoxzolamide. We have now recorded end-plate potentials (EPP) in the rat diaphragm incubated in the presence of the CA inhibitor acetazolamide (Diamox). The muscles were incubated in Krebs-Ringer solution (pH 7.4, 25°C, 0.1 µM tubocurarine to prevent twitching), and EPPs were evoked by supramaximal electric nerve stimulation with trains (4 or 10 Hz) of short pulses. After dissection, the diaphragm was incubated for 1 hr before Diamox was added (or not: control); after 1 hr of exposure, Diamox was washed out again. The transmission ratio (= number of EPPs per number of stimuli applied to the nerve) decreased from 0.80 before, to 0.2 at the end of 1 hr exposure to Diamox, and returned to 0.8 after wash-out of the inhibitor. For the controls, the respective values were: 0.8, 0.8, 0.75. These results show that CA inhibitors interfere with the synaptic transmission at the neuromuscular junction. In face of unaltered miniature endplate potentials (frog cutaneous pectoris, Pflügers Arch. **406**, 1986, R 29), this suggests an involvement of CA in the presynaptic transmitter release.

Institut für Physiologie, Ruhr-Universität, Universitätsstraße 150, D-4630 Bochum.

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AGONISTS CAN DEPOLARIZE SMOOTH MUSCLE CELLS ISOLATED FROM HUMAN MESENTERIC ARTERY VIA A Ca^{2+}-ACTIVATED CHLORIDE CURRENT

U. Klöckner

Vascular myocytes were enzymatically isolated from human Aa. mesenterica inf. (surgical preparations). The cells were voltage clamped with single patch electrodes filled with 150 mM CsCl, 20 µM EGTA, 10 mM HEPES/CsOH, adjusted to pH 7.3 at 35 °C. When the cells were held at -50 mV (≈resting potential) bath application of agonists like acetylcholine, endothelin or the stable thromboxane analogue U 46619 induced an inward current. The inward current had an amplitude of 200-2000 pA per cell, peaked within 1-2 s and decayed in the following 6 s. Inward currents of similar amplitude could be evoked by agonist application in the presence of 5 mM e.c. Ni^{2+} or when the e.c. Na^+ were replaced by $Tris^+$. Both results argue against a major contribution of the Na-Ca exchanger to the inward current.

The reversal potential of the inward current was close to 0 mV and insensitive to changes in e.c. cation concentration. But it shifted as predicted by the Nernst equation when the e.c. or i.c. chloride concentration was changed, suggesting chloride to be the charge carrier of this current.

Bath application of 20 mM caffeine induced a current similar to the one described above. The current was not seen when the i.c. Ca^{2+} was buffered with 10 mM EGTA and 5 mM BAPTA. In the presence of caffeine, agonists were no longer able to evoke an inward current and vice versa. From these results it is concluded that the chloride current is Ca^{2+}-activated. Most likely the Ca^{2+}- ions are released by caffeine or agonist application from the same intracellular calcium store in these cells.

The present experiments demonstrate that agonists can depolarize smooth muscle cells from human mesenteric artery by induction of a Ca^{2+}-activated chloride current.

Zentrum für Physiologie, Robert-Koch-Str. 39, 5000 Köln 41

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DIHYDROPYRIDINE-SENSITIVITY OF THE TRANSIENT, CALCIUM INDEPENDENT POTASSIUM OUTWARD CURRENT IN SINGLE SMOOTH MUSCLE CELLS OF GUINEA PIG PORTAL VEIN

Th. Noack, P. Deitmer, K. Golenhofen, E. Lammel

Under voltage clamp, single cells from guinea pig portal vein responded to positive going step voltage commands from a holding potential of -70 mV with an initial inward current (I_{Ca}) which inactivated in approximately 20 ms. It was followed by an outward current consisting of three components: a transient outward current (I_{TO}) inactivating within 5-10 s, a long lasting 'steady state current' (I_{SS}), and, superimposed on I_{TO} and I_{SS}, brief current peaks called 'spontaneous transient outward currents' (STOCs). I_{TO} was calcium independent, it was not altered by e.c. calcium removal and addition of 1 mmol/l EGTA. I_{TO} was also unaffected by increasing i.c. EGTA from 1 mmol/l to 10 mmol/l. The reversal potential of I_{TO} was at the potassium equilibrium potential, and classical potassium channel blockers like TEA (10 mmol/l) and 4-aminopyridine (5 mmol/l) had a suppressing effect on this current. Caffeine (1 mmol/l e.c.) selectively blocked I_{TO}. Nifedipine (NIF) at a concentration of 10^{-6} mol/l (sufficient to block the greater part of the calcium inward current) had a negligible effect on I_{TO} and I_{SS}. However, at a concentration of $3 \cdot 10^{-5}$ mol/l, in calcium-containing as well as in calcium-free solution with EGTA (1 mmol/l), NIF completely blocked I_{TO} without affecting I_{SS}. This effect was reversible. In normal calcium-containing solution, the calcium agonistic dihydropyridine, the (-)-enantiomer of BAY K 8644 (BAY K), increased I_{Ca} and STOCs, and at high concentration ($3 \cdot 10^{-5}$ mol/l) selectively inhibited I_{TO}, similarly to NIF. The inhibitory effect of the dihydropyridines NIF and BAY K at high concentrations is therefore independent of the typical effect of these substances on calcium channels. Moreover, it is not an unspecific effect on membrane channels, since I_{SS} persisted and STOCs even increased in frequency and amplitude during BAY K-treatment.

Physiologisches Institut der Universität Marburg
Deutschhausstr. 2, D-3550 Marburg/Lahn

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WHOLE-CELL RECORDING OF HORMONALLY REGULATED CALCIUM CURRENT IN SMOOTH MUSCLE

Andrea Welling[1], Jochen Felbel[2], Franz Hofmann[2] and Klaus Peper[1]

Calcium currents were examined in single, freshly isolated bovine tracheal smooth muscle cells by the whole-cell patch-clamp technique. After blocking potassium currents by cesium- and tetraethylammonium-chloride (TEA-Cl) small inward currents were detected and identified as L-type calcium currents by the I-V relationship and organic and inorganic calcium channel blockers.
The calcium peak current (I_{Ca}) was increased 2.7 ± 0.2 (n = 54) fold by isoproterenol (EC_{50} ≈ 3 nM). The isoproterenol effect was mediated by the β_2- but not by the α-adrenergic receptor. Carbachol decreased the isoproterenol enhanced I_{Ca}. The inhibition disappeared with atropine.
Internal dialysis of the cell with cAMP (100 µM) or cAMP-kinase had no effect on basal or isoproterenol stimulated I_{Ca}.

Conclusion: L-type calcium current can be regulated by the ß-adrenergic receptor in freshly isolated bovine tracheal smooth muscle. Apparently, this regulation does not involve cAMP or cAMP-kinase.

1: Physiologisches Institut
2: F.R. Medizinische Biochemie

Medizinische Fakultät, Universität des Saarlandes, D-6650 Homburg-Saar

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ENDOTHELIN INDUCED CONTRACTIONS MAY BE RELAXED WITHOUT DE-PHOSPHORYLATION OF MYOSIN LIGHT CHAINS.

S. Katoch, T. Watanabe*, and G. Pfitzer

Endothelin (ET, $3x10^{-10}$–10^{-6} M) elicits potent contractions in smooth muscle strips from lamb trachea. Low concentrations (10^{-8} M) induce a slow, monotonous rise in tension attaining its steady state value after 30 min comparable to contractions induced by high K^+. Myosin light chain phosphorylation (MLCP) rises to 0.53 mol Pi/mol MLC within 2 min and declines to 0.42 mol Pi/mol MLC after 30 min. In contrast, 10^{-6} M ET elicits a biphasic tension response showing an initial rapid increase in force followed by a further slow rise until steady state force is reached after 20-30 min. Two minutes after onset of stimulation, MLCP amounts to 0.63 mol Pi/mol MLC which does not decline thereafter. Both at low and high concentrations of ET, the phosphorylation pattern differs from carbachol induced contractions, since about 15% of the phosphorylated MLC appear to be diphosphorylated, a pattern that is also observed after inhibition of MLC phosphatase with okadaic acid (Obara et.al. Pflügers Arch 414:134,1989) suggesting that ET might indirectly affect phosphatase activity. ET induced contractions are relaxed by Verapamil (2 µM), Nitrendipine (2-10 µM), D 600 (2 µM) without dephosphorylation of MLC. W-7 (200 µM), and H-7 (25-100 µM) induce relaxation which is associated with the disappearence of the di- but not of the monophosphorylated MLC species. In contrast, relaxation with a K-channel agonist (EMD 52692, E. Merck, Darmstadt) is associated with the dephosphorylation of MLC. According to Hai and Murphy (Annu Rev Physiol 51:285,1989), the phosphorylation theory accounts for tension maintenance both at high and low levels of MLC phosphorylation but would not account for relaxation at high levels of MLC phosphorylation. Thus an additional, phosphorylation independent relaxation mechanism must be postulated.

*Central Research Division, Takeda Chemical Industries, Osaka 532, Japan and II. Physiologisches Institut, Universität Heidelberg, INF 326, D-6900 Heidelberg

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SPONTANEOUS TONIC CONTRACTIONS OF PORCINE DUODENUM AND INHIBITORY INNERVATION WITH VIP AS A PUTATIVE TRANSMITTER

K. Mandrek and K. Milenov

Mechanical activity was recorded in isolated preparations from the circular and longitudinal layers of different sections of porcine duodenum. Strong spontaneous tonic contractions were observed in the circular preparations. These tonic duodenal contractions (TDC) were weak or absent in the first 1 or 2 cm postpyloric (pp), reached a maximum in the region 3 to 10 cm pp, and disappeared further distally. In the longitudinal preparations no spontaneous tonic contractions could be observed. Strips with strong TDC also possess a strong inhibitory innervation. Electrical field stimulation (EFS) with a single pulse of 10 V and 3 ms duration produced a short lasting inhibition to near zero tone. Phasic rhythmical contractions of both circular and longitudinal preparations could be suppressed by application of the calcium channel blocker nifedipine. The TDC, however, were resistant to nifedipine and could be suppressed by sodium nitroprusside, similar to tonic contractions of fundic preparations from the stomach.
Vasoactive intestinal peptide (VIP) inhibited both the spontaneous tonic and rhythmic activity (half suppression at $2 \cdot 10^{-9}$ mol/l).
Neither the inhibitory effect of VIP nor that of EFS were influenced by guanethidine, atropine, propranolol, phentolamine, apamin and methysergide. This suggests that the transmitter system is neither cholinergic, nor adrenergic, nor serotonergic, nor purinergic (ATP had no effect).
Conclusion: Strong tonic contractions exist in circular preparations of porcine duodenum, which are similar in nature to fundic tone of the stomach and different in their mechanism from phasic rhythmic contractions. Duodenal tone is probably part of a control process for the regulation of duodenal transit which has not yet been described in the literature. A strong inhibitory innervation is observed in the tonic regions of the duodenum with VIP as a putative transmitter.

Institut für Normale und Pathologische Physiologie, Philipps-Universität Marburg, Deutschhausstraße 2, D-3550 Marburg

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MODULATION OF URETERIC MOTILITY BY CAPSAICIN AND SENSORY NEUROPEPTIDES IN GUINEA-PIGS AND CHICKEN

K. Hammer, W. Rössler, H. Sann and Fr.-K. Pierau

Ureteric motility of rodents can be modulated by a release of substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) from capsaicin sensitive afferents. Since birds are highly resistant to different actions of capsaicin, we have compared the effect of sensory peptides and capsaicin on the ureteric motility of guinea-pigs and chicken. Freshly dissected ureters were cannulated and superfused with oxygenated artificial interstitial fluid at 37 °C. Changes in intraluminal perfusion pressure indicated ureteric motility. The distribution of SP and CGRP in the ureter were examined using immunohistochemistry.
In the guinea-pig ureter NKA (0.005-2.5 µM) and SP (0.1-2.5 µM) evoked dose dependant rhythmic contractions, whereas CGRP (0.1 µM) inhibited spontaneous or provoked rhythmic peristalsis. Contractions were also produced by capsaicin 1 µM. This effect was completely desensitized upon repeated applications of capsaicin. After desensitization, however, capsaicin in concentrations >10 µM inhibited contractions induced by NKA, indicating an unspecific direct effect of the drug on smooth muscle. In the chicken ureter neither SP or NKA (up to 5 µM) nor capsaicin (0.1-100 µM) evoked contractions. Rhythmic peristalsis, however, was produced by KCl (10-30 mM) or Noradrenalin (1-10 µM) and was suppressed by CGRP (>0.3 µM) or capsaicin (10-100 µM). The demonstrated species differences appear to be not due to the absence of peptidergic afferents, since SP and CGRP containing nerve fibres were found in the ureter of both species.
The data suggest the lack of a specific action of capsaicin on afferent neurones (release of tachykinins) in the chicken.

MPI für physiol. und klin. Forschung, W.G. Kerckhoff-Inst., Parkstr. 1, D-6350 Bad Nauheim

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MECHANICAL RESPONSES OF ISOLATED PORCINE CORONARY ARTERIES TO INTRACELLULAR ALKALINE OR ACID LOADING

H. Nguyen-Duong

As previously reported (Nguyen-Duong, Pflügers Arch., 411, R203, 1988 and Nguyen-Duong, Bamosa and Spurway, Proc. of the Int.Union of Physiol.Sciences, Helsinki, p.398,1989), the pattern of contractile responses of isolated porcine coronary arteries (PCA) to application and removal of weak acids or bases at constant external pH_o coincides exactly with expected pH_i changes, pH_i decrease leading however to contraction and pH_i increase to relaxation. The phasic, amiloride-sensitive propionate-induced contractions were strongly enhanced in Na-free PSS and after treatment with ß-methyldigoxin; they were unaffected by incubation in Ca-free, EGTA containing PSS. The more complex patterns of mechanical responses to removal and reintroduction of external CO_2 or to addition and removal of NH_4^+ though very similar, differed however in that, in the first case the initial relaxation was maintained, while transient in the latter case, i.e. the tension recovery following the transient relaxation overshooted the initial tension. The rate of this SITS-sensitive recovery phase was slowed down in CO_2-free, HEPES-buffered PSS and strongly enhanced by TEA, Ba ions, Na-orthovanadate and ß-methyldigoxin. NH_4^+ removal induced a rebound, generally phasic contraction, which could be made tonic by removal of external Na^+ or by applying inhibitors of the Na-H exchange. The NH_4^+ induced effects were completely abolished after 2 h incubation in Ca-free EGTA-containing PSS. All aforementioned effects could also be observed with several weak acids (Na acetate, Na butyrate) and bases (Methylamine, Trimethylamine) in PCA preparations stimulated by various agonists (5-HT, Histamine, ACh). The results are consistent with the assumption of a dual pH_i regulatory mechanism involving the activation of a $Cl\text{-}HCO_3$ exchanger for recovery from alkaline loads and of a H-Na exchanger for recovery from acid loads and may be interpreted on the basis of a competition between Ca^{2+} and H^+ ions for common intracellular binding sites.

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Abt. für Allg. Physiologie, Uni. Ulm, Fed. Rep. of Germany.

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CA-INFLUX THROUGH ATP-OPERATED NON-SELECTIVE CATION CHANNELS IS LARGE ENOUGH TO INACTIVATE VOLTAGE-OPERATED CALCIUM CURRENTS

P. Schneider and G. Isenberg

In myocytes isolated from the urinary bladder of the guinea-pig, we studied the effect of ATP on membrane currents with the voltage-clamp method. K-currents were reduced with CsCl-electrodes. At 22°C, the isolated myocytes were continuously superfused by solutions containing 3.6 mM $CaCl_2$.

At -60 mV, bath-application of ATP induced an inward current $I_{in,ATP}$ that rapidly activated. It decayed in the constant presence of ATP, recovery from desensitization required about 5 min. 1 μM ATP induced 50% of the maximal $I_{in,ATP}$ 520 ± 120 pA (mean ± S.E., n=15) which was obtained using a saturating concentration (50 μM ATP). $I_{in,ATP}$ had a reversal potential at -6 mV ± 2.2 mV. Changes in external Na- or Ca-concentration modified amplitude and reversal potential of $I_{in,ATP}$ in a comparable extent as expected from a permeability ratio pNa:pCa ≈ 1 :3 (myocytes from ear artery, [1]). These properties predict $I_{in,ATP}$ to increase the cytosolic Ca-concentration $[Ca^{2+}]_c$.

We monitored effects of $I_{in,ATP}$ on $[Ca^{2+}]_c$ through the Ca-mediated inactivation of voltage-operated Ca-current I_{Ca} (160 ms long pulses from -60 to 0 mV). Bath-application of ATP reduced I_{Ca} by 50-90%. Reduction was not seen when e.c. $CaCl_2$ was replaced by $BaCl_2$. Reduction of I_{Ca} was prevented by 40 mM EGTA in the pipette solution. Reduction of I_{Ca} was mimicked by 20 mM caffeine, most likely due to Ca-release from the SR. In the continuous presence of caffeine, when the SR can no longer relase Ca, ATP still induced $I_{in,ATP}$ and also reduced I_{Ca} as in the absence of caffeine. The results suggest that an ATP-induced increase in $[Ca]_c$ is due to influx and not to Ca-release from the SR. Hence, Ca-influx through ATP-operated channels is large enough for increase in $[Ca^{2+}]_c$ and contractile activation.

[1] Benham,C.D. and Tsien,R.W., Nature 328, 275-278 (1987)

Zentrum für Physiologie, Robert-Koch-Str. 39, 5000 Köln 41

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ACETYLCHOLINE INCREASES INTRACELLULAR CALCIUM IN SINGLE CELLS OF CULTURED HUMAN CILIARY MUSCLE CELLS

F. Stahl, A. Lepple-Wienhues, C. Korbmacher, H. Haller, M. Wiederholt

The ciliary muscle is known to play an important role in regulation of accommodation and aqueous humor outflow. In order to get more insight into cellular mechanisms involved in ciliary muscle contraction we performed measurements of free intracellular calcium, $(Ca)_i$, in single cells of confluent monolayers of an established human ciliary muscle cell line (H7CM). $(Ca)_i$ was measured using the fluorescence properties of the calcium indicator FURA-2.

Application of acetylcholine lead to a typical biphasic increase of intracellular free calcium. First, a transient peak elevation of $(Ca)_i$ occured, followed by a recovery to a sustained elevated $(Ca)_i$ level. In most of the experiments oscillations of $(Ca)_i$ occured during the recovery phase and the sustained elevation of $(Ca)_i$. The acetylcholine induced increase of $(Ca)_i$ was dose dependent (10^{-8} to 10^{-3}) and blocked in the presence of atropine.

The initial calcium peak was not abolished either in the presence of verapamil (10^{-4} M) or in the absence of extracellular calcium (1 mM EGTA). In contrast, the sustained elevation of $(Ca)_i$ could be completly blocked by withdrawal of extracellular calcium and was partly inhibited by verapamil.

We conclude that the initial $(Ca)_i$ peak is due to calcium release from intracellular stores and the sustained $(Ca)_i$ elevation is most probably mediated by opening of Ca-channels of the muscle cell membrane.

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Institut f. Klin. Physiologie, Universitätsklinikum Steglitz, Hindenburgdamm 30, D-1000 Berlin 45.

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ENDOTHELIN-EVOKED CONTRACTIONS IN ISOLATED BOVINE CILIARY MUSCLE STRIPS

A. Lepple-Wienhues, F. Stahl, M. Gebauer, U. Willner, C. Korbmacher and M. Wiederholt

Endothelin was found to mediate contractions in many different types of vascular, uterine, intestinal, and bronchial smooth muscle tissue. Recently, we described changes in the membrane potential of a human ciliary muscle cell line evoked by endothelin.

Small ciliary muscle strips (5 x 0.5 mm) were prepared from freshly enucleated bovine eyes. Force measurement was performed using a "position clamp" device similar to that described by Brutsaert et al. (Circ.Res 1988;62:358). A coil was suspended in a magnetic field, and shortening of the muscle strips resulted in a small rotation of the coil. This rotation was instantaneously corrected by an optoelectronic feed-back mechanism. Coil current was calibrated in Micronewton (µN). Maximal contractions by 10^{-4}M/l acetylcholine were adjusted near 100 µN.

Endothelin evoked concentration-dependent contractions in bovine ciliary muscle. 10^{-9}Mol/l had no effect. Related to the maximal acetylcholine response, the maximal contraction amounted 274 ± 2.8 % (n=8), and was reached at $5 \cdot 10^{-8}$Mol/l. $1 \cdot 10^{-7}$ Mol/l evoked no further contraction. In contrast to cholinergic agents the effect of endothelin was slow in onset, the maximum was reached after 10 min. In presence of the drug tension decreased to the basal level within 30 min. Repeated stimulation diminished the answer to endothelin without apparent reduction of the acetylcholine response. Recovery of the endothelin response could not be seen before 60 min.

Endothelin seems to be a most potent contracting agent in ciliary muscle. The observed action of endothelin in this tissue is comparable to that in other smooth muscle tissues. Endothelin may play a role in accommodation and regulation of the intraocular pressure.

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Institut für Klinische Physiologie, Freie Universität Berlin, Klinikum Steglitz, Hindenburgdamm 30, D-1000 Berlin 45

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REGULATION OF MYOSIN ISOENZYME EXPRESSION IN THE RAT HEART AND UTERUS DURING PREGNANCY

I. Morano, C. Bialojan, M. Lengsfeld and V. Kyrtatas

We studied myosin isoenzymes (MI) of rat ventricle and uterus during pregnany and compared these MI with those from rat aorta and portal veine using the native pyrophospate polyacrylamide gelelectrophoresis and western blot analysis with antibodies directed against smooth muscle myosin heavy chains and filamin. All MI of smooth muscle revealed a higher mobility in the gel than the three ventricular MI (V1, V2, and V3). In the uterus of non-pregnant and early pregnant rats we observed two bands designated with increasing mobility as F and SMu_1. In late pregnant rats (18-21 d gravid) SMu_1 disappeared and a second myosin band (SMu_2) appeared with an even higher electrophoretic mobility than all the other smooth muscle MI. F but not SMu_1 and SMu_2 reacted with the filamin antibody, while SMu_1 and SMu_2 but not F reacted with the antibody against smooth muscle myosin.
SMu_1 revealed the same electrophoretic mobility as the main MI band of aorta and portal veine.
In the late stage of pregnancy ventricular MI shifted to the V3-Form the V1/V3-ratio being 57/15 in the non-pregnant and early pregnant (up to 12 d gravid) and 31/35 after 20 d of pregnancy.
We conclude that especially during late stages of pregnancy regulation of MI expression in uterus and ventricle is distinct from non-or early pregnant stages.

II. Physiologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg

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The composition and organization of the cytoskeleton of cultured vascular smooth muscle cells and the influence of culture conditions

Peter C. Dartsch

The cytoskeleton components of smooth muscle cells can be divided into three categories: 1. Microfilaments, i.e. actin and actin-binding proteins; 2. intermediate-sized filaments; and 3. microtubules. The pattern of distribution of these components in stationary cultivated smooth muscle cells is presented in detail. Alterations of this pattern are observed when cells are subjected to a mechanical stimulus (= cyclic and directional stretching) imitating the volume pulse of the abdominal aorta. Under serum-containing cell culture conditions, the α-actin expression of smooth muscle cells is influenced by the growth state of the cells and has a maximum expression at confluency. While the expression of smooth muscle myosin, smooth muscle tropomyosin and 140 kD-caldesmon is decreased during phenotypic modulation from contractile to synthetic phenotype, smooth muscle α-actin is also expressed in cells which have been subcultivated for several times. Smooth muscle cells cultivated with a fibroblast-conditioned medium exhibit a decreased α-actin expression. Moreover, under serum-free culture conditions using various serum replacers alterations in cytoskeletal organization occur due to cell attachment. In addition, in serum-free cultures a significant decrease in smooth muscle γ-actin content is observed. The findings are discussed in relation to atherogenesis in vivo and the alterations in smooth muscle cell cytoskeleton composition and organization during migration and proliferation from the media into the subendothelial space.

Physiologisches Institut I der Universität Tübingen, Gmelinstr. 5, D-7400 Tübingen 1, FRG

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BOTH MYOSIN LIGHT CHAIN PHOSPHORYLATION-DEPHOSPHORYLATION AND ACTIN POLYMERIZATION-DEPOLYMERIZATION MAY INDEPENDENTLY REGULATE ACTIVE FORCE IN SKINNED MESENTERIC ARTERIOLES (MA)

P.J. Boels and G. Pfitzer

MA (diameter, 50-100 µm) and the main mesenteric artery (MMA) were skinned (1% Triton X, 4 hrs) and mounted in relaxing solution (Ca^{2+}≤10nM, pH=6.7) to measure circumferential force development. Raising free Ca^{2+} (≥1µM) in presence of calmodulin (0.1-0.3µM) caused active force development to similar maxima (MA, 1.169 ±0.128; MMA, 1.363 ±0.114 mN/mm²; Ca^{2+}=40µM) although MA were more sensitive to Ca^{2+} than MMA. Okadaic acid (OA, 1µM), a phosphatase inhibitor, further increased force at free Ca^{2+} ≥1µM. Phalloidin (PH, 1-100µM) induced slow (1hr), dose-dependent relaxations at free Ca^{2+}≥1µM. Maximal force (Ca^{2+}=40µM) after PH-exposure and wash-out was still reduced as compared to maximal force before exposure except when PH was added at Ca^{2+}≤10nM. PH-relaxations were still observed in presence of OA and PH did not inhibit myosin light chain kinase activity. Cytochalasin D (CD, 20nM-20 µM) caused rapid (<20'), dose-dependent relaxations (free Ca^{2+}≈1µM); maximal force after CD-exposure and wash-out was also reduced. It is therefore concluded that: (i) contraction in MA depends on Ca^{2+}-dependend phosphorylation-dephosphorylation processes; the relative predominance of either process may cause the difference of Ca^{2+}-sensitivities between MA and MMA; (ii) actin metabolism plays a decisive role in tension maintenance as filament stabilization or destruction cause loss of active force; (iii) PH and CD may still directly affect actomyosin interactions.

II Physiologisches Institut der Universität, In Neuenheimer Feld 326, 6900 Heidelberg

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LATCH-LIKE CONTRACTIONS IN INTACT AND SKINNED SMOOTH MUSCLE STRIPS OF CHICKEN GIZZARD

G. Pfitzer and W. Fischer

In skinned chicken gizzard, latch like contractions can be induced at submaximal Ca^{2+} (1.7 µM) and low calmodulin (CaM, 0.15 µM), which are characterized by high force (95% of the maximum elicited at 15 µM Ca^{2+}, 1 µM CaM), low levels of myosin light chain phosphorylation (MLCP: 0.1 mol Pi/mol MLC) and low shortening velocity (v_{us}: 0.22 Li/sec). Inhibition of MLC-phosphatase by okadaic acid reverses the latch state, i.e. v_{us} increases 2-fold (0.44 Li/sec) in concert with MLCP (0.85 mol Pi/mol MLC) but force is barely affected (117% of max.). In intact smooth muscle strips from chicken gizzard, physiological stimuli (carbachol, norepinephrine, membrane depolarization either electrically or with high K^+) elicit only phasic contractions preceded by a rapid phosphorylation transient; e. g. in electrically stimulated preparations, the tension transient lasts only 15 sec (time to peak tension 6 sec) and the MLCP transient lasts 10 sec (time to peak MLCP 3 sec). However, slow tonic contractions may be elicited by decreasing the K^+-conductance with 3,4-diaminopyridine (Kirsch and Narahashi, Biophys J 22:507,1978) which are not associated with an increased MLCP. The active state in these contractions, as estimated from quick release experiments, is low. The experiments in the skinned gizzard preparations are in line with the hypothesis of a dominant role of MLC phosphatase in the development of a latch state (Hai and Murphy, Am J Physiol 254:C99,1988). However, at least in the chicken gizzard, certain potassium channels appear to be involved in the control of the expression of the latch state, i.e. tonic contractions.

II. Physiologisches Institut der Universität, Im Neuenheimer Feld 326, D-6900 Heidelberg

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MINOR ROLE OF THYROXINE AS AN INHIBITOR OF CALMODULIN/MLCK INTERACTION IN SMOOTH MUSCLES

T. Lenz, P.J. Boels, U. Theiß, I. Morano, and V.A.W. Kreye

Thyroxine has been shown to act as a competitive antagonist on calmodulin-dependent myosin light chain kinase (MLCK) activation in human platelets (Mamiya et al., J.Biol.Chem. 264:8575, 1989). This leads to inhibition of collagen-induced aggregation response and release reactions as well as to marked reduction of phosphorylation rate of the 20-kDa myosin light chain. Since calmodulin and MLCK are also involved as essential biochemical mediators in the contraction processes of vascular and nonvascular smooth muscle, it is conceivable that thyroxine exerts comparable inhibitory effects in these tissues. Therefore we studied the influence of thyroxine (10^{-5} - 10^{-4}M) on contraction responses induced either by noradrenaline or by KCl in intact smooth muscle preparations obtained from uterus of the guinea-pig and from the thoracic aorta of the rabbit. Chemically skinned fibers from resistence vessels of the guinea-pig (diameter of 0.1mm), contracted with 10^{-6}M Ca^{2+} either in the absence or presence of calmodulin (0.1 and 0.3µg), were used to study more directly the effect of thyroxine on calmodulin/MLCK interaction. Force development induced with noradrenaline (10^{-9} - 10^{-6}M) and KCl (5 - 80mM) in guinea-pig uterus and rabbit aorta were not appreciably altered by thyroxine (n=4). There was also no significant difference in the Ca^{2+}-induced contraction response of skinned fibers with thyroxine if calmodulin was added. If, however, no calmodulin was added (nominally calmodulin-free) thyroxine (10^{-4}M) was capable of reducing force production by about 50%, indicating a minor inhibitory effectiveness of thyroxine.

Conclusion: in all smooth muscle preparations tested we were unable to demonstrate a significant physiological role for thyroxine as a competitive antagonist of calmodulin/MLCK interaction, such as has been described for human platelets. Whether this discrepancy is due to a cell- or species-specific variant of MLCK isoenzyme remains to be elucidated.

2nd Department of Physiology, University of Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg.

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CHANGES IN FATTY ACID COMPOSITION IN THE RAT AORTA AFTER PHYSICAL EXERCISE

T. Ohkubo, H. Rupp and R. Jacob

Physical exercise is known to reduce the high blood pressure in SHR. Although the underlying mechanisms are not known, prostaglandins (PG) are likely to be involved. Because arachidonic acid (C20:4) is the essential precursor of PG, the effect of swimming (max. 2x90 min/day, 35°C, 6 wk) on C20:4 was studied. Fatty acids were determined in the aorta by gas chromatography (Carlo Erba GC 6000; CP-Sil.88 capillary column; 100-227°C; methyl esters/BF3/MeOH method). Essentially, the swimming rats exhibited an increased percentage of C20:4, whereas linoleic acid (C18:2) was markedly reduced; gamma-linolenic acid (C18:3) was not affected. Noteworthy is that also eicosapentaenoic acid (C20:5) was increased and alpha-linolenic acid (C18:3) was reduced:

FA	male sedentary	male swimming
C18:2 (n6)	20.2+2.6	10.8+1.9*
C20:4 (n6)	11.3+1.3	14.3+1.8*
C18:3 (n3),alpha	1.6+0.2	1.2+0.4*
C18:4 (n3)	0.5+0.1	0.4+0.1
C20:5 (n3)	0.5+0.2	0.7+0.1*
C22:6 (n3)	1.9+0.5	2.0+0.6

The changes in FA composition resemble those seen after norepinephrine injections, suggesting that this type of exercise is associated with a marked adrenergic drive. In accordance, the norepinephrine or epinephrine stores of heart and adrenal glands were increased which is indicative of an enhanced biosynthetic capacity. The reduced C18:2 cannot be attributed to a lower intake of C18:2 because in female swimming rats where the food intake was not reduced, comparable changes were seen. The reduced C18:2 level suggests that the biosynthetic pathways leading to C20:4 and PG are greatly stimulated. The data show for the first time that changes in FA composition of the vessel wall can occur not only after dietary interventions or administration of catecholamines, but also after physical exercise.

Physiological Institute II, University of Tübingen, Gmelinstr. 5, D-7400 Tübingen

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MEASUREMENTS OF INTRACELLULAR PH IN SMOOTH MUSCLE CELLS OF THE RABBIT EAR ARTERY

M. Vonderlage and V. Schreiner

The pH-sensitive dye 2',7'-bis-(2-carboxyethyl)-5(6')-carboxyfluorescein (BCECF) was used to measure intracellular pH (pH_i) in everted segments of the rabbit ear artery (REA). To measure fluorescence, segments were investigated in a manner as described previously (Vonderlage and Schreiner, Am.J.of Physiol.257,H649-657,1989). The wavelength for excitation was 506 nm, bandwidth 0.2 and for emission 530 nm, bandwidth 8.0. There was a loss of dye with time, which however was very slow (decrease in fluorescence < 3%/5 min). By using the K^+-H^+- ionophore nigericine fluorescence signals were calibrated. Resting level of pH_i was found at 7.2 in a bicarbonate-free and at 6.8 in a bicarbonate- containing buffer solution. After a latency of about 5 sec, norepinephrine (1 µM) induced a decrease in pH_i down to 7.0 probably due to hydrolysis of ATP. After reaching this minimum, pH_i increased and returned more or less to its initial value. Using a sodium-free solution pH_i decreased continuously. Due to these procedures only slight changes in pH_i could be observed when a bicarbonate- containing solution was used. It is suggested that in cells of the REA there is a Na^+/H^+- exchange which is directly and/or indirectly activated by norepinephrine.

Physiologisches Institut der Universität Hamburg, Grindelallee 117 , D-2000 Hamburg 13

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ENHANCED RUNNING ACTIVITY CAUSES INCREASE IN TRANSMITTER RELEASE IN MOUSE EDL MUSCLE

M. Dorlöchter, M. Brinkers, A. Irintchev and A. Wernig

Activity possibly influences structural and functional parameters in neuromuscular junctions. We studied effects of longterm (2-8 months) running in wheels on transmitter release in leg muscles of mice. In the trained group each animal was provided with a running wheel which was used ad libitum to an average amount of 5-15 km per day. Intracellular recordings in Mg-blocked preparations (2.75 mM Mg, 0.4 mM Ca) at 10 Hz stimulation revealed that quantal content of endplate potentials (EPPs) was higher in trained than in control EDL ($\bar{m}$ = 1.75 ±0.19 versus 1.35 ±0.35, n=7 each, $p<.05$). In curare-block the amplitudes of the first, maximum and plateau EPPs in a train of 100 Hz for 400 ms or 1 s were increased in trained EDL by 28% each ($p<.05$). Resting membrane potentials and muscle fiber diameters measured from frozen cross-sections did not differ significantly in trained and control EDLs. Training effects were particularly evident in two pairs of monozygotic twins in which beside a drastic increase in quantal content and EPP amplitudes the time course of facilitation and depression in a 100 Hz train were changed: in trained EDL the maximum EPP was reached earlier (in the average at 2.0 impulses versus 2.6 in controls, $p<.01$) and was followed by a somewhat earlier decline below the value of the first EPP (at 3.8 impulses versus 5.0, $.1>p>.05$). In isometric tension measurements block resistance in Mg-block was higher in trained than control EDL for single twitches, depression in a train of four single twitches at 2 Hz (Mg- and curare-block) was lower ($p<.05$). The results suggest that prolonged elevated activity causes a marked increase in transmitter release and safety margin of transmission in EDL muscles. Histochemical studies showed mainly an increase in Type IIB (in the mouse FOG) fibers and a decrease in Type IIA (FG) fibers ($p<.05$) which can be considered as a rise in oxidative capacity caused by endurance exercise.

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Dept.of Physiology, Univ.Bonn, Wilhelmstr. 31, D-5300 Bonn 1

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REPEATED OBSERVATION IN VIVO OF IDENTIFIED FROG NM.JUNCTIONS

A. Wernig and B. Langenfeld

Recently dyes have been used for repeated staining of endplates in the living animal. In the present experiments we compared vitally stained endplates in adult frogs at intervals of 4-5 weeks. Before the second in vivo staining, transcutaneus stimulation of the c.p.nerve stem was performed (3-5 h per day for 6-13 days, 10 Hz, 0.5 ms pulses in anaesthesia). Twelve (14) cutaneus pectoris muscles of six stimulated and one unstimulated R.pipiens (rump to nose 6-7 cm) were investigated. In deep anaesthesia (MS 222) the outer muscle surface was stained with 10 µM 4-Di-2-ASP (Molecular Probes) and viewed with epifluorescence (40/0.75 w, HBO 50, BP 450-490 nm filter) through a coverglass. Images were video-taped and lateron drawn on lucite sheaths. From a total of 92 synapses 47% showed growth, 27% growth and retraction, 12% retraction only and 14% no change. Individual length changes were as large as 20% of the total synapse length (median=5%). The occurrence of both, sprouting and retraction even within a single junction and in the unstimulated frog suggests the existence of junctional remodelling. Stimulation had no obvious effects on synaptic length changes. In three stimulated and three control animals (without stimulation and in vivo stainings) signs of sprouting (spotted arrangement of AChR) and retraction were quantified after staining the muscles with rh-αBGTx and for cholinesterase in vitro. Sprouts were present in both, but less frequent in untreated controls ($p<0.01$, t-test). This indicates that the experimental procedures might enhance but do not initiate the structural changes. Sprouts were more and abandoned gutters less frequent in the unstimulated contralateral than in stimulated muscles ($p<0.05$). This indicates that stimulation reduced sprouting and enhanced retraction without significantly affecting junctional size. This is consistent with previous findings that stimulation caused reduction in transmitter release (Hinz and Wernig, 1988) and a slight increase in abandoned gutters relative to contact length.

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Dept.of Physiology, Univ.Bonn, Wilhelmstr.31, D-5300 Bonn 1

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DECLINE OF QUANTAL AND VESICULAR STORES OF TRANSMITTER AT NERVE-MUSCLE SYNAPSES OF CRAYFISH IN THE PRESENCE OF VERATRIDINE

W. Finger, H. Wolburg* and A. Beer**

At the crayfish neuromuscular junction on application of veratridine the quantal release rate increases instantaneously to several thousand quanta per second and afterwards declines exponentially with a time constant of about 50 s. This effect of veratridine can be induced only once in a single nerve-muscle preparation suggesting that veratridine largely depletes the presynaptic store of transmitter quanta (Finger and Martin 1989, Pflügers Arch. 414:437-442).

In the present study we employed morphological techniques to find out whether a correlation exists between the veratridine-induced decline in the rate of quantal release and the number of vesicles stored in the nerve terminals. After about 5 min when veratridine-evoked quantal release had declined to a low level preparations were fixed in glutaraldehyde. For control, preparations not treated with veratridine were also fixed. Electron microscopy was performed on ultrathin sections obtained from control muscles and muscles treated with veratridine. The results show that compared to nerve terminals in control muscles nerve terminals in preparations treated with veratridine were largely depleted of their synaptic vesicles. This suggests that in the presence of veratridine the decline of quantal secretion results from the loss of vesicles. In addition, our results suggest that during or after excessive quantal release evoked by veratridine synaptic vesicles not only have the ability to fuse with the presynaptic membrane but also may fuse with each other and with other intraterminal organelles.

Naturwissensch. und Medizin. Institut an der Univ. Tübingen in Reutlingen, Gustav-Werner-Str. 3, D-7410 Reutlingen; *Patholog. Institut der Univ. Tübingen; **Physiolog. Institut der Techn. Univ. München.

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BLOCK OF CA-INFLOW BY Cd^{2+} DEPRESSES SYNAPTIC QUANTAL RELEASE WITH LITTLE EFFECT ON ITS TIME COURSE.

J. Dudel

The effect of cadmium was studied at nerve terminals of frog muscle measuring quantal transmitter release by means of a perfused macro-patch-clamp electrode (Dudel 1989, 1990; Pflügers Arch. 415, 289-298 and in press). The Ca-current component of presynaptic action potentials was suppressed almost completely by 20 µM Cd^{2+}, and half-block was attained by about 5 µM Cd^{2+}. With Cd containing Ringer solution perfused through the electrode release elicited by depolarizing pulses through the electrode was depressed by factors of 10 to 100 by 20 to 50 µM Cd^{2+}, while twin pulse facilitation (due to 'residual Ca') was totally suppressed. Depression of release and facilitation were readily reversible on washing. It seems that appreciable release can be elicited by depolarization of the terminal in absence of significant local Ca inflow.

The time course of release was determined at 5 terminals at 5 °C, using up to 16000 stimuli to construct distributions of delays at low release rates. While the total rate of release dropped by factors of 10 to 35 in presence of 20 to 250 µM Cd^{2+}, the minimum latency of release was not altered at all in 2 of the 5 experiments, and increased insignificantly from 2.2 to 2.3 ms on average. Cd^{2+} also increased the average delay of peak release from 2.7 to 3.4 ms, and lengthened the post-peak decay of the rate of release from an average time constant of 0.9 ms to 1.2 ms. If release should be elicited in presence of Cd^{2+} by very low inflow of Ca^{2+}, one would expect a considerable increase in the delay of first releases and a shortening of the period of release. The absence of such effects is compatible with our hypothesis (Parnas et al. 1986; Pflügers Arch. 406, 121-137) that the time course of release is largely controlled by the timing of depolarization, and that the resting Ca^{2+} concentration in the terminal may be sufficient to support considerable release without phasic inflow of Ca^{2+}.

Physiologisches Institut der Techn. Univ. München, Biedersteiner Straße 29, 8000 München 40, F.R.G.

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PROLONGATION OF ACTION POTENTIAL INCREASES THE DELAY OF QUANTAL RELEASE.

Ch. Schwejda, J. Dudel

It was shown in Dudel (1986, Pflügers Arch 407: 134-144) that prolonged depolarization of nerve terminals by applied current increased the delay of first releases and of the median delay of release. Therefore we investigated the correlation between lengthened action potentials and the time course of release in crayfish muscle. The K^+ channel blockers 4-aminopyridine, 3,4-diaminopyridine (3,4 DAP) and tetraethylamonium (TEA) were applied in order to prolongate action potentials. The motor axon was excited via a suction electrode, and quantal currents were recorded by a macro-patch-clamp electrode which was perfused with the drug-containing solution (Dudel 1989, Pflügers Arch 415: 289-298). This limited the action of the drug to about 20 um diameter within the terminal. Recordings of quantal release per stimulus (m) before and after application of K^+ channel blockers show an increase of m on average by a factor 7 which was presumably due to the lengthened action potentials. Distributions of release times were determined for >300 quanta. After K^+ channel block, the delay of first releases and of the median of the distribution of releases were increased by up to 1.6 ms and on average by 1.3 ms $\pm$0.35 ms (7 experiments). Most efficient was a combination of 0.1 mM 3,4-DAP und 1 mM TEA.

If release after membrane depolarization were only controlled by the increased intracellular Ca^{2+} concentration, the initial delay of the transmitter release should be independent of the duration of the action potential. The present results indicate that although Ca inflow starts during the upstroke of the action potential and Ca concentration rises within the terminal simultaneously, prolongation of the action potential can repress release for more than the usual minimal delay. It seems that depolarization has a repressing effect on the onset of release (Dudel 1986).

Physiologisches Institut der Technischen Universität München, Biedersteinerstr. 29, D-8000 München 40, Federal Republic of Germany

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IN VIVO INVESTIGATION OF THE PARTICIPATION OF ASPARTATE AND GLUTAMATE IN THE GENERATION OF SPREADING DEPRESSION.

D. Scheller, U. Heister and F. Tegtmeier

Spreading depression (SD) may be a correlate of migraine. A possible role of the excitatory amino acids (EAA) aspartate (asp) and glutamate (glu) in the generation of SD has been demonstrated in hippocampal slice preparations. The present study was undertaken to determine in vivo whether the extracellular concentrations of these EAA's vary during such attacks. Therefore, a microdialysis (MD) probe was implanted into the cerebral cortex of anaesthethized rats. Dialysate fractions were collected in one min periods and the glu and asp content determined by HPLC. A microelectrode for recording of DC or extracellular ion changes was placed in a distance of 100 to 200 µm from the tip of the MD probe. Single SD's were induced by a 1 - 2 min application of a perfusate containing a high K-CSF (128 mmol/l K^+). SD's were characterized at the remote electrode by a negative shift of the field potential (fp) of about 1 min duration and 19.6 ± 2,31 mV amplitude (n = 8) and by large shifts in $[K^+]o$, $[Ca^{2+}]o$ or by alkaline/acidic shifts of the pH. Parallel to the negative fp shift the EAA content in the perfusate increased by a factor of 18.4 for asp and by a factor of about 9.9 (n = 8) for glu. Local application of the NMDA antagonists APV (0.1 mmol/l in perfusate) or ketamine (1 mmol/l in perfusate) blocked the K^+-induced SD. The K^+-induced rise in EAAs was somewhat reduced. A triggering role of the K^+-induced glu and asp release for the generation of SD was also suggested by the fact that local application of NMDA (10 mmol/l in perfusate, 30'') caused SD's which were sensititive to APV or ketamine. However, ketamine had to be applied in higher concentrations (20 mmol/l in perfusate). These results demonstrate in vivo, that K^+-induced glu and asp release produce SD similarly to NMDA. We conclude, that asp and/or glu release can be a trigger for the generation of SD in vivo.

JANSSEN RESEARCH FOUNDATION, Raiffeisenstraße 8, D-4040 Neuss 21, FRG

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HYPOGLYCAEMIA-INDUCED HYPERPOLARIZATION PRECEDES RISE IN CYTOSOLIC FREE CALCIUM IN HIPPOCAMPAL PYRAMIDAL CELLS

Thomas Knöpfel, Andreas Spuler, Peter Grafe and Beat Gähwiler

The mammalian brain relies on a persistent supply of glucose. Lack of this energy delivering subtrate results in a loss of conciousness and an isoelectric electroencephalogram. These symptoms are reversible depending on the duration of the hypoglycaemia. In hippocampal pyramidal cells glucose deprivation as well as anoxia induces a membrane-hyperpolarization by inducing a potassium conductance (g_K). It has been proposed that this hyperpolarization results from a rise in cytosolic free calcium ($[Ca^{2+}]_i$) which appears to be one of the causes of cell death following anoxia. The g_K might, therefore, be activated by a rise in $[Ca^{2+}]_i$ and would then simply reflect the onset of cell detoriation.

We used intracellular recordings combined with microfluorometric measurements of $[Ca^{2+}]_i$ to investigate the relation between $[Ca^{2+}]_i$ and the g_K induced by glucose deprivation in slice cultured CA3 pyramidal cells. We found that this g_K does not result from a rise in $[Ca^{2+}]_i$ but, on the contrary, might serve as an emergency measure to lower further energy consumption otherwise leading to calcium accumulation and cell death.

Brain Research Institute, August-Forel-Strasse 1, CH-8029 Zürich, Switzerland

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MODULATION OF LIGAND-GATED POTASSIUM CONDUCTANCE IN HIPPOCAMPAL CA3 NEURONS.

M. Bijak, U. Misgeld and W. Müller

In the hippocampus acetylcholine (ACh) and norepinephrine (NE) are considered to produce an excitatory and inhibitory effect, respectively. Yet the depolarization evoked by ACh and hyperpolarization induced by NE are small unless they are applied in high concentrations. Under single electrode voltage and current clamp carbachol (Cch, 0.05-0.5 µM) significantly reduces (by 40-80%) hyperpolarization and outward current induced by adenosine (5-10 µM), serotonin (5-10 µM) and baclofen (0.05-0.5 µM) in CA3 cells of guinea pig hippocampal slices. The effect of Cch is antagonised by pirenzepine (0.1 µM), suggesting an involvement of the M1 muscarinic receptor subtype. In contrast to Cch, NE (5-10 µM) and α-adrenergic agonists phenylephrine and clonidine (0.5-5 µM) potently augment (up to 300%) the ligand activated K-conductance. The effects of Cch and NE are observed after blockade of Na spikes with TTX, suggesting a direct postsynaptic interaction. Cch and α-adrenergic agonists modulate ligand-gated K-conductance without having any effect of their own on membrane potential or holding current. The potency of both modulatory effects on the baclofen-induced K-conductance increase depends on concentrations of Cch and noradrenergic-agonists as well as on the magnitude of the baclofen response. In conclusion, Cch and α-adrenergic agonists can exert strong excitatory and inhibitory effects via suppression and enhancement, respectively, of ligand-gated potassium conductance increase in the hippocampus.

Max-Planck-Institute for Psychiatry, Dept. of Neurophysiology, Am Klopferspitz 18A, D-8033 Planegg-Martinsried

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EFFECTS OF PENTYLENETETRAZOL ON SYNAPTIC ACTIVITY AND $[Ca^{2+}]_i$ IN RAT HIPPOCAMPAL SLICES

G. Rausche, E M. Leweke and U. Heinemann

Effects of pentylenetetrazol (PTZ) on synaptic activity of dentate granule cells and CA1 pyramidal cells were investigated with intra- end extracellular recording techniques. PTZ bath-applied with doses of 0.5 to 20.0 mM/l induced spontaneous epileptiform field potential transients in area CA1 (concentration optimum of 2 mM/l) but not in dentate gyrus. Disinhibitory effects of PTZ on paired pulse stimulus induced responses were already seen with concentrations of 0.5 mM/l in both areas. PTZ augmented paired pulse potentiation in area CA1 and reversed frequency habituation into potentiation in dentate gyrus at all stimulus intervals investigated (25, 50, 200 ms). This effect was strongest at 50 ms interval with 5 mM/l PTZ as quantified with a coastline index. With larger concentrations and intense stimulations, however, the second response could become depressed in both areas when evoked at stimulus intervals of more than 100 ms. Antidromically evoked field potentials were unaffected in dentate gyrus but enhanced in area CA1. Repetitive stimulation (20/s, 10s) induced decreases in extracellular calcium concentration were enhanced by PTZ. This effect was optimal with 5 mM/l PTZ in granule cell layer as well as in stratum moleculare of dentate gyrus. In area CA1 the effect was optimal in pyramidale cell layer with a PTZ concentration of 5 mM/l while in stratum radiatum optimal effect was seen with 7 mM/l PTZ. Intracellularly four effects of PTZ could be seen: 1. block of the early part of the synaptically induced IPSP (optimal effect with 2 mM/l PTZ) while the late part was unaffected, 2. dose dependent increases in input resistance (effect became apparent between 1 and 2 mM/l PTZ) and as a result of this 3. facilitated bursting during depolarizing current injection with a reduced frequency accomodation and afterhyperpolarization, 4. prolongation of action potentials. All effects were fully reversible and could be seen in both CA1-pyramidal cells and dentate gyrus granule cells.

Institut für Neurophysiologie, Robert-Koch Str. 39, D-5000 Köln 41

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CAFFEINE INDUCED PAROXYSMAL DEPOLARIZATIONS IN HIPPOCAMPAL NEURONS IN VITRO (GUINEA PIG)

D. Bingmann[1], I. Moraidis[1] and E.-J. Speckmann[2]

It is well known that caffeine intoxication of patients may lead to epilepsy. The present experiments aimed to study this epileptogenic effect in hippocampal slices (400 µm thick).

When the slices were superfused by a 32°C warm saline, a spontaneous aperiodic bioelectric activity predominated in granule cells and in CA1-CA3 neurons. After adding caffeine to the superfusate (final concentrations: 0,2-5 mmol/l) the following reactions were observed: (1) At caffeine concentrations exceeding 0,3 mmol/l all CA3 neurons (n=28), but only 2 out of 9 CA1 neurons started to generate paroxysmal depolarizations (PD) which periodically occurred 60 to 3 times per min. The latency until the onset of PD typically ranged between 2 and 5 min. In granule cells PD were absent. During washing with control saline, PD of CA neurons disappeared within two hours. (2) To study, whether transmembraneous calcium currents contribute to the generation of PD, effects of the organic calcium antagonists verapamil (40-80 µmol/l bath concentration; n=14) and flunarizine (40 µmol/l added to the bath; n=4) on caffeine induced PD were tested. Both calcium antagonists reversibly reduced PD in amplitude, duration and frequency of occurrence until failure. During a first application of these drugs, PD disappeared after 20-60 minutes. A second application reduced this latency markedly. When tetrodotoxin (TTX 0,2 µmol/l; n=5) was added to the caffeine containing saline, PD stopped as soon as the amplitude of action potentials was reduced. However, even after abolition of sodium spikes, intracellular current injections evoked PD, which again were reduced reversibly in amplitude and duration by verapamil.

As a whole, the findings indicate that caffeine induced PD are generated endogeneously and that verapamil sensitive calcium currents contribute to these endogeneous mechanisms.

[1] Institut f. Physiologie, Hufelandstr. 55, 43 Essen
[2] Institut f. Physiologie, Robert-Kochstr. 27 a, 44 Münster

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CURRENTS IN THE NATIVE OOCYTES OF XENOPUS LAEVIS ELICITED BY THE EPILEPTOGENIC AGENT PENTYLENETETRAZOL

M. Madeja, U. Mußhoff, H. Straub, A. Lehmenkühler, D. Kuhlmann and E.-J. Speckmann

The effects of the epileptogenic agent pentylenetetrazol (PTZ) on membrane currents of the native oocytes of Xenopus laevis were studied.

Oocytes (stage V or VI; up to 10 days after preparation) were investigated in a modified Barth solution (Na: 88; K: 1; Mg: 0.8; Ca: 0.7; Cl: 90.4; SO_4: 0.8; Hepes: 5mmol/l; pH 7.4) at a temperature of 20°C. Two-needle voltage-clamp technique was used. PTZ (1 to 100mmol/l) was applied for 30 to 120s using a concentration-clamp technique (exchange of solutions in less than 30ms).

With administration of PTZ an inward current with an amplitude of up to 30nA was elicited at holding potentials of -50mV. The current remained constant or slightly increased during application (up to 10nA). The time t_{10-90} of rise and decay was ca. 4s and ca. 40s, respectively. Sucrose solutions of comparable concentration failed to elicit currents.

With PTZ application the conductance was reduced for up to 30% and reincreased with ongoing application nearly to initial values. The equilibrium potential of the inward current ranged between -85 and -95mV. With elevating extracellular potassium concentration to 10mmol/l the equilibrium potential was shifted for 20 to 30mV in positive direction. With administration of the anion channel blocking substance 4-Acetamido-4'-isothiocyanatostilben-2,2'disulfonsäure (SITS; 1mmol/l) the amplitude of the inward current was reduced.

From these observations the conclusion may be drawn that PTZ reduces the conductance for potassium ions and increases the conductance for chlorid ions.

Institut für Physiologie der Universität Münster, Robert-Koch-Str. 27a, D-4400 Münster

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A versatile highly sensitive CCD camera system for quantitative fluorescence microscopy

M. Hans, D. Swandulla and H. D. Lux

Fluorescent probes have been proven to be a useful tool in the investigation of cellular functions (Tsien, R.Y., in: Methods in Cell Biology, D. L. Taylor and Y.-L. Wang (eds), Vol. 30, 1989, Academic Press, San Diego, CA.). We have developed a system for simultaneous measurements of low-level fluorescence emission signals and membrane currents from biological samples. The image aquisition and processing system consists of an epiilluminated Zeiss fluorescence microscope, a solid-state detector unit (Photometrics) and an image processing unit (ITEX151). The nitrogen-cooled detector nitrogen-cooled CCD detector with a 384x576 pixel array which has a very low intrinsic noise (dark current < 5e-/pixel/sec) and shows linear response over the full dynamic range with a quantum efficiency of 30% (300-700 nm). An analog processor digitizes the signals with high resolution (14 bit/50 kHz). The main feature of this system is that fluorescence signals as low as five photons can be reliable detected. This allows detection of emission signals from single fluorescent labelled ligands such as monoclonal antibodies. The small pixel size of the detector results in a spatial resolution of 0.2 μm^2/pixel (40x objective), a value which enables resolution of the spatial distribution of free ion concentrations (Ca^{2+}, H^+, etc.) or membrane potential in single cells using appropriate fluorescent probes. In order to minimize photodecomposition processes, the fluorescence excitation light path is controlled by an electronic shutter. For dual wavelength measurements, excitation filters are changed rapidly using a stepper motor. All camera functions and components of the imaging system are operated by a microcomputer (Intel 80386), which allows selection of subareas of the detector. For a 100x100 pixel area frames are obtained at a frequency of 10 Hz. The maximal temporal resolution of the system is less than 5 ms/frame (100x100 pixel area with 25x25 pixel binning).

Max-Planck-Institut für Psychiatrie, Abteilung Neurophysiologie, Am Klopferspitz 18a, 8033 Martinsried

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SUPPRESSION OF BICUCULLINE-INDUCED PAROXYSMAL ACTIVITY IN HIPPOCAMPAL AND NEOCORTICAL NEURONS (IN VITRO) AFTER APPLICATION OF THE ORGANIC CALCIUM ANTAGONIST VERAPAMIL

H. Straub, D. Bingmann, E.-J. Speckmann, J. Walden, R.E. Baker

The organic calcium antagonist verapamil has been shown to suppress epileptic activities in archicortical and neocortical neurons elicited by pentylenetetrazol (PTZ; cf. Bingmann and Speckmann, Exp. Brain Res. 64: 99-104, 1986, Bingmann et al., Exp. Brain Res. 72: 439-442, 1988). The present investigations tested whether verapamil is also able to block epileptic discharges elicited by the GABA-A antagonist bicuculline.

The investigations were performed on hippocampal slices (CA3, guinea pig) and on organotypical explants (neonatal rat). The membrane potential was recorded by microelectrodes filled with 2 mol/l KCH_3SO_4. Bicuculline was applied to the control bath solution (10 µmol/l). Verapamil was added to the bicuculline-containing bath solution (40, 60 and 80 µmol/l).

Verapamil reduced amplitude, duration and frequency of appearance of PDS in hippocampal and in neocortical neurons and led eventually to an abolition of the epileptic discharges. In both preparations verapamil blocked PDS after 10-70 min. After withdrawal of verapamil from the bicuculline-containing bath solution PDS reappeared within a period of up to 3 h.

The investigations show that the organic calcium antagonist verapamil suppresses PDS elicited by bicuculline as well as those induced by PTZ. Thus calcium currents are also involved essentially in bicuculline PDS.

Institut für Physiologie, Robert-Koch-Str. 27a, D-4400 Münster

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BDNF-SUPPORTED RETINAL GANGLION CELLS FROM THE POSTNATAL RAT REACCEPT SYNAPTIC CONNECTIONS IN VITRO: A PATCH CLAMP STUDY OF GABA-ERGIC SYNAPTIC CONNECTIONS

R. Grantyn, M. Perouansky and K. Kraszewski

The regenerative capacity of longaxonal projection neurons from the mammalian central nervous system is limited under the condition of dissociated cell culture. This could, at least in part, be related to the dependency of these neurons on neurotrophic factors from their target areas. Since embryonic rat retinal ganglion neurons (RGNs) were shown to be supported by Brain Derived Neurotrophic Factor BDNF (Johnson and Barde 1986), it was asked to which extent older RGNs would be rescued as well, and if so, whether regenerating postnatal RGNs could be reinnervated by their afferents.

Dissociated cell cultures were prepared from P3 rat retinae and grown on laminin in a serum-free medium containing the N_2-supplements, bFGF (2 ng/ml) and BDNF (10 nM/ml). RGNs were maintained in these mixed cultures for 7 to 14 days and identified by an antiserum against Thy 1.1. Under these conditions RGNs displayed not only ionic conductance changes in response to application of the neurotransmitters glutamate, acetylcholine and GABA, but also spontaneous synaptic activity. The properties of $GABA_A$-receptor-mediated synaptic chloride currents will be described in detail.

Neurobiologische Projektgruppe des BMFT und Abt. Neurophysiologie, Max-Planck-Institut für Psychiatrie, Am Klopferspitz 18A, 8033 Martinsried

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NMDA-INDUCED NEGATIVE FIELD POTENTIAL CHANGES IN THE RAT MOTORCORTEX IN VIVO: REDUCTION BY NORADRENALINE

Chr. Lehmenkühler, J. Walden, E.-J. Speckmann

The excitatory glutamate subreceptor agonist N-methyl-D-aspartate (NMDA) is suggested to be involved in the generation and spread of epileptic discharges. Since the neurotransmitter noradrenaline (NA) has been described to reduce epileptic activity (D.I. Barry et al., Proc. Natl. Acad. Sci. 84: 9712-8715, 1987; R.S. Neumann, Epilepsia 27, 359-366, 1986), the influence of NA on NMDA induced negative cortical field potential changes (CFP) was tested.

The experiments were carried out in anesthetized and artificially ventilated rats. Transmitters were ejected by pressure pulses with a 3-barrelled micropipette which was glued in parallel to the recording microelectrode. CFP were led from different laminae of the rat motorcortex.

NMDA induced negative CFP were reduced in amplitude when NA was applied together with or 10 to 30 s before the excitatory amino acid. This effect was mimicked by the alpha-1 agonist phenylephrine, but not the beta agonist isoproterenol. The alpha-2 agonist clonidine de- or increased the NMDA response.

In summary, the results suggest that the reduction of glutaminergic effects is involved in the antiepileptic action of NA.

Institut für Physiologie, Universität Münster, Robert-Koch-Str. 27a, 4400 Münster

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INFRARED DIC-VIDEO MICROSCOPY OF LIVING BRAIN SLICES

H.U. Dodt and W. Zieglgänsberger,

In the present study we tried to visualize single neurons in an in vitro slice preparation. We exploited a nearly neglected property of the brain slices, namely their translucence. For this purpose a newly developed brainslice chamber with a coverslip bottom was mounted on an inverted microscope (Zeiss Axiovert) equipped with differential interference contrast (DIC) optics. The slice could thus be inspected from below. The image was projected on the target of a video camera and displayed on a TV monitor. The Hamamatsu videosystem used allows analog and digital contrast enhancement and background subtraction. As the DIC optics provide optical sectioning, cells in brain slices of 300 µm could be visualized to a depth of 50-100 µm if illuminated with light of λ>750 nm. The contrast enhancement provided by the video system proved to be crucial for visualization of unstained single cells in slices of this thickness. The method was applied to hippocampus and neocortex of the rat. Intracellular and field potentials elicited by orthodromic stimulation were recorded with standard electrodes to show the viability of the preparation.

In the neocortex pyramidal and nonpyramidal neurons could be differentiated. Dendritic bundling of assemblies of pyramidal cells was clearly visible with medium magnification (20X-40X objectives). It was most prominent in rat visual cortex. Small neuronal networks in the neocortex could be visualized as well at medium magnification. The use of an objective with high numerical aperture (63X, N.A. 1.4) allowed the visualization of spinelike structures in both neocortex and hippocampus. With this resolution even fibers running across somata of neurons were visible.

In combination with electrophysiological recordings infrared DIC-video microscopy should facilitate the search for morphological changes underlying neuronal plasticity and the characterisation of neuronal networks.

Max-Planck-Institute for Psychiatry, Clinical Neuropharmacology, 8000 München 40, FRG.

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SINGLE CELL ACTIVITY ELICITS SYNAPTIC POTENTIALS IN NEOCORTICAL NEURONS AS REVEALED BY SPIKE-TRIGGERED AVERAGING IN RAT BRAIN SLICES IN VITRO

H. Pawelzik, H.U. Dodt and W. Zieglgängsberger

A prerequisite for the understanding of higly complex structures like the neocortex is the evaluation of single cell-to-cell interactions. The in vitro brain slice technique allows stable intracellular recording and pharmacological manipulation of synaptic transmission. However conventional electrical stimulation of an in vitro preparation simultaneously activates heterogeneous synaptic inputs.

To study potentials elicited by excitation of single cells we employed the method of spike-triggered averaging: a neocortical neuron (layer II/III) was recorded intracellularly; the discharge activity of an adjacent neuron which was recorded extracellularly was increased by microiontophoretic application of glutamate or NMDA. The spikes of the extracellularly recorded unit (presumptive presynaptic) were used to trigger averages of the intracellularly recorded postsynaptic potential. In 68 intracellularly and 166 extracellularly recorded neurons 19 pairs of synaptic connections were revealed. In 17 cases spikes in the presynaptic cell resulted in excitation of the follower cell with amplitudes of 162 $\pm$ 111 µV (mean $\pm$ S.D.). Only two purely inhibitory connections were found. The excitatory postsynaptic potentials were followed in 9 out of 17 neurons by hyperpolarizing potentials with time to peak of about 60 ms and amplitudes of 27 $\pm$ 16.3 µV. Time to peak of this potential lies between the time to peak of fast (20 - 25 ms) and slow (150 - 250 ms) inhibitory postsynaptic potentials in neocortical pyramidal cells. Thus this potential may represent a novel synaptic component which can only be revealed by spike-triggered averaging. One possible origin of this potential may be the co-release of a second neuroactive substance, e.g. a neuropeptide together with the classical neurotransmitter glutamate.

Max-Planck-Institute for Psychiatry, Clinical Neuropharmacolgoy, 8000 München 40, FRG.

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MEMBRANE CURRENTS IN MYELINATED NERVES OF RATS WITH EXPERIMENTAL ALLERGIC NEURITIS

J.R. Schwarz, K. Mann[1] and H. Wiethölter[1]

Experimental allergic neuritis (EAN) is an acute polyneuritis confined to the peripheral nervous system. The morphological changes of the nerve fibres are characterized by segmental demyelination and are similar to those found in the Guillain-Barré-Strohl-syndrome in humans. EAN can be induced in Lewis rats by immunization with bovine myelin plus Freund's complete adjuvans. Within 12-16 days motor deficits occur ranging from limp tail to tetraparesis. In diseased rats conduction velocity is decreased and latency of the H-reflex is increased (Wiethölter, Springer 1989).
Single myelinated nerve fibres, isolated from the sciatic nerve of rats suffering from EAN, exhibited morphological changes ranging from widening of the nodal gap to extensive para- and internodal demyelination. In voltage-clamped fibres with paranodal demyelination the amplitude of action potentials was decreased and the threshold increased. Accordingly, the Na^+ currents were reduced in these fibres and the membrane capacity measured with negative potential steps was increased. The main difference was observed in K^+ currents. In intact nodes two populations of K^+ channels could be distinguished with either slow or fast gating kinetics. Slow K^+ channels are predominantly located in the nodal membrane and fast K^+ channels in the paranode (Röper & Schwarz, J Physiol 1989). In nerve fibres from rats with EAN there was a large increase in K^+ current. However, the relative increase in fast K^+ channels was less than in acutely demyelinated nerve fibres and suggests that the chronically demyelinated paranodal axolemma acquires properties similar to those of the nodal membrane.

Physiologisches Institut, Universität Hamburg, Martinistr. 52,
D-2000 Hamburg 20 & Neurol. Klinik, Universität Tübingen[1]

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ONTOGENETIC ASPECTS OF LOW MAGNESIUM INDUCED EPILEPTIFORM ACTIVITY IN RAT HIPPOCAMPAL SLICES

D. Albrecht and U. Heinemann

Mg^{2+}-free solutions evoke spontaneous epileptiform activity in area CA1 of hippocampal slices from adult and young rats at the age of 7/8, 14-16 and 23-25 days.
Extracellular measurements with K^+ and Mg^{2+} reference electrode pairs reveal a large variability in the incidence of spontaneous epileptiform changes and on average a higher variability in the frequency than in older animals (7/8 days: 9-32; 14-16: 12-26; 23-25: 16-20; adult: 19-24 per min.). The equilibration curves for changes in $[Mg^{2+}]_o$ reveal a faster exchange in young than in adult slices, suggesting that the extracellular space is wider. The low Mg^{2+} epileptiform activity starts also earlier after onset of washout and at a higher Mg^{2+} level than in older and adult rats. In the youngest age-group the interictal activity can synchronize to periodic clustered bursts. While in older aminals spreading depressions (SD) sometimes emerge from epileptiform activity, this is not the case in the youngest age-group. At the age of 14-16 50% of all slices are capable to produce SD in response to afferent stimulation. In this age-group and the age-group of 23-25 days sometimes spontaneous SD develops. The increased probability to produce SD in the age of 14-25 days corresponds to an enhanced NMDA receptor expression in area CA1.

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Institut für Neurophysiologie, Robert-Koch-Str. 39
5000 Köln 41, FRG

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DIFFERENCES IN EPILEPTOGENICITY IN THE VARIOUS SUBFIELDS OF RAT TEMPORAL LOBE STUDIED IN VITRO.

J. Dreier and U. Heinemann

It is well known that lowering $[Mg^{2+}]$ produces in area CA1 and CA3 of rat hippocampus spontaneous epileptiform discharges with a mean duration of 40 to 80 ms and an average frequency of 20/min. In order to compare the epileptiform activity with other areas of rat temporal lobe we developed a technique to prepare a combined slice of the temporal lobe containing the rhinal, perirhinal and entorhinal cortex as well as the subicular areas and the hippocampus proper. Measurements were performed with three ion selective reference microelectrodes positioned into various parts of the preparation. Lowering of $[Mg^{2+}]$ induced also in this preparation epileptiform activity. The aspects of this epileptiform activity varied from area to area.
In the rhinal cortex the activity was characterized by clonic and tonic like electrographic activity associated with considerable rises in $[K^+]_o$ and slow negative fp shifts. Between seizure like events interictal like events developed. These were depressed immediately after a seizure and then slowly increased in amplitude, duration and frequency. In the EC and the subiculum similar tonic clonic episodes developed but interictal discharges were rare. The ictal activity could begin in any of these three areas. The hippocampus did not actively participate in the electrographic activity of the remainder of the temporal lobe although this activity is propagated through the alvear path to area CA1 and CA3 on one hand and through the perforant path to the dentate gyrus. In the CA1 stratum oriens, large potassium rises were occasionally seen together with large negative field potentials probably due to activation of local inhibitory interneurons. The dentate gyrus began to participate in this activity only when small amounts of picrotoxin were added to the perfusion fluid. When the dentate gyrus became active also the CA3 field participated in active tonic clonic activity.
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Inst. f. Neurophysiologie, Univ. zu Köln, Robert Kochstr. 39, 5 Köln 41, FRG

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VARIATION OF POTASSIUM CURRENT EXPRESSION WITH CULTURE CONDITIONS

E. Ficker, H. Beck and U. Heinemann

Previous studies in peripheral and central neurons usually show a characteristic sequence of expression of voltage-dependent currents. Here we describe potassium currents in granule cells of the dentate gyrus and CA1 cells acutely isolated at different developmental stages. We compared these currents to potassium currents in neurons of CA1 or dentate gyrus grown for several days in primary cell culture.
Hippocampal cell cultures of embryonic rats were prepared at embryonic days 18-19, whereas the cultures of dentate gyrus neurons were established from dentate gyrus of 4-5 day-old rat pups. Acutely isolated cells from hippocampal subfields of CA1 or dentate gyrus of 7-24 day-old rats were prepared with trypsin. Whole cell currents were measured with patch electrodes under conditions suitable for isolation of potassium currents.
Whole cell currents elicited in acutely isolated CA1 neurons showed in addition to sustained outward currents an initial transient component. In the embryonic cultures of 'CA1 precursor cells' delayed currents were present in all cells under investigation. Only 50% of day 1-7 cells possessed a transient outward current. The pharmacological characterization of the transient currents in both preparations revealed a remarkable insensitivity to 4-AP. This finding could be interpreted as a delayed expression of a transient potassium current during differentiation of early postmitotic CA1 cells, but the situation might be complicated by the heterogenity of the embryonic culture preparation. In contrast to CA1 no marked transient current could be elicited in the acutely isolated dentate gyrus cells of different ages. In cultures of dentate gyrus cells, however, in additon to the sustained, moderately inactivating currents an initial transient component could be observed with properties similar to the transient currents in the other preparations.Thus we observed in the cultured dentate gyrus cells the expression of a transient current that could not be demonstrated in cells differentiating in their natural environment. This unique developmental sequence may be initiated by factors present only under culture conditions.

Institut für Neurophysiologie, Universität Köln,
Robert-Koch-Str. 39, 5000 Köln 41

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DENDROTOXIN BLOCKS ONE TYPE OF PARANODAL FAST K^+ CHANNEL IN RAT MYELINATED NERVE

B.J. Corrette, F. Dreyer[1], H. Repp[1] and J.R. Schwarz

As in the frog, slow and fast K^+ channels have been shown to occur in the rat node of Ranvier, but the distribution of these channels is different. Slow K^+ currents predominate in the nodal membrane, whereas large fast K^+ currents can only be recorded after paranodal demyelination (Röper & Schwarz, J Physiol 1989). We have investigated the effect of dendrotoxin (DTX), a specific blocker of one type of fast K^+ channel in the frog (Benoit & Dubois, Brain Res 1986), on the K^+ currents before and after demyelination.
Single myelinated nerve fibres were mechanically isolated from rat sciatic nerve and voltage clamped. K^+ currents were measured in isotonic KCl solution. The relative contributions of fast and slow K^+ channels were derived from kinetic analysis of tail currents. In intact nodes, the ratio of slow to fast K^+ channels was found to be as high as 9:1. This ratio could be inverted by paranodal demyelination with pronase. 140 nM DTX blocked 85-100% of fast K^+ currents in the intact node. After demyelination the same concentration was only able to block 73-86%. 14 nM DTX blocked about 50% of the toxin-sensitive K^+ channels. No consistent effect of DTX on slow K^+ currents was observed. Binding studies with nerve fibre membranes indicated high affinity binding sites for ^{125}I-labelled DTX. Furthermore, iodinated DTX could be completely displaced from its sites by DTX I, MCD-peptide and charybdotoxin. Immunocytochemical methods showed that binding sites for DTX are only present following paranodal demyelination.
Our results show that the fast K^+ channels present in the rat paranode can be further subdivided into DTX-sensitive and DTX-insensitive channels.

Physiologisches Institut, Universität Hamburg, Martinistr. 52, D-2000 Hamburg 20 & Inst. für Pharmakologie, Universität Gießen[1]

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INTRACELLULAR CALCIUM TRIGGERS LONG-TERM DEPRESSION IN THE CEREBELLUM

A. Konnerth, J. Dreessen and G.J. Augustine

Synaptic transmission between parallel fibers and cerebellar Purkinje cells (PC) is depressed by concurrent activation of the climbing fibers. This form of synaptic depression can last for hours or longer and has been termed long-term depression (LTD). We have performed two tests of the hypothesis that LTD is initiated by a rise in free calcium concentration ($[Ca]_i$) in PC. First, we asked whether activation of climbing fibers raises $[Ca]_i$ in PC. Whole-cell patch clamp methods were used to record from PC in thin cerebellar slices of 12-18 d old rats. Synaptic currents were elicited in these neurones by extracellular stimulation of parallel and/or climbing fibers. The recording pipette contained fura-2 (100-400 µM) and the fluorescence of this dye was measured with a SIT video camera or a photomultiplier to determine $[Ca]_i$. We found that climbing fiber activation produced a large rise in $[Ca]_i$ in PC. This rise was transient, usually lasting less than 5 s, and was much more prominent in dendrites than in somata. The rise in $[Ca]_i$ appeared to be due to the climbing fibers activating voltage-gated Ca channels, because the $[Ca]_i$ changes were small when Ca spikes were absent. The second test of the hypothesis was to examine whether raising $[Ca]_i$ depressed transmission at the parallel fiber-PC synapse. Elevating $[Ca]_i$, by depolarizing the membrane potential of the PC to open voltage-gated Ca channels, often produced a dramatic reduction in the magnitude of the synaptic current evoked by parallel fiber activation. This depression was long-lasting (> 30 min), greatly exceeding the duration of the $[Ca]_i$ rise that triggered it. In conclusion, both tests support the hypothesis that climbing fiber synapses initiate LTD by raising $[Ca]_i$ in PC.

AG Zelluläre Neurophysiologie, Max-Planck Institut für biophysikalische Chemie, 3400 Göttingen, FRG.

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DECLINE OF EXTRACELLULAR FREE CALCIUM CONCENTRATION DURING CAFFEINE-INDUCED PAROXYSMAL DISCHARGES OF CA3 NEURONS IN HIPPOCAMPAL SLICES (GUINEA PIG)

I. Moraidis, A. Lehmenkühler, H. Straub and D. Bingmann

Recently, Kostyuk et al. (J. Membrane Biol. 110, 11-18, 1989) have described caffeine-induced periodic increases of the free intracellular calcium concentration ($[Ca^{2+}]_i$) in snail neurons. The caffeine concentration used induced paroxysmal depolarizations in snail (Kostyuk, pers. communication) and hippocampal neurons (cf.Bingmann et al.; this volume). The present experiments were carried out to analyze whether calcium entry from the extracellular space contributes to increases of $[Ca^{2+}]_i$. Therefore, changes of extracellular free calcium concentration ($[Ca^{2+}]_o$) and field potentials (FP) were recorded with double-barreled liquid membrane micro-electrodes in hippocampal slices (n=8) during exposure to a saline containing caffeine (0.5 mmol/l; T=32°C).
The experiments revealed: (1) In the stratum pyramidale of the CA3 region FP periodically occurred at a rate of 5 to 20 per min. They consisted of an initial large positive deflection (amplitude: 0.5-1 mV; duration: 0.2 s) followed by a small negative one (amplitude: 0.1 mV; duration: 0.6 s). FP reversed in polarity in the stratum radiatum. (2) The bioelectrical events were accompanied by decreases in $[Ca^{2+}]_o$ of 30-50 µmol/l. Minimum calcium values were observed after 0.3 s. $[Ca^{2+}]_o$ recovered within 6 s.
The findings indicate that caffeine-elicited epileptic bioelectric phenomena are associated with transmembraneous calcium currents which may contribute to oscillations of $[Ca^{2+}]_i$.

Inst. f. Physiologie, Hufelandstr. 55, 4300 Essen

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ELECTROPHYSIOLOGICAL STUDIES ON CO-CULTURES OF THE HIPPOCAMPUS AND THE HYPOTHALAMUS

D. Büsselberg, B. Heimrich[1] and H. L. Haas

Histaminergic neurons in the tuberomammillary nucleus (TM) project to different brain regions, including the hippocampus. We used organotypic co-cultues of the hypothalamus and the hippocampus in order to study their neuronal connections in vitro.
400 µm thick slices of the TM region in the posterior hypothalamus and the hippocampus were prepared from 3-5 day old rat pups. The two slices were placed on coverslips at a distance of about 1 mm. The surface of the hypothalamus was orientated to the stratum oriens of the hippocampus. The slices were embebbed in a clot of chicken plasma and were cultured for 15-35 days with the roller-tube technique. The histaminergic neurons were identified by histidine-decarboxylase immunoreactivity.
CA1 neurons were impaled with glass microelectrodes (filled with 3 M KCl; 40-70 MΩ) while the TM region was electrically stimulated at 0.02-0.1 Hz. Signals were recorded, digitized and analyzed using "pclamp" software. Both structures displayed spontanous action potential firing.
The response was a complex depolarizing synaptic potential which appeared about 3-7 msec after the stimulus artefact and declined over twenty to several hundred msec, sometimes triggering action potentials. Spontaneous postsynaptic potentials (PSPs) were regularly observed. Adding Pb^{2+} (10 µM; a blocker of synaptic transmission) to the bath abolished all potentials. Bicuculline (10 µM), a GABA A-antagonist, only partially reduced the spontaneous and evoked PSPs. Histamine H_1-antagonists did not affect the potentials but H_2-antagonists enhanced their size and number significantly. Thus excitatory and probably inhibitory (gabaergic) as well as modulatory (histaminergic) projections were established between hypothalamus and hippocampus in vitro.

Institut für Physiologie und Pathophysiologie der Johannes-Gutenberg Universität Mainz, Duesbergweg 6, D-6500 Mainz; [1]Anatomisches Institut der Universität Frankfurt, Theodor-Stern-Kai 7, D-6000 Frankfurt.

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LONG-TERM POTENTIATION (LTP) IS NOT SUPPRESSED BY ANTIDEPRESSANTS AND ANTIEPILEPTICS

S. Birnstiel and H.L. Haas

Recent investigations indicate an inhibitory role of tricyclic antidepressants and antiepileptics on the N-methyl-D-aspartate (NMDA) receptor complex (Neuron 2:1221; Brit.J.Pharmacol. 95:95, Naunyn-Schmiedeb.Arch. 339:613). Therefore we studied the impact of the antidepressant agents imipramine, (+)-oxaprotiline and (-)-oxaprotiline as well as midazolam and phenytoin on tetanus-induced LTP in the CA 1 region of hippocampus.

Recordings were obtained from Sprague-Dawley rats weighing 150-200 g. Stimulation was at 50% or 67% of maximum strength at 0.1 Hz, tetanic stimulation consisted of 4 trains at 100 Hz and the same stimulation strength. Drugs were added 11 min before tetanus and were present until the end of the experiment (22 min after tetanus). At 67% of maximum stimulation, neither Phenytoin (10 µM, n=7) nor Midazolam (0.1 µM, n=9) at their ED 50 to inhibit NMDA-mediated discharges in Mg-free medium influenced LTP. Midazolam was equally ineffective at 1 µM (n=8). Similar results were obtained with the antidepressants at 10 µM and 67% maximum stimulation. When tested at halfmaximal stimulation however, all antidepressants significantly enhanced LTP ($p < 0.05$).

Department of Physiology and Pathophysiology, Saarstr. 21, 6500 Mainz, FRG

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SEROTONIN ACTION ON TUBEROMAMMILLARY NEURONES

B. Schönrock, D. Büsselberg and H.L. Haas

The tuberomammillary nucleus (TM) contains large neurones, the only histaminergic nerve cells in the brain, which project to most regions of the central nervous system and receive inputs from many sources including serotonergic neurones in the raphe nucleus. Both nuclei exhibit congruent marked changes in firing rate across behavioural states.

We report here the responses of TM histamine cells and other mammillary neurones of the rat in vitro to serotonin. Histamine cells fire spontaneously and display two characteristic A-currents and an inward rectifier current (J.Physiol. 399:633-646) while neighbouring mammillary neurones often have a low threshold Ca-spike. Both types of neurones could be depolarized (n=16) or hyperpolarized (n=8) by serotonin 1-10 µM. Biphasic actions consisting of an initial hyperpolarization followed by a longer lasting depolarization were also observed. These actions were accompanied by an increase or decrease of membrane resistance respectively and occurred in the presence of tetrodotoxin too. In some neurones, 5-HT induced a long lasting depolarization or a membrane potential oscillation (ca 1 second) after a hyperpolarizing pulse, the low threshold Ca-spike was markedly prolonged and enhanced.

Department of Physiology and Pathophysiology, J. Gutenberg-Universität, Saarstr.21 ,6500 Mainz,FRG

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SHIFT OF THE STEADY-STATE INACTIVATION CURVE OF THE A-CURRENT BY VALPROATE-SODIUM

J. Walden, U. Altrup, H. Reith, E.-J. Speckmann

Valproate-sodium (VPA) has been found to decrease the slope of depolarization of paroxysmal discharges and to suppress paroxysmal depolarization shifts of single neurons. It was studied whether the A-current (I_A) is responsible for these effects.
In identified neuronal individuals in the buccal ganglia of Helix pomatia membrane currents were measured using conventional voltage clamp techniques. VPA was administered extracellularly (dissolved in control medium; 10 mmol/l) or was applied intracellularly (dissolved in KCl solution (150 mmol/l); 0,7 mol/l; pH= 7.4) by pressure pulses. The final intracellular concentration of VPA was estimated to be ca. 1 mmol/l.

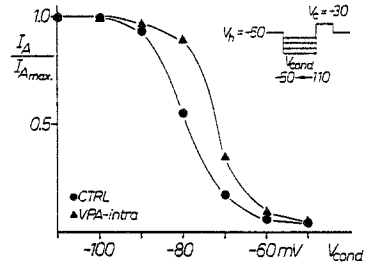

Fig. 1

Extra- or intracellular applications of VPA had no effects on the current-voltage relation and the time to peak of I_A. The steady state inactivation function (b_∞) was shifted to more positive potentials after VPA application (Fig. 1). Thus the value of half inactivation ($I_A/I_A max$= 0.5) changed from -78.5 ± 3.8 mV to 69.3 ± 2.8 mV (n= 5).
In summary, the findings may explain the flattening of paroxysmal depolarization shifts after VPA application.

Institut für Physiologie, Universität Münster, Robert-Koch-Str. 27a, D-4400 Münster

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DIFFERENTIAL EXPRESSION OF FUNCTIONAL SEROTONIN, GLUTAMTE, GABA, AND GLYCINE RECEPTORS IN XENOPUS OOCYTES INJECTED WITH RAT BRAIN RNA

U. Muβhoff, M. Madeja, H. Straub, D. Kuhlmann, A. Lehmenkühler and E.-J. Speckmann

Oocytes from Xenopus laevis are able to express functional receptors for a variety of neurotransmitters after injection of RNA from nervous tissues. The rate of co-expression was examined after injection of rat brain RNA.
RNA was prepared by a guanidin/LiCl method (Cathala et al., DNA 2: 329, 1983). Poly $(A)^+$ mRNA was isolated by affinity chromatography on oligo (dT)-cellulose. Oocytes (stage V or VI) were pressure-injected with 75µg total RNA or mRNA and kept for up to 14 days in a modified Barth medium. Membrane currents were measured by conventional voltage-clamp technique. Transmitters were applied by the concentration-clamp technique: serotonin (5-HT) 10 µmol/l, glutamate 200 µmol/l, GABA 200 µmol/l, glycine 1 mmol/l. Oocytes injected with total RNA or mRNA developed differential sensitivity to the transmitters. 75% of oocytes responded to 5-HT (60 of 80 tested). Only oocytes sensitive to 5-HT responded also to glutamate (14 of 60 tested; 23%), GABA (14 of 60 tested; 23%) and glycine (11 of 45 tested; 24%). Co-expression of three and four receptors appeared in 15% and 7%, respectively. Control oocytes (non-injected or water-injected; n=30) showed no responses.
One possible reason for the different rate of expression might be due to the different complexity of receptors.

Institut für Physiologie der Universität Münster, Robert-Koch-Str. 27a, D-4400 Münster

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The kappa opioid-receptor agonists, U 50488H and U 69593, reduce postsynaptic potentials, but increase direct excitability in hippocampal CA3 neurons in vitro.

C. Alzheimer and G. ten Bruggencate

Within the hippocampal formation, CA3 neurons receive a strong peptidergic innervation by mossy fibers of the dentate granule cells which is mediated by prodynorphin-derived peptides. Dynorphin has been shown to act as an endogenous ligand at the kappa opioid-receptor subtype, which is also present in the CA3 subfield. In order to examine whether the activation of kappa opioid-receptors might affect neuronal excitability in this area, we tested the electrophysiological actions of two kappa opioid-receptor agonists, U 50488H and U 69593, on guinea pig CA3 neurons in vitro using intracellular recording techniques.

Within 30-40 min following bath-application of U 50488H (30-100 μM), the neurons' direct excitability was substantially enhanced as indicated by the following observations: (1) U 50488H dose-dependently increased the neurons' input resistance by 10-38% (50 μM: 16.7 $\pm$ 2.5% [$\bar{x} \pm$ S.D.] n=6, 100 μM: 32.7 $\pm$ 4.7% n=3), whereas the resting membrane potential was not significantly altered. (2) Measurements of the neurons' I/V relations revealed a reduction of inward rectification in the hyperpolarizing direction in the presence of U 50488H. (3) Spike repolarization was found to be impaired by U 50488H causing a broadening of the spike and the appearance of a depolarizing afterpotential. In contrast to the excitatory actions on direct excitability, U 50488H, within the same concentration range, reduced mossy fiber-evoked postsynaptic potentials. Similar effects on both intrinsic properties and postsynaptic potentials of CA3 neurons were observed in the presence of U 69593, although the concentrations required were moderately higher (100-200 μM). The electrophysiological actions of the kappa opioid-receptor agonists were at least partially reversibel following prolonged superfusion (60-90 min) with normal bathing solution. None of the effects were blocked by naloxone (2-50 μM, n=4). However, since naloxone is only a weak antagonist at the kappa opioid-receptor and apparently exerts agonistic effects at higher concentrations, it remains to be determined whether the actions produced by the two kappa opioid-receptor agonists are mediated by the corresponding opioid receptors, or whether they are due to mechanisms unrelated to opioid receptors.

Dept. of Physiology, University of Munich
D-8000 Munich 2, Pettenkoferstr.12

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CENTRAL ALPHA ADRENOCEPTORS AND REM SLEEP REGULATION

B. Fruhstorfer[1], E. Mignot[2], S. Nishino[2], W.C. Dement[2] and C. Guilleminault[2]

It has generally been accepted that brainstem cholinergic mechanisms play a major role in REM sleep but a monoamine involvement in REM sleep regulation was also proposed. Narcolepsy is a disorder of REM sleep, and canine narcolepsy is an animal model of the human disease with essential homologies, that allow the investigation of brain neurotransmitter abnormalities potentially involved in this disorder. The present study aimed at investigating a possible involvement of central alpha-2 receptors in the narcoleptic symptomatology.

Alpha-2 receptors in the canine brain were pharmacologically characterized with 3[H]yohimbine binding. Tissue samples from five normal and five narcoleptic Doberman brains were used. The receptor density was compared between normal and narcoleptic dogs by Scatchard analysis in frontal cortex, hippocampus, locus coeruleus, nucleus caudatus and thalamus. The main result was a significant difference in the number of alpha-2 receptors in the locus coeruleus: narcoleptic dogs had a higher number of receptors in this region ($p<.01$) while the other investigated brain areas revealed no such differences. The locus coeruleus is the brain area with the highest number of norepinephrine containing cell bodies with many alpha-2 receptors in the soma-dendritic region. An increase in the number of these receptors could result in impaired norepinephrine release.

[1]Physiologisches Institut der Universität, Deutschhausstr. 2, 3550 Marburg, FRG, and [2]Sleep Research Center, Stanford School of Medicine, CA 94305, USA

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CENTRAL EFFECTS OF NOREPINEPHRINE (NE) AND ADRENERGIC AGONISTS ON ADH RELEASE, RENAL AND CIRCULATORY RESPONSES IN COMPARISON TO HYPEROSMOTIC STIMULI.

Christa Simon-Oppermann and Olaf Günther*.

Perfusion of the 3rd cerebral ventricle (3rdV) in conscious diuretic ducks with hypertonic saline (400 mOsm/kg) stimulates ADH (AVT, arginine vasotocin in birds) from 3.5 ±0.5 to 4.7 ±0.9 pg/ml in plasma, causes antidiuresis, and slightly elevates mean arterial pressure (MAP) and heart rate (HR). Responsiveness of the antidiuretic system to hyperosmotic solutions is restricted to the rostro-dorsal part of the 3rdV and was used as a test stimulus for adaequate localization of a chronically implanted perfusion device in 23 ducks. Perfusion of NE (750 ng/min) for 10 min also increased plasma AVT (from 3.2 ±0.2 before to 4.6 ±0.2 pg/ml 3 min after end of perfusion) and induced antidiuresis, but simultaneously depressed MAP and HR. Both antidiuretic and circulatory effects were dose-dependent. - To evaluate the type of adrenergic receptors involved, 3 different adrenergic agonists were perfused at similar concentrations (each at 188 ng/min for 10 min). The α_1-agonist phenylephrine (Phe), the α_2-agonist clonidine (Clo) and the ß-agonist isoproterenol (Iso) all depressed MAP and HR although to different degrees. Phe and Clo had not significantly affected plasma AVT 3 min after the end of perfusion, in contrast to Iso which significantly increased plasma AVT (from 4.4 ±0.7 to 5.5 ± 1.0 pg/ml) at this time. Clo had the strongest and longest lasting effect on the circulatory parameters. Despite pronounced depression of MAP and HR by about 25%, Clo lowered plasma AVT significantly from 5.5 ±0.7 to 3.7 ±0.2 pg/ml 20 min after the end of perfusion. - It is concluded that 1.) ADH release is stimulated preferably by ß-adrenergic receptors in the duck. 2.) Adrenergic hypothalamic modulation of circulatory control in the lower brain stem involves inhibitory α_2-adrenergic synapses and is independent of the baroreceptor reflex mechanism.

Max-Planck-Institut, W. G.Kerckhoff-Institut, D-6350 Bad Nauheim; *Physiol. Inst. Med. Akademie, DDR-301 Magdeburg.

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VASOPRESSIN: ASPECTS OF ITS MODULATORY ROLE FOR PINEAL FUNCTION

J. Stehle*, S. Reuss, A. Seidel, R. Riemann & L. Vollrath

Previous studies have shown that intraarterial (i.a.) application of the endogenous nanopeptide arginine-vasopressin (AVP) inhibits the nocturnally elevated activity of the rate-limiting enzyme for pineal melatonin synthesis, N-acetyl-transferase (NAT), in Sprague Dawley (SD) rats. This effect was mimicked in SD but not in AVP-deficient Brattleboro (BB) rats by electrical stimulation of the paraventricular nucleus (PVN) which is the main source of AVP-secretion into circulation. We therefore sought to prove the assumption of a modulatory role of AVP within the melatonin-producing system and to determine the site of AVP action. We found that (1) i.a. application of AVP in BB rats decreased NAT activity as it did in SD rats. (2) the AVP agonist dDAVP which is devoid of pressor activity mimicked the effects of AVP in both SD and BB rats. (3) The noradrenaline-induced stimulation in NAT activity of in vitro cultered pineal glands was potentiated by both AVP or its major metabolite VP4-9 in a dose-dependent manner while both peptides given without NE were without effect. AVP receptors are present within the retino-hypothalamo-pineal pathway (RHPP) in the suprachiasmatic nucleus and the superior cervical ganglion. Additionally, VP4-9 binding sites are highly concentrated in the pineal gland. We therefore suggest that the synergistic effects of either PVN stimulation in SD rats or the i.a. application of AVP in SD and BB rats is due to the activation of V1 receptors in the suprachiasmatic nucleus which in turn lead to an inhibition of neural transduction within the RHPP. Thus, the suppression of pineal NAT activity upon PVN stimulation does not occur exclusively in BB rats since no AVP can be released. The in vitro effects of both peptides and the overall lower levels of pineal melatonin synthetis in BB rats during nighttime as compared to SD rats lead to the assumption that the quantity of secreted melatonin is modulated via AVP receptors within the RHPP as well as via VP4-9 receptors on membranes of pinealocytes.

Dept. Anat., University of Mainz, Saarstr. 19, 6500 Mainz, FRG. *Present address: Dept. Physiol.,University of Mainz

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PENTYLENETETRAZOLE KINDLING CAUSES A SLOWLY-REVERSIBLE DECREASE IN DYNORPHIN GENE EXPRESSION IN THE RAT HIPPOCAMPUS

Susanne Reimer and Volker Höllt

Kindling is a phenomenon in which repeated administration of subconvulsive electrical or chemical stimuli result in a progressive development of seizure activity. We have previously reported that kindling induced by electrical stimulation of hippocampal granule cells resulted in a decrease in the level of prodynorphin (PDYN) mRNA in the dentate gyrus of rat hippocampus.
We now report that chemical kindling induced by prolonged treatment with pentylenetetrazole (PTZ) causes a decrease in PDYN mRNA levels. Rats received 30 mg/kg doses of PTZ for up to 15 days and were killed one hour after the last injection. One group of rats was allowed to recover 4 weeks after chronic administration for 15 days. PDYN and c-fos proto-oncogene mRNAs were measured using in-situ hybridization and Northern blot analysis.
PTZ treatment diminished PDYN mRNA levels by 50% after one and by 70% after 15 days. At the latter time the rats showed stage 4 kindling seizures. Following cessation of the drug treatment PDYN levels slowly returned back to normal but were still 30% below control levels after 4 weeks. At that time rekindling again produced stage 4 kindling seizures. The reversibility of the change of PDYN mRNA levels together with unaltered behavioural characteristics implies that dynorphins may trigger the establishment of the kindling phenomenon, but not its maintenance. Administration of PTZ resulted in rapid transient induction of c-fos mRNA levels at all time points indicating that each PTZ injection triggers the induction of early-onset genes in hippocampal granule cells.

Physiologisches Institut der Universität München, Pettenkoferstr. 12, D-8000 München 2

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CHANGES IN THERMAL RESPONSIVENESS OF RAPHE NEURONES DURING PROESTRUS LIKE PHASES

P. HINCKEL, H.B.H. DICK, C. HIEMKE and A. JUSSOFIE

In female mammals the preovulatory phase is characterized by surges of luteinizing hormone (LH) and a shift in the basal body temperature. The stimulation of gonadotropin release is triggered primarily by estradiol via the central nervous system. The elevation of body temperature seems to be due to increased production of progesterone in the ovary. The present study examines the effects of s.c. progesterone application on thermoresponsive neurones in the nucleus raphe magnus (NRM), a circumscribed area in the lower brain-stem, during proestrus like phases in ovariectomized rats.

Spike rate maxima of warm-responsive neurones decreased and peak activity of cold-responsive neurones increased 20 - 50 minutes after progesterone injection, whereas no significant change in spike rate was seen after sham or control injection. After pretreatment with estradiol the progesterone induced inhibitory effect on warm-responsive units and the excitatory effect on cold-responsive units was amplified. Serum concentrations of LH and the two steroids were measured by using specific radioimmunoassays.

It is concluded that the hormonal control of basal body temperature change is mediated by complex effects of the steroids on thermal neurones in the lower brain-stem.

Physiologisches Institut, Universität Giessen, Aulweg 129, D-6300 Giessen 1, F.R.G., Institut für Physiologische Chemie, Universität Essen, Hufelandstrasse 55, D-4300 Essen 1, F.R.G., and Psychiatrische Klinik der Universität Mainz, Untere Zahlbacher Strasse 8, D-6500 Mainz, F.R.G.

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THE COBALT-EPILEPSY, A PHENOMENON OF A MODIFIED SODIUM CHANNEL.

U. Sonnhof, J. Krupp and H. Claus
SPI GmbH Wormser Str. 32 D-6504 Oppenheim

Cobalt ions when applied to the cortex evoke epileptic reactions. Cobalt has been used more than twenty years as a model to study the basic mechanisms of epilepsy. We have investigated its effects on the membranes of hippocampal neurons using conventional intracellular recordings and "direct" patch-clamp records from cells of the CA1 region in hippocampal slices. Cobalt was added to the bath in concentrations of 1-5mM. The action of cobalt consists of a short term effect, that contrasts markedly with a long term effect of this ion. The short term effect is completed after an application period of 10 to 20 minutes with the concentrations used. It is characterized by a complete suppression of synaptic transmission and an increased frequency of action potentials during a depolarizing current puls. These effects are consistent with the well known suppression of voltage dependent calcium conductances by cobalt. The increased excitability appears to result mainly from blockade of Ca-activated K-conductances. The long term effect of cobalt is observed after more than one hour of continuous application as a plateau like deformation of the action potential. The duration of this plateau is strongly potential dependent, it increases up to several hundred milliseconds, when the membrane potential is depolarized. TTX does completely suppress these prolonged action potentials. In cell attached- and in outside-out membrane patches single Na-channels were investigated. In cobalt free solutions the mean open time of single Na-channels was in the range of few milliseconds and only in rare cases repetitive openings were observed. In the presence of cobalt Na-channels tended to open in bursts of repetitive long openings interrupted by very brief closures. When depolarizing pulses of a duration up to 4 seconds were used, there was no indication for inactivation. From these results we conclude, that the epileptic action of cobalt results from a complete abolishment of the inactivation of Na-channels. The termination of the action potential in the absence of Na-inactivation results from the activation of slow potassium conductances in the course of depolarization.

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PATTERNS OF INTERICTAL ACTIVITY IN NEOCORTICAL EPILEPTIC FOCI OF RATS

T. Dorn, S. Uhlig and O.W. Witte

Penicillin-induced interictal spikes in the motor cortex of the rat are followed by different phases of refractoriness. An absolute refractory period lasting 200 to 300 ms is followed by two relative refractory phases lasting about 700 and 2000 ms, respectively (Dorn, Uhlig, Witte, Eur. J. Neurosci. Suppl. 2, 1989, 53). In order to elucidate the influence of these refractory phases on interictal activity we analyzed the rhythms of spontaneously occuring interictal discharges.
Experiments were carried out on motor cortex of anaesthetized and relaxed rats. The DC potential was recorded from the cortical surface. Focal interictal activity was induced by penicillin. The spike patterns were analyzed off-line. Three different types of interictal activity were observed. Spikes occured in irregular, composed or regular patterns. In case of irregular patterns, the mean interval lengths between successive spikes ranged from 1700 to 4800 ms. The lengths of successive intervals varied to a great extent. The composed patterns consisted of two classes of intervals with lengths of about 250 to 500 ms and 1700 to 4000 ms, respectively. One long interval was followed by 1 to 3 short ones or by a further long interval. The slow component of this composed pattern resembled the irregular rhythm. The third pattern was characterized by very regular intervals ranging from 650 to 1200 ms in different experiments. During such sequences of regular discharges spike frequency gradually decreased. Within the experiments the foci switched between different patterns; a certain pattern could persist for several seconds, minutes or hours.
The investigations show that penicillin-induced interictal spikes can occur in three different patterns. There is a correspondence between the mean interval lengths in the spontaneous discharge patterns, the durations of different refractory phases following interictal spikes and the time courses of intrinsic and synaptic inhibitory processes following paroxysmal depolarization shifts in neurons (Witte, Uhlig, Valle, Neurosci. Lett 1989, 101, 51-56). It is suggested that interictal spike rhythms and refractoriness of epileptic foci are determined by cellular inhibitory processes.

Neurologische Klinik d. Universität., Moorenstr. 5, D-4000 Düsseldorf, FRG

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EEG-MAPPING IN NEONATES - EXPERIMENTAL AND CLINICAL STUDIES

Rother, M.; Eiselt, M.; Witte, H.; Zwiener, U.
Institute of Pathological Physiology, Friedrich-Schiller-University Jena, Löbderstr.3,G.D.R.

EEG mapping is commonly used today in cerebral function analysis of adults. Local disturbances like seizures, cerebral infarction, cortical bleedings and disturbed regional cerebral blood flow demand better topographical description of cortical electrical activity in neonates, too. Multichannel (8 or 16) EEG were recorded simultaneously at different anatomical structures (dura, os, skin) in neonatal piglets. It could be shown, that EEG maps recorded from surface position represent fairly good the cortical EEG-topography.
In the clinical study 8 healthy term newborns were examined by 16 channel unipolar recordings related to the international 10/20 system. Different EEG-topography were found for different typical EEG-patterns occuring in the term newborn. Low intra- and interindividual variability for the maximum of electrical activity was found, if the typical patterns were considered. Thus, we had introduced a new methode of pattern recognition. This maximum were located in the centroparietal region coresponding to the region of the maximum of the regional cerebral blood flow.
Topographic changes of epileptogenic discharges were studied by experimental seizures (penicillin-induced) in newborn piglets and rabbits by dynamic EEG-mapping. The results were compared to the dynamic EEG-topography of benign epileptogenic patterns of the human neonate (frontal sharp transients) and discharges during neonatal status epilepticus.

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EFFECT OF NEONATAL SEIZURES ON VEGETATIVE BRAIN STEM FUNCTION

Rother, M., Eiselt, M.
Institut of Pathological Physiology, Friedrich-Schiller-University, Jena, G.D.R.

Epileptogenic discharges may play an important role in autonomic dysfunction and for sudden unexplained death in adults with epilepsy. We tried to examine whether neonatal seizures may influence vegetative brain stem function.
Seizures were defined by polygraphic recordings of human neonates including at least EEG, ECG and respiratory movements. Cardiorespirography was inspected visually to determine gross changes of mean heart rate and respiration related to seizure activity, e.g. bradycardia, tachycardia, apnoea or tachypnoea. Furtherly, spectral analysis of cardiorespirography were performed during periods with and without seizures to detect subtle changes. Experimental seizures were induced by focal cortical penicillin application in neonatal rabbits. Spectral analysis was performed during control and periods with penicillin induces seizures.
32 out of about 300 newborns were found to show neonatal seizures. 8 out of these 32 showed gross changes of vegetative parameters. Subtle changes of heart rate fluctuations were found by spectral analysis, wheras both an increase and decrease of such parameters as Respiratory Sinus Arrhythmia may occur. Only an increase of the mean heart rate was found during experimental seizures indicating an increase of the sympathicotonus. No direct sign of brain stem involvement could be shown in that study.
In summary, we concluded that neonatal seizures may affect vegetative brain stem functions and may be partly responsible for their poor prognosis.

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THE INFLUENCE OF DIFFERENT RATIOS OF RARE TO FREQUENT VISUAL STIMULI ON VISUAL P-300 COMPLEX

A. Taghavy, A. Krätzer

Visual P-300 Complex was elicited in 21 male subjects 18 - 30 years old by flashing two different kinds of 1.1/s randomly presented checkerboard patterns of 100 ms duration on a viewing screen 1 m in front of the subjects' eyes. Both, frequent A-stimulus (16x16 caskets) and rare B-stimulus (64x64 caskets), were always kept constant in size. Every subject was investigated with 5 different B/A ratios in a random sequence (I: 10:90, II: 20-80, III: 30-70, IV: 40-60, V: 50-50). In three instances during an extra session, the 5 ratios were presented vice versa (A-stimuli being rare). The potentials were derived from Oz to Fz, Cz was ground. The subjects counted silently only the rare stimuli.

Results: (1) The N-250 latencies from I through V of frequent A-stimuli as well as of the rare B-stimuli are not significantly different (Friedmann-Test; = 0.05). (2) The N-250-P-300 amplitudes are highest at the ratio I and show a steep decrease to II, also from II to III, but little difference in III-IV and in IV-V. The differences I-II, I-III, I-IV, I-V are significant (Wilcoxon-Wilcox-Test; = 0.05). (3) With A-stimuli being the rare ones the same results were obtained as above in respect to P-300 Complex

Conclusions: The finding, that N-250 latency is independent of the ratio of frequencies of the two stimuli to be differentiated, is additional evidence of the hypothesis of N-250 latency being a discrimination-potential (Taghavy and Kugler 1988, Intern. J. Neuroscience, 179-188). The impact of the reversal of the ratio of A/B-stimuli on N-250-P-300 amplitudes as well as systematic changes of ratios of visual stimuli support the hypothesis of N-250-P-300 being an endogenous task processing potential.

Neurologische Universitäts-Klinik Erlangen, Schwabachanlage 6, 8520 Erlangen

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DIFFERENCES IN THE CONTRIBUTION OF NMDA RECEPTORS TO SYNAPTIC TRANSMISSION BY LATERAL AND MEDIAL PERFORANT PATH AND COMMISSURAL FIBERS IN RAT DENTATE GYRUS

H. Clusmann, J. Stabel and U. Heinemann

The degree of NMDA receptor participation in synaptic transmission in dentate gyrus has become controversial. We therefore studied the relative distribution of NMDA receptors versus quisqualate receptors in the area dentata of rat hippocampal slices by measuring the Quis- and NMDA-induced decreases in extracellular Na^+ concentation ($[Na^+]_o$). Previous studies have shown that part of these dose dependent decreases persist in the presence of TTX. They can therefore be attributed to Na^+ fluxes through the respective receptor related ionophores. Quis- and NMDA- induced $[Na^+]_o$ decreases were small in the hilus and stratum granulare. Quis induced large $[Na^+]_o$ decreases in all of stratum moleculare (SM), whereas amplitudes of NMDA-evoked $[Na^+]_o$ decreases declined from the inner to the outer SM. Since the lateral perforant path (LPP) innervates the outer SM, the medial perforant path (MPP) the medial SM and the commissural fibers (CF) predominantely the inner SM, it appeared likely that the NMDA receptors would contribute to synaptic transmission in the different pathways to a different degree. This was tested by lowering extracellular Mg^{2+} concentration ($[Mg^{2+}]_o$) and then applying the NMDA receptor antagonist ketamine during selective stimulation of the three pathways. In low $[Mg^{2+}]_o$, field potentials evoked by CF stimulation were enhanced to the greatest degree, while those evoked by MPP stimulation were less, and LPP-evoked potentials were least enhanced. All enhancements were sensitive to ketamine.
These findings show that during synaptic transmission NMDA receptors can be activated to a different amount by the three dentate gyrus input pathways with the greatest amount of NMDA receptor participation in CF, a lesser amount in MPP and the least in LPP. Controversial findings indicating NMDA receptor participation in synaptic transmission in dentate gyrus can be explained by various stimulation sites within these three pathways.
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Institut für Neurophysiologie, Zentrum Physiologie und Pathophysiologie, Universität zu Köln, Robert-Koch-Str. 39, 5000 Köln 41, FRG

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ELECTROPHYSIOLOGICAL AND BEHAVIOURAL CHANGES PRODUCED BY 2 VESSEL OCCLUSION (2VO) IN THE RAT

F. Block and K.-H. Sonatg

In contrast to experimental models of ischemia 2VO does not lead to consistent neuronal damage in vulnerable structures of the brain (Heim et al, Eur J Neurosci Suppl 1,1988,58). The metabolism, measured by local PO_2 and lactate, was markedly influenced during occlusion and early reperfusion (Block et al, Eur J Neurosci Suppl 2,1989,151). To gain more information about this model electrophysiological measurements and behavioural observations were made.
Induction of 2VO was done in pentobarbital anesthesia (60mg/kg i.p.) by transient (60 min) occlusion of both common carotid arteries. The electrophysiological experiments were carried out during the occlusion and the first hour of reperfusion. By electrical stimulation of a forepaw somatosensory evoked potentials (SEP) could be recorded from the corresponding somatosensory cortex. Visual evoked potenials (VEP) were elicited by photic stimulation using a strobe and responses were recorded from the primary visual cortex. The behaviour was monitored during the night (12 h) at intervals of 20 min using an automatic system. One group of animals was observed 2 days after 2VO, the other one 1 year afterwards. The SEP's were not altered by 2VO. VEP's revealed a significant reduction in amplitude and increase in latency during the occlusion compared to sham-operated controls. These changes were reversible since during reperfusion no differences between the groups were seen. The acute effect in behaviour, measured 2 days after 2VO, resulted in an increase of stereotypic movements and rearing at the occluded animals. One year afterwards 2VO animals had significant less resting periods (defined by distance travelled = 0, ambulation = 0, rearing = 0) compared to controls. These results suggest that besides reversible changes during occlusion 2VO leads to long-lasting alterations. They might be interpreted as early aging.

Max-Planck-Institut für experimentelle Medizin, Hermann-Rein-str. 3, D-3400 Göttingen, F.R.G.

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ERPS ASSOCIATED WITH CORRECTED AND UNCORRECTED ERRORS IN A CHOICE REACTION TASK.

M.Falkenstein, J.Hohnsbein, and J.Hoormann

Recently we observed a severe amplitude reduction in the P300 latency range for ERPs associated with incorrect compared to correct reactions in a two-alternatives forced-choice reaction task (Falkenstein et al., EPIC IX, 1989). This reduction was caused by a deflection that we named error negativity (Ne). In our interpretation the Ne reflects an (automatic) mismatch between the (incorrect) overt reaction (based on insufficient imformation from the response selection process) and the (correct) result of sufficient response selection. In order to rule out an alternative explanation, namely, that the Ne could reflect a correction (i.e., a press of the correct key within 300 ms after the incorrect reaction), we conducted this study. Spoken letters "F" or "J" (synthetic speech) were presented one at a time in random order with a randomized ISI of about 1500 ms to 10 subjects. The subjects had to react to each letter by pressing the appropriate key of a keyboard. The error rate was enhanced by a time pressure regimen. The EEG was recorded from six electrodes (Fz, Cz, C3, C4, Pz, Oz). ERPs were obtained separately for incorrect trials with and without corrections. The Ne was seen in both classes of trials. We conclude that the Ne is indeed not caused by correction procedures, and therefore, that our earlier interpretation still holds.

Institut für Arbeitsphysiologie, Abt. Sinnes- und Neurophysiologie, Ardeystr.67, D-4600 Dortmund

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ON THE LOCALIZATION OF CEREBRAL GENERATORS OF LONG LATENCY EVOKED POTENTIALS AND FIELDS

A. Frieling, I.M. Tarkka, W. Kullmann*, B. Bromm

First results of a comparison of methods for the localization of the generators of late evoked potential components are presented. Intracutaneous electrical stimuli (20 ms, 2- and 3-fold individual pain threshold, tip of left middle finger) and auditory stimuli (0.5s, 500 Hz, 65dB$_{SPL}$) were given in blocks of 40 stimuli with interstimulus intervals from 10 to 30s, and the reactions were averaged. The electroencephalogram (EEG) was recorded from 14 positions (10-20 system) with reference to the left earlobe, the magnetoencephalogram (MEG) was recorded with a 1-channel SQUID system (2nd order gradiometer) from 49 positions (spacing 2cm) normal to a sagittal plane over the right hemisphere. Magnetic resonance tomograms were taken with the electrode positions marked.

The table gives latencies (ms) of the main negativities (N) and positivities (P) in one subject.

stimuli	EEG		MEG	
somatosens.	N170	P275	N185m	P270m
auditory	N110	P220	N110m	P180m

Brain electrical source analysis (BESA) of EEG and a least squares fit of MEG field maps localized, in case of auditory stimuli, the equivalent dipoles bilaterally in the proximity of the auditory cortex. Following somatosensory stimuli, EEG source analysis located equivalent dipoles close to the midline for the main negativity and nositivity, whereas MEG fieldmaps where complex and could not be explained by a singular current dipole. (Supported by the Deutsche Forschungsgemeinschaft Br 310/16)

Physiologisches Institut der Universität Hamburg,
Universitätskrankenhaus Eppendorf, Martinistr.52, 2000 Hamburg 20
*Philips Forschungslaboratorium Hamburg

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COMPUTER ANALYSIS OF NEURONAL SPIKE TRAINS WITH SPECIAL REGARD TO IMPULSE GROUPS

M.Chr. Hirsch, H. Wissing and H.A. Braun

Grouping of impulse pattern has been observed in a variety of excictable cells, ranging from peripheral sensory receptors to central cortical and subcortical neurons. These impulse groups sometimes occur rather irregular but often exhibit a distinct periodicity. The periodicity might be due to synaptic interaction within neuronal networks but could also be produced by intrinsic membrane properties of the individual cell. The occurence of impulse groups depend on environmental conditions and can experimentally be modified by external stimuli and pharmacological substances. Such experiments in combination with an analysis of the impulse patterns could considerably contribute to a better understanding of the spike-triggering mechanisms.
The computer program presented here includes standard techniques of impulse pattern analysis like PSTHs, intervall histograms etc. but has additional features dealing with the analysis of impulse groups. Impulse groups (bursts) are identified by different criteria which are adjustable to the specific features of a given impulse pattern. Distribution and time course of different burst characteristics can be presented numerically and graphically. Additionally, bursts can be selected by these characteristics. Intraburst intervalls can be examined in dependence of their position in the burst.
These methods, for example, allowed to distinguish between different principles of burst generation in sensory cells and might also be a useful tool to examine spike trains in the central nervous system.

Physiologisches Institut der Universität Marburg, Deutschhausstraße 2, 3550 Marburg, FRG

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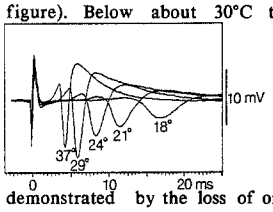
EFFECTS OF TEMPERATURE ON SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPAL SLICES

P. Igelmund, U. Heinemann and F.W. Klußmann

We are interested in comparing the effects of temperature on neuronal functions in hibernators, which survive body temperatures down to 2°C, and in non-hibernators. In the present study we have investigated the effects of cooling on synaptic transmission in area CA1 of rat hippocampal slices. Evoked field potentials were recorded extracellularly in stratum radiatum (SR) and stratum pyramidale (SP). Stimulation electrodes were positioned in SR for orthodromic stimulation and in the alveus for antidromic stimulation of the pyramidal cells. The temperature was varied between 37°C and 8°C.

In normal artificial CSF and with constant stimulus intensities the latency and duration of orthodromically induced population spikes of the pyramidal cells increased continuously with cooling. In contrast, the amplitude of these spikes first increased between 37°C and approximately 28°C and then decreased with further cooling (see figure). Below about 30°C the ability of the tissue to follow high frequency stimulation was impaired. At temperatures below 20°C the stimulus threshold increased strongly and in the range of 17-13°C the synaptic transmission from Schaffer collaterals and commissural fibers to the pyramidal cells was completely blocked. This was demonstrated by the loss of orthodromically evoked population spikes, even with very high stimulus intensities; with direct antidromic stimulation pyramidal cells were excitable down to temperatures below 10°C. The effects were largely reversible. The temperature at which synaptic transmission was blocked was strongly dependent on extracellular cation concentrations. For example, it increased from approximately 15°C in the presence of 5 mM K^+ to about 25°C in 10 mM K^+.



Differences between rats (non-hibernators) and hamsters (hibernators) are currently investigated.

Institut für Neurophysiologie der Universität zu Köln, Robert-Koch-Str. 39, D-5000 Köln 41, FRG

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FASTER FOVEOPETAL THAN FOVEOFUGAL MOTION EXTRAPOLATION REVEALED IN A VISUO-MOTOR TASK

W.H. Ehrenstein*, N. Yakimoff**, S. Mateeff**, J.Hohnsbein*

Six subjects indicated, by pressing a button, the moment at which a moving target (direction of motion: left to right; velocity 6 deg/s) "passed" a stationary reference line, marked by two bright dots 1 deg above and below the horizontal motion path (12 deg long). The reference line was randomly presented at seven positions. For two of the positions the alignment between the moving target and the reference was visible while in the other five positions an imaginary alignment had to be based on motion extrapolation. Two conditions of binocular fixation were tested: (A) at the point of target disappearance; (B) at the actual position of the reference. Extrapolation of motion was thus away from fovea (foveofugal) in condition A and towards fovea (foveopetal) in condition B.

A linear relationship between response time and physical duration of extrapolated motion (both measured from the moment of target disappearance) was obtained. Whereas no systematic differences between conditions A and B were found for the indicated moments of visual alignment, response times, indicating imaginary alignment, were consistently longer in A. The differences increased with extrapolation distance. The results suggest that motion is faster (23% on the average) for foveopetal as compared to foveofugal extrapolation. The data are discussed in relation to recent electrophysiological findings of foveopetal-foveofugal asymmetries in the responses of visual neurons in primate parietal cortex.

*Institut für Arbeitsphysiologie, Dortmund, FRG
**Institute of Physiology, Sofia, Bulgaria

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CORTICOSTERONE DECREASES INHIBITORY POST-SYNAPTIC POTENTIALS IN RAT NEOCORTICAL AND HIPPOCAMPAL CELLS *IN VITRO*

M. L. Zeise, J. Arriagada[1], A. Teschemacher and W. Zieglgänsberger

Steroid hormones are known to act through the genom inducing mRNA synthesis. Recent evidence suggests that steroids may also affect membrane responses of neurons directly. We investigated the action of corticosterone (CT) on membrane properties and synaptic potentials in neurons of the rat hippocampus and neocortex.

Conventional intracellular recordings were performed in slices of the frontal neocortex and the hippocampus (CA1). CT (10^{-7} - 10^{-5} M) was dissolved in ethanol and added to the perfusion fluid. The excitability of the neurons decreased due to a diminuition of the inward going rectification. CT (10^{-5} M) did not change resting membrane potentials (RMPs) or thresholds for eliciting postsynaptic potentials. Early and late IPSPs were both reduced under CT. At low stimulation intensities (EPSPs not contaminated by IPSPs) EPSPs were not affected by the steroid. In about half of the hippocampal neurons, CT increased the afterhyperpolarization (AHP) following repetitive spiking. When CT was applied locally close to hippocampal neurons, IPSPs were reduced almost immediately. This implies that in central neurons CT acts not only through a genomic mechanism mediated via cytosolic receptors but exerts effects also at membrane receptors. The present data are in line with an action at GABA receptors.

Max-Planck-Institute for Psychiatry, Clinical Neuropharmacology, D-8000 München 40 and [1]Department of Cellular and Molecular Biology, Catholic University, Santiago de Chile

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DC RECORDINGS AT THE SURFACE OF THE SKULL DURING CORTICAL SPREADING DEPRESSION AND GENERALIZED SEIZURE ACTIVITY

A. Lehmenkühler, T. Pöppelmann and E.-J. Speckmann

Characteristic negative DC potential shifts can be led from the surface of the brain cortex during spreading depression (SD) and generalized seizure activity (cf. Caspers, H, Speckmann E.-J., Lehmenkühler, A., Rev. Physiol. Biochem. Pharmacol. 106: 127-178, 1987).

The present experiments on anesthetized and artificially ventilated rats tested whether and to what extent these cortical DC shifts appear at the intact surface of the skull. SD waves were produced by continuous local application of KCl solution (0.5 to 1 mol/l) to the surface of the brain skull lying over the motorcortex. This was done via a recording outflow electrode with a porous ceramic membrane. The first SD started ca. 1 hour after positioning of the electrode and was associated with a negative transient DC shift (amplitude: 4 to 6 mV; duration: 5 to 7 min). Generalized tonic-clonic seizures were produced by systemic administration of pentylenetetrazol. Generalized seizures were associated with negative DC shifts (amplitude: up to 3 mV). This type of DC shift had a steep ascending slope while the return to baseline was rather slow and nearly monoexponential.

The results demonstrate that SD and generalized seizures are associated with negative DC transients not only at the surface of the brain cortex but also of the skull.

Institut für Physiologie der Universität Münster, Robert-Koch-Str. 27a, D-4400 Münster

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SUPERFUSION OF THE BRAIN CORTEX WITH VERAPAMIL DURING PARTIAL AND GENERALIZED EPILEPTIC ACTIVITY

R. Köhling, E.-J. Speckmann, A. Lehmenkühler, J. Walden, D. Bingmann and P. Boerrigter

Focal and generalized tonic-clonic seizures have been suppressed by intracerebroventricular perfusion of verapamil (Walden, J., Speckmann, E.-J., Witte, O., Electroencephal. clin. Neurophysiol. 61: 299-309, 1985 and 69: 353-362, 1988). The present experiments tested whether partial and generalized seizure activity can be suppressed by verapamil with superfusion of the cortical surface.
Experiments were performed on anesthetized and artificially ventilated rats. Partial and generalized seizure activity was elicited by cortical superfusion of penicillin (PEN) and i.p. injections of pentylenetetrazol.
1) Partial and generalized seizures were neither suppressed nor prevented by superfusing verapamil (100 µmol/l).
2) Extracellular concentration of verapamil could be measured in brain cortex using an ion-selective microelectrode (valinomycin; acidification of tissue: pH=6.5).
3) Superfusion of the brain with verapamil (10 mmol/l; pH=6.5) resulted in a standing concentration gradient of the drug of 50:1 per 100 µm depth.
The missing effect of verapamil is attributed to a very low entry of the drug into the extracellular space of the cortex.

Institut für Physiologie der Universität Münster, Robert-Koch-Str. 27a, D-4400 Münster

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EFFECTS OF VALPROATE SODIUM ON EPILEPTIC DISCHARGES IN A MODEL NERVOUS SYSTEM (BUCCAL GANGLIA, HELIX POMATIA)

U. Altrup, H. Reith, E.-J. Speckmann and J. Walden

The elementary mechanisms underlying the effects of antiepileptic drugs in the nervous system are generally not fully understood. Presently, the effects of valproate sodium (VPA) were studied in identified neuronal individuals of a model nervous system.

The experiments were performed on neurons B1 to B4 of the buccal ganglia of Helix pomatia. The ganglia were kept in an experimental chamber continuously perfused with a conventional saline. Epileptic activities were induced by pentylenetetrazol (40 mmol/l) or etomidate (0.5 mmol/l). VPA was added to the saline (0.1 to 20.0 mmol/l) or injected into the soma of neurons.

VPA (0.5 to 10.0 mmol/l) suppressed paroxysmal depolarization shifts (PDS) and desynchronized epileptic activities. Comparable effects were obtained with 0.5 mmol/l VPA applied for ca. 15 h and 10 mmol/l VPA for ca. 2 h. With the begin of extracellular VPA-treatment PDS were accentuated transiently, PDS then decayed. After termination of VPA-application the decay accelerated and reversed only in part during several hours of washing. With intracellular injection of VPA the antiepileptic effects appeared immediately.
VPA (above 10 mmol/l) induced typical PDS when applied extracellularly in high concentrations without pentylenetetrazol or etomidate in the saline. PDS were facilitated at acidic pH levels (ca. 6.9). Intracellular injection of VPA did not induce PDS.

Results suggest that VPA slowly crosses the cell membrane and that it exerts its antiepileptic and epileptogenic effects from the intracellular and extracellular space, respectively.

Institut für Physiologie der Universität Münster, Robert-Koch-Str. 27a, 4400 Münster

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SYNAPTIC CONTACTS BETWEEN LUNG STRETCH RECEPTOR AFFERENTS AND BETA-NEURONES IN CAT

K. Anders, W. Ohndorf*, R. Dermietzel* and D.W. Richter

Light- (LM) and electronmicroscopical (EM) analysis revealed terminal arborization of slowly adapting lung stretch receptor afferents (SARs) within various subnuclei of the solitary tract (TS). These regions are the localization of somata and dendrites of inspiratory beta-neurones (Rß) which receive monosynaptic inputs from SARs as shown electrophysiologically. In the present experiments, we have double-labelled SARs and Rß-neurones to verify the synapses and further describe their morphological characteristics.
Experiments were performed in pentobarbitone (40 mg/kg) anaesthetized and artificially ventilated cats. SARs were identified within the TS by lung inflation and excitation following vagal nerve stimulation. Rß- neurones were identified by their depolarization during sustained lung inflation and central inspiration, as indicated by phrenic nerve activity. One Rß-neurone and 1-7 SARs per experiment were double-labelled by intracellular HRP injection.
In LM, we found 40 boutons of SARs terminating on proximal dendrites of Rß-neurones; only one SAR ended on the soma. Boutons were of the "en passant" or "terminal" type. 12 presumed contacts were analyzed in EM. Single axodendritic synapses were verified for 2 SARs terminating on Rß-neurones. Multiple synaptic contacts became evident in 2 terminal branches of a single SAR synapsing on proximal dendrites of the same Rß-neurone. The boutons had a diameter ranging between 1.5-3 µm, contained round, clear vesicles of a diameter of 40-50 nm and formed asymmetrical synapses. There was only one active zone per synapse.
The findings verify monosynaptic connections between SARs and Rß-neurones, partly forming multiple synapses.

Physiol. Inst. Univ., 3400 Göttingen, Humboldtallee 23
* Anat. Inst. Univ. Klinikum, 4300 Essen, Hufelandstr. 55

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PROTO-ONCOGENE *C-FOS* INDUCTION IN THE SPINAL CORD: DIFFERENTIAL MODULATION BY MORPHINE, ANTI-EPILEPTIC DRUGS AND NMDA ANTAGONISTS

T.R. Tölle, J.M. Castro-Lopes and W. Zieglgänsberger

The proto-oncogene *c-fos* is expressed in dorsal horn neurons of the rat spinal cord following peripheral noxious stimulation (NS). Excitatory amino acids, such as L-glutamate, serve as transmitters of primary afferent fibers and involve actions mediated by one or more combinations of receptors (quisqualate, kainate, NMDA).
Seventy rats anesthetized with halothane were injected i.v. with morphine (5, 7.5, and 10 mg/kg) or the NMDA antagonists ketamine (4 and 10 mg/kg) or MK-801 (1 mg/kg). In another set of experiments the anti-epileptic drugs carbamazepine (50 mg/kg) or valproate (300 mg/kg) were injected i.p.; these agents have an analgesic effect in some forms of chronic pain, such as trigeminal neuralgia. The spinal cord lumbar segments L_4-L_5 were immunohistochemically processed for the detection of the nuclear *c-fos* protein 2 hrs after stimulation. Following NS *c-fos* postitive neurons were found in the medial half of the ipsilateral laminae I-II and to a smaller extent also in deeper laminae (III-VI,X). Morphine and to a smaller extent carbamazepine and valproate reduced the number of stained neurons. Of the drugs tested morphine (10 mg) caused the most extensive depression; 70 % in superficial and 85 % in deep neurons. Morphine dose-dependently (antagonized by naloxone) depressed *c-fos* expression when injected 10 min before NS, but not 10 min after finishing the NS. Naloxone per se (5 and 10 mg/kg) slightly increased the number of stained neurons. The NMDA antagonists ketamine and MK-801 produced no differences in the number or distribution of labelled neurons, although both drugs showed analgesic potency verified in behavioural tests at this dose-level.
NMDA receptor mediated processes are involved in somato-sensory processing at the first stage in the dorsal horn. However, the present study shows that NMDA receptor blockade does not prevent *c-fos* induction. Since an increase in intracellular Ca^{2+} induces *c-fos* expression in vitro, it remains to be shown whether an activation of the inositol phosphate metabolism by L-glutamate or co-released substance P leading to an increase in intracellular Ca^{2+} is the adequate trigger for *c-fos* induction in the spinal cord.

T.R. Tölle, Klinische Neuropharmakologie, Max-Planck-Institut für Psychiatrie, Kraepelinstr. 2, 8000 München 40.

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PRIMING OF PROTO-ONCOGENE EXPRESSION IN NEURONES OF THE SPINAL DORSAL HORN BY CONTRALATERAL NOXIOUS SKIN STIMULATION

J. SANDKÜHLER, J.D. LEAH AND T. HERDEGEN

Nociceptive inflow to the spinal cord may be followed by facilitation of spinal nociception at the contralateral site. Here we report that the induction of the proto-oncogene *c-fos* in neurones of the spinal dorsal horn is 'primed' by contralateral noxious stimulation.

Rats which were either awake or lightly or deeply anaesthetized with thiopental or α-chloralose were used. A 20 min noxious mechanical (pinch), or radiant heat (56 ° C for 10 s at 2 min intervalls) stimulus, or a chemical (formalin 2%, or capsaicin 10^{-7} M, 50 nl injected s.c.) stimulus was applied to the central pad at one hindpaw. Sixty minutes later this stimulus was followed by an identical one applied to the corresponding skin area at the *contra*lateral hindpaw. The rats were killed one hour after the second stimulus, perfused transcardially with PBS and paraformaldehyde and the lumbar spinal cord was removed. Transverse sections were processed with conventional immunocytochemical techniques to detect the nuclear c-fos like protein (primary antibody at 1:10.000; avidin-biotin method).

The number of labelled neurones in the superficial and in the deeper dorsal horn was 80–200% higher at the site of the second stimulus, irrespective of the level of anaesthesia, the anaesthetic used or the type of noxious stimulation. The only exception was capsaicin induced *c-fos* expression which was equally rare on both sites of the cord even though these animals were unanaesthetized. The time dependent decline of the c-fos protein accumulation could, in itsself, not account for these large differences in labelling.

This priming of the expression of the *c-fos* gene in neurones of the spinal dorsal horn may be involved in longterm changes in the central nervous system following peripheral trauma.

II. Physiologisches Institut, Universität Heidelberg, Im Neuenheimer Feld 326, 69 Heidelberg. Supported by the DFG (grant Sa 435/3-3).

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NOXIOUS TRANSYNAPTIC STIMULATION OF SPINAL NEURONS IS FOLLOWED BY COMPLEX NUCLEAR EVENTS: INDUCTION OF IMMEDIATE EARLY GENES IN THE RAT.

T.Herdegen, J.D.Leah and M.Zimmermann

Jun, JunB, JunD, Krox-24, FosB and Fos proteins belong to the group of immediate early genes (IEGs) which are supposed to play a pivotal role in foundamental processes as cell division, differentiation or tumorgenesis. All proteins exert their function in the control of transcription of genes. Here, we have investigated the expression of these proteins as induced by transynaptic activation of nerve cells in spinal cord. The sciatic nerve of pentobarbital anesthetised adult rats was stimulated electrically and the immunoreactivity (IR) of the proteins was investigated in lumbar spinal cord. Stimulation of Aß-fibers (0.7 V, 20 Hz, 20 min) did not induce for gene expression. Stimulation of Aß- and C-fibers (20 V, 5 Hz, 10 min) induced the expression of all 6 proteins in neurons of the dorsal and ventral horn, except motoneurons. A minimum stimulation period of 2 min was required for expression of all proteins. The increase of intensity of stimulation was followed by an increased number and intensity of IR. All proteins appeared ipsilaterally between 25-30 min following the onset of stimulation. The maximum of IR was observed between 1-2 h, then the number of labeled cells decreased within 15 h. Contralaterally there was an increase of labeled cells after 6h. All proteins showed a similar IRpattern of appearance resp. disappearance. Repetition of a stimulus (Aß- and C-fiber stimulation 2 h to 24 h following the first stimulation) provoked changes in the temporal and spatial pattern of IR: compared to the first stimulus the number of labeled cells was increased and the presence of the proteins was prolonged. It is conceivible that the alteration of induction and of stability of proteins following repetition of noxious stimulation reflects genetic mechanisms which might underly lasting changes in the nervous system such as memory formation.

II. Institute of Physiology, INF 326, 6900 Heidelberg, Germany. Supported by the Deutsche Forschungsgemeinschaft.

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STIMULATION OF HIGH- AND LOW-PRESSURE RECEPTORS INFLUENCES CNS IN HUMANS

D. Vaitl and H. Gruppe

Previous studies in animals have shown that stimulation of carotid baroreceptors increases cortical slow wave activity. In order to investigate this effect in humans high- and low-pressure receptors were systematically stimulated by changing body position (head-up tilt: 30^0, 45^0; head-down tilt: -6^0, -12^0) and preventing venous blood from pooling (by an anti-G-suit) during orthostasis. In three experiments, scalp EEG was recorded from occipital, parietal, and frontal sites during consecutive 4 second epochs during different postures with and without lower body compression (duration: 4 minutes each).

Spectral power analyses of EEG of fourty-four subjects revealed consistent changes in the EEG-theta frequency band which were mainly brought about by changes in body position and less influenced by lower body compression. A linear relationship between increases in EEG-theta power and circulatory changes could be observed within the range of 30^0 head-up tilt and -6^0 head-down tilt. Above and below these limits (i.e. -12^0 and 45^0) no further changes in EEG-theta activity could be obtained. These systematic changes in EEG did not occur in other frequency bands and cannot be explained by decreased vigilance or drowsiness. Changes in hemodynamics and high-pressure receptors appear to influence slow wave brain activity. These effects observed in humans are similar to those found in animals.

Klinische Psychologie, Fachbereich Psychologie, Universitaet Giessen, Otto-Behaghel-Strasse 10, D-6300 Giessen, F.R.G.

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REDUCTION OF PAIN PERCEPTION TO NOXIOUS STIMULI BY ACTIVATION OF CAROTID SINUS BARORECEPTORS IS NALOXONE REVERSIBLE.

M.K. Herbert[1], M.K.C. Mengel[2], K.-D. Kniffki[2]

Aim of investigation: As shown on previous experiments stimulation of carotid sinus baroreceptors (CSBs) reduces experimentally evoked pain in healthy volunteers as well as pain perception in chronic pain patients. The aim of the present study was to explore whether this pain reducing effect in healthy humans is naloxone sensitive and may be mediated by endogenous opioids.

Methods: In 6 male and 5 female untrained healthy volunteers experimental pain was induced by applying constant pressure on a 2.6 mm^2 area to the third and fourth finger for 20 s. One of two different stimuli randomly presented in intervals of 4 min to each of the 4 spots on the 2 fingers was of noxious intensity. During pressure stimulation subjects rated their pain sensation continuously on a 50 point category scale. CSBs were activated by negative pressure applied bilaterally to the neck using a suction chamber beginning 15 s before and ending with the painful stimulus. Trials of 3-5 stimuli of either intensity were performed under (a) control conditions, (b) activation of CSBs and (c) activation of CSBs following i.v. application of 1 mg naloxone or saline solution.

Results: In 7 of 11 subjects tested CSBs activation with an individually tolerated strength of -55 mmHg to -95 mmHg lowered the magnitude of pain ratings significantly as compared to control trials. The pain reducing effect of CSBs stimulation was naloxone sensitive and fully reversible in 4 of these 7 subjects. Saline solution had no effect at all. Pain ratings under CSBs activation were not influenced in one and enhanced in 3 of 11 subjects. Efficiency of neck suction in activating CSBs was confirmed in all cases by the reduction of blood pressure and heart rate monitored during CSBs stimulation.

Conclusion: Neck suction induced activation of CSBs is capable of reducing pain ratings to experimental mechanical noxious stimuli in 7 of 11 subjects tested. This effect was naloxone reversible in 4 of these 7 and therefore might be mediated by endogenous opioids released during CSBs activation.

Institut für Anaesthesiologie[1] und Physiologisches Institut[2], Universität Würzburg, D-8700 Würzburg, FRG.

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DISTRIBUTION OF CALCITONIN GENE-RELATED PEPTIDE-LIKE IMMUNOREACTIVITY IN THE CAT'S THALAMUS

R. Linke, C. Vahle-Hinz and K.-D. Kniffki

Calcitonin gene-related peptide (CGRP) is a widely distributed neuromediator which is thought to be involved in processing visceral, cardiovascular, olfactory, gustatory, and noxious stimuli. We studied the distribution of CGRP at diencephalic sites involved in these functions.

Adult cats not pretreated with colchicine were perfused transcardially under deep anesthesia with 4% paraformaldehyde and 2% picric acid. Serial frontal sections of the thalami were reacted with an antibody against CGRP (Peninsula) using the peroxidase-antiperoxidase (PAP) method (2nd antibody: goat-anti-rabbit, Dakopatts; 3rd antibody: PAP complex, Dakopatts).

A dense terminal field of CGRP-immunoreactive (CGRP+) fibers was observed in the ventral part of the principal ventromedial nucleus adjacent to the dorsal hypothalamic area. More caudally a network of fibers of intermediate density was seen in the subparafascicular nucleus. A second dense terminal field was located in the anterior hypothalamic area mediad the fornix. Here small and large boutons outlined the somata and primary dendrites of unstained cells. Less dense fiber populations occurred in the dorsal part of the rostral submedial nucleus, in the mediodorsal nucleus, in the lateral part of the lateral habenula, in the dorsal and lateral hypothalamic areas, and in the ventral tegmentum. A few fibers were found in the medial part of the ventral periphery of the ventrobasal complex. However, these fibers displayed distinct terminal boutons in some cases situated on somata. CGRP+ cells were also found but their number might be underestimated due to the lack of colchicine pretreatment. Scattered immunopositive cells were located at the caudal border of the basal ventromedial nucleus in a row running in a caudal and lateral direction to the peripeduncular nucleus. Some cells were also found at the medial border of the medial geniculate body. A third location was in the anterior hypothalamic area surrounding the dense terminal field of CGRP+ fibers.

The distribution of CGRP+ fibers and cells in the cat's brain appears to be similiar to that in the rat (Kruger et al., J. Comp. Neurol. 273:149, 1988). The results suggest that at the thalamic level CGRP is more likely a neuromediator of ascending gustatory and visceral pathways and to a lesser degree involved in processing noxious information.

Physiologisches Institut, Universität Würzburg, Röntgenring 9, D-8700 Würzburg

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DIVERGING EFFECTS OF BOMBESIN ON THE TEMPERATURE SENSITIVITY OF NEURONES IN DIFFERENT HYPOTHALAMIC AREAS

J. Schenda, K. Matsumura and Fr.-K. Pierau

Previous investigations have demonstrated that bombesin increases the temperature coefficient (TC) of neurones in the preoptic area/anterior hypothalamus (PO/AH) *in vitro*. Since bombesin affects temperature regulation only after injection into the PO/AH area but has no effect by injection into the posterior hypothalamus (PH), we have studied the action of bombesin upon the TC of neurones in different nuclei of the AH and PH. Extracellular recordings were accomplished from rat hypothalamic slices in a perfusion chamber (artificial cerebrospinal fluid, 95% O_2/5% CO_2 atmosphere) with periodic temperature changes between 35 and 41 °C. Bombesin was injected as a bolus (0.1 mg in 0.1 ml).

The proportion of warm sensitive units in different areas of the AH varied between 30 and 50% (threshold TC: 0.6 imp/s). Bombesin increased the number of warm sensitive neurones in 3 of the 4 investigated AH areas (median preoptic area - MPA, median preoptic nucl. - MPN and ant. periventricular nucl. - aPVN), indicating a conversion of temperature insensitive neurones into warm sensitive ones. By contrast, bombesin decreased the TC of some of the neurones in the suprachiasmatic nucleus (SCN) resulting in a 5% reduction of warm sensitive neurones. Similarly, the number of warm sensitive neurones was reduced by about 8% in the arcuate nucleus (AN) of the PH, in which however, only 20% of the neurones were warm sensitive. The neurones of the other PH area investigated - the pPVN - resembled the AH neurones, in that the proportion of 40% warm sensitive neurones was increased to 50% by bombesin. The results might suggest that the functional role of hypothalamic neurones in temperature regulation is not simply defined by their affiliation to the AH or PH.

MPI für physiol. und klin. Forschung, W.G. Kerckhoff-Inst. Parkstr. 1, D-6350 Bad Nauheim

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AXOTOMY AND BLOCK OF AXONAL TRANSPORT INDUCE EXPRESSION OF PROTEINS ENCODED BY THE JUN PROTO-ONCOGENE FAMILY: MOLECULAR GENETIC CHANGES DEPENDENT ON THE SUCCESS OF NERVE REGENERATION

T.Herdegen, J.D.Leah and M.Zimmermann

Proteins of jun and fos proto-oncogene families (JUN and FOS) control transcription of genes and belong to the immediate early genes whose expression plays a central role in development, differentiation or tumorgenesis. We have studied the expression of JUN and FOS proteins in nerve cells following axotomy and block of axonal transport (axT). In pentobarbital anesthetised adult rats, the sciatic nerve was transected. Both JUN- and FOS- immunoreactivity (IR) appeared 30 min after the sciatic axotomy in nuclei of numerous dorsal horn cells which correspond somatotopically to the termination area of sciatic nerve fibers. 24 hours later JUN- and FOS-IR was nearly absent. After 4 days, JUN, but not FOS, appeared ipsilaterally in motoneurons (MN) and dorsal root ganglion cells (DRG). In MN the increased JUN-IR persisted for up to 50 days. In DRG, JUN-IR persisted over 15 months if the regeneration was prevented by ligation and removal of the distal sciatic nerve. After crush of the sciatic nerve the DRG cells revealed a similar JUN-IR as after transection for up to 20 days but was almost absent between 50 and 60 days when the nerve had successfully regenerated. Injection of the tracer fast blue to the proximal nerve stump showed that all those MN and DRG cells expressed the JUN which were labeled with the fast blue. Transection of the dorsal columns, vagus nerve and preganglionic sympathetic nerve fibers provoked JUN-IR in the neurons of origin of the transected axons. Block of sciatic axT by colchicine induced JUN in MN and DRG. Injection of colchicine into lumbar spinal cord and of vinblastine into the putamen induced JUN-IR in numerous neurons of the injected areas. We suppose that JUN-IR following axotomy and block of axT is induced by absence of trophic factors signaling the intact neuron-target axis and may play an important role in the strategy of survival of axotomized neurons to prevent cell death.

II.Institute of Physiology, INF 326, 6900 Heidelberg, Germany. Supported by the Deutsche Forschungsgemeinschaft.

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HOW REPRESENTATIVE ARE THE RESPONSES OF SINGLE NOCICEPTIVE DORSAL HORN NEURONS?

J. SANDKÜHLER, Q.-G. FU AND C. FORSTER

It is not known as to whether recordings from single nociceptive spinal dorsal horn neurones provide representative informations. We have addressed this question by recording action potentials of multiple individual neurones in the spinal cord simultaneously.

In rats, deeply anaesthetized with pentobarbital (60 mg kg^{-1} i.p. initially and 10–20 mg kg^{-1} h^{-1} i.v. for maintenance), extracellular recordings were made with tungsten microelectrodes from multireceptive lumbar spinal dorsal horn neurones responding to noxious radiant heating (50 °C, 10 s) of the skin at the ispsilateral hindpaw. Action potentials of individual neurones were identified in a multineuron recording by off-line analysis with our spike-discrimination programm.

For simultaneously recorded neurones the magnitude of responses to noxious heat stimuli given at 2 min intervalls displayed synchronous fluctuations. The time course and the efficacy of descending inhibition induced by blockage of $GABA_A$-receptors in the midbrain periaqueductal grey was also highly correlated for simultaneously recorded spinal neurones. In contrast, the efficacy of *tonic* descending inhibition determined by the increase in heat-evoked activity during reversible cold blocking of the lower thoracic cord varied largely between neurones.

Thus, the variability of nociceptive responses does not reflect low retest reliability of the single cell recordings but may be due to a time dependent modulation of spinal nociception. Representative data about time course and efficacy of stimulation-produced but not tonic descending inhibiton may be obtained by single neuron recordings.

II. Physiologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg. Supported by the Deutsche Forschungsgemeinschaft (grant Sa 435/3-3).

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LACK OF EFFECT OF BACLOFEN ON RELEASE OF SUBSTANCE P FROM THE CAT SUBSTANTIA GELATINOSA IN VIVO

W. D. Hutchison* and C. R. Morton

Baclofen reduces the presynaptic release of excitatory transmitter from large diameter primary afferent fibres terminating in the spinal cord. Analgesia results upon intrathecal administration in animals and humans, suggesting a similar reduction in release of neurotransmitters from small diameter nociceptive fibres. The antibody microprobe technique (Duggan et al. J. Neurosci. Methods 23: 241, 1988) was used to measure the intraspinal release of immunoreactive substance P (irSp) in barbiturate anaesthetized cats before and after the administration of (+/-) baclofen. Glass microelectrodes bearing antibodies to the C-terminal end of Sp are inserted into the lower lumbar spinal cord during noxious thermal or mechanical cutaneous stimulation of the ipsilateral hind paw or electrical stimulation of the tibial nerve at an intensity sufficient to excite C-fibres. Microprobe tips were then incubated in radioiodinated Sp and zones of release identified as deficits in tracer binding seen on autoradiographic images. A video camera and computer were used to scan the images and calculate average scans. In addition, extracellular recordings of dorsal horn neurones allowed the gated C-fibre response to tibial nerve stimulation to be measured before and after (+/-)baclofen administration.

Noxious cutaneous stimulation of the hindpaw or electrical stimulation of the tibial nerve evoked irSp release at a level of the dorsal horn corresponding to the substantia gelatinosa. There was no effect on the height or shape of the zone of release of irSp induced by tibial nerve stimulation following administration of 4 mg/kg of baclofen. A similar result was found for release of irSp induced by noxious cutaneous stimuli. There appeared a slightly elevated zone of release at the spinal cord surface in both stimulation groups after baclofen administration. This dose of baclofen, however, strongly depressed the gated C-fibre responses of the dorsal horn interneurones to tibial nerve stimulation. These findings suggest either that the subpopulation of Sp-containing C-fibres plays a minor role in nociceptive transmission or that baclofen does not induce analgesia by a presynaptic reduction in irSp release from these C fibres.

Div. Neurosci, Austral. Natl. Univ., Canberra, Australia.
*Physiol. Instit., Rõntgenring 9, D-8700 Würzburg.

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CONTROL OF LOCOMOTOR ACTIVITY DURING ISOLATION

J. Aschoff

Human subjects who live in isolation without tempo-ral cues usually develop circadian rhythms which "free-run" with periods close to 25 h. However, in about 30% of the subjects the sleep-wake cycle is suddenly lengthened to more than 28 h, or shortened to less than 20 h. If this happens, the rhythms of other functions such as body temperature continue to free-run with a period of about 25 h. During such states of internal desynchronization, subjects can be awake for up to 30 h, or for only 10 h. In-spite of these drastic changes in the duration of wakefulness α , the subjects adhere to their usual number of meals and stretch or compress the intervals between meals proportionally to α . The caloric intake per meal remains constant, and body weight neither decreases on long "days", nor increases on short "days". This is possible only if the energy expenditure is adjusted to the duration of α .

Since muscular work contributes substantially to the total energy expenditure, it could be assumed that activity is negatively correlated with the duration of α . In search of such a relationship, locomotor activity was measured by means of contact plates installed below the carpet within the isolation unit. Electric impulses elicited by steps on the plates were recorded continuously and averaged in 1-h bins. Protocols from 14 subjects revealed a negative correlation between the hourly means of activity and α . On the average, the mean activity decreased by 4.9% per 1 h increase in α . As a consequence, the total amount of "daily" activity remained almost constant within a range of α -values from 12 to 22 h.

Max-Planck-Institut für Verhaltensphysiologie
D -8138 Andechs

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MEDULLARY RESPIRATORY NEURONES IN RAT

Z. Wilhelm*, S.W. Schwarzacher**, K. Anders and D.W. Richter

Many studies of respiratory control and respiratory substrates have been performed in the rat. The information about the organization of the respiratory network in the rat brainstem is, however, sparse. Our present knowledge derives from extracellular recordings that revealed the existence of a dominant ventral group (VRG) and a small dorsal group of respiratory neurones (RNs) similar to the cat (Ezure et al., Brain Res. 455: 262-270, 1988; Saether et al., Brain Res. 419: 87-96, 1987).

To obtain more detailed information about the organization of the respiratory network, we recorded from 70 RNs within VRG and analyzed the postsynaptic activity in 41 RNs in urethane (1.2 g/kg) or pentobarbitone (60 mg/kg) anaesthetized Wistar rats. Phrenic and recurrent laryngeal nerve discharges were used to determine the central respiratory rhythm (CRR) and to functionally identify RNs. Some RNs were identified as bulbospinal by their antidromic response to spinal cord stimulation at C4. The RNs examined were not antidromically excited by vagal nerve stimulation.

Six classes of RNs were identified: early-inspiratory (e-I), throughout-inspiratory (r-I), late-inspiratory (l-I), post-inspiratory (p-I), late-expiratory (E-2) and phase-spanning E-I neurones. Intracellular analysis of postsynaptic activities, IPSP reversal following chloride injection and changes in input resistance revealed e-I and p-I inhibition in E-2 neurones, e-I and E-2 inhibition in p-I neurones, and p-I and E-2 inhibition in r-I neurones. E-2 inhibition was weak but p-I inhibition strong in E-I neurones. Tonic excitatory inputs not shunted by weak E-2 inhibition, therefore, might explain the discharge pattern of E-I neurones.

The results reveal that in the rat the CRR is organized in three (I, p-I and E-2) phases and that synaptic interaction between RNs occurs similarly as described for the cat (Richter et al., NIPS 100: 109-112, 1986).

Physiol. Inst. Univ., 3400 Göttingen, Humboldtallee 23;
* Dept. Physiol. Univ. Brno, CSSR; ** Anat. Inst. Univ. A-1090 Wien

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THE INFLUENCE OF LOCATION AND NUMBERS OF REINNERVATING MOTONEURONS ON MUSCLE COORDINATION AFTER PERIPHERAL NERVE TRANSECTION

M. Wasserschaff

The dependence of muscle coordination on the reorganization of the spinal cord motor nucleus following peripheral nerve repair has been examined in 24 adult mice. 14 weeks after transection of the right common peroneal nerves the EMG activity patterns of the reinnervated tibialis anterior (TA) and the medial gastrocnemius muscles (MG) were recorded simultaneously during free running. The motoneurons supplying the TA were labelled bilaterally with HRP.

In a control group of 5 animals the TA motoneuron numbers varied considerably, but were bilaterally symmetrical (right side: 123.2 ± 17.9; left side: 127.8 ± 28.0 cells). The distributions of the cells were also symmetrical. After nerve regeneration muscle coordination between the reinnervated TA and the MG was significantly impaired, the degree of coordination averaging 0.44 ± 0.31 (mean ± SD) on a scale between 0 (TA activity without any correspondence to the antagonistic MG activity phases) and 1 (normal coordination as seen in the control animals). The number of TA motoneurons was reduced to 70.2 ± 24.0% of the regular contralateral number (134.2 ± 28.9 cells). The longitudinal distribution of the cells was significantly shifted, the mean position being located 283 ± 197 µm caudal of that of the contralateral side. This indicated inappropriate innervation of the TA by motoneurons previously supplying the peroneal muscles, whose motor nucleus was localized in another group of 6 mice.

Multiple regression analysis revealed that muscle coordination was significantly influenced by the number of motoneurons reinnervating the TA ($p<0.05$) and their mean longitudinal position ($p<0.001$) independently of one another. Loss of muscle coordination is due mainly to reinnervation by inappropriate neurons. Some central correction of muscle activation seems to be independent of the amount of inappropriate innervation but is the more efficient, the more appropriate cells are involved in muscle reinnervation.

Institut für Neurophysiologie der Universität zu Köln, Robert-Koch-Str.39, 5000 Köln 41, FRG

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TOPOGRAPHY OF SHOULDER MOTOR NUCLEI AFTER RETROGRADE LABELING WITH FLUORESCENT DYES IN THE CAT SPINAL CORD

M. Hörner, H. Kümmel

Detailed studies on the neuronal organisation of forelimb motor control have so far focused on the proximal and distal muscle groups, excluding the shoulder girdle. Behavioural experiments, however, indicate that the shouldermuscles are specifically activated during different movements of the forelimb such as locomotion or target reaching. To reveal the structural basis for further studies on the functional interaction between forelimb muscles we used the retrograde axonal transport of exogenous fluorescent tracers to demonstrate the intraspinal localisation of motor nuclei projecting to the cat's shoulder muscles.

The fluorescent dyes (bisbenzimide, fast blue, fluorogold, propidium-iodide, rhodamine-b-isothiocyanate) were pressure-injected into the proximal ends of transected nerves of the different shoulder muscles. The animals, deeply anaesthetized during dye injection, recovered within 1 day after operation and survived another 3-5 days before perfusion and histological processing. In one animal up to four different tracers were injected in different nerves to establish the topographical relation between different motor nuclei within the same spinal cord. Due to their characteristic emission spectra all fluorochromes were unequivocally identified in the epifluorescence microscope. The position of labeled neurons was digitized for quantitative data evaluation and later reconstruction (Fritz, N. et al., J. Comp. Neurol.: 244, 286-301, 1986).

All motor nuclei of the shoulder investigated so far are distributed between the caudal part of C5 and the margin between C7/8. Thus, they extend to more rostral regions than the motoneurons of the deep radial (DR) nerve the projection of which was used as a standard landmark. Within the grey matter the shoulder nuclei are arranged in longitudinal layers, M. infraspinatus and spinodeltoideus in most lateral regions near the DR, M. supraspinatus and teres minor in latero-medial position and M. subscapularis and acromiodeltoideus in medial parts. A comparison of the dorso-ventral localisations showed that motor nuclei located in medial regions (M. subscapularis and acromiodeltoideus) reached more ventral areas than those in lateral positions. The topographical map established in this study will be used for electrophysiological experiments on the functional organisation of shoulder muscles active in different motor behaviors.

Physiologisches Institut der Christian-Albrechts-Universität, Olshausenstr. 40-60, D-2300 Kiel

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SYNAPTIC INTERACTION BETWEEN RESPIRATORY NEURONES AFTER BLOCKADE OF NMDA-RECEPTORS IN CAT

U. Windhorst, J.L. Feldman*, K. Anders and D.W. Richter

Termination of inspiration seems to be mediated by two mechanisms: "reversible" inhibition during late-inspiration and "irreversible" inhibition during postinspiration. Small lesions in the rostral pons disturb this inspiratory termination when lung afferents are inactive and, in anaesthetized animals, cause apneusis during "non-inflation". Similar disturbances to the rhythm were observed after systemic application of the NMDA-receptor antagonist MK 801 (Foutz et al., Neurosci. Lett. 87: 221, 1988). To study NMDA-receptor activating mechanisms, we analyzed the postsynaptic activity of medullary late-expiratory (E-2) and post-inspiratory (p-I) neurones before and after MK 801 application.

Experiments were performed in pentobarbitone (40 mg/kg) anaesthetized, thoracotomized cats which were ventilated with a cycle-triggered pump. Membrane potential (MP) trajectories of 46 E-2 and 8 p-I neurones were analyzed before and/or after MK 801 (0.3-0.7 mg/kg) was administered intravenously. The neurones were tested for their response to inflation and non-inflation.

MK 801 had no effect on mean values and periodic fluctuations of MPs of E-2 and p-I neurones when the lungs were ventilated. Non-inflation that caused augmentation and lengthening of inspiratory activity under control, resulted in apneusis after MK 801 was administered. During apneusis, MP of E-2 and p-I neurones remained slightly hyperpolarized by continuation of chloride-mediated synaptic inhibition. Superior laryngeal nerve stimulation stopped apneusis and MP hyperpolarization of E-2 neurones.

The findings reveal that synaptic interactions between medullary respiratory neurones are not normally mediated through NMDA-receptor activating pathways. The latter are also not used by pulmonary and laryngeal afferents. NMDA-receptor controlled pathways seem to be activated only during non-inflation and to originate in the pons.

Physiol. Inst. Univ., Göttingen; * Dept. Kinesiol. UCLA

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DISCHARGE PATTERN OF FELINE MEDULLARY RESPIRATORY NEURONS DURING AMINO ACID-INDUCED EXCITATION

K. Schmid*, A.S. Foutz** and M. Denavit-Saubié**

Discharge patterns or trajectories of membrane potential of different types of medullary respiratory neurons (RN) probably result from afferent input and from intrinsic cell properties, both of which may be responsible for respiratory rhythm generation. The temporal pattern of excitability of RN during the active and the normally silent phase was examined by tonic synaptic activation. Cats were anaesthetized with pentobarbitone, paralyzed and ventilated. Multi-barrel glass electrodes were used for ejection of drugs (glutamate 0.5 M; 20-100 nA, quisqualate 5 mM; 5-20 nA), current balancing and extracellular recording. RN of the dorsal and ventral respiratory groups were identified for spinal projections. Cycle-triggered averaging was performed simultaneously on single unit and phrenic nerve activity. The following patterns of excitability were obtained (grade of excitability indicated by -/+; I=inspiratory; E=expiratory):

Neuron type	Inspiration early	mid	late	Expiration early	mid	late
early burst I	++	++	+	--	+	++
early onset I	++	++	++	+	+++	--
early onset I	++	++	+	--	-	+
late onset I	++	+	++	+	-	+
transitional IE	-	+	++	+++	++	+
post-I	--	-	+	+++	+++	++
early burst E	-	--	-	+	++	+++
late onset E	---	--	-	+	++	+++
"rostral" E	+	++	+++	+++	+++	+++

Discharge patterns of most types of medullary RN during amino acid-induced excitation resembled membrane potential trajectories as revealed by intracellular studies.

* Dept. Physiol., University Mainz, 6500 Mainz, F.R.G.
** Lab. Neurophysiol., CNRS, Gif-sur-Yvette, France.
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THE EFFECT OF INTERLEUKIN-1 AND OTHER CHONDROCYTE ACTIVATING FACTORS ON NERVEFUNCTION

M. A.. Pak[1], P. Wehling[2], C. Evans[3], G. Bandara[3]

Radiculopathy in degenerative lumbar spine disease is not clearly understood. Clinically it is well known that in patients radicular syndromes can be present without space occupying signs. We hypothesized that radicular syndromes can also be produced by a release of chondrocyte activating factors (CAF) e.g. Interleukin-1 that changes nerve function around the facets and in nerve roots. We were interested in possible neurophysiological changes after CAF contact.

In 5 rats we observed the effect of CAF on the function of the sciatic nerve. Therefore the nerve was visualized by an operation microscope, 0.1 ml of a defined CAF solution from rabbit synovial cells also containing Interleukin-1 was injected under the epineurium of the right sciatic nerve. On the left sciatic nerve saline was applied identically. 5 rats served as control by using culture media from non activated synovial cells. After injection the animals were examined over a period of 6 days by neurophysiogical and clinical observation (Rivlin/Tator). For the neurophysiological evaluation we used the evoked potential technique. A branch of the sciatic nerve was stimulated with supramaximal voltage impulses (40 V) of constant duration (0.1ms). The responses were recorded at the dorsal root entry zone L1. 64 responses were averaged.The results can be summarized as follows:

1.CAF causes a significant (t-test $p<0.009$) decrease in the amplitude of the evoked potential.

2. The motor capacity of the hind limb that received CAF was lowered compared to the control side.

3. The neurophysiological findings indicate a Wallerian degeneration.

Although it is not yet clear if IL-1 is increased in degenerative spinal diseases, our previous results indicate that IL-1 and other chondrocyte activating factors could play an important role by a direct damaging effect on nerve funtion. We suggest that radiculopathy and pain in degenerative spinal diseases are due to the effect of IL-1 and other CAF`s on nerve tissue.

1) Physiologisches Institut, Abteilung Neuro-und Sinnesphysiologie und 2) Orthop. Klinik derHeinrich-Heine-Universität Düsseldorf, Moorenstr 5, D-Düsseldorf, 3) A. B. Ferguson Lab. for Orthopaedic Research, Pittsburgh, U.S.A.

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Properties of the "silent period" of human α-motoneurons evoked by magnetic brain stimulation

F. Awiszus, H. Feistner‡ and H.-J. Heinze‡

Magnetic brain stimulation elicits a (presumably) monosynaptic excitation of an α-motoneuron which is followed by a time interval during which the motoneuron has a low probability to fire an action potential (Day et al., *J. Physiol.*, **412**:449, 1989). The following experiments on healthy volunteers were performed to characterize some properties of the period following monosynaptic excitation evoked by magnetic brain stimulation.

Spike trains of 31 single motor units were recorded from the first dorsal interosseus muscle using a conventional concentric needle electrode. The units were made active by a slight voluntary contraction. Each unit was exposed to 50–120 magnetic brain stimuli (Dantec Stimulator) with the stimulating coil positioned over the vertex.

It was found that one interspike interval of the investigated motor units exhibited a rapid decrease at monosynaptic latency, i.e. 24–34 ms after the stimulus. During the following time interval the probability to fire an action potential was so low that it could be regarded as a "silent period". The duration of this period varied from unit to unit (range 28–64 ms) in a characteristic way as it was found to be linearily correlated to the amount of interspike interval shortening at monosynaptic latency.

This result is consistent with the notion that the "silent period" appears merely as a consequence of a stimulus-induced motoneuron excitation and does not require motoneuronal inhibition as proposed by Boniface and Mills (*J. Physiol.*, **412**:8P, 1989).

Abteilung Neurophysiologie and ‡Neurologische Klinik mit klinischer Neurophysiologie, Medizinische Hochschule Hannover, Postfach 610180, D-3000 Hannover 61

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DOES THE LACK OF RECURRENT INHIBITION IN FORE-LIMB MOTONEURONES TO THE EXTRINSIC DIGIT EXTENSORS CORRELATE WITH ABSENCE OF RECURRENT AXON COLLATERALS ?

M. Hörner, M. Illert, H. Kümmel

When Hahne et al. (1988) investigated the distribution of recurrent inhibition (RCI) in motor nuclei to cat forelimb muscles with electrophysiological techniques, they found pronounced RIPSPs in elbow motor nuclei, smaller effects in wrist motor nuclei and no effects in the motor nuclei to the extrinsic digit extensors. To verify the latter results and to correlate the electrophysiological data with their morphological substrate, we labelled identified motoneurones (MNs) with HRP.
The experiments were performed in anaesthetized, immobilized cats whose dorsal roots were cut. For antidromic identification of spinal MNs the nerves to elbow, wrist and digit extensors and other forelimb muscles were mounted on stimulating electrodes. Intracellularly recorded MNs were tested for RCI and injected with a 15% solution of HRP by depolarizing pulses and DC current. After histological processing, morphological identification was done by camera lucida reconstruction from serial sections of the spinal cord.
So far, 18 MNs are reconstructed in detail, which are all strongly labelled throughout their projection into the ventral roots. Nine of them displayed axon collaterals. All MNs to the elbow extensor muscles showed several axon collaterals together with RCI patterns in accordance to the results of Hahne et al. (1988). On the other hand the MNs to extrinsic digit extensors neither displayed axon collaterals nor RIPSPs. On the basis of our preliminary results the MNs to the wrist extensors belong to an intermediate group. Some of them either have a single axon collateral with a relatively small projection field with or without RIPSPs, others have no collaterals and receive only heteronymous IPSPs.
The demonstrated lack of recurrent axon collaterals in the motor nuclei to the extrinsic digit extensors confirms the electrophysiological findings by Hahne et al. (1988). This raises the question as to the role of RCI in the differentiated movement repertoire of the distal forelimb.
M. Hahne, M. Illert and D. Wietelmann: Brain Res., 456 188–192 (1988)

Physiologisches Institut der Christian-Albrechts-Universität, Olshausenstr. 40-60, D-2300 Kiel

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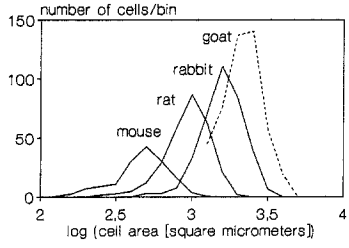
COMPARATIVE MORPHOMETRIC MEASUREMENTS ON LUMBAR MOTONEURONS IN ANIMAL SPECIES OF DIFFERENT BODY SIZE

M. Wasserschaff, P. Igelmund, D. Kleinebeckel, F.W. Klußmann (with the technical assistance of K. Wehner)

Motoneurons of the tibialis anterior muscle of 2 adult mice, 3 rats, and 2 rabbits were labelled by intramuscularly injected horseradish peroxidase. For direct comparison sections of the appropriate spinal segments of mouse, rat, and rabbit were subjected to the same histological procedure. Cell areas were determined with a Morphomat 30. In addition the dimensions of the lumbar spinal neurons of an adult goat were determined after perfusion and Nissl-staining.
The figure shows the distribution of cell areas of the different animals, divided into bins with a bin-width of 0.1 logarithmic units. It is obvious that the distribution curves of motoneuron area (mean number of cells per bin) are shifted to greater soma areas from mouse to goat. The values of 450, 880, 1480, and 1700 μm^2 mean soma areas in mouse, rat, rabbit, and goat, respectively, are significantly different (t-test, $p<0.001$). The calculated mean value of the goat contains also soma areas of the interneurons, the distribution of which is omitted in the figure.



Institut für Neurophysiologie der Universität zu Köln, Robert-Koch-Straße 39, D-5000 Köln 41, FRG

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NMDA- AND NON-NMDA-RECEPTORS ARE INVOLVED IN SYNAPTIC EXCITATION OF PHRENIC MOTONEURONS.

G. Böhmer, K. Schmid and W. Schauer

The involvement of endogenous excitatory amino acid neurotransmitters in the excitation of motoneurons of the phrenic nerve was examined in urethane-anaesthetized, bilaterally vagotomized, paralyzed and artificially ventilated rabbits. The activity of the phrenic nerve (PNA) was recorded from branches of spinal segments C3 and C4, and was digitally integrated (iPNA). The dorsal surface of the cervical spinal segments C2 - C5 was exposed by dorsal laminectomy. The dorsal roots of these segments were cut. The involvement of excitatory amino acid neurotransmitters in the excitation of motoneurons of the phrenic nerve (PMN) was studied by pneumatic injection of 50 - 100 nl of solutions of NMDA- or non-NMDA-receptor antagonists into the ventral horn of the cervical spinal segments C3 - C5. The blocking action of D-2-amino-5-phosphonovaleric acid (APV),ketamine, γ-D-glutamylaminomethylsulphonic acid (GAMS) or 6,7-dinitro-quinoxaline-2,3-dione (DNQX) was studied. After injection of the competitive NMDA-receptor antagonists APV or of the non-competitive NMDA-receptor antagonist ketamine the peak amplitude of iPNA, i.e. the neuronal equivalent of the tidal volume, was reduced. The same was true when GAMS or DNQX, both acting on non-NMDA-receptors, were administered. Effects of all antagonists were dose-dependent. High doses of NMDA-receptor antagonists caused total suppression of respiratory modulation of PNA. Suppression of PNA by high doses of ketamine was irreversible. Results indicate that NMDA- as well as non-NMDA-receptors are present in PMN. Excitation of NMDA- and non-NMDA-receptors crucially determines the level of activity of PMN. This excitation is accomplished by the release of endogenous excitatory amino acids. Whether the synaptic excitation of phrenic motoneurons by descending inputs from bulbo-spinal inspiratory neurons is induced by the release of excitatory amino acids can not be answered on the basis of the present results.

Institut für Physiologie und Pathophysiologie der Universität Mainz, Saarstr. 21, D-6500 Mainz

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DIFFERENTIAL RELATION OF SINUS-ARRHYTHMIA TO RESPIRATORY PARAMETERS

H.-H.Abel, D.Klüßendorf and H.P.Koepchen
Inst. für Physiologie, FU Berlin, F.R.G.

Heart rate fluctuations in the frequency range of respiration originate from an interaction of central cardiovascular-respiratory coupling, respiratory phase-dependent baroreceptor processing and respiration modulated afferent inputs. We analysed different components of heart rate fluctuations in relation to different components of respiration at rest. In 17 sitting, young volunteers, ECG and respiratory movements were recorded during 45'. Means of RR-interval (RR), beat to beat differences (D-variability), and rhythmicity at 0.1 and 0.25 Hz, as well as respiratory cycle time (TT), tidal volume (VT) and mean inspiratory flow were computed from successive 2' periods. After relative transformation of the 2 min means, mean RR was classified in three ranges. For each RR range, D-variability and both rhythmicities were sorted according to respiratory parameters which were also classified in three ranges. For each RR range, each triplet of D-variability or rhythmicity was tested (Kruskal-Wallis, α=0.01). Comparison of D-variability and both rhythmicities with all respiratory parameters resulted in only two significant relations. At middle RR, D-variability is related to VT and rhythmicity at 0.25 Hz is related to TT. In conclusion, during stable, low level of activity, heart rate fluctuations in a restricted RR range show no significant dependency on spontaneous changes in the respiratory pattern. This indicates an absence or weakness of coupling of the respective parameters. When there is common activation of the cardiorespiratory system, a decrease in heart rate fluctuations is likely to be caused primarily by common central nervous excitation. Corresponding changes in heart rate pattern do not occur obligatorily with each change in respiratory parameters.

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pH-SENSITIVITY OF NEURONS IN SLICES OF THE RAT VENTRAL MEDULLA OBLONGATA

W. Jarolimek, U. Misgeld and H.D. Lux

Hypercapnia enhances ventilation even when peripheral chemoreceptors are denervated, however, cells in the medullary chemosensitive area are poorly characterized (E.N. Bruce J appl Physiol 62:389 1987). Therefore, the effects of extracellular pH ($[H^+]_o$) changes on neurons were investigated in slices of rat ventral medulla oblongata. Changes in discharge rate were compared to changes in the actual $[H^+]_o$ in the tissue as measured by H^+-selective microelectrodes. pH was altered by varying the bicarbonate concentration ($[HCO_3^-]$) in the superfusion solution. In 138 "pH-sensitive" neurons, the discharge rate increased with increasing $[H^+]_o$ and decreased with decreasing $[H^+]_o$, while in 180 neurons, the discharge rate was depressed or unaffected by increasing $[H^+]_o$. Changes of only 0.01 to 0.04 pH units in either direction affected pH-sensitive neurons, but the response was always transient, lasting 1.5-10 min. The pH-sensitivity persisted in the presence of 0.5μM atropine, 20μM bicuculline and after replacing Ca^{2+} by Mg^{2+} in the superfusion solution to reduce synaptic transmission. The H^+-induced response was significantly stronger with increased pCO_2 than with reduced $[HCO_3^-]_o$ and increased during hypoxia. In conclusion, central pH-sensitive neurons respond to small $[H^+]_o$ changes in both directions, the response is transient and independent from peripheral or local network synaptic inputs. The reactivity of pH-sensitive neurons, however, depends on influences of local pCO_2 and pO_2.

Max Planck Institut für Psychiatrie, Abt. Neurophysiologie. Am Klopferspitz 18a, D-8033 Planegg-Martinsried

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CHEMOSENSITIVITY OF SYMPATHOEXCITATORY NEURONES IN THE ROSTROVENTROLATERAL MEDULLA OF THE CAT

St. König, J. Czachurski, H. Seller

The region of sympathoexcitatory neurones within the rostroventrolateral medulla (RVLM) is highly vascularized with a high local blood flow and glucose metabolism (Göbel et al., Pflügers Arch 1988,411:R142). These features and the location of this group of neurones in the intermediate chemosensitive zone described by Schläfke (Schläfke, Loeschcke, Pflügers Arch 1967, 297:201-220) led to the suggestion that the activity of these neurones might be directly affected by pCO2 and/or pH of the arterial blood. This hypothesis was tested in chloralose anesthetized cats by artificial perfusion of the RVLM via the left vertebral artery. The baro- and peripheral chemoreceptors were denervated by bilaterally dissecting the carotid sinus and vagus nerves. Either WR-T3 or renal n. were recorded as indication of sympathetic activity (SA). Perfusion with saline or Ringer solution bubbled with CO2 produced a marked increase in SA and BP. This effect was significantly smaller with solutions of the same pH, achieved with HCl, but without CO2. Unbuffered CO2-bubbled solutions were more effective in stimulating SA than buffered CO2-bubbled solutions. A linear relationship between pCO2 of the perfused solution and SA was found. During prolonged perfusion (90 s) SA returned to control level after 40 s and was reduced well below control levels after the end of perfusion. The SA response to perfusion with solutions bubbled with CO2 was unchanged after blockade of synaptic input by local microinjection of COCl2 into the RVLM whereas spontaneous SA and the supraspinal somato-sympathetic reflex from IC-T4 to WR-T3 were abolished. From these results it is concluded that sympathoexcitatory, bulbospinal neurones within the RVLM are directly chemosensitive to changes of arterial pCO2 and pH. (This work was supported by the DFG within the SFB 320.)

I. Physiologisches Institut der Universität Heidelberg
Im Neuenheimer Feld 326, D-6900 Heidelberg

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ROSTROCAUDAL GRADIENT OF NEURONAL SUBTYPES IN GUINEA PIG PREVERTEBRAL SYMPATHETIC GANGLIA.

R.L. Meckler and E.M. McLachlan

Sympathetic neurones have distinctive discharge characteristics based on populations of K^+ channels in their membranes. Three types of neurones have been identified: phasic (P), tonic (T), and LAH (long afterhyperpolarizing) neurones. The present experiments assessed the distribution of these cell types in the coeliac ganglion (CG), superior mesenteric ganglion (SMG), and inferior mesenteric ganglion (IMG), and compared their passive electrical properties and the afferent synaptic input arising from stimulation of intestinal nerve trunks.

Intracellular recordings were made from ganglia *in vitro*. Neurones were classified and passive properties were determined. Synaptic and antidromic responses were evoked by stimulation of the coeliac and/or colonic nerves.
The proportion of T neurones increased progressively from CG to IMG, whereas LAH neurones decreased in number towards the IMG and numbers of P neurones were relatively consistent. P neurones had higher input resistances (R_{in}) rostrally relative to T neurones, whereas in the caudal direction the input time constant of T neurones increased as a result of their greater R_{in}.

Multiple excitatory synaptic potentials (e.s.ps) were evoked in CG T neurones but not in P neurones. In contrast, e.s.ps were produced in SMG and IMG P as well as T neurones. LAH neurones normally received no peripheral inputs. Equivalent proportions of all three neurone types could be activated antidromically.

We conclude that the distributions and characteristics of the three types of sympathetic neurone vary in relation to their rostrocaudal location. This may reflect the functions regulated by these neurones (phasic≡vasoconstriction; tonic≡motility; LAH≡secretion), although this remains to be proven.

Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Qld 4067, AUSTRALIA

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WHAT IS THE INFLUENCE OF THE SACRAL AFFERENT SUPPLY ON VESICO-SYMPATHETIC REFLEXES IN THE CAT ?

A. Boczek-Funcke, H.-J. Häbler, W. Jänig, M. Michaelis

The urinary bladder receives a dual afferent innervation projecting through the pelvic nerves to the sacral spinal cord and through the hypogastric nerves to the lumbar spinal cord. Both populations of visceral afferents are activated in a graded manner by distension and contraction of the urinary bladder (Bahns et al., Pflügers Arch. 407: 510-518, 1986; Bahns et al., Pflügers Arch. 410: 296-303, 1987). The sacral vesical afferents are essential for organ regulation, whereas the functions of the lumbar vesical afferents are unknown. Distension and contraction of the urinary bladder evoke reflexes in neurones of the sympathetic outflow to the cat hindlimb: muscle vasoconstrictor (MVC) neurones are excited and cutaneous vasoconstrictor (CVC) neurones inhibited (Jänig, W., Rev. Physiol. Biochem. Pharmacol. 102:119-213, 1985). We tested by cutting the cauda equina, to what extent the sacral vesical afferents participate in these reflexes.

Experiments were performed on chloralose-anaesthetized, immobilized and artificially ventilated cats. Multiunit activity was recorded from strands isolated from both the deep and superficial peroneal nerves (innervating muscle and skin). Vesico-sympathetic reflexes were elicited by isotonic distensions of the urinary bladder in a graded manner. In four of the experiments a sacral laminectomy was performed and during the experients the cauda equina was cut to eliminate the sacral afferent supply leaving the lumbar vesical afferents intact.

The reactions of MVC and CVC neurones induced by distension of the urinary bladder are dramatically diminished or abolished after elimination of the sacral afferent supply. Blood pressure responses are also attenuated, although the increases are still significant at intravesical pressure steps above 60 mmHg. This blood pressure rise may be due to vasoconstriction in other parts of the body. We conclude that the vesico-sympathetic reflexes examined in the present experiments are almost exclusively mediated by excitation of sacral vesical afferents.

Physiologisches Institut, Christian-Albrechts-Universität, Olshausenstr. 40-60, D-2300 Kiel

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RESPIRATORY MODULATION OF SYMPATHETIC PREGANGLIONIC NEURONES IN THE THORACIC SPINAL CORD OF THE CAT

K. Dembowsky, S. König, J. Czachurski

The on-going activity in sympathetic nerves is rhythmically modulated by the central respiratory drive, the maximum of activity occurring during inspiration synchronous to phrenic nerve activity (PN). In the present study the respiratory modulation of sympathetic preganglionic neurones (SPN) in the upper thoracic spinal cord has been studied by conventional intracellular recording techniques in chloralose-anaesthetized, artificially ventilated and vagotomized cats. At an end-tidal CO_2 concentration between 3.8 and 4.5% less than 10% of all SPNs displayed an inspiratory-related modulation of their membrane potential (MP) synchronous to PN activity, the modulation showing either one of the following three types: (1) In some SPNs a burst of small, discrete EPSPs occurred during PN activity; the synaptic activity was minimal during the post-inspiratory phase (PI) of the respiratory cycle. (2) In other SPNs a ramp-like depolarization of the MP was observed synchronous to PN activity which decayed slowly synchronous to declining PN activity during PI. In these SPNs MP was highest during stage II expiration. (3) A third group of SPNs was characterized by a ramp-like inspiratory depolarization and an additional also ramp-like, albeit smaller expiratory MP depolarization, both depolarizations being separated by a brief hyperpolarization. In these SPNs MP was highest during PI. Any manoeuver which affected PN activity also caused corresponding changes in the inspiratory ramp-like depolarization of the last two groups of neurones. Irrespective of the type of respiratory modulation, however, the discharge of action potentials was always inhibited during PI. An exclusively expiratory-related modulation of the MP, unaccompanied by an inspiratory-related modulation, has not as yet been observed in SPNs.

In conclusion, these data indicate a differential coupling of individual SPNs to the central respiratory network the nature of which will be the subject of future experiments.

I. Physiologisches Institut, Universität Heidelberg, Im Neuenheimer Feld 326, 6900 Heidelberg, F.R.G.

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RESPONSES OF THE ECG T-WAVE INDICATE BETA-ADRENERGIC ACTIVATION

Harald Rau

The amplitude of the T-wave (TWA) of the electrocardiogram (ECG) varies with psychological stimulation and provides inportant information for psychophysiological work. The present paper presents additional evidence that phasic changes in TWA depend on task conditions and beta-adrenergic drugs. Three experiments were designed to test the sensitivity of TWA to manipulations in sympathetic arousal. In the first experiment TWA was recorded during an active behavioral task in which 32 subjects believed they could control the duration of an aversive white noise and during a passive behavioral task in which another 30 subjects knew they had no control. TWA decreased to a significantly greater extent in the active behavior group than in the passive group ($t(60)=3.87;p<0.001$). In the second experiment 9 subjects receiving beta-adrenergic blockers and 10 subjects receiving placebo completed the active task. TWA decreases were significantly blocked in the beta blocker group ($t(17)=2.91;p=0.01$). In the third experiment 12 subjects received placebos and 24 received one of two different beta-blockers. All subjects performed a mental arithmetic task. Subjects receiving placebos responded with a significant reduction in TWA. Beta-blockers blocked this reduction significantly ($F(2,27)=5.3;p<0.02$). Results show that TWA responds distinctly to psychological as well as to pharmacological manipulations. Since in all cases TWA changes were blocked through beta-blockade it follows that beta-adrenergic activity is related to phasic TWA responses.

Psychologisches Insitut der Universität Tübingen; Klinische & Physiologische Psychologie. Gartenstrasse 29, D-7400 Tübingen

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RESPONSES OF SYMPATHETIC PREGANGLIONIC NEURONES TO ELECTRICAL STIMULATION OF THE SUPERIOR LARYNGEAL NERVE

A. Boczek-Funcke, H.-J. Häbler, W. Jänig, M. Michaelis

Activity in sympathetic postganglionic neurones innervating kidney, heart (Bainton et al, J Auton Nerv Syst 12, 77, 1985) and resistance vessels (Boczek-Funcke et al, J Physiol 409, 61P, 1988) is modulated within the respiratory cycle: the neurones are activated during inspiration, followed and sometimes preceded by a depression of activity during postinspiration (pI) and early inspiration (e-I), respectively. Both inhibitions are probably related to excitation in respiratory interneurones in the brain stem that are active during pI and e-I. Synaptic inhibition arising from these neurones is thought to be important in the generation of the respiratory rhythm. Electrical stimulation of the afferents in the superior laryngeal nerve (SLN) activates the pI interneurones and leads to a respiratory arrest in pI (Remmers et al, Pflügers Arch 407, 190, 1986). If the pI interneurones mediate the pI depression in the sympathetic neurones (e.g. via the bulbospinal neurones in the rostral ventrolateral medulla) it might be hypothesized that SLN stimulation reduces the activity in the sympathetic neurones.

We have investigated the responses of preganglionic neurones that projected in the cervical sympathetic trunk and behaved like muscle vasoconstrictor neurones to electrical stimulation of the SLN in anaesthetized, immobilized and artificially ventilated cats. The stimulus strength was adjusted to abolish the phrenic nerve discharges. The overall activity in the sympathetic neurones was not depressed but slightly enhanced, though the respiratory modulation was abolished. This finding is at variance with the above hypothesis: the inhibition in pI is either not mediated via the pI interneurones or the afferents in the SLN have additionally excitatory effects on the sympathetic premotor neurones via other interneurones in the medulla oblongata.

Physiologisches Institut, Christian-Albrechts-Universität, Olshausenstr. 40, 2300 Kiel, FRG

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ALTERATION OF FEVER BY MANIPULATION OF NORADRENERGIC INPUT INTO THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS IN THE GUINEA PIG

M.Unger, G.Merker, J.Roth and E.Zeisberger

Evidence for the release of arginine-vasopressin (AVP) in the brain septum during endogenous antipyresis was recently reviewed (N. W. Kasting, Brain Res Rev 14: 143, 1989). Based on our immunocytochemical studies in guinea pigs (Merker et al., Experientia 45: 722, 1989) vasopressinergic terminals in the septum originate partly from parvocellular neurons of the hypothalamic paraventricular nucleus (PVN). The PVN and other thermoregulatory structures in the hypothalamus may be influenced by aminergic afferents from the lower brain stem (Brück & Zeisberger, Pharmac Ther 35: 163, 1987). Therefore we investigated in guinea pigs whether the febrile responses to bacterial endotoxin could be altered by manipulation of the noradrenergic input into the PVN. Electrical stimulation of the PVN neurons by implanted microelectrodes reduced the febrile responses to 45% of the control values (n = 11). This confirms the antipyretic role of these neurons. Chronical destruction of noradrenergic afferents to the PVN by microinjected 6-hydroxydopamine (6-OHDA) also resulted in a significant reduction of febrile responses to 38% of the control values (n = 10), whereas a microinfusion of noradrenaline into the PVN enhanced febrile responses by 39% in comparison to animals microinfused with the solvent (0.9% NaCl; n = 6). Since the febrile response was increased by microinfused noradrenaline and decreased by 6-OHDA we assume an inhibitory influence of noradrenergic brain stem afferents on the antipyretic vasopressinergic system of PVN.

Physiologisches Institut, Klinikum der JLU Giessen, Aulweg 129, D-6300 Giessen, F.R.G. (Supported by the Deutsche Forschungsgemeinschaft)

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THE EFFECT OF ELECTRICAL STIMULATION OF THE ACUPUNCTURE POINT K_3 ON BLOOD PRESSURE AND THE ACTIVITY OF THE RENAL SYMPATHETIC NERVE IN ANAESTHETIZED CATS

H. Gao, M. Hunold, F. Kirchner and K. Takano

Stimulation of the skin or peripheral nerves has effects on visceral organes, and this might explain the effects of acupuncture (for review see SATO and SCHMIDT, Jap. J. Physiol. 37,1-17,1987). In an attempt to combine the experiences of acupuncture and neurophysiology we studied the effect of electrical stimulation of an acupuncture point, which is used in China for controlling the blood pressure e.g. during acupuncture analgesia. In anaesthetized cats (60 mg/kg chloralose) repetitive stimulation (1 Hz with an intensity so that a small movement of the foot is observed) of the acupuncture point produced a decrease in blood pressure as well as in the activity of the renal sympathetic nerve. The decrease of the activity of the renal sympathetic nerve was produced by an inhibition which began 50 ms after the single acupuncture stimulus. With higher stimulus intensity a supraspinal reflex discharge was also observerd which began about 90 ms after the stimulus. The effect of acupuncture on blood pressure as well as on sympathetic activity disappeared after transsection of the sciatic nerve. In some experiments a minor decrease of sympathetic activity and blood pressure was observed during insertion of the acupuncture needle into the point. Stimulation on points on the leg and on the ventral skin, which are not used in acupuncture, did not produce any effect except the small movement of neighboured muscles which was used for adjusting the stimulation intensity.

Physiologisches Institut der Universität Göttingen, Abteilung Pathoneurophysiologie, Humboldtallee 23, 34 Göttingen

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INFLUENCE OF OSMOTIC STIMULATION ON PERIPHERAL AND CENTRAL RELEASE OF ARGININE VASOPRESSIN IN THE GUINEA-PIG

J.Roth, K.Schulze, E.Simon* and E.Zeisberger

Arginine vasopressin (AVP), synthesized in hypothalamic neurons, is transported in axons either to the pituitary for release into the circulation or to different brain areas. In contrast to the peripheral release of AVP, the central release has not yet been clarified. From our experiments we proposed central antipyretic AVP-pathways from the hypothalamus to the limbic septal area (Zeisberger, in: Thermal Physiol., ed. J.B. Mercer, Elsevier, pp. 117-122, 1989). In the present study we investigated, if osmotic stimulation is able to activate peripheral and central release of AVP concurrently and if the antipyretic pathways are influenced by this kind of stimulation. In dehydrated (24 h water deprivation) and hyperhydrated (intraarterial infusion of a hydrating solution) guinea-pigs blood plasma levels of AVP, osmolality of plasma and urine and febrile response to bacterial pyrogen were compared to control animals. The control values of 4 pg AVP/ ml, 278 mosm/kg (plasma) and 790 mosm/kg (urine) were altered to 9 pg AVP/ml, 287 mosm/kg and 1470 mosm/kg in dehydrated and to 3 pg AVP/ml, 266 mosm/ kg and 200 mosm/kg in hyperhydrated guinea-pigs. Fever was sinificantly reduced in dehydrated and enhanced in hyperhydrated animals. In another group of dehydrated guinea pigs the AVP concentrations in push-pull perfusates of the lateral septum were significantly higher (5.6 pg AVP/ml) than in controls (2.6 pg AVP/ml). From these data we assumed a simultaneous activation of peripheral and central release of AVP with antidiuretic and antipyretic effects by osmotic stimulation.

Physiologisches Institut, Klinikum der JLU Giessen, Aulweg 129, D-6300 Giessen, F.R.G. und *Max-Planck-Institut für Physiol. und Klin. Forschung, D-6350 Bad Nauheim, F.R.G. (Supported by the DFG)

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DIFFERENT HYPOTHALAMO-SEPTAL NEUROPEPTIDE SYSTEMS PARTICIPATING IN ENDOGENOUS ANTIPYRESIS

E. Zeisberger

The febrile elevation in body temperature seems to be limited, and sometimes even prevented, by the action of endogenous antipyretic substances liberated within the brain septum during fever (for review cf. Kasting NW, Brain Res Rev 14:143-153,1989). Different neuropeptides were proposed for this function by several authors working with diverse species and injecting mostly large doses into distinct septal or hypothalamic sites. To assess the proposed antipyretic function of the respective neuropeptides the following experiments were made. In several series in conscious freely moving guinea pigs the febrile responses to i.m. injections of bacterial endotoxin (E. coli, 20 µg/kg) have been compared (several times in each animal at 1-week intervals) during a continuous microinfusion (0.1 µl/min for 6 h) of solutions of either arginine vasopressin, α-melanocyte stimulating hormone, angiotensin II and adrenocorticotrophic hormone at nearly physiological doses (8 pmol in 6 h) or of a solvent (sterile saline) into a site in the ventral lateral septum. Here all these substances have been found effective in suppressing the febrile increase of body temperature. Since the immunohistochemical studies revealed that all these peptides are present in the nerve fibers projecting from different hypothalamic cell nuclei to the septum, it is concluded that several neuropeptide systems participate and cooperate in the endogenous antipyresis. This cooperation may explain the different responsivity to febrile agents in various physiological situations ranging from the control of maximum febrile temperature at about 41°C to a complete suppression of temperature increase.

Physiologisches Institut, Klinikum der JLU Giessen, Aulweg 129, D-6300 Giessen, Fed. Rep. Germany
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HEART RATE RESPONSES TO CHEMOSENSORY STIMULI IN THE CORD INJURED

M. Pokorski*, Y. Sakakibara, A. Masuda, T. Morikawa, B. Ahn, S. Takaishi, P-E. Paulev, and Y. Honda

Transection of the cervical spinal cord, disrupting the descending vasomotor drive, offers an opportunity of studying the effect of missing supraspinal influences on heart rate (HR) control. In this study we investigated the hypothesis that the stimulatory HR responses to hypoxia and hypercapnia would be less in the cord injured. We determined the HR responses to acute progressive isocapnic hypoxia and hyperoxic hypercapnia from the linear slopes of HR on oxygen saturation (SaO_2) or on alveolar PCO_2 in 16 conscious patients with chronic, complete, traumatic spinal cord transection at C_4-C_7, who consented to study procedure. We found that the HR responses were unattenuated; $\Delta HR/\Delta SaO_2$ was 0.83 ± 0.14 (SE) beats/min per 1% decrement in SaO_2 and $\Delta HR/\Delta P_ACO_2$ 0.30 ± 0.13 beats/min per 1 Torr increase in P_ACO_2. Arterial blood pressure was increased by ~10 Torr during both hypoxia and hypercapnia. Since pulmonary reflexes mediated by pulmonary stretch receptors are stimulatory for HR, we compared the contribution of tidal volume (V_T) to HR increase in each gas condition. The $\Delta HR/\Delta V_T$ slope was 12.6 ± 10.1 beats per 1 l of hypoxic V_T and 6.8 ± 2.2 beats per 1 l of hypercapnic V_T. The higher HR response in hypoxia should thus neither be ascribed to the function of intact baroreceptor reflexes nor to the pulmonary reflexes activated by lung hyperinflation. We conclude that supraspinal vasomotor influences are not indispensable for the tachycardic response to chemical stimuli.

Dept. of Physiology, Chiba University School of Medicine, Chiba, 280 Japan and *Polish Academy of Sciences Medical Research Center, Warsaw, Poland.

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ALPHA2-BINDING SITES IN THE MEDULLA OBLONGATA OF MAMMALS

G. Flügge and E. Fuchs

In the medulla oblongata (MO), alpha2-adrenoceptors appear to be involved in the mechanisms regulating blood pressure since in this brain area, hypertensive rats show different α2-adrenoceptor characteristics than normotensive rats. To investigate whether there are species differences in the patterns and characteristics of α2-adrenoceptors we visualized and quantified binding of the α2-antagonist 3H-rauwolscine (3H-RAUW) in the MO of the marmoset monkey, the rat, and the tree shrew (Tupaia belangeri) by in vitro-autoradiography.

In tree shrews and marmosets, the n. dorsalis nervi vagi (nX), the n. tractus solitarii (NTS), the n. nervi hypoglossi, part of the n. reticularis parvocellularis, and the area of the adrenergic cell group C1 were distinctly labelled, which is in clear contrast to the rat, where binding of 3H-RAUW was only quantifiable in the NTS and nX. Scatchard analysis revealed one type of high affinity binding sites in each nucleus of the tree shrew, except the NTS where high and low affinity binding was measured. Competition experiments demonstrated that 3H-RAUW bound specifically to α2-adrenoceptors in all investigated areas. In the nXII and the area of group C1, 3H-RAUW binding sites also interacted with the α1-antagonist prazosin.

In summary, there species as well as regional differences with respect to the patterns and pharmacological properties of α2-binding sites in the MO of the mammals investigated. These different types of receptors may be related to different physiological mechanisms in the distinct medullary regions.

Deutsches Primatenzentrum, Kellnerweg 4, D-3400 Göttingen

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SYMPATHETIC AND RESPIRATORY NERVE ACTIVITIES IN THE CAT DURING HYPOXIA AND SUPERIOR LARYNGEAL NERVE STIMULATION

K. Dembowsky, S. König, J. Czachurski

The inspiratory peak activity in sympathetic nerves is usually followed by a brief silent period during the postinspiratory (PI) phase when phrenic nerve (PN) activity slowly declines. The mechanism of this silent period in sympathetic nerves during the PI phase, however, is poorly understood. In chloralose-anaesthetized and artificially ventilated cats sympathetic activity in the cardiac nerve (CN) and respiratory activity in PN and recurrent laryngeal nerve (RLN) were simultaneously recorded and their activity profiles were analyzed during hypocapnia and superior laryngeal nerve (SLN) stimulation. During normocapia RLN activity displayed an inspiratory and a marked PI component; hypocapnia silenced PN and abolished the inspiratory, but not the PI activity in RLN and caused the appearance of a prominent expiratory component in RLN. CN activity lost its inspiratory peak component during hypocapnia and became more irregular. Although PI activity was still present in RLN during hypocapnia, the silent period in CN activity during PI phase was either abolished or greatly reduced. SLN stimulation (20-50Hz) silenced PN completely, but resulted in a tonic activity in RLN without any respiratory modulation. Prolonged SLN stimulation (up to 1-2 min) caused rapid burst increases in RLN, but not PN activity at irregular intervals. When the muscle relaxans wear off it was verified that this RLN burst activity coincided with the cat swallowing. SLN stimulation abolished the respiratory modulation of CN activity and, in addition, caused slight de- or increases in tonic CN activity depending on the stimulation parameters. During swallowing, when SLN stimulation caused a burst increase in RLN activity, CN activity was completely abolished, the effect being less pronounced or abolished during hypocapnia. Intracellular recordings of sympathetic preganglionic neurons in the spinal cord have revealed that this abolition of CN activity during swallowing is due to spinal post-synaptic inhibition.

I. Physiologisches Institut, Universität Heidelberg, Im Neuenheimer Feld 326, 6900 Heidelberg, F.R.G.

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PATHOLOGICAL NEURONAL RESPONSES IN THE CAT VISUAL CORTEX AFTER FOCAL HEAT LESIONS

U.Th. Eysel and R. Schmidt-Kastner

Pathological neuronal activity at the border of cerebral infarcts has recently gained increasing attention. As a simple model for focal brain damage we produced a small heat lesion (1 mm diameter) in the cat visual cortex. Visually evoked responses and spontaneous activity of single cells were recorded with tungsten-in-glass electrodes at different distances (0.5-2.5 mm) from the center of the lesions on postoperative days 1, 2, 7 and 30 under halothane/N_2O/O_2 anesthesia. Physiological parameters were continuously monitored throughout the experiments.
No single cell activity was obtained within the lesions. The neuronal responses in the surrounding included spontaneously inactive cells only weakly responding to maximal stimuli [IW], inactive cells with spontaneous or stimulus evoked high frequency (500-1000 spikes per second) bursts [IB], and cells with maintained high frequency activity modulated by the visual stimulus [HM]. Normal responses [N] were always present far from the lesion. Epileptic discharges have not been observed. During the first post-lesion day IW cells were found at 1.0 mm distance, IB and IM at 1.5 mm and N cells at 2.0 mm. A similar pattern was observed on the second day but in addition to N cells, IM cells were still found at 2.5 mm. After 7d IW cells were observed at 1.0 mm, IB responses at 1.5 mm and N cells again at 2.0 mm. After 30d IW cells were observed at 0.5 mm, IM and N cells at 1.0 mm. Histological and immunohistochemical analysis of the lesioned cortex revealed a demarcated area of coagulative necrosis accompanied by various signs of neuronal damage and glial reactions. Vasogenic edema surrounded the lesion and spread into the underlying white matter. Interestingly, the border region of the focal cortical lesion was characterized by cells with reduced excitability as well as hyperactive cells. (Supported by DFG Ey 7/14-1).

Institut für Physiolgie, Ruhr-Universität Bochum, Universitätsstraße 150, D-4630 Bochum 1.

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MONOCULARLY AND BINOCULARLY EVOKED POTENTIAL FIELD TOPOGRAPHY: EFFECTS OF RETINAL STIMULUS LOCATION AND SPATIAL FREQUENCY

W. Skrandies

Most neurones of the primate visual cortex receive input from both eyes, and we invesitgated electrophysiological correlates of differences between monocularly and binocularly elicited brain activity related to stimulus parameters like spatial frequency and retinal location.
Electric brain activity was recorded in eighteen healthy adults from an array of 21 electrodes covering the occipital areas. Vertical black and white grating patterns of different spatial frequency were presented in the center, or lateralized to the left or right hemiretina. Computation of "global field power" determined component latency independent of the reference electrode, and topographic characteristics were examined at individual component latency using statistical comparisons between experimental conditions.
We found no effects on component latency while the strength of the potential fields was significantly larger with binocular stimuli. Significant differences occurred in the potential field distribution of brain electric activity: With 2.5 c/d topographic differences between monocularly and binocularly evoked activity were obtained showing more anterior and more lateralized potential fields with binocular stimuli. In addition, when the gratings were presented binocularly significant differences in topography were observed when low and high spatial frequency stimuli were compared confirming our earlier observations on topography of VEPs evoked by stimuli of different spatial frequency (**Brain Topography, 1988, 1, 107-116**).
These data show that the topographical relation of evoked components to retinal location and spatial frequency is different for monocular and binocular stimuli giving further evidence that binocular information processing triggers different neuronal processes in the human visual cortex.

Max-Planck-Institut für Physiologische und Klinische Forschung, 6350 Bad Nauheim

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EFFECT OF LUMINANCE AND CONTRAST ON THE THRESHOLD OF BINOCULAR DEPTH PERCEPTION

T. Geib and C. Baumann

Stereoscopic acuity was investigated in 11 healthy volunteers. The three-rod arrangement of Helmholtz was employed in which three equally-spaced rods are used, with the middle one made movable in and out of the plane of the other two. The middle rod was shown in one of nine different positions and the presentations were repeated in random order until each of the positions had been presented to the subject 20 times. A psychometric curve was fitted to the data by the method of probits. Thresholds were expressed as angular disparities and referred to 75 % correct responses. Points of subjective equality were also determined. Both the luminance of the rods and that of the background could be adjusted independently. This allowed fixing the contrast when the effect of luminance was studied or fixing the luminance when the effect of contrast was investigated. Observation distance was 40 cm.

Lowest thresholds (2.85 ± 0.67 sec of arc) were found for a moderate contrast of 0.5 whereas low (0.05) and high (0.95) contrast both produced higher thresholds (luminance 250 cd/m²). The differences were statistically significant. Altering the field luminance (50, 250, 1600 cd/m²) under constant contrast conditions (0.95) did not measurably influence stereocopic acuity.

The threshold-raising effect of low contrast is attributable to a diminished visual acuity while that of high contrast may have to do with the central processing of depth signals.

Physiologisches Institut der Justus-Liebig-Universität
Aulweg 129, D-6300 Gießen

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EFFECTS OF DOPAMINE ON THE KINETICS OF AMINO ACID SENSITIVE ION CHANNELS IN RETINAL HORIZONTAL CELLS OF THE WHITE PERCH (*Roccus americana*)

K.-F. Schmidt, A. G. Knapp and J. E. Dowling

Two effects of dopamine on cone horizontal cells, both mediated by cAMP-dependent phosphorylation, have been demonstrated: an uncoupling of the electrical junctions of the cells and a reduction in the light responsiveness. This reduction can be explained by an enhancement of the cells' responses to the proposed photoreceptor transmitter L-glutamate and kainate (Knapp & Dowling, Nature 325, 437, 1987). We have sought to determine which physiological properties of horizontal cell glutamate receptors are modified by dopamine. In single channel recordings from cell attached patches with agonist in the patch pipette, the frequency of 5-10 pS unitary events, but not their amplitude, increased by as much as 150 % following application of dopamine. The duration of channel openings (0.7-1 ms) also increased by 20-30 %. Analysis of whole-cell current recorded during slow superfusion of voltage clamped horizontal cells with agonist with and without dopamin also indicated that dopamine increased the channel open probability but not the amplitude (6-8 pS) of unitary events. In the case of kainate, noise analysis additionally demonstrated that dopamine did not alter the number of functional channels. The power spectra of whole-cell currents elicited by glutamate or kainate consisted of two Lorentzian components with time constants near 1 and 9 ms. The contribution of the slower component increased following dopamine treatment, suggesting a change in the open-time kinetics of the channels. This effect was more pronounced for currents induced by glutamate than for those induced by kainate. We conclude that dopamine potentiates the activity of horizontal cell glutamate receptors by altering the kinetics of the ion channel so as to favor the open state.

Physiologisches Institut der Justus-Liebig-Universität
Aulweg 129, 6300 Gießen

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VISUAL EVOKED RESPONSES TO MOTION-REVERSAL STIMULATION

M. Kuba, N. Toyonaga and Z. Kubová

Direction changes of motion of a patterned visual stimulus (motion-reversal) produce visual evoked responses (VERs) which were described to be similar to the pattern-appearance VERs but they were classified as "genuine responses to changes in the motion as such" (P.G.H. Clarke, Exp Brain Res 18:156-164, 1973).

In our group of 20 repeatedly examined persons (recordings from lead O_z - A_1 and symmetrical lateral occipital leads 5 cm from O_z) the distinct motion-reversal VERs were obtained in all cases. The average amplitude of 2 Hz horizontal motion-reversal VERs (motion velocity 23 deg/s) was significantly larger (13.1 ± 5.1 µV) than the amplitude of comparable 2 Hz pattern-reversal VERs - 8.2 ± 4.9 µV (black-white checkerboard, check size 25', contrast 0.95, average luminance 25 cd/m², stimulated area - central 20°). However, the shape of curves of these VERs was interindividually very different (the opposite polarity of peaks in some cases). Besides, using a low contrast non-structured stimulus (variant of random dots) the shape of the VERs to motion-reversal was changed in some persons to the form of motion-onset VERs (dominant negative peak with a latency of about 170 ms) and the maximum of response was localized over the right occipital area (as it was the case in our previous experiments with motion-onset VERs). We suppose the existence of different types of motion reversal VERs with predominance of either motion or pattern-on/off related components.

Max-Planck-Inst., Parkstr. 1, 6350 Bad Nauheim

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THE HIGH FREQUENCY LIMITS IN THE SPATIAL VISION OF THE PIGEONS.

M. A. Pak

The spatial contrast transfer function of the visual system of the pigeon was determined by recording from the optic tectum evoked potentials or the extracellular unit responses to a pattern stimulus. The spatial contrast transfer function, determined as "response function", describes the relationship between the contrast of the pattern, whose intensity varies sinusoidally with position, and the amplitude of the response at various spatial frequencies (c/deg). The transfer function supplies an estimate of the high frequency limit, which is a measure visual resolving power.

The highest spatial frequency detectable in a visual system is limited by many factors, in particular the diffraction of light at the pupil and the anatomical spacing of the photoreceptors. The pupil factor can be controlled in experiments in a suitable way.

In this paper the electrophysiologically determined high-frequency limit was compared with the theoretical resolution limit imposed by the photoreceptor mosaic. The experimental results show that the visual system of the pigeon has a high frequency limit at spatial frequency of 15.5 c/deg, which corresponds to a visual acuity of 1.9 min of arc. My attempts to relate visual acuity in the pigeon to the anatomical spacing of the photoreceptors show that the Nyquist frequency of the photoreceptor mosaic, the theoretical upper bound of the spatial resolution, agrees with measurement.

Physiologisches Institut der Heinrich-Heine-Universität Düsseldorf, Abteilung Neuro-und Sinnesphysiologie.4000 Düsseldorf.

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DISTANCE, DURATION, AND VELOCITY OF MOTION ESTIMATED DURING OCULAR PURSUIT

W.H. Ehrenstein*, S. Mateeff**, J. Hohnsbein*

Eight subjects pursued binocularly a target that moved 24 deg, either from left to right or vice versa, at 8 and 16 deg/s. Accurate tracking was controlled by an afterimage method. During the target motion a test stimulus that moved at the same speed as the target, in either the same or opposite direction, was exposed 0.6 deg above the midpoint of the trajectory for 200, 400, or 600 ms. Subjects estimated the extent of test stimulus motion (in cm), reproduced its duration by pressing a key connected to a clock, and made magnitude estimations of its velocity.

Compared to a control condition (= 100%) in which the subjects fixated a steady point, the perceived distance (d) and velocity (v) of motion was underestimated when the stimulus moved with the eyes and overestimated when it moved opposite to the eyes:

	8 deg/s		16 deg/s	
d	81.1%	123.8%	73.4%	108.2%
v	81.2%	124.4%	71.6%	120.7%

Duration estimates were not affected by stimulus direction.

The results suggest that the distance and velocity estimates are independent of the perceived duration of the motion. Both over- and underestimation of the extent and the velocity of motion may be explained assuming that the velocity of eye movements is underregistered by the visual system.

* Institut für Arbeitsphysiologie, Dortmund, FRG
** Institute of Physiology, Sofia, Bulgaria

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EFFECT OF INTRACELLULARLY APPLIED ORGANIC ANIONS ON THE DARK VOLTAGE OF ISOLATED RETINAL RODS.

P. Jacobi, K.-F. Schmidt, G.N. Nöll and Ch. Baumann

The specific effects of various anions that have been used for intracellular perfusion of excitable cells are poorly understood at present. We have therefore investigated the effect of anions on the dark voltage of retinal rods of the frog *Rana esculenta*. By means of the whole-cell patch-clamp technique we tested the effects of a number of potassium salts. When the recording pipette contained a medium with potassium as the principle cation and chloride as the principle anion, a slow spontaneous hyperpolarization of the receptor potential due to a diffusional loss of cGMP and GTP was observed (Schmidt et al., Visual Neuroscience 2, 101-108, 1989). When 80 of 100 mM chloride was replaced by organic anions the speed of the hyperpolarization was diminished to 78 % by acetate (mol.wt. 59), to 50 % by L-aspartate (mol.wt. 133), to 43 % by gluconate (mol.wt. 196) or to 15 % by D-aspartate. As these data indicate, the pure size of the anions may contribute to its stabilizing effects, but the different effects of L- and D-aspartate suggest an additional influence on the cell's metabolism. To investigate this further we used various anions in combination with 1mM GTP, the precursor of the photoreceptor's intracellular transmitter, which by itself slows down the speed of hyperpolarization to about 30 %. When L-aspartate or gluconate were used together with GTP, the stabilizing effects of anions and nucleotides on the dark currents were almost additive while acetate, on the other hand, diminished the stabilizing effect of GTP. Beside the effects on the dark voltage, aspartate had a profound effect on the configuration of the light responses. Replacement of the normally used HEPES-buffer by 10 mM phosphate-buffer reduced the speed of the hyperpolarization to 53 %, because phosphate appears to stimulate the triphosphate synthesis. Accordingly, the appplication of GTP was less effective with, than without, phosphate.

Physiologisches Institut der Justus-Liebig-Universität
Aulweg 129, 6300 Gießen

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TEMPERATURE REGULATES NEURAL ACTIVITIES IN THE PHOTOSENSITIVE PINEAL ORGAN OF THE TROUT.

M. Tabata, Ch. Martin and H. Meissl

In ectotherms the pineal organ contributes, in addition to its photoneuroendocrine function, to the regulation of body temperature by behavioral thermoregulation. So far little is known how the pineal organ receives and transmits environmental information about temperature. We report here on the effect of temperature on the neural activity of a photosensitive pineal organ maintained in flow-through organ culture conditions. Single dark-adapted achromatic ganglion cells were extracellularly recorded from the isolated pineal organ of the rainbow trout, *Salmo gairdneri*, while the temperature was changed between 5° and 37°C at a rate of 1°C per min. At 5°C in darkness all ganglion cells showed spike discharge frequencies below 8/s, an irregular firing interval and the lowest spike amplitude. At increasing temperatures the impulse frequency increased almost linearly, the discharge pattern became more regular and the amplitude larger. However, increase of temperature above a certain range caused a sudden reduction, followed by an interruption of firing. Ganglion cells could be classified into two types according to this critical temperature range. The first type showed a reduction of spike activity at 20-22°C, with interruption of firing at 24-25°C, the second type at 29-30°C and 35-36°C, respectively. Decrease of temperature resulted in a reappearance of spike activity. The Q_{10} values for spike activity ranged between 1.8 and 2.2. The intensity-response relation displayed a distinct intensity dependent inhibitory effect of light within an intermediate temperature range approx. between 10°C and 20°C. Measurement of spectral sensivity at 10°C indicated slight higher values (0.4 log unit) than at 20°C and the λ_{max} shifted about 10nm toward longer wavelengths. The findings indicate that environmental temperature influences underlying neural activity of the photosensitive pineal organ in fishes.

Max-Planck-Institut für Physiol. und Klin. Forschung, W.G. Kerckhoff-Institut, Parkstr. 1,.D-6350 Bad Nauheim.

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OPPONENT-COLOUR VISION AND THE ABNEY EFFECT

Th.Knottenberg and H.Scheibner

A classical description of the Abney effect says that the hues of spectral lights change when white light is added to them.

By means of a visual tristimulus colorimeter (GUILD-BECHSTEIN), the two chromaticity loci were determined on which the perceptual criteria "neither green nor red" or "neither blue nor yellow" hold for normal trichromatic observers. Additionally, chromaticity loci were determined which obey the abstract criterion "neither positively bright nor negatively bright". Experimentally, these latter loci were measured from vectorial differences of colours that obey the perceptual criterion "heterochromatically equally bright". The three loci form curves that deviate more or less from straight lines, deviations being an expression of the Abney effect. By linearising two of them and piece-wise linearising one of them, opponent-colour triangles were obtained, with which corresponding opponent-colour spaces are associated. By construction, such opponent-colour spaces are, first of all, related to the instrumental colour space defined by the colorimeter used. By introducing a fundamental colour space, physiologically relevant spectral opponent-colour functions were derived that take some features of the Abney Effect into account.

Physiologisches Institut der Universität Düsseldorf, Abt.Neuro-und Sinnesphysiologie, Moorenstr.5, D-4000 Düsseldorf

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ACTIVE MICROMECHANICS OF ISOLATED MAMMALIAN VESTIBULAR HAIR CELLS

Hans P. Zenner, Ulrike Zimmermann and Alfred H. Gitter

The vestibular sensory cells (hair cells) in the mammalian inner ear transduce acceleration of the head or acceleration due to gravity into bioelectrical and biochemical signals. A vestibular hair cell (VHC) responds to acceleration due to mechanical displacement of its sensory hair bundle. Vestibular hair cells (VHC) were isolated from the guinea pig inner ear. Using the whole cell variant of the patch recording technique a cell potential of -63.1 ± 9.9 mV was measured in macular hair cells.

The angle of hair bundle displacement during VHC stimulation is thought to be determined by the energy of the stimulus and the passive micromechanical properties of the VHC and its accessory structures (e.g. endolymph fluid, otolith, cupula). They can be modelled by a mass-spring-dashpot system. Thus, the prevailing view is that VHCs have a passive micromechanical role in vestibular transduction with the stereocilia serving as passive plungers. By isolating living VHCs from the guinea pig inner ear, however, we have found that the cells produce spontaneous shape changes. Furthermore, depolarizing conditions evoked reversible motile responses of solitary vestibular sensory cells.

VHCs were gradually depolarized when subjected to increasing extracellular $[K^+]_e$ > 25 mM K^+/gluconate or K^+/Cl^- (25 - 137 mM). A decrease of $[K^+]_e$ to the normal value of 5.7 mM induced a repolarization of the sensory cells. Under these conditions in 44 % of the cells visible active mechanical responses were evoked. Both, type I (46 %) and type II (40 %) VHCs responded with a shortening when depolarized and responded with a subsequent elongation, when repolarized. The response amplitude produced by type II VHCs appeared to be smaller than that by type I cells. After elongation a cell could be restimulated to shorten. Individual VHCs were able to undergo several cycles of shortening and elongation when subjected to cycles of de- and repolarization. Detachment of the kinocilium from the hair bundle had no influence on the motile responses. Pretreatment (30 min.) of VHCs in the presence of cytochalasin B or colchicine (100 µM) did not inhibit the evoked motile VHC responses.

The evoked active process, presently demonstrated, might contribute to regulate the precise angular hair bundle position. It could actively damp the angular motion near the adequate equilibrium position of the displaced hair bundle for a given acceleration. Moreover an active mechanical mechanism could prevent damage to hair bundles and accessory structures by inhibiting ciliary displacements following profound stimuli.

The cellular mechanisms underlying the observed active VHC motions remain unknown. The experiments, however, give some information that the mechanical responses are controlled by the intracellular potential or related internal parameters. Hence, they might be induced by the receptor potential and could be electromechanically operated processes.

HNO Hearing Research Laboratories, University of Tübingen, Silcherstraße 5, D-7400 Tübingen

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FREQUENCY MAPPING ON THE BASILAR MEMBRANE OF THE PIGEON.

D. Ding, J.W.T. Smolders and R. Klinke.

In order to determine hair-cell characteristic frequency-gradients over the basilar papilla the frequency-intensity response area of single primary auditory neurons in the pigeon was determined, followed by dye-marking of the neurons with hexamminecobaltchloride (HCC) or horseradish-peroxidase (HRP). With HCC 17 stained fibres from 40 injections in 14 ears were identified. Only the unmyelinated part of the nerve fibres and the ganglion cell body were stained. The nerve endings did not branch. Each fibre contacted a single hair cell on the part of the basilar membrane overlying the neural limbus. With HRP 18 stained fibres from 45 injections in 7 ears were identified. As with HCC only the unmyelinated part of the nerve fibres was stained. Ganglion cell bodies, however, were faintly stained in 2 cases only. 14 of the fibres contacted hair cells over the neural limbus. One of these branched to contact two adjacent hair cells, the remainder contacted a single hair cell. 4 fibres contacted hair cells on the free part of the basilar membrane; 2 of these branched and made contact with two adjacent hair cells. The frequency-intensity response areas of the fibres (characteristic frequencies 0.38 - 0.7 kHz) contacting hair cells over the free basilar membrane (the intermediate or short hair cell area) were not different from the pool of response areas of fibres contacting hair cells over the neural limbus (the tall hair cell area). The distribution of hair-cell characteristic frequency along the length of the basilar papilla can be fitted with a single exponential. The mean mapping constant over the frequency range 0.16 - 4.2 kHz for a mean basilar membrane length of 3.97 + 0.20 mm (n = 19) is 0.79 mm/octave. This value is in agreement with the mechanical frequency mapping constant over the basal 1.33 mm of the basilar membrane (Gummer et al., Hearing Res 29:63, 1987).

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Zentrum der Physiologie, Theodor-Stern-Kai 7, D-6000 Frankfurt.

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MOLECULAR ASPECT OF OLFACTORY SIGNAL TRANSDUCTION

I. Boekhoff, K. Raming, J. Krieger, E. Tareilus, J. Strotmann and H. Breer

The chemo-electrical transduction in olfactory receptor cells comprises a chain of events including the transfer of hydrophobic odorant molecules to the perceptive dendritic membranes where they interact with specific receptors inducing the generation of receptor potentials; second messengers are supposed to provide the critical link between the initial receptor binding and subsequent ion channel activity.
Towards an elucidation of the reaction cascade underlying olfactory signal transduction, pheromone binding proteins, which are supposed to catalyse the transfer of odorant molecules, as well as G-proteins, which may couple the receptors to intracellular effector systems (enzymes) have been cloned, sequenced and expressed.
The process of signal transduction has been analysed using preparations from insect antennae and from rat olfactory epithelia. In a modified stop flow approach the formation of second messengers induced by pheromones or odorants was studied on the msec time range. It was established that in insect antennae pheromones activate phospholipase C via specific G-proteins leading to elevated IP_3-levels within 50 msec after stimulus application. In vertebrate preparation most of the odorants analysed induced a significantly increased level of cAMP immediately after application; however, several odorants were found to activiate the PIP_2-system.

Institut für Zoophysiologie, Universität Stuttgart-Hohenheim, Garbenstraße 30, D-7000 Stuttgart 70

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DUAL RESPONSE OF XENOPUS OLFACTORY RECEPTOR CELLS UPON APPLICATION OF cAMP AND NATURAL STIMULI

D. Schild, J.A. DeSimone, S. Hellwig

cAMP has been suggested to gate directly generator channels in olfactory receptor neurones (Nakamura and Gold, 1987). Here we report patch clamp and Ussing chamber experiments indicating a more complex action of cAMP.
The voltage gated currents in these receptor neurones have been analysed (Schild, 1989). However, recording of generator currents in the whole cell mode upon stimulation with natural odorants was not successful. In order to study the reasons for this, we measured voltage stimulus responses across the whole olfactory mucusa in a current clamp loop using an Ussing-type chamber. Responses to a food extract solution as well as to phobol 12,13-dibutyrat were positive and phasic-tonic pulses, while the responses to amino acids or Br-cAMP showed two phases, a negative wave followed by a positive one. Responses occurred only if the stimuli were applied to the apical side of the mucosa, and the positive responses were abolished by Amiloride. In the whole cell mode of the patch clamp technique, only one of the above stimuli, Br-cAMP, elicited a response the shape of which was virtually identical to the whole mucosa response. It consisted of a slow hyperpolarization followed by a depolarization. Cells which had lost their cilia did not show the depolarizing part of the response. From the comparison of the whole mucosa and the single cell responses, it can be concluded that the hyperpolarization as well as the depolarization have their origin at the apical side of the neurone. The first part of the response can be interpreted as a cAMP-induced adaptation mechanism accompanying the ciliar depolarization.

Physiologisches Institut der Universität Göttingen
Humboldtallee 23, D-34 Göttingen

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ACETYLCHOLINE- AND GABA-RECEPTORS IN OUTER HAIR CELLS OF THE GUINEA-PIG COCHLEA

A.H. Gitter, P.K. Plinkert and H.P. Zenner

In the mammalian inner-ear cochlea active mechanical processes give rise to amplification and sharp tuning of the sound-induced vibrations of the cochlear partition. Efferent nerve endings at the basal end of one type of auditory receptor cells, outer hair cells (OHC), may participate in the regulation of these processes. There is evidence for putative neurotransmitters, acetylcholine (ACh) and τ-aminobutyric acid (GABA), but direct demonstration of receptor molecules was absent so far.
Monoclonal antibodies allowed us to visualize epitopes of postsynaptic ACh receptors and GABAA/benzodiazepine receptors at the basal end of guinea-pig OHC of all cochlear turns, but the local distribution of the receptors types was different. GABA-receptor immunoreactivity was higher in apical than in basal turns of the cochlea, whereas ACh-receptor immunoreactivity was more pronounced in basal turns. However, in isolated living OHC application of ACh or other agonists did not induce the significant depolarization expected from nicotinic ACh receptors. By contrast, simultaneous application of GABA and flunitrazepame elicited significant hyperpolarization of OHC membranes, which was reversible when the drugs were removed. Whole-cell recordings with different chloride concentration in the pipette showed an inwardly directed chloride current as electrochemical basis for the observed cell membrane hyperpolarization.

Universitätsklinik für Hals-Nasen-Ohrenheilkunde,
7400 Tübingen, FRG

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A QUANTITATIVE COMPARISON OF THE STIMULATION RESPONSE CURVES OF SACRAL PRIMARY AFFERENTS RESPONDING TO FILLING AND DISTENSION OF THE URINARY BLADDER.

H.-J. Häbler, W. Jänig, M. Koltzenburg

Mechanosensitive primary afferents innervating the urinary bladder are activated by increases of intravesical pressure evoked by both isotonic distension and isovolumetric contractions of the bladder. However, it is thought that the stimulation response curves differ under both conditions and that the excitability of afferents is modulated by parasympathetic efferents supplying the bladder. Here, using standard techniques, we have quantitatively compared for both stimuli the responses of single fibres that were recorded from the dorsal root S_2 of anaesthetized and paralysed cats. Intravesical pressure was continuously recorded through an apex catheter, and through an urethral catheter the bladder was either slowly filled with an infusion pump (2ml/min) or distended using a pressure reservoir. Myelinated vesical afferents had no resting activity and thresholds below 25 mmHg. At an intravesical pressure of 50 mmHg they discharged at 1.2-19.1 Hz during filling and 2.2-17.3 Hz during distension. In 22/28 units the response curves for both type of stimuli were virtually identical and only in 6 units did slow filling produce a slightly steeper curve than isotonic distension. After normalization of all 28 stimulation response curves, using the discharge at 50 mmHg as a reference, there was neither a significant difference between the two groups of curves nor between the threshold distribution in both stimulation protocols. To exclude the reflex contraction of the detrusor muscle that accompanies intravesical pressure increases, 8 units were studied prior and after a complete transsection of all spinal roots distal to L_6. However, this did not significantly change the average response curve of afferents with respect to the intravesical pressure although the volume pressure curve of the bladder was flatter after rhizotomy. Only few unmyelinated vesical afferents were activated by the pressure stimuli used. They were not spontaneously active and had significantly higher thresholds than myelinated units. For 3 afferents tested there was no clear difference in the stimulation response curves during filling or distension.
In conclusion, mechanosensitive afferents innervating the urinary bladder encode the actual intravesical pressure regardless of the initiating type of stimulus used and regardless whether the efferent parasympathetic innervation of the bladder is intact or not.

Physiologisches Institut, Universität, Olshausenstr. 40-60, 2300 Kiel, FRG.

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DIFFERENT TYPES OF ANTIDROMIC VASODILATION IN THE CHICKEN SKIN

E. Pinter, Fr.-K. Pierau, and H. Sann

Vasodilation in response to antidromic stimulation is part of the neurogenic inflammation reaction in the skin, which is abolished after capsaicin pretreatment. Although birds are highly insensitive to capsaicin, antidromic stimulation induces vasodilation in the skin of the wing which was, however, much shorter than in mammals. To further analyse this vasoreaction, changes of capillary blood flow in response to electrical stimulation of the sciatic nerve were measured by the laser Doppler technique in anesthetized (nembutal) and immobilized (gallamine) chicken, pretreated with 20 mg/kg guanethidin on the previous day.
Electrical stimulation of the peripheral part of the sciatic nerve (1Hz, 40V, 2ms, 32 imp) produced a 2 phasic response in the lower leg, starting with a short initial vasodilation which exceeded the stimmulation period only by 10-20 sec and was abolished by atropin (1mg/kg). The second phase lasted 3-5min and was simmilar to the 1 phasic reaction in the skin of the foot. Both long duration responses were not affected by the histamin H_1 receptor antagonist diphenhydramine (5-10 mg/kg) and the ganglion blocking agent hexamethonium (5-10 mg/kg), while the vasodilation (flux x duration) was reduced by 10-25 % after the dopamine D_2 receptor antagonist metoclopramide (10-40 mg/kg). Pronounced reduction of antidromic vasodilaton was observed during close arterial infusion of substance P (SP 0.15-0.8 µg/kg/min). By contrast, the SP antagonists spantide and D-Arg^1, D-Pro^2 ,D-Trp^{7,9}, Leu^{11})-SP did not effect antidromic vasodilation, which might indicate species specificity of SP antagonists. It remains to be elucidated, wether the different configuration of antidromic vasodilation in different skin areas is due to morphological and/or molecular differences.

MPI für physiol. und klin. Forschung, W.G. Kerckhoff-Inst.
Parkstr. 1, D-6350 Bad Nauheim

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DYNAMIC COMPONENTS IN SENSORY TRANSDUCTION OF THERMAL AND ELECTRICAL STIMULI

H. Wissing, K. Schäfer and H.A. Braun

The frequency-stimulus-response of sensory receptors is generally described as a proportional-derivative (PD) transfer characteristic. A rapid step-like stimulus induces a dynamic frequency overshoot or undershoot (D) which is followed by adaptation to a new steady-state (P). In thermosensitive afferents, however, the initial dynamic frequency change is often followed by a second dynamic component of inverse direction. Such a biphasic response have particularly been observed in two of the most sensitive receptor populations: in cold sensitive afferents of the ampullae of Lorenzini (dog fish) and in warm afferents of boa constrictor. We examined the dynamic responses of these receptors using ramp-shaped instead of step-like stimuli. For comparison we stimulated the ampullary electroreceptors with linear current ramps.
The onset of a slow temperature ramp induced a fast initial frequency change, but the receptors already adapted during the ongoing ramp. The end of the ramp evoked a second dynamic component of inverse direction. The occurence of a second dynamic component depended on stimulus conditions and was generally more pronunced in ampullary cold afferents. It is therefore of particular interest that electrical ramps never induced a second dynamic component. Sensory transduction of temperature stimuli obviously induced specific membrane mechanisms which are not activated by electrical stimuli. These mechanisms enables the receptor to detect not only absolute temperature and temperature changes but also temperature acceleration.

Zentrum für Anästhesiologie und Wiederbelebung der Universität, Theodor-Stern-Kai 7, 6000 Frankfurt, FRG

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SOMATOSENSORY PATHWAYS FOR FLIGHT CONTROL IN BIRDS

R. Necker

It is well known that stimulation of cutaneous receptors are important for flight control in birds (M. Gewecke and M. Woike, Z. Tierpsychol. 47:293, 1978; D. Bilo and A. Bilo, Naturwiss. 65:161, 1978). Interactions between sensory and motor systems may occur both at the spinal and at brainstem levels (vestibular system, cerebellum). Possible pathways of the mechanosensory system in the pigeon were studied both with electrophysiological and anatomical means (tracer technique).

Feather follicles are richly supplied with mechanoreceptors (Herbst corpuscles, Merkel cells). In the spinal cord cutaneous mechanoreception is processed in the n. proprius (mainly lamina IV) of the dorsal horn where a distinct somatotopy could be demonstrated both with electrophysiological and with anatomical studies. N. proprius neurons of the cervical cord (wing) project to the dorsal column nuclei in the brainstem which otherwise receive a direct input from primary afferent fibers. However, cervical n. proprius neurons have descending projections also. Furthermore, lumbar n. proprius neurons (leg) seem to terminate largely in the cervical cord. This suggests that n. proprius neurons may be involved in coordination of limb movements during walk (legs) and flight (wings). There is no evidence of a direct cutaneous input to vestibular nuclei. However, the dorsal column nuclei project to the cerebellum via the inferior olive thus reaching supraspinal motor systems.

Institut für Tierphysiologie der Ruhr-Universität Bochum, Postfach 102148, D-4630 Bochum 1, FRG

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DISTRIBUTION OF SOMATOSTATIN IN PRIMARY ARTICULAR AFFERENTS IN CAT

U. Hanesch, B. Heppelmann, and R.F. Schmidt

Somatostatin (SOM), a recently isolated and characterized neuropeptide, has been demonstrated immunohistochemically in afferent nerve fibers of various tissues and predominently in small dorsal root ganglion cells giving probabely rise to unmyelinated and thinly myelinated fibers. The knee joint of the cat is also mainly innervated by fine afferent nerve fibers: in the medial articular nerve (MAN) 70% belong to Group IV and 21% to Group III (Heppelmann et al, Somatosens Res 5, 273-281, 1988). It could be shown that a portion of MAN neurons contains the peptides substance P and calcitonin gene-related peptide (CGRP) (Hanesch et al, Europ J Neurosci Suppl 2, 69, 1989). The aim of this study was to investigate whether SOM also occurs in primary afferent perikarya of the MAN.

For retrograde tracing the MAN was cut and exposed to the fluorescent dye Fast blue. After 4 days the corresponding dorsal root ganglia were treated with colchicin. After a further day the cats were fixed by perfusion, the ganglia were cut in 50 μm sections and fluorescent cell bodies were photographed. After visualizing SOM-like immunoreactivity with the PAP-method, the percentage of peptide containing articular neurons was determined by comparing the photographs of labelled perikarya with the immunopositive cells. A morphometrical analysis followed.

After exposure of the MAN with Fast blue, labelled perikarya were found in dorsal root ganglia of segments L5 and L6. Their soma size were in a range from 26 to 88 μm. About 21% of these perikarya showed a SOM-like immunoreactivity. Their diameter ranged from 30 to 50 μm.

Conclusion. Somatostatin has been shown to be present in dorsal root ganglia cells of the MAN. Their diameter distribution suggests that SOM is nearly exclusively contained in small sized neurons, which are thought to give rise to group III and IV nerve fibers. In addition to the neuropeptides substance P and CGRP, SOM was shown to be a further candidate probably acting as a neurotransmitter or -modulator in articular afferent neurons.
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Physiologisches Institut der Universität Würzburg, Röntgenring 9, D-8700 Würzburg

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INFLUENCE OF LEUKOTRIENE D4 ON THE DISCHARGES OF SLOWLY CONDUCTING AFFERENT UNITS FROM NORMAL AND INFLAMED MUSCLE IN THE RAT

S. Mense and U. Hoheisel

Previous experiments have shown that in carrageenan-inflamed muscle of cats and rats, many group III and IV muscle receptors exhibit signs of a sensitization (increase in resting discharge and/or decrease in mechanical threshold). Among other mediators the leukotrienes (LTs) are currently discussed as possible candidate substances that might elicit these changes.

In the present study, the impulse activity of single muscle receptors with group III and IV afferent fibres (conduction velocities 0.5-5.4 m/s) was recorded in anaesthetized SD rats. All the afferent units had mechanosensitive receptive fields (RFs) in the gastrocnemius-soleus muscle. The RFs were stimulated with quantitative mechanical stimuli and the response magnitudes determined before and after infiltration of the RF with 100 ng-1 µg LTD4 in 0.1 ml Tyrode solution.

Changes in the resting discharge of the units were not observed following LTD4 application. However, receptors in normal muscle exhibited a decrease in response magnitude under the influence of LTD4. The effect became significant 30 min after injection of LTD4 and was present in both low- and high-threshold mechanosensitive units. In the mechanically induced responses of the receptors in inflamed muscle such a change in responsiveness was not detected. The results suggest that LTD4 has a desensitizing rather than a sensitizing action on muscle receptors.

Institut für Anatomie und Zellbiologie der Universität, Im Neuenheimer Feld 307, D-6900 Heidelberg

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SENSITIZATION AND SELECTIVE EXCITATION BY PROTONS OF NOCICEPTIVE NERVE ENDINGS IN RAT SKIN, *IN VITRO*

K.H. Steen, F. Anton, P.W. Reeh and H.O.Handwerker

In ischemic and inflamed regions pH levels down to 5.4 have been measured (Häbler: Archiv f. klin. Chirurgie 156, 1929, 20-42) and this may contribute to pain and hyperalgesia. Therefore, in a skin-saphenous nerve *in vitro* preparation, receptive fields of identified primary afferents were superfused at the corium side with CO_2 saturated synthetic interstitial fluid (SIF at pH 6.1) followed by a series of superfusions with phosphate buffered carbogen gassed SIF at acid pH levels down to 4.2 (duration 5 min; interval 10 min). Mechanical thresholds were repeatedly monitored by v.Frey-hair stimulation.

Mechanosensitive Aβ (n=12) and Aδ fibres (n=11) were not excited nor sensitized by acid pH levels. In 16 out of 58 mechano-heat sensitive (MH), "polymodal", C and Aδ fibres and 8 out of 26 mechano- and mechano-cold sensitive C-fibres irregular low frequency discharge was induced. However, a distinct subpopulation of C- and Aδ-MH units (n=24; 40%) showed stimulus-related responses increasing with proton concentration and encoding the time course of the pH-change. All such fibres responded to CO_2 (pH 6.1) as well as to phosphate buffered solution at the same pH. The CO_2-responses, however, displayed shorter latencies (n=8, means: CO_2 44 s, phosphate SIF 91 s) and more pronounced dynamic responses. With phosphate buffered SIF set to different pH values, we found threshold levels ranging from pH 6.9 to 6.1. This closely agrees with a recent report on the activation of a sustained sodium inward current by equally low pH in rat DRG neurones (Bevan & Yeats: Soc. Neurosci. Abstr. 15, 1989, 1152). This paper also proposed a close similarity to the action of capsaicin. In our preparation, however, many, but certainly not all fibres sensitive to protons were driven by capsaicin (10^{-6}M, 10^{-5}M) and vice versa. Prolonged or repeated application of CO_2 or low pH induced a marked and long lasting decrease (≈ 30 min) of the v.Frey thresholds in almost all C-MH fibres tested (n=16; mean before 34.8 mN, after 15.9 mN; p=0.001 Wilcoxon) and this occurred whether or not a fibre was excited by protons. The sensitizing effect was the more pronounced the higher the v.Frey thresholds had previously been (0.75 rank correlation).

This is so far the only chemical condition which in our preparation produced a sensitization to mechanical stimulation, since previously, even an extensive combination of inflammatory mediators only induced a sensitization to heat. Interactions between pH and those mediators remain to be elucidated.

Institut für Physiologie und Biokybernetik, Universität Erlangen-Nürnberg Universitätsstr. 17, D-8520 Erlangen (DFG grant Re 704/1-5).

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STIMULATION OF UNMYELINATED PRIMARY AFFERENTS INCREASES BLOOD FLOW IN SKIN AND SPINAL CORD

M. Koltzenburg, G.R. Lewin, S.B. McMahon*

Activation of unmyelinated primary afferents produces vasodilatation and plasma extravasation in the skin. Here, we have asked whether C-fibre stimulation precipitates the same phenomena in the spinal cord. In anaesthetized (1.25 g/kg urethane, i.p.) and paralysed (40 mg/kg gallamine, i.p.) rats the sciatic nerve was stimualted supramaximally (4mA, 1ms). Relative blood flow changes and plasma extravasation were measured in the ipsi- and contralateral hairy skin of the foot and the dorsal root entry zone of the lumbar enlargement of the spinal cord using the laser-Doppler (Perimed PF3) and the Evans blue technique (50mg/kg,i.v.). Baseline line blood flow gave readings of 73.2±17.5 and 19.9±6.2 arbitrary units ($p<0.05$, mean±SEM, n=5) for spinal cord and skin, respectively. C-fibre stimulation for 30s at different frequencies (0.5-10 Hz) produced graded blood flow increases in both spinal cord and hairy skin. At a frequency of 10 Hz, vasodilatation over and above the baseline increased to a peak flow of 175±50 % in the skin contrasting with only 50±14 % in the spinal cord. While blood flow increases were sustained in the skin outlasting the stimulation by several minutes, they were short-lived in the spinal cord and returned to the pre-stimulus level within 30s when the stimulation ceased. Changes in the skin were seen only ipsilaterally, but bilaterally in the spinal cord. Moreover, flow increases in the spinal cord, but not in the skin, corresponded to the C-fibre evoked rise of the systemic blood pressure, and spontaneous fluctuations of the blood pressure also caused parallel changes of the flow in the spinal cord while they did not significantly affected cutaneous flow. After 10 min. of C-fibre stimulation at 10 Hz the Evans blue content of the skin rose from 6.3±3.4 to 228.9±12.7 μg/g ($p<0.001$; n=5) while it did not change in the ipsilateral spinal cord and remained always very low averaging 6.7±3.1 μg/g. No significant difference was detected between the ipsi- and contralateral side of the lumbar spinal cord. Thus, whilst the same substances are apparently released from the peripheral and central terminals of primary afferent fibres, their ability to produce vasodilataion and extravasation is severly restricted in the spinal cord. The blood flow increases in the spinal cord are probably a passive effect following the corresponding changes of the systemic blood pressure.

*Physiologisches Institut, Olshausenstr. 40-60, D-2300 Kiel, FRG, Dept. Physiology, St. Thomas's Hospital Medical School, London SE1 7EH, UK.

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IMPAIRED SKIN REACTIONS TO INTRADERMALLY INJECTED SUBSTANCE P AND TOPICALLY APPLIED MUSTARD OIL IN ATOPIC DERMATITIS PATIENTS

G. Heyer, O.P. Hornstein, H.O. Handwerker

We have previously demonstrated an inability of atopic dermatitis (AD) patients to distinguish different levels of iontophoretically applied histamine concentrations as shown by their diminished vascular reactions and itch responses. Since the results indicated an impaired function of small cutaneous nerve fibers, we conducted a further study on skin reactions and on sensations of itch and burning pain after intradermal injection of substance P (SP) and topical application of mustard oil (MU) in 20 AD patients and 20 healthy controls. As in the previous study we measured the change in skin blood flow with a Laser Doppler flowmeter (LDFM), quantified the sizes of wheal and flare responses and assessed itch and pain sensations by verbal report on a category partitioning scale. SP evoked dose-dependent wheal and flare reactions. Stronger SP stimuli elicited smaller flares and weaker itch ratings compared to those of the controls. However, identical wheal reactions in both groups indicate undisturbed direct plasma-extravasation due to SP in AD. In contrast, the indirect effect of SP, release of histamine from cutaneous mast cells, which induces flare and itch sensations, is diminished in AD. In both groups MU elicited similar inflammatory reactions, seen as erythema and increase of skin blood flow by LDFM measurements. However, burning pain sensations were significantly delayed in AD patients, although these patients show a decreased skin barrier function. The observed diminished flare, itch, and burning pain responses may result from histamine receptor down-regulation in AD patients impeding the "cascade reaction".

Institut für Physiologie und Biokybernetik, Universitätsstr.17, D-8520 Erlangen und Universitäts-Hautklinik

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PAIN BUT NOT TEMPERATURE SENSATION IS EVOKED BY THERMAL STIMULATION OF VEINS IN MAN

W. Klement and J.O. Arndt

Free nerve endings in the intima constitute the afferent innervation of veins (Staubesand: VASA 6 (1977) 208-210). These uniform structures are claimed to sense both temperature and pain (Fruhstorfer & Lindblom: PAIN 17 (1983) 235-241). To see if cutaneous perception may have confounded this study, we explored thermally evoked sensations before and after blockade of cutaneous afferents.

Hand veins, vascularly isolated in 8 subjects, were exposed to temperatures between 0 and 52°C via an indwelling thermode or by the injection of saline. We evaluated pain intensity ratings (visual analogue scale) and temperature sensations when either skin (application of 20 % benzocaine) or veins (0.1 - 1.0 g% procaine intravenously) were numbed.

With the skin innervation intact, subjects sensed both temperature and pain but pain only after numbing the skin. Contrariwise, the subjects sensed temperature without pain after intravenous application of procaine.

Therefore, the intimal receptors of superficial veins can be qualified as nociceptors.

Institut für Experimentelle Anaesthesiologie der Universität Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf 1

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PAIN EVOKED BY INTRAVASCULAR ELECTROSTIMULATION OF SUPERFICIAL VEINS OF MAN

J.O. Arndt and W. Klement

Veins, that are innervated afferently by uniform free nerve endings, are said to respond to thermal, mechanical, and chemical stimuli. We explored what kind of sensation could be evoked by intravenous electrostimulation.

Electrical stimuli (bipolar, constant current, intensity 0 - 10 mA, pulse width 0.1 - 20 msec) were applied within a vascularly isolated segment of superficial hand veins in man (6 informed and consenting physicians). We valued the subjects' sensations, thresholds, and tolerance maxima of pain, and also the blocking concentration of intravenously administered procaine.

Painful sensations described as sharp and tearing at the site of stimulation had thresholds at 1.5 mA and tolerance maxima at 2.5 mA at 20 msec pulse width. These sensations were blocked by intravenous procaine concentrations of 0.6 g% within 5 min.

Intraluminal electrostimulation of superficial veins evokes pain in man which can be abolished by intravenous concentration of 0.6 g% procaine.

Institut für Experimentelle Anaesthesiologie der Universität Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf 1

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CHEMOSENSITIVITY OF AFFERENT FIBRES INNERVATING THE GUINEA-PIG'S URETER *IN VITRO*

H. Sann, K. Hammer and Fr.-K. Pierau

The guinea-pig ureter provides a good model to study visceral afferent fibres involved in nociception. It is innervated by two types of mechanosensitive afferents: U-1 units (<10%) have low thresholds and respond to contractions, whereas high threshold U-2 units (90%) are not excited by contractions (Cervero & Sann, J.Physiol. 412:245-266, 1989).
To study the chemosensitivity of these afferents electrophysiological recordings were made from small branches of the hypogastric nerve using an *in vitro* model. The cannulated ureter was placed into an organ bath and perfused externally and internally with oxygenated artificial interstitial fluid at 37 °C. Most mechanosensitive U-2 units were excited by intraluminal (i.l.) application of at least one of the chemicals used (bradykinin: 0.1-10 µM; capsaicin: 0.03-33 µM or nondiuretic urine). Some of these units expressed only weak response to high i.l. pressure (> 40 mmHg) but could be repeatedly excited by strong probing. The response thresholds of the units for bradykinin (0.1-1 µM) and capsaicin (<0.03 µM) were lower than those necessary to induce contractions. During chemical stimulation, however, the discharge pattern appeared to be modulated by ureteric contractions. Repeated application of 3-33 µM capsaicin resulted in a desensitization of its contractory effect and a reduction of the evoked discharges. In contrast, the chemically induced responses of the few U-1 units studied, appeared to be predominantly evoked by contractions.
The results demonstrate that like in other visceral organs most ureteric afferent fibres can be classified as polymodal receptors. In addition, chemical stimulation appears to sensitize U-2 units to mechanical stimulation.

MPI für physiol. und klin. Forschung, W.G. Kerckhoff-Inst., Parkstr. 1, D-6350 Bad Nauheim

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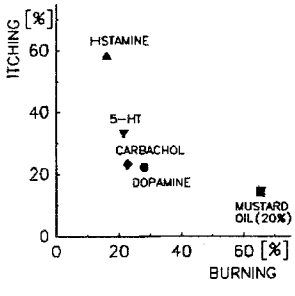
SENSATIONS AND LOCAL INFLAMMATORY RESPONSES INDUCED BY APPLICATION OF CARBACHOL, DOPAMINE, 5-HT, HISTAMINE AND MUSTARD OIL TO THE SKIN IN HUMANS

W. Magerl, G. Grämer and H.O. Handwerker

Mediators of inflammation (histamine and 5-HT) and substances related to the action of postganglionic sympathetic nerves (dopamine and the acetylcholine analog carbachol (carbamoylcholine)) were compared to the effects of 20 % mustard oil in the skin of human subjects. The polar substances were iontophoresed into the skin (5, 20 and 80 mC for 5-HT, dopamine and carbachol, and 1 mC for histamine, respectively) by means of a constant current stimulator (WPI A 360), the apolar mustard oil was aplied in a standardized soaked filter paper to the skin surface. Inflammatory responses were measured as wheal, flare and degree of erythema (flux). The latter was measured by Laser Doppler flowmetry. 5-HT, dopamine and carbachol were equipotent. All substances induced dose-dependent inflammatory reactions and sensations.
However, the qualities of sensations were quite different. The substances could be ranked from itching to burning in the order of histamine, 5-HT, dopamine and mustard oil.



Histamine predominantly induced itching and mustard oil predominantly induced burning. All other substances significantly elicited more burning/less itching than histamine and less burning/more itching than mustard oil. It is concluded that skin nociceptors may belong to groups of differential chemosensitivity, respective activation of which elicits different sensory perceptions. Tissue hormones released from sympathetic postganglionic nerve endings may possibly contribute to the early stages of inflammation in the skin.

Institut für Physiologie und Biokybernetik, University of Erlangen-Nürnberg, Universitätsstr. 17, D-8520 Erlangen (DFG grant Ha 831/8-5)

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PROSTAGLANDIN I2 ENHANCES THE MECHANOSENSITIVITY OF FINE AFFERENTS FROM THE KNEE JOINT OF THE CAT

K. Schepelmann, K. Meßlinger, H.-G. Schaible and R.F. Schmidt

In the cat, an experimental arthritis of the knee joint as well as close arterial injection of inflammatory mediators, in particular Prostaglandin E2, lead to ongoing activity and sensitization to passive movements of slowly conducting articular afferents of the medial articular nerve[1,2].
In order to study the effects of Prostaglandin I2 (PGI2), a further mediator of inflammation, extracellular single unit recordings were made from units of the medial articular nerve in cats anesthetized with α-chloralose. We recorded from 48 units with low conduction velocities (CV), 30 belonging to group III (CV 2.5-20 m/s) and 18 to group IV (CV below 2.5 m/s) and monitored their responses to passive movements of the knee joint. PGI2 was applied intraarterially close to the joint in concentrations from 3.0 to 30 µg per bolus injection.
We observed an excitatory effect in 50% of the group III and in 50% of the group IV units. A sensitization to passive movements occured in 66% of the group III and 44% of the group IV units. In 9 of the units studied, a sensitizing effect took place without an excitation and 2 were excited without any sign of sensitization. We conclude that PGI2 increases the mechanosensitivity in a large proportion of articular afferents in a similar way to an artificial arthritis. Thus, PGI2 may be another important mediator of inflammation which plays a major role in generating arthritic pain.

[1]Schaible,H.-G. and Schmidt,R.F., J.Neurophysiol. 54 (1985): 1109;
[2]Schaible,H.-G. and Schmidt,R.F., J.Physiol. 403 (1988): 91.

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Physiologisches Institut der Universität Würzburg, Röntgenring 9, D-8700 Würzburg, FRG

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PRIMARY NOCICEPTIVE AFFERENTS IN MONKEY THAT MAY SIGNAL FIRST PAIN

R.-D. Treede*§, R. A. Meyer§, J. N. Campbell§

First pain to a stepped heat stimulus is perceived within 0.5 s of stimulus onset and is characterized by a sharp pricking sensation. The myelinated fibers that signal first pain sensation should therefore have a short activation latency and respond with a high discharge rate at the stimulus onset.
We studied the response properties of 39 A-fiber nociceptors (AMHs) that were sensitive to mechanical and to heat stimuli. Standard teased-fiber techniques were used to record from single AMHs that innervated the hairy skin of pentobarbital anesthetized monkey. Radiant heat stimuli with a rise time of 0.1 s were delivered to the receptive field by a laser thermal stimulator. AMHs were classified based on the response to a 30 s, 53°C stimulus. Two parameters were evaluated: the latency to first action potential and the latency to peak discharge.
Three distinct types of responses were observed: One group of AMHs had a mean activation latency of 124 ms, a peak discharge within 1 s, a median heat threshold of 47°C, and a mean conduction velocity in the slow Aδ range (16 ± 3 m/s, mean and s.e.m., n=12). Another group (n=14) had longer activation latencies (570 ms) and a peak discharge close to the end of the stimulus. The third group did not respond to short (1 s) heat stimuli up to 53°C. Activation latencies to the 30 s, 53°C stimulus were between 5 and 29 s, and the mean conduction velocity was 28 ± 4 m/s (n=13). This group may more appropriately be considered as high-threshold mechanoreceptors that sensitize to heat.
In conclusion, AMHs can be classified into three distinct groups based on their heat response properties. One group (previously termed type 2 AMHs) has properties expected of fibers that signal first pain sensation.

*:Physiologisches Institut, Univ.-Krankenhaus Eppendorf D-2000 Hamburg 20
§:Department of Neurosurgery The Johns Hopkins University, Baltimore, MD 21205, U.S.A.

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FORCED CHOICE TESTING AND METHOD OF LIMITS - IS THERE A SUBSTANTIAL DIFFERENCE IN MEASURING RESULTS?

D. Claus, B. Neundörfer

Thermal testing was carried out in 55 healthy subjects in order to establish normal results. A modified Marstock thermode was used and tests were repeated on three consecutive days in order to investigate reproducibility of warm and cold thresholds. The sensitivity of different testing procedures was investigated in 33 patients with diabetes mellitus but without disabling polyneuropathy. Measuring procedures were carried out at the right ankle in random order.
Forced choice testing takes 6 times longer than the method of limits; however, the reliability and variability of the results are not considerably different between forced choice and method of limits. When performed in diabetics, forced choice testing is not more sensitive (pathological results m.limits/forced choice, warm: 7/8; cold: 12/10).
It is thought that the forced choice algorithm does not provide a method appropriate for clinical routine investigations. The limitations of the assessment of perception thresholds are not affected by painstaking forced choice testing.

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Neurologische Klinik, Univ. Erlangen-Nürnberg, Schwabachanlage 6, D-8520 Erlangen

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NEUROGENIC INFLAMMATION IN THE PIG'S SKIN: CAPSAICIN EFFECT AND INTERACTION WITH RUTHENIUM RED

R. Ernst, Fr.-K. Pierau and H. Sann

The pig's skin appears to be the only animal model in which the neurogenic inflammation in response to local irritants resembles the triple response reaction in humans. As in the human skin intradermal electrical stimulation produces antidromic vasodilation and flare reactions which can be measured with the laser Doppler technique and planimetry of the flare area recorded on transparent foil. The magnitude and duration of the vasodilation is directly related to the number of impulses but demonstrates a frequency optimum at about 1 Hz. The reaction depends on capsaicin sensitive peptidergic afferents since repeated previous painting with 1% capsaicin solution prevents the neurogenic vasoreaction. While the flare response to local injections (20 µl) of tachykinins and calcitonin gene-related peptides (CGRP) is only small (maximal concentration 100 µM and 10 µM, respectively) capsaicin produces dose depended flare reactions in a threshold concentration of 300 nM, attaining areas of 10 to 20 cm^2 at a concentration of 3 mM, lasting for 15-30 min.

It has been demonstrated in the rat urinary bladder that ruthenium red specifically interacts with the capsaicin induced activation and desensitization of sensory nerves (Maggi et al. Neurosc Lett 88:201, 1988). Constant perfusion of an experimental blister (cantharidin, 0.4%) in the pig's skin with ruthenium red (5 µM) protects the desensitization to repeated capsaicin perfusions. The vasoreaction to capsaicin is blocked by a ruthenium red concentration of 10 µM. The results suggest that the mechanism responsible for the local efferent function of afferent nerves containing sensory peptides is similar in different organs and species.

MPI für physiol. und klin. Forschung, W.G. Kerckhoff-Inst. Parkstr. 1, D-6350 Bad Nauheim

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PROCESSING IN AN AFFERENT THERMAL PATHWAY: RESPONSES OF COLD RECEPTORS AND HUMAN SENSATION INDUCED BY RAMP-SHAPED SPOT-LIKE STIMULI

A.M. Tick-Waider, K. Schäfer and H.A. Braun

Identical thermal stimuli were applied either to receptive fields of single facial receptors in the rat or to human single cold spots of the forearm by means of an electronically controlled thermode of 4 mm tip diameter. The stimuli were linear temperature changes of either 0.05, 0.1 or 0.5 °C/s from 34 °C adapting temperature to 24 °C and back. Afferent neuronal activity was recorded by conventional means from the infraorbital nerve; human thermal sensation was recorded continuously in arbitrary units by setting of a gliding bar.

Cold receptors responded with excitation followed by inhibition, which was only complete during warming with 0.5 °C/s. In no case the dynamic responses showed a sign of the presence of a second order component, i. e. the responses consisted only of a proportional and a differential component. The peak frequency during the dynamic response increased with faster rates of cooling, whereas the number of impulses, which were induced additionally to the background discharge during the complete cooling-warming stimulus, was maximal at the lowest rate of temperature change. The peak value of the cool sensation induced by the stimulus was independent of the rate of temperature change, as has been observed earlier (*Molinari at al, Sens. Process. 1*: 354, 1977), and was reported during the warming part of the stimulus at 0.5 °C/s, or at the end of the cooling part at 0.1 and 0.05 °C/s. In general, the cool sensations lasted longer than the complete cooling-warming stimulus. Identical stimuli with thermodes of 10 and 24 mm tip diameter did not qualitativly alter the sensation.
The results indicate that information from peripheral cold receptors is processed considerably, that sensation is more likely to reflect the stimulus-induced number of impulses than dynamic peak discharges, and that a single cold spot provides qualitativly the same information as a greater number of such spots.

Institut für Zoophysiologie, Universität Hohenheim, D-7000 Stuttgart 70

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OUABAIN, AN INHIBITOR OF THE SODIUM PUMP, MODULATES PERIODIC DISCHARGE PATTERNS OF COLD RECEPTORS

K. Schäfer and H.A. Braun

The effect of ouabain on periodic discharge pattern of feline cold receptors was studied in order to substantiate a possible contribution of neuronal Na-K pump activity to signal transduction. Afferent activity was recorded from specific cold fibres of an isolated preparation of the tongue. Under test conditions, the preparation was perfused with solutions containing 10^{-7} to 10^{-6} M ouabain, which in all receptors induced excitatory responses. These consisted of a short vigorous increase of activity, occuring repeatedly in some receptors, which without exception was followed by complete inhibition. Thus the receptors never stabilized to or maintained a new static level of activity. The peak discharge rate during the ouabain-induced responses was a function of temperature and increased with warming. During ouabain treatment, cooling evoked dynamic responses in several cold receptors; inverse responses (i.e. to warming) were never observed. The ouabain-induced responses were associated with a pronounced burst discharge and analysis of several such responses showed that the changes of activity were produced by remarkable stereotyped modifications of both the burst frequency and the number of impulses per burst. During the response, both parameters passed through a maximum; their different temporal course indicated that they may be controlled independently. The oscillation frequency attained values which increased monotonically with warmer temperatures and which were considerably greater than peak control values. However, intervals within impulse groups (bursts) were always shorter than the burst period, indicating that the process of impulse generation is not the limiting factor for the oscillation frequency.
These data allow the conclusion that an electrogenic Na-K pump in fact contributes to the transducer process of cold receptors, and that inhibiton of this pump evidently gives rise to a depolarizing imbalance of the membrane potential, accelerating the oscillation frequency to a maximal value. Thus the oscillation frequency seems to be controlled by temperature and by membrane potential in cold receptors.

Institut für Zoophysiologie, Universität Hohenheim, 7000 Stuttgart 70.

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COMPARATIVE ANALYSIS OF BURST DISCHARGES IN ELECTRORECEPTORS OF CATFISH AND IN MAMMALIAN THERMORECEPTORS

F. Bretschneider, H.A. Braun, R.C. Peters, K. Schäfer and P.F.M. Tennis

Afferent activity of peripheral cold receptors is known to exhibit an unorthodox temporal pattern: repetitive (beating) activity at high and burst (grouped) discharges at low temperatures. This pattern, which has been observed in all cold receptor populations, is characterized by its precise timing of impulse generation. It is viewed to be produced by temperature- and calcium- dependent periodic receptor events. It has been demonstrated that the temporal arrangement of the interspike intervals within single bursts reflects the shape of the burst generating receptor potential oscillation.
We have observed similar bursts during recording from mammalian and reptilian warm receptors, but - in contrast to cold receptors - at the warmer end of the activity range. The temporal arrangement of the intraburst intervals likewise reflects the shape of the underlying receptor potential wave, which is represented rather faithfully even by bursts consisting of a different number of action potentials, indicating that the impulses itself do not contribute appreciably to the cyclic process.
Additionally, we have studied the response characteristics of electroreceptors of *Ictalurus* (Teleostei) at various skin temperatures. The sensory system consists of about 20 neuroepithelial receptor cells innervated by a single afferent axon. Interestingly, we observed a regular burst discharge at the high-temperature end of afferent activity (30 - 35°C) with characteristics corresponding to that of thermoreceptor burst discharges. The timing of these impulse patterns in electroreceptors suggests that they originate in the afferent nerve fibre and that they are related to impulse generation. Apparently, temperature-dependent periodic events are part of the encoding process in sensory afferents.

Laboratory of Comparative Physiology, University of Utrecht, Padualaan 8, NL - 3584 CH Utrecht

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TEMPERATURE DEPENDENCE OF STIMULUS TRANSDUCTION IN AMPULLARY ELECTRORECEPTORS OF TELEOSTS

R.C. Peters, F. Bretschneider, P.F.M. Teunis, H.A. Braun and K. Schäfer

The response properties of ampullary electroreceptors to sinusoidal electrical stimulation were studied in the catfisch *Ictalurus nebulosus* at local temperatures between 5 and 35°C. A unimodal relation of background activity to temperature was obtained. There were no dynamic responses to temperature changes in either the warming or cooling direction. The sensitivity to electrical sinusoidal stimulation (change of discharge rate related to current density) showed an identical temperature dependence as the background discharge. The sensitivity was dependent on the stimulus frequency as well as on temperature. Generally, there was a shift of the peak sensitivity with higher temperatures from low to high stimulus frequencies (range 1 to 20 Hz). The shape of the frequency characteristics varied with temperature: peak sensitivity and zero phase shifted with warming to higher frequencies, thus increasing the phase lead at lower and decreasing the lag at higher frequencies. Sensitivity was maximal at zero degree phase angle at all temperatures. Latency to supramaximal rectangular electrical stimuli was temperature-dependent, ranging from 16.4 ms (5°C) to 5.6 ms (35°C). Application of the specific calcium channel blocker menthol (0.2 mM) suppressed afferent activity, the effect becoming more prominent with higher adapting temperatures. Sensitivity to sinusoidal electrical stimulation was also impaired, but to a lesser degree and mainly at lower temperatures. We conclude that the bandpass filter properties of the receptor system depend on temperature; the resonant frequency is shifted to higher values by warming. We suspect this behavior to be partly determined by the temperature sensitivity of the activation parameters of the transducer channels. Our results indicate the contribution of a calcium conductance to sensory transduction in these receptors.

Laboratory of Comparative Physiology, University of Utrecht, Padualaan 8, NL - 3584 CH Utrecht

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DISTRIBUTION OF DIFFERENT CELL TYPES WITHIN SPINAL GANGLION L4 IN THE RAT

U. Hoheisel, R. Scherotzke and S. Mense

The study was designed to find out whether somata of different size or function are distributed differentially within a spinal ganglion. Two sets of data were sampled, one concerning the size distribution of the somata along a medio-lateral axis, and another one concerning the intraganglionic location of single cells connected to hair follicle receptors and muscle spindle primary endings, respectively. The impulse activity of the latter cells was recorded intracellularly with glass micropipettes filled with Lucifer Yellow. The dye was inontophoretically injected into the cells. The somata were tested for the presence of calcitonin-gene related peptide-immunoreactivity (CGRP-IR) using the indirect PAP method.

The medio-lateral size distribution showed clear differences in that the medial and lateral thirds of the ganglion contained a significantly higher proportion of large cells than the centre. The proportion of cells exhibiting CGRP-IR was smallest in the medial third.

The somata of the hair follicle receptors and muscle spindle primary endings did not show a preferred location within the ganglion. There was likewise no topographical relationship between the location of the soma within the ganglion and the location of the receptive ending in the hind-limb. Units showing CGRP-IR were missing among the cells connected to muscle spindle primary endings and were underrepresented among the hair follicle receptor cells.

Institut für Anatomie und Zellbiologie der Universität, Im Neuenheimer Feld 307, D-6900 Heidelberg.

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ON THE CORRELATION OF BODY FLUID CHANGES AND THE ATRIAL NATRIURETIC PEPTIDE (ANP) AT HIGH ALTITUDE.

A. Bub, G. Manzl and W.G. Forssmann

The influence of altitude hypoxia on changes in plasma volume (% PV), body weight (BW), fractional excretion of sodium ($FE_{Na}+$), and ANP was investigated. Eleven healthy volunteers were studied at the "Hochalpine Forschungsstation Jungfraujoch" (3450 m) under resting conditions during a period of 12 days. Control values were taken at sea level, 110 m (sl). % PV was calculated from hematocrit and hemoglobin measurements using the "Strauss"-equation.

Plasma volume was found to be unchanged at day 1 at altitude (0.59%) but decreased at day 3 (-4.58%), 4 (-5.10%), and 12 (-9.31%). ANP rose from 91±11 pg/ml (sl) to 967±322 pg/ml (day 1), 787±173 pg/ml (day 3), 354±153 pg/ml (day 4), and 574±200 pg/ml (day 12). BW increased significantly from 68.45±1.54 (sl) to 69.91±1.84 (day 3), however BW decreased again to 68.59±1.56 at day 4. $FE_{Na}+$ was unchanged until day 3 and increased at day 4 to 1.46±0.19% (vs 1.29±0.2% at sl) and day 12 to 1.79±0.21%.

Our findings demonstrate that altitude hypoxia leads to a reduction in plasma volume which is accompanied by elevated plasma ANP. With respect to the increase in BW we suppose that the loss of plasma volume within the first three days of exposure to altitude is due to a fluid shift from the intravascular to the extravascular compartment, since $FE_{Na}+$ and urinary output (data not shown) were found to be unaltered during this period. The increase in $FE_{Na}+$ at day 4 might contribute to the loss of BW. As ANP and $FE_{Na}+$ are correlated at day 4 ($r=0.552$, $p=0.077$) it is likely that ANP is involved in hypoxia induced sodium excretion and the reduced plasma volume in the following period at altitude.

Hence, we conclude that the reduction of plasma volume within the first days at altitue reflects an initial hemoconcentration which enhances oxygen uptake and delivery. The unchanged urinary excretion until day 3, despite elevated plasma ANP levels, might be explained by an increased sympathetic activity overdriving the renal ANP actions.

Institut für Anatomie und Zellbiologie III der Universität Heidelberg, INF 307, D-6900 Heidelberg

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THE METABOLIC RESPONSE TO REPEATED COLD EXPOSURES IN GOATS

G. Kuhnen and C. Jessen

The effects of repeated cold exposures on the relationship between skin and core temperatures (Tskin, Tcore) in control of metabolic rate (MR) were investigated in 119 experiments on three conscious goats. Tskin was manipulated by a rapidly circulating shower bath, while Tcore was controlled via extracorporeal heat exchangers in a chronic arterio-venous shunt. After the relationship between Tskin, Tcore and MR had been determined by a first standard test, the animals were exposed to combinations of low Tcore and high Tskin, and vice versa. These counteracting stimuli maintained MR close to resting values inspite of a definite cold load imposed on either the skin or the body core. After 10 to 15 exposures the standard test was repeated. The thresholds of Tcore, at which MR began to increase, were found to be significantly lowered. The second standard test was followed by 10 to 15 experiments with exposures to severe cold stress, in which Tskin and Tcore were simultaneously lowered to induce major and long lasting increases of MR. Two exposures were performed per day. No systematic differences between the responses to the first and the second exposure were observed. A subsequent third standard test revealed no significant change in threshold temperatures relative to tests I and II. The slope of MR over Tcore, however, decreased. It is concluded that repeated cold exposures without manifest shivering can induce tolerance adaptation to cold.

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Physiolog. Inst., Aulweg 129, 6300 Giessen (FRG)

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CROSSFOSTERING PHASE-SHIFTS THE JUVENILE CIRCADIAN CORE TEMPERATURE RHYTHM IN RATS.

B. Nuesslein and I. Schmidt

During the first week of life isolated rat pups develop an endogenous circadian core temperature (Tc) rhythm (Mumm et al., J. Comp. Physiol. B 159 (5), 1989). To find out whether or not the phase of this rhythm is determined by postnatal cues, pups were crossfostered 12 to 24 h after birth. Foster mothers were maintained in a light cycle 12 h out of phase with that of the natural mothers (LD-colony: lights on from 07:00 to 19:00; DL-colony: lights on from 19:00 to 07:00). Control pups were raised by their original mothers. On day 4 pups from both light-cycles were isolated and artificially reared in dim light (LL) until day 12. They received a continuous infusion of synthetic rat milk via a chronically inserted esophageal tube. Core temperature was continuously recorded from thermocouples inserted 1.8 to 2.4 cm beyond the anal sphincter. After the 5 th day of life all pups showed a clear Tc rhythm with a period length close to 24 h. The marked Tc minima characteristic of the juvenile Tc rhythm occured in control pups 2 to 3 h after lights went on for their mothers (LD-pups: 08:31 ± 0.24 h, N = 32 minima; DL-pups: 21:59 ± 0.35 h, N = 52 minima). Crossfostered pups reached their minima about 6 h later than the control pups (pups born to LD-mothers: 13:55 ± 0.33 h, N = 45; pups born to DL-mothers: 03:21 ± 0.45 h, N = 26). In pups raised by foster mothers until day 10 of age the phase of the Tc rhythm was equal to that of control pups born and reared in the colony of the foster mother. We conclude that the phase of the Tc rhythm is set prenatally and/or within 24 h after birth, but can gradually be phase-delayed by postnatal influences to match the phase of pups from a 180° phase-shifted colony after 9 days of crossfostering.

W. G. Kerckhoff-Institut, Max-Planck-Institut, Parkstrasse 1, D-6350 Bad Nauheim, FRG.

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EFFECTS OF OFF-CENTER ROTATION OF THE HEAD ON PERCEPTION OF THE SUBJECTIVE VERTICAL

J. Wetzig, M. Reiser, N. Bregenzer and R. J. von Baumgarten

Ten healthy subjects were eccentrically rotated with constant speed on a Bárány chair. Setting of a luminous line (LL) to the subjective vertical was evaluated. During eccentric position rotation subjects consistently reported illusory rotation and set the luminous line to an angle correlating to centrifugal force. At the same time an OCR of opposite direction was measured.
In one patient, labyrinthectomised on the right side, only counterclockwise rotation of the luminous line was observed.
Differences between "inner" and "outer" eye were evident for luminous line settings in some subjects.
The results indicate that eccentric rotation is a promising method to test for bilateral otolith asymmetries in normal and pathological subjects.

Physiologisches Institut der Johannes-Gutenberg-Universität, Saarstrasse 21, D 6500 Mainz

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CORE-TEMPERATURE RHYTHMS IN THE SHEEP AND THEIR POSSIBLE RELATIONSHIPS TO ORGANIC FUNCTIONS

E. Mohr and H. Krzywanek

Regarding the time course of core temperature during daytime, it is remarkable that the basic circadian rhythm is superimposed by oscillations of much higher frequencies (ultradian rhythms). Furthermore, the variations of these ultradian rhythms let us presume that the intensity of these rhythms is changing within daytime. Because internal as well as external factors could modify these temperature variations , different origins for the various rhythms seem to be possible. To examine potential relationships between several rhythms and organic functions, thermoprobes were implanted at various sites in the abdomen of sheep; sheep as animals large enough to expect a temperature gradient between the different points of measurement. To minimise any influences due to measurement or experimental conditions, a telemetric system was used for registration and all animals were kept without restraint. With regard to the various intensities of the rhythms, long-time temperature records were analysed by Fast-Fourier-Transformation. Shifting the beginning of the analysis, the changes which occur during daytime could be taken into consideration.

Independent from the point of measurement and the time of day, ultradian rhythms with wave-lengths of about 140- and about 90-min were found. For these cycles, an oscillator located in the central nervous system can be supposed. Because other rhythms were not stable in time and occurred at the different points of measurement with unequal intensities, they seemed to be triggered by local processes. Especially for rhythms with wave-lengths in the range between 12- and 2.5-hours, a comparison with the used feeding-schedule points to intestinal activity as a possible origin for their variability . (Supported by DFG Mo 450/1-1)

Institut für Veterinär-Physiologie der Freien Universität Berlin
Koserstr.20, D-1000 Berlin 33

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DIURNAL THERMAL AND ELECTROMYOGRAPHIC RESPONSES IN HUMMINGBIRDS (AMAZILIA TZACATL, COLIBRI CORUSCANS) TO CHANGING AMBIENT TEMPERATURES

G. Warncke, K.-L. Schuchmann+, W. Linow and F.W. Klußmann

In two neotropical hummingbird species (Amazilia tzacatl, average body mass: 3,9 g, Colibri coruscans: 7,9 g) core temperature (Tco), electromyographical activities (EMG) and respiratory frequency were simultaneously and continously recorded under laboratory conditions (LD 12:12, ambient temperatures (Ta): day time, 22-24°C; night time, 6-8°C; food ad lib.) over 7 to 14 consecutive days. Both trochilids revealed a taxon and/or body mass related regulatory pattern in all parameters studied. During the day Amazilia tzacatl exhibited average electromyographical activities of 590 µV (max. amplitude). In the larger Colibri coruscans, these values reached 470 µV during light regimes. Amazilia tzacatl did not enter torpor at night, a numbness-like state, indicated by a relatively high core temperature of about 36,2°C and by continous muscle activity (bursts) reaching up to 280 µV. Whereas, the heavier Colibri coruscans, underwent torpor at night. Prior to this energy saving state, core body temperature dropped in this species from 42,1 to 36,3°C (shallow sleep), and decreased steadily to 8,2°C (time span required to drop from maximum to minimum core temperature: 2.5 h). This lowest temperature value was regulated by means of a sporadically appearing but then low and continous EMG of 40 µV. During the noctural stages from shallow sleep to torpor Colibri coruscans lowered the respiration frequecy from 120·min^{-1} to 38·min^{-1}. Arousal from torpor to shallow sleep phase was accompanied by strong shivering (4200 µV, time span 1.5 h). Finally awaking from sleep was induced by light, after which daytime EMG-activity was resumed by bird (470 µV).

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Institut für Neurophysiologie der Universität zu Köln, Robert-Koch Str. 39, D-5000 Köln 41;
+Zoologisches Forschungsinstitut Museum Alexander Koenig, Konrad-Adenauerallee 150-164, D-5800 Bonn 1.

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ACTIVATION OF THYROXINE 5'-DEIODINASE IN BROWN ADIPOSE TISSUE OF DJUNGARIAN HAMSTERS DURING COLD ACCLIMATION

U. Redlin and G. Heldmaier

Apart from the presence of a high K_M thyroxine 5'-deiodinase (5'-D) in liver and kidney (type I) a quite different low K_M 5'-D (type II) can be found in CNS, placenta, and brown adipose tissue (BAT). The intracellular deiodination of thyroxine to triiodothyronine in BAT is necessary for the optimal thermogenic function of this tissue. As the 5'-D in BAT can be cold-activated we studied the changes of the 5'-D activity during a 28 d lasting cold exposure (5 °C, 16 h light, 8 h dark). 5'-D activity was measured in vitro by quantifying the release of radioactive iodide from L-[3',5'-^{125}I]-thyroxine.

Hamsters living in 23 °C exhibited a 5'-D activity of 17.8±1.5 fmol I^-/mg protein. The cold exposure resulted in a sharp increase in 5'-D activity after a lag phase of approx. 2 h. After 24 h the maximum value was reached with a six-fold increase above the control value (109.9±12.1 fmol I^-/mg protein). Further cold exposure led to a decrease of the elevated 5'-D level. At 7 d 5'-D activity was 38.7±6.1 fmol I^-/mg protein and remained on this level until 28 d (47.9±7.8 fmol I^-/mg protein).

The rapid activation and the overshoot in activity of 5'-D indicate that this enzyme is significant for the initial phase of the cold response. The decrease of the 5'-D activity after 24 h of cold exposure can be interpreted as a functional cold response of 5'-D i.e. the process of cold acclimation of BAT can continue with only a moderately elevated 5'-D activity.

Philipps University, Department of Zoology, D-3550 Marburg, and W.G. Kerckhoff Institute, Max-Planck-Institute, D-6350 Bad Nauheim, FRG

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CARDIOVASCULAR AND METABOLIC RESPONSES TO ADRENOCEPTOR AND CALCIUM CHANNEL BLOCKADE DURING SUBMAXIMAL AND MAXIMAL EXERCISE IN YOUNG HEALTHY MEN

G. Koch and A. Bamler

Previous studies in middle-aged men have shown that certain agents interfering with autonomic cardiovascular control increase muscle fatigue and reduce exercise performance. The present study aims at assessing whether young trained individuals show the same pattern of response and analyze some mechanisms underlying this response. Eight volunteers aged 22 - 26 ys were studied at rest and during submaximal steady state and maximal bicycle ergometer exercise before and after equivalent doses of metoprolol (M, β_1-antagonist, A), pindolol (P, unselective β-blocker with intrinsic sympathetic activity), labetalol (L, α/β-blocker), and nifedipine (N, Ca-antagonist). Heart rates, blood pressures, ventilation, oxygen uptake, plasma catecholamines, plasma glucose and blood lactate were simultaneously measured.

Maximal heart rates and systolic pressures under control conditions were 185 ± 19 b.p.m. and 230 ± 9 mmHg respectively. Under all conditions, heart rates were reduced by M, P and L; they were unaffected by N. Blood pressures were significantly reduced by all agents; the hypotensive effect of N was, however, less pronounced than that of M, P and L. Ventilation during heavy exercise was reduced by the adrenoceptor blockers M, P and L ($p < 0.05$); maximal oxygen uptake under control conditions was 3.26 ± 0.61 l; it tended to be lower with M and P. Exercise plasma glucose and blood lactate were significantly lowered by the non-selective β-blocker P only, suggesting reduced (β_2-receptor mediated) glycogen breakdown. Plasma adrenaline was raised ($p < 0.05$) by P and N under heavy exercise, noradrenaline only by N. Total work performed was not affected by any agent.

It is concluded that young trained men maintain unaltered aerobic and endurance capacity despite substantial cardiovascular and metabolic changes induced by adrenergic blockade, in particular by non-selective β-receptor antagonists. This clearly contrasts with the response of middle-aged men to the same interventions.

Physiologisches Institut, Freie Universität Berlin, Arnimallee 22, 1000 Berlin 33

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EXERCISE MYOPATHY: MAGNETIC-RESONANCE-IMAGING (MRI)

A. Koller, S. Felber, C. Haid, K. Wicke and E. Raas

Previous work indicates that the type of exercise performed leads to transient changes in muscle MR signal intensity immediately after exercise (Fleckenstein et al., AJR, 151: 231-237, 1988).

In the present study, imaging of thigh muscles was performed after a single bout of eccentric exercise using one leg. The exercise bout consisted of seven sets of 10 eccentric contractions of the quadriceps muscle group.

We find that eccentric exercise leads to delayed increases in skeletal muscle MR signal intensity, which were most apparent in the m. vastus intermedius and deep part of m. vastus lateralis.

These signal intensity changes are likely related, at least in part, to increases in extra- and/or intracellular water content and allow improved definition of specific muscles and may prove useful in the evaluation of exercise and clinical situations in which improved definition of muscle boundaries is required. Our results question the general applicability of the needl biopsy techique and electromyography through surface electrodes (both of these techniques are generally restricted to superficial muscles) to study the pathogenesis of the delayed type of exercise injuries in human skeletal muscle.

Institut für Sport- und Kreislaufmedizin, Univ.-Kliniken Innsbruck, Anichstraße 35, A-6020 Innsbruck

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POST-OPERATIVE VARIATIONS OF ULTRADIAN CORE-TEMPERATURE RHYTHMS IN THE SHEEP

H. Krzywanek and E. Mohr

It is well known that surgery is followed by a disruption of circadian rhythms which lasts for about 4 to 5 days. This study examines possible modifications of the ultradian, short-time fluctuations after surgical treatment. Therefore, core temperatures were measured - by telemetry - in 5 sheep at various locations in the abdomen (sheep kept without restraint). Immediately after implantation of the thermoprobes as well as after sham-operation, time courses of core temperatures were registered every 30 seconds throughout a minimum of 3 weeks each. Data were analysed by Fast-Fourier-Transformation.

Independent from the point of measurement, in the first 6 days following surgery, the highest intensities were expressed by ultradian rhythms with wave lengths between 2- and 4-hours. It took a minimum of 14 days until power-spectra returned to the typical splitting in different maxima: a) wave-length of about 12-hours, b) wave-lengths between 2- and 4-hours, c) wave-lengths in the range between 80- and 100-minutes. Earlier investigations in sheep pointed to intestinal activity as a trigger for wave-lengths larger than 2 hours. Therefore the changes in this zone may caused by varying appetite. Beyond that, our experiments show, that the 80- to 100-minute-rhythms are altered by surgical treatment, and that this influence can last for about 14 days. Because other physiological functions such as blood-pressure or heart-rate also show ultradian rhythms with wave-length of about 90-minutes, a common oscillator for these rhythms could be supposed. In this case, surgical treatment has also an influence on other physiological parameters, lasting much longer than it can be admitted according to circadian disruption.

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Institut für Veterinär-Physiologie der Freien Universität Berlin Koserstr.20, D-1000 Berlin 33

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An increasing oxidative phosphorylation coupling in rat liver and heart during the early phase of burn injury

Wang Xuemin, Chen Keming, Shi Ying and Shi Hanping

We have recently found an initial increase in oxidative phosphorylative coupling in succinate respiratory chain in rat-liver mitochondria during the early phase of burn injury (1).

Using glutamate + malate and α-ketoglutarate as substrates, the respiratory control ratio of liver mitochondria from rats with full skin thickness burns covering 20 per cent of body surface area were increased at 45, 60, 75 and 90 min after burn, the peak being at 75 min post-burn. The ADP/O ratio and the rate of ATP formation were also increased at about 75 min after burn and the ATP content in liver was increased at 120 min following burn. The ATP and creatine phosphate content in heart was increased at 105, 120 and 135 min post-burn respectively. A sham group acted as control. All the results suggested an increased oxidative phosphorylation coupling in some organs during the early phase of burn injury.

(1) Wang X. M., Chen K. M. et al. (1986) Functional changes in rat-liver mitochondria during the early phase of burn injury. Burns 12, 461.

Dept. Biochem. 2nd Mil. Med. College, Shanghai

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ISOFLUORANE NITROUS-OXIDE OXYGEN INHALATION ANESTHESIA IN EXTREMELY SMALL BIRDS (HUMMINGBIRDS) WITH HIGH METABOLIC RATE.*

Günther Warncke

The use of inhalation anesthesia in small birds, particularly very small species, is considered to be difficult. Experiments were carried out on two hummingbirds (Trochilidae) species (Amazilia tzacatl, n=5, average body mass: 3.9 g; Colibri coruscans, n=5, body mass: 7.9 g) in order to determine whether anesthetics and gas mixtures, which had already been tested on larger birds and mammals, could be used on extremely small birds. Simple and reliable control of the depth of anesthesia, including electromyogram (EMG), was also tested. How large is the anesthetic safety and what influence do the various body positions have on the physiological parameters of the bird? The investigations have shown: 1) The systems of inhalation anesthesia applied up till now on larger birds, can also be used on extremely small birds. 2) The classical stages of anesthetic depth has only a limited use. 3) A reliable control of the depth of anesthesia in hummingbirds is possible through the monitoring of heart rate, respiratory frequency, EMG, and the control of the reflexes of eyelid, cornea, pupil and toes. Owing to the smallness of the eyes the pupilar reflex cannot be observed. 4) During anesthesia up to 2 hours with surgical intervention the inspiratory concentration of Isofluorane ranged from 0.7-1.9% for small hummingbirds and 0.5-1.5% for larger ones. In prelimenary experiments without operation the birds tolerate slightly higher concentrations of Isofluorane: 0.9-2.1% for small birds and 0.7-1.7% for larger ones. 5) Unusual body-positions during anesthesia would cause a fatal slowing of spontaneous respiration. 6) Isofluorane has only slight effect on respiratory depression but should only be used in combination with nitrous-oxide/oxygen in the ratio 2:1.

*In co-operation with K.-L. Schuchmann, D-5300 Bonn 1

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Institut für Neurophysiologie der Universität zu Köln, Robert-Koch Str. 39, D-5000 Köln 41

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The Effect of Fibrinolytic Agents in Vitro on Plaque Cells Derived from Human Peripheral and Coronary Arteries

R. Voisard, P.C. Dartsch, E. Betz

Primary stenosing plaque tissue from 19 patients (age: 64 ± 12 years) and restenosing plaque tissue from 5 patients (age: 64 ± 9 years) was extracted either by a percutaneous Simpson atherectomy device from femoral arteries (extraction was done by Prof. Höfling and Dr. Bauriedel, Klinikum Großhadern, Munich) or by thrombendarterectomy in case of coronary arteries (Prof. Unger, Dr. Hutter, Landeskrankenanstalten Salzburg, Österreich). The obtained plaque tissue was encymatically disaggregated by a collagenase/elastase enzyme mixture. Isolated cells could be identified as smooth muscle cells (SMC) by positive reaction with antibodies against smooth muscle alpha-actin. Plaque derived cells were routinely cultivated in a mixture of Waymouth's MB 752/1 and Ham F-12, supplemented with 15% fetal calf serum. Growth rates were determined by a cell analyzer system (CASY 1; Schärfe Systems, Reutlingen).

Growth Rates in Population Doublings/Day (PD/Day): The growth rates of SMC from restenosing plaque tissue (0.64 ± 0.15 PD/day) were highly increased in comparison to SMC from primary stenosing tissue (0.16 ± 0.04 PD/day, $p<0.001$).

The Effect of Fibrinolytic Agents on SMC from Primary Stenosing Tissue: Streptokinase (Kabi), urokinase (medac) and human plasminogen-activator (Thomae) were added 24 hours after cell seeding to SMC cultures of primary stenosing tissue at the following concentrations: 1, 10, 100, and 1000 IE/ml. At each medium exchange the agents were added at the appropriate concentration. Control dishes were incubated without drugs. At clinically relevant concentrations (approximately 20 IE/ml for streptokinase and urokinase and approximately 100 IE/ml for tissue plasminogen-activator), streptokinase and urokinase had no significant effect on proliferative activity, whereas tissue plasminogen-activator caused 20% inhibition. At higher concentrations all three drugs reduced the cell proliferation rates significantly.

Conclusions: The highly increased growth rates of SMC from restenosing lesions might be the in vitro-equivalent to the rapid progression of restenosing events after angioplasty. Therefore, the influence of drugs on SMC proliferation is of clinical interest. As demonstrated by our results, all three drugs routinely used for a fibrinolytic therapy do not stimulate plaque SMC proliferation and, thus, may not be an unspecific stimulus for restenoses.

Physiologisches Institut I der Universität Tübingen, Gmelinstr. 5, D-7400 Tübingen 1, FRG.

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MICROENVIRONMENT, CELL PROLIFERATION AND NECROSIS IN TUMOR SPHEROIDS

U. Karbach, S. Walenta, M. W. Gross, W. Mueller-Klieser

Multicellular tumor spheroids, cultured **in vitro** in stirred media, are cellular aggregates that mimic the **in vivo** situation in malignant tumors with regard to nutrition, oxygen supply, proliferation, and differentiation (Mueller-Klieser: J. Cancer Res. Clin. Oncol. 113: 101, 1987). Histological sections show a central necrotic area surrounded by a viable cell rim of 150 to 250 µm thickness depending on the cell line and the growth condition. The thymidine labeling index in EMT6 spheroids with diameters < 460 µm, i.e., before the onset of necrosis, decreases from 47.4 % in superficial cell layers to 16.3 % in inner regions at a radial distance from the surface of around 100 µm. PO_2 profiles obtained in EMT6 spheroids by microelectrode measurements show values decreasing from the surface to the center and reaching average central values of 0 mmHg only in spheroids > 800 µm in diameter. At the onset of necrosis central PO_2 values are often > 50 mmHg. Furthermore, the oxygenation status of these spheroids can be characterized by ^{3}H-misonidazole labeling of cell regions with PO_2 values < 10 mmHg. The local distribution of glucose, lactate, and ATP is obtained using a bioluminescence method (Mueller-Klieser et al.: J. Natl. Cancer Inst. 80: 609, 1988). Central ATP values during spheroid growth decrease from 1.1 mM to 0.1 mM, yet the emergence of necrosis precedes this drop in ATP to very low levels. The data summarized are indicative of pronounced changes in the microenvironment of tumor cells during spheroid growth. However, the specific metabolic milieu is apparently not directly related to proliferation arrest and massive cell death in the inner regions of the aggregates. For further studies on these phenomena, serumfree culture techniques for spheroids and new spheroid models with differentiated tumor cell clones have been established.

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Inst. of Physiology and Pathophysiology, Univ. Mainz, FRG

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^{31}P NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF MURINE TUMORS

P. Vaupel[+], P. Okunieff[*]

^{31}P nuclear magnetic resonance spectroscopy (^{31}P-NMR), a noninvasive and nondisturbing technique which allows repetitive measurements in nonanesthetized animals has been applied to subcutaneous murine tumors. In a first attempt, tumor size dependent changes in the bioenergetic status and in the apparent intracellular pH of murine fibrosarcomas (FSaII) and mammary adenocarcinomas (MCaIV) have been investigated. In a second series of experiments, tumor energy status and pH_{NMR} have been evaluated upon therapeutic measures (e.g., irradiation, hyperthermia) and during modulation of the metabolic status (hyperoxia, hyperglycemia). Finally, ^{31}P-NMR spectroscopy-derived parameters have been verified in murine tumors growing in an irradiated tissue and, thus mimicking tumor regrowth after unsuccessfull radiation therapy. From these experiments there is clear evidence that tumor bioenergetics reflect the efficiency of microcirculation in these malignancies. Furthermore, there is a close correlation between tissue oxygenation and energy status suggesting that the microcirculation in these tumors yields an O_2-limited energy metabolism.

[+]Institute of Physiology & Pathophysiology, University of Mainz, D-6500 Mainz, W.-Germany
[*]Dept. Radiation Medicine, Massachussetts General Hospital Cancer Center, Harvard Medical School, Boston, MA 02114, USA

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MODIFICATION OF MICROCIRCULATORY FUNCTION AND ENERGY STATUS OF SUBCUTANEOUS TUMORS BY HEAT AND/OR GLUCOSE

W.-K. Mayer[#], M. Stohrer[*], S. Walenta[*], C. Schaefer[*], W. Krüger[#], W. Müller-Klieser[*], and P. Vaupel[*]

The effects of hyperglycemia (12.5 mg/g BW/2.5 h i.v., 40% glucose solution) and/or local water bath hyperthermia (tumor temperatures: 42.7-44.0°C for 2 h) on microcirculatory function and energy status of s.c. rat tumors (0.4 ± 0.20 ml) were studied using laser Doppler flowmetry, HPLC techniques, standard enzymatic assays for tissue extracts, and single photon imaging with quantitative bioluminescence. Control animals were given respective volumes of a 0.9% NaCl solution (tumor temperatures: 35.5-36.2°C).

Laser Doppler flow significantly decreased in all treated tumors to about 20% of initial values. During **hyperglycemia** (blood glucose level: ≈35 mM), estimated glucose availability and tumor tissue glucose levels were elevated. Tissue ATP concentrations were unaffected. During **hyperthermia**, glucose availability did not change during the first hour, then finally declined below starting level. Despite relatively constant glucose levels, tumor ATP significantly dropped upon heating, though some regions with "normal" ATP levels could still be detected. Following **hyperthermia/hyperglycemia**, glucose availability and tissue glucose levels were elevated. Tissue ATP concentrations were significantly lower than in untreated tumors. From the results it is concluded that hyperthermia alone or in combination with hyperglycemia leads to a significant ATP drop which cannot be compensated by an increased glucose supply.

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[*]Institute of Physiology & Pathophysiology, University of Mainz, D-6500 Mainz, FRG
[#]Research Institute M. von Ardenne, DDR-8051 Dresden, GDR

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IS RESISTANCE TO ISCHEMIA OF MOTOR AXONS IN DIABETIC SUBJECTS DUE TO MEMBRANE DEPOLARIZATION?

M. Strupp[1], P. Weigl[1], H. Bostock[2], K. Piwernetz[3], R. Renner[3] and P. Grafe[1]

Peripheral nerves of patients with diabetes show an abnormal resistance to ischemia. The mechanisms of this alternation are not yet clear. We have now explored whether axonal depolarization is involved, as has been proposed by several authors. For this purpose axonal superexcitability was used as an indirect measure of membrane potential. This phenomenon is potential-dependent, i.e. depolarization abolishes superexcitability (Bostock H and Grafe P, J Physiol 1985; 365: 239-257). The peroneal nerve was stimulated at the popliteal fossa; compound muscle action potentials (CAP) were recorded from m. ext. dig. brev. Superexcitability was measured as an amplitude ratio: the change in the amplitude of a test potential (30 % of max. CAP) without and with a supramaximal conditioning stimulus (distance 10 - 30 ms) was observed. The susceptibility to ischemia was determined by threshold tracking (Weigl P et al., EEG and Clin Neurophysiol 1989; 73: 369-371): the strength of stimulation (0.2 ms current pulse once/s) over the peroneal nerve was adjusted by feedback to maintain a constant CAP (30 % of maximum), and recorded.

Our data reveal a striking resistance to ischemia (10 min period) of the peroneal nerve in diabetic subjects (n = 11) compared with a control group (n = 14): 5 min after the onset of ischemia threshold current fell to 59.2 ± 7.1 % (mean ± S.D.) of pre-ischemic values in the control group and to 82.4 ± 7.9 % in diabetics ($p < 0.001$). Additionally, the post-ischemic increase of threshold current was in diabetics (117.6 ± 14.9 % of pre-ischemic value) significantly smaller ($p < 0.001$) than in the control group (200.6 ± 23.8 %). However, no difference in the post-spike superexcitability was found between controls and diabetics. The post-spike superexcitability (20 ms distance) was 133.5 ± 25.4 % in diabetics and 138.7 ± 26.8 % in controls (mean ± S.D.). This observation indicates that membrane depolarization is not involved in the resistance to ischemia of motor axons in diabetic subjects.

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[1] Physiologisches Institut der Universität München, Pettenkoferstr. 12, D-8000 München 2, F.R.G.
[2] Institute of Neurology; University of London; U.K.
[3] III. Medizinische Klinik, Klinikum Bogenhausen, München, F.R.G.

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FIRST AND SECOND PAIN IN POLYNEUROPATHY

J.Lankers, A.Frieling, W.Zangemeister*, K.Kunze*, B.Bromm

Cutaneous CO_2 laser stimuli (e.g. 20 ms duration, 5 mm diameter, 20 Watt) activate Aδ- and C-fibres. Aδ-fibre mediated first pain is reflected by late evoked potentials with latencies between 250 and 430 ms (EP, vertex vs. linked earlobes). These potentials were studied in 5 patients with polyneuropathy of various etiology with disturbances of mechanosensibility and impairment of pain and temperature sense. In 4 cases the late EP was completely missing, if the laser pulses were applied to the most affected body site. In one case the peak latency was increased from 430 ms (mean value) to 585 ms. At the less affected site in one patient the EP was missing and in 4 patients it was delayed. C-fibre mediated second pain is reflected by ultralate EP with latencies of more than 1000 ms. Since cerebral activity is focussed upon the first arriving information mediated by Aδ-fibres, in healthy men ultralate EP can be observed only with experimental A-fibre block. However, if illness has affected myelinated fibres, ultralate EP appear (in 4 of 5 patients). But, prolonged latency of late EP may not only be discussed in terms of selective disturbance of myelinated fibres, but also in terms of plasticity of spinal cord function. In conclusion, measures of EP in response to cutaneous heat pulses proved to be a sensitive tool to assess disturbances of pain and temperature sensitivity.

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Physiologisches Institut der Universität und *Neurologische Universitätsklinik, Martinistraße 52, 2000 Hamburg 20

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INFLUENCE OF LDL AND ITS OXIDATIVELY FORMED DERIVATIVES ON ARTERIAL CONTRACTION - MODULATION BY ENDOTHELIUM

S. Tries, H. Heinle

Products formed by lipid peroxidation, expecially by the oxidation of low density lipoproteins can be regarded as important pathophysiological components impairing cellular function and integrity. In order to obtain further information on the effects of LDL and its oxidized derivatives on vascular smooth muscle, we applied LDL, oxidized LDL, arachidonic acid hydroperoxide (AAOOH; 5-20 μM), hydrogen peroxide (≤ 1 mM), 4hydroxynonenal (HNE, ≤ 20 μM) and malondialdehyde (MDA; 1-20 μM), to segments of rabbit carotid artery together with KCl-enriched solution (48 mM). To investigate the influence of hypercholesterolemia on smooth muscle contractility we used also carotid artery segments of rabbits fed with a 0.5% cholesterol diet for 4-5 weeks. Furthermore we examined the importance of endothelium as modulator of contraction response under oxidative stress; i.e. in one series of experiments endothelium was mechanically removed from the segments prior to stimulation.

The results show, that oxidatively modified LDL, hydrogen peroxide, AAOOH, HNE and MDA, but not native LDL cause a concentration-dependent increase in contraction response of carotid artery segments of normal fed rabbits. Endothelial cell damage of these segments does not show a significant influence on the contraction responses.

Vessel segments of hypercholesterolemic rabbits show clearly lower contraction responses than those of normal fed animals when stimulated by AAOOH, HNE and MDA, whereas these segments show a striking increase in contraction force upon stimulation with both forms of LDL. Deendothelialization in segments of cholesterol-fed rabbits leads to a significant increase in the induced contraction responses.

This means that under the condition of high cholesterol levels intact endothelium seems to be important in protecting the arterial wall from the contraction enhancing effects of LDL and its oxidatively formed derivatives.

Physiologisches Institut (I) der Universität Tübingen, Gmelinstraße 5, D-7400 Tübingen

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THE ROLE OF GLIBENCLAMIDE-SENSITIVE POTASSIUM CHANNELS IN REGULATING HYPOXIC AND POST-ISCHEMIC VASODILATION IN ISOLATED GUINEA-PIG HEART

N. v. Beckerath, W. Maier-Rudolph, G. Mehrke, K. Günther and L. Goedel-Meinen and J. Daut

The coronary arteries of isolated guinea-pig hearts were perfused at a constant rate (5-10 ml/min) with bicarbonate-buffered physiological salt solution. Coronary perfusion pressure (CPP) and isovolumetric left ventricular pressure (LVP) were measured. When the P_{O2} of the perfusate was changed from > 500 to < 10 mm Hg a decrease of CPP by about 50% was observed within 30 s, indicating a change in coronary resistance. After a 30 s period of no-flow ischemia a transient change in coronary resistance of similar magnitude was found. Both hypoxic and post-ischemic vasodilation were completely abolished in the presence of 1 μM glibenclamide (n=20 hearts), a blocker of ATP-sensitive K^+ channels. Application of 100-500 mM cromakalim (BRL 34915), an opener of ATP-sensitive K^+ channels, produced the same reduction of CPP as hypoxia (n=30). The effect of cromakalim could also be blocked by glibenclamide. Substances known to lower intracellular ATP (200 μM cyanide or 50 μM 2,4- dinitrophenol) also produced a glibenclamide-sensitive vasodilation (n=7). On the other hand, the change of coronary resistance produced by the endothelium-dependent vasodilator bradykinin was not affected by glibenclamide. Our results suggest that early hypoxic vasodilation in guinea-pig heart is mediated by the opening of ATP-sensitive potassium channels in vascular smooth muscle cells. This leads (1) to a hyperpolarization, (2) to a reduction of Ca influx through potential-sensitive Ca channels, (3) to a reduction of free intracellular Ca, and thus (4) to a relaxation of coronary resistance vessels.

PHYSIOLOGISCHES INSTITUT DER TECHN. UNIVERSITÄT MÜNCHEN, BIEDERSTEINER STR. 29, D-8000 MÜNCHEN 40

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A PATHOPHYSIOLOGICAL APPROACH TO AN INDIVIDUALIZED TUMOR THERAPY BY DETECTION OF TUMOR HETEROGENEITY

W. Mueller-Klieser and S. Walenta

The existence of various cell populations with different biological properties within tumors is associated with a large variability in the response of cancers to treatment. The detection of pathophysiological parameters that may characterize such heterogeneities in each individual malignancy may contribute to an "individualization" of strategies in cancer therapy. Therefore, a method using quantitative bioluminescence and single photon imaging has been developed in our laboratory allowing for the assessment of local ATP-, glucose-, and lactate-concentrations in absolute terms with high resolution. Unlike previous procedures, the new technique includes a glass slide with a flat casting-mold that has been filled with an enzyme cocktail being kept at -20 °C. The cocktail contains enzymes to link the substrate of interest to the bioluminescent reaction of luciferase. For measurement, a cover glass with a frozen tissue cryostat section adhered to its upper side is placed upside down upon the glass with the mold, and the enzyme reactions are started by raising the temperature of the sandwich above the melting point. The concomitant light emission is registered with a microscope and an imaging photon counting system (Hamamatsu Europe, Herrsching, FRG).
The application of the technique in multicellular spheroids demonstrated a spatial resolution at the single cell level ($\leq$ 20 µm). Multiple determinations and validation with HPLC resulted in a coefficient of variation for ATP measurements with bioluminescence of 5%.

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Institute of Physiology & Pathophysiology, University of Mainz, D-6500 Mainz, FRG.

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EFFECTS OF ADENOSINE AND ATP ON THE MEMBRANE POTENTIAL OF CULTURED CORONARY ENDOTHELIAL CELLS ISOLATED FROM GUINEA-PIG HEART

G. Mehrke and J. Daut

The release of adenosine from hypoxic cardiomyocytes is assumed to play an important role in mediating hypoxic and post-ischemic vasodilation in the heart. Adenosine released into the perivascular space may act on coronary smooth muscle cells and on coronary endothelial cells. However, the relative importance of these two effects is not yet clear. We have measured the effects of adenosine and ATP on the membrane potential of confluent monolayers of cultured coronary endothelial cells. At 37 °C the monolayers had a membrane potential of -20 to -40 mV. Adenosine (0.2-2 µM) produced a transient hyperpolarization of up to 20 mV which, after 1-2 min, was followed by a depolarization of up to 12 mV. The transient hyperpolarization, but not the depolarization, could be inhibited by 10 µM theophylline, an antagonist of the P1 subtype of purinoceptors. Application of ATP (0.2-2 µM), which primarily acts on P2 purinoceptors, produced an even stronger transient hyperpolarization than adenosine, but no subsequent depolarization. The effects of ATP were not reduced by 10 µM theophylline.
The transient hyperpolarization is probably due to the phosphoinositide-mediated relase of intracellular Ca and the subsequent opening of Ca-activated K channels. Since the release of prostacyclin and nitric oxide from the endothelium appears to be always associated with a rise in intracellular Ca and a transient hyperpolarization, our results suggest that both adenosine and ATP can elicit release of vasoactive substances from coronary endothelium.

PHYSIOLOGISCHES INSTITUT DER TECHN. UNIVERSITÄT MÜNCHEN, BIEDERSTEINER STR. 29, D-8000 MÜNCHEN 40

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MITOCHONDRIAL ATP-SYNTHETASE ACTIVITY IN ANOXIC CARDIOMYOCYTES

H.M. Piper, A. Koop, T. Noll

It has been suggested that in the oxygen-deficient myocardial cell the (oligomycin inhibitable) mitochondrial ATP-synthetase turns into an ATP-hydrolase and contributes to ATP depletion. To test this hypothesis, isolated cardiomyocytes from adult rats were incubated in substrate-free Tyrode's solution (37°C,pH 7.4) at defined lowered pO_2 (0.1 torr) using the "oxystat" (Biochem J 236: 765, 1985). The oxygen consumption was halfmaximal at 0.5 torr, at 0.1 torr metabolism was anaerobic. When oligomycin (18 µM) was added at 0.1 torr, ATP depletion of the cardiomyocytes was accelerated by 10 % and did not slow down, as expected for a prevailing mitochondrial ATP-hydrolase activity.

Conclusion: In anoxic cardiomyocytes, (oligomycin-sensitive) mitochondrial ATPase activity does not contribute to energy exhaustion. Instead, mitochondrial ATP-Synthetase is still the source for a small amount of ATP. This may be because electron flux through complex I with a coupling to ATP-synthetase is maintained, enabled by an inverted succinate dehydrogenase reaction.
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Physiologie I, Universität Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf

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TUMOR ENERGY STATUS AND GLYCOLYTIC ACTIVITY EVALUATED BY ^{31}P - NMR SPECTROSCOPY, HPLC, ENZYMATIC ASSAYS, AND SINGLE PHOTON IMAGING WITH QUANTITATIVE BIOLUMINESCENCE

C. Schaefer*, P. Okunieff+, M. Stohrer*, W. Mueller-Klieser*, and P. Vaupel*

Energy status and glycolytic activity of s.c. murine fibrosarcomas (FSaII) were evaluated as a function of tumor size (range: 50 - 550 mm^3). Bioenergetic status was evaluated in vivo by ^{31}P nuclear magnetic resonance spectroscopy (^{31}P-NMR). In addition, mean tissue concentrations of adenine nucleotides (ATP, ADP, AMP) were determined by HPLC techniques. Regional ATP distribution on a microscopic level was studied by single photon imaging and quantitative bioluminescence. Glucose and lactate concentrations were assessed using standard enzymatic assays for tissue extracts.
Results: Tumor **ATP levels** evaluated by HPLC decreased marginally at tumor sizes > 350 mm^3 (1.0 to 0.8 mM). The sum of ATP, ADP, and AMP did not vary substantially with tumor size. Concomitantly, adenylate **energy charge** significantly declined from 0.72 to 0.60 with the latter figure representing the lowest value possible for living cells. The **regional ATP distributions** measured by quantitative bioluminescence were heterogeneous in all tumors investigated. ^{31}P-NMR spectroscopy exhibited a progressive drop of PCr/P_i, NTP/P_i, and pH_{NMR} as the tumors increased in size which was paralleled by an increasing deterioration of the tumor tissue oxygenation (Vaupel et al., 1989). The drop in the ratios was mostly due to a drastic increase in the P_i resonance. Tissue **glucose concentrations** were 1.65 mM in small tumors and significantly dropped to 0.8 mM in larger malignancies, whereas tumor **lactate levels** steadily increased during growth from 8 to 12 mM.
The experiments show that the glycolytic activity in FSaII is enhanced during growth, which can guarantee a rather constant level of high-energy phosphates for a certain growth period.

*Inst. of Physiol. & Pathophysiol, Univ. Mainz, D-6500 Mainz, FRG
+Dept. Radiat.Med., Mass. General Hospital, Boston, MA 02114, USA

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MICROCHEMICAL ANALYSIS OF GLUCOSE LEVELS IN MULTICELLULAR EMT6/RO TUMOR SPHEROIDS

A. Goellner[1], S. Gerlach[2], W. Mueller-Klieser[1], and H.F. Teutsch[2]

Multicellular tumor spheroids serve as in-vitro models of tumor microregions and avascular stages of tumor growth. They are characterized by the development of concentrically arranged regions of proliferative, quiescent and necrotic cell populations. Regional differences in the availability of nutrients are considered a major factor responsible for the development of cellular heterogeneity. While it has been shown that the thickness of the viable cell rim depends upon the glucose concentration in the culture medium (Mueller-Klieser et al.: Br. J. Cancer 53,345,1986), little is know about glucose levels within the spheroid. We have therefore developed a microprocedure of high analytical sensitivity (0.5-1.5 x 10^{-12} Moles) to measure regional glucose levels within the spheroid (H.F. Teutsch : Histochemistry 82,159, 1985). Small tissue samples were isolated from lyophilized cryosections of spheroids, and their size and location within the sections were graphically documented. The dry weight of samples (30-50ng) was determined on a quartz-fiber balance, and glucose was measured microchemically according to the following procedure: glucose was first converted to glucose-6-P with hexokinase and then to 6-P-gluconate with glucose-6-dehydrogenase, resulting in the formation of NADPH. Using an enzymatic amplification system, 19000 molecules of 6-P-gluconate were accumulated for each molecule of NADPH. Finally, 6-P-gluconate was converted to ribulose-5-P with 6-P-gluconate-dehydrogenase, leading to the formation of NADPH, that was measured fluorometrically. Data on glucose distribution revealed for the first time 30% lower glucose concentrations in the central necrosis as compared to the viable regions.

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[1]Inst. of Physiol. & Pathophysiol., Univ. Mainz, D-6500 Mainz, FRG; [2]Dep. of Anatomy, Univ. Ulm, D-7900 Ulm, FRG

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ANGIOGENESIS WITH HUMAN RECOMBINANT TNF-α : DEVELOPMENT OF A THERAPEUTIC SYSTEM FOR STIMULATION OF BLOOD VESSEL GROWTH.

K. Schlenger*, R. Schwab#, M. Höckel#, and P. Vaupel*

A variety of causative factors (e.g., systemic diseases, inflammation, traumata, operations, radiotherapy) can lead to a local rarefication of the microvascularization and, as a consequence, to a restriction of nutritive blood flow. The mandatory results are necrosis, ulcer and fistula formation, fibroatrophy and/or impaired wound healing, which can all result in severe disturbances of tissue or organ functions. For unrestricted wound healing a well orchestrated formation of microvessels is a crucial precondition.

Recent results prove the possibility to specifically induce local angiogenesis with the help of angiogenic biologic response modifiers. Hypovascularization and/or hypoperfusion could possibly be avoided by this concept.

A newly designed system for therapeutical angiogenesis in combination with recombinant human tumor necrosis factor-α (STA/rh TNF-α) is presented that may be suitable for clinical application. The system induces dose dependent angiogenesis in the rat cornea bioassay. Furthermore, a novel rat uterotomy model has been developed using the uterus bursting pressure as a test parameter. In the latter model, the uterus is cut by a longitudinal 10 mm incision and sutured thereafter. The bursting pressure is measured on the 3rd day after wounding. Compared to the controls, the implantation of STA/rhTNF-α at the site of the incision led to a significant elevation of the bursting pressure (433 ± 34 mmHg vs. 280 ± 39 mmHg, 2p = 0.014).

*Institute of Physiology & Pathophysiology, and #Department of Gynecology & Obstetrics, University of Mainz, D-6500 Mainz, FRG

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THE INHIBITION OF SMOOTH MUSCLE CELL PROLIFERATION FOLLOWING BALLOON INJURY IN THE CAROTID ARTERY BY THE CALCIUM-CALMODULIN ANTAGONIST, FENDILINE.

Z. Fotev and E. Betz

The proliferation of smooth muscle cells (SMC) in arteries is a prime cause of stenosing lesions. Events prior to proliferation involves calcium and calmodulin activation in SMC. We have examined whether the calcium-calmodulin antagonist, Fendiline, could inhibit the proliferation of SMC in the ballooned carotid artery of male Sprague-Dawley rats. Intimal lesion size was determined by measuring the DNA content in the left (ballooned) and right (non-ballooned) carotids (µg DNA/5 mm vessel).

Animals recieving only the vehicle (water) had an intimal DNA content of 4.07 ± 0.17 (n=13; mean ± SD) 14 days after surgery. Fendiline (given orally) at concentrations of 40, 20 and 10 mg/kg/day displayed a dose-dependent inhibition of intimal lesion formation; 2.01 ± 0.46, 2.30 ± 0.34 and 2.70 ± 0.21 (n=4), respectively. Even at the high concentration of 40 mg/kg/day no obvious toxic side-effects could be seen. The mitotic rate of SMC in the media is maximal 48 hrs after ballooning, therefore we investigated the effect of Fendiline with bromo-desoxyuridine, an analogue of thymidine. Fendiline (20 mg/kg) was able to inhibit SMC proliferation by 40% already 48 hrs after ballooning, which is very similar to the inhibition 14 days after ballooning (43%).

Interestingly, endothelial regrowth of the denuded vessel was unimpeded by Fendiline 28 days after ballooning. Furthermore, Collagen Types I and IV were reduced in the neo-intima while Collagen Types III remained unaffected.

In conclusion, Fendiline was able to inhibit specifically SMC proliferation and its production of extracellular matrix components, suggesting that it may be useful to prevent restenosis after angioplasty and bypass surgery.

Physiologisches Institut der Universität Tübingen, Gmelinstr. 5, D-7400 Tübingen

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PATHOPHYSIOLOGY OF SODIUM AND WATER RETENTION DUE TO MECHANICAL VENTILATION WITH POSITIVE AIRWAY PRESSURE IN CONSCIOUS DOGS.

M.Nienhaus, Gabriele Kaczmarczyk, Dinah Richter, Gabriele Korte, J.Förther and H.W.Reinhardt.

Positive airway pressure(as used in intensive care) reduces sodium and water excretion (UNaV, µmol/min/kg bw; V, µl/min/kg bw). Haemodynamic (i.e.decrease of mean arterial pressure MAP, mmHg) and hormonal mechanisms (i.e. increase of ADH) maybe involved.-5 conscious chronically tracheotomized, well-trained dogs were breathing spontaneously with Continuous Positive Airway Pressure =CPAP of 4 or 20 cm H_2O or were ventilated, Controlled Mechanical Ventilation =CMV, with 20 cm H_2O airway pressure. An iv infusion of 0.5ml Ionosteril/min/kg bw was applied throughout.

Results: * p<0.05, refers to 1.h
§ p<0.05, refers to CPAP 4 (2. and 3.h)

	CPAP 4 (1.h)	CPAP 4 (2.h)	CPAP 4 (3.h)
MAP	106±2	108±2	110±1*
V	189±23	366±35*	393±28*
UNaV	11.4±2.7	30.4±3.4*	44.4±4.5*
	CPAP 4	**CMV 20**	**CMV 20**
MAP	109±1	110±1	111±1
V	222±28	224±20 §	261±24 §
UNaV	14.5±1.4	23.1±2.1	29.4±2.8 §
	CPAP 4	**CPAP 20**	**CPAP 20**
MAP	115±2	122±2*	121±2*
V	210±49	211±26 §	312±47*
UNaV	15.9±3.4	23.0±3.0	34.0±4.8* (x±SE)

Plasma ADH (RIA,1st hr.:1.4±0.1 pg/ml) did not change throughout.

Positive airway pressure of 20 cm H_2O decreases UNaV and V in conscious, volume expanded dogs without a decrease of MAP and changes of ADH.

AG Exptl. Anästhesie, Klinik für Anästh.and Op.Intensivmedizin, FU Berlin, Spandauer Damm 130, 1000 Berlin 19, UKRV-Charlottenburg.

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IMMUNOHISTOLOGICAL STUDY OF THE INDUCTION OF STROMELYSIN AND COLLAGENASE IV IN SMC IN VITRO

R.Schreiber,J.Rupp,G.Murphy*,E.Betz,Z.Fotev and J.Fingerle

The migration of smooth muscle cells (SMC) and their excessive production of extracellular matrix are important steps for the formation of intimal thickening and vessel stenosis. This may require that the extracellular matrix in the media be reorganized by specific SMC proteinases. We have investigated the induction of stromelysin (str) and collagenase IV (coIV) in SMC cultures. Rabbit SMC were cultured in serum free medium (SFM) containing insulin, transferrin and thyroglobulin. Following a 48 hr adaption period in SFM, cultures were incubated with various stimulants. Proteinase induction was examined by immunofluorescence 24 hr and 72 hr after incubation. Cells positive for str or coIV were counted and expressed as percentage from total cell number.

stimulant		str 24 hr	coIV	str 72 hr	coIV
SFM (control)		0%	0%	0%	0%
SFM+PDGF (B-B)	25 ng/ml	0%	10%	0%	16%
	10 ng/ml	0%	10%	0%	9%
SFM + PMA	10^{-8} M	4%	26%	3%	14%
	10^{-9} M	3%	8%	-	0%

Il-2, TNF-α, TGF-β, have no effect on proteinase induction. These results show that SMC can be induced by phorbol myristate acetate (PMA) and platelet-derived growth factor (PDGF) but not by Il-2, TNF-α, TGF-β. However, only a subset of SMC (16%) responded to PDGF stimulation. PDGF is known as a mitogen and a chemotactic factor for mesenchymal cells. Our study suggest an involvement of PDGF in collagenase IV expression in a subset of SMC which could be important for SMC migration.

Physiologisches Institut I der Universität Tübingen, Gmelinstraße 5, 7400 Tübingen
* Strangeways Research Laboratory, Cambridge, UK

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NMR-SPECTROSCOPY AND DOPPLER FLOWMETRY OF RAT BRAIN AFTER GLOBAL ISCHEMIA

O. Kloiber, T. Miyazawa, M. Höhn-Berlage and K.-A. Hossmann

The relationship between post-ischemic restoration of blood flow and recovery of energy metabolism was studied in 10 rats submitted to 30 min forebrain ischemia produced by 4-vessel occlusion. Blood flow and blood volume were measured in the parietal cortex by laser Doppler flowmetry, and energy metabolism by NMR-spectroscopy, using a surface coil and a 4.7 T horizontal magnet. Animals were anaesthetized with halothane, mechanically ventilated and thermostabilized at 37°C. Ischemia was induced in the magnet, and continuous measurements were carried out for 3 h into the recirculation period. After vascular occlusion blood flow completely ceased in all animals, ATP and creatine phosphate were depleted in less than 6 min, and pH declined within 4 min. Post-ischemic recirculation was successful in 9 out of 10 rats. Blood volume (measured in 9 animals) consistently returned to control level within a few seconds after opening of vessels. Return of blood flow to control, in contrast, depended on blood pressure and varied between 30 sec and 29 min. Recovery of energy metabolism was flow dependent and varied accordingly.

The results demonstrate that the limiting factor in this model for post-ischemic recovery is post-ischemic blood flow which, in turn, depends on post-ischemic blood pressure. Post-ischemic reanimation, in consequence, can be greatly accelerated by stabilising post-ischemic systemic pressure.

Max-Planck-Institut für neurologische Forschung, Abteilung für experimentelle Neurologie, Gleueler Str. 500, D-5000 Köln 41

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EFFECT OF INTRACORONARY PAPAVERINE ON MYOCARDIAL FUNCTION OF AREAS WITH REDUCED CORONARY FLOW RESERVE

H. Schad, W. Heimisch, R. Blasini, F. Haas, N. Mendler

Intracoronary papaverine (PAPA) is used to assess coronary flow reserve in patients suffering from coronary artery disease. The present study in 9 anaesthetized dogs was aimed to investigate effects of PAPA on myocardial performance. Aortic (AoP) and left ventricular end-diastolic pressure (LVedP), dP/dt, stroke volume (SV), and ECG were monitored to describe global ventricular function. Performance of a subendocardial wall segment supplied by the left circumflex coronary artery (LCX) was assessed by sonomicrometry. Physiological LCX flow reserve was determined by the reactive hyperaemia following 15 s LCX-occlusion. Peak reactive hyperaemia was 145% (mean) of base line flow (41 ml/min) indicating reduced coronary reserve. PAPA was injected into the LCX (.3, .6, 1.2, 2.5, 5.0 mg/ml, 1 ml in 15 s). Qmax following PAPA 0.3 was comparable to peak reactive hyperaemia, Qmax induced by PAPA 0.6-5.0 was about 10% higher than after LCX-occlusion and was not dose-dependent, but recovery to base line flow was increasingly delayed. Systolic shortening of the affected myocardium (control: 17.5% of end-diastolic length) became dose-dependently reduced (by 5% to 25%) up to 2 min. During ventricular relaxation segment length atypically increased as seen during coronary occlusion. This disturbance of wall motion was accompanied by an S-T-depression or T-inversion in the ECG. Maximal -dP/dt (1800 mmHg/s) transiently decreased (-20%), SV (1 ml/kg) slightly reduced (-10%), and heart rate (130 1/min) slightly increased (+5%). The 5% decrease in AoP (90 mmHg) was not due to a systemic effect of PAPA but to the impaired ventricular performance as indicated by the systemic vascular resistance. LVedP (7 mmHg) showed no significant change following PAPA. The observed effects of intracoronary PAPA are consistent with a transient myocardial ischaemia arising from a redistribution of myocardial blood flow from subendocardial to subepicardial layers.

Klinik für Herz- und Gefäßchirurgie, Deutsches Herzzentrum München, Lothstraße 11, D-8000 München

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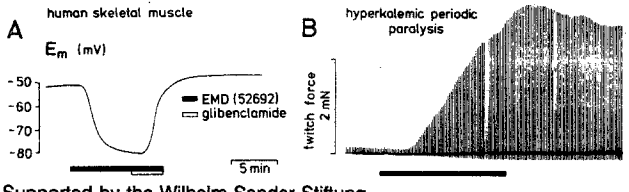
ENHANCEMENT OF K^+ CONDUCTANCE IMPROVES IN VITRO THE CONTRACTION FORCE OF SKELETAL MUSCLE FROM PATIENTS WITH PERIODIC PARALYSIS.

S. Quasthoff, M. Strupp, A. Spuler, F. Lehmann-Horn* and P. Grafe

An abnormal ratio between Na^+ and K^+ conductances seems to be the cause for the depolarization and paralysis of skeletal muscle in hypo- and hyperkalemic periodic paralysis. Recently, we have shown that K^+ channels openers" hyperpolarize mammalian skeletal muscle fibers (S. Quasthoff et al., Pflügers Arch. 414:S179, 1989; see Fig. A). Now, we studied the effects of such drugs on the twitch force of muscle biopsies from normal and diseased human skeletal muscle. Specimens of fiber segments (4-6 cm long) were dissected from biceps brachii, deltoid, vastus medialis or vastus lateralis muscles under local anesthesia. The preparations were superfused at 36° C in a perspex chamber and silver plates on both sides of the chamber were used for direct ("field") stimulation (2 ms, 100 V; 0.1 Hz). Cromakalim (100 µmol/l), EMD 52692 (10 µmol/l) and pinacidil (300 µmol/l) had little effect on the twitch force of normal muscle whereas these drugs strongly improved the contraction force of fibers from patients suffering from hypo- or hyperkalemic periodic paralysis (see Fig. B). These experiments were supplemented by measurements of intracellular K^+ and Cl^- activities. The results of these recordings are in accordance with the view that cromakalim, EMD 52692, and pinacidil enhance the membrane K^+ conductance. Glibenclamide (1 µmol/l) completely blocked mechanical and electrophysiological effects of the "K^+ channels openers" indicating the involvement of ATP-sensitive K^+ channels. The data show that enhancement of membrane K^+ conductance may have a beneficial effect in hypo- and hyperkalemic periodic paralysis.



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Physiologisches Institut der Universität München, Pettenkoferstr. 12, D-8000 München 2, F.R.G.
* Neurologische Klinik, Technische Universität München, F.R.G.

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EEG AND EP RECORDINGS IN THE STUDENTS LABORATORY SESSIONS: AN APPROACH COMBINING A DEMONSTRATION WITH PC BASED DATA ANALYSIS

M. ILLERT, H. WIESE AND U.WOLFRAM

We developed a teaching program which supplements a demonstration of EEG and EP recordings. For the EEG and visual EP recordings a volunteer is seated in a Faraday cage. An EEG amplifier and chart recorder is used. Visual EPs are evoked with a switching chequered pattern on a PC monitor. The EPs are on-line averaged and shown on a PC screen. To give all students an impression of the signals and of the ongoing experiment the analog traces of the recordings (from the chart recorder or the PC screen) are displayed with aid of a video projector onto a wall . In the second part of the laboratory session 18 students share six PCs to analyze EEG and EP records. These data from healthy subjects under ideal measuring conditions resemble the demonstration experiment. Additional data deal with the different sleep phases and with pathologic potentials of an epileptic patient. After A-D conversion the data had been stored on the hard disks of the PCs from where they are loaded during the execution of the teaching program.
The EEG analysis section offers the following experiments: pick up of remote physiological signals, analysis of alpha- and beta-waves, desynchronisation and habituation of cortex activity, different sleep phases and epilepsy. The EP analysis section offers the experiments: principle of the averaging method and visual EPs in different areas of the cerebral cortex.
The experiments can be started by selecting the particular item from the main menu. An oscilloscope (EP) or a chart recorder (EEG) are simulated on the terminal. For measuring frequencies, latencies and amplitudes of the different EEG or EP components a cursor function is offered which reads relative and absolute values.
This combined approach of recording EEG and EP data from a volunteer together with the possibility of a PC based data analysis -including sleep and epilepsy- has proved very valuable for a thorough understanding of integrative processes in the central nervous system.

Christian-Albrechts-Universität Kiel, Hermann- Rodewald- Str. 5, D-2300 Kiel 1

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FLUID INTAKE AND THE SYMPATHO-ADRENOMEDULLARY SYSTEM IN THE GUINEA PIG: INFLUENCE OF NEW HOUSING CONDITIONS

M.Fenske and N.Sachser

Guinea pigs show a strong reduction of fluid intake if exposed to new housing conditions. However, if allowed to adapt to the new situation, they exhibit a normal fluid intake, irrespectively of the spout size (1.4 or 0.7 mm) of the drinking bottles (M Fenske, Z.Versuchtierkd. 32:119,1989).To test whether catecholamine (CA) plasma levels are changed during fluid deprivation, adult male guinea pigs (body weight: 700-900 g) were exposed to new housing conditions for three days,having access to drinking bottles with normal (1.4 mm:group A) or unfamiliar,reduced (0.7 mm,group B) spout size.

	Day 1	Day 2	Day 3
Body weight[1)]	819/890	814/850	820/838
Fluid Intake[2)]	53/9*	87/25*	119/44*
Food Intake[3)]	44/26*	41/19*	54/24*
Hematocrit[4)]	49/56*	48/58*	50/56*
Norepinephrine[5)]	0.8/1.0	0.8/1.0	1.1/1.0
Epinephrine[5)]	0.3/0.5	0.2/0.2	0.3/0.2
Dopamine[5)]	1.1/1.3	0.8/0.6	0.7/1.0

Group A/B;[1)]g; [2)]ml/day; [3)]g/day; [4)]%; [5)]ng/ml; *p<0.01.
The table shows that a marked loss of body weight occurred in animals of group B (p<0.01) and that both fluid and food intake were reduced. On the other hand, an increase of hematocrit values was observed in animals of group B. Surprisingly, CA plasma levels were very similar in both experimental groups, indicating that CA levels do not serve as a sensitive index for stress induced by fluid deprivation.

Lehrstuhl Tierphysiologie, Universität Bayreuth, Postfach 101251, 8580 Bayreuth

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"SERUM LIPIDS IN CHILDHOOD AND ADOLESCENCE WITH REGARD TO SOCIOECONOMIC FACTORS"

Ch.Weis,W.Marktl,B.Kopta,N.Klammer and B.Rudas

In 1974/75 a longitudinal study concerning risk factors of atherosclerosis was started with 10-12 years old girls and boys(VIENNA STUDY).In 1986/87 174 women and 102 men of the first study could be reexamined.Serum levels of triglycerides and cholesterol were determined and brought in connection with anthropometric data (height and weight) as well as with socioeconomic factors (Working class,College, University degree),familiar risk constellations(insult, myocardial infarction,hypertension,overweight,metabolic diseases) and recreational activities (sports).Mean cholesterol levels in children had been 206+/-31mg/dl ($\bar{x}$ +/- s_x;n=276)in adults the mean values were 174+/-31mg/dl.64% of the girls but only 21% of the women had cholesterol levels higher than 200 mg/dl. The differences of the cholesterol levels between childhood and adolescence were statistically significant (p<0.001).Mean triglyceride levels in children were 83+/-35mg/dl and in adults 84+/-39mg/dl.4% of the children,5% of the women and 8% of the men had triglyceride levels exceeding 150 mg/dl.Completely regarded adults had higher triglyceride levels than children (p<0.05). As well in children as in adults there had been no sex linked influence on cholesterin levels;in the same manner no influence of social class,familiary risks,bodyweight and sports could be found.Only adults with positive familiary risks (myocardial infarction,insult) had higher cholesterol levels than those with negative familiary risks (p=0.059).In childhood triglyceride levels showed generically differences - girls always had higher levels than boys. Familiary risks showed no influence on triglyceride levels (children and adults).Triglyceride levels decreased with higher social class (children and adults;p<0.05).In children bodyweight had no influence on triglyceride levels but in adults the levels increased with bodyweight (p<0.05).

Institut für Medizinische Physiologie der Universität Wien, Schwarzspanierstraße 17, A-1090 Wien.

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DIAMETER AND WALL COMPOSITION OF MESOMETRIAL ARTERIES DURING LONGTERM ESTRADIOL TREATMENT AND EARLY PREGNANCY IN GUINEA PIGS.

H. Pohl, A. Nienartowicz, and W. Moll

During pregnancy the mesometrial arteries widen and undergo extensive changes in mass and composition in order to adjust placental blood flow to fetal growth. The controlling signals are not yet identified. We have tested, in guinea pigs, whether estradiol (E_2) is capable of inducing the arterial changes seen in early pregnancy.
Nonpregnant guinea pigs were treated with 250 µg E_2 undecylate s.c.. After 1, 2, 3 and 4 weeks, the animals were killed and 20 arteries were excised from the fat-free mesometrium. External and internal diameters, weight, collagen, desmosin (a measure of elastin) and DNA content were determined. The same measurements were also made in pregnant guinea pigs in the first weeks of pregnancy.
E_2 plasma concentration rose from 10 pg/ml before treatment to around 80 pg/ml and fell with a half time of 4 weeks. During the first 3 weeks, internal diameter, weight, length and DNA content increased 2-times, as did uterine weight. Desmosin concentration fell, while collagen concentration remained constant. Apart from collagen concentration which decreased, similar findings were obtained in the first 3 weeks of pregnancy. In the 4th week, however, no further changes of the measured variables were observed under estradiol treatment while the changes in pregnant animals accelerated 10times.
It is concluded that most changes seen in early pregnancy can be induced by estradiol. It must be doubted, however, that estradiol can account for the arterial changes seen in later pregnancy.

Institut für Physiologie der Universität Regensburg, Universitätsstraße 31, D-8400 Regensburg

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ENDOCRINE OSMOREGULATION DURING EMBRYONIC DEVELOPMENT IN THE CHICK

M. Klempt, F. Ellendorff and R. Großmann

The aim of the present study was to investigate the development of the response of arginine-vasotocin (AVT)-system to osmotic stimulation during late embryonic life.
In a first experiment blood samples were collected by heartpuncture or decapitation in chick embryos after 15 to 20 days of incubation (E15-E20) and in newly hatched chicks (D1).
AVT measured by RIA (in collaboration with Prof. Simon, MPI, Bad Nauheim) is first detectable at E16 (5.9 ± 2.1 pg/ml; mean ± SEM; n=5) peaks at E19 (27.3 ± 4.2 pg/ml; n=11)and drops subsequently to 10.0 ± 1.0 pg/ml (n=13) at D1.
Plasma osmolality increases continuously from 279.3 ± 5.6 mosmol/kg (n=5) at E15 to 301.3 ± 1.4 mosmol/kg (n=13) at D1.
In a second experiment E18 embryos and D1 chicks were anaesthetized with urethane (3 mg/kg BW i.p.) and catheterized into the jugular vein. Blood samples were taken before, and 15 and 30 min. after osmotic challenge (0.1 ml, 1.5 M NaCl; i.p.). Plasma osmolality was increased to similar extent (10.0 vs 9.2 mosmol/kg) in E18 and D1. However, plasma AVT concentrations exceed the basal values 10 fold in D1 (3.8 vs 35.4 pg/ml) and 1.5 fold in E18 (29.8 vs 45.3 pg/ml).

We conclude that the efficiency of osmoregulation in chicks rapidly matures during late embryonic development.
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Forschungsbereich Physiologie, Institut für Kleintierzucht (FAL), Dörnbergstr. 25-27, 3100 Celle

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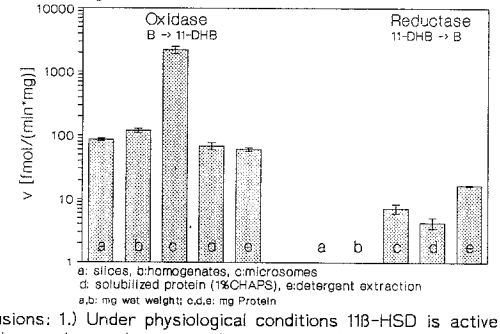
OXIDATIVE AND REDUCTIVE ACTIVITY OF 11ß-HYDROXYSTEROID DEHYDROGENASE (11ß-HSD) OBTAINED FROM HUMAN PLACENTA

S Blum, H Bühler, I Lichtenstein, A Novak, H Siebe and K Hierholzer

11-ß-HSD catalyses the reaction: Corticosterone(B)↔11-ß-Dehydrocorticosterone (11-DHB) in many target tissues. It is a membrane bound protein localized in the endoplasmic reticulum. In all but one available studies it was observed that 11-ß-HSD exerts only oxidative acivity in the trophoblastic tissue of the human placenta. Expression of reductive activity is of interest since the 11-ß-HSD might serve important biological functions in the placental barrier. Expression of only oxidative activity might point to the existence of two seperate enzymes, an oxidase and a reductase (dual-enzyme-hypothesis Monder, Shackleton, 1989).
In slices and homogenates of term placentas high ox activity was found, but no significant red activity. Preparation of microsomes, solubilization with 1% CHAPS and detergent extraction revealed significant red activity (Fig.). The red/ox-ratio increased from 0.003 (microsomes) to 0.06 (solubilized enzyme + detergent) and 0.27 respectively (solubilized enzyme after removal of detergent).



Conclusions: 1.) Under physiological conditions 11ß-HSD is active only as an oxidase and may, thus, control the activity of glucocorticosteroid passing from maternal to fetal circulation; 2.) Red-activity of the intracellularly bound enzyme was virtually absent, however, 11ß-HSD can be unmasked by solubilization as an oxidoreductase system (E.C.1.1.1.146).
Inst. Klin. Physiologie, Klinikum Steglitz, FUB, Hindenburgdamm, 1 Berlin 45

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DATA-ACQUISITION AND REAL TIME EXPERIMENT CONTROL WITH THE PERSONAL COMPUTER (AT)

K. Peper

Whereas abundant software is available for Data Analysis with the Personal Computer (AT), the hardware for quick Data aquisition and real time experiment control has become very sophisticated and expensive and its development is normally beyond the possibilities of a scientist. The reason is that the AT was developed as an office instrument and is, from its structure, mostly unsuitable for real time experiments. For upmost performance it is quite often necessary to build a dedicated interface between computer and experiment. However, the AT-Bus is not well designed for this purpose, and DMA and Interrupt techniques have very poor time responses. For this reason, real time applications have to be constructed in hardware rather than in software.
What is needed is a processor with an open, quick bus structure and a well designed real time structure. This is now available as the Harris RTX 2000. A rather cheap card with this RISC-Prozessor is on the market. Here the use of this (slightly changed) one platine computer is discussed for a general purpose real time system with a quick link to the AT. Data input and output cards can easily be constructed. The CPU has 10 Mips and is directly programmed in a higher level language: FORTH. By this preprozessor arrangement it appears much more easy to optimize the equipment to the experiment without tradeoffs.

Physiologisches Institut der Universität des Saarlandes, D-6650 Homburg/Saar