

The Role of Extracellular Adenosine in Chemical Neurotransmission in the Hippocampus and Basal Ganglia: Pharmacological and Clinical Aspects

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Abstract: Now there is general agreement that the purine nucleoside adenosine is an important neuromodulator in the central nervous system, playing a crucial role in neuronal excitability and synaptic/non-synaptic transmission in the hippocampus and basal ganglia. Adenosine is derived from the breakdown of extra- or intracellular ATP and is released upon a variety of physiological and pathological stimuli from neuronal and non-neuronal sources, i.e. from glial cells and exerts effects diffusing far away from release sites. The resultant elevation of adenosine levels in the extracellular space reaches micromolar level, and leads to the activation A_1 , A_{2A} , A_{2B} and A_3 receptors, localized to pre- and postsynaptic as well as extrasynaptic sites. Activation of presynaptic A_1 receptors inhibits the release of the majority of transmitters including glutamate, acetylcholine, noradrenaline, 5-HT and dopamine, whilst the stimulation of A_{2A} receptors facilitates the release of glutamate and acetylcholine and inhibits the release of GABA. These actions underlie modulation of neuronal excitability, synaptic plasticity and coordination of neural networks and provide intriguing target sites for pharmacological intervention in ischemia and Parkinson's disease. However, despite that adenosine is also released during ischemia, A_1 adenosine receptors do not participate in the modulation of excitotoxic glutamate release, which is nonsynaptic and is due to the reverse operation of transporters. Instead, extrasynaptic A_1 receptors might be responsible for the neuroprotection afforded by A_1 receptor activation.

Keywords: Adenosine, A_1 receptor, A_{2A} receptor, ATP, hippocampus, striatum.

INTRODUCTION

Since the first suggestion by Drury and Szent-Györgyi in 1929 [1] that extracellular adenosine may have a physiological effect on living cells, forty-one years elapsed before it was shown that adenosine acts at the subcellular level to increase cAMP [2]. Studies have also shown that adenosine is released [3] from cortical slices and inhibits acetylcholine (ACh) release from cholinergic terminals evoked by axonal stimulation via activation of theophylline-sensitive receptors [4, 5]. The finding [5] that theophylline competitively inhibited the presynaptic inhibitory effect of adenosine ($K_i = 21 \mu\text{M}$) and nucleotides has triggered studies using purinergic transmission and changed the dogma that nucleotides might have exclusive effects on postsynaptic sites.

In 1978, Burnstock proposed [6] that there were two purinergic receptors: adenosine-sensitive P_1 and ATP/ADP-sensitive P_2 receptors. Burnstock concluded that P_1 receptors were located presynaptically and P_2 receptors were located on the postsynaptic site. A couple of years later, studies found that both ATP and its non-metabolising compound α, β -methylene ATP were able to act presynaptically on P_{2X} receptors on the nerve terminals to potentiate transmitter release [7-9].

The general view is that adenosine is not a transmitter substance. It is not synthesised and/or stored in vesicles and

is not released from nerve terminals in response to depolarisation followed by Ca^{2+} -influx, although recent studies raised the possibility that adenosine could be also released in a vesicular fashion [10]. It is the degradation product of ATP in the extracellular space and may act as a chemical messenger at ambient concentrations. Therefore, it is generally accepted that the presence of ATP and its metabolite adenosine in the extracellular space may function in the central nervous system as nonsynaptic signalling molecules that diffuse far away from the cells where they originated from and tonically influence chemical neurotransmission [11], inflammation [12, 13] and immune responses [14]. Although the role of ATP in immunity is closely related to the role of its breakdown products, i.e., the nucleoside adenosine, as far as the chemical neurotransmission is concerned, adenosine and nucleotides such as ATP, ADP and AMP all have their own presynaptic effects on chemical neurotransmission [5, 9]. These molecules can inhibit or facilitate the release of transmitters via activation of P_1 or P_{2X} receptors. To date, two families of purinergic receptors have been defined, P_1 and P_2 receptors [15]. Adenosine acts on P_1 receptors, which are subdivided into G-protein-coupled A_1 , A_{2A} , A_{2B} and A_3 receptor subtypes [16]. The primary second messenger of all four subtypes of adenosine receptors is adenylate cyclase, which is either activated or inhibited depending on the type of receptor that is stimulated. A_1 and A_3 receptors are coupled to G_i , and their activation results in a decrease of cAMP levels, increase of K^+ conductance, and decrease of transient Ca^{2+} conductance important in transmitter release. These effects are analogous to the effect of presynaptic M_2 acetylcholine receptors or α_2 adrenergic receptors, both of which

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are G_i-coupled receptors. Adenosine A_{2A} and A_{2B} receptor subtypes, however, are coupled to G_s and increase levels of cAMP.

SOURCE OF EXTRACELLULAR ADENOSINE

The role of extracellular space has been emphasized in nonsynaptic chemical interactions [11]. Extracellular adenosine is thought to be generated in the extracellular space either as a breakdown product of released ATP [17] or as the released adenosine, depending on the type of stimulus (e.g. during ischemia, [18]). Because ATP is ubiquitous, all metabolically active cells of the nervous system are able to synthesize ATP, which provides a potential pool for the release of adenosine. Therefore, the cellular source of released purines in the hippocampus and basal ganglia could be any cell type present in these brain areas (i.e., nerve terminals, astrocytes and other types of glia, microglial cells and endothelia). The majority of ATP is formed in the mitochondria by oxidative phosphorylation, which results in approximately 5-10 mM ATP concentration in the cytoplasm under normal metabolic conditions. In addition, ATP has also been shown to be taken up and stored in synaptic vesicles of nerve terminals [19] and astrocytes [20]. There is, however, intense controversy about whether astrocytes can exocytose transmitters *in vivo* [21]. Conversely, the basal extracellular adenosine concentration was much lower (in the low-micromolar to high-nanomolar range), and the majority of adenosine was taken up into cells and rapidly reincorporated into ATP stores or deaminated by adenosine deaminase under normal metabolic conditions [21, 22].

A wide variety of stimuli are known to release ATP and/or adenosine to the extracellular space, which could theoretically lead to sufficient purine levels to activate either ATP or adenosine receptors [19, 22, 23]. Indeed, ATP and adenosine have been shown to be released in response to KCl depolarisation [24] low- [25, 26] and high-frequency [25, 27, 28] electrical stimulation from acute hippocampal and striatal slices and in response to glutamate receptor activation [29]. Interleukin-1 β (IL-1 β), a cellular mediator of inflammation is able to release ATP from hippocampal slices [30]. Generally, it appears that the proportion of adenosine derived from the extracellular breakdown of ATP is higher when the stimulation frequency is higher [25]. ATP and various transmitters are released from nerve terminals when exocytosis occurs in response to neuronal firing because neurotransmitters are stored together with ATP in the vesicles. ATP could also be released from postsynaptic sites in response to activation of receptors by primary transmitters [31]. Various purines, including ATP and adenosine, are also released from the hippocampus and striatum *in vivo* by the quasi-physiological stimuli mentioned above [32-35]. It is also important to mention that depolarising stimuli lead to the extracellular accumulation of not only adenine nucleotides and nucleosides but also guanine and pyrimidine nucleosides, such as uridine, which might also act as neuroactive substances under certain circumstances [36-38].

Although the stimulation-dependent release of ATP and adenosine by conventional neuronal activity is well documented, these stimuli probably result in a spatially restricted, localised increase in extracellular purine levels, which con-

tribute to the synaptic transmission and the modulation of pre- and post-synaptic functions within the synaptic cleft. Thus, ATP-metabolising ectoenzymes, present on the nerve terminal membrane, and nucleoside transporters may strongly limit further purine availability.

Pathological events are also known to powerfully stimulate purine release. These signals include mechanical [20, 39, 40] and hypotonic [41] stimuli, hypoxia/ hypoglycemia/ ischemia and consequent energy deprivation [18, 26, 42-44], inflammatory signals, such as bacterial lipopolysaccharide (LPS) [45, 46], and cytotoxicity. Among pathological stimuli, hypoxia and ischemic-like conditions are well-known causes of adenosine release in the striatum and hippocampus both *in vitro* [18, 47-53] and *in vivo* [54-58] (for further reference see [22]). However, unlike extracellular adenosine accumulation detected in response to physiological stimuli, the source of adenosine released by metabolic distress is mainly intracellular [59]. Pathological signals could release purines from neurons and non-neuronal cells. This release would result in a purine-rich extracellular milieu that could lead to a widespread activation of receptors that may reach neighbouring or distant cells, such as astrocytes and microglia. Moreover, nucleotides and nucleosides themselves may promote further release of purines, by a homo- or heteroexchange mechanism, if they reached relatively high concentrations in the extracellular space [60].

If purines are in the extracellular space, then their extrasynaptic concentrations are controlled by the enzymes that catalyse their conversion [61]. Several enzyme families are responsible for the extracellular degradation of ATP in the nervous system. The first step of the inactivation of ATP is mediated by the family of ectonucleoside triphosphate diphosphohydrolases (E-NTPDases, EC 3.6.1.5, also known as ectoATPase or apyrase), which are able to hydrolyse ATP and ADP to AMP [61]. These enzymes show widespread distribution in the brain [62, 63] and have micromolar K_m for ATP and ADP, which causes rapid and highly effective hydrolysis of ATP in almost all neuronal tissues (Table 1). In addition to the E-NTPDase family, ATP can also be dephosphorylated by ecto-nucleotide pyrophosphatases (E-NPPs) and alkaline phosphatases, which both have broader substrate specificity and widespread tissue distribution [61]. The next step of extracellular inactivation is the hydrolysis of AMP by the ecto-5' nucleotidase (EC 3.1.3.5) (CD73) enzyme, which is the rate-limiting step in the formation of adenosine [19, 64]. Ecto-5' nucleotidase exhibits a micromolar K_m for AMP and is feed-forwardly inhibited by ATP, which results in a delayed, 'burst-like' adenosine production [65]. It is also widely expressed in the brain, and it is predominantly associated to glial cells [66, 67]; however, its expression has also been demonstrated in purified hippocampal and striatal nerve terminals [68, 69]. Adenosine deaminase and adenosine kinase are the key enzymes in adenosine metabolism. Adenosine deaminase catalyses the deamination of adenosine to inosine [70], and adenosine kinase catalyses the phosphorylation of adenosine to AMP. Microglial cells are brain-specific macrophages that are able to release adenosine, produce it through ATP breakdown and respond to extracellular adenosine Fig. (1). Microglial cells are also equipped with different adenosine receptors [71]. They express functional A₁, A_{2A} and A₃ but not A_{2B} adeno-

sine receptors [71], which regulate different aspects of the microglial response [13].

Table 1. Kinetic Parameters of ATP, ADP and AMP Hydrolysis in Rat Hippocampal Slices (Data are Taken From [63])

	K_m	V_{max}
ATP	$322 \pm 95.24 \mu\text{M}$	$14.5 \pm 3.4 \text{ nmol/min/prep}$
ADP	$321.5 \pm 94.4 \mu\text{M}$	$22.6 \pm 3.8 \text{ nmol/min/prep}$
AMP	$267 \pm 52.88 \mu\text{M}$	$4.99 \pm 0.44 \text{ nmol/min/prep.}$

Adenosine-mediated actions can be terminated by its uptake into the cells via specific nucleoside transporters, which are widely expressed in the nervous system. Specific nucleoside transporters consist of two families: equilibrative transporters (ENTs) and concentrative transporters (CNTs). ENTs are driven by the concentration gradient, and CNTs are driven by the sodium gradient [72, 73]. ENTs can carry different nucleosides, including adenosine and inosine, but not nucleotides, across the cell membrane in both directions. They are regarded as the dominant nucleoside transporters of the brain. Because the intracellular adenosine level under normal metabolic conditions is in the low micromolar range

[22], ENTs loaded from the extracellular space by excess adenosine mediate adenosine uptake into the nerve terminals. When adenosine is taken up, the adenosine kinase and adenosine deaminase enzymes convert it to AMP and inosine, respectively, thereby maintaining the driving force of the carrier. However, the ENTs can also act in a reverse direction under certain circumstances, which mediates the release of adenosine into the extracellular space. Excessive accumulation of adenosine could occur during energy deprivation or metabolic distress when ATP stores are depleted and AMP is generated intracellularly. Cytosolic 5' nucleotidase, which has a relatively high K_m for AMP (1–14 μM), becomes active under energy deprivation and accumulates adenosine intracellularly. This adenosine then flows out to the extracellular space in a transporter mediated manner [22, 65].

EXTRACELLULAR CONCENTRATIONS OF ADENOSINE

The availability of extracellular adenosine is determined by a balance between the speed of decomposition of ATP by ectonucleotidases [64] and the rate of release of ATP and adenosine. Extracellular ATP is decomposed to adenosine in the hippocampus with a half-life of about 200 msec [17]. The maintenance of an ambient concentration of adenosine in the extracellular space is mainly dependent on ATP re-

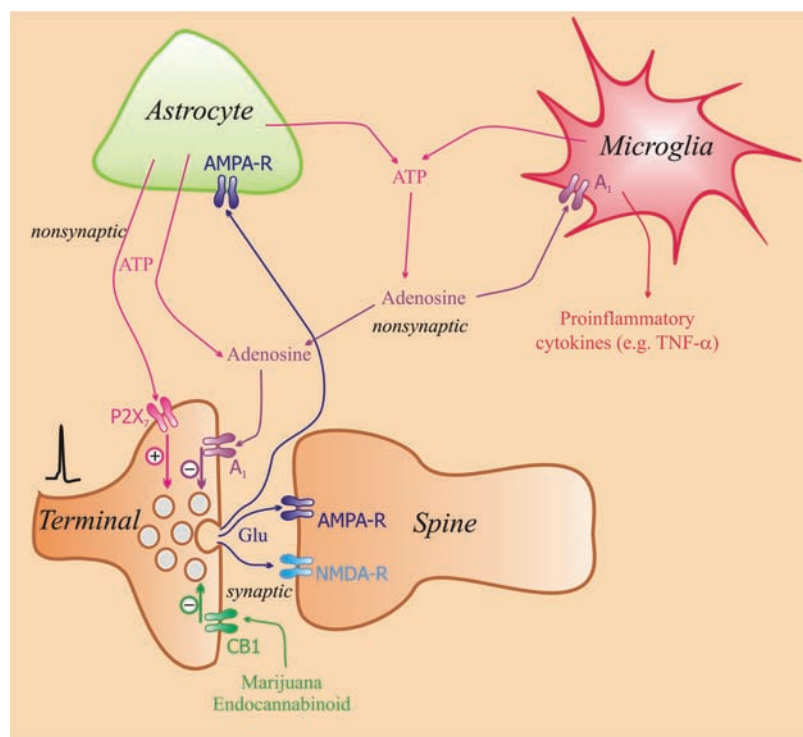


Fig. (1). A schematic model of the interaction of P_{2X7} , CB1 and A_1 receptors located on glutamatergic terminals. Activation of P_{2X7} receptors facilitates and those of A_1 receptors reduces the release of glutamate. Glutamate (Glu) released into synaptic gap activates AMPA and NMDA receptors on the postsynaptic site. ATP released from astrocytes [20] and microglia [172] acts on P_{2X7} receptors located on the terminal of glutamatergic neurons and facilitates the release of Glu ([173] for review see [174]). Adenosine decomposed from ATP acts on A_1 receptors inhibiting the release of Glu [168, 175]. This inhibitory effect of A_1 receptor activation may be mediated by inhibiting voltage-dependent Ca^{2+} channels, which reduces Ca transients measured in the bouton [176]. CB1 cannabinoid receptors together with A_1 receptors are also expressed on glutamatergic terminals [80] and activation of both of these receptors results in a decrease of Glu release. Extremely high concentrations of adenosine act on A_{2A} receptors to increase the release of Glu [77].

lease from astrocytes [74]. Once ATP is released into the extracellular space, it can be rapidly metabolised (within seconds, [17]) to adenosine, which is normally present in a concentration between 10 and 30 nM. Under hypoxia or ischemia, adenosine concentrations can reach 20 [17] and 30 μ M [75], respectively, in the extracellular space of the hippocampus. According to Zetterström *et al.* (1982) [58], adenosine concentrations under resting conditions can be as high as 1-2 μ M. One *in vivo* study observed adenosine levels around 19- to 23-fold compared with resting levels in response to ischemia [55]. Extracellular concentrations of adenosine and ATP of neuronal or glial origin can increase markedly in response to inflammation [22]. Once released into the extracellular space, adenosine may diffuse far away and reach nonsynaptic A₁ receptors as well as high-affinity nonsynaptic A_{2A} receptors. Therefore, the balance between A₁-receptor-mediated neuroprotective and A_{2A} receptor-mediated excitotoxic effects plays an important role in the outcome of adenosine action [76]. At lower concentrations of adenosine, the A₁ receptors were mainly tonically activated and A_{2A} receptors were not stimulated [77]. However, neuronal activity increased the bursting rate and the concentration of adenosine in the extracellular space, which caused both receptors to get tonically activated. It was observed that the stimulation of A_{2A} receptors by adenosine, formed from ATP at high-frequency neuronal firing, caused a down-regulation of the A₁ receptors. This finding supports observations that A_{2A} receptor blockade potentiates the neuroprotective actions of A₁ receptor activation [78].

EFFECT OF ADENOSINE ON TRANSMITTER RELEASE

It is generally accepted that adenosine plays a crucial role in neuronal excitability and synaptic transmission in the CNS. Electrophysiological and neurochemical evidence showed that presynaptic A₁ receptor activation reduced the release of different transmitters in the CNS including the hippocampus and basal ganglia (Table 2). The adenosine A₁ receptors are widely distributed in the CNS, and their activation induces several responses. These responses include inhibition of voltage dependent Ca²⁺ channels, activation of K⁺ channels resulting in hyperpolarisation and inhibition of adenylyl cyclase, all of which may cause inhibition of transmitter release. Furthermore, evidence was observed that nerve terminals were equipped with A_{2A} receptors, and stimulation of these receptors increased the release of different transmitters.

Electrophysiological Evidence

Activation of A₁ receptors decreases synaptic transmission in the hippocampus [79]. The stimulation of A₁ receptors located on Schaffer collateral axon terminals by adenosine decomposed from ATP inhibited the field EPSPs and EPSCs in hippocampal slices [80]. This finding indicated that the release of glutamate (Glu) Fig. (1) was inhibited from the hippocampus [81, 82]. Other studies also shed light on that endogenous adenosine, involved in the tonic A₁ receptor mediated inhibition of glutamatergic transmission, has largely astrocytic origin. Astrocytes are able to release ATP in a vesicular fashion [20], which then broke down to adeno-

sine by the ectonucleotidase cascade [74]. Activation of hippocampal astrocytic network leads to heterosynaptic depression of excitatory transmission, a mechanism, whereby the stimulation of a particular pathway could suppress the activation of a nearby pathway and coordinate the activity of a given network [83]. Heterosynaptic depression is mediated by A₁ adenosine receptors and selective gliotoxins [84] or selective knockdown of exocytotic machinery in astrocytes [74] leads to the disappearance of A₁-receptor mediated inhibition of excitatory transmission and the heterosynaptic depression in the hippocampal slices.

In this preparation, A₁ and CB1 cannabinoid receptors were colocalised on glutamatergic terminals [80]. A₁ receptor activation by endogenous adenosine (e.g., increased in concentration during ischemia or hypoxia) prevented the CB1 receptor-mediated reduction of Glu release. Accordingly, A₁ receptor antagonism with caffeine potentiated the effects of endocannabinoids [80], which indicated that cannabinoid signalling and presynaptic control of glutamatergic transmission in the hippocampus was limited by the tonic modulation mediated by the ambient levels of adenosine present in the extracellular space. Interestingly, this study concluded [80] that the effects of marijuana on hippocampal-dependent memory and cognition in humans might be potentiated during the simultaneous consumption of marijuana and caffeine, which happens frequently.

By contrast, in the striatum, A_{2A} receptor activation seems to play a permissive role on CB1 receptor mediated depression of excitatory transmission [85]. This effect, however, is related with the activation of postsynaptic A_{2A} receptors, as the effects of cannabinoid agonists were reduced in slices from mice lacking post-synaptic striatal A_{2A} receptors.

A₁ receptor mediated inhibition of excitatory transmission could serve as fine tuning mechanism to decrease the noise of excitatory transmission but also as a break of excessive synaptic activation under pathological conditions and is involved in the regulation of widespread physiological and pathological phenomena, from the regulation of arousal to neuroprotection and seizure susceptibility [81].

Activation of A₁ receptors also inhibits excitatory transmission in the striatum [86], while excitatory A_{2A} receptors participate in NMDA receptor dependent long-term potentiation of synapses between cortical pyramidal neurons and principal striatal medium spiny neurons [87, 88]. Moreover, a recent study showed that co-activation of A_{2A} receptors and fibroblast growth factor (FGF) receptor not only facilitates corticostriatal long term potentiation but also induce morphological plasticity, by inducing increasing spine density and neurite formation by the activation of MAPK/ERK signalling pathway [89].

Neurochemical Evidence

The first neurochemical evidence for a presynaptic site of action of adenosine was shown on cholinergic terminals in the cerebral cortex and ileal Auerbach plexus [5]. In addition, the study by Vizi and Knoll [5] observed a presynaptic inhibitory effect of nucleotides (ATP, ADP and AMP) via their degradation product, adenosine and discussed the impact of this effect on transmitter release. They described a

Table 2. Neurochemical Evidence of Presynaptic Effect of Adenosine on Transmitter Release in the CNS

	Transmitter Release	Effect	Receptor Mediated	Reference
Cerebral cortex	ACh	inhibition	A ₁	[5, 158]
		facilitation	A _{2A}	[166, 167]
Hippocampus	GABA	facilitation	A _{2A}	[101]
	ACh	facilitation	A _{2A}	[92, 94]
		inhibition	A ₁	
	NA	inhibition	A ₁	[93, 99]
Striatum	Glutamate	inhibition	A ₁	[168]
	Glutamate	inhibition	A ₁	[169]
	5-HT	inhibition	A ₁	[170]
	GABA	inhibition	A _{2A}	[103]
	ACh	no effect inhibition	A _{2A} A ₁	[93]
	DA	inhibition no effect	A ₁ A _{2A}	[93, 105, 171]

phenomenon that was different from the generally accepted view that the transmitter of purinergic nerves, ATP, has exclusive actions on P₂ receptors of the postsynaptic sites [90]. Stimulation of A_{2A} receptors resulted in an increased release of different transmitters, such as glutamate [65, 91] and acetylcholine [92, 93].

Following these pioneering investigations, subsequent studies revealed that the release of almost all transmitters of the CNS is regulated by inhibitory A₁ and/or facilitatory A_{2A} adenosine receptors (Table 2). For example, in acute hippocampal slices the electrically evoked release of acetylcholine is subject to dual modulation: it is inhibited by the activation of A₁ receptors [92, 94] and facilitated by A_{2A} receptors [92, 93]. However, the spatial extension and intensity of the two kind of modulation is not equal. Whilst A₁-receptor mediated inhibition is detected in all subregions of the hippocampus, A_{2A} receptor mediated facilitation is manifested only in the CA3 regions and in the dentate gyrus and not the whole hippocampus [92]. It also has been shown that endogenous adenosine, if released as such, preferentially activates A₁ receptors, whereas it is formed from the released ATP preferentially activates A_{2A} receptors [77]. Moreover aging and other pathological conditions seems to differentially affect inhibitory and facilitatory modulation. The level of endogenous ATP and adenosine is increased during aging [95, 96] and in parallel with these changes the density of A₁ receptors is decreased [96]. Consequently, a lower degree of inhibitory effect of adenosine agonists on acetylcholine release is detected in hippocampal slices prepared from aged rats [95, 96] but see [97]. On the other hand, the number and functional responsiveness of A_{2A} receptors is increased during aging [95, 97, 98].

In addition to acetylcholine, other transmitters such as glutamate [65, 91] are also subject to dual modulation by A₁

and A_{2A} receptors, respectively, whereas the release of noradrenaline (NA) [93, 99] and 5-HT [100] appears to be exclusively modulated by A₁ receptors and that of GABA by A_{2A} receptors [101] in the hippocampus. Adenosine inhibits the release of [³H]Glu from hippocampal slice preparation, an effect mediated via A₁ receptors Fig. (2).

Similar rules seems to be valid for the striatum, where the release of acetylcholine is regulated by both inhibitory A₁ and facilitatory A_{2A} receptors [93, 102] and the release of GABA by A_{2A} receptors [103], while the release of dopamine and 5-HT is modulated by A₁, but not A_{2A} receptors [93, 104, 105].

During ischemia and/or hypoxia, the release of glutamate [106], NA, dopamine (DA) [107] and adenosine [26, 108] was enhanced. During ischemia, the release of transmitter was [Ca²⁺]_o-independent [11] and mainly originated extrasynaptically from varicose nerve terminals without synaptic contact due to the reverse operation of transporters. Therefore, it seems very unlikely that adenosine is able to reduce transmitter release during ischemia Fig. (3). In support of this assumption, we showed that [³H]glutamate release from the hippocampus in response to oxygen-deprivation was increased and cannot be inhibited by adenosine and the selective A₁ receptor antagonist 1,3-dipropyl-8-cyclopentyl-xanthine (DPCPX) [109] failed to enhance the release Fig. (3). However, low temperature completely prevented the effect of glucose and oxygen deprivation (OGD) to release glutamate Fig. (3), which act nonsynaptically on NR2B receptors Fig. (4).

SYNAPTIC AND NONSYNAPTIC ADENOSINE RECEPTORS

Whilst A₁ adenosine receptors are highly expressed in many brain regions including the neocortex, hippocampus

cerebellum and brain stem, A_{2A} receptors display a more restricted localization, with high expression level in the striatum and olfactory bulb and lower expression in other brain regions [110]. By contrast, the expression of A_{2B} and A_3 receptors is moderate or low in most areas of the brain [111].

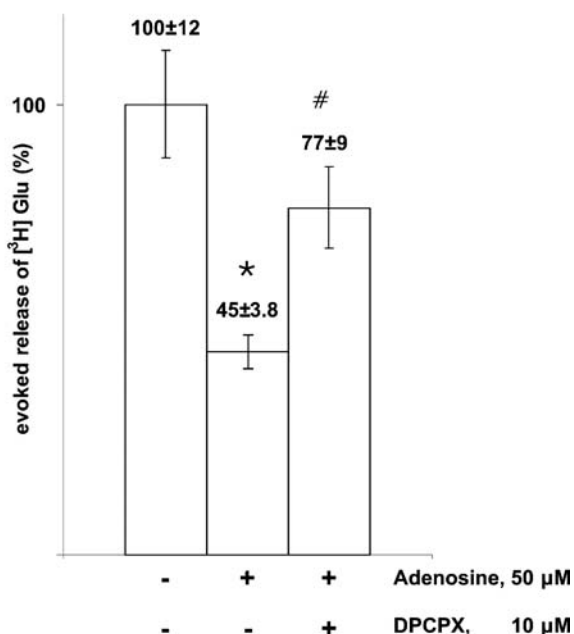


Fig. (2). Inhibitory effect of adenosine on glutamate release evoked by axonal stimulation. Hippocampal slice preparation of the rat. For Methods see [147]. Electrical field stimulation was used. Note that A_1 adenosine receptor antagonist DPCPX prevented the effect of adenosine to reduce Glu release. *, $p < 0.01$ (compared to control); #, $p < 0.05$ (comparison of the effect of adenosine and adenosine plus DPCPX).

Using quantitative autoradiography, both pre- and post-synaptic localization of A_1 receptors was shown in the CA1 region of rat hippocampus [112]. More recent investigations with postembedding immunogold electron microscopy revealed that A_1 receptors are co-localized with P_{2Y1} receptors in hippocampal synapses and distributed to the pre- and post-synaptic membrane as well as to the surrounding astroglial membrane [113]. In glutamatergic synapses of hippocampus, where both A_1 and A_{2A} receptors are presynaptically expressed [114], the inhibition of synaptic transmission by tonic activation of A_1 receptors was insurmountable with increasing concentrations of adenosine [79, 115]. There was an interaction between the two adenosine receptors, and activation of presynaptic A_{2A} receptors by an agonist may lead to a decrease in the affinity of A_1 receptors on the terminals [116, 117].

In the basal ganglia, the typical localization of A_{2A} receptors are the dendrites and somata of striatopallidal GABAergic neurons, where it is colocalized with D_2 receptors. However, immune electron microscopy studies showed that it is also expressed, although less abundantly on axons and nerve terminals in both asymmetric and symmetric synapses, which implicate the role of A_{2A} receptors in the modulation

of excitatory transmission [118, 119]. Indeed, A_1 receptors are coexpressed with A_{2A} receptors on the same glutamatergic terminals in the striatum and form heterodimers, when they are co-transfected [120]. Activation of A_{2A} receptors reduce the affinity of the A_1 receptors for agonists, and provides a switch mechanism by which low and high concentrations of adenosine inhibit and stimulate, respectively, glutamate release [120]. A_1 - A_{2A} heteromers are also recognized as targets for caffeine and it is presumed that chronic caffeine treatment leads to modifications in the function of the A_1 - A_{2A} heteromer that could underlie the strong tolerance to the psychomotor effects of caffeine [121]. On the other hand, the glial expression of A_{2A} receptor is less prevalent than that of A_1 receptors.

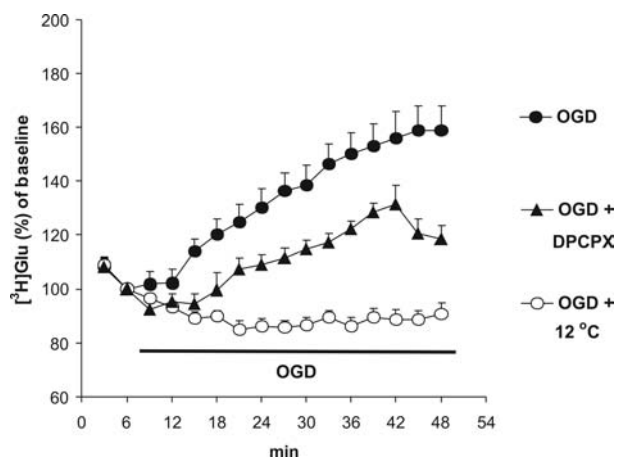


Fig. (3). The effect of low temperature (12 °C) and the selective A_1 receptor antagonist DPCPX (50 nM) on combined oxygen glucose deprivation (OGD)-evoked [3 H]glutamate efflux from rat hippocampal slices. Hippocampal slices were preloaded with [3 H]glutamate and then superfused. After a 60-min preperfusion, perfusate samples were collected and the slices were exposed to OGD by the omission of the glucose and the replacement of 95% O_2 +5% CO_2 with 95% N_2 + 5% CO_2 from the perfusion solution according to the horizontal bar. Low temperature and DPCPX were applied from 30 min before the beginning of the sample collection period till the end of the sample collection period. The release of glutamate is expressed as a percentage of baseline. The curves show the mean \pm S.E.M of 16 (OGD), 7 (OGD + 12 °C) and 8 (OGD + DPCPX) experiments.

CLINICAL ASPECTS

It is generally accepted that adenosine present in extracellular space and acting on A_1 receptors reduces excitatory transmission. Adenosine has a neuroprotective action in brain injuries, like hypoxia, ischemia, epileptic seizures, and neuroinflammation. Therefore, adenosine A_1 receptors have been suggested as a potential target for the treatment of neurodegenerative diseases [122]. In agreement with this suggestion it was found that inhibition of A_{2A} receptors improves neuronal recovery on brain injury [123].

Parkinson's Disease

Corticolimbic and thalamic glutamatergic neurons and mesencephalic dopaminergic neurons innervate the

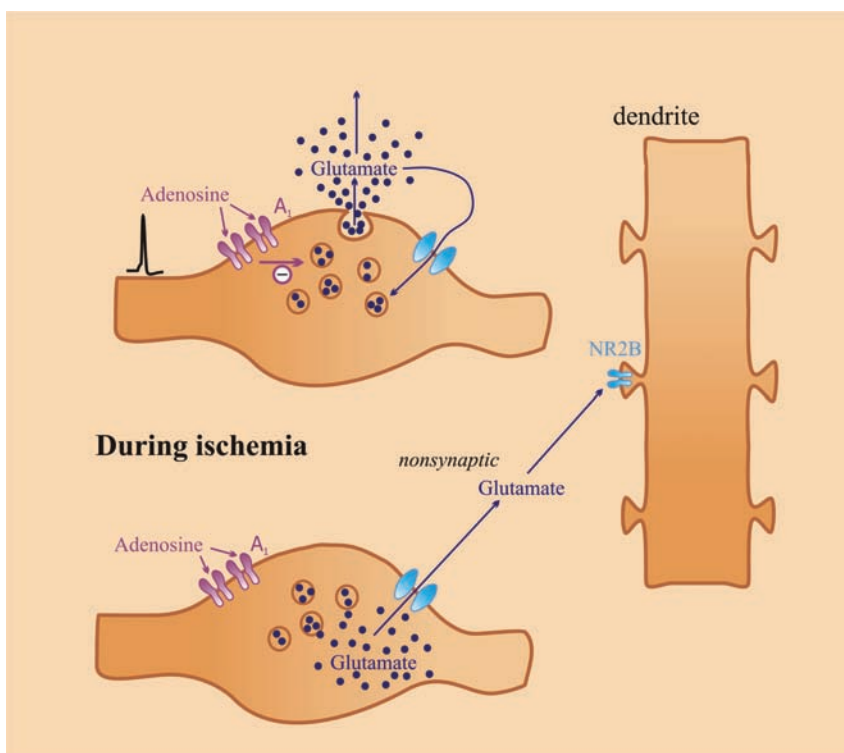


Fig. (4). Scheme of exocytosis of glutamate in response to axonal firing and its release during ischemia. Note that adenosine inhibits the release of Glu under physiological condition. This type of release is $[Ca^{2+}]_o$ -dependent. Glutamate is taken up by glutamatergic terminals through plasma membrane transporters. During ischemia the ion gradients that power Glu uptake run down and axonal firing fails to release Glu, but in response to reverse operation of Glu transporter Glu release occurs in $[Ca^{2+}]_o$ -independent way. Glu released in this way diffuses far away and activates non-synaptic NR2B receptors inducing excitotoxicity [11]. Under this condition cooling inhibits the excessive release of Glu.

GABAergic medium-sized spiny neurons of the striatum [124]. These GABAergic pathways are the striatal efferent neurons (“indirect” pathways), which project via the globus pallidus and the subthalamic nucleus to output nuclei (substantia nigra and entopeduncular nucleus). The “direct” pathway sends axons directly to the GABAergic neurons [124]. D_1 -type receptors belong to the “direct” pathway and D_2 -type receptors signal to the “indirect” pathway [124]. The balance between these two pathways is essential for proper functioning of the extrapyramidal motor system [125]. Overactivity of the “indirect” striatal pathway plays an important role in generating parkinsonian symptoms [125]. The degeneration of the nigrostriatal dopaminergic pathway results in striatal dopamine depletion, which consequently impairs the function of the basal ganglia circuits producing akinesia, bradykinesia, tremor, and rigidity [126]. Adenosine exerts its effects in the basal ganglia by acting through A_1 and A_{2A} adenosine receptors. The A_{2A} receptors are co-localised and interact functionally with D_2 receptors on the medium-sized spiny neurons of the “indirect” pathway. In addition, A_{2A} receptors also interact with NMDA receptors present on a subpopulation of medium spiny neurons, in a negative way [127, 128].

In the basal ganglia, A_{2A} receptors are prevalently and selectively localised in dendrites, dendritic spines and axons of GABAergic neurons of the indirect pathway projecting from the caudate putamen to the external globus pallidus [118, 119]. Adenosine influences striatal output pathways

known to be involved in motor symptoms and the onset of dyskinesia in Parkinson’s disease (PD). Therefore, inhibition of A_{2A} receptors seems to be a potential target for neuroprotection in PD. Indeed, in rodent models of PD, A_{2A} antagonism exerts antiparkinsonian actions [119, 129, 130]. Similarly, this treatment proved to be effective against experimentally-induced tremor [131]. The anti-tremor effect might be explained by the fact that A_{2A} receptor antagonists reduced the release of ACh from the striatum [132]. Recently, it was shown that expression level and functionality of A_{2A} adenosine receptors on human lymphocytes correlate with the severity of parkinsonian motor symptoms as scaled by the Unified Parkinson’s Disease Rating Scale (UPDRS) [133], implicating that peripheral A_{2A} receptors could also play a role in disease progression. Preclinical studies with istradefylline (an A_{2A} selective antagonist) suggest that these antagonists prevent the development of dyskinesia induced by a dopamine agonist [134]. This mechanism offers a rationale for the A_{2A} receptor antagonist treatment as a monotherapy of concurrent administration with levodopa or a dopamine receptor agonist [119, 135]. Unfortunately, clinical trials with istradefylline failed to fulfil expectations [119].

Nevertheless, the potential role of A_{2A} receptors in neurodegeneration of the nigro-striatal dopaminergic pathway is supported by a 30-year follow-up study [119, 136], which reported that there was an inverse relationship between consumption of the non-selective adenosine receptor antagonist caffeine and the risk of PD. The neuroprotective action of

A_{2A} receptor antagonism is further supported by preclinical studies. Administration of caffeine prevents MPTP-induced damage of dopaminergic terminals [137-139]. DPCPX, an A₁ receptor antagonist treatment [137] or genetic deletion [137] of A_{2A} receptors proved to be effective against the MPTP model of PD. However, inhibition of A_{2A} receptors expressed on glial cells was also involved in the neuroprotective actions [140]. Cunha *et al.* [141] have shown that A_{2A} receptors in the hippocampus and cerebral cortex are high-affinity receptors, whereas those in the striatum are different. Drugs that were effective in animal models of PD proved to be ineffective as neuroprotective agents in clinical trials [142, 143].

Ischemia

Glutamate is removed from the extracellular space by nonsynaptically localised glutamate transporters. Astrocytes possess two forms of these transporters, but glutamate is released from glial cells under ischemia due to a reversal of transporter operation. The cascade of events in response to hypoxia or cerebral ischemia is still being debated. However, it is generally accepted that a large amount of glutamate release causes excitotoxic effects Fig. (4). The excessive amount of glutamate leads to over-depolarization and the subsequent depression of evoked population potential size, and both A₁ and A_{2A} receptor activation has been shown to modulate the recovery from the loss of fEPSP in response to glutamatergic insult in the CA1 region of the hippocampus [144]. Evidence has accumulated that neuroprotection is also related to activation of nonsynaptic A₁ receptors [78]. The A₁ receptor-mediated effect of adenosine on the release of glutamate has been assumed to play a major protective role against post-ischemic damage [145]. However, this conclusion was drawn from *in vitro* experiments under normoxic conditions e.g. [79, 146]. There is strong neurochemical evidence that the release of transmitters under ischemic conditions is mainly due to the reverse operation of transporters. The release was [Ca²⁺]_o-independent, did not result from neuronal activity and was not subjected to presynaptic modulation [11]. Lowering the temperature inhibited transporter operation [147, 148] and completely inhibited the extracellular flood of transmitter release, which indicated that the transporter was responsible for the release. To avoid excitotoxicity due to extremely high concentrations of glutamate in the extracellular space and its possible effect on nonsynaptic high affinity NR2B receptors [11], local cooling or inhibition of transporter operation would be an efficient treatment. In addition, it was shown that damage after middle cerebral artery occlusion was not significantly altered by the lack of A₁ receptor genes [149]. An interesting observation is that A₁ receptor activation decrease brain energy metabolism [56] and the neuroprotective action of the chemokine fractalkine is mediated by the release of adenosine from microglia and a subsequent action on A₁ receptors [150, 151]. All these data support the emerging view that neuroprotection, afforded by A₁ receptor activation, is related with extrasynaptic, rather than synaptic receptors.

Immune Responses

The original view that the brain is not involved in adaptive and innate immune reactions is no longer accepted. The

innate immune system has been implicated in variety of neurodegenerative disorders [152]. Multiple acute and chronic neurodegenerative disorders of the CNS are accompanied by activation of microglia and increased production of proinflammatory cytokines (IL-1, IL-6, IL-12, TNF α) and chemokines [153]. Adenosine possesses anti-inflammatory properties and inhibited proinflammatory cytokine production via activation of A₃ receptors [154]. However, it has also been shown [155] that A₃ receptor stimulation had detrimental neurotoxic effects in cerebral ischemia. The case is similar to stimulation of A_{2A} receptors as their activation may produce anti-inflammatory and proinflammatory actions [156]. Interestingly, the balance between the anti- and proinflammatory actions of A_{2A} receptor is governed by extracellular glutamate levels in the brain, and the increase in glutamate levels results in a shift of A_{2A} effects from anti-inflammatory to proinflammatory direction [157]. Adenosine involvement in immune response is supported by findings that evidence was obtained that there is a significant change in ectonucleotidase activities during experimental autoimmune encephalitis [158].

The combined effects of adenosine on neurotoxicity and inflammatory processes have also led to considerations of its role in Lesch-Nyhan syndrome, and multiple sclerosis [159].

Epilepsy

It is known that during epileptic seizures, endogenous adenosine accumulates in significant amounts in the extracellular space and suppresses epileptic seizure activity [59, 160, 161]. Adenosine exerts an anticonvulsant effect on the A₁ adenosine receptors by modulating ionic currents postsynaptically and reducing excitatory neurotransmitter release presynaptically [162]. In addition, A_{2A} receptors modulate the stability of currents mediated by GABA_A receptors microtransplanted into *Xenopus* oocytes from neurosurgically resected epileptic human nervous tissues [163]. In the model of chronic temporal lobe epilepsy there is an upregulation of the A₁ receptors [162]. An interesting observation is that a single convulsive episode in early life causes a delayed memory deficit in adulthood accompanied by a glutamatergic synaptotoxicity that was prevented by caffeine or adenosine A_{2A}R antagonists [160].

Psychiatric Disorders

There is growing evidence that A_{2A} receptors able to interact with D₂ dopamine receptors [164] which are major targets of psychoactive drugs. Therefore these receptors are promising candidate target for therapy applied in mood disorders [165].

CONCLUDING REMARKS

It is now clear that adenosine is one of the most important neuromodulators in hippocampus and basal ganglia, which regulates a wide variety of neuronal functions pre-, post- and non-synaptically, including synaptic transmission, neuromodulation, glia-neuron interactions and neuroimmunomodulation. Nevertheless, there are a number of aspects which needs further investigation. Despite of the wealth of data on adenosine receptor mediated signaling at the molecu-

lar and cellular level, the present knowledge is still limited at the systems level. This holds true to any aspects of purinergic mechanisms including the release and inactivation mechanisms of adenosine and to presynaptic adenosine-receptor mediated responses as well, which are well characterized in *in vitro* systems, but poorly extrapolated to *in vivo* conditions. The progress along this line together with the utilisation of more adequate disease models might lead to the therapeutic utilization of purinergic signaling system, which offer a number of potential target sites for pharmacological intervention in the CNS pathology.

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ABBREVIATIONS

ACh	=	Acetylcholine
CNT	=	Concentrative transporter
DA	=	Dopamine
DPCPX	=	1,3-dipropyl-8-cyclopentylxanthine
E-NPP	=	Ecto-nucleotide pyrophosphatase
ENT	=	Equilibrative transporter
E-NTPDase	=	Ectonucleoside triphosphate diphosphohydrolase
FGF	=	Fibroblast growth factor
Glu	=	Glutamate
IL-1 β	=	Interleukin-1 β
LPS	=	Bacterial lipopolysaccharide
NA	=	Noradrenaline
OGD	=	Oxygen and glucose deprivation
PD	=	Parkinson's disease
UPDRS	=	Unified Parkinson's disease rating scale

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