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REVIEW

NEUROPROTECTIVE ROLE OF ADENOSINE IN THE CNS

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It is well established that in the CNS, endogenous adenosine plays a pivotal role in neurodegeneration. A low, nanomolar concentration of adenosine is normally present in the extracellular fluid, but it increases dramatically during enhanced nerve activity, hypoxia or ischemia. In these pathological conditions, adenosinergic transmission-potentiating agents, which elevate adenosine level by either inhibiting its degradation (adenosine deaminase and kinase inhibitors) or preventing its transport, offer protection against ischemic or excitotoxic neuronal damage. The directly acting adenosine A_1 receptor agonists are known to mediate neuroprotection, mostly by the blockade of Ca^{2+} influx, which results in the inhibition of glutamate release and reduction of its excitatory effects at a postsynaptic level. More recent data have shown that antagonists of adenosine A_{2A} receptors markedly reduce cerebral ischemic damage in animal models of focal and global ischemia. Moreover, these compounds attenuate the neuronal loss induced by excitatory amino acids (EAA). A neuroprotective effect of adenosine A_{2A} receptor antagonists was also shown in animal models of Parkinson's disease (MPTP, 6-OHDA, methamphetamine). Hence, it might be suggested that adenosine A_{2A} receptor antagonists may represent a novel strategy in the therapeutic approach to pathologies characterized by acute or chronic neurodegenerative events, since they not only reverse motor impairment but can act as neuroprotective compunds by promoting cell survival.

Key words: *adenosine, adenosine A_1 receptors, adenosine A_{2A} receptors, excitotoxicity, ischemia, neuroprotection, Parkinson's disease*

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Abbreviations: AC – *adenylate cyclase,* ADA – *adenosine deaminase,* ADAC – *adenosine amine congener,* AKA – *adenosine kinase,* APNEA – *N^6-2-(4-aminophenyl)ethyladenosine,* CADO – *2-chloroadenosine,* cAMP – *cyclic adenosine monophosphate,* CCPA – *2-chloro-N^6-cyclopentyladenosine,* CGS 15943 – *5-amino-9-chloro-2-(2-furyl)-1,2,4-triazolo[1,5-c]quinazoline,* CGS 21680 – *2-[4-(2-carbonylethyl)-phenethylamino]-5'-N-ethylcarboxamidoadenosine,* CHA – *N-cyclohexyladenosine,* Cl-IB-MECA – *2-chloro-N^6-(3-iodobenzyl)-adenosine-5'-N-methyluronamide,* CPA – *N^6-cyclopentyladenosine,* CP 66713 – *4-amino-1-phenyl-[1,2,4]-triazolo[4,3-a]quinoxaline,* CPT – *8-cyclopentyltheophylline,* CSC – *8-(3-chlorostyryl)caffeine,* DA – *dopamine,* DCF – *2'-deoxycoformycin,* DMPX – *3,7-dimethyl-1-propargylxanthine,* DPCPX – *8-cyclopentyl-1,3-dipropylxanthine,* EHNA – *erythro-9-(2-hydroxy-3-nonyl)adenine,* Glu – *glutamic acid,* GP 683 – *4-(N-phenylamino)-5-phenyl-7-(5'-deoxy-β-D-ribofurazonyl)pyrrolo[2,3-d]-pyrimidine,* 2-HE-NECA – *2-hexyl-5'-N-ethylcarboxamidoadenosine,* IB-MECA – *N^6-(3-iodobenzyl)-adenosine-5'-N-methyluronamide,* IOT – *5-iodotubercidin,* KF 17837 – *(E)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-1H-purine-2,6-dione,* KO – *knock-out mice,* KW 6002 – *(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,* MRE 30008F20 – *5-N-(4-methoxyphenylcarbamoyl)amino-8-propyl-2-(2-furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c-]pyrimidine,* MRS 1220 – *9-chloro-2-(2-furyl)-5-[(phenylacetyl)amino][1,2,4]-triazolo[1,5-c]pyrimidine,* MTH – *methamphetamine;* NH_2dAD – *5'-amino-5'-deoxyadenosine,* NECA – *adenosine-5'-N-ethyluronamide,* 6-OHDA – *6-hydroxydopamine,* R-PIA – *R-N^6-phenylisopropyladenosine,* 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-ylamino]ethyl)phenol*

Introduction

Adenosine is present in all tissues of a mammalian organism where it modulates a variety of important physiological processes. Adenosine is formed within cells as a result of hydrolysis of AMP through an action of ecto-5'-nucleotidase [117], hence its formation depends upon ATP breakdown and synthesis. In the extracellular compartment, the level of adenosine also depends on the rate of hydrolysis of ATP which is released from either neurons or glial cells. Extracellularly, adenosine concentrations are kept in equilibrium by specific reuptake mechanisms working *via* specialized bidirectional transporters [103]. It has been estimated that levels of adenosine in the CNS range between 30 and 300 nM [38]. Adenosine is then catabolized by the action of enzymes such as adenosine kinase and adenosine deaminase [117].

Adenosine receptors and their localization in the CNS

The action of adenosine is mediated by specific receptors located on cell membranes, which belong to the family of G protein-coupled receptors. Currently, four adenosine receptors have been cloned and characterized: A_1, A_{2A}, A_{2B} and A_3 [25]. The main intracellular signaling pathways involve the formation of cAMP, with A_1 and A_3 receptors causing inhibition of adenylate cyclase, and A_{2A} and A_{2B} receptors activating it (Tab. 1). Other transduction mechanisms, e.g. voltage-sensitive Ca^{2+} channels, are also involved in signal transduction by each of the adenosine receptors. Molecular characteristics of the receptors and the intracellular signaling have been described in detail elsewhere [25, 38, 71]. The profile and distribution of adenosine receptors in the CNS are shown in Table 1.

A_1 receptors are widely distributed in the brain and are present on neurons and glial cells. They are localized both pre- and postsynaptically. The highest expression of A_1 receptors has been found in the cortex, cerebellum, thalamus and hippocampus (Tab. 1) [38]. Moreover, the mRNA encoding A_1 receptor is also present in basal ganglia structures including the striatum, globus pallidus, subthalamic nucleus [16, 38]. It is also known that in the striatum, A_1 receptors are present on both dopaminergic nigrostriatal and glutamatergic corticostriatal terminals. Moreover, they are co-localized with dopamine D_1 receptors on GABA/dynorphin output neurons which send their terminals to the substantia nigra pars reticulata [28, 38].

A_{2A} receptors are predominantly located in several basal ganglia structures such as the striatum, globus pallidus, nucleus accumbens and tuberculum olfactorium (Tab. 1) [41, 85]. However, using more sensitive techniques, lower levels of expres-

Table 1. Characteristics of adenosine receptors and their distribution in the CNS

Receptor subtype	Second messengers	Distribution in the CNS	Selective agonists	Selective antagonists
A_1	G_i, G_o AC PLC, PLD K^+, Ca^{2+}	cerebral cortex, hippocampus, striatum, thalamus, cerebellum	CPA CCPA CHA R-PIA	DPCPX CPT
A_{2A}	G_s, G_{olf} AC	striatum, n.accumbens, olfactory tubercle, globus pallidus, cerebral cortex, hippocampus	CGS 21680 2-HE-NECA	SCH 58261 KF 17837 KW6002 ZM 241385, CSC
A_{2B}	G_s AC	low level in the brain hippocampus, thalamus, hypothalamus, striatum	not available	not available
A_3	G_i, G_q AC	low level in the brain hippocampus, thalamus, cerebral cortex, cerebellum	2-Cl-IB-MECA	MRS 1220, MRE 3008F20

AC – adenylyl cyclase; Ca^{2+} – calcium channel; G – G-protein inhibiting AC (Gi, Go, Gq) and stimulating AC (Gs, Golf); K^+ – potassium channel; PLC – phospholipase C; PLD – phospholipase D; – stimulation; – inhibition

sion of A_{2A} receptors and mRNAs have also been demonstrated in several other brain areas, e.g. the hippocampus, cerebral cortex, thalamic nuclei, some differences being found between human brain and that of other animal species (Tab. 1) [16, 38, 101]. It is noteworthy that using different methodological approaches, all the abovementioned studies are consistent in describing high levels of A_{2A} receptors in the striatum [73]. With regard to specific neuronal populations in the striatum, A_{2A} receptors are present in striatopallidal enkephalin-expressing neurons [23, 92]. The same cells also express dopamine D_2 receptors, hence both A_{2A} and D_2 receptors are distributed on the same neuronal pathway [23, 28, 38]. In contrast, there are no A_{2A} receptors on neurons expressing D_1 receptors, substance P and dynorphin, which project from the striatum to the substantia nigra [23, 28, 92]. It is noteworthy that A_{2A} receptors are also present on glial cells.

Preliminary studies showed that A_{2B} receptors were mainly present in peripheral organs such as the bowel, bladder, lung, vas deferens as well as in the spinal cord and brain. [16, 38]. In the brain, both A_{2B} receptors and their mRNA were found in hippocampal CA1 and CA3 neurons and glial cells [16]. Moreover, they were also present in a small amount in the hypothalamus, thalamus and striatum (Tab. 1) [16, 38].

A_3 receptors are widely distributed in peripheral organs (mainly in the testis and lung). However, their distribution and physiological functions in the brain are still unclear. It has been shown that A_3 receptors and their mRNA are present in a relatively small amount in the hippocampus and cerebellum (Tab. 1) [16, 38]. However, recent studies have not corroborated their presence in the human hippocampus [38].

The role of A_1 receptors in neuroprotection

Several lines of evidence indicate that adenosine may be an endogenous neuroprotective agent in the CNS, since it prevents the damage caused by ischemia, excitotoxicity or epileptic seizures [15, 74, 91, 105]. A low (nanomolar) concentration of adenosine is normally present in the extracellular fluid in the CNS, but it rises dramatically following hypoxia or ischemia (up to a μmolar level). Hence, adenosine-potentiating agents which elevate endogenous adenosine levels by either inhibiting its degradation (adenosine deaminase and kinase inhibitors) or preventing its transport offer protection against ischemic or excitotoxic neuronal damage (Fig. 1) [15, 74, 91]. A growing body of evidence also supports the role of both A_1 and A_{2A} receptors in the neuroprotective mechanisms. Moreover, re-

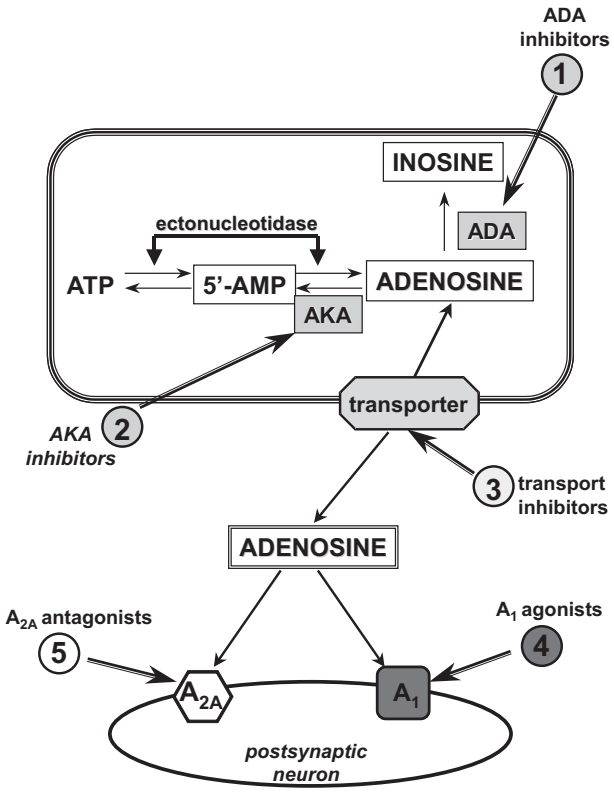

Fig. 1. The main mechanisms by which compounds may afford neuroprotection by modulating adenosine transmission. A_1 – adenosine A_1 receptor; A_{2A} – adenosine A_{2A} receptor; ADA – adenosine deaminase; AKA – adenosine kinase; 5'-AMP – adenosine monophosphate

cent data have shown that the same net results can be achieved by either stimulating A_1 receptors or blocking A_{2A} ones (Fig. 1) [15, 74, 91].

Effects of A_1 receptor agonists in ischemia/hypoxia

A_1 receptor agonists were conclusively shown to attenuate ischemic or excitotoxic neuronal damage both *in vitro* (cell cultures, brain slices) and *in vivo* in different models of ischemia/hypoxia [15, 74, 79]. Using primary cortical or hippocampal cell cultures subjected to hypoxia or glucose deprivation, it was demonstrated that both adenosine and the selective A_1 receptor agonist CHA reduced the neuronal damage evaluated morphologically by alteration of the lactate dehydrogenase release or deoxyglucose transport [11, 30, 54], whereas adenosine receptor antagonists (theophylline, CPT) enhanced the hypoxia-induced cell damage in the same models [11, 54]. A similar, neuroprotective effect was observed in another model of histotoxic anoxia, where adenosine and CPA, a selective A_1 receptor agonist, attenuated neuronal cell death in the hippocampal cell cultures exposed to potassium cyanide (KCN, an inhibitor of the mitochondrial respiratory chain) [99]. The neuroprotective effect of CPA and adenosine in that model was blocked by CPT, a selective antagonist of A_1 receptors [99].

Studies conducted on brain slices, mainly cortical or hippocampal ones, also indicated that both adenosine and A_1 receptor agonists (R-PIA, CHA, CPA) could attenuate the neuronal cell loss and degeneration evoked by superfusion with a hypoxic or a hypoglycemic medium [20, 53, 69, 70]. Furthermore, in organotypic hippocampal slices, stimulation of A_1 receptors by CHA reduced the cell death induced by a glucose-deprived medium [36]. In contrast, the adenosine analogue CADO increased cell death in organotypic hippocampal slices subjected to hypoxia and glucose deprivation [5]. However, the above-described effect was seen in the case of a very high concentration of CADO (10–100 μM), and was attributed to nitric oxide release under those experimental conditions [5].

The majority of the well-known A_1 receptor agonists show neuroprotective properties in animal models of global or focal ischemia. It has been demonstrated that local administration of CADO, an adenosine analogue, can attenuate cell loss in the CA1 region of the rat hippocampus in a model of global forebrain ischemia (temporary occlusion of carotid arteries) [21]. Moreover, acute systemic or intracerebroventricular injection of CHA attenuates the neuronal loss in the hippocampus and improves neurological deficits in gerbils or rats subjected to global forebrain ischemia [106, 107, 112, 114, 116]. Similarly, CPA and CCPA, other A_1 receptor agonists, reduce mortality and the loss of neurons in gerbils after global forebrain ischemia [90, 111]. Furthermore, R-PIA injected acutely to rats improves neurological deficits, but does not prevent memory impairment or damage to hippocampal neurons, measured histologically [17, 34]. The protective effect of adenosine and A_1 agonists has also been shown in other models, e.g. in a model of retinal ischemia in rats, where R-PIA can attenuate changes seen in electroretinogram and retinal histology [48]. It is interesting, however, that R-PIA does not reduce the infarct size, nor does it ameliorate neurological deficits in a model of focal cerebral ischemia (middle cerebral artery occlusion) in spontaneously hypertensive rats [87]. Therefore,

it may be suggested that under the above experimental conditions, some changes in the neuromodulatory role of adenosine or in the control of vascular tone occur, which may account for the described results. It is noteworthy, however, that a decreased affinity of A_1 receptors has been described in spontaneously hypertensive rats [63] which might account for the lack of R-PIA effect in these rats.

All the abovementioned studies confirm that the stimulation of A_1 receptors exerts the neuroprotective effect both *in vitro* and in animal models of hypoxia/ischemia. It seems, however, that in *in vivo* experiments numerous factors, not exclusively direct effects at the cellular level, may contribute to the protective role of adenosine. For example, adenosine is a crucial factor in the control of cerebral circulation, moreover, by causing vasodilation of cerebral arteries, it can ameliorate the consequences of ischemia. Adenosine can also prevent the activation and adhesion of leukocytes on endothelial cells in blood vessels. The decrease in body temperature produced by adenosine and its analogues is also likely to contribute to neuroprotection [10, 15, 78].

Effects of A_1 receptor antagonists in animal models of ischemia/hypoxia

Adenosine receptor antagonists, both nonselective ones such as caffeine or theophylline and selective for A_1 receptors (CPT, DPCPX), were also thoroughly studied in different models of ischemia/hypoxia. In general, each of those compounds given acutely exacerbates or does not influence the changes induced by ischemia. Both, theophylline and aminophyline administered acutely potentiated the mortality or degeneration of hippocampal neurons in rats and gerbils subjected to global forebrain ischemia [12, 40, 88, 89, 91, 116]. Similar effects (degeneration of hippocampal cells, memory impairment) were also observed after acute DPCPX and CPT in animal models of global or focal ischemia [6, 79, 91, 111]. Moreover, DPCPX deteriorated the changes induced by retinal ischemia, measured histologically and electroretinographically in rats and cats [50, 76].

Interestingly, a reverse effect appeared after chronic administration of adenosine receptor antagonists. Thus, both caffeine and DPCPX given chronically for 2–3 weeks before an ischemic insult lowered the neuronal injury assessed by magnetic resonance and histopathological examination in rats and gerbils [100, 111]. A similar neuroprotective effect was also observed after caffeine given to gerbils for 4 weeks in a model of global forebrain ischemia [88, 90]. It has been suggested that the beneficial effects seen after chronic administration of adenosine antagonists may be due to e.g. the up-regulation of A_1 receptors, though such an effect has not been adequately described [39].

A_1 receptors and excitotoxicity

It has been suggested that EAAs are involved in the induction of ischemic/hypoxic damage in the brain [86, 96]. EAAs are released immediately after ischemic injury, and they induce a sequence of changes ranging from excessive membrane depolarization to a rise in intracellular Ca^{2+} level which lead to cell death [62, 74, 97, 104]. On the other hand, however, the released glutamate enhances the liberation of adenosine which, in turn, is likely to act as an endogenous neuroprotectant. Hence, a question arises whether adenosine and A_1 receptor agonists can exert neuroprotective effects in experimental models of the EAA-induced excitotoxicity.

It has been shown in a number of animal models that selective agonists of A_1 receptors, such as CADO, R-PIA, CHA or CPA, given either systemically or directly into the striatum or hippocampus attenuate the neuronal loss induced by NMDA, kainate, quisqualate or ibotenate [3, 4, 24, 55–61, 65]. On the other hand, adenosine receptor antagonists (DPCPX, CPT) potentiate the neurotoxic effect of kainic acid in the hippocampus [55–61, 65]. Therefore, it seems that, like in animal models of ischemia/hypoxia, A_1 receptor agonists exert neuroprotective effects on the EAA-induced excitotoxicity, a process which has been implicated in a variety of neuropathological conditions.

Putative mechanisms of the neuroprotective action of A_1 receptor agonists

There are several ways in which adenosine may act as a neuroprotector. It has already been postulated that the ischemia-induced cell death may be partly mediated by excitotoxic actions of some EAAs such as glutamate or aspartate. The excessive activation of NMDA receptors by EAAs released during ischemia results in membrane depolarization, elevated intracellular Ca^{2+} concentration and cell death, caused by various Ca^{2+}-mediated processes [15, 74, 91, 104]. There is also evidence

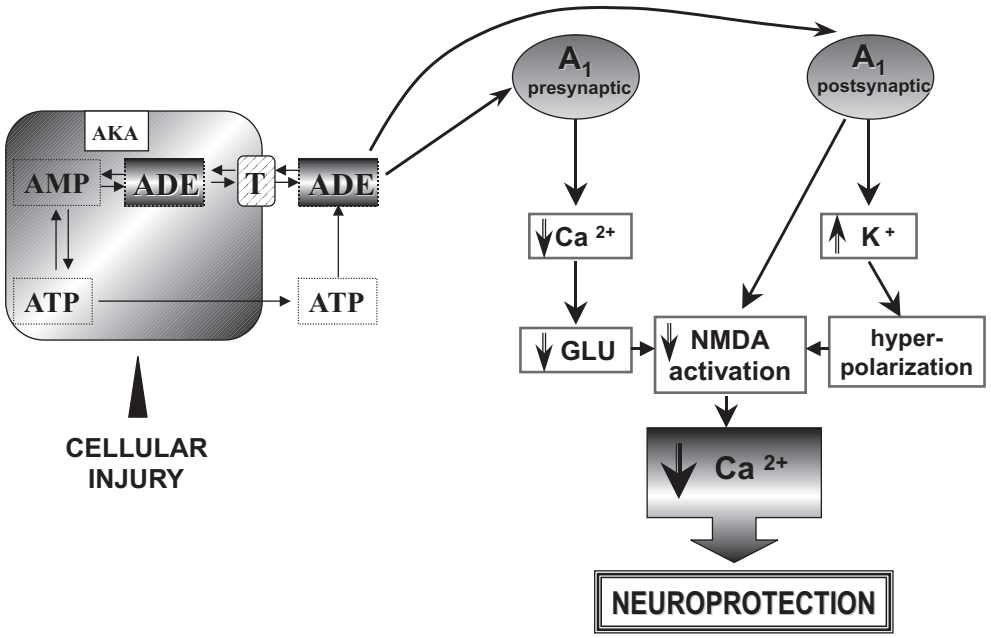

Fig. 2. Schematic representation of possible mechanisms responsible for the neuroprotective action of adenosine A_1 receptor agonists. ADE – adenosine; A_1 – adenosine A_1 receptor; AKA – adenosine kinase; Ca^{2+} – calcium ion; GLU – glutamate; K^+ – potassium ion; NMDA – N-methyl-D-aspartate receptor; T – bidirectional nucleoside transporter; – decrease; – increase. Following cellular injury, extracellular level of adenosine is markedly increased *via* an enhanced release of intracellular ADE and/or ATP, which is rapidly degraded to ADE by ectonucleotidases. Once in th extracellular space, adenosine acting through presynaptic A_1 receptors may attenuate the influx of Ca^{2+} through voltage-dependent calcium channels, and thus decrease the release of glutamate. On the other hand, adenosine acting through postsynaptic A_1 receptors may activate K^+ channels, which leads to hyperpolarization of postsynaptic neurons and directly inhibits NMDA receptor activation. All these effects limit the opening of voltage-dependent Ca^{2+} channels and the neuronal Ca^{2+} influx, which contributes to neuroprotection (also see the text)

that adenosine levels rise rapidly in cortical areas after interruption of cerebral blood flow in a variety of animal species [15, 74, 91]. The released adenosine, which acts *via* presynaptic A_1 receptors, may attenuate the influx of Ca^{2+} through voltage-dependent calcium channels (mainly N-type) and may thus decrease the release of glutamate (Fig. 2) [15, 74]. By inhibiting this release, adenosine decreases the excitability of NMDA receptors and in consequence hinders the NMDA-mediated Ca^{2+} influx to neurons, the latter being the major mechanism that underlies neuroprotection (Fig. 2) [15, 74].

Another putative mechanism of the neuroprotective action of adenosine is related to postsynaptic adenosine A_1 receptors (Fig. 2). By stimulating postsynaptic A_1 receptors, adenosine counteracts excessive membrane depolarization by the activation of K^+ channels and increases in the efflux of K^+, which leads to hyperpolarization of postsynaptic neurons [15, 74]. In consequence, adenosine action diminishes the opening of voltage-dependent Ca^{2+} channels and neuronal Ca^{2+} influx. By antagonizing membrane depolarization, adenosine elevates the threshold for the opening of NMDA receptor-operated channels, which possibly contributes to its neuroprotective action. Therefore, by stabilizing membrane potentials and maintaining intracellular Ca^{2+} homeostasis in postsynaptic neurons, adenosine may act as a neuroprotector (Fig. 2).

Neuroprotective role of adenosine deaminase, kinase and transport inhibitors in ischemia/hypoxia

It is expected that elevation of the extracellular level of adenosine during ischemia/hypoxia may reduce neuronal damage. Such elevation might be achieved by the inhibition of adenosine deaminase and kinase, or by the blockade of adenosine uptake (Fig. 1). It has been shown that 2'-deoxycoformycin, an inhibitor of adenosine deaminase (ADA), prevents histological changes in the hippocampus by decreasing the infarct area and neuronal degeneration in global forebrain ischemia or focal ischemia in rats and gerbils [29, 51, 80, 81]. EHNA,

another ADA inhibitor, given directly to a rat's eye attenuated both the histological changes and changes in the electroretinogram in a model of retinal ischemia in rats [48]. Similarly, adenosine kinase (AKA) inhibitors, such as 5'-deoxy-5-iodotubercidin, 5-iodotubercidin or GP683 displayed a neuroprotective action in different models of global or focal ischemia in rats and gerbils [42, 66, 82, 102]. A possible limitation to the use of AKA inhibitors in ischemia may be connected with the fact that the activity of adenosine kinase is already diminished under ischemic/hypoxic conditions [54]. It has already been suggested that AKA is effective mainly under physiological conditions; however, in pathological states, when extracellular level of adenosine is increased, adenosine deaminase seems to play a pivotal role [15].

Another way to raise the extracellular level of adenosine is to block adenosine uptake. However, this approach has also some limitations due to the fact that adenosine carriers are mainly bidirectional [25, 103, 117], and, hence, their blockade may limit not only the uptake to the cell, but also the transport of adenosine formed in the cell during ischemia to the extracellular space. Such a mechanism may indicate, at least to some extent, that dipyridamole reduces the extracellular level of adenosine and accelerates neuronal death in a cell culture of neurons under ischemic-like conditions [52]. However, propentophylline, another uptake inhibitor which has been extensively studied in different animal models of global or focal ischemia, attenuates degeneration of the hippocampal and cortical neurons in rats and gerbils [12, 13, 19, 43, 46, 47, 64, 77].

Only few studies have examined the role of ADA, AKA or transport inhibitors in animal models of excitotoxicity. Nonetheless, it has been shown that 2-deoxycoformycin, an inhibitor of ADA, as well as propentophylline, an uptake inhibitor, attenuate the neurological deficits and mortality induced by NMDA or ibotenate in mice [26, 108].

Methamphetamine and MPTP-induced neurotoxicity

Adenosinergic compounds have also been examined in animal models of the neurotoxicity induced by methamphetamine (MTH) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). It is well known that MTH given repeatedly at high doses produces a massive release of dopamine in the striatum, measured by an *in vivo* microdialysis, as well as long-lasting changes in the dopaminergic nigro-striatal system, measured 5–8 days after MTH administration [14, 32, 33]. The MTH-induced neurotoxicity is characterized by decreases in striatal dopamine concentration, in the number of dopamine transporters (DAT) and in the activity of tyrosine hydroxylase (TH) [14, 32, 33]. It has been proposed that the abovementioned changes are related to the reversal of DAT function, which leads to dopamine shift from the inside to the outside of the cell, as well as to disturbance of dopamine metabolism [33].

Administration of the selective A_1 receptor agonist CPA or the nonselective one, NECA, reversed the MTH-induced changes in the dopaminergic system in mice, having diminished the release of dopamine in the striatum (an *in vivo* microdialysis), and having elevated the extracellular concentration of striatal dopamine and TH activity [14, 32]. A similar neuroprotective action was observed after administration of inhibitors of adenosine deaminase (DCF, EHNA) and kinase (IOT, NH_2dAD), as well as after a transport inhibitor (dilazep) [33]. Both short- (dopamine release) and long-lasting (striatal dopamine concentration) changes in the dopamine system were reversed by all the abovementioned treatments [32, 33].

Moreover, when CHA, a selective agonist of A_1 receptors, was examined in mice in a model of the MPTP-induced neurotoxicity (which closely resembles biochemical, neuropathological and clinical features of Parkinson's disease), a neuroprotective effect was observed [49].

Neuroprotective role of A_{2A} receptor antagonists

Much less is known about the role of A_{2A} receptors in neuroprotection. Nonetheless, some existing data indicate that the stimulation of A_{2A} receptors by the selective agonist CGS21680 reduces ischemic or excitotoxic hippocampal damage [44, 95]. Furthermore, it has been suggested that these neuroprotective properties may be due to effects occurring in the periphery rather than directly at neuronal sites. The major mechanisms that may account for the A_{2A}-mediated protection include: vasodilation, inhibition of platelet aggregation and supression of neutrophil superoxide generation [15, 72, 74].

Results of recent studies have substantiated the neuroprotective properties of A_{2A} receptor antagonists in different models of neurodegeneration, but the mechanisms underlying these properties are hardly known so far. The selective A_{2A} receptor antagonists, CSC and ZM241385, as well as the less selective ones, CGS15943 and CP66,713, were able to ameliorate hippocampal cell injury following global forebrain ischemia in gerbils or rats [27, 35, 79, 110]. Similarly, the selective A_{2A} receptor antagonist SCH58261 reduced cortical infarct volume in a focal cerebral ischemia model of permanent middle cerebral artery occlusion [67]. The latter compound also decreased brain damage in a model of unilateral carotid artery occlusion in neonatal rats [7]. Likewise, administration of SCH58261, ZM241385 or CSC decreased the extent of neuronal cell death observed in hippocampal regions following kainic-, kynurenic- and quinolinic acid-induced lesions in rodents [44, 45, 98]. In summary, selective adenosine A_{2A} antagonists exerted a neuroprotective effect in a fairly effective manner.

Studies in genetically manipulated mice confirmed the role of A_{2A} receptors in mediating hypoxic/ischemic damage [8]. Cerebral infarction and neurological deficits were attenuated in A_{2A} receptor knock-out mice (A_{2A} KO) subjected to a focal ischemia model of temporary middle cerebral artery occlusion in comparison with their wild-type littermates [8].

Moreover, in animal models of Parkinson's disease (MPTP-treated mice and 6-OHDA-lesioned rats), a neuroprotective effect was demonstrated using both genetic and pharmacologic approaches [9, 37, 68]. The MPTP-induced depletion of dopamine and dopamine transporter levels was significantly attenuated in the striatum of A_{2A} KO mice, as well as in mice pretreated with the selective A_{2A} antagonist CSC [9]. Since an analysis of striatal MPTP metabolites revealed elevated levels of MPTP, but diminished levels of its oxidation products, $MPDP^+$ and MPP^+, in A_{2A} KO mice and in mice treated with CSC, it was suggested that the observed attenuation of neurotoxicity might be due to the inhibition of monoamine oxidase activity [9]. However, this suggestion seems to be questioned by recent results of Ikeda et al. [37] who have shown that the blockade of A_{2A} receptors by KW6002 did not inhibit monoamine oxidase or dopamine transporter *in vitro*. Ikeda et al. [37] observed also a neuroprotective effect of A_{2A} receptor antagonist in two experimental models of Parkinson's disease – after MPTP in mice and 6-OHDA in rats. Since KW6002 decreased the MPTP-induced gliosis and morphological changes in the striatum, it seems that *in vivo* A_{2A} receptor antagonists may inhibit the effect of dopamine neurotoxins (MPTP, 6-OHDA) *via* a direct action on glial cells [37].

The abovementioned results, together with some recent data showing an antiparkinsonian action of selective adenosine A_{2A} receptor antagonists in different animal models of Parkinson's disease [22, 38, 68, 72, 84, 115], suggest that A_{2A} receptor antagonists seem to be very promising compounds in the treatment of Parkinson's disease, since they not only reverse the motor impairment but can also slow down or stop the progress of the disease by promoting cell survival.

Putative multiple mechanisms that underlie the neuroprotective properties of A_{2A} receptor antagonists include neuronal, vascular and microglial elements. It has been shown that the stimulation of A_{2A} receptors enhances the release of glutamate under both ischemic and non-ischemic conditions [75, 83, 97], as well as the release of other neurotransmitters such as acetylcholine [93, 94]. The above effect of A_{2A} receptor agonists can be attributed to positive coupling to a second messenger system. Therefore, the blockade of A_{2A} receptors may afford neuroprotection after ischemia due to a reduced glutamate release and excitotoxicity (Fig. 3). Apart from the reduction of glutamate release, there are also several other mechanisms by which A_{2A} receptor antagonists can contribute to neuroprotection, e.g. diminution of the activation of microglia and astrocytes which can promote cell survival, diminution of cytokine release (TNF-α, IL-1β) or some vascular mechanisms [74] (Fig. 3). However, the results obtained with A_{2A} KO mice speak against a vascular mechanism underlying neuroprotection, observed in the latter model [8].

Interaction between A_1 and A_{2A} receptors

Functional evidence indicates that both A_1- and A_{2A}- subtypes of adenosine receptors can coexist in the same nerve terminals [15, 93]. It has been shown that the stimulation of A_{2A} receptors decreases the binding at A_1 receptors in hippocampal and striatal synaptosomes and attenuates the ability of A_1 receptor agonists to inhibit excitability and

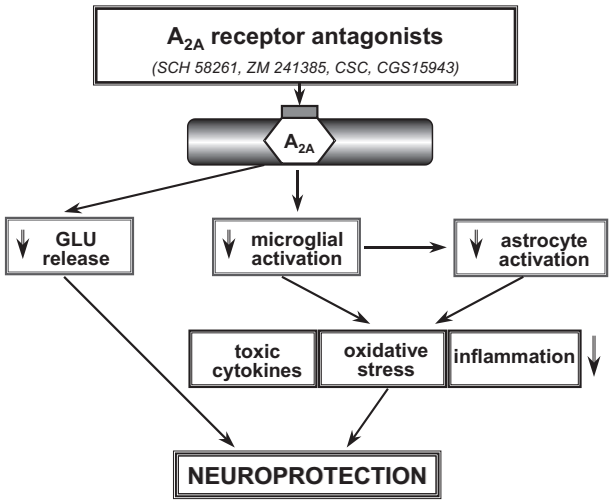

Fig. 3. Some important mechanisms relevant to the neuroprotective action of A_{2A} receptor antagonists. A_{2A} – adenosine A_{2A} receptor; GLU – glutamate; – decrease. A_{2A} receptor antagonists may afford neuroprotection by reducing the release of glutamate and the glutamate-induced excitotoxicity. There are also several other mechanisms important to neuroprotection that are mediated by A_{2A} receptor antagonists. One of them is inhibition of the pathological activation of glial cells. It is well known that resting microglial cells start to proliferate under pathological conditions and adopt a number of immune functions (e.g. production of prostaglandins, release of toxic cytokines). This may cause inflammation and neurotoxic effects due to microglial cell activation and consecutive reactive astrocyte changes. Therefore, the blockade of A_{2A} receptors by diminishing the activation of microglial cells and astrocytes (inhibition of the release of toxic cytokines and inflammation, depression of free radical formation) may be neuroprotective

synaptic transmission in the hippocampus [15, 93]. Thus, in general, activation of A_{2A} receptors leads to diminution of the effects mediated by A_1 receptors. That functional interaction between both these subtypes of adenosine receptors seems to suggest that the action of endogenous adenosine which is mediated by A_1 receptors may be attenuated if concomitant activation of A_{2A} receptors takes place. Therefore, on the basis of above-quoted studies, it has been suggested that the beneficial effect of selective A_{2A} receptor antagonists might be, at least in part, due to the relief of tonic inhibition upon A_1 receptors [15, 74].

Moreover, it is well known that these two receptors affect differently the release of glutamate which determines the risk of excitotoxic nerve cell damage, whereas stimulation of A_1 receptors inhibits, and that of A_{2A} ones stimulates the release of glutamate [15, 74, 75, 83, 97]. Therefore, the opposite regulation of presynaptic glutamate release may partly account for the neuroprotective effect of A_1 receptor agonists and A_{2A} receptor antagonists.

A_3 receptors and neuroprotection

Recently, several new compounds acting selectively on A_3 receptors have become available. However, the results of very few studies on the role of A_3 receptors in neuroprotection are rather contradictory. It has been found that the stimulation of A_3 receptors by the selective agonist Cl-IB-MECA at low concentrations inhibits cell death measured in glial cell cultures, whereas high concentrations of this agonist increase cell death in cerebellar granule cell cultures [1, 2]. On the other hand, *in vivo* studies have shown that IB-MECA, another selective agonist of A_3 receptors, administered acutely exacerbates the loss of hippocampal neurons in a model of global forebrain ischemia in the gerbil; nonetheless, beneficial effects were also reported after its chronic administration in the same model [109, 113]. Moreover, in a model of the MTH-induced neurotoxicity, Gołembiowska and Żylewska [31] showed that the putative agonist of A_3 receptors, APNEA, enhanced the neurotoxic effect of MTH measured by an *in vivo* microdialysis in rats. Therefore, it seems that, depending on experimental conditions which have not been defined as yet, the stimulation of A_3 receptors exerts either neuroprotective or neurotoxic effects. It is, however, noteworthy that agonists of A_3 receptors may also activate A_1 receptors [15]. In accordance with this assumption, some effects of A_3 receptor stimulation e.g. by Cl-IB-MECA may be blocked by the selective A_1 receptor antagonist DPCPX [15]. Furthermore, the stimulation of A_3 receptors by Cl-IB-MECA attenuates the A_1 receptor-mediated inhibition of synaptic transmission in hippocampal slices [18]. Therefore, it is speculated that deleterious effects of A_3 receptor agonists may be due, at least in part, to the attenuation of the beneficial effects of A_1 receptor activation on hypoxia or ischemia.

Conclusions

In recent years, data have been generated showing the crucial role of adenosine as a modulator of neurotransmission and neuroprotective agent against ischemic and excitotoxic neuronal injury. The results obtained in animal models of ischemia/hypoxia and EAA-induced excitotoxicity suggest that

the blockade of adenosine catabolism or uptake might provide a new approach to neuroprotection. Similar neuroprotective effects may be produced by the stimulation of A_1 receptors and blockade of A_{2A} ones. It seems, however, that elucidation of the role of A_3 receptors in the neuroprotection needs further studies, as stimulation of A_3 receptors can mediate both cell protection and cell death, depending upon the degree of receptor activation and/or specific pathophysiological conditions. Unfortunately, the development of selective A_1 receptor agonists as antiischemic agents has been hampered by their major cardiovascular side effects. However, recent studies have proved that A_{2A} receptor antagonists exert a neuroprotective effect in animal models of Parkinson's disease, which suggests that these compounds may be very promising in the treatment of neurodegenerative diseases since they not only relieve motor disabilities but also can stop the progress of the disease by promoting cell survival.

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