Purinergic Signalling (2005) 1: 111–134

Review

Neuroprotection by adenosine in the brain: From A_1 receptor activation to A_{2A} receptor blockade

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Received 3 September 2004; accepted in revised form 10 November 2004

Key words: A_1 receptors, A_{2A} receptors, adenosine, adenosine kinase, brain, neuroprotection

Abstract

Adenosine is a neuromodulator that operates via the most abundant inhibitory adenosine A_1 receptors (A_1Rs) and the less abundant, but widespread, facilitatory A_{2A}Rs. It is commonly assumed that A_1Rs play a key role in neuroprotection since they decrease glutamate release and hyperpolarize neurons. In fact, A_1R activation at the onset of neuronal injury attenuates brain damage, whereas its blockade exacerbates damage in adult animals. However, there is a down-regulation of central A_1Rs in chronic noxious situations. In contrast, A_{2A}Rs are up-regulated in noxious brain conditions and their blockade confers robust brain neuroprotection in adult animals. The brain neuroprotective effect of A_{2A}R antagonists is maintained in chronic noxious brain conditions without observable peripheral effects, thus justifying the interest of A_{2A}R antagonists as novel protective agents in neurodegenerative diseases such as Parkinson's and Alzheimer's disease, ischemic brain damage and epilepsy. The greater interest of A_{2A}R blockade compared to A_1R activation does not mean that A_1R activation is irrelevant for a neuroprotective strategy. In fact, it is proposed that coupling A_{2A}R antagonists with strategies aimed at bursting the levels of extracellular adenosine (by inhibiting adenosine kinase) to activate A_1Rs might constitute the more robust brain neuroprotective strategy based on the adenosine neuromodulatory system. This strategy should be useful in adult animals and especially in the elderly (where brain pathologies are prevalent) but is not valid for fetus or newborns where the impact of adenosine receptors on brain damage is different.

Abbreviations: Aβ – β-amyloid peptide; A_1Rs – A_1 receptors; A_{2A}Rs – A_{2A} receptors; CGS 21680 – 2-[4-(2-*P*-carboxyethyl)phenylamino]-5′-N-ethylcarboxamidoadenosine; CGS 15943 – 9-chloro-2-(2-furyl)(1,2,4)triazolo(1,5-c)quinazolin-5-amine; CPA – N^6-cyclopenthyladenosine; CSC – 8-(3-chlorostyryl)caffeine; DMPX – 3,7-dimethyl-1-propargylxanthine; DPCPX – 1,3-dipropyl-8-cyclopentyladenosine; KW6002 – (E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-1H-purine-2,6-dione; MPTP – 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SCH 58261 – 7-(2-phenylethyl)-5-amino-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-ylamino]ethyl)phenol

Introduction

Adenosine is a purine nucleoside that exists in all cells, where it is a metabolite involved in key pathways of primary metabolism such as nucleotide and nucleoside metabolism, sulphur-containing amino acid metabolism, *trans*-methylation reactions and handling of ammonia. Its intra-cellular concentration in basal conditions is typically around 10–50 nM in the cell types where it was so far quantified (reviewed in Cunha [1]). This intracellular concentration of adenosine is tightly linked to the energy charge of the cells, in the sense that small decreases in the energy charge (sometimes considered equivalent to the concentration of ATP) cause a disproportionate larger increase in the intracellular levels of adenosine (reviewed in Cunha [1]). The impact of changes in the intracellular levels of adenosine on the primary metabolism has not yet been explored. However, because all cell types so far investigated possess bi-directional non-concentrative nucleoside transporters, the intracellular levels of adenosine equilibrate with the extracellular levels of adenosine. Thus, if the levels of intracellular adenosine rise in a particular cell, a gradient of increased levels of extracellular adenosine will build-up in the surroundings of this cell. This extracellular adenosine is then able to act on metabotropic adenosine receptors located in the cell membrane of neighbouring cells (as well as of the cell that released adenosine). The

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activation of the different types of adenosine receptors (reviewed in the section "Adenosine receptors") can then modify cell metabolism according to the set-up of adenosine receptors and to the primary metabolism of each particular cell type. In general, most mammalian cell types are equipped with adenosine A_1 receptors (A_1Rs), whose activation causes a decrease in the rate of metabolism, allowing the cell to cope better with noxious stimuli. Thus, adenosine fulfils an homeostatic role in most cell types (see [2]), whereas a noxious stimuli reaching a particular cell causes a slight drop in energy charge that is converted into a large change in the intracellular levels of adenosine that diffuses out of the cell and signals to neighbouring cells via A_1Rs the presence of a noxious stimuli, preparing neighbouring cells to handle better this noxious stimuli (by generally decreasing their rate of metabolism).

This review will concentrate on the role of adenosine in the realm of neuroprotection, focusing on the brain. It should be kept in mind that the homeostatic role of adenosine described above occurs in the brain as it occurs in most mammalian tissues. But adenosine fulfils other particular roles in the brain, apart from its general homeostatic role. In fact, the brain is the tissue by far with the greatest density of the most abundant adenosine A_1Rs, which play key roles in controlling neuronal excitability and in particular neurotransmitter release, as will be discussed in the section "Modulatory roles of adenosine." And several lines of evidence (reviewed in Cunha [1]) indicate that this particular neuromodulatory role of adenosine is independent of changes in the energy charge of brain cells, i.e. the neuromodulatory role of adenosine is independent of the homeostatic role of adenosine.

It is important to emphasise that adenosine behaves as a typical neuromodulator in the brain. In fact, adenosine signalling is designed to control the flow of information between neurons in the brain, rather than to directly transfer information between neurons, as occurs for neurotransmitters. And in contrast to typical neurotransmitters, there is no evidence available (well on the contrary) for an accumulation of adenosine in synaptic vesicles or for a release of adenosine in a quantal manner. However, as shall be discussed in the section "Generation of extracellular adenosine," the build-up of extracellular adenosine in synapses is closely connected with the release of neurotransmitters and is tightly linked to the frequency and intensity of neuronal firing.

Adenosine receptors

There are four types of membrane-bound adenosine receptors, named A_1, A_{2A}, A_{2B} and A_3 receptors. These receptors have been cloned in different species and are all seven membrane-spanning metabotropic receptors that have, so far (but see [3]), been shown to couple via G proteins (reviewed in [4]). Traditionally, adenosine receptors were divided into two broad groups: A_1Rs and A_3Rs that would negatively couple to adenylate cyclase and A_{2A}Rs and A_{2B}Rs that would positively couple to adenylate cyclase. However, this classification is now difficult to accept because all adenosine receptors have been shown to couple to different G proteins and to different transducing systems in different cell types. Thus, it appears that all adenosine receptors are fundamentally pleiotropic receptors, i.e. receptors with the potential to couple to different G proteins and to different transducing systems according to their degree of activation and with their particular cellular and sub-cellular localization. It is important to emphasise that the long-time assumed relation between brain adenosine receptors and cAMP is mostly of historical interest. For instance, the more widespread effect of adenosine in the brain (inhibition of neurotransmitter release by the most abundant A_1Rs) is now well established to be independent of the control of cAMP levels. This obviously does not exclude that some effects of adenosine receptors in the brain are actually mediated by cAMP, in particular important effects of adenosine like the A_1R-mediated neuronal hyperpolarization or A_{2A}R-mediated signal integration in striato-pallidal neurons.

Out of the four adenosine receptors, the A_1R is the most abundant and widespread in the brain (see, e.g., [5]). A_{2A}Rs are concentrated in the basal ganglia (reviewed in [6]), but they are also present throughout the brain albeit with a considerably lower density (discussed in "Modulatory roles of adenosine"). For these two main adenosine receptor subtypes, there are now good pharmacological tools as well as receptor knockout mice strains to probe their role in the brain. Thus, the role of A_1Rs is explored taking advantage of the selectivity of agonists such as CPA and antagonists such as DPCPX, whereas the role of A_{2A}Rs has been investigated based on the use of its selective antagonist SCH 58261 (and also other less selective antagonists such as ZM 241385, CSC or KW6002). Activation of A_{2A}Rs can be achieved with its agonist CGS 21680 but care is required to exclude the involvement of A_1Rs to which CGS 21680 also binds [7, 8] and activates (e.g., [9]).

Because of their low abundance in the brain, the role of A_{2B}Rs and A_3Rs has received considerably less attention. Thus, the role of adenosine in the brain is currently considered to be mediated by a balanced activation of A_1 and A_{2A} receptors, as shall be detailed in this review. It is hoped that greater experimental efforts as well as novel tools will allow a novel perspective on the eventual relevance of the less abundant A_{2B}Rs and A_3Rs.

It is also relevant to mention that the traditional idea of A_1Rs and A_{2A}Rs as individual signalling systems may also need to be revised. As illustrated in Figure 1, molecular studies have opened the possibility of conceiving novel entities involving adenosine receptors, which may either be homodimers of either A_1Rs [10] or A_{2A}Rs [11] or heterodimers. The heterodimers so far identified can involve A_1Rs with either $P2Y_1$Rs (a metabotropic receptor for ATP) [12] or metabotropic glutamate type 1 receptors [13] as well as A_{2A}Rs with either dopamine D_2Rs [14] or metabotropic glutamate type 5 receptors [15, 16]. Although the functional relevance of most of these dimers involving adenosine receptors remains to be determined, this notion of receptor dimerization clearly wideness the possible impact of adenosine on brain function and demands a re-

evaluation of our current pharmacological classification of adenosine receptor-mediated effects.

Subcellular localization of adenosine receptors

Although A_1Rs, and also A_{2A}Rs, are widespread in the brain, they are not homogeneously located in neurons and other cell types in the brain. Thus, the investigation of their cellular and subcellular localization may provide a first insight on the possible modulatory role(s) that may be fulfilled by adenosine in the brain.

Although A_1Rs are most abundant in limbic and neocortical regions, they are also abundant in the basal ganglia and cerebellum and are also present in most nuclei in the diencephalon and brain stem (see [5] for a detailed mapping of the relative densities of brain A_1Rs). A_1Rs are considerably more abundant in neurons, but they are also present in astrocytes [17], microglia [18] and oligodendrocytes [19], albeit with a much lower density. The localization of A_1Rs in neurons is also highly asymmetric. Immunohistochemical studies of A_1R localization in brain preparations mostly concluded that A_1Rs displayed a predominant axonal localization (see [20]). This probably reflects the general low accessibility of antibodies to extracellular epitopes located in the synaptic cleft, which is densely packed with adhesion molecules [21]. In fact, when using radioligands or antibodies following cellular fractionation, it is concluded that A_1Rs are most abundant in synapses [22], in particular in the presynaptic active zone and post-synaptic density [23]. Functional data also indicate that the efficiency of A_1R activation (and probably A_1R density) is also different amongst different types of nerve terminals. In fact, A_1Rs efficiently control the release of glutamate, acetylcholine and serotonin, but several studies have documented the inability of A_1Rs to modulate the release of GABA and of noradrenaline in the brain (reviewed in [1]). This leads to the idea (detailed in "Modulatory roles of adenosine") that A_1Rs mainly fulfil a synaptic neuromodulatory role in particular in excitatory nerve terminals in the brain.

In contrast to the widespread distribution of A_1Rs in the brain, A_{2A}Rs are highly concentrated in the basal ganglia (reviewed in [24]), where their density is about 20 times greater than elsewhere in the brain [7]. In the basal ganglia, A_{2A}Rs are predominantly located in dendritic spines [25] and post-synaptic densities [26] of asymmetric contacts between cortico-thalamic glutamatergic projections and medium spiny GABAergic neurons, as well as symmetric contacts between medium spiny neurons (see [25, 27]). In fact, striatal A_{2A}Rs are almost segregated to a particular sub-type of medium spiny neurons, the enkephalin-containing medium spiny neurons that constitute the indirect pathway (see [24]), where they are located post-synaptically controlling the integration of signal responses in these neurons (see reviews in [6, 24, 28]). This high density of striatal A_{2A}Rs has lead to the erroneous idea that A_{2A}Rs were *exclusively* located in the basal ganglia. However, both *in situ* hybridization [29], binding [7], immunological [30, 31] and functional studies [31, 32] have concurred to the conclusion that A_{2A}Rs are located in limbic and neocortical regions in the brain. And in these regions, A_{2A}Rs have a localization fundamentally different from that found in the striatum. In fact, cortical A_{2A}Rs are predominantly located in synapses, in particular in the presynaptic active zone [33]. And as discussed in "Modulatory roles of adenosine," this localization of A_{2A}Rs is in agreement with the predominant role of extra-striatal A_{2A}Rs in the control of the release of neurotransmitters (reviewed in [1]). Again, in contrast to A_1Rs, A_{2A}Rs seem to have a broader localization (although at a considerably lower density) in different types of nerve terminals. In fact, extra-striatal A_{2A}Rs can not only control the release of glutamate (e.g., [32]), acetylcholine (e.g., [30, 34–36]), but can also control the release of GABA [37] and of noradrenaline [38], which are mostly insensitive to A_1R activation. Apart from their presynaptic localization, A_{2A}Rs are also located in astrocytes [39, 40] and microglia cells [41, 42] as well as in brain blood vessels [43, 44], most likely in endothelial cells.

In conclusion, throughout the brain, there is predominant synaptic localization of both A_1 and A_{2A} receptors. Both A_1Rs and A_{2A}Rs are mostly located presynaptically and A_1Rs also have a dense post-synaptic localization. The striatum is clearly the exception, where A_{2A}Rs are most densely located post-synaptically. Apart from this predominant neuronal localization, both A_1Rs and A_{2A}Rs are also located in astrocytes and microglia and A_1Rs are located in oligodendrocytes and A_{2A}Rs in blood vessels.

The rest of the review will mostly concentrate on the role of A_1 and A_{2A} receptors in mediating the effects of adenosine in the brain. In fact, because of the lack of selective pharmacological tools and of their low density in the brain, the possible roles of A_3Rs and A_{2B}Rs in the brain are still largely unexplored. However, it should always be kept in mind that both A_3Rs and A_{2B}Rs are also present in the brain. Thus, the presence of A_3Rs has been defined in neurons, both with binding [45], immunological [46] and functional studies [47, 48] but their function seems more evident in astrocytes (e.g., [49]) and eventually in microglia [50]. With respect to A_{2B}Rs, they are not present in microglia [50] but are mainly located in astrocytes (e.g., [51]). Some functional studies also indicate their possible presence in neurons (e.g., [52]) although molecular evidences for their neuronal location are still lacking.

Modulatory roles of adenosine

As inferred from the presentation made on the localization of adenosine receptors, it is expectable that the main effect of adenosine on brain function might be a presynaptic control of the release of neurotransmitters. Also, based on the considerably greater density of A_1Rs compared to other adenosine receptor subtypes in the brain (excepting the basal ganglia), it is also expectable that the predominant effect of adenosine in the brain might be an inhibition of neurotransmitter release. And, in accordance with these

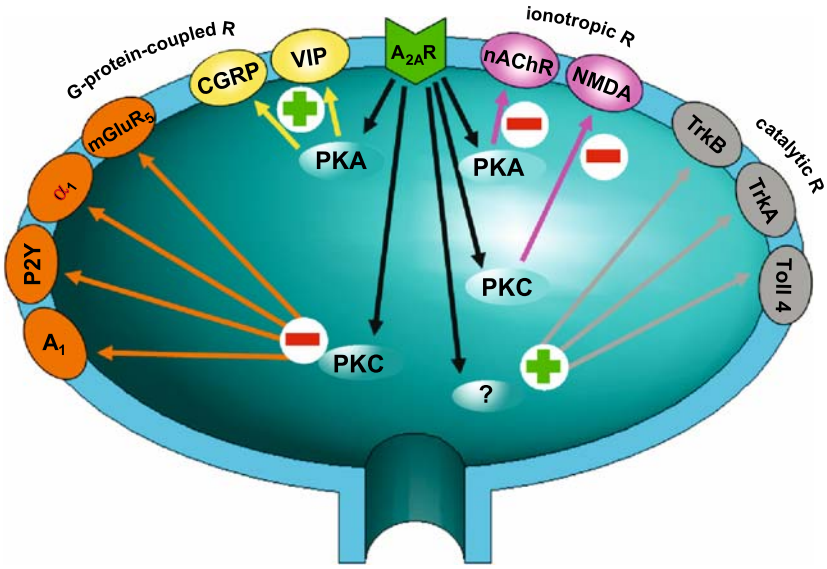

Figure 1. Ability of adenosine A_{2A} receptors (A_{2A}Rs) to fine-tune the efficiency of functioning of different other modulatory systems operated by either metabotropic, ionotropic or catalytic receptors. Activation of A_{2A}Rs can either inhibit (−) or facilitate (+) the receptors using either protein kinase A (PKA) or C (PKC) as transducing pathways. Note that this modulation of different systems is a compilation and does not imply that A_{2A}Rs affect all these transducing pathways in the same cell or that each target is always under the control of A_{2A}Rs.

predictions, electrophysiological studies of the role of adenosine in brain slices have concluded that adenosine mainly inhibits neuronal excitability and synaptic transmission (reviewed in [53–55]). This inhibitory effect of adenosine is mediated by A_1Rs and, accordingly, adenosine is nearly devoid of effects of synaptic transmission and neuronal excitability in brain slices in the A_1R knockout mice [56].

The adenosine A_1R-mediated inhibition of neuronal excitability and synaptic transmission is a dual role exerted in different neuronal compartments, both concurring to refrain neuronal activity. In fact, A_1R activation inhibits excitatory synaptic transmission, mostly through a presynaptic inhibition of glutamate release [57, 58]. This tonic presynaptic control by A_1Rs has recently been shown to be a key factor in defining the release probability of different hippocampal synapses [59]. In parallel, A_1R activation also inhibits potassium conductances at the postsynaptic level, leading to neuronal hyperpolarization (reviewed in [55]). This latter effect is of uppermost importance to control the bursting of neuronal firing, but has a minor importance for the control of synaptic transmission at lower frequencies of nerve stimulation (see [58]). However, it has been questioned whether the A_1R-mediated inhibition of glutamate release (which appears to be the predominant action of adenosine at lower frequencies of nerve stimulation) might be of relevance for the control of neuronal firing at higher frequencies of nerve stimulation (see [60]). The high density of A_1Rs in the post-synaptic density also anticipates an important role of adenosine in the control of signal integration at the post-synaptic level. Accordingly, the pioneering work of de Mendonça revealed that the tonic activation of A_1Rs controls the amplitude of synaptic plasticity in excitatory circuits (reviewed in [61]). This might result from the ability of A_1Rs to efficiently control NMDA receptors [62, 63] as well as post-synaptically located voltage-sensitive calcium channels [52, 63].

In contrast to these multiple actions of A_1Rs to inhibit neuronal function, our knowledge about the role of A_{2A}Rs in modulating neuronal activity is more limited. This is

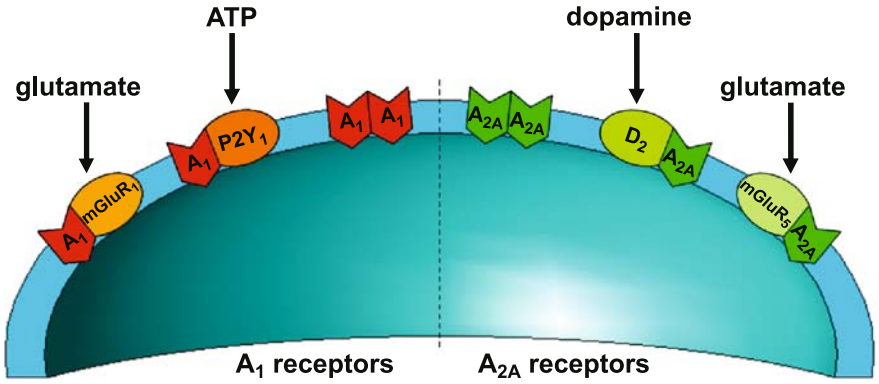

Figure 2. Adenosine A_1 and A_{2A} receptors (A_1R, A_{2A}R) can form dimers with different metabotropic receptors operated by other neurotransmitters. This opens the possibility that adenosine may operate hitherto unrecognised entities that may display novel pharmacological properties.

mainly because most of the studies have focused in the basal ganglia where A_{2A}Rs are by far more abundant because of their 'abnormal' large expression in the medium spiny neurons of the indirect pathway. Thus, the study of these A_{2A}Rs, which have a particular density and subcellular localization in this particular set of neurons, might not be representative of the more general role of A_{2A}Rs in the most regions of the brain. Outside the basal ganglia, the function that has mostly been ascribed to A_{2A}Rs is the control of neurotransmitter release, in accordance with the preponderant presynaptic localization of extra-striatal A_{2A}Rs (see [33]). As previously discussed (see the section "Subcellular localization of adenosine receptors"), A_{2A}Rs have been shown to facilitate the release of most neurotransmitter types (glutamate, GABA, glycine, acetylcholine, noradrenaline, serotonin) in different extra-striatal brain regions. Although it is also known that extra-striatal A_{2A}Rs are also located in the post-synaptic density (although with a density lower than in nerve terminals), only one report has so far described the ability of A_{2A}R activation to depolarise hippocampal neurons by a mechanism that remains to be unravelled [64].

In conclusion, in relation to the two main adenosine receptors in the brain, both seem to cause opposite effects on the release of excitatory neurotransmitters in the brain. In particular, in glutamatergic nerve terminals, it has been shown that A_1 and A_{2A} receptors are co-located in a subset of these terminals in the hippocampus [65] and there is a functional interaction between these two adenosine receptors with opposite effects on glutamate release [32, 66]. Furthermore, extra-striatal A_{2A}Rs can also facilitate the release of some neurotransmitters that are not controlled by A_1Rs, like GABA or noradrenaline. Apart from these presynaptic effects, A_1Rs can also concur to inhibit neuronal activity by acting post-synaptically, both in distal dendrites (mainly at the post-synaptic density) as well as in proximal dendrites.

Whereas most available work provides a strong support for the effects of A_{2A}Rs in controlling neurotransmitter release and of A_1Rs to control both neurotransmitter release and neuronal excitability, it is important to also consider other more subtle or indirect mechanisms by which adenosine receptors might control neuronal function. One of the subtle effects of adenosine might be to fine-tune other systems controlling neurotransmitter release [67]. The work of Correia-de-Sá was instructive to understand the key role of A_{2A}Rs in resetting the modulatory systems able to come into play at the neuromuscular junction. Thus, the increased activation of A_{2A}Rs with increasing frequencies of nerve stimulation, can shut down the presynaptic nicotinic autofacilitatory system [68], reset the muscarinic acetylcholine receptors [69] and allow the peptidergic presynaptic modulatory systems to come into play [70, 71]. Further work by Sebastião has extended this idea to the brain, where the peptidergic modulation of excitatory synaptic transmission in the hippocampus by G protein coupled receptors (operated by CGRP and VIP) is strictly dependent on the activation of A_{2A}Rs [72, 73]. More recent work has also found that the effects of BDNF on hippocampal synaptic transmission are also abolished by blocking adenosine receptors [74] and A_{2A}Rs are able to trans-activate TrkB receptors [75]. This places A_{2A}Rs in a key position to shut on and off the important effects of neurotrophins in the brain. Figure 2 summarises the known modulatory systems whose efficiency is controlled by A_{2A}Rs and shows that receptors from all classes (metabotropic, ionotropic and catalytic) are under control by A_{2A}Rs. These results also prompt the need to always evaluate the status of adenosine receptors when studying any other presynaptic modulatory systems, since the functioning of most of the presynaptic neuromodulatory systems are in fact under a tight control by adenosine receptors (see [76]).

It should also be kept in mind that the effects of adenosine receptor activation are most likely not restricted to the direct control of neuronal activity. In fact, adenosine receptors are also located in other cell types in the brain (astrocytes, microglia, oligodendrocytes) that can indirectly influence neuronal activity. Figure 3 illustrates the localization of A_1Rs and A_{2A}Rs in different cell types and compartments in the brain, showing that adenosine is indeed well positioned to participate in neuron–glia communication. Astrocytes are equipped with all four types of adenosine receptors [77–82] that control astroglyosis [83–84] and the release of different substances that can impact on neuronal activity [39, 85–88]. As occurs for the presynaptic control of neurotransmitter release, adenosine receptors in astrocytes also fine-tune the action of several other receptor systems in astrocytes, like metabotropic glutamate receptors [89, 90], histamine [91], α1-adrenergic receptors [92] and ATP P2Y receptors [93, 94]. One particular exciting action of adenosine receptor activation in glial cells is the control of the expression and release of cytokines [85, 95, 96]. Furthermore, adenosine receptors also control microglia reactivity [18, 97–99]. This prompts the hypothesis, initially raised by Schubert [100] that adenosine might play an important role in the control of neuro-inflammation, an issue that will be discussed latter in more detail in the realm of the neuroprotective role of adenosine.

Finally, the last topic that should be considered when discussing the actions of adenosine in the brain is the ability of adenosine receptors to control metabolism. In fact, the activation of adenosine receptors can modify the primary metabolism of most cell types (see [1]) and this is also true for both neurons and astrocytes [101, 102] and in particular for the control of glygogen metabolism [51, 103, 104]. However, it still remains to be explored if this modulation of brain metabolism by adenosine receptors is implicated in the neuromodulatory or neuroprotective properties of adenosine or if it might be related with the trophic effects of purines (see [105]).

Generation of extracellular adenosine

The general presentation of the different roles operated by adenosine in the brain clearly illustrates that adenosine

causes different and most often opposite actions by activating different receptors. Furthermore, it is now becoming clear that adenosine receptors causing opposite effects can be co-localised, at least in nerve terminals. Thus, it becomes of uppermost importance to understand how the differential activation of the different adenosine receptors can be effectively controlled to meet the needs of the system. One possibility would be that the different adenosine receptors might have different affinities for their endogenous ligand, i.e. adenosine. However, because it is not possible to completely eliminate endogenous adenosine (which is present in all biological preparations) one can only estimate rough affinities of the different adenosine receptors for adenosine. And most results suggest that the affinity of adenosine for A_1Rs and A_{2A}Rs is similar, in the low nanomolar range. Thus, one has to assume that there might be different ways of generating adenosine to activate either A_1Rs or A_{2A}Rs (reviewed in [1]). The correlate of this assumption is that one cannot define an "extracellular concentration of adenosine," but one should instead discuss an "extracellular gradient of adenosine."

The data obtained in hippocampal nerve terminals provides probably one of the few clear pictures of the extracellular metabolism of adenosine (which is particularly relevant given that the predominant role of adenosine is a presynaptic control of neurotransmitter release). Two main mechanisms have been identified in nerve terminals for the generation of extracellular adenosine: one is based on the release of adenosine as such through bi-directional non-concentrative (or equilibrative) nucleoside transporters [106]. In fact, inhibition of equilibrative nucleoside transporters can actually decrease the extracellular levels of adenosine in nerve terminals [107, 108], in accordance with a build-up of extracellular adenosine involving its release through equilibrative nucleoside transporters in this particular compartment of the brain. The second mechanism for the extracellular build-up of adenosine is its formation from released ATP, after its extracellular catabolism by ecto-nucleotidases (reviewed in [109]). Thus, ATP is stored in synaptic vesicles and nerve terminals release ATP on stimulation (reviewed in [110]). This release of ATP is larger the higher the frequency of nerve stimulation [111, 112] and the contribution of ATP-derived adenosine increases with increasing frequencies of nerve stimulation [112, 113]. In contrast, the contribution of adenosine released as such through equilibrative nucleoside transporters predominates at lower frequencies of nerve stimulation ([112]; see also [114]). Thus, in these hippocampal excitatory nerve terminals, there are two mechanisms responsible for the formation of extracellular adenosine and two adenosine receptors (A_1Rs and A_{2A}Rs) with opposite effects on glutamate release. And electrophysiological studies at these synapses revealed that the inhibitory effects of A_1Rs clearly predominate at low frequencies of nerve stimulation, since blockade of A_{2A}Rs, but not A_1Rs, is devoid of effects [31, 115]. However, stimulation with burst of high frequencies reveals a tonic activation of A_{2A}Rs [116] and inhibition of ecto-5′-nucleotidase blunts the tonic activation of A_{2A}Rs [113, 117].

In conclusion, in this particular compartment of the brain, the available data provide a rationale to understand the differential activation of A_1Rs and A_{2A}Rs as a function of the intensity of functioning of the nerve terminals (reviewed in [1, 109]), which is based on the different relative contributions of two possible pathways for the build-up of extracellular adenosine, as summarised in Figure 4. This general mechanism of controlling A_1R *versus* A_{2A}R activation according to the levels of released ATP seems to be valid for hippocampal excitatory nerve terminals (reviewed in [1, 109]) and phrenic nerve endings [113]. However, it is important to stress that different types of nerve terminals are likely to have different organizations of extracellular adenosine metabolism and adenosine receptors as found, for instance, in cortical or hippocampal cholinergic nerve terminals (reviewed in [109]). One key aspect of the mechanistic explanation coupling the extracellular metabolism of released ATP with the preferential activation of A_{2A}Rs is the proximal localization of ecto-5′-nucleotidase (responsible for the formation of ATP-derived adenosine) and A_{2A}Rs. This has, so far, not been directly demonstrated to occur in hippocampal nerve terminals. However, it is striking to note that several physiological [117, 118] and pathological situations [119, 120] cause a parallel increase of the activity of ecto-5′-nucleotidase and of the density of A_{2A}Rs, in contrast to A_1Rs. Furthermore, in different models, it has been shown that noxious stimuli cause a parallel increase of the expression of ecto-5′-nucleotidase and of A_{2A}Rs [121–123], strongly supporting the view that these two molecules are tightly interconnected.

In more integrated brain preparations, the relation between A_1R and A_{2A}R activation is less well defined. Here, it is unlikely that it might be the extracellular metabolism of adenosine that governs the relative activation of A_1Rs and A_{2A}Rs, because there is scarce evidence to support the co-localization of these two receptors outside excitatory nerve terminals. Furthermore, the source of extracellular adenosine is less well defined in more integrated brain preparations. Thus, most cell types and compartments in the brain are equipped with equilibrative nucleoside transporters. However, in integrated brain preparations, the inhibition or blockade of equilibrative nucleoside transporters causes an *increase* rather than a *decrease* of extracellular adenosine (reviewed in [124]). This means than the role of nucleoside transporters is to clear-up adenosine rather than to promote its release. It is now well accepted that all cell types release ATP, namely, neurons, astrocytes or microglia cells, by mechanisms that remain controversial [125]. Likewise, all cell types are endowed with ecto-nucleotidases, forming an efficient enzymatic pathway to convert ATP into adenosine (reviewed in [126]). However, in most integrated brain preparations (and in contrast to nerve terminals), the prototypical inhibitor of ecto-5′-nucleotidase, α,β-methylene ADP, fails to modify the extracellular levels of adenosine (reviewed in [124]). It is possible that in more integrated preparations, the conversion of adenine nucleotides into adenosine cannot be effectively prevented. In

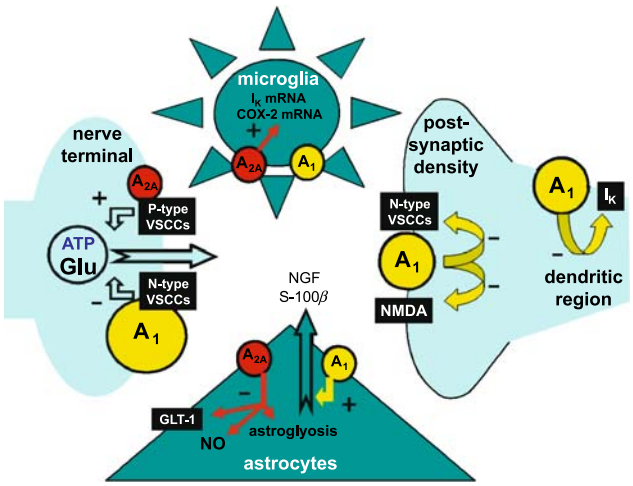

Figure 3. All cell types and sub-cellular compartments in the brain are endowed with different relative proportions (indicated by the relative size of the circles) of adenosine A_1 and A_{2A} receptors (A_1R, $A_{2A}R$) that fulfil different roles according to their localization. Since all the represented compartments can also release both ATP and adenosine and are endowed with ecto-nucleotidases to convert extracellular ATP into adenosine, this nucleoside is ideally positioned to mediate all conceivable forms of neuron–glia communication. This scheme also illustrates that the effects of adenosine in the brain might always result from a balanced modulation of all cell types and compartments in the brain. COX, cyclooxygenase; GTL, glutamate transporter; Glu, glutamate; NGF, nerve growth factor; VSCCs, voltage-sensitive calcium channels.

fact, α,β-methylene ADP is a competitive inhibitor of ecto-5′-nucleotidase, but does not affect other ecto-enzymes able to metabolise AMP (reviewed in [126]). It should also be kept in mind that the ecto-nucleotidase system is a notable efficient system, probably organised in a channelled manner [127] and able to generate adenosine to act on its receptors in a few milliseconds [128], a time course faster than the K_{cat} of soluble enzymes. Thus, in more integrated preparations, either the concentration of α,β-methylene ADP fails to equilibrate with ecto-5′-nucleotidase or enzymes other than ecto-5′-nucleotidase are mostly responsible for the formation of extracellular adenosine or there are other (still unknown) pathways of extracellular adenosine formation that are still to be identified. Note that in purified nerve terminals, which only account for 1%–2% of the volume of more integrated preparations [129], spatial restrains are decreased and it is the only preparation where it has been demonstrated that ecto-5′-nucleotidase was the predominant enzymatic activity responsible for the formation of extracellular adenosine from adenine nucleotides [130]. Thus, in nerve terminals, α,β-methylene ADP is able to decrease the formation of extracellular adenosine [107, 108, 112, 117, 118].

In conclusion, in more integrated brain preparations, it appears that the role of equilibrative nucleoside transporters is to clear-up adenosine. Hence, the formation of extracellular adenosine should result from the extracellular metab-

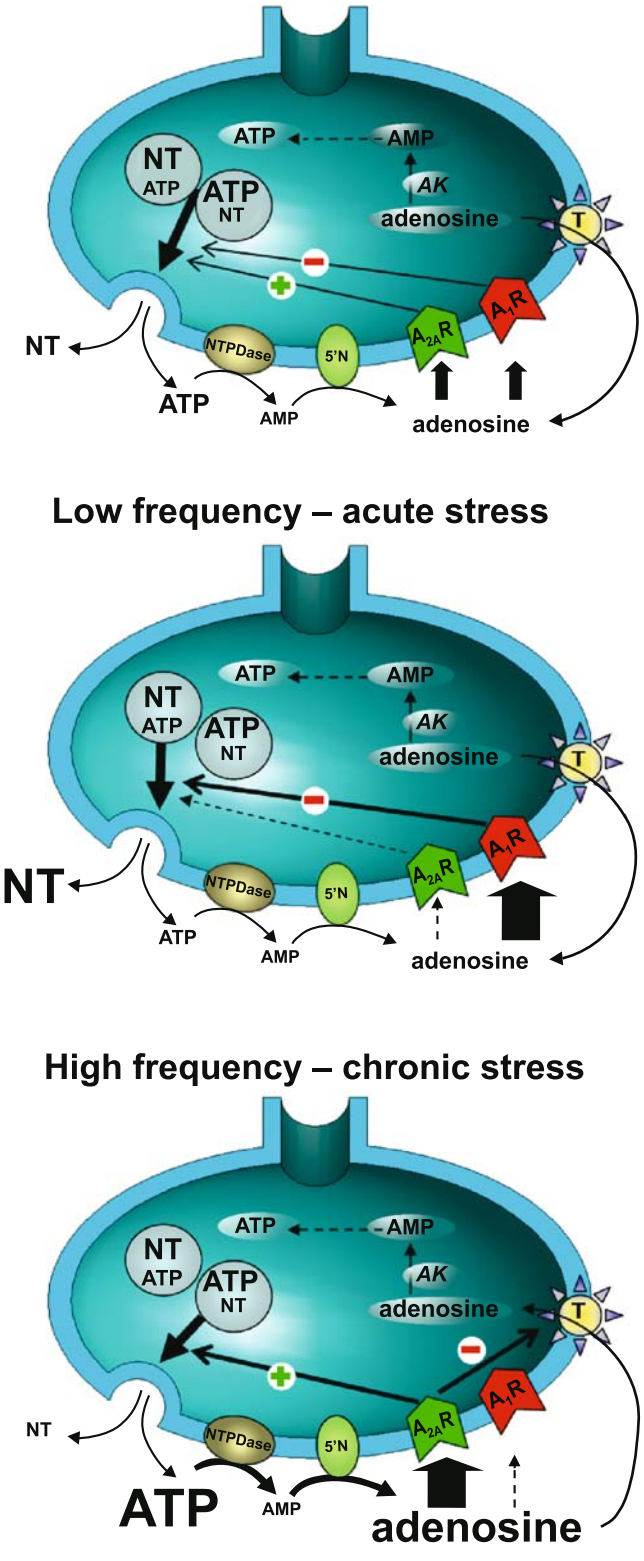

Figure 4. Schematic representation of the ability of the extracellular metabolism of adenosine (and ATP) to determine which adenosine receptor-mediated effect will be preponderant in glutamatergic nerve terminals of the rat hippocampus. Thus, at lower frequencies of nerve stimulation, there is a lower release of ATP, which will form low amounts of adenosine allowing the nucleoside transporters to contribute for the accumulation of extracellular adenosine, favouring the activation of A_1 receptors. At higher frequencies of stimulation, the release of ATP is disproportional larger, the extracellular levels of adenosine will be essentially derived from ATP (after its catabolism by ecto-nucleotidases), leading to a preferential activation of A_{2A} receptors that are in close proximity of ecto-5′-nucleotidase. ADO, adenosine; AK, adenosine kinase; NT, neurotransmitter (in this case, glutamate); NTDPase, ecto-nucleotidases able to convert ATP and/or ADP into AMP; 5′-N, ecto-5′-nucleotidase; T, equilibrative nucleoside transporters.

olism of released adenine nucleotides, but direct evidence for this is lacking. Extracellular ATP is the strongest candidate to act as a primary source of adenosine since it is released in a controlled manner from neurons (reviewed in [110]) and from astrocytes [131–141] as well as from activated microglia [142]. It has also been proposed that cAMP could be released from neurons [143], but its contribution is at best limited [144]. Clearly further work is required to elucidate the pathways of generation of extracellular adenosine in the brain, which is a pre-requisite to understand the dynamics of activation of adenosine receptors in different physio-pathological conditions.

Modification of adenosine metabolism on stressful conditions

When considering the relevance of adenosine in the realm of neuroprotection, it is most important to first consider the consequences of noxious stimuli on the extracellular metabolism of adenosine. This will allow gaining insight on the neuroprotective role of endogenous adenosine and it is also instructive to understand if it makes sense to pharmacologically manipulate the effects mediated by adenosine receptors when their tonic activation might be dramatically modified.

The energy charge is one of the fundamental parameters (together with the redox status) to define the status of the primary metabolism of cells. Hence, it is one of the earliest parameter to be re-adjusted by any stressful stimuli in all cell types. Based on the tight relation between the levels of adenosine and the energy charge, it is expectable that the tissue levels of adenosine will be modified by noxious stimuli. In fact, the intracellular concentration of ATP is in the range of 3–10 mM, i.e. about 100,000 times greater than that of adenosine (between 10 and 50 nM). Thus, slight changes in the concentration of ATP will cause several-fold changes in the intracellular concentration of adenosine (discussed in [1]). Therefore, it is not surprising that stimuli ranging from increased neuronal firing to hypoxia, ischemia or cell poisoning will cause increases in the extracellular levels of adenosine (reviewed in [124]; [1]). However, the mechanism responsible for coupling the expected increase in the intracellular levels of adenosine and the observed increased levels of extracellular adenosine is still not clear.

The simplest explanation would be that the adenosine formed intracellularly would be released through equilibrative nucleoside transporters thus leading to increased levels of extracellular adenosine. According to this scenario, there is a good correlation between the graded intensity of the noxious stimuli applied, the drop in energy charge of the studied brain tissue and the extracellular levels of adenosine (e.g., [145, 146]). Also, blockade of the intracellular enzymatic pathways responsible for the consumption of intracellular adenosine (mainly adenosine deaminase and adenosine kinase) increase the extracellular levels of adenosine under different noxious conditions in brain preparations (e.g., [147, 148]; reviewed in [124]). However, the inhibition of equilibrative nucleoside transporters increases the extracellular levels of adenosine. This tells us that the role of equilibrative nucleoside transporters is mostly to take up rather than to release adenosine, as occurs during non-stressful conditions (reviewed in [1]; [124]). The obvious alternative is that the formation of extracellular adenosine should result from the extracellular catabolism of released adenine nucleotides triggered by noxious stimuli. Some few studies have reported a release of ATP as such during stressful stimulation of brain preparations [149–153]. Also, both axonal depolarization and increased glutamate levels, both of which are hallmarks of potential neurotoxic conditions, are effective triggers of ATP release [154–156]. And, although it remains to be tested if this stress-induced ATP release contributes for the extracellular build-up of adenosine, it is interesting to note that there is an up-regulation of ecto-nucleotidases upon noxious brain conditions [120, 157–159].

This presentation and discussion of the data available on purine release during noxious brain conditions clearly tells us how little is known about the pathways of generation of extracellular adenosine in stressful conditions. In fact, neither the pathways leading to the build-up of extracellular adenosine nor the major cell type (or sub-cellular compartments) contributing for this elevation of the extracellular levels of adenosine caused by noxious stimuli have been experimentally tackled.

In spite of our ignorance on how extracellular adenosine is formed, it is never the less evident that noxious stimuli trigger a robust increase in the extracellular levels of adenosine that reaches micromolar concentrations in the extracellular fluid of stressed brain preparations (reviewed in [124]). Given that the affinity for adenosine of the most abundant adenosine receptors (A_1Rs and A_{2A}Rs) is in the low nanomolar range, the obvious question that pops up is whether these increased levels of endogenous adenosine are enough to saturate A_1Rs and A_{2A}Rs. In fact, if this was to occur, it would make little sense to devise any therapeutic neuroprotective strategy based on the use of agonists of adenosine receptors. This does not appear to be the case since inhibition of the key enzymatic activities thought to control the availability of adenosine still potentiates the neuroprotective effects of endogenous adenosine. In fact, inhibition of adenosine kinase is strongly neuroprotective in different animal model of brain injury [160–166], and some, but not all studies (see [167, 168]), also found that inhibitors of adenosine deaminase were neuroprotective [169, 170]. This clearly indicates that it is justified to invest a greater effort into understanding the changes of the metabolism of adenosine caused by noxious stimulation.

Acute A_1 receptor activation increases the threshold for acute neurodegeneration

When considering which adenosine receptor plays a major role in affording neuroprotection in the brain, the strongest

candidate is obviously the A_1R. This is because A_1Rs have a major inhibitory effect on synaptic transmission and neuronal excitability (see "Modulatory roles of adenosine") and also because they are, by far, the most abundant adenosine receptor subtype in the brain. And, in fact, a diversity of studies in different brain preparations of different species using different noxious stimuli, consistently found that the acute activation of inhibitory A_1Rs is neuroprotective (elegantly reviewed in [171]). Thus, in isolated neurons and in brain slices, A_1R activation reduces damage to neurons, whereas A_1R antagonists potentiate damage (reviewed in [171–174]). Likewise, in whole animals subject to ischemia or other type of brain noxious stimuli (e.g., epileptic models, trauma, exposure to excitotoxins), it is also concluded that the acute activation of A_1Rs is neuroprotective, whereas A_1R antagonists potentiate damage (reviewed in [171–176]).

In conclusion, the data available are notably consistent in establishing a neuroprotective role for A_1R acute activation in noxious brain conditions in adult animals. Likewise, the worsening effect caused by A_1R antagonists, as well as the beneficial effect caused by enhancing the extracellular levels of adenosine (reviewed in [171–174]) or using allosteric enhancers of A_1Rs [177], also indicates that the tonic activation of A_1Rs is an endogenous neuroprotective system in stressful brain situations.

However, the therapeutic interest of A_1R agonists has several limitations that hamper its usefulness as novel neuroprotective drugs. The first major drawback is due to the profound cardiovascular effects of A_1R agonists (e.g., [178, 179]), which are most worrying because A_1R agonists have a poor brain permeability [180, 181]. The second limitation is related to the short 'window of opportunity' of A_1R agonists, which is limited to a few hours, at most, after the initiation of the brain insult (reviewed in [171, 173, 174]; but see [182]). This is aggravated by the fact that is not conceivable to administer A_1R agonists chronically (as a preventive strategy) because it causes an effect inversion, i.e. chronic A_1R stimulation actually exacerbates neuronal loss caused by noxious brain stimulation (reviewed in [183]). Finally, the last major limitation to develop A_1R agonists as neuroprotective drugs is the observation that the effect of A_1R activation desensitize in chronic stressful brain conditions, as shall be discussed in more detail in "Long-term desensitization of A_1 receptors and up-regulation of A_{2A} receptors by chronic noxious conditions."

In conclusion, it appears that the activation of A_1Rs is an endogenous neuroprotective system, but its usefulness is limited to *acute* noxious brain conditions, i.e., to control the onset or enhance the threshold of neuronal damage.

Long-term desensitization of A_1 receptors and up-regulation of A_{2A} receptors by chronic noxious conditions

Adenosine A_1Rs belong to the G protein-coupled receptor family but, unlike most in their family, A_1Rs have a long half-life (e.g., [184]) and seem to be resilient to desensitization [185]. In fact, several works suggest that neuronal A_1R desensitization occurs in large time frames (12–24 h) of exposure to exogenously added A_1R agonists *in vitro* (e.g., [184, 186, 187]) as well as *in vivo* (e.g., [188, 189]). The time course of desensitization of A_1Rs is particularly critical to understand if adenosine maintains its neuroprotective efficiency in chronic noxious brain situations.

In animal models of epilepsy as well as in patients with temporal lobe epilepsy, i.e., in situations causing a long-lasting enhanced release of adenosine (e.g., [190–192]), there is a long-term decrease in the density of A_1Rs in different brain regions [120, 193–195], which is also observed in patients with mesial sclerosis ([196]; but see [197]). This decreased density of A_1Rs is in general agreement with the development of tolerance in relation to the anti-convulsive effects of A_1 receptor agonists [198, 199] that is accompanied by a reduced potency of A_1R agonists [120]. In other chronic neurodegenerative conditions, such as Alzheimer's disease, the density of A_1Rs is also reduced ([206, 397, 398]; but see [207]). Likewise, several studies showed that short periods of brain ischemia, which also trigger a robust increase in the extracellular levels of adenosine ([121]; reviewed in [172]), produce a long-lasting decrease in the density of A_1Rs in several brain regions (e.g., [200–202]). Again, this hypoxia-induced homologous desensitization of A_1Rs [203] is accompanied by a loss of efficiency of A_1R agonists when applied more than one hour after the hypoxia period (reviewed in [171, 173, 174]; but see [182]). This homologous desensitization of A_1Rs has also been documented in other situations which trigger the release of adenosine. Thus, the implementation of long-term potentiation, which triggers a robust release of adenosine (see [112]), also decreases the efficiency of A_1R modulation of synaptic transmission [204, 205].

In conclusion, several physio-pathological conditions able to generate endogenous extracellular adenosine, cause a long-term down-regulation of A_1Rs that contributes to hamper the neuroprotective effectiveness of the A_1R system in chronic noxious brain conditions. This is in agreement with the idea that the activation of A_1Rs is important to control the *acute onset* of neuronal dysfunction and/or neurodegeneration, but that these A_1Rs suffer a long-term desensitization making the A_1R system less appealing as a target for the development of neuroprotective agents aimed at interfering with *long-term chronic* noxious brain conditions.

Considerably fewer studies have been performed to investigate the long-term effect of noxious stimuli to the density and efficiency of $A_{2A}Rs$ in the brain. Most studies have focused on the striatum, where both the density and the role of $A_{2A}Rs$ are biased by their particular localization with high density in a particular subset of neurons, the medium spiny neurons of the indirect pathway. Interestingly, in clear contrast with what occurs for A_1Rs, chronic stressful stimuli directed to the basal ganglia cause an increased expression and density of $A_{2A}Rs$, as observed in animal models of Parkinson's disease (e.g., [208, 209]) and

in Parkinsonian patients ([210]; see also [211, 212]). It is also possible that the expression of A_{2A}Rs might also increase in animal models and patients with Huntington's disease, since the expression of A_{2A}Rs is increased in neurons over-expressing huntingtin [213]. However, the major loss of medium spiny neurons in this condition may mask the increased density of A_{2A}Rs in the remaining viable neurons [211, 214, 215]. Interestingly, this idea that brain A_{2A}Rs might be up-regulated by noxious stimuli was most evident in a recent study focusing on extra-striatal regions. It was observed that convulsive behaviour caused a long-term robust enhancement of the density of cortical A_{2A}Rs, which contrasted with the decreased density of cortical A_1Rs [195]. Likewise, in brain sections from patients with Alzheimer's disease, a greater density of A_{2A}Rs was also observed, which was reported to be confined to microglia processes [207]. This is in agreement with the recently reported increased in the density of A_{2A}Rs in activated microglia cells [216] and with the ability of cytokines to up-regulate A_{2A}Rs [217]. This increase in A_{2A}R density and efficiency is also observed in other cell models systems, like PC12 cells (see [121, 218]) or activated lymphoid cells [219–223]. Finally, an up-regulation of A_{2A}Rs was also reported in schizophrenic patients [224].

In conclusion, the balance between A_1Rs and A_{2A}Rs in the brain appears to be modified by stressful stimuli. In fact, stressful stimuli cause a decrease of A_1R density and efficiency whereas there is an increased expression and density of A_{2A}Rs. This confirms the idea that the neuroprotective effect of A_1Rs is probably most relevant at the onset of brain damage, whereas A_{2A}Rs might come into play at latter stages of brain damage and in particular in chronic noxious brain conditions that are characteristic of neurodegenerative diseases.

A_{2A} receptor blockade confers robust neuroprotection

The presentation of the available evidence to suggest an up-regulation of A_{2A}Rs by noxious stimuli makes it logical to conceive that the manipulation of the activity of this receptor might affect the outcome of brain damage. But the first report by Phillis' group that a non-selective A_{2A}R antagonist (CGS 15943) attenuated cerebral ischemic injury [225], in contrast to A_1R antagonists [226], appeared as a serendipitous observation. Similar observations were made by von Lubitz in a similar gerbil model of brain ischemia [227]. However, this concept of A_{2A}R blockade as a neuroprotective strategy was difficult to understand at the time and consequently was not widely accepted until the group of Ongini and the group of Chen demonstrated that both the pharmacological blockade of A_{2A}Rs with a selective antagonist (SCH 582610) [228] as well as the genetic inactivation of A_{2A}Rs (using A_{2A}R knockout mice) [229] conferred a robust neuroprotection in animal models of focal ischemia.

In the last three years, numerous studies by different groups using different noxious brain stimuli have systematically confirmed this ability of A_{2A}R blockade to confer robust neuroprotection (reviewed in Table 1). Interestingly, this neuroprotection afforded by A_{2A}R blockade was more robust in cortical regions than in the basal ganglia, where these A_{2A}Rs are considerably more abundant [228, 229, 231]. This emphasises again that the 'abnormal' high density of A_{2A}Rs in striatal medium spiny neurons of the indirect pathway fulfils a very particular role in the control of striatal circuitry and that our knowledge about striatal A_{2A}Rs should not be extrapolate to understand the general role of A_{2A}Rs in the brain. Nevertheless, although A_{2A}R blockade is particularly effective in preventing cortical and hippocampal damage [225, 229, 231–237], it is also effective in attenuating striatal damage following brain ischemia [229, 231], exposure to quinolinic acid [230, 238], 3-nitropropionic acid [239, 240], malonate [241] or MPTP [242, 243].

One particular interesting aspect related with the neuroprotection derived from the blockade of A_{2A}Rs is that the effects of A_{2A}Rs do not seem to desensitize with prolonged administration of A_{2A}R antagonists [244, 245]. This contrasts with the neuroprotection based on the activation of A_1Rs, which desensitises over time (see "Long-term desensitization of A_1 receptors and up-regulation of A_{2A} receptors by chronic noxious conditions"). In particular, long-term exposure of caffeine leads to a rapid A_1R desensitization but maintenance of A_{2A}R-mediated responses [246]. Thus, the maintenance over long periods of the central effects of A_{2A}R antagonists, probably related to the stress-induced up-regulation of A_{2A}Rs (see "Long-term desensitization of A_1 receptors and up-regulation of A_{2A} receptors by chronic noxious conditions"), makes this receptor an attractive target for prolonged manipulation of brain injury. The use of A_{2A}R antagonists as novel neuroprotective drugs is also favoured by their beneficial pharmacokinetic profile. In fact, the doses of A_{2A}R antagonists that afford neuroprotection are considerably lower than these producing peripheral effects, namely cardiovascular effects (see [247, 248]). In fact, the neuroprotective properties of A_{2A}R antagonists are lost on increasing their dosage [237–240], but it is still unclear if this is related to an increased contribution of peripheral effects, to a differential blockade of different populations of A_{2A}Rs or to a loss of selectivity of the currently available A_{2A}R antagonists. Finally, the neuroprotective effective doses of A_{2A}R antagonists are also devoid of other measurable central effects, which is in accordance with the lack of evident secondary effects in the on-going clinical trials with A_{2A}R antagonists (unpublished results). In conclusion, the lack of peripheral or other evident central effects together with the maintenance over time of A_{2A}R-mediated responses make A_{2A}Rs particular interesting targets to develop novel and effective neuroprotective drugs.

One important aspect that remains to be unravelled is the mechanism(s) by which A_{2A}R blockade affords such robust neuroprotection. This is particularly intriguing given that cortical and hippocampal A_{2A}Rs have a low abundance (in the range of 20 fmol/mg of protein compared to a 50-times greater density of A_1Rs; [7]) and the amplitude of the effect resulting from A_{2A}R activation is discrete, especially when

evaluating neuronal activity. Probably because several groups reported an ability of A_{2A}Rs to control the release of glutamate both in the cerebral cortex [249–251], hippocampus [31, 32, 252] and striatum [26, 230, 253–257], the hypothesis that the A_{2A}R-mediated control of glutamate release might be the explanation for the neuroprotective effects of A_{2A}Rs became popular [238, 258]. Indeed, some studies reported that A_{2A}R activation was involved in enhancing the extracellular levels of glutamate triggered by noxious stimuli [231, 238, 249, 251]. However, in more simplified models of neuronal dysfunction, it was not possible to confirm that the presynaptic modulation of glutamate release by A_{2A}Rs was related to their control of neuronal damage. In fact, when studying hypoxia- or ischemia-induced depression of synaptic transmission, where the presynaptic control of glutamate release is related to the post-hypoxic recovery of synaptic transmission (see [259]), blockade of A_{2A}Rs is essentially devoid of effects [259, 260]. An interesting alternative to reconcile the control of extracellular glutamate levels by A_{2A}Rs with the neuroprotective role of A_{2A}R blockade would be a control by A_{2A}Rs of the release and clearance of glutamate by astrocytes. Thus, A_{2A}R activation can inhibit glutamate transport into astrocytes [261], in particular GLT-1 [40] and enhance the release of glutamate from astrocytes [39, 40]. However, strong arguments have been provided to support the view that the role of glutamate transporters during stressful stimuli is to contribute for the extracellular build-up of glutamate rather than for its removal (see, e.g., [262]). In conclusion, it remains to be determined what might be the contribution of the A_{2A}R modulation of astrocytic glutamate transporters in the realm of the neuroprotection afforded by A_{2A}R antagonists in noxious brain conditions.

It is also important to consider that A_{2A}R antagonists are effective in preventing neurotoxicity in isolated neurons in culture. In fact, both caffeine and antagonists of A_{2A}Rs (SCH 58261 or ZM 241385) effectively prevent the neurotoxicity induced by exposure of cerebellar [263] or hippocampal neurons [264] to the fragment 25–35, 1–40 or 1–42 of β-amyloid protein (Aβ), a putative causative factor of Alzheimer's disease [265]. And there is no evidence to suggest that Aβ enhances glutamate release, since the effect of Aβ is the opposite, i.e. to depress glutamatergic transmission (reviewed in [266]). Thus, one has to assume that A_{2A}Rs might have direct effects on neurons to control their susceptibility to neurotoxic stimuli. The possible control by A_{2A}Rs of one the receptor systems most frequently involved in neurodegeneration, the NMDA receptor, does not appear to be a likely candidate. In fact, it has been shown that A_{2A}R activation actually *inhibits* NMDA receptors in striatal neurons [267 –269] and A_{2A}R blockade *increased* NMDA-dependent neurotoxicity in the hippocampus [270]. Alternatively, A_{2A}Rs might control the apoptotic machinery in neurons and other cell types in the brain (see [264]), in a manner similar to the control by A_{2A}Rs of apoptosis in PC12 cells [271–273] or in neutrophils [274–276].

Another hypothesis, first advanced by Schubert to understand adenosine neuroprotection would be the possi-

Table 1. Neuroprotective effects afforded by A_{2A}R blockade/inactivation in *in vivo* models of adult brain toxicity.

Experimental model	Manipulation	Effect	Reference
Global ischemia, gerbil	CGS 15943 0.1 mg/kg i.p.	Protection (HIP cell loss)	[225]
Global ischemia, gerbil	CSC 0.1 mg/kg i.p.	Protection (HIP cell loss)	[226]
Forebrain ischemia, gerbil	CSC 1 mg/kg i.p.	Protection (HIP cell loss)	[227]
Forebrain ischemia, rat	SCH58261 0.01 mg/kg i.p.	Protection (CTX infarct vol.)	[228]
Forebrain ischemia, mouse	A_{2A}R knockout	Protection (CTX, STR infarct vol.)	[229]
Forebrain ischemia, rat	SCH58261 0.01 mg/kg i.p.	Protection (CTX, STR infarct vol.)	[231]
Forebrain ischemia, rat	SCH58261 0.01 mg/kg i.v.	Protection (CTX c-*fos* density)	[233]
Ischemia + hyperglycemia, rat	ZM241385 1 mg/kg i.p.	Protection (HIP cell loss)	[234]
KA intra-HIP, rat	ZM241385 2.5 pmol intra-HIP	Protection (HIP cell loss)	[235]
KA i.c.v., mouse	DMPX 20 μg i.c.v.	Protection (HIP cell loss)	[236]
KA i.p., rat	SCH58261 0.01–0.05 mg/kg i.p.	Protection (HIP cell damage)	[284]
LPS i.c.v., rat	SCH58261 5 pmol, i.c.v.	Protection (HIP LTP)	[285]
QA + X/XO intra-HIP, rat	ZM421385 25 pmol intra-HIP SCH58261 50 pmol intra-HIP CSC 100 pmol intra-HIP	Protection (HIP cell loss)	[232]
QA intra-STR, rat	DMPX0.2 μg intra-STR	Protection (CTX EEG)	[237]
QA intra-STR, rat	SCH58261 0.01 mg/kg i.p.	Protection (CTX EEG, STR glyosis)	[238]
3-NP i.p., rat, mouse	A_{2A}R knockout MSX-3 5 mg/kg i.p.	Protection (STR lesion vol.)	[239]
3-NP i.p., mouse	A_{2A}R knockout CSC 5–20 mg/kg i.p.	Protection (STR lesion vol.)	[240]
Malonate intra-STR, rat, mouse	DMPX 5 mg/kg i.p.	Protection (STR TH, DA levels)	[241]
MPTP i.p., mouse	A_{2A}R knockout	Protection (STR DAT, DA levels)	[242]
MPTP i.p., rat 6-OHDA intra-STR	KW6002 1–10 mg/kg p.o.	Protection (STR DAT, DA levels)	[243]

CTX, cortical; DA, dopamine; DAT, dopamine transporters; EEG, electroencephalography; i.c.v., intracerebroventricular; i.p., intraperitoneal; i.v., intravenous; HIP, hippocampal; KA, kainate; KO, knockout; LPS, lipopolyssacharide; LTP, long-term potentiation; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 3-NP, 3-nitropropionic acid; p.o., oral administration; QA, quinolinic acid; STR, striatal; TH, tyrosine hydroxylase; vol., volume; X, xanthine; XO, xanthine oxidase.

bility that A_{2A}Rs might control the process of neuro-inflammation [277]. The process of neuro-inflammation is known to contribute for the spreading of neuronal damage in different noxious brain conditions and particular attention is currently focused on the therapeutic possibilities of controlling neuro-inflammation as a strategy to attenuate the neurological consequences of neurodegenerative diseases (reviewed in [278, 279]). Since the brain is not subject to the actions of the inflammatory system under physiological conditions (immune privilege of the brain; reviewed in [280]), it is generally accepted that the initial trigger of neuro-inflammation is dependent on the recruitment and activation of microglia cells, which are brain-resident (reviewed in [281]). Accordingly, activated microglia are found in most brain noxious conditions such as ischemia, trauma, brain infections, epilepsy, Parkinson's, Alzheimer's or Huntington's disease (reviewed in [278]; [279, 282]) and activated microglia are even considered one of the most sensitive sensors for pathological events in the brain (see [283]). Some evidences suggest that A_{2A}Rs can control the detrimental neuronal consequences associated with neuro-inflammation. Blockade of A_{2A}Rs completely prevented the recruitment of activated microglia to the CA3 region of the hippocampus in rats injected with kainate [236]. However, this might either be due to a direct effect of A_{2A}Rs known to be present in microglia cells [41, 42, 216] or because A_{2A}R antagonists control the evolution of the severity of convulsion and associated neurotoxicity triggered by cumulative sub-threshold amygdala kindling or by kainate injection [284], which pre-date microglia recruitment. It was also recently shown that an A_{2A}R antagonist (SCH 58261) can prevent the hippocampal neuronal dysfunction and neurotoxicity triggered by the direct administration of lipopolyssaccharide (LPS) [285], a potent inflammatory trigger and activator of microglia cells (see e.g., [286, 287]). Interestingly, SCH 58261 also attenuated the LPS-induced neuro-inflammation, as evaluated by the abolishment of LPS-induced increase in interleukin 1β levels and the recruitment of activated microglia [285]. This shows that A_{2A}R blockade effectively control neuro-inflammation and the neuronal dysfunction and damage resulting from a neuro-inflammatory status in the brain. However, it does not allow stating that microglial A_{2A}Rs play a key role in controlling the involvement of A_{2A}Rs in neuronal damage. In fact, studies with cultured cells showed that adenosine can directly attenuate neuronal damage caused by administration to neurons of medium from activated microglia cultures [98]. The development of a neuro-inflammatory process depends not only on microglia activation, but also on the participation of astrocytes (e.g., [288]) and neurons (e.g., [289]), as well as on the involvement of infiltrating myeloid cells (see [282, 290, 291]). Interestingly, a recent elegant study by the group of Jiang-Fan Chen showed that brain resident A_{2A}Rs might only play a minor role in controlling neurodegeneration, at least in an animal model of focal ischemia [292], which causes a major disturbance of the blood–brain barrier with the easier invasion of circulating myeloid cells (reviewed in [293]). By comparing the infarcted area in the cerebral cortex of γ-irradiated wild-type mice receiving a bone marrow transplant from A_{2A}R knockout mice and γ-irradiated A_{2A}R knockout mice transplanted with bone marrow from wild-type mice, they found that a superior neuroprotection was observed in the second group (i.e., which possessed A_{2A}Rs in myeloid cells but not in the brain). It remains to be tested if A_{2A}R in myeloid cells also play a key role in other brain noxious conditions where the infiltration of these cells into the brain is less well documented.

This proposal that the effect of A_{2A}Rs in myeloid cells is most important for the role of A_{2A}Rs in the control of brain damage is surprising based on the well established robust role of adenosine in *attenuating* (rather than *exacerbating*) inflammation in the periphery ([294]; reviewed in [295, 296]). In fact, adenosine is a potent anti-inflammatory agent with A_{2A}Rs triggering "OFF" signals in activated immune cells, which constitutes one of the most fundamental and immediate tissue-protecting mechanisms (reviewed in [295, 296]). A_{2A}R agonists were even named "the most potent anti-inflammatory drug known to mankind." Accordingly, activation of A_{2A}Rs has been shown to confer a robust protection against tissue damage from ischemia–reperfusion injury in different organ such as heart [297–299], blood vessels [300], kidney [301, 302], liver [303, 304], lung [305, 306], joints [307], skin [308, 309], and even in the spinal cord [310, 311] and in the brain following hemorrhage [312] or acute infection [313]. Thus, it is the activation (rather the blockade) of A_{2A}Rs that confers protection against damage triggered by inflammation in peripheral tissues, precisely the opposite of what is observed in the damaged adult brain. Moreover, A_{2A}R blockade actually exacerbates tissue damage involving inflammatory reactions in the periphery (reviewed in [295, 296]), instead of the tissue protection, as observed in noxious brain conditions. Furthermore, all studies available indicate that the activation of A_{2A}Rs inhibits the release of pro-inflammatory cytokines from inflammatory cells, such as macrophages (e.g., [223, 314–316]), dendritic cells [220, 221], monocytes [317–319] or T cells [320, 321]. This latter cell type, in particular $CD4^+$ T cells, are most relevant since it was recently shown that the key role of A_{2A}Rs in attenuating peripheral tissue damage from ischemia–reperfusion injury is due to the activation of A_{2A}Rs in $CD4^+$ T cells [322]. Since the depletion of $CD4^+$–$CD25^+$ regulatory T cells has been shown to promote survival of neurons after brain insults [323], in a manner regulated by metabotropic receptors such as dopamine D_1 receptors [324], it would be interesting to test if A_{2A}Rs might affect this particular population of T cells to control brain injury. However, the urgent need remains to provide a logical explanation for the fundamentally opposite effects of A_{2A}Rs in cell death involving inflammatory reactions in the brain and in the periphery.

In conclusion, there are currently five concurring hypothesis to explain the robust neuroprotective effects afforded by A_{2A} receptors in noxious brain conditions in adult animals: (1) presynaptic control of glutamate release; (2) control of astroglyosis and of glutamate uptake and re-

lease by astrocytes; (3) direct control of neuronal viability by interference with pathways of cell death; (4) control of microglia reactivity; (5) control of the reactivity of infiltrating lymphoid cells. As discussed, all these hypotheses have pitfalls that limit their acceptance. Most likely, it may be that different conjunctions of these mechanisms might come into play according to the type of insult, since different insults are likely to operate different demises of neuronal damage.

Therapeutic neuroprotective potential based on adenosine in the adult brain

The results discussed so far allow to draw a general picture of the role of adenosine in the control of brain damage involving A_1 and A_{2A} receptors: activation of A_1Rs would have an important role to control the early onset of brain damage, whereas blockade of A_{2A}Rs would have a preponderant effect in the latter stages of brain damage. And since the efficiency of A_1Rs decreases upon chronic noxious stimuli (see "Acute A_1 receptor activation increases the threshold for acute neurodegeneration"), whereas the efficiency of A_{2A}R antagonists seems to be preserved (probably due to the down-regulation of A_1Rs and up-regulation of A_{2A}Rs by noxious stimuli), the use of A_{2A}R antagonists currently seems to be the most promising brain neuroprotective strategy based on the adenosine neuromodulatory system in adult animals [325]. Accordingly, A_{2A}R antagonists are being developed as novel anti-parkinsonian drugs [326–328], which are currently under clinical trials with promising results (e.g., [329]).

However, it might still be possible to further refine the efficiency of a neuroprotective strategy based on adenosine neuromodulation. In fact, although there is a general down-regulation of A_1Rs caused by noxious stimuli, this does not mean that the ability of A_1Rs to control neuronal damage is eliminated. In fact, some reports indicate that A_1R-mediated neuroprotective effects may still be achieved in chronic brain noxious conditions in adult animals, such as in animal models of epilepsy [165, 330, 331], multiple sclerosis [332], paroxysmal dystonia [333] or 3-nitropionic acid-induced neurotoxicity [334]. It has already been discussed that the use of A_1R agonists as neuroprotective tools *in vivo* may be of limited use because of their profound peripheral effects (see "Acute A_1 receptor activation increases the threshold for acute neurodegeneration"). However, it might be possible to explore the potential of inhibitors of the main enzymatic pathway controlling the consumption of adenosine in the brain, i.e. adenosine kinase. Some concerns were initially raised with respect to the efficiency of this approach, because it was reported that ischemia might cause a down-regulation of this enzymatic activity in cultured cells [122, 335, 368]. However, a recent careful study carried out *in vivo* established the maintenance of the activity of adenosine kinase after convulsions and most importantly, showed that the inhibition of adenosine kinase was effective over long periods of time (up to 1 month in rodents), without observable peripheral side effects [165]. Thus, it is now proposed that the simultaneous enhancement of the extracellular levels of adenosine by inhibition of adenosine kinase (to burst A_1R activation) together with antagonists of A_{2A}Rs might be the most efficient neuroprotective strategy based on adenosine neuromodulation to prevent brain damage in adult animals. It is hoped that this hypothesis might be confirmed in animal models of disease in the future.

The consequences of adenosine neuroprotection differ in the newborns

Whereas it is now possible to draw a consistent picture about the roles of A_1 and A_{2A} receptors in controlling neuronal damage in adult animals, the same is not true in the case of fetus or newborns animals. Brain A_1Rs and A_{2A}Rs are ontogenically regulated and, although A_1 and A_{2A} receptors are already present at early developmental timepoints, their expression and density undergo a striking burst at birth and major increases until P9–P15 [336–340]. Some groups [338, 341] suggested that the efficiency of A_1Rs might be reduced in the immature brain. However, several central A_1R-mediated effects, like the control of epileptogenesis [342–344], of axonal growth [345, 346], of brain metabolism [347] and of synaptic transmission [348] have been documented to occur in pups or newborn animals, suggesting that A_1Rs are functional (reviewed in [349]). However, A_1R agonists seem to be essentially ineffective in protecting against ischemia-induced damage in the immature brain [341, 350]. The activation of A_1Rs might even be detrimental for the immature brain, since A_1R activation inhibits neurite outgrowth [351, 352], which may be the reason for the ability of caffeine and A_1R antagonists to prevent the prevalent condition of periventricular leukomalacia in newborns [346]. Interestingly, the acute increase of extracellular adenosine affords neuroprotection against ischemic insults in the immature brain [353]. This may make sense if one considers that the role of A_{2A}Rs in controlling neuronal damage is also the opposite in the immature brain and in adult animals. In fact, whereas A_{2A}R blockade is a consistent neuroprotective strategy against brain damage in adult animals, brain damage is aggravated in immature A_{2A}R knockout mice [354].

In conclusion, it appears that the roles of adenosine in the control of brain damage are fundamentally the opposite in fetus/newborns and in adult animals. Thus, whereas A_1R activation affords neuroprotection in the adult animals, its role in the immature brain might be predominantly deleterious [346]. In contrast, whereas blockade of A_{2A}Rs confers brain neuroprotection in adult animals, it aggravates damage in the immature brain. This opposite impact of adenosine receptor activation on neuronal viability might not necessarily be due to a different functioning of A_1Rs and A_{2A}Rs in immature and mature neurons, but instead to an opposite effect of intracellular calcium levels in immature and mature neurons. In fact, whereas the

activation of NMDA receptors and the rise in intracellular free calcium concentrations are two well defined hallmarks of neurotoxicity in neurons from adult animals (e.g., [355]), these features appear to be fundamental for the survival of immature neurons [356]. In fact, A_1R activation in cultured cortical neurons (from newborn rats) decreases NMDA receptor function and voltage activated calcium channels in a manner analogous to that found in adult rats [356–358] but this lead to a decreased neuronal viability [356] rather than a neuroprotection, as observed in adult brain preparations. Thus, it appears that neuronal adenosine receptors maintain their way of functioning in newborn neurons, but it is the mechanism of neuronal death that is fundamentally the opposite in mature and in immature neurons.

These modifications of the effects mediated by adenosine and eventually of the coupling of adenosine receptors should be kept in mind when considering the use of cultured brain cells as experimental models to study purinergic modulation, since these preparations are obtained from embryos or newborns. Some studies in cultured brain preparations found that A_1R activation or $A_{2A}R$ blockade were neuroprotective [263, 264, 359–361], whereas other found patterns of control of neuronal death by manipulation of A_1Rs [362–364] or $A_{2A}Rs$ [365, 366] different from these observed in adult animals. Furthermore, there is evidence to suggest that the metabolism of adenosine in preparations from newborn animals [348] or in neuronal cell cultures [367, 368] also differs from adenosine metabolism in brain preparations of adult animals.

Adenosine neuroprotection and neurodegenerative diseases on aging

The word of caution on the possible limitations of the extrapolation of the usefulness of the adenosine system for neuroprotection, from adult animals to the immature brain, obviously raises the question of the applicability of the proposed strategies of adenosine neuroprotection in the elderly. This is particularly relevant since most of the proposed therapeutic applications based on the manipulation of the adenosine system (Parkinson's and Alzheimer's disease, brain ischemic conditions, epilepsy, sleep disorders) are prevalent in the elderly, but the experimental work is largely carried out in young adult animals. Thus, one issue that needs to be established before translation of animal work into disease in humans is whether there are changes in the adenosine neuromodulatory system on aging, as it was found to occur for the immature brain.

In aged animals, it is observed that there is a decreased expression [369] and density of A_1Rs in cortical and hippocampal regions ([370–375]; but see [376]), whereas the density of $A_{2A}Rs$ increases [31, 372, 377]. This is paralleled by a decreased efficiency of A_1R agonists [375, 378–380] and by an increased G protein coupling [377] and efficiency of $A_{2A}Rs$ in limbic regions [31, 377]. Interestingly, the modification of the status of $A_{2A}Rs$ in aged animals is different in the basal ganglia from that in cortical regions. In fact, there is a decreased expression [381] and density of $A_{2A}R$ in the striatum of aged rats [376, 382–384]. However, since the role of $A_{2A}Rs$ in motor control is tightly linked to dopaminergic signalling (reviewed in [6, 28]) and there is also a reduction in the density of dopamine D_2 receptors in the striatum of aged rats even greater than that of $A_{2A}Rs$ [382, 383], the motor effects of $A_{2A}R$ antagonists are increased in aged animals [385]. This again stresses the differences between striatal and extra-striatal $A_{2A}R$ and the limitations in extrapolating any information from striatal $A_{2A}Rs$ to our understanding of the role of extra-striatal $A_{2A}Rs$. It is interesting to note that the changes in aged animals of the relative density of A_1Rs *versus* $A_{2A}Rs$ in cortical regions is similar to that found upon noxious brain conditions (i.e., down-regulation of A_1Rs and up-regulation of $A_{2A}Rs$). This opens the question of whether the change of status of A_1/A_{2A} receptors in aged animals results from aging viewed as a physiological stress condition or whether the modification of the adenosine receptor system evolves to compensate the general loss of efficiency of functioning of brain circuits [386, 387]. Future aging studies (rather than comparisons between groups of adult and aged animals) will be required to elucidate these issues. This might be particularly relevant in view of the observation that A_1Rs seem to control the life-span of mice, since the life expectancy of A_1R knockout mice is significantly lower than that of their wild-type littermates [388].

In aged animals, there is not only a modification in the densities of A_1Rs and $A_{2A}Rs$ in the brain, but there is also a modification of the extracellular metabolism of adenosine (see data and references in [118]). The most surprising observation is that there is a major decrease in the tonic A_1R activation in hippocampal preparations from aged rats ([380]; in agreement with the results in [375, 379, 389]; but see [390–392]). This might eventually contribute for the greater susceptibility of brain tissue from aged animals to stressful stimuli, since the main role of A_1Rs appears to be the control of the threshold of onset of neuronal damage.

However, these studies comparing the extracellular metabolism and A_1 *versus* A_{2A} receptor-mediated effects between young adult and aged animals allow anticipating that the currently proposed best adenosine-based strategy for neuroprotection seems particularly adequate for aged animals. In fact, in aged animals there is a decrease in the extracellular levels of adenosine acting on A_1Rs and there are increased levels of $A_{2A}Rs$ that are more efficient that in the brain from young adult animals. Thus the combination of adenosine kinase inhibitors with antagonists of $A_{2A}Rs$ might be particularly effective in aged animals. It is hoped that future work will allow confirming the neuroprotective efficiency of the combination of these drugs in aged animals. Also, careful parallel studies will be required to test if there are unexpected secondary effects that may appear only in aged animals. In fact, the pharmacokinetics of most drugs is considerably changed in aged animals, and some pharmacodynamic characteristics are also modified, like, for instance, the cardiovascular effects of adenosine in aged rats [393–395].

Acknowledgements

The experimental work carried out by my group 'Purines at CNC' (http//:cnc.cj.uc.pt/lab_lef/) was supported by Fundação para a Ciência e para a Tecnologia (grant no. 44740/2002) and by Sociedade Portuguesa de Neurociências. I am indebted to the members of my group for helpful suggestions, and particularly to Lisiane Porciúncula and Nelson Rebola for carefuly reviewing the MS and to Attila Kofalvi for his generous help in drawing the pictures of this review. Most of the ideas presented result from discussions with our mentor Bertil Fredholm (Karolinska Institutet, Sweden) and with Jiang-Fan Chen (Harvard University, USA) to whom I am in debt.

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