

The role of ATP and adenosine in the brain under normoxic and ischemic conditions

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Abstract By taking advantage of some recently synthesized compounds that are able to block ecto-ATPase activity, we demonstrated that adenosine triphosphate (ATP) in the hippocampus exerts an inhibitory action independent of its degradation to adenosine. In addition, tonic activation of P2 receptors contributes to the normally recorded excitatory neurotransmission. The role of P2 receptors becomes critical during ischemia when extracellular ATP concentrations increase. Under such conditions, P2 antagonism is protective. Although ATP exerts a detrimental role under ischemia, it also exerts a trophic role in terms of cell division and differentiation. We recently reported that ATP is spontaneously released from human mesenchymal stem cells (hMSCs) in culture. Moreover, it decreases hMSC proliferation rate at early stages of culture. Increased hMSC differentiation could account for an ATP-induced decrease in cell proliferation. ATP as a homeostatic regulator might exert a different effect on cell trophism according to the rate of its efflux and receptor expression during the cell life cycle. During ischemia, adenosine formed by intracellular ATP escapes from cells through the equilibrative transporter. The protective role of adenosine A₁ receptors during ischemia is well accepted. However, the use of selective A₁ agonists is hampered by unwanted peripheral effects, thus attention has been focused on A_{2A} and A₃ receptors. The protective effects of A_{2A} antagonists in brain ischemia may be largely due to reduced glutamate outflow from neurones and glial cells. Reduced activation of

p38 mitogen-activated protein kinases that are involved in neuronal death through transcriptional mechanisms may also contribute to protection by A_{2A} antagonism. Evidence that A₃ receptor antagonism may be protective after ischemia is also reported.

Keywords Adenosine · Adenosine A_{2A} receptors · ATP · Ecto-ATPase inhibitors · Ischemia · P2 purinergic receptors

Abbreviations

ATPγS	Adenosine-5'-o-(3-thio)triphosphate
α,β-meATP	Alpha,beta-methylene ATP
ARL 67156	6-N,N-diethyl-D-β,γ-dibromomethylene ATP
BBG	Brilliant blue G
BGO 136	1-Hydroxynaphthalene-3,6-disulfonate
CGS 15943	9-Chloro-2-(2-furanyl)-[1,2,4] triazolo[1,5-c]quinazolin-5-amine
CGS 21680	2-p-(2-Carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine hydrochloride
Cl-IB-MECA	1-[2-Chloro-6[[[3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-N-methyl-β-D-ribofuranuronamide
DPCPX	8-Cyclopentyl-1,3-dipropylxanthine
fEPSP	field extracellular postsynaptic potential
GIRK	Kir 3.2 and 3.4 channels: potassium inward rectifiers
hMSCs	Human mesenchymal stem cells
LTP	Long-term potentiation
LTD	Long-term depression
MAPKs	Mitogen-activated protein kinases
MRS 1191	3-Ethyl 5-benzyl-2-methyl-6-phenyl-4-phenylethynyl-1,4-(6)-dihydropyridine-3,5-dicarboxylate

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MRS 2179	2'-Deoxy-N ⁶ -methyladenosine 3',5'-bisphosphate
NTPDase	Nucleoside triphosphate diphosphohydrolase
OGD	Oxygen/glucose deprivation
PPADS	Pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid
PS	Population spike
PV4	Hexapotassium dihydrogen monotitanoundecatungstocobaltate(II) tridecahydrate, K ₆ H ₂ [TiW ₁₁ CoO ₄₀]·13H ₂ O
RB2	Reactive blue 2
SCH 58261	5-Amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine
ZM 241385	4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]ethyl)phenol

Historical overview

The physiological roles of adenosine triphosphate (ATP) and its metabolite adenosine have been historically linked to cell metabolism since ATP is an ubiquitous intracellular energy source in a number of enzymatic processes. However, over the years both metabolites have emerged as very versatile molecules of biological systems, being implicated in a variety of cell processes, from platelet aggregation to neurotransmission. The term 'purinergic signalling' was first introduced in the scientific literature by Burnstock [1]. An implicit concept for sustaining the hypothesis of purinergic neurotransmission proposed in the 1970s was the existence of purinergic receptors. The first evidence in this direction suggested the existence of two different subfamilies of such 'purinoceptors', identified as P1 and P2 receptors, selective for adenosine and ATP respectively [2]. Four different subtypes of P1-G-protein-coupled receptors: A₁, A_{2A}, A_{2B} and A₃ are known at present [3], whereas P2 purinoceptors belong to two major families: P2X ligand-gated ion channel receptors and P2Y G-protein-coupled receptors [4]. Cloning experiments supported this classification and helped to subdivide P2 receptors into seven P2X and eight P2Y subtypes [5] (plus the recently deorphanized GPR17 receptor [6]).

Roles of ATP in neurotransmission under normoxic conditions

The first studies on the role of extracellular ATP in hippocampal neurotransmission indicated prominent inhibitory action of this purinergic nucleotide on synaptic activity [7, 8]. However, several lines of evidence led to the hypothesis that ATP-mediated inhibition of hippocampal neurotransmission was probably mediated by adenosine acting on A₁

receptors. In fact, this response was theophylline-sensitive [7–9] and absent in A₁ knockout mice [10]. This assumption was supported by the fact that, in the hippocampus, extracellular ATP is rapidly converted into adenosine by ecto-ATPases and ecto-nucleotidases [11] and that adenosine acting on A₁ receptors exerts a well-described inhibitory role on CA1 excitatory neurotransmission [3]. In recent years, a more detailed observation of ATP-evoked effects, supported by the synthesis of new pharmacological tools, has helped to clarify the effective role of purinergic nucleotides in the hippocampus. In particular, it emerged that ATP mediates inhibitory effects by P2 receptor activation [12–14].

In a recent paper, we contributed to elucidating the role of ATP on CA1 hippocampal neurotransmission, and we reinforced the concept that ATP-mediated effects are not necessarily linked to adenosine formation in this brain region [15]. ATP is hydrolysed by ecto-NTPDase, enzymes located on the cell surface in the CNS that limit ATP, ADP and AMP spatio-temporal activity [11, 16]. There are three different known NTPDases: NTPDase1 hydrolyzes ATP and ADP equally well, NTPDase2 has a high preference for ATP, NTPDase3 is a functional intermediate, preferably hydrolyzing ATP [17]. In our study, we took advantage of some recently synthesized compounds: ARL 67156, which, at micromolar concentrations, inhibits rat NTPDase1 and 3 transiently transfected in Chinese hamster ovary cells, showing negligible activity on NTPDase2 [18]; BGO 136, a new inhibitor described as a selective NTPDase1 and 2 blocker with *K_i* values in the high micromolar range [19]; and the recently synthesized PV4, which strongly inhibits rat NTPDase1, 2 and 3 with *K_i* values in the nanomolar range [20]. By using these inhibitors that are able to block NTPDase activity without interfering with P2 receptor activation, we demonstrated that ATP exerts an inhibitory action, independent of its degradation to adenosine. As shown in Fig. 1, the application of ATP during a NTPDase activity blockade still elicits a decrease in evoked synaptic responses which is even more pronounced than that evoked by ATP alone.

An excitatory effect of exogenous ATP on hippocampal neurotransmission has also been reported by different authors. This effect, which was observed after drug removal, was described to persist in *in vitro* preparations for a relatively prolonged period, up to 1 h [21–25]. This 'long-lasting' potentiation of synaptic responses was compared to electrically evoked LTP, firstly described in the hippocampus by Abrams and Kandel [26] and called 'ATP-induced LTP'. In agreement, we observed a potentiation of the synaptic responses after drug removal when slices were superfused in the presence of the metabolically stable ATP-analogue ATPγS (Fig. 2) [15]. In addition to this 'long-lasting' excitatory effect evoked by the exogenous application of P2 agonists, we also demonstrated an excitatory tone exerted by endogenous ATP.

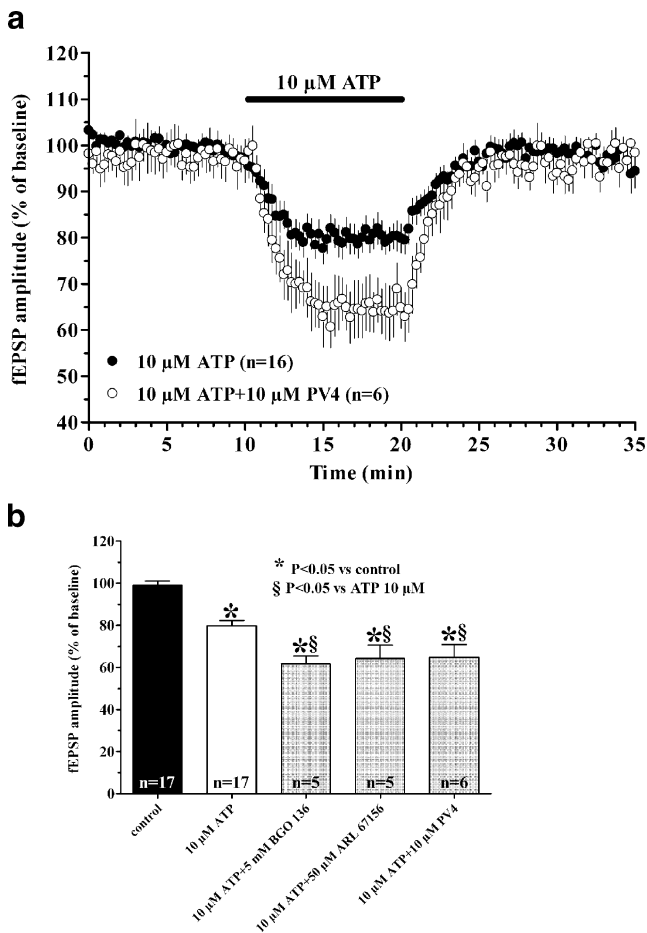


Fig. 1a, b The inhibitory effect induced by ATP on fEPSP amplitude is potentiated in the presence of different NTPDase inhibitors. **a** Time-course of fEPSP amplitude before, during and after the application of ATP in the absence or in the presence of the NTPDase1,2,3 inhibitor PV4. Each point in the graph represents the mean \pm SE of fEPSP value measured as percent of baseline, pre-drug level. **b** Columns in the graph summarize the average amplitude (mean \pm SE) of evoked fEPSP recorded from CA1 hippocampal region in control conditions, 5 min after superfusion of ATP alone and 5 min after ATP superfusion in the presence of different ecto-ATPases inhibitors. Note that the inhibitory effect of ATP on fEPSP amplitude is potentiated by BGO 136, PV4 and ARL 67156. * $P < 0.05$ one-way ANOVA, Newman-Keuls multiple comparison post-hoc test versus pre-drug value. § $P < 0.05$, one-way ANOVA, Newman-Keuls multiple comparison post-hoc test versus 10 μ M ATP treated slices. (Modified from [15])

Slices superfused with P2 antagonists show a small but significant reduction in synaptic transmission (Fig. 3). These data demonstrate that tonic activation of P2 receptors contributes to glutamatergic excitatory neurotransmission in the hippocampus, an observation that is in line with previous work [27].

Roles of ATP in ischemic conditions

The role of ATP may become critical during pathological conditions such as ischemia, when extracellular ATP concen-

trations increase. An enhanced outflow of radioactive ATP from hippocampal slices during in vitro ischemic-like insults was first reported by Juranyi and co-workers [28], and the first demonstration that ATP outflow increases in vivo during the induction of focal ischemia in the rat was reported by Melani and colleagues [29]. Thus, at the ischemic site, levels of extracellular nucleotides may remain elevated for long periods of time after injury. Evidence supports the idea that, under such pathological conditions, released ATP may exert an excitotoxic role by acting on its receptors, thus enhancing Ca^{2+} inward currents and altering synaptic activity and cellular plasticity. Non-selective antagonists of P2 receptors, suramin and PPADS, and the selective antagonists, BBG and MRS 2179 of P2X₇ and P2Y₁ receptors respectively, prevent the irreversible failure of neurotransmission induced by a prolonged period of OGD in hippocampal slices [15]. Moreover, they protect from development of anoxic depolarization (AD), which is a rapid and regenerative wave of depolarization that propagates in tissue and represents an unequivocal sign of suffering [15]. Antagonists of P2 receptors have been proved protective against cell death induced by either ATP itself, hypoglycemia or glutamate exposure in primary cultures of brain-derived neurones [30–33]. Intrastriatal ATP injection in rats induced, 24 h later, a clearly lesioned area [34]. The ATP-induced damage was concentration-dependent, mimicked by ATP γ S and α,β meATP (but not by ADP or adenosine) and blocked by RB2, a non-specific P2 antagonist [34]. Suramin, another non-specific P2 receptor antagonist, administered 30 min before occlusion of the middle cerebral artery, resulted in a significant decrease in infarct and oedema volume 6 h after brain injury [35]. In agreement, it was demonstrated that RB2 [36] and PPADS [37] improve neurological deficit and

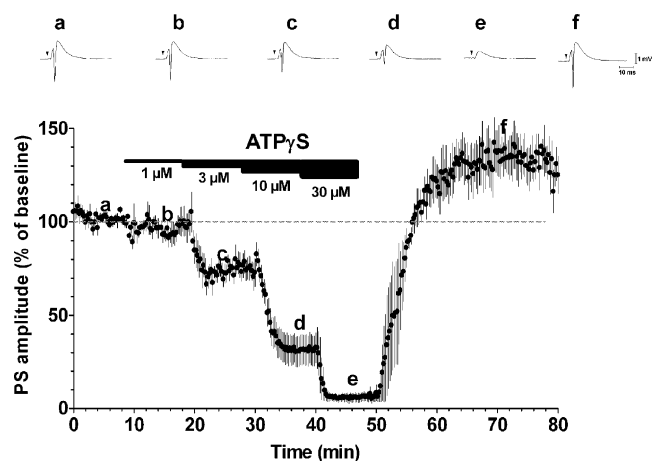
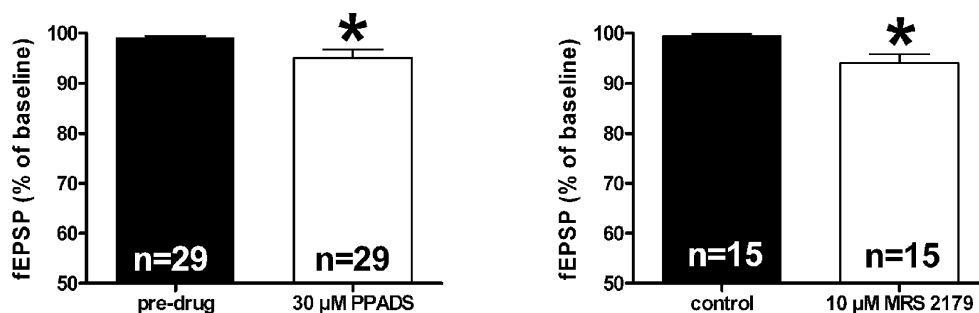


Fig. 2 Inhibitory and excitatory effects of the stable ATP analogue ATP γ S. Averaged time-course ($n=4$) of PS amplitude before, during and after the application of different concentrations of ATP γ S. PS amplitude (mean \pm SE) is measured as percent of baseline level. Upper panels represent single traces recorded in a typical experiment before, during and after ATP γ S application at different concentrations

Fig. 3 Excitatory effects of endogenous ATP. Bars in the graphs represent the average of fEPSP amplitude in the presence of P2 antagonists: PPADS (30 μ M) and MRS 2179 (10 μ M). * P <0.05, paired Student's t -test. (Modified from [15])



reduce the damage induced in rats in a model of focal ischemia in vivo. Moreover, RB2 induces the expression of P2X₇ receptors on reactive microglia in the remote ipsi and contralateral cingulate and medial frontal cortex and striatum. Although a pro-apoptotic role has been attributed to the P2X₇ receptor, results suggest that microglial cells expressing the P2X₇ receptor can be implicated in tissue damage as well as in the defence and repairing processes in the remote ipsi and contralateral undamaged areas [36].

In considering the effect of ATP during ischemia, it must be taken into account that ATP itself is involved in control of cerebrovascular regulation [38] and that the metabolite of ATP, ADP, that interacts with P2Y₁/P2Y₁₂ receptors, is a potent platelet aggregator. However, clinical studies that checked the antiplatelet therapy of the selective P2Y₁₂ receptor antagonist clopidogrel reported a response variability [39, 40].

Although evidence suggests that ATP exerts a detrimental role under ischemia, there are reports that it may exert a trophic role in terms of cell division and differentiation in both differentiated and undifferentiated cells [41–43], including

adult neural stem cells [44]. Interesting data correlate these trophic effects with the occurrence of spontaneous Ca²⁺ waves that propagate among adjacent cells in a self-renewing manner. Kawano et al. [45] reported that ATP-induced initiation and propagation of intracellular Ca²⁺ waves in human mesenchymal stem cells (hMSCs) promote activation of transcription factors (e.g. NFAT) that are involved in cell differentiation. In the same study, they demonstrated that ATP-induced Ca²⁺ waves disappear in the fully differentiated adipogenic phenotype. We recently reported that ATP is spontaneously released from hMSCs during the early stages of culture (P0-P5). Moreover, we reported that ATP decreases proliferation rate (Fig. 4) and modulates specific ionic current hMSCs [46]. A high extracellular ATP concentration at earlier cell culture passages suggests an important role of ATP in regulating cell differentiation. Increased hMSC differentiation may account for an ATP-induced decrease in cell proliferation.

Therefore, it can be envisaged that ATP, as an autocrine/paracrine homeostatic regulator, exerts different effects on

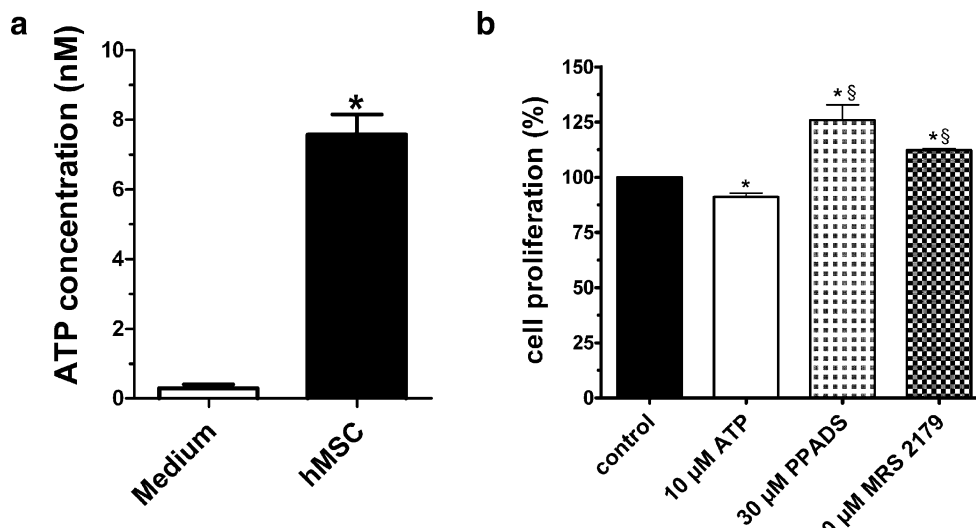


Fig. 4a, b Human mesenchymal stem cells in culture spontaneously release ATP that modulates cell proliferation. **a** Extracellular concentrations of ATP were measured in the medium containing hMSCs and in control medium not containing cells. Data are expressed as mean \pm SE, n =11, unpaired Student's t -test: * P <0.0001 vs medium alone. **b** Effect on hMSC proliferation after daily application of ATP (10 μ M) and P2 antagonists, PPADS (30 μ M) and MRS 2179 (10 μ M). Data are

expressed as percentage of proliferation. Proliferation of untreated cells was assumed as 100%. The cell number was determined after 5 days of culture by a culture counter. Each column bar represents the mean \pm SE of n =4 for each experimental condition. Paired Student's t -test: * P <0.05 vs respective control; one-way ANOVA, Newman-Keuls post-test: § P <0.05 vs 10 μ M ATP-treated cells. (Modified from [46])

cell trophism according to its extracellular concentrations, distinct cell populations involved, differential expression and recruitment of P2 receptors and of more or less sustained stimulation of the same receptor. Balancing these effects may be relevant in the post-ischemic brain, when a neuroregenerative process could promote tissue repair [47]. On this basis, determination of ATP extracellular concentrations at various times after ischemia induction may help to identify which types of receptors may be stimulated under ischemia and explain the role of detrimental versus trophic ATP during ischemia. ATP present in the extracellular space is rapidly metabolised by membrane-bound NTPDase. Extracellular ATP concentrations, evaluated in the brain to date, have been underestimated, since it has not been possible to selectively inhibit ecto-NTPDase. In fact, the selective inhibitor ARL 67156, tentatively used thus far to inhibit ecto-NTPDase, interferes with the ATP assay method [29].

Role of adenosine in cerebral transmission under normoxic conditions

Adenosine exerts an important tonic modulation of synaptic transmission in the brain. This tonic inhibition of synaptic transmission is evoked by stimulation of A₁ receptors, as demonstrated in several brain regions, such as the hippocampus, striatum and olfactory cortex [48, 49]. The inhibitory effect of adenosine A₁ receptor stimulation has a pre- and postsynaptic component. Activation of the presynaptic A₁ receptors reduces Ca²⁺ influx through the preferential inhibition of N-type and, probably, Q-type channels [50, 51]. Inhibition of presynaptic calcium currents decreases transmitter release [52], and adenosine, by stimulation of A₁ receptors, has been found to inhibit the release of virtually all classical neurotransmitters: glutamate, acetylcholine, dopamine, noradrenaline and serotonin (see in [53]). In particular, powerful suppression of glutamate release from presynaptic terminals has been described in the hippocampus [54, 55], where adenosine A₁ receptor activation reduces the number of quanta released (but not the size of individual quanta nor postsynaptic glutamate receptor sensitivity) in the Schaffer collateral-commissural pathway [56]. The postsynaptic effect of A₁ receptors consists of direct hyperpolarisation of neurones via activation of GIRK channels (Kir 3.2 and 3.4 channels: potassium inward rectifiers) [57, 58]. Endogenous adenosine exerts tonic inhibition of excitatory neurotransmission. The selective A₁ antagonist, DPCPX, causes a 15% increase in synaptic potential amplitude in *in vitro* brain slices [59]. This is an expected result in a brain region where the adenosine concentration at a receptor level was calculated around 200 nM [59, 60] and A₁ receptors, whose affinity for adenosine is in the low nanomolar range, are highly expressed. These data are confirmed by the fact that, in slices

taken from homozygous A₁ receptor knockout mice, no evidence was found for an endogenous inhibitory action by adenosine in the Schaffer collateral pathway in the CA1 region of the hippocampus or at the mossy fibre synapses in the CA3 region [61].

Opposite effects from A₁-mediated synaptic inhibition are elicited by A_{2A} receptor activation, which has been shown to mediate excitatory actions in synaptic function [62–64]. In the hippocampus *in vitro*, A_{2A} receptor stimulation results in a Ca²⁺-dependent release of acetylcholine [65, 66]. Furthermore, the application of CGS 21680, a selective A_{2A} receptor agonist, decreases the ability of A₁ receptor agonists to inhibit excitatory neurotransmission [9, 67]. This effect suggests that A_{2A} receptor stimulation increases synaptic transmission through A₁ receptor desensitisation [68, 69]. However, there is also evidence that A_{2A} receptors increase excitatory amino acid release. In fact, the selective stimulation of adenosine A_{2A} receptors augments the amount of glutamate released in the hippocampus and striatum of young rats [70–72].

In spite of the excitatory role in neurotransmission brought about by A_{2A} receptors, the net effect of adenosine is an inhibitory tonus on neurotransmission, in accordance with observations suggesting that activation of A_{2A} receptors requires protracted stimulation to induce evident effects on synaptic transmission [59]. It is worth noticing that the role of A_{2A} receptors in the striatum is recently gaining interest in light of their heterodimerisation with D₂ dopamine receptors. The association between A_{2A} and D₂ receptors results in an antagonistic interaction that provides a rationale for evaluating A_{2A}-selective antagonists in Parkinson's disease, supported by epidemiological evidence indicating an inverse relationship between caffeine consumption and the risk of developing this pathology [73, 74]. It was suggested that A_{2A} antagonists not only provide symptomatic relief but also decelerate dopaminergic neurone degeneration in patients.

Discrepancies about the role of adenosine A₃ receptors in the brain are present in the literature. Activation of this receptor subtype has been associated with both excitatory and inhibitory effects, even in the same brain region. An excitatory role of A₃ receptors has been supported by evidence indicating that, in the rat hippocampus, their activation attenuates LTD and allows induction of LTP elicited by a subliminal weak-burst protocol [75]. In addition, in the same brain area, A₃ receptor activation through a selective adenosine A₃ agonist has been shown to antagonize the adenosine A₁ receptor-mediated inhibition of excitatory neurotransmission [8]. Moreover, A₃ receptor stimulation always attenuates the inhibition of hippocampal slice neurotransmission, in a PKC-dependent manner, caused by presynaptic metabotropic glutamate receptors [76]. Whole-cell patch clamp recordings in CA3 hippocampal pyramidal neurones demonstrate that A₃ receptor activation results in a significant potentiation of high threshold hippocampal Ca²⁺

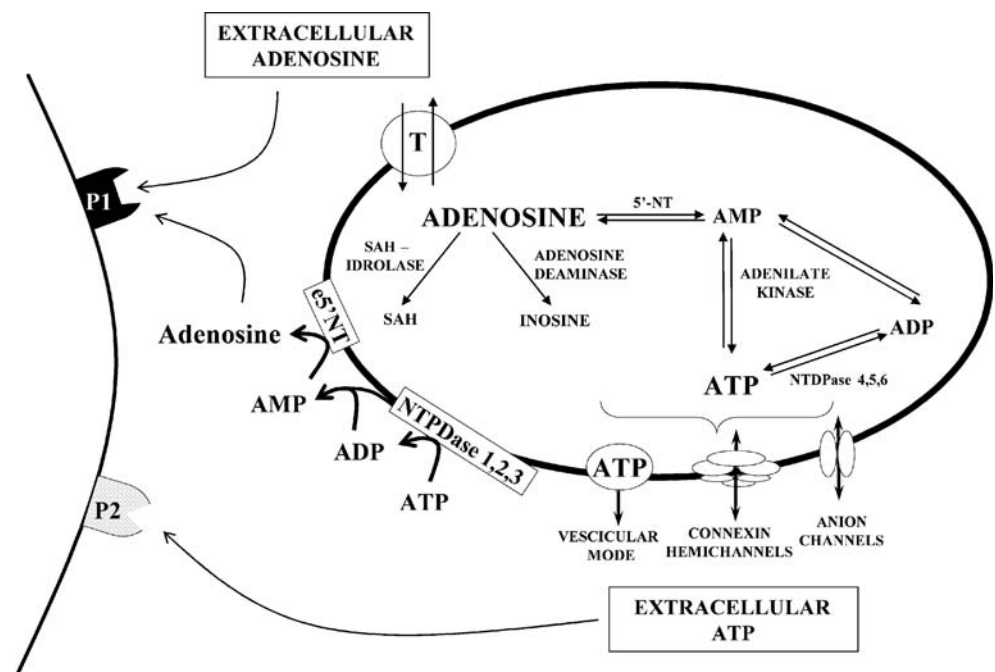
currents by a PKA-dependent mechanism [77]. Finally, facilitation of the onset of epileptiform discharge has been observed in the presence of the selective A_3 receptor agonist CI-IB-MECA [78], and a reduction in such epileptic activity was observed when A_3 receptors, activated by endogenously released adenosine during seizures, were blocked by the selective antagonist MRS 1191 [79].

Contrary to previous results, an inhibitory action has been attributed to A_3 receptors by Brand and colleagues [80], who demonstrated that, in rat cortical neurones, the selective activation of this adenosine receptor subtype is involved in inhibition of excitatory neurotransmission, suggesting a synergistic action with the inhibitory effect of adenosine brought about by A_1 receptor activation. Despite results obtained by A_3 receptor stimulation, evidence that a selective block of A_3 receptors does not affect neurotransmission in the CA1 region of the hippocampus under normoxic conditions indicates that endogenous adenosine at physiological concentrations does not exert tonic activation of A_3 receptors [8, 81]. This is in line with evidence that A_3 receptor activation requires micromolar levels of extracellular adenosine, which can be reached only during pathological conditions of impairment in energy supply (for example during hypoxia or ischemia [82–85]).

Role of adenosine in ischemic conditions

Extracellular adenosine concentrations increase dramatically during ischemia [82, 86, 87]. During ischemia, following the imbalance between ATP degradation and resynthesis, the intracellular concentration of adenosine increases.

Fig. 5 Schematic drawing of intracellular and extracellular adenosine formation. In the extracellular space, adenosine and ATP act on own purinergic receptor subtypes: $P1$ and $P2$ receptors, respectively. *ADP* adenosine diphosphate, *AMP* adenosine monophosphate, *ATP* adenosine triphosphate, *e5'-NT* ecto-5'-nucleotidase, *5'-NT* 5'-nucleotidase, *NTDPase* ecto-nucleoside triphosphate diphosphohydrolases, *P1* adenosine receptor, *P2* ATP receptor, *SAH* S-adenosylhomocysteine, *T* bidirectional nucleoside transporter. (Modified from [142])



Therefore, increased adenosine concentrations in the extracellular space during ischemia are likely due to the equilibrative transporter that carries adenosine out of cells. Adenosine formation can also take place at the extracellular level, through the hydrolysis of extracellular ATP operated by NTPDases and ecto-5'-nucleotidase (e5'-NTs) (see Fig. 5). Hence, these enzymes have a dual function in modulating purinergic neurotransmission: (1) they rapidly interrupt ATP-mediated signalling by degrading extracellular nucleotides and (2) they give rise to extracellular adenosine, which activates $P1$ receptors. However, recent evidence in vitro suggests that released ATP does not substantially contribute to the adenosine concentration in the extracellular milieu during ischemia [88].

Adenosine-potentiating agents, which elevate endogenous adenosine levels by either inhibiting its metabolism by adenosine deaminase or kinase [89, 90] or preventing its transport [91, 92], offer protection against ischemic neuronal damage in different in vivo ischemia models. Moreover, adenosine infusion into the ischemic striatum during transient focal ischemia proves to significantly ameliorate the neurological outcome and reduce infarct volume [93].

A temporal correlation exists between adenosine outflow and synaptic potential inhibition in rat hippocampal slices during ischemia-like conditions [84, 88]. Synaptic inhibition during ischemia is greatly dependent on adenosine, which, by stimulating A_1 receptors, exerts a protective role by reducing the Ca^{2+} influx, thus counteracting the presynaptic release of excitatory neurotransmitters [55, 94] and, in particular, glutamate, which exerts an excitotoxic role during ischemia mainly by overstimulation of NMDA receptors [95]. By directly increasing the K^+ and Cl^- ion conductan-

ces, adenosine stabilises the neuronal membrane potentials, thus reducing neuronal excitability [96]. Consequent reductions in cellular metabolism and energy consumption [97] and moderate lowering of the body/brain temperature [98] are protective in ischemia.

A₁ receptor agonists are shown to attenuate ischemic or excitotoxic neuronal damage in both in vitro and in vivo models of cerebral ischemia (for review, see [99, 100]). In accordance, adenosine A₁ antagonists given acutely exacerbate the damage induced by ischemia in different animal models of ischemia. An unselective A₁ receptor antagonist, theophylline, increased mortality [101, 102]. Unlike acute treatment, chronic administration of A₁ agonists worsened survival and increased neuronal loss [103], a phenomenon thought to depend on A₁ receptor desensitization.

Although data converge in demonstrating a neuroprotective effect of adenosine through A₁ receptors during ischemia, the use of selective A₁ agonists is hampered by unwanted peripheral effects, e.g. sedation, bradycardia, hypotension [104]. Von Lubitz and co-workers [105] have reported that post-ischemic administration of the A₁ receptor agonist adenosine amine congener (ADAC), which induces fewer undesirable effects, increases survival in gerbils. Moreover, we may consider that administration of agents that elevate the local concentration of adenosine at the injury site, by inhibiting its metabolism to inosine or rephosphorylation to AMP or reuptake, may have the advantage of restricting the effect of such inhibitors to areas of injury-induced adenosine release [106].

More recently, the role of A_{2A} receptors in ischemic neuroprotection has been studied. Gao and Phillis [107] demonstrated for the first time that the non-selective A_{2A} receptor antagonist CGS 15943 reduces cerebral ischemic injury in the gerbil following global forebrain ischemia. Subsequently, many reports have confirmed the neuroprotective role of A_{2A} receptor antagonists in different models of ischemia. The selective A_{2A} receptor antagonist SCH 58261 reduced ischemic brain damage in neonatal [108] and adult [83, 109] rat models of focal cerebral ischaemia. The same antagonist, subchronically administered, was protective against both brain damage and neurological deficit in the adult rat model of focal cerebral ischemia [110, 111]. Studies in A_{2A} receptor knockout mice supported the neuroprotective role of A_{2A} receptor antagonists on ischemic brain damage [112]. The beneficial effects of A_{2A} antagonists in stroke were mainly attributed to reduced glutamate outflow [83, 110, 113]. Prolonged application of the A_{2A} selective agonist CGS 21680 significantly reduced synaptic depression brought about by OGD in the hippocampus [59] and the same agonist promotes glutamate release under normoxic and ischemic conditions [70, 71, 114, 115]. In addition to neurones, adenosine A_{2A} receptors are located on microglia [116, 117] and astrocytes [118]. On glial cells, A_{2A} receptors

mediate inhibition of the glutamate uptake transporter, GLT-1, and stimulate glutamate outflow [119]. A_{2A} antagonists prevent the increase in glutamate levels induced by glutamate uptake inhibitors [120]. Therefore, the protective effects of A_{2A} antagonists in brain ischemia may be largely due to reduced glutamate outflow from neurones and glial cells.

Further support of a major role for glia in the neuroprotective effect of A_{2A} antagonism in ischemia comes from the observation that subchronic administration of the A_{2A} receptor antagonist SCH 58261 reduces p38 mitogen-activated protein kinase (MAPK) activation in striatal and cortical microglia 24 h after permanent focal ischemia [111]. Evidence indicates that p38 MAPK is activated in glia up to 24 h after ischemia [121, 122] and is involved, through transcriptional mechanisms, in neuronal death [123, 124]. Since SCH 58261 reduces glutamate outflow in the first hours after ischemia [83], reduced p38 MAPK activation may be due to a direct effect on glial A_{2A} receptors or may be secondary to a reduction in the excitotoxic cascade that primes p38 activation [125]. The importance of A_{2A} receptors under ischemia is highlighted by the observation that A_{2A} receptor expression increases on neurones and microglia after ischemia [126]. Selective inactivation of A_{2A} receptors on bone-marrow-derived cells (wild-type mice transplanted with A_{2A} receptor knockout bone marrow cells) attenuates infarct volumes and ischemia-induced expression of several proinflammatory cytokines in the brain [127]. Therefore, protective effects of A_{2A} antagonists may be attributed to inhibition of inflammation product production.

In several studies, A_{2A} receptor agonists have been found to be protective in the global ischemia model in the gerbil [128, 129]. Jones and co-workers [130] show that peripheral administration of the A_{2A} receptor agonist CGS 21680 protects the hippocampus against kainate-induced excitotoxicity. However, the direct injection of CGS 21680 into the hippocampus failed to afford protection, while the A_{2A} antagonist ZM 241385, when injected directly into the hippocampus, reduced kainate-induced neuronal damage [131]. These data suggest that the neuroprotective properties of A_{2A} agonists are mainly due to peripherally mediated effects. Major mechanisms that may account for A_{2A}-mediated protection include inhibition of platelet aggregation and vasodilation [89] and anti-inflammatory actions. A_{2A} receptors on neutrophils may account for inhibition of adhesion to endothelial cells and ensuing production of free radicals [132, 133].

On this basis, adenosine and its receptors are considered targets for therapeutic implementation in the treatment of stroke. At the moment, a possible adenosinergic therapeutic strategy after ischaemia that is worth consideration is that of increasing adenosine concentrations at the ischemic sites by inhibitors of adenosine metabolism or reuptake in association with adenosine A_{2A} antagonists. When considering the

possible use of adenosine kinase inhibitors, it should be taken into account that adenosine represents only a small percentage of nucleotide content [134], therefore inhibition of its rephosphorylation to ATP by adenosine kinase inhibitors does not weigh upon the ATP content.

The few studies present in the literature concerning the role of A_3 receptors in the pathophysiology of cerebral ischemia are rather contradictory. We have demonstrated that selective antagonism of A_3 receptors facilitates the recovery of synaptic activity induced by ischemic preconditioning in rat hippocampal slices [81]. A harmful role of A_3 receptors during *in vitro* OGD was confirmed by our observation that blocking the A_3 adenosine receptor consistently abolishes or delays the occurrence of anoxic depolarization (AD) and significantly protects from the irreversible disruption of excitatory neurotransmission caused by a severe ischemic episode [135]. These results are in agreement with the observation that acute administration of a selective adenosine A_3 agonist exacerbates the damage elicited by global ischemia in the gerbil [136]. On the contrary, it was demonstrated that chronic pre-ischemic administration of an A_3 agonist protects against ischemic neuronal damage [136]. This effect may be attributed to desensitisation of A_3 receptors. In fact, both human and rat A_3 receptors are desensitised within a few minutes after agonist exposure [137, 138].

Contrary to the above information, Hentschel and colleagues [139] demonstrated that under hypoxic conditions, selective activation of A_3 adenosine receptors brings about an inhibition of excitatory neurotransmission on cortical neurones, indicating that A_3 receptors may sustain the neuroprotective action of adenosine induced by A_1 receptors. Consistent with these reports, mice lacking A_3 adenosine receptors show increased neurodegeneration in response to repeated episodes of moderate hypoxia [140] or an increase in cerebral infarction after transient ligation of the middle cerebral artery [141]. These opposite results regarding an excitatory or inhibitory role of A_3 receptors on synaptic activity under hypoxia/ischemia may be reconciled by our recent data [135], suggesting that in a first phase of ischemia, A_3 receptors play a protective synergistic role with A_1 receptors. Severe ischemia would transform the A_3 receptor-mediated effects from protective to injurious.

Taken together, these data suggest that the outcome of A_3 receptor stimulation on synaptic transmission during hypoxic/ischemic phenomena depends on the intensity and duration of stimulation.

Concluding remarks

Purinergic signalling, e.g. adenosine and ATP, by activating specific membrane receptors (P1 and P2 respectively), is strictly correlated in orchestrating brain cell functions either

under physiological normoxic or ischemic circumstances. However, the respective contribution of each single element is hard to discern from the total outcome, due to the rapid and ubiquitous enzymatic interconversion of these molecules.

In the brain, both compounds are likely to play a role in neuronal activity, and the inhibitory action of endogenous adenosine in excitatory neurotransmission (mainly due to A_1 receptor activation) is well documented. Conversely our data, in line with previous observations in the literature, suggest that tonic activation of P2 receptors contributes to excitatory transmission in the hippocampus. Such an effect is modest, but it is unmasked by the application of P2 purinergic antagonists that reduce evoked synaptic responses.

An inhibitory effect elicited by exogenous ATP application has been frequently described and is mostly due to enzymatic degradation to adenosine and the subsequent activation of A_1 receptors. However, our evidence demonstrates that ATP itself may inhibit hippocampal synaptic transmission since an even more pronounced effect is observed when enzymatic ATP degradation is blocked. Moreover, in our studies in the hippocampus, a potentiation of neurotransmission by the ATP analogue, ATP γ S, develops a few minutes after the inhibitory effect has been washed out, and it persists for 45 min to 1 h. Within the brain, ATP is involved in the synaptic plasticity phenomenon and may be an important mediator of long-lasting effects in synaptic plasticity.

Either adenosine or ATP seems to become a particularly important signalling molecule under pathological conditions, such as ischemia, when the extracellular concentration of either compound drastically rises. It is well known that adenosine exerts important neuroprotective effects during brain ischemic insults by activating adenosine A_1 receptors, which profoundly inhibit synaptic transmission and in particular the release of glutamate, known to contribute to ischemic damage. Under severe ischemia, a prolonged stimulation of both A_{2A} and A_3 receptors may be deleterious. The role of endogenously released ATP during cerebral ischemia is mainly deleterious, as found in our work and in the literature, since the application of P2 antagonists always results in a reduction in ischemic damage. The mechanisms underlying these effects are still unknown, but they can be correlated to the tonic excitatory action of endogenous ATP found in many brain regions (for example in the hippocampus and prefrontal cortex).

Purinergic signalling has an ancient phylogenetic origin. Expression of P2 receptors on cell membrane and responses to extracellular ATP are found in primitive prokaryotic species up to evolved animals and plants, suggesting an important and highly conserved role of extracellular purine nucleotides during evolution. Similarly, ontogenetic development in several species seems to involve purinergic signalling, especially during its first stages. Our observation that hMSCs,

an undifferentiated line of cells able to originate a number of different cell lineages (adipocytes, chondrocytes, osteoblasts, neurones), spontaneously release ATP into the extracellular space and express functional purinergic P2 receptors, which modulate different kinds of membrane currents, and that ATP, during the early stages of culture, inhibits proliferation indicates an autocrine/paracrine mechanism of action for extracellular ATP in modulating cell functions of undifferentiated stem cells at early developmental stages. Such effects may be important in brain neurogenesis during development and in responses to neurodegenerative stimuli.

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References

- Burnstock G, Dumsday B, Smythe A (1972) Atropine resistant excitation of the urinary bladder: the possibility of transmission via nerves releasing a purine nucleotide. *Br J Pharmacol* 44:451–461
- Burnstock G, Cocks T, Kasakov L et al (1978) Direct evidence for ATP release from non-adrenergic, non-cholinergic (“purinergic”) nerves in the guinea-pig taenia coli and bladder. *Eur J Pharmacol* 49:145–149
- Fredholm BB, IJzerman AP, Jacobson KA et al (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 53:527–552
- Abbracchio MP, Burnstock G (1994) Purinoceptors: are there families of P2X and P2Y purinoceptors? *Pharmacol Ther* 64:445–475
- North RA (2002) Molecular physiology of P2X receptors. *Physiol Rev* 82:1013–1067
- Ciana P, Fumagalli M, Trincavelli ML et al (2006) The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. *EMBO J* 25:4615–4627
- Cunha RA, Sebastiao AM, Ribeiro JA (1998) Inhibition by ATP of hippocampal synaptic transmission requires localized extracellular catabolism by ecto-nucleotidases into adenosine and channeling to adenosine A1 receptors. *J Neurosci* 18:1987–1995
- Dunwiddie TV, Diao L, Proctor WR (1997) Adenine nucleotides undergo rapid, quantitative conversion to adenosine in the extracellular space in rat hippocampus. *J Neurosci* 17:7673–7682
- O’Kane EM, Stone TW (1998) Interaction between adenosine A1 and A2 receptor-mediated responses in the rat hippocampus in vitro. *Eur J Pharmacol* 362:17–25
- Masino SA, Diao L, Illes P et al (2002) Modulation of hippocampal glutamatergic transmission by ATP is dependent on adenosine A1 receptors. *J Pharmacol Exp Ther* 303:356–363
- Zimmermann H, Braun N (1996) Extracellular metabolism of nucleotides in the nervous system. *J Auton Pharmacol* 16:397–400
- Rodrigues RJ, Almeida T, Richardson PJ et al (2005) Dual presynaptic control by ATP of glutamate release via facilitatory P2X1, P2X2/3, and P2X3 and inhibitory P2Y1, P2Y2, and/or P2Y4 receptors in the rat hippocampus. *J Neurosci* 25:6286–6295
- Mendoza-Fernandez V, Andrew RD, Barajas-Lopez C (2000) ATP inhibits glutamate synaptic release by acting at P2Y receptors in pyramidal neurons of hippocampal slices. *J Pharmacol Exp Ther* 293:172–179
- Luthardt J, Borvendeg SJ, Sperlagh B et al (2003) P2Y(1) receptor activation inhibits NMDA receptor-channels in layer V pyramidal neurons of the rat prefrontal and parietal cortex. *Neurochem Int* 42:161–172
- Coppi E, Pugliese AM, Stephan H et al (2007) Role of P2 purinergic receptors in synaptic transmission under normoxic and ischaemic conditions in the CA1 region of rat hippocampal slices. *Purinergic Signalling* 3:203
- Zimmermann H, Braun N, Kegel B et al (1998) New insights into molecular structure and function of ectonucleotidases in the nervous system. *Neurochem Int* 32:421–425
- Zimmermann H (2000) Extracellular metabolism of ATP and other nucleotides. *Naunyn Schmiedebergs Arch Pharmacol* 362:299–309
- Iqbal J, Vollmayer P, Braun N et al (2007) A capillary electrophoresis method for the characterization of ecto-nucleoside triphosphate diphosphohydrolases (NTPDase) and the analysis of inhibitors by in-capillary enzymatic microreaction. *Purinergic Signalling* 1:349–358
- Kukulski F, Komoszynski M (2003) Purification and characterization of NTPDase1 (ecto-apyrase) and NTPDase2 (ecto-ATPase) from porcine brain cortex synaptosomes. *Eur J Biochem* 270:3447–3454
- Muller CE, Iqbal J, Baqi Y et al (2006) Polyoxometalates—a new class of potent ecto-nucleoside triphosphate diphosphohydrolase (NTPDase) inhibitors. *Bioorg Med Chem Lett* 16:5943–5947
- Wieraszko A, Seyfried TN (1989) ATP-induced synaptic potentiation in hippocampal slices. *Brain Res* 491:356–359
- Fujii S, Kato H, Furuse H et al (1995) The mechanism of ATP-induced long-term potentiation involves extracellular phosphorylation of membrane proteins in guinea-pig hippocampal CA1 neurons. *Neurosci Lett* 187:130–132
- Fujii S, Kato H, Kuroda Y (1999) Extracellular adenosine 5'-triphosphate plus activation of glutamatergic receptors induces long-term potentiation in CA1 neurons of guinea pig hippocampal slices. *Neurosci Lett* 276:21–24
- Fujii S, Kato H, Kuroda Y (2002) Cooperativity between extracellular adenosine 5'-triphosphate and activation of N-methyl-D-aspartate receptors in long-term potentiation induction in hippocampal CA1 neurons. *Neuroscience* 113:617–628
- O’Kane EM, Stone TW (2000) Characterisation of ATP-induced facilitation of transmission in rat hippocampus. *Eur J Pharmacol* 409:159–166
- Abrams TW, Kandel ER (1988) Is contiguity detection in classical conditioning a system or a cellular property? Learning in *Aplysia* suggests a possible molecular site. *Trends Neurosci* 11:128–135
- Pankratov Y, Castro E, Miras-Portugal MT et al (1998) A purinergic component of the excitatory postsynaptic current mediated by P2X receptors in the CA1 neurons of the rat hippocampus. *Eur J Neurosci* 10:3898–3902
- Juranyi Z, Sperlagh B, Vizi ES (1999) Involvement of P2 purinoceptors and the nitric oxide pathway in [3H]purine outflow evoked by short-term hypoxia and hypoglycemia in rat hippocampal slices. *Brain Res* 823:183–190
- Melani A, Turchi D, Vannucchi MG et al (2005) ATP extracellular concentrations are increased in the rat striatum during in vivo ischemia. *Neurochem Int* 47:442–448
- Amadio S, D’Ambrosi N, Cavaliere F et al (2002) P2 receptor modulation and cytotoxic function in cultured CNS neurons. *Neuropharmacology* 42:489–501
- Cavaliere F, D’Ambrosi N, Ciotti MT et al (2001) Glucose deprivation and chemical hypoxia: neuroprotection by P2 receptor antagonists. *Neurochem Int* 38:189–197
- Volonte C, Merlo D (1996) Selected P2 purinoceptor modulators prevent glutamate-evoked cytotoxicity in cultured cerebellar granule neurons. *J Neurosci Res* 45:183–193
- Volonte C, Ciotti MT, D’Ambrosi N et al (1999) Neuroprotective effects of modulators of P2 receptors in primary culture of CNS neurones. *Neuropharmacology* 38:1335–1342

34. Ryu JK, Kim J, Choi SH et al (2002) ATP-induced in vivo neurotoxicity in the rat striatum via P2 receptors. *Neuroreport* 13: 1611–1615
35. Kharlamov A, Jones SC, Kim DK (2002) Suramin reduces infarct volume in a model of focal brain ischemia in rats. *Exp Brain Res* 147:353–359
36. Melani A, Amadio S, Gianfriddo M et al (2006) P2X7 receptor modulation on microglial cells and reduction of brain infarct caused by middle cerebral artery occlusion in rat. *J Cereb Blood Flow Metab* 26:974–982
37. Lammer A, Gunther A, Beck A et al (2006) Neuroprotective effects of the P2 receptor antagonist PPADS on focal cerebral ischaemia-induced injury in rats. *Eur J Neurosci* 23:2824–2828
38. Marrelli SP, Khorovets A, Johnson TD et al (1999) P2 purinoreceptor-mediated dilations in the rat middle cerebral artery after ischemia-reperfusion. *Am J Physiol* 276:H33–H41
39. Lahiri P, Chaudhuri U, Chattopadhyay A et al (2005) Structural insights in platelet receptor synergism-antiplatelet therapy in post-ischemic cerebrovascular events. *Blood Cells Mol Dis* 34:248–256
40. Ziegler S, Schillinger M, Funk M et al (2005) Association of a functional polymorphism in the clopidogrel target receptor gene, P2Y12, and the risk for ischemic cerebrovascular events in patients with peripheral artery disease. *Stroke* 36:1394–1399
41. Neary JT (2000) Trophic actions of extracellular ATP: gene expression profiling by DNA array analysis. *J Auton Nerv Syst* 81: 200–204
42. Rathbone MP, DeForge S, Deluca B et al (1992) Purinergic stimulation of cell division and differentiation: mechanisms and pharmacological implications. *Med Hypotheses* 37:213–219
43. Franke H, Illes P (2006) Involvement of P2 receptors in the growth and survival of neurons in the CNS. *Pharmacol Ther* 109:297–324
44. Mishra SK, Braun N, Shukla V et al (2006) Extracellular nucleotide signaling in adult neural stem cells: synergism with growth factor-mediated cellular proliferation. *Development* 133: 675–684
45. Kawano S, Otsu K, Kuruma A et al (2006) ATP autocrine/paracrine signaling induces calcium oscillations and NFAT activation in human mesenchymal stem cells. *Cell Calcium* 39:313–324
46. Coppi E, Pugliese AM, Urbani S et al (2007) ATP modulates cell proliferation and elicits two different electrophysiological responses in human mesenchymal stem cells. *Stem Cells* 25:1840–1849
47. Scholzke MN, Schwaninger M (2007) Transcriptional regulation of neurogenesis: potential mechanisms in cerebral ischemia. *J Mol Med* 85:577–588
48. Latini S, Pedata F (2001) Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J Neurochem* 79:463–484
49. von Lubitz DK, Lin RC, Bischofberger N et al (1999) Protection against ischemic damage by adenosine amine congener, a potent and selective adenosine A1 receptor agonist. *Eur J Pharmacol* 369: 313–317
50. Yawo H, Chuhma N (1993) Preferential inhibition of omega-conotoxin-sensitive presynaptic Ca²⁺ channels by adenosine autoreceptors. *Nature* 365:256–258
51. Wu LG, Saggau P (1994) Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus. *Neuron* 12:1139–1148
52. Prince DA, Stevens CF (1992) Adenosine decreases neurotransmitter release at central synapses. *Proc Natl Acad Sci USA* 89:8586–8590
53. Fredholm BB, Dunwiddie TV (1988) How does adenosine inhibit transmitter release? *Trends Pharmacol Sci* 9:130–134
54. Burke SP, Nadler JV (1988) Regulation of glutamate and aspartate release from slices of the hippocampal CA1 area: effects of adenosine and baclofen. *J Neurochem* 51:1541–1551
55. Corradetti R, Lo CG, Moroni F et al (1984) Adenosine decreases aspartate and glutamate release from rat hippocampal slices. *Eur J Pharmacol* 104:19–26
56. Lupica CR, Proctor WR, Dunwiddie TV (1992) Presynaptic inhibition of excitatory synaptic transmission by adenosine in rat hippocampus: analysis of unitary EPSP variance measured by whole-cell recording. *J Neurosci* 12:3753–3764
57. Takigawa T, Alzheimer C (1999) G protein-activated inwardly rectifying K⁺ (GIRK) currents in dendrites of rat neocortical pyramidal cells. *J Physiol* 517(Pt 2):385–390
58. Takigawa T, Alzheimer C (2002) Phasic and tonic attenuation of EPSPs by inward rectifier K⁺ channels in rat hippocampal pyramidal cells. *J Physiol* 539:67–75
59. Latini S, Bordoni F, Corradetti R et al (1999) Effect of A2A adenosine receptor stimulation and antagonism on synaptic depression induced by in vitro ischaemia in rat hippocampal slices. *Br J Pharmacol* 128:1035–1044
60. Latini S, Bordoni F, Pedata F et al (1999) Extracellular adenosine concentrations during in vitro ischaemia in rat hippocampal slices. *Br J Pharmacol* 127:729–739
61. Moore KA, Nicoll RA, Schmitz D (2003) Adenosine gates synaptic plasticity at hippocampal mossy fiber synapses. *Proc Natl Acad Sci USA* 100:14397–14402
62. Pedata F, Pepeu G, Spignoli G (1984) Biphasic effect of methylxanthines on acetylcholine release from electrically-stimulated brain slices. *Br J Pharmacol* 83:69–73
63. Latini S, Pazzagli M, Pepeu G et al (1996) A2 adenosine receptors: their presence and neuromodulatory role in the central nervous system. *Gen Pharmacol* 27:925–933
64. Sebastiao AM, Ribeiro JA (1996) Adenosine A2 receptor-mediated excitatory actions on the nervous system. *Prog Neurobiol* 48:167–189
65. Spignoli G, Pedata F, Pepeu G (1984) A1 and A2 adenosine receptors modulate acetylcholine release from brain slices. *Eur J Pharmacol* 97:341–342
66. Cunha RA, Johansson B, Fredholm BB et al (1995) Adenosine A2A receptors stimulate acetylcholine release from nerve terminals of the rat hippocampus. *Neurosci Lett* 196:41–44
67. Cunha RA, Johansson B, van der Ploeg I et al (1994) Evidence for functionally important adenosine A2a receptors in the rat hippocampus. *Brain Res* 649:208–216
68. Dixon AK, Widdowson L, Richardson PJ (1997) Desensitisation of the adenosine A1 receptor by the A2A receptor in the rat striatum. *J Neurochem* 69:315–321
69. Stone TW, Nikbakh M-R, O’Kane EM (2004) Adenosine and purines. In: Riedel G, Platt B (eds) *From messengers to molecules: memories are made of these*. Kluwer Academic/Plenum, New York, p 196–223
70. Popoli P, Betto P, Reggio R et al (1995) Adenosine A2A receptor stimulation enhances striatal extracellular glutamate levels in rats. *Eur J Pharmacol* 287:215–217
71. Corsi C, Melani A, Bianchi L et al (1999) Striatal A_{2A} adenosine receptor differentially regulate spontaneous and K⁺-evoked glutamate release in vivo in young and aged rats. *Neuroreport* 10:687–691
72. Corsi C, Melani A, Bianchi L et al (2000) Striatal A2A adenosine receptor antagonism differentially modifies striatal glutamate outflow in vivo in young and aged rats. *Neuroreport* 11:2591–2595
73. Ross GW, Abbott RD, Petrovitch H et al (2000) Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA* 283:2674–2679

74. Ascherio A, Zhang SM, Hernan MA et al (2001) Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. *Ann Neurol* 50:56–63
75. Costenla AR, Lopes LV, de Mendonca A et al (2001) A functional role for adenosine A3 receptors: modulation of synaptic plasticity in the rat hippocampus. *Neurosci Lett* 302:53–57
76. Macek TA, Schaffhauser H, Conn PJ (1998) Protein kinase C and A3 adenosine receptor activation inhibit presynaptic metabotropic glutamate receptor (mGluR) function and uncouple mGluRs from GTP-binding proteins. *J Neurosci* 18:6138–6146
77. Fleming KM, Mogul DJ (1997) Adenosine A3 receptors potentiate hippocampal calcium current by a PKA-dependent/PKC-independent pathway. *Neuropharmacology* 36:353–362
78. Laudadio MA, Psarropoulou C (2004) The A3 adenosine receptor agonist 2-Cl-IB-MECA facilitates epileptiform discharges in the CA3 area of immature rat hippocampal slices. *Epilepsy Res* 59:83–94
79. Etherington LA, Frenguelli BG (2004) Endogenous adenosine modulates epileptiform activity in rat hippocampus in a receptor subtype-dependent manner. *Eur J Neurosci* 19:2539–2550
80. Brand A, Vissienon Z, Eschke D et al (2001) Adenosine A(1) and A(3) receptors mediate inhibition of synaptic transmission in rat cortical neurons. *Neuropharmacology* 40:85–95
81. Pugliese AM, Latini S, Corradetti R et al (2003) Brief, repeated, oxygen-glucose deprivation episodes protect neurotransmission from a longer ischemic episode in the *in vitro* hippocampus: role of adenosine receptors. *Br J Pharmacol* 140:305–314
82. Melani A, Pantoni L, Corsi C et al (1999) Striatal outflow of adenosine, excitatory amino acids, gamma-aminobutyric acid, and taurine in awake freely moving rats after middle cerebral artery occlusion: correlations with neurological deficit and histopathological damage. *Stroke* 30:2448–2454
83. Melani A, Pantoni L, Bordoni F et al (2003) The selective A2A receptor antagonist SCH 58261 reduces striatal transmitter outflow, turning behavior and ischemic brain damage induced by permanent focal ischemia in the rat. *Brain Res* 959:243–250
84. Latini S, Bordoni F, Corradetti R et al (1998) Temporal correlation between adenosine outflow and synaptic potential inhibition in rat hippocampal slices during ischemia-like conditions. *Brain Res* 794:325–328
85. Pearson T, Damian K, Lynas RE et al (2006) Sustained elevation of extracellular adenosine and activation of A1 receptors underlie the post-ischaemic inhibition of neuronal function in rat hippocampus *in vitro*. *J Neurochem* 97:1357–1368
86. Dux E, Fastbom J, Ungerstedt U et al (1990) Protective effect of adenosine and a novel xanthine derivative propentofylline on the cell damage after bilateral carotid occlusion in the gerbil hippocampus. *Brain Res* 516:248–256
87. Phillis JW, Smith-Barbour M, O'Regan MH et al (1994) Amino acid and purine release in rat brain following temporary middle cerebral artery occlusion. *Neurochem Res* 19:1125–1130
88. Frenguelli BG, Wigmore G, Llaudet E et al (2007) Temporal and mechanistic dissociation of ATP and adenosine release during ischaemia in the mammalian hippocampus. *J Neurochem* 101:1400–1413
89. Phillis JW, O'Regan MH (1989) Deoxycoformycin antagonizes ischemia-induced neuronal degeneration. *Brain Res Bull* 22:537–540
90. Lin Y, Phillis JW (1992) Deoxycoformycin and oxypurinol: protection against focal ischemic brain injury in the rat. *Brain Res* 571:272–280
91. DeLeo J, Schubert P, Kreutzberg GW (1988) Protection against ischemic brain damage using propentofylline in gerbils. *Stroke* 19:1535–1539
92. Parkinson FE, Xiong W, Zamzow CR (2005) Astrocytes and neurons: different roles in regulating adenosine levels. *Neurol Res* 27:153–160
93. Kitagawa H, Mori A, Shimada J et al (2002) Intracerebral adenosine infusion improves neurological outcome after transient focal ischemia in rats. *Neurol Res* 24:317–323
94. Pedata F, Latini S, Pugliese AM et al (1993) Investigations into the adenosine outflow from hippocampal slices evoked by ischemia-like conditions. *J Neurochem* 61:284–289
95. Choi DW (1990) Possible mechanisms limiting N-methyl-D-aspartate receptor overactivation and the therapeutic efficacy of N-methyl-D-aspartate antagonists. *Stroke* 21:III20–III22
96. Greene RW, Haas HL (1991) The electrophysiology of adenosine in the mammalian central nervous system. *Prog Neurobiol* 36:329–341
97. Tominaga K, Shibata S, Watanabe S (1992) A neuroprotective effect of adenosine A1-receptor agonists on ischemia-induced decrease in 2-deoxyglucose uptake in rat hippocampal slices. *Neurosci Lett* 145:67–70
98. Gourine AV, Dale N, Gourine VN et al (2004) Fever in systemic inflammation: roles of purines. *Front Biosci* 9:1011–1022
99. von Lubitz DK (2001) Adenosine in the treatment of stroke: yes, maybe, or absolutely not? *Expert Opin Invest Drugs* 10:619–632
100. Pearson T, Currie AJ, Etherington LA et al (2003) Plasticity of purine release during cerebral ischemia: clinical implications? *J Cell Mol Med* 7:362–375
101. Jarrott DM, Damer FR (1980) A gerbil model of cerebral ischemia suitable for drug evaluation. *Stroke* 11:203–209
102. Rudolphi KA, Keil M, Fastbom J et al (1989) Ischaemic damage in gerbil hippocampus is reduced following upregulation of adenosine (A1) receptors by caffeine treatment. *Neurosci Lett* 103:275–280
103. Jacobson KA, von Lubitz DK, Daly JW et al (1996) Adenosine receptor ligands: differences with acute versus chronic treatment. *Trends Pharmacol Sci* 17:108–113
104. Williams M (1993) Purinergic drugs: opportunities in the 1990s. *Drug Dev Res* 28:438
105. von Lubitz DK, Lin RC, Paul IA et al (1996) Postischemic administration of adenosine amine congener (ADAC): analysis of recovery in gerbils. *Eur J Pharmacol* 316:171–179
106. Phillis JW, Goshgarian HG (2001) Adenosine and neurotrauma: therapeutic perspectives. *Neurol Res* 23:183–189
107. Gao Y, Phillis JW (1994) CGS 15943, an adenosine A2 receptor antagonist, reduces cerebral ischemic injury in the Mongolian gerbil. *Life Sci* 55:L61–L65
108. Bona E, Aden U, Gilland E et al (1997) Neonatal cerebral hypoxia-ischemia: the effect of adenosine receptor antagonists. *Neuropharmacology* 36:1327–1338
109. Monopoli A, Lozza G, Forlani A et al (1998) Blockade of adenosine A2A receptors by SCH 58261 results in neuroprotective effects in cerebral ischaemia in rats. *Neuroreport* 9:3955–3959
110. Pedata F, Gianfriddo M, Turchi D et al (2005) The protective effect of adenosine A2A receptor antagonism in cerebral ischemia. *Neurol Res* 27:169–174
111. Melani A, Gianfriddo M, Vannucchi MG et al (2006) The selective A2A receptor antagonist SCH 58261 protects from neurological deficit, brain damage and activation of p38 MAPK in rat focal cerebral ischemia. *Brain Res* 1073–1074:470–480
112. Chen JF, Huang Z, Ma J et al (1999) A2A adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. *J Neurosci* 19:9192–9200
113. Marcoli M, Raiteri L, Bonfanti A et al (2003) Sensitivity to selective adenosine A1 and A2A receptor antagonists of the release of glutamate induced by ischemia in rat cerebrocortical slices. *Neuropharmacology* 45:201–210
114. O'Regan MH, Simpson RE, Perkins LM et al (1992) The selective A2 adenosine receptor agonist CGS 21680 enhances excitatory transmitter amino acid release from the ischemic rat cerebral cortex. *Neurosci Lett* 138:169–172

115. Simpson RE, O'Regan MH, Perkins LM et al (1992) Excitatory transmitter amino acid release from the ischemic rat cerebral cortex: effects of adenosine receptor agonists and antagonists. *J Neurochem* 58:1683–1690
116. Fiebich BL, Biber K, Lieb K et al (1996) Cyclooxygenase-2 expression in rat microglia is induced by adenosine A2A-receptors. *Glia* 18:152–160
117. Saura J, Angulo E, Ejarque A et al (2005) Adenosine A2A receptor stimulation potentiates nitric oxide release by activated microglia. *J Neurochem* 95:919–929
118. Lee YC, Chien CL, Sun CN et al (2003) Characterization of the rat A2A adenosine receptor gene: a 4.8-kb promoter-proximal DNA fragment confers selective expression in the central nervous system. *Eur J Neurosci* 18:1786–1796
119. Nishizaki T, Nagai K, Nomura T et al (2002) A new neuro-modulatory pathway with a glial contribution mediated via A2A adenosine receptors. *Glia* 39:133–147
120. Pintor A, Galluzzo M, Grieco R et al (2004) Adenosine A2A receptor antagonists prevent the increase in striatal glutamate levels induced by glutamate uptake inhibitors. *J Neurochem* 89:152–156
121. Irving EA, Barone FC, Reith AD et al (2000) Differential activation of MAPK/ERK and p38/SAPK in neurones and glia following focal cerebral ischaemia in the rat. *Brain Res Mol Brain Res* 77:65–75
122. Wu DC, Ye W, Che XM et al (2000) Activation of mitogen-activated protein kinases after permanent cerebral artery occlusion in mouse brain. *J Cereb Blood Flow Metab* 20:1320–1330
123. Barone FC, Irving EA, Ray AM et al (2001) Inhibition of p38 mitogen-activated protein kinase provides neuroprotection in cerebral focal ischemia. *Med Res Rev* 21:129–145
124. Gao Y, Signore AP, Yin W et al (2005) Neuroprotection against focal ischemic brain injury by inhibition of c-Jun N-terminal kinase and attenuation of the mitochondrial apoptosis-signaling pathway. *J Cereb Blood Flow Metab* 25:694–712
125. Kawasaki H, Morooka T, Shimohama S et al (1997) Activation and involvement of p38 mitogen-activated protein kinase in glutamate-induced apoptosis in rat cerebellar granule cells. *J Biol Chem* 272:18518–18521
126. Trincavelli ML, Melani A, Guidi S et al (2007) Regulation of A2A adenosine receptor expression and functioning following permanent focal ischemia in rat brain. *J Neurochem*. DOI 10.1111/j.1471-4159.2007.04990.x
127. Yu L, Huang Z, Mariani J et al (2004) Selective inactivation or reconstitution of adenosine A2A receptors in bone marrow cells reveals their significant contribution to the development of ischemic brain injury. *Nat Med* 10:1081–1087
128. Sheardown MJ, Knutsen LJS (1996) Unexpected neuroprotection observed with the adenosine A2A receptor agonist cgs 21680. *Drug Dev Res* 39:108
129. von Lubitz DK, Lin RC, Jacobson KA (1995) Cerebral ischemia in gerbils: effects of acute and chronic treatment with adenosine A2A receptor agonist and antagonist. *Eur J Pharmacol* 287:295–302
130. Jones PA, Smith RA, Stone TW (1998) Protection against kainate-induced excitotoxicity by adenosine A2A receptor agonists and antagonists. *Neuroscience* 85:229–237
131. Jones PA, Smith RA, Stone TW (1998) Protection against hippocampal kainate excitotoxicity by intracerebral administration of an adenosine A2A receptor antagonist. *Brain Res* 800:328–335
132. Sitkovsky MV, Ohta A (2005) The 'danger' sensors that STOP the immune response: the A2 adenosine receptors? *Trends Immunol* 26:299–304
133. Mayne M, Fotheringham J, Yan HJ et al (2001) Adenosine A2A receptor activation reduces proinflammatory events and decreases cell death following intracerebral hemorrhage. *Ann Neurol* 49:727–735
134. Latini S, Corsi C, Pedata F et al (1995) The source of brain adenosine outflow during ischemia and electrical stimulation. *Neurochem Int* 27:239–244
135. Pugliese AM, Coppi E, Spalluto G et al (2006) A3 adenosine receptor antagonists delay irreversible synaptic failure caused by oxygen and glucose deprivation in the rat CA1 hippocampus in vitro. *Br J Pharmacol* 147:524–532
136. von Lubitz DK, Lin RC, Popik P et al (1994) Adenosine A3 receptor stimulation and cerebral ischemia. *Eur J Pharmacol* 263:59–67
137. Palmer TM, Benovic JL, Stiles GL (1995) Agonist-dependent phosphorylation and desensitization of the rat A3 adenosine receptor. Evidence for a G-protein-coupled receptor kinase-mediated mechanism. *J Biol Chem* 270:29607–29613
138. Trincavelli ML, Tuscano D, Marroni M et al (2002) A3 adenosine receptors in human astrocytoma cells: agonist-mediated desensitization, internalization, and down-regulation. *Mol Pharmacol* 62:1373–1384
139. Hentschel S, Lewerenz A, Nieber K (2003) Activation of A(3) receptors by endogenous adenosine inhibits synaptic transmission during hypoxia in rat cortical neurons. *Restor Neurol Neurosci* 21:55–63
140. Fedorova IM, Jacobson MA, Basile A et al (2003) Behavioral characterization of mice lacking the A3 adenosine receptor: sensitivity to hypoxic neurodegeneration. *Cell Mol Neurobiol* 23:431–447
141. Chen GJ, Harvey BK, Shen H et al (2006) Activation of adenosine A3 receptors reduces ischemic brain injury in rodents. *J Neurosci Res* 84:1848–1855
142. Pedata F, Pugliese AM, Coppi E et al (2007) Adenosine in the central nervous system: effects on neurotransmission and neuroprotection. *Immunol Endocr Metab Agents Med Chem* 7:304–321