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Respiratory and pulmonary pharmacology
(14.30–15.30)

C025

Evaluation of antibiotic prescribing in upper respiratory tract infections

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The purpose of this study was to evaluate trends in antibiotic prescribing at Family and Community Health Clinics. Males, females and children who attended for the treatment of upper respiratory tract infections from November to April were included. A data collection form that included question items on type of antibiotic, duration of treatment, medical conditions, other medications, performance of culture tests, sensitivity results, number of antibiotics and duration, adverse drug reactions and use of generic versus trade names. The number of prescriptions was 300. SPSS was used for statistical analysis. Forty three percent who received antibiotics were less than 18 years of age, 20% were between 18–30 years and 16% were above 30 years. Males were 43% and females 47%. Most patients had one course of antibiotics (94%), 6% had two courses. There was no documentation of adverse drug reactions. In 45% of prescriptions generic names were used and 55% used trade names. The duration of treatment for 66% of patients was five days, 7 days for (21%) and less than 5 days in (9%). No data on antibiotic sensitivity was available. Co-amoxiclav accounted for (62%), followed by amoxicillin 37% and azithromycin 12%. Antibiotics were largely prescribed to children. Antibiotics ranked 5th highest prescribed drugs in infants (Al Khaja *et al.*, 2006). In our study most prescriptions were empirical. More co-amoxiclav prescribing compared with amoxicillin indicates resistance. The trend of prescribing beta lactam antibiotics for upper respiratory tract infections by primary care physicians has also been observed in other parts of the Arabian Gulf. (Al Khaja *et al.*, 2008).

References:

Al Khaja KA *et al.* P J Trop Pediatr. 2006 Dec; 52 (6): 390–393.
Al Khaja KA et al, Pharmacoepidemiol Drug Saf. 2008 Mar 5.

C026

Oral antibody to interferon gamma in ultra low doses: clinical efficacy and interferon stimulation in patients with upper respiratory viral infections

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Inducers of interferons production are considered as new potential anti-virals. Anaferon (pediatric formulation) is a novel anti-viral and immunomodulating drug developed in Russia. Anaferon (AF) contains antibodies to gamma-interferon, ultra-low doses, for peroral use. The aim of the study was to assess AF's clinical efficacy and it influence on interferons production in patients with upper respiratory viral infections (URVI). Two randomized double blind placebo-controlled trials of AF were conducted in Research Institute for Influenza (St. Petersburg) and Russian State Medical University (Moscow). 255 children aged 0.5–14 years with URVI of various etiology (influenza A/B, coronavirus, parainfluenza, RS virus and others) were enrolled. At a baseline all patients had body temperature ≥ 37.5°C. Therapy with AF (n = 160)/placebo (n = 95), 3–7 tablets daily, was started on day 1–2 of the onset as an add-on to symptomatic therapy and continued for 5–14 days. Clinical signs and symptoms were registered daily. Influence of AF on interferons production/activity: interferons (gamma and alpha) level (serum level, spontaneous and mitogen-induced production by peripheral blood leukocytes) was measured (baseline, 2–3 and 6–7 days of treatment) by ELISA in 139 patients (93 were given AF, 46 – placebo). Activity of interferons (IFNs) was assessed by their ability to inhibit virus cytopathic effect. Treatment with AF resulted in statistically significant (P < 0.001 vs. placebo) reduction in duration of clinical signs by more than 1 day vs. placebo: time to no fever was 2.4 ± 0.1 vs. 3.5 ± 0.1 days; time to no catarrh was 5.0 ± 0.2 vs. 6.9 ± 0.4 days. The adverse events rate in AF group was equal to placebo. AF significantly (P < 0.001 vs. placebo) stimulated IFNs production/activity on day 2 to 3 of treatment (see Table 1). IFNs levels in placebo group were decreased or remained unchanged. Contrary to placebo, AF prevented depression of IFNs production/activity on day 6–7.

The studies revealed AF ability to stimulate production/activity of IFNs and prevent its decrease on recovery. AF was effective and safe treatment of URVI of various etiologies. The stimulation of IFNs production may be the key mechanism of AF anti-viral activity

Table 1. Effects of AF on IFNs production in patients with URVI measured by ELISA

IFN production	Anaferon, n = 93		Placebo, n = 46	
	baseline	2–3 days	baseline	2–3 days
Serum IFNα, pg/mL	44.9 ± 3.5	66.3 ± 3.5*	40.8 ± 4.7	34.5 ± 4.6
Serum IFNγ, pg/mL	59.6 ± 2.9	78.9 ± 3.6*	54.4 ± 4.7	54.0 ± 5.4
Spontaneous IFNα production, pg/mL	77.3 ± 4.5	91.6 ± 4.6*	73.3 ± 5.9	77.3 ± 7.3
Spontaneous IFNγ production, pg/mL	44.9 ± 2.5	63.5 ± 2.9*	43.3 ± 4.1	44.3 ± 3.8
Mitogen-induced IFNα production, pg/mL	103.5 ± 6.0	143.2 ± 8.3*	107.9 ± 8.9	97.0 ± 11.7
Mitogen-induced IFNγ production, pg/mL	91.2 ± 6.6	162.5 ± 22.0*	86.4 ± 6.5	78.9 ± 5.9

The data represent the mean values ± SE; *P < 0.001 vs. placebo.

C027

Effect of etanercept in a strain-dependent mouse model of allergen-induced airway hyperresponsiveness and lung inflammation

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The aim of these studies was to develop an allergen-induced murine model of airway inflammation, characterised by airway hyperresponsiveness (AHR) and an upregulation of TH_1 and TH_2 cytokines. The role of TNF in this model was investigated using etanercept; a recombinant human soluble TNF receptor fusion protein reported to have efficacy in patients with persistent asthma and known to neutralise mouse TNF. C57bl/6 or Balb/c mice (male, 20–25 g, n = 8/group) were sensitised subcutaneously on day 0 with saline or 100 μg of house dust mite [*Dermatophagoides pteronyssinus* (Der p)] extract in complete Freund's adjuvant. On day 14, mice were exposed to saline or Der p (100 μg or 25 μg) via intranasal instillation. 48 hours post-challenge, non-invasive whole body plethysmography was used to assess AHR stimulated by doubling concentrations of aerosolised methacholine (MCh, 3.125–50 mg/ml). Bronchoalveolar lavage fluid (BALF) was retrieved for measurement of inflammatory cells and cytokine profile. In subsequent studies using Balb/c mice (n = 10/group), the effect of etanercept (30 mg/kg, i.v.) or vehicle (PBS, i.v.), administered on days 13 and 14, was assessed. Allergen-induced AHR was more profound in Balb/c as compared to C57bl/6 mice. Immunisation and challenge of Balb/c mice with Der p induced AHR (> 2-fold increase in Penh at all concentrations of MCh), whereas AHR in C57bl/6 mice was less robust (> 2-fold increase only at 6.25 and 12.5 mg/ml MCh). The AHR was accompanied by BALF neutrophilia (Balb/c: 83.66 ± 17.31 x 10^4 cells/mL; C57bl/6: 47.10 ± 21.62 x 10^4 cells/ml) and eosinophilia (Balb/c: 17.51 ± 5.07 x 10^4 cells/ml; C57bl/6: 11.43 ± 5.58 x 10^4 cells/mL). Treatment of Balb/c mice with etanercept during the challenge phase significantly inhibited AHR by 52% (12.5 mg/ml MCh: Penh reduced from 3.70 ± 0.34 to 2.49 ± 0.38, P < 0.05). Etanercept significantly (P < 0.05) altered the BALF cytokine profile, inhibiting IL-1β (52%), IL-5 (54%), IL-10 (36%), IL-12 (76%) and IFNγ (68%). Etanercept had no significant effect on BALF neutrophilia or eosinophilia measured 48 h after allergen challenge. The sensitivity of components of this model to etanercept, a clinically used anti-TNF therapeutic, demonstrates the potential for utilising this model to investigate the mechanisms of persistent asthma and in the assessment of novel therapeutics.

C028

Functional changes in background K^+ channels during the culture of pulmonary arteries

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The culture of blood vessels is gaining interest for use with transfection-based techniques (Gurney and Hunter, 2005), but has been shown to alter the contractile properties of the vessels (Guibert *et al.*, 2005). The present study tested the effects of culture on the intrinsic tone of rat pulmonary arteries (PAs) and examined the function and expression of K^+ channels involved in regulating the resting membrane potential (E_m) and tone of pulmonary artery smooth muscle cells (PASMCs). Male Sprague–Dawley rats (250–300 g) were sacrificed according to Schedule I of the UK Scientific Procedures (Animals) Act 1986. Intrapulmonary arteries were isolated and cultured as previously described (Gurney *et al.*, 2005). Contractile responses of fresh and cultured PA to various drugs were measured using vessel myography. The E_m of PASMC was recorded using the perforated patch technique and K^+ channel expression quantified using real time RT-PCR. The contractile response to 1 μM phenylephrine did not change during culture, but subsequent relaxation to 1μM carbachol was significantly reduced. Over 4 days in culture, contractile responses to 15 mM KCl, 1 mM 4-aminopyridine and 10 mM tetraethylammonium increased, while the vessels developed an uncharacteristic relaxation response to 1 μM nifedipine, 10 μM levcromakalim and Ca^{2+}-free solution. These changes were associated with depolarisation of the PASMC E_m and down regulation of Kv1.5, Kv2.1 and TASK-1 mRNA expression. Bubbling the culture medium with 95% O_2 partially reversed the changes observed. The changes in PA function that developed during culture are consistent with a progressive depolarisation of PASMC, probably resulting from reduced expression of background K^+ channels, especially TASK-1. O_2 enrichment appears to limit the changes induced by culture.

References:

Guibert, C. *et al.* Br J Pharmacol. 2005; 146: 692–701.
Gurney, A.M. and Hunter, E. J Pharmacol Toxicol Methods. 2005; 51: 253–62.

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Pulmonary and cardiovascular pharmacology (16.00–17.00)

C029
Chronic exposure to fibrin enhances agonist-induced calcium release in human pulmonary artery smooth muscle cells

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Acute pulmonary embolism occurs in more than 500 000 patients a year in the US. Chronic thromboembolic pulmonary hypertension (CTEPH) develops in approximately 4% of the patients (or ~20 000 cases) with acute pulmonary embolism each year because of unresolved thromboemboli. CTEPH is a relatively common, progressive and potentially fatal disease process. One currently proposed theory for such poor resolution advocates that modification of fibrin in CTEPH patients causes resistance of emboli to fibrinolysis, which could lead to pulmonary vascular remodeling. Control of intracellular calcium is central to the regulation of cell migration, proliferation and contraction and contributes to the pathophysiological process of vascular remodeling. The current study investigates the regulation of intracellular calcium by chronic exposure of pulmonary artery smooth muscle (PASMC) and endothelial (PAEC) cells to thrombin and fibrin. PASMC or PAEC were plated on control, fibrinogen (FNG), thrombin or fibrin coated cover slips for 72 h. Cells were loaded with a fluorescent calcium sensitive dye, Fura-2-AM, and changes in intracellular calcium in response to thrombin (5 nM) were recorded. Chronic exposure of PAEC to thrombin, an agonist of proteinase-activated receptors, significantly reduced the agonist-induced peak calcium transient (F/F_0 ratio) from 1.3 ± 0.07 (n = 27) to 0.97 ± 0.06 (n = 43), $P < 0.001$. However, in PASMC a substantial increase was observed. The peak calcium was significantly increased in PASMC chronically exposed to 4 μg/ml fibrin and the recovery rate of the agonist-induced calcium transients decelerated ($P < 0.05$). In PAEC, only higher FNG and fibrin concentrations (40 μg/ml) significantly affected the calcium transients; decreasing the peak calcium transient and the calcium transient peak width at 50% of the peak height ($P < 0.05$) when compared to control in PAEC. In conclusion, chronic exposure of cells to fibrinogen, fibrin and thrombin caused changes in intracellular calcium which could stimulate accelerated smooth muscle cell proliferation, migration and contraction. Prolonged exposure to thrombin had a potent effect reducing agonist-induced calcium transient in PAEC, suggesting a desensitization of receptors and intracellular calcium regulation in these cells.

C030
Activation of Toll-like receptor (TLR) 3 and TLR 8 lead to endothelin-1 release from pulmonary vascular smooth muscle: relevance to pulmonary hypertension

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Endothelin-1 (ET-1) is an important mediator in pulmonary hypertension. Usually released by the endothelium; it can be released by pulmonary vascular cells, and act in an autocrine manner (Wort *et al.*, 2001) to cause vasoconstriction and smooth muscle hypertrophy. Pathogens, via pathogen-associated molecular patterns (PAMPS), can mediate profound vascular responses, e.g. the shock induced by lipopolysaccaride. These responses are mediated by Toll-like receptors (TLRs), (Mitchell *et al.*, 2007). Our aim was to determine if bacterial and viral PAMPS can lead to endothelin-1 release from pulmonary vascular smooth muscle, and thus whether pathogens can be triggers to pulmonary hypertension. Human pulmonary artery smooth muscle (HPASM) cells were grown in 96 well plates and were stimulated with a range of PAMPS. Supernatants were collected at 24 h. In addition, primary human endothelial (HE) cells were also stimulated with PAMPs. ET-1 was measured by ELISA.

Our results showed that synthetic ligands for viral PAMPS, Poly I:C, (activates TLR 3), and LyoVec (ligand for TLR 8), cause ET-1 release from HPASM cells. Importantly, bacterial PAMPs did not cause ET-1 release in these cells. By contrast, activation of TLR8 or TLR3 did not elevate ET-1 in HE cells. These observations are the first to indicate a role for viral pathogen sensing in pulmonary hypertension.

References:

Mitchell, JA. *et al.* Biochem Soc Trans. 2007; 35(Pt 6): 1449–1452.
Wort, S. J. *et al.* Am J Respir Cell Mol Biol. 2001; 25: 104–110.

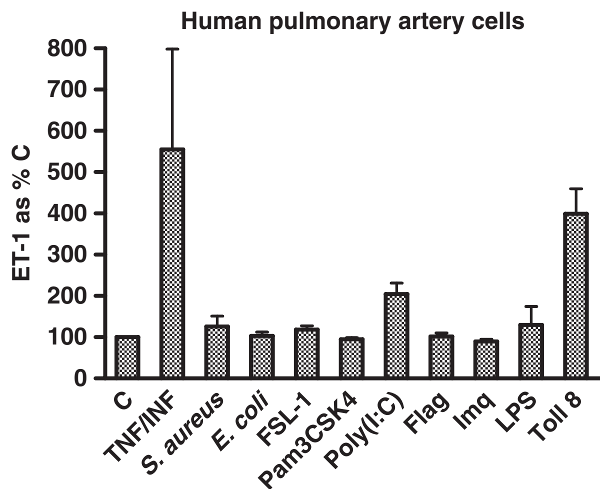

Figure 1 for Abstract C030.

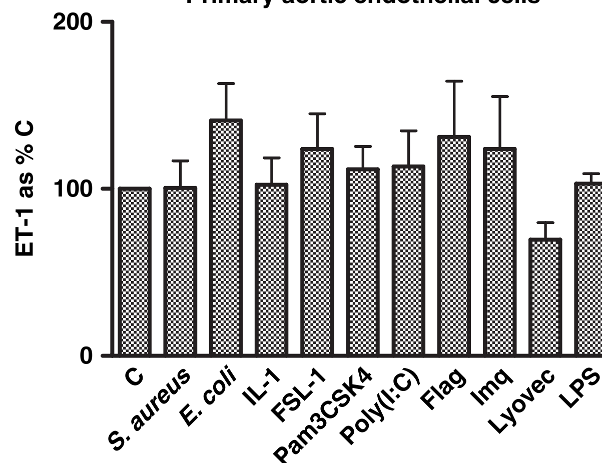

Figure 2 for Abstract C030.

C031
Antisense oligonucleotide against endothelin receptors modulate ET-1-induced vasoconstriction of rat pulmonary arteries

S Sauvageau, J Dupuis, E Thorin *Montreal Heart Institute, Montreal/Quebec, Canada*

Most G protein-coupled receptors (GPCR) form homo and/or hetero-oligomeric complexes. This dimerization concept raises the problem of how two different receptors can influence the coupling of each other and determine the ultimate function of the complex. We previously reported an interaction between the ET_A and ET_B receptors (ET-R) in rat pulmonary arteries, which may modify endothelin-1-(ET-1) induced vasoconstriction (Sauvageau *et al.* 2007). To test this hypothesis we developed a novel non-pharmacological vessel culture technique combined with gene therapy. Small isolated pulmonary arteries (100–150 μM) from male wistar rats (200–250 g) were harvested and pooled for standard protein extraction. Heterodimerization of receptors was evaluated by immunoprecipitation of the ET_B-R, followed by Western blotting for the expression of the ET_A receptor in both denaturating and non-denaturating conditions. For gene therapy experiments, pulmonary arteries were incubated and exposed for 24 (ET_A-R) or 72 h (ET_B-R) to selective antisense (AS) oligonucleotides. A total of two different AS oligodeoxy-ribonucleotides (ON) phosphorothioate (PTO) sequences were used targeting either the rat ET_A receptor (ET_A-AS) or the rat ET_B receptor (ET_B-AS) mRNA sequences. The corresponding 2 scrambled (Sc) ON sequences were used as negative controls (Sc-ET_A-AS; Sc-ET_B-AS). Following the incubation period pulmonary arteries were mounted on a microvessel myograph to assess ET-1-induced constriction. Heterodimerization of both receptors was observed. Western blotting confirmed that each AS reduced protein expression of its targeted receptor. The use of ET_B-AS significantly reduced the sensitivity to ET-1 (−7.90 ± 0.09; $logEC_{25}$ ± SEM, $P < 0.05$) compared to control vessel (−8.59 ± 0.17) without affecting the maximal vasoconstriction. In contrast, ET_A-AS significantly increased the vascular sensitivity (−8.36 ± 0.04, $P < 0.05$) and the maximal vasoconstriction induced by ET-1 (100 ± 3, Emax ± SEM, $P < 0.05$) compared to controls (−7.83 ± 0.05, 89 ± 3%). In conclusion, suppression of ET_B receptors reduced vascular sensitivity to ET-1 while suppression of ET_A receptors increased the vascular sensitivity to this peptide. Hence, there is an interaction between the ET_A and ET_B receptors to induce the ET-1 response in rat pulmonary arteries. Furthermore, these results confirm the functional importance of ET_B receptors in ET-1-induced vasoconstriction of small pulmonary arteries.

Reference:

Sauvageau, S *et al.* J Vasc Res. 2007; 44: 375–381.

C032
IP-10 (CXCL10) release following viral sensing by pulmonary artery cells-role of Toll like receptors

R Badiger, J Mitchell, M Paul-Clark, S Wort, P De Sousa, S Johnson, M Edwards *Imperial College, London, UK*

The aetiology of most types of pulmonary hypertension is unknown. Pathogens, via pathogen-associated molecular patterns (PAMPs), can mediate profound vascular responses, for example, the shock induced by lipopolysaccharide. These responses are mediated by Toll-like receptors (TLRs) (Mitchell *et al.*, 2007). Our aim was to determine if viruses can be 'sensed' by pulmonary artery smooth muscle cells, what cellular responses may occur, and thus whether viral infection could be an aetiological factor in pulmonary hypertension. Human pulmonary artery (HPASM) cells were grown in 24 well plates and inoculated with respiratory syncitial virus, (multiplicity of infection of 1 $TCID_{50}$) at 100, 20 and 10% dilutions for 8, 24 and 48 h after which the supernatant was removed. In addition, the cells were treated with the viral PAMPS, Poly (I:C), (TLR3) and Lyovec (TLR8). IP-10 was measured by ELISA.

Our results show that infection of HPASM cells with RSV leads to marked release of IP-10, and that these cells sense viruses via Toll-like receptors. These observations are the first to indicate a role for viral pathogen sensing in pulmonary hypertension

Reference:

Mitchell, JA. *et al.* Biochem Soc Trans. 2007; 35(Pt 6): 1449–1452.

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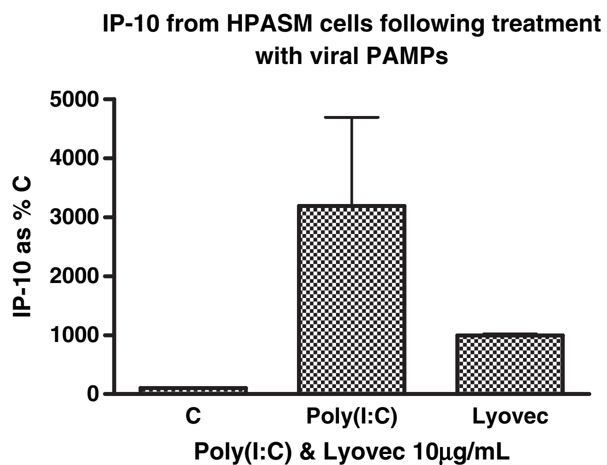

Figure 1 for Abstract C032.

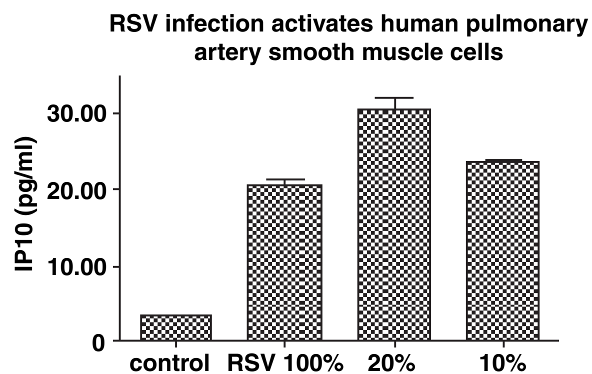

Figure 2 for Abstract C032.

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Molecular pharmacology (14.30–15.30)

C033

Role of key transmembrane residues in the pharmacological actions of isoprenaline and CGP 12177 at the human β1 adrenoceptor

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Functional studies with catecholamines and CGP 12177 at the human beta-1-adrenoceptor have provided evidence for two different active agonist conformations that have markedly different pharmacological properties (Baker *et al.*, 2003). Here, key transmembrane (TM) residues in TM 3, 5 and 7 have been mutated to provide structural insights into the nature of these two conformations. Mutations (D138A, D138S, S228A, S229A, S232A and N363A) were generated using the Strategene QuickChange mutagenesis kit. These constructs were then transfected into a CHO cell line stably expression a CRE-SPAP reporter gene. The cells were selected for 3 weeks using G418 for the receptor and hygromycin for the CRE-SPAP reporter. These stable mixed populations were then used in ^{3}H-CGP 12177 whole cell binding and CRE-SPAP reporter assays as previously described (Baker *et al.*, 2003). Agonist responses to isoprenaline and CGP 12177 had different sensitivities to β1-antagonists (e.g. CGP 20712A; log K_D = −8.65 and −7.26 respectively). CGP 12177 acted as a high affinity antagonist (log K_D = −9.18) at the 'catecholamine site' and as a lower affinity agonist at the 'CGP 12177 site' (log EC_{50} = −8.12). Mutations to D138 abolished all ^{3}H-CGP 12177 binding and all functional responses. N363 mutations abolished all CGP 12177 binding and responses and markedly reduced responses to isoprenaline and cimaterol. Each of the TM5 serine residues (S228A, S229A, S232A) were found to contribute to catecholamine binding, but none was singularly responsible. Cimaterol binding (a non-catechol agonist) however was minimally affected. The S228A and S229A mutations reduced both the affinity of CGP 12177 for the catecholamine site (25.5 and 7.1-fold) and also its agonist potency (15.5 and 6.8-fold) at the 'CGP 12177 site'. Thus D138 and N363 are essential for binding and function of both conformations of the β1-adrenoceptor and each of the TM5 serine mutants retained the pharmacology of the two conformations of the β1-adrenoceptor.

Reference:

Baker JG *et al.* Mol Pharmacol. 2003; 63: 1312–1321.

C034

Long and short distance movements of β2-adrenoceptor in cell membrane assessed by photoconvertible fluorescent protein dendra- β2-adrenoceptor fusion

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In the present study we addressed the following questions: 1) Is the local diffusion of the receptor in the cell membrane restricted to small domains?, 2) if the long distance diffusion of the receptor in the membrane is not macroscopically bounded in local domains, can local diffusion explain this large scale diffusion quantitatively?, 3) what is the nature of the compartmental connectivity in the cell in terms of receptor trafficking, when the cellular distribution of receptor is in the steady-state?. We used HEK293 cells permanently transfected with human β2 adrenoceptor fused to the N-terminal of the dendra protein (a GFP-like fluorescent protein that undergoes an irreversible spectral conversion from {Ex. 488, Em.507 nm} to {Ex. 558, Em.575 nm} upon subsecond irradiation with 488 nm ~1 W/cm-sq argon laser) as a model system. This construct allowed us to locally (and instantaneously) label the receptors in a small region of the membranes in living cells. We used a confocal microscope (Leica) equipped with appropriate lasers, thermostatic baths and software for data collection. Solutions of two-dimensional diffusion equation (analytical or numerical) for appropriate boundary and initial conditions were used for quantitative evaluation of the data. We found that 1) functional integrity of the dendra-tagged receptor remains intact (as assessed by agonist stimulated cyclase activation or radioligand binding experiments), 2) inward or outward flux of the receptor to, or from a small membrane patch (~ 4 micron-sq) can be symmetrically explained by the same simple diffusion process with a diffusion coefficient of ~0.1 micron-sq/s (with an average mobile fraction of 85%), 3) this process is found to be independent of the activity state of the receptor, as assessed by using constitutively active mutants of the receptor, 4) only a part of the large scale movement of the receptor in the membrane can be explained by the same local diffusion process, implying the presence of large-scales diffusion barriers in the membrane, 5) cell-wide re-distribution of the receptor protein in the membrane is governed by the same local diffusion process (i.e. the entire cell membrane is apparently available to the receptor molecules, and 6) all the visible compartments (within the spatial resolution of the microscope) in the cell are interconnected within the time frame of hours. These are the first experiments that demonstrate these points.

C035

Role of key residues in TM2 and TM6 in the two agonist conformations of the human β1-adrenoceptor

J Baker, R Proudman, N Hawley, S Hill *University of Nottingham, Nottingham, UK*

Functional studies with CGP 12177 at the human β1-adrenoceptor have provided evidence for two active conformations that have markedly different pharmacological properties (Baker *et al.*, 2003). In the β-2 adrenoceptor Asp79 in TM2 and residues in TM6 (Asn 293 and Phe290) have been implicated in receptor activation mediated by agonists (Kobilka, 2007). Here, the equivalent residues in the β-1 adrenoceptor have been mutated to investigate the contribution of these residues to the two agonist conformations of the β1-adrenoceptor. Mutations (wild-type, (WT), D104A, D104N, N341A and F343A) were generated using the Strategene QuickChange mutagenesis kit. These constructs were then transfected into a CHO cell line stably expression a CRE-SPAP reporter gene. The cells were selected for 3 weeks using G418 for the receptor and hygromycin for the CRE-SPAP reporter. These stable mixed populations were then used in ^{3}H-CGP 12177 whole cell binding and CRE-SPAP reporter assays as previously described (Baker *et al.*, 2003). Responses to isoprenaline and CGP 12177 were inhibited in the WT receptor by CGP 20712A to give a log K_D values of −8.65 and −7.26 respectively, indicative of the two conformations of the β1-adrenoceptor. Mutations of Asp104 had very little effect on the affinity of ligands however functional responses including that to CGP 12177 were greatly reduced. Mutations in TM6 (N341A and F341A) slightly reduced the binding affinity of isoprenaline and CGP 12177 but had little effect on their agonist actions. Antagonism by CGP 20712A of these two responses was similar (−log K_D values = −7.6 and −7.6 for F341A; −8.1 and −8.2 for N344A with isoprenaline and CGP 1277 as agonist respectively). This suggests that CGP 20712A was no longer able to discriminate between the two agonist conformations of the receptor. These studies suggest that F341 and N344 may have important roles in defining the two conformations of the β1-adrenoceptor.

References:

Baker JG *et al.* Mol Pharmacol. 2003; 63: 1312–1321.

Kobilka B Biochim Biophys Acta. 2007; 1768: 794–807.

C036

Regulation of G-protein-coupled receptor kinase 2 (GRK2) by calmodulin and protein kinase C

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G-protein-coupled receptor kinases (GRKs) mediate the first step of homologous desensitisation of G-protein-coupled receptors, namely phosphorylation of agonist-activated receptors. It has been previously shown that one of the GRKs, GRK2 is subject to inhibition by calmodulin. We have previously postulated that this inhibition can be relieved by protein kinase C which phosphorylates GRK2 on Ser-29, within a putative calmodulin-binding site. We have now investigated this regulatory mechanism further. To directly assess GRK2-calmodulin interaction, we covalently labelled calmodulin with dansyl chloride and investigated its interaction with various concentrations of GRK2, GRK2(1-53) and GRK2(552-689) by measuring dansyl fluorescence at 486 nm. Fluorescence increases in a saturable manner when calmodulin interacts with increasing concentrations of a binding partner. GRK2 activity was assessed in the presence and absence of calmodulin by measuring phosphorylation of rhodopsin (membrane-bound substrate) or tubulin (soluble; a kind gift from Jens Müller, DESY, Hamburg, Germany) using ^{32}P-ATP. To mimick phosphorylation of GRK2 at Ser-29, we constructed a Ser29Asp mutant (S29D). GRK2 interacted with calmodulin in the presence of 5 mM EGTA and the interaction increased at 1 mM Ca^{2+}. Calmodulin was able to interact with both GRK2(1–53) and GRK2(552-689), in agreement with predictions from the calmodulin target database (http://calcium.uhnres.utoronto.ca/ctdb/), but interaction with the N-terminus occurred only in the presence of 1 mM Ca^{2+} whereas interaction with the C-terminus was evident in the presence of 5 mM EGTA. GRK2(1–53) S29D showed reduced interaction with calmodulin compared to GRK2(1–53). Calmodulin inhibited GRK2 in a strictly calcium-dependent manner. Rhodopsin phosphorylation was inhibited with higher potency (IC_{50} ~2 μM) than GRK2-mediated tubulin phosphorylation (IC_{50} ~100 μM). In a different set of experiments, calmodulin inhibited GRK2 S29D activity approximately 10-fold less potently than GRK2 activity. These experiments support the hypothesis that calmodulin is able to inhibit GRK2 interaction with membrane-bound substrates by interacting with the GRK2 N-terminus. This interaction (and thus the inhibition) is relieved if Ser-29 within the calmodulin binding site is phosphorylated by protein kinase C.

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C037

Structural determinants regulating P2Y12 purinergic internalization into a distinct population of clathrin-coated pits

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Upon activation many G protein-coupled receptors (GPCRs) internalise by clathrin-mediated endocytosis and are subsequently sorted to undergo recycling or lysosomal degradation. Recent research from our laboratory (Mundell *et al.*, 2006) and others (Puthenveedu and von Zastrow 2006) has suggested that there may be multiple populations of clathrin-coated pits (CCPs) available to selectively sort GPCR cargo. In this present study we examined the structural determinants present in the COOH tail of the $P2Y_{12}$ receptor that regulate their internalization into CCPs. These studies were undertaken either in CHO cells stably transfected with receptor constructs or in HEK293 cells. N-terminal tagged (either HA or FLAG) receptor constructs were co-transfected into cells using Lipofectamine. Surface receptor surface loss was measured by ELISA and cellular distribution of HA-tagged or FLAG-tagged receptor examined by immunofluorescence microscopy as previously described (Mundell *et al.*, 2006). Our initial studies confirmed that deletion of the last four amino acids (E^{339}stop), a PDZ motif (ETPM), of the $P2Y_{12}$ receptor attenuated receptor internalization. Interestingly mutation of P341 to alanine in this motif also attenuated receptor internalization. Subsequent immunofluorescent studies revealed that both P341A and E^{339}stop trafficked to clathrin-coated pits but did not colocalize with full length $P2Y_{12}$ receptor at these sites. In addition colocalization with arrestin, which regulates $P2Y_{12}$ receptor entry into CCPs was not evident for E^{339}stop. Subsequent experiments revealed that following internalization both E^{339}stop and P341A did not traffic normally back to the cell surface but were retained in an as yet uncharacterised endosomal compartment. This intracellular retention attenuated receptor resensitization. In conclusion this study demonstrates that the presence and integrity of a PDZ motif on the $P2Y_{12}$ receptor is essential for correct targeting to distinct populations of CCPs and subsequent receptor internalization and traffic.

References:

Mundell *et al.*, Traffic. 2006; 7(10): 1420–1431.

Puthenveedu and von Zastrow Cell. 2006; 127(1): 113–124.

C038

The analysis of group effects (AGE) on the binding of drugs to receptors: what you can do with a library of values of log.K (pA_2)

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Published values of log.K (pA_2) for 496 competitive antagonists acting at muscarinic (M_3) receptors in isolated guinea-pig *ileum* at 37°C, collected in the package DIXDATA, have been used to calculate the 'group effect' (ΔlogK) for large numbers (24–89) of pairs with and without a particular group. Values of group effects, analyzed from their cumulative frequency curves, in some instances fit a single population but most can be resolved into at least two components, indicating different areas of binding. Groups can have positive effects in some pairs of compounds but negative effects in others suggesting there may be a limit to the size of the group which can be accommodated. For hydroxyl groups and others which have only small apparent molal volumes it is likely to involve interactions with water. Cumulative frequency curves for the effects of temperature on affinity for these muscarinic receptors show that an appreciable fraction of the group effect on binding is associated with changes in entropy, even though the compounds are paired. From the geometry of the drugs it is possible to construct crude models showing areas (or pockets) in the receptor with which the drugs may interact but these need to be checked against models derived from aminoacid sequences. The validity of these models likewise needs to be checked by comparing values of log.K which they may provide against the experimental ones. Comparisons can be made between receptor types: the effect of replacing hydrogen by an aromatic ring in antagonists acting at nicotinic receptors in the frog *rectus abdominis* is smaller than for muscarinic receptors in ileum. This paper shows what you can do with a library of values of log.K (pA_2). It would be particularly interesting to compare group effects for muscarinic receptors with those for histamine H1 receptors in ileum.

C039

Effects of rosiglitazone and sumatriptan in human isolated small and large coronary arteries

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Recently, the FDA expressed concerns over a potential increased risk for heart attacks in relation to rosiglitazone (www.fda.gov/bbs/topics/NEWS/2007/NEW01636.html) (PPARγ agonist used in diabetes management). Coronary vasoconstriction, a known side effect of some drugs such as triptans, has been demonstrated in human isolated tissues (MaassenVanDenBrink, A *et al.*, 1998). Therefore, in the current study, coronary constrictor potential of rosiglitazone was compared to sumatriptan and 5-HT in human isolated small and large coronary arteries (HCA). HCA were taken from three different non-diseased hearts obtained from ethically approved organ procurement organisations. Small HCA (SHCA, 0.3–0.5 mm internal diameter (i.d.)) and large HCA (LHCA, 1–2 mm i.d.) rings were dissected out, suspended in organ baths containing Krebs' physiological salt solution, gassed with 95% O_2/5% CO_2 and maintained at 37°C. After initial viability assessments with KCl, compounds were assessed either under basal or endothelin-1 (ET-1) pre-contracted conditions (LHCA only). Subsequently, function of the endothelium was assessed pharmacologically. In rings from all three donors, KCl (30–100 mM), $PGF_{2\alpha}$ (1 μM) and ET-1 (0.1–10 nM) caused contraction, whereas sodium nitroprusside (100 μM) caused relaxation. Substance P (1–10 nM) caused relaxation in the majority of rings tested demonstrating a functional endothelium. Sumatriptan and 5-HT but not rosiglitazone (1 nM–10 μM) caused concentration-dependent contractions that were similar in both SHCA and LHCA. Compared to sumatriptan, 5-HT was ~3-10-fold more potent and caused approximately double the contraction magnitude. Vehicle (DMSO) caused small but variable relaxation. In conclusion, rosiglitazone did not contract HCA in these preliminary studies. Further studies are ongoing to understand the significance of these findings in relation to the reported clinical side effects.

References:

MaassenVanDenBrink, A *et al.* Circulation 1998; (98): 25–30.

www.fda.gov/bbs/topics/NEWS/2007/NEW01636.html.

Table 1. Effect of rosiglitazone, sumatriptan and 5-HT in human isolated coronary arteries

Treatment CEC	Small HCA		Large HCA			
	Basal tone		Basal tone		ET-1 elevated tone	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}	pEC_{50}	E_{max}
Rosiglitazone	–	8.3 ± 9.8	–	−21.6 ± 15.3	–	−9.8 ± 3.8
Sumatriptan	6.4 ± 0.4	42.7 ± 13.9	6.5 ± 0.1	45.8 ± 17.4	6.2 ± 0.1	31.6 ± 9.9
5-HT	7.4 (7.1–7.6)[a]	95.7 (79–112)[a]	6.9 ± 0.1	89.6 ± 13.3	6.9 (6.5–7.3)[a]	85.2 ± 23.5
Vehicle	not tested	not tested	–	−1.9 ± 1.6	–	−18.1 ± 13.4

Data are mean ± s.e.mean (n = 3–6 rings, 3 donors), or [a]range (n = 2 rings, 2 donors). E_{max} = % KCl 100 mM

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Cell signalling (14.30–15.15)

C040

L-carnitine protects neuroblastoma (SH-SY5Y) cells from oxidative stress by mitochondria, stress response proteins and GADD genes

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Oxidative stress plays an important role in neurodegenerative disorders and H_2O_2-induced oxidative toxicity is a well-described model of oxidative stress-induced neurodegeneration. L-carnitine (LC) is an endogenous mitochondrial membrane compound and some studies have reported that LC could effectively protect various functions of mitochondria and cells against oxidative injury both *in vitro* and *in vivo*. However, the exact molecular mechanism of LC on oxidative stress in neurodegeneration is unclear. In the present study we used the human neuroblastoma SH-SY5Y cell line as an *in vitro* model and assessed the effect of L-carnitine on hydrogen peroxide (H_2O_2)-mediated oxidative stress and neurotoxicity. Cells in culture were treated for 24 h with 100, 200, 300, 400, 500 μM H_2O_2 alone or pretreated with 0.1, 1, 10, 100, 300 and 1000 μM L-carnitine. H_2O_2 produced a dose-related decrease in cell viability as measured by Trypan blue exclusion assays, a reduction in the mitochondrial metabolism of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and an increase in DNA fragmentation analysis. Pretreatment with LC 2 h inhibited H_2O_2-induced cell death in a concentration-dependent manner. And analysis of DNA fragmentation showed that LC could prevent H_2O_2-induced DNA damage and apoptosis. Western blot analysis showed that LC could inhibit the release of of cytochrome c from mitochondria and upregulated the expression of heat shock proteins (HSP27, HSP 70 and HSP 90) levels compared with untreated control cells. Meanwhile, the expression of two endogenous anti-oxidant defense components, growth arrest and DNA damage-inducible (GADD) mRNA, GADD45 and GADD153, were observed at the early phase during cell death in 400 μM H_2O_2 –treated control groups and LC could elevate the expression of GADD genes to protected DNA from oxidative damage. Taken together, these results demonstrate that LC exerts protective effects against oxidative stress in part by protect mitochondria and up-regulating the levels of endogenous anti-oxidant defense components and stress proteins, GADD genes and HSPs. This evidences support the pharmacological potential of LC in the management of oxidative stress, neurotoxicity and neurodegenerative diseases.

C041

Rosiglitazone inhibits GPVI-stimulated platelet activation through non-genomic signalling

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Rosiglitazone, the most potent of the currently marketed thiazolidinediones, acts by activation of the peroxisome proliferator-activated receptor-γ (PPAR γ). The PPARs are members of the nuclear hormone receptors that heterodimerise with the retinoid X receptor (RXR) and then modulate transcription of many target genes (Moraes *et al.*, 2005). Although platelets are anucleate cells, recent reports demonstrate that they express the intracellular receptors PPARγ (Akbiyik, 2004), PPARβ/δ (Ali 2006), GR (Moraes *et al.*, 2005) and the RXR (Moraes *et al.*, 2007). Previous work has demonstrated that the treatment of diabetes with PPARγ agonists is associated with a reduced risk of some cardiovascular complications. We have, therefore, examined the effects of PPARγ agonists on platelet function. Washed platelets were stimulated with PPARγ ligands and collagen-induced aggregation was measured using optical aggregometry. Calcium levels were measured by spectrofluorimetry in Fura-2AM loaded platelets. Tyrosine phosphorylation levels of early signalling components of the GPVI signalling pathway were measured using imunoblot analysis. The role of PPARγ agonists in thrombus formation was assessed using an *in vitro* flow system, where fluorescently labelled whole blood was perfused through a collagen coated capillary at a shear rate of 1000/s in the presence or absence of PPARγ agonists. Thrombus volume was quantified by confocal microscope using Leica Sp2 software. In this study, we report that PPARγ ligand rosiglitazone inhibits collagen-stimulated platelet aggregation. This was accompanied by a reduction in collagen-stimulated intracellular calcium mobilization and reduction of thrombus formation on immobilised collagen under arterial flow conditions in a concentration-dependent manner. This was accompanied by inhibition of collagen-stimulated tyrosine phosphorylation of phospholipase Cγ2. We propose that the PPARγ ligands may have beneficial clinical actions through inhibition of platelet activation. Furthermore our results demonstrate a novel non-genomic mode for nuclear receptor action, and functional cross-talk between the collagen receptor GPVI and nuclear receptor signalling families in platelets.

C042

Identification of the anti-apoptosis activity of nerve growth factor on cardiac myocytes

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Neurotrophins (NTs) control the survival and regeneration of neurons. Recent research showed that NTs posses cardiovascular actions. In this study, we investigated the hypothesis that the NT nerve growth factor (NGF) prevents cardiomyocyte apoptosis. We demonstrated that cultured rat neonatal cardiomyocytes (RNCMs) produce NGF and express its trkA receptor. RNCMs given a neutralizing antibody for NGF or the trkA inhibitor K252a underwent apoptosis, thus suggesting that NGF is an endogenous pro-survival factor for cardiomyocytes. Recombinant NGF induced trkA phosphorylation, followed by Ser473-phosphorylation and nuclear translocation of Akt in RNCMs. In response to Akt activation, Forkhead transcription factors Foxo-3a and Foxo-1 were phosphorylated and excluded from the nucleus. Adenovirus (*Ad*)-mediated *NGF* over-expression RNCMs protected RNCMs apoptosis induced by either hypoxia/reoxygenation or angiotensin II. Inhibitory approaches using K252a, LY294002 (a pan-phosphatidyl inositol 3-kinase -PI3K- inhibitor), and adenoviruses carrying a dominant negative mutant form of Akt (*Ad.DN.Akt*) or an Akt-resistant Foxo-3a (*Ad.AAA-Foxo-3a*) demonstrated that the pathway encompassing trkA, PI3K-Akt, and Foxo is essential for the pro-survival effect of *NGF*. The anti-apoptosis action of NGF was confirmed in adult myocytes extracted from the mouse heart, which were submitted to the angiotensin II apoptosis test in the presence of recombinant NGF. Finally, intramyocardial *NGF* gene transfer prevented cardiomyocyte apoptosis in a murine model of myocardial infarction.

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Education (16.00–16.30)

C043

Communicative methods of teaching: A structuralistic model of the construction of knowledge in problem based learning

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Although methodology of teaching has been developed extensively the last years, teachers of science and biological sciences usually use a methodology of teaching, which is about 70-years-old. During the last few years, an effort has been made in medical, to use contemporary communicative methods of teaching, like problem based teaching. Nevertheless, it is obvious that lack of theoretical knowledge on the procedure of learning and on methodology of teaching restricts the application of communicative methods of teaching. The aim of this work is to present the theories of constructivism in learning, and to develop a new structuralistic model of the construction of knowledge in integrated methods of teaching, which may serve as the theoretical basis for problem based learning (PBL) and problem based teaching (PBT). The psychological constructivism of Piaget, the social constructivism of Durkheim and the radical constructivism of von Glaserfeld have given the basis for the development of communicative methods of teaching. Neo–Piazetian theories have developed models of learning process and have suggested new strategies for solving problems in teaching and learning. Understanding memory and its organization can make teaching and learning easier. Factors influencing memory and learning are: motivated attention, grade of familiarity with new information, the way of classifying and relating new information with old data, chunking of perceived information, repetition, and knowledge of rules of memory processing and data storing. Problem based teaching is a communicative, student oriented method of teaching, which offers strong motivation for learning and memorizing. In this work, as well as presenting the theoretical background, models of the construction of knowledge are presented briefly, as well as the new structuralistic model of the construction of knowledge in PBL, which has been developed by the author. All the data are presented in a simple, interactive and friendly to the audience manner, with a lot of images and in a way that provides strong motivation for participation of the audience. Structuralistic theories of cognitive development have given new tools in methodology of teaching, and lead to the development of communicative and very effective methods of teaching and learning, like PBL and PBT.

C044

Preparation of list of essential cardiovascular drugs for medical school teaching

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An essential task facing those teaching medical pharmacology is to decide what drugs must be covered. This study aimed to prepare a list of essential cardiovascular drugs using evidence-based medicine. We first determined the CVS drugs in the top 200 drugs in 2006 used in hospitals and prescribed by trade or generic names in the US. There were marked differences in the drugs listed in these three categories. CVS drugs totalled about 70. We next listed all drugs included in Guidelines of the American College of Cardiology for management of hypertension, chronic & unstable angina, myocardial infarction, cardiac failure, atrial fibrillation, ventricular arrhythmias, and peripheral vascular disease. Because such Guidelines do not always name individual drugs but rather classes of drugs (e.g. ACE Is; ARBs; CCBs etc), we used the drugs listed in the top 200 drugs in 2006 in the US to determine members of such groups. This combined approach yielded a list of about 60–70 drugs. We next examined the number of these 60–70 drugs covered in latest editions of commonly used US textbooks. Major texts (e.g., Goodman & Gillman, 2005; Katzung, 2006 and Golan *et al.*, 2008) covered essentially all the drugs. Shorter texts designed to be less comprehensive not surprisingly covered fewer of these drugs, the chief difference being the number of individual drugs in major classes covered. We next examined whether the number of drugs within such groups (e.g., ACE Is; ARBS; CCBS; beta blockers etc) could be reduced by comparing relative efficacies of such drugs using data from Evidence-based Practice Centers, listed by the Agency for Healthcare Research and Quality. However, with the exception of beta-blockers in cardiac failure, little or no significant differences in the efficacies of drugs within a given group have been found. The Consumers Reports Best Buy Drugs Program is distinguishing between such groups of drugs based on other factors (e.g. safety, ease of administration, cost etc). Using these combined approaches a list of about 30–35 essential cardiovascular drugs was prepared. Whenever possible USAN stems for drugs with similar mechanisms of action (e.g.-olol for beta blockers; -pril for ACE Is; –sartan for ARBs; -asozin for alpha$_1$ antagonists; -vastatin for HMG-CoA inhibitors) should be pointed out to students to simplify their study of CVS pharmacology and therapeutics.

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Pain and inflammation (14.30–15.15)

C045

Lipopolysaccharide augments the contractions of rat prostatic *vas deferens* via mechanisms that do not involve the endothelin receptors

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Lipopolysaccharide (LPS)-induced hyporesponsiveness of isolated smooth muscles to vasoconstristors is a hall-mark of experimental sepsis. Contrarily, alpha adrenoceptor-induced contractions of mesenteric arteries from LPS-treated mice were reported to be enhanced *via* the activation of Rho-kinase pathway which involves also the endothelin peptides (Buyukafsar *et al.*, 2004).Thus, we investigated the effect of LPS on the contractile responsiveness of rat prostatic vas deferens with special emphasis on the role of endothelin peptides. Wistar albino rats (250–350 g) pretreated with LPS (4 mg/kg, ip) was then given bosentan (30 mg/kg, ip twice at 2nd and 12th hour after LPS). Parallel controls received saline (0.9% NaCl, ip). At 24th hour, prostatic sections of vas deferens were isolated into organ baths containing Krebs-Henseleit solution at 37°C and contracted by electrical field stimulation (EFS, 0.1–100 Hz, supramaximal voltage, 2 min duration for 10 s) or by cummulatively added phenylephrine (0.1 μM–0.1 mM). All data were expressed as means ± SEM of number (n) of observations. Ordinary one-way ANOVA or two-way ANOVA for repeated measures were used where appropriate and statistical significance was accepted when $P < 0.05$. LPS significantly augmented the contractile responses to EFS (e.g. mg contraction to 3 Hz, control: 3.6 ± 0.4; LPS: 5.1 ± 0.4, $P = 0.0076$, $n = 15$) and to phenylephrine (i.e. two-way ANOVA for repeated measures applied to curves obtained from control versus LPS-treated animals revealed $P = 0.0486$). However, bosentan had no significant effect on neither EFS nor phenylephrine-induced contractions. Therefore, we conclude that LPS augments the EFS- or alpha adrenoceptor-mediated contractions of rat prostatic vas deferens *via* mechanisms that do not involve the endothelin receptors.

Reference:

Buyukafsar *et al.*, Eur J Pharmacol. 2004; 498: 211–217.

C046

A potential inhibitory role of hydrogen sulphide on carrageenan-induced acute arthritis in rats

E Ekundi-Valentim, A Barreto, S Teixeira, M Muscará, S Costa *University São Paulo, Department of Pharmacology, São Paulo, Brazil*

Hydrogen sulphide (H_2S), a well known environment pollutant, has been proven to be produced endogenously in mammalian tissues and now seems to play an emerging role in physiological and pathophysiological conditions (Szabó, 2007). This study was undertaken to evaluate the relevance of H_2S in carrageenan (CGN)-induced experimental acute arthritis. Male Wistar rats (180–220 g) were subjected, under halothane anaesthesia, to intra articular injection (i.art. injection) of 3% CGN or saline (50 μl; control group). Sixty min before CGN injection, either an inhibitor of H_2S formation, DL-propargylglycine (PAG; 53 mmol/knee joint), or an H_2S donor, Lawson's reagent (3.6 μmol/knee joint), was injected i.art. in the ipsilateral (IPSI) knee joint. Following 4 h CGN injection, functional assays revealed that the IPSI knee of vehicle-treated rats exhibited a potent oedema associated with pain scored behaviour and high contents of myeloperoxidase (MPO) and inducible nitric oxide synthase (iNOS) activity in the synovial fluid. Treatment with Lawson's reagent significantly attenuated the CGN-induced oedema (3.32 ± 0.1 and 2.47 ± 0.2 mm** for control and treated, respectively; $n = 7–8$), pain score (1.7 ± 0.2 and 0.87 ± 0.2* for control and treated) and MPO activity (281 ± 41.6 and 74 ± 9.6** U/cavity for control and treated), but failed to reduce increased iNOS activity (0.44 ± 0.2 and 2.4 ± 0.3 pmol/min/mg protein for control and treated). In contrast, the treatment of rats with PAG had no effect on CGN-induced arthritic signs and further potentiated synovial iNOS activity (4.45 ± 0.24*** pmol/min/mg protein; $n = 4$). We show for the first time that exogenous supply of H_2S in the knee joint allowed a significant reduction of CGN-induced acute arthritis signs and symptoms, although did not prevent the up-regulation of iNOS, which may contribute to the pathogenesis of arthritis.

FAPESP and CNPq for financial support. Ekundi is a recipient of a grant from University Agostinho Neto, Angola.

Reference:

Szabó C. Nat Rev Drug Discov. 2007; 6(11): 917–35.

C047

Up-regulation of histamine H1R receptor and TNF-α gene expression by exogenous administration of histamine in the rat paw

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In addition to provoking acute symptoms of inflammation, histamine may be involved in late phase allergic responses, as implied by reports of H1R up-regulation in the nasal mucosa of patients with allergic rhinitis, as well as in animal models thereof (Dinh *et al.*, 2005; Murata *et al.*, 2004). In following recent reports indicating that histamine can induce H1R expression in various types of cells in culture (Das *et al.*, 2007), we recorded the time course of H1R gene expression, as well as that of TNF-α, following intradermal administration of histamine in the rat paw. Male Wistar rats (250–300 g) were used in this study. Acute reaction to local subcutaneous histamine administration (100 μl, 5 mM) was followed by measuring paw oedema formation, with the use of a plethysmometer. H1R and TNF-α gene expression at various time-points following histamine administration was determined semi-quantitatively by conventional, end-point, RT-PCR on RNA isolated from subcutaneous tissue excised from rat paws. Histamine induced a significant increase in steady state mRNA levels of both H1R and TNF-α in the rat paw. The time course of this up-regulation was specific for the two transcripts. H1R expression was relatively low during the first 3 h, peaked at hours four to five, and then fell again at hour six, post-histamine. TNF-α gene expression responded in biphasic manner, with a steady increase during the first 3 h, followed by a reduction at hour four and a new increase, but at a lower rate, over hours five and six following the injection of histamine into the paw. This complex pattern of H1R and TNF-α gene expression is suggestive of histamine's ability to elicit late phase allergic responses.

FAPESP and CNPq for financial support. Ekundi is a recipient of a grant from University Agostinho Neto, Angola.

References:

Das AK *et al.*, J Pharmacol Sci. 2007; 103: 374–382.
Dinh QT *et al.*, Clin Exp Allergy. 2005; 35: 1443–1448.
Murata Y *et al.*, Inflamm Res. 2004; 53 (Suppl 1): S11–12.

Obesity (16.00–16.30)

C048

Age-related changes in cardiovascular risk factors among type 2 diabetic patients compared to age- matched healthy control subjects

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Type 2 diabetes mellitus (T2DM) accounts for about 95% of diabetic patients (over 190 million globally). In the UK it costs the NHS system round £5.2 billion annually to diagnose, treat and care for DM patients with long- term complications. Cardiovascular dysfunctions are a major cause of morbidity and mortality in DM patients. This study attempted to identify biochemical risk factors in the plasma of type II diabetic patients compared to healthy male and female subjects employing different age groups (15–25 years, 26–40 years, 41–60 years and 61–80 years). Levels of insulin, glucose, triglycerides (TG), total cholesterol, High density lipoprotein (HDL), low density lipoprotein (LDL), C-reactive protein (CRP), homocysteine (HCys), tumour necrosis factor (TNF-α) and interleukin-6 (Il-6) were measured using established commercial assay procedures. The results show no significant differences in the age groups of T2 diabetes compared to controls. T2 diabetics have slightly elevated plasma insulin and body mass index compared to healthy controls. There were significant ($P < 0.05$) increases in plasma glucose levels in T2 diabetics compared to healthy controls in all four age groups. Both TG and total cholesterol were significantly ($P < 0.05$) elevated in diabetics compared to health subjects in all four age groups. Diabetics have elevated LDL and decreased HDL compared to controls. The levels of CRP and HCys increased significantly ($P < 0.05$) in all diabetic subjects compared to controls. In contrast, TNF-α remained more or less constant in control and diabetic subjects in all age groups, In contrast, IL-6 increased gradually in diabetic subjects compared to controls but this was only significant at age groups, 41–60 years and 61–80 years. The results clearly indicate that all diabetic subjects display signs of obesity, hyperglycemia, dyslipidemia and low grade inflammation compared to healthy controls, but inflammatory cardiovascular risk factors seem to be more age-dependent.

C049

The effect of montelukast in metabolic syndrome in rats

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Metabolic syndrome is associated with systemic inflammatory status and increased coronary heart pathology. Leukotrines (LTs), lipid mediators, are involved in atherosclerosis and other inflammatory cardiovascular diseases. This study investigates the effect of montelukast (MK, cysteinyl-LT1 R antagonist) in metabolic syndrome in rats. We worked on 4 groups of 8 male Wistar rats each (4 weeks of age) weighing between 40–45 g which received as follows: group I (control) - saline, group II - MK 10 mg/kg/day for 15 weeks, group III - high fat diet (HF, 58% saturated fatty acids) for 15 weeks, group IV - MK (the same dose as group II) and HF diet for 15 weeks. After 15 weeks, we determined body weight, glycemia, blood pressure, insulin, glucose and insulin resistance, lipid profile, uric acid, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione peroxidase (GPx) in blood and in liver homogenate. A histopathological exam of liver, kidney and aorta was performed. For statistical analysis we used analysis of variance (ANOVA one way) followed by Tukey's multiple comparison tests. Compared to control group, group III exhibited statistical significant increased levels of uric acid (2.35 ± 0.84 vs. 3.71 ± 0.85 mg/dL, $P = 0.017$), total cholesterol, low density lipoprotein cholesterol, triglycerides and oxidative stress parameters. The group IV presented statistical significant reduced levels of triglycerides (161.33 ± 46.51 vs. 215.83 ± 55.84 mg/dL, $P = 0.002$), MDA (2.85 ± 0.17 vs. 4.32 ± 0.41 nmol/mL, $P = 0.001$), increased levels of GSH (49.72 ± 15.02 vs. 27.38 ± 11.52 microg/mL, $P = 0.048$) and SOD (46.88 ± 2.85 vs. 33.2 ± 4.43% inhibition, $P = 0.0007$) in liver homogenate, CAT in blood (12481.04 ± 1202.81 vs. 8953.33 ± 614.09 U/min/mL, $P < 0.0001$) compared to group III. The glucose intolerance was observed in group III but not in other groups (199.33 ± 5.88 glucose blood levels in group III vs. 111.33 ± 11.21 mg/dL in control group, 1 h after glucose administration). The admnistration of MK reduced the hepatic steatosis induced by HF feeding. In conclusion, MK has a partial protective effect in metabolic syndrome in Wistar rats.

C050

***PAX4* enhances differentiation of human embryonic stem cells to insulin-secreting cells**

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Guiding human embryonic stem cells (hESC) to differentiate into functional insulin-secreting β-cells *in vitro* can serve as an unlimited renewable source for cell-transplantation to treat diabetes. Since the transcription factor PAX4 initiates terminal beta-cell differentiation during murine pancreatic development, we investigated whether constitutive expression of PAX4 in hESCs could promote progression towards a functional beta-cell phenotype *in vitro*. H7 hESC stably-transfected with *Pax4* (H7.Px4) and untransfected H7 controls (H7) was allowed to spontaneously differentiate over a 3 week period following embryoid body (EB) formation. Expression of genes important during hESC maintenance, pancreatic development and mature β-cell function were assessed using standard RT-PCR and quantitative PCR at each differentiation time-point. Mature EBs were enzymatically dissociated and subjected to fluorescence-activated cell-sorting (FACS) using Newport Green to isolate a Zn^{2+}-positive population of cells. The Zn^{2+}-positive cells were subsequently assayed for c-peptide secretion using an ELISA kit. *OCT4*, *ISL1*, *NEUROD1*, *KRT19*, *SLC2A1*, *GCK*, *ABCC8* and *KCNJ11* transcripts were expressed at all time points in both control and H7.Px4 EBs ($n=3$ for each). Interestingly, Q-PCR revealed substantially higher levels of expression of *PDX1* and *INS* mRNA in H7.Px4 EBs than H7 control EBs ($n = 3$) at the mid- to late- stages of differentiation. Following FACS, the Zn^{2+} positive cells were found to be positive for *INS* expression by RT-PCR and Q-PCR, they also contained c-peptide protein (77 ± 7 pg/10^4 cells; $n = 3$) and secreted c-peptide in response to stimulation with the insulin-secretagogue, tolbutamide (100 μM; basal 23 ± 5 pg/10^4 cells/15 min vs. tolbutamide 68 ± 4 pg/10^4 cells/15 min; $n = 3$; Student's *t*-test $P < 0.001$). These studies describe for the first time the enhancement of beta-cell differentiation in hESC by constitutive expression of PAX4, and also a novel method to separate differentiating insulin-secreting cells from undifferentiated precursors.

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