ADENOSINE IN THE CENTRAL NERVOUS SYSTEM

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ABSTRACT

Besides being a metabolite of nucleotides like ATP, adenosine is a mediator of neuronal function in the central nervous system. Its actions are mediated by at least three extracellular receptors. In this review different aspects of adenosine such as biosynthesis, release, inactivation and its receptors are discussed. It also covers pre- and postsynaptic effects as well as postreceptor mechanisms of adenosine. Finally, therapeutic aspects of this neuromodulator have been discussed.

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INTRODUCTION

Adenosine has effects on almost all kinds of mammalian tissue including the central nervous system. As a neuromodulator adenosine has a profound depressant action in the central nervous system. The mechanism of adenosine’s action is not fully understood. Its action may be mediated pre- and postsynaptically through receptor-mediated mechanisms including effects on second messenger systems, transmembrane ion fluxes and neurotransmitter release.

1. Historical perspective of adenosine

A definite action of adenosine was first demonstrated by Drury and Szent-Györgyi about seventy years ago. They showed that adenosine in extracts of heart muscle and other tissues such as brain have a specific action upon the heart. Satin and Rall (1970) later reported an effect of adenosine on cAMP accumulation in the CNS. Phillis et al. (1975) showed that adenosine and several adenine nucleotides depressed the excitability of cerebral cortical neurons of the rat in vivo. The depressive effect of adenosine in different areas of the brain was later demonstrated by Kostopoulos and Phillis (1977). In 1978 Burnstock proposed the terms P1 for nucleoside receptors like adenosine and P2 for nucleotide receptors like ATP. The adenosine receptors were divided by Van Calker et al. (1979) into A1 and A2 based on the stimulation or inhibition of cAMP. The 1980s witnessed the more detailed characterization of adenosine receptors. These years were also spent elucidating the second messengers by which adenosine may act to elicit its array of biological responses. The 1990s will lead this field into the realm of molecular pharmacology, with determination of the amino acid sequences for adenosine receptors and ultimately the genes coding for these receptors.

2. Biosynthesis, release and inactivation of adenosine

The two likeliest sources of adenosine are the dephosphorylation of 5'-AMP by 5'-nucleotidase and the action of S-adenosylhomocysteine hydrolase upon S-adenosylhomocysteine. Adenosine as a nucleoside of adenosine can also be synthesized de novo from 5-phosphoribosyl-1-pyrophosphate and glutamine.

Adenosine can be formed largely within the cytosol, especially under conditions of metabolic stress. Adenosine levels in the brain are regulated by the balance of energy supply and demand. There is equilibrium between the cytoplasmic concentrations of ATP, ADP and AMP, and because the ATP concentration in resting cells is much more
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than the AMP concentration, a very small decrease in the percentage of ATP concentration can increase cytoplasmic AMP concentration substantially, with the formation of adenosine.11-12

The concentration of adenosine is increased several times during seizures,13 hypoxia or ischemia.14-17 Chemical agents such as excitatory amino acids or veratridine, or electrical brain stimulation can also release adenosine.18,19 Potassium can also evoke a calcium-dependent extrasynaptosomal accumulation of endogenous adenosine.20

Adenosine can be inactivated by uptake into neurons and neighbouring cells through a nucleoside transporter by a facilitated diffusion process which is largely regulated by the concentration gradient for adenosine. It is also inactivated either by phosphorylation to AMP by adenosine kinase or deamination to inosine by adenosine deaminase.11,21 Nucleoside transport inhibitors such as dipyridamole or nitrobenzyl-thioinosine enhance the effect of adenosine.11

In contrast to the sites and mechanisms of adenosine, the sites and mechanisms of adenosine formation and subsequent release are still subject to much argument and controversy.23 Although released extracellular ATP is broken down to AMP by adenosine kinase or deamination to inosine by adenosine deaminase,24 Nucleoside transport inhibitors such as dipyridamole or nitrobenzyl-thioinosine enhance the effect of adenosine.25

Adenosine is formed predominantly intracellularly and released into the extracellular space. Although adenosine can be taken up by synaptosomes and released following depolarization, there is no evidence that adenosine is stored in synaptic vesicles and no clear cut adenosinergic pathways have been established in the brain.25 Adenosine is also poorly released by potassium from preparations in comparison with neurotransmitters.23 Therefore the term neuromodulator may be better used for adenosine.

3. Adenosine Receptors

A- Classification

The finding of Sattin and Rall that methylxanthines such as theophylline can block the effects of adenosine was the first evidence for specific adenosine receptors.2 In 1978 Burnstock divided purine receptors into P, for the nucleoside receptor and P, for nucleotide receptors.5 The P, receptors are most readily characterized as sites at which xanthines act as competitive antagonists and adenosine has higher affinity than ATP.26,27

P, receptors are further subdivided into A, and A, based on the inhibition or stimulation of adenylate cyclase, respectively. Adenosine has nanomolar affinity for A, receptors and micromolar affinity for A, receptors.6 In another study P, receptors were subdivided into R and R for A, and A, respectively, the subscripts of which refer to the inhibition (i) and activation (a) of adenylate cyclase activity. R refers to the ribose group of adenosine which is necessary for agonist activity.28,29 Because some physiological effects of adenosine are not mediated via a cAMP-dependent mechanism,30 the general use of the A,/A, nomenclature which does not inherently imply any activation or inhibition for adenylate cyclase was recommended.31 Based on ["H"]NECA binding, adenosine A, receptors are also further divided into A, (high affinity) and A, (low affinity) receptors.32 Both these subtypes increase adenylate cyclase activity.

There is also an intracellular P-site which mediates inhibition of adenylate cyclase and requires integrity of the purine ring for activity but can tolerate compounds with a modified ribose moiety.33 Some adenosine derivatives, e.g. dideoxadenosine, are specifically active at this P-site. Inhibition of adenylyl cyclase via the P-site is most efficient when the enzyme is activated.34

Besides extracellular (A, and A, receptors) and intracellular (P-site) adenosine receptors, recently Zhou et al. reported the cloning, expression and functional aspects of a new adenosine receptor, which they called the A, receptor.35 They showed that this receptor is coupled to a pertussis toxin-sensitive G protein, and can inhibit adenylate cyclase. This receptor is different from an earlier proposed A, site which was proposed by Ribeiro and Sebastião.36 The first A, receptor was based on pharmacological tools. The non-selective adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA) is equipotent to the non-selectively A, receptorligands cyclohexyladenosine (CHA) and R-N6-phenylisopropyladenosine (R-PIA), and more potent than 2-chloroadenosine. This receptor was not linked to adenylyl cyclase but involved inhibition of Ca" influx and/or mobilization. The major difference between this receptor and the subsequently cloned receptor is the fact that the latter receptor is not sensitive to alkylxanthine antagonists.37

B- Distribution

Adenosine A, receptor

A quantitative autoradiographic study in the human brain showed that adenosine A, receptors were heterogeneously distributed throughout the brain and essentially localized to the gray matter. The highest receptor densities were found in the stratum oriens, pyramidale and radiatum of the hippocampus. High densities were also found in the cerebral cortex and the striatum. The hypothalamus had low receptor densities.37 Ex vivo autoradiographic distribution of ["H"]DPCPX in the brain also showed high levels of adenosine A, receptor in tissues such as the cerebellum and hippocampus and a lower density in the brain stem and hypothalamus.38

Adenosine A, receptors

With the autoradiographic study of distribution of
Adenosine A<sub>2</sub> receptor

There are limited studies concerning adenosine A<sub>2</sub> receptors. There was relatively low expression of this novel receptor in the central nervous system, but high expression was observed in the testis. 44

C- Ligands for adenosine receptors

Many agonist ligands for the adenosine A<sub>1</sub> receptor have been investigated over the past 30 years. These include the N<sub>6</sub>-substituted analogues cyclohexyladenosine (CHA), cyclopentyladenosine (CPA), phenylisopropyladenosine (PIA) and 2-chloroadenosine (2-CADO). 44-45

There has been less success in developing A<sub>2</sub> adenosine ligands. For a period of time, the 5-substituted adenosine analogue 5'-N-ethylcarboxamidoadenosine (NECA) was used to define tissue responses mediated by A<sub>2</sub> receptor activation. However, this ligand is non-selective and approximately equipotent at both A<sub>1</sub> and A<sub>2</sub> receptors. A recently produced compound, CGS21680, is 70-140-fold selective on A<sub>2</sub> receptors in binding assays. 44 At present for the A<sub>2</sub> receptor, N'-2-(4-aminophenyl)ethyladenosine (APNEA) is the most useful agent for activation of this receptor. 45-47

The agonist potency orders for different adenosine receptors are: 48

A<sub>1</sub>: CPA>R-PIA=CHA≥NECA>2-CADO>S-PIA
A<sub>2</sub>: CGS21680=NECA>2-CADO>R-PIA=CHA>CPA>S-PIA
A<sub>2</sub>: NECA>2-CADO>R-PIA=CHA>S-PIA≥CGS21680
A<sub>2</sub>: APNEA>R-PIA=NECA>CGS21680

The most famous antagonists of adenosine receptors are theophylline and caffeine which share a xanthine structure. These agents cannot discriminate between A<sub>1</sub> and A<sub>2</sub> adenosine receptors. 49-50 8-phenyl substituted xanthine molecules like 8-phenyltheophylline show more selectivity for A<sub>1</sub> receptors. Alterations in the substituents at the 1- and 3- positions alter both the activity and pharmacological selectivity of the xanthines. 1,3-Diethyl-8-phenylxanthine (DPX) is a potent A<sub>1</sub> antagonist with 18-fold selectivity. The cyclopentyl xanthine, 8-cyclopentyl-1,3-dipropylxanthine (CPX or DPCPX) has subnanomolar affinity for the A<sub>2</sub> receptor and is 740-fold selective. Cyclopentyltheophylline (CPT) is 130-fold selective for the A<sub>1</sub> receptor. 44 CPT is 40 times more soluble than DPCPX. 51

There is no selective A<sub>2</sub> adenosine receptor ligand available. There are a few A<sub>2</sub> antagonists such as 8-(3-chlorostyryl) caffeine (CSC) which is 520-fold selective for this receptor. 52

Recently, an A<sub>1</sub> receptor antagonist, 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)-1-propylxanthine (BW-A522) has been introduced which blocks adenosine A<sub>1</sub> receptor-mediated hypotensive responses in the rat. 53

D- Functional effects of adenosine receptors

Generally, adenosine has depressant actions in the hippocampus, cerebral cortical neurons, olfactory cortex, and different areas of the brain. 4
The A<sub>1</sub> and A<sub>2</sub> receptors mediate somewhat different effects in most tissues. A<sub>1</sub> adenosine receptors have inhibitory action. 56-58 In guinea pig hippocampal slices, the highly selective adenosine A<sub>1</sub> receptor antagonist, DPCPX, produced parallel, rightward shifts of the concentration-response curve for CPA-induced decreases in orthodromically-evoked population spikes of CA1 neurons. 60
An increasing volume of data indicates that excitatory actions of adenosine, such as depolarization and enhancement of transmitter release, can be mediated by A<sub>2</sub> receptors. 56 Sebastian and Ribeiro showed that nanomolar concentrations of CGS21680 reversibly increased in a concentration-dependent manner the amplitude of orthodromically-evoked population spikes recorded from the CA1 pyramidal cell layer of rat hippocampal slices. 64

E- Allosteric enhancers

Recently, a new class of compounds has been shown to enhance adenosine receptor binding. These compounds originated from a series of 2-amino-3-benzoyl-thiophenes. PD81,723 enhanced the inhibitory effect of exogenously applied adenosine, with no effect alone, in hippocampal brain slices. 55 In another study, this group showed that in low magnesium-induced bursting, which induces adenosine release, this agent alone reduced the duration of epileptiform bursting. 66

4. Presynaptic actions of adenosine

There is abundant documentation that adenosine diminishes transmitter release via an action at the presynaptic terminal. Adenosine or adenosine analogues inhibit release of glutamate, 67-68 acetylcholine, 69-71 noradrenaline, 72-73 dopamine, 74 GABA and serotonin 75 in the central nervous system. Adenosine also peripherally inhibits release of neurotransmitters. This may be consistent with the idea that adenosine receptors are located on excitatory terminals. 76-81

The mechanism of the inhibitory effect of adenosine on transmitter release is largely dependent on calcium influx into nerve terminals and the mobilization of intracellular...
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calcium.\textsuperscript{82,83} Therefore, one possible mechanism of adenosine to inhibit transmitter release may be blocking of the calcium influx or an effect on the calcium sensitivity of the release process.

Inhibitory effect(s) of adenosine on Ca\textsuperscript{2+} influx are controversial. There are some positive effects of adenosine on the uptake of labelled \textsuperscript{45}Ca into synaptosomes. In rat brain synaptosomal preparations, adenosine modulated calcium uptake by potassium depolarized nerve terminals.\textsuperscript{84,85} By contrast, other laboratories reported that adenosine and adenosine analogues had no effect on calcium uptake by potassium or veratridine depolarization.\textsuperscript{86-88}

2-Chloroadenosine decreased calcium currents in cultured rat hippocampal pyramidal or dorsal root ganglion neurons under whole-cell voltage clamp.\textsuperscript{89,90} The effect of adenosine analogues was relatively weak in these experiments. In acutely isolated pyramidal neurons from the CA3 region, the calcium channels which were blocked by activation of adenosine A\textsubscript{1} agonists were of the N-type.\textsuperscript{91}

With ion sensitive microelectrodes, Schubert et al. showed that adenosine decreased pre- and postsynaptic calcium signals of rat hippocampal cells in low calcium medium.\textsuperscript{92} In another report Schubert demonstrated that endogenous adenosine also, via A\textsubscript{1} receptors, inhibits calcium influx in the synaptic and pyramidal cell soma layer in the CA1 area of rat hippocampal slices.\textsuperscript{93} However, they did not clearly indicate into which compartment, neuron or glia, calcium moves.

Silinsky proposed that adenosine impairs transmitter secretion by reducing the affinity for calcium at a site beyond the external orifice of the calcium channel.\textsuperscript{94}

5. Postsynaptic actions of adenosine

There is a relatively large amount of evidence that adenosine hyperpolarizes postsynaptic cells via potassium channels. Adenosine hyperpolarized CA1 neurons of rat hippocampus and decreased input resistance in normal and low calcium medium when synaptic activity was blocked. Adenosine also decreased excitatory postsynaptic potentials (EPSP) by a presynaptic effect, without any effect on resting membrane potential.\textsuperscript{95} Okada and Ozawa (1980) also showed a hyperpolarizing action of adenosine in guinea pig hippocampal slices.\textsuperscript{96}

In more precise experiments, using the patch clamp technique, Trussell and Jackson also confirmed that adenosine can activate potassium channels in postsynaptic neurons from rat striatum.\textsuperscript{97} The same group also showed this effect in the hippocampus and implicated a G protein in the activation of potassium channels.\textsuperscript{98} Recently Li and Henry suggested that adenosine induces opening of potassium channels in the postsynaptic membrane of CA1 rat neurons, including K\textsubscript{ATP} channels. Glibenclamide, a blocker of K\textsubscript{ATP}, reversibly depressed 2-chloroadenosine-induced hyperpolarization.\textsuperscript{99}

Besides acting on potassium, adenosine via an A\textsubscript{1} adenosine receptor and pertussis-sensitive effect can induce a steady-state inward current by a voltage-dependent chloride conductance in cultured hippocampal neurons. This current was blocked by application of DIDS, a putative Cl\textsuperscript{-} channel blocker.\textsuperscript{100}

In the absence of extracellular calcium, adenosine-evoked inhibition of pyramidal neuron excitability appears to be lost, probably through some form of desensitization or resulting from the increased sodium conductances in calcium-free solution.\textsuperscript{102}

6. Post-receptor mechanism of adenosine

A- G protein

GTP-binding proteins are closely related proteins which transduce extracellular signals into effector responses such as ion channels, adenylate cyclase and phospholipase C.\textsuperscript{103}

In whole-cell patch-clamp, adenosine evoked an outward potassium current in cultured mouse hippocampus and striatum and in low-resistance patch electrodes lost its action. GTP in the patch electrode filling solution restored the adenosine effect. Thus a G protein is involved in the coupling between adenosine receptors and a potassium channel.\textsuperscript{99} This potassium channel mediates the postsynaptic effects of adenosine and is sensitive to barium and coupled to a pertussis toxin-sensitive GTP binding protein.\textsuperscript{80}

B- Cyclic AMP

Although the original classification of adenosine receptors was based on the changing cAMP level\textsuperscript{16-28} the functional role of this second messenger in relation to adenosine in the CNS is not clear.

Raising [cAMP] either with bath applied forskolin or 8-bromo cAMP did not change the adenosine-evoked potassium outward current in the hippocampus and striatum and, as mentioned above, the potassium channel was coupled via a G protein.\textsuperscript{98} In rat hippocampal slices, application of PbCl\textsubscript{2}, which has a disruptive effect on adenylate cyclase, had no significant effect on depressant responses to adenosine. Isoprenaline, which increases cyclic AMP levels, had no effect on the amplitude of adenosine-mediated depressant responses, whereas noradrenaline potentiated the very modest inhibitory effects of adenosine. Thus, adenosine-mediated cyclic AMP accumulation can be either inhibited or facilitated without markedly affecting the electrophysiological responses to adenosine; this suggests that accumulation of cyclic AMP is not directly involved in such responses.\textsuperscript{104}

C- Phosphatidylinositol turnover and calcium mobilization

Adenosine can modulate (decrease or increase) phospholipase activity depending upon the animal species.
the tissue and the nature of activation of this enzyme. Adenosine inhibited inositol phospholipid hydrolysis elicited in rat cortical slices by mM histamine concentrations. This modulation was selective for histamine; adenosine has no effect on either basal or carbachol-, glutamate-, quisqualate- and noradrenaline-stimulated inositol phosphate generation. The rank order of potency of adenosine agonists and inhibition of the adenosine effect by DPCPX, an A₃ receptor antagonist, indicated the involvement of A₃ receptors.¹⁰⁵

D- Arachidonic acid

The inhibitory actions of adenosine on hippocampal responses were unaffected by a phospholipase inhibitor (p-bromophenacyl bromide), lipoxygenase inhibitor (nordihydroguaiaretic acid=NDGA) and a cyclo-oxygenase inhibitor, indomethacin.⁴ The lipoxygenase inhibitor NDGA also failed to antagonize adenosine inhibition of the release of acetylcholine evoked by electrical pulses.⁴⁹

7. Therapeutic aspects of adenosine

In the CNS, the potential therapeutic uses⁶ of adenosine consist of: anti-convulsant,⁶ anti-ischemic,¹⁰⁷-¹⁰⁹ anti-Parkinson’s disease,¹¹¹ antipsychotic, anxiolytic, sedative, analgesic and Alzheimer’s disease.⁴,¹¹³-¹¹⁶ The failure of existing entities and the perceived disadvantages of classical medicinal chemical approaches for adenosinereceptor drugs has led a number of laboratories to produce prodrugs, and indirectadenosineagonists such as adenosine uptake blockers and allosteric enhancers.¹¹⁶

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