Medical Journal of the
Islamic Republic of Iran

Review Article

Volume 9
Number 4
Winter 1374
February 1996

ADENOSINE IN THE CENTRAL NERVOUS SYSTEM

H. HOSSEINZADEH, AND T.W. STONE*

*From the Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Islamic Republic of Iran, and * the Department of Pharmacology, Glasgow University, Glasgow, Scotland, U.K.*

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.

Keywords: Adenosine, Purine, Central nervous system, Review.

MJIRI, Vol. 9, No. 4, 361-368, 1996.

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.[1] They showed that adenosine in extracts of heart muscle and other tissues such as brain have a specific action upon the heart. Sattin and Rall (1970) later reported an effect of adenosine on cAMP accumulation in the CNS.[2] Phillis et al. (1975) showed that adenosine and several adenine nucleotides depressed the excitability of cerebral cortical neurons of the rat *in vivo*.[3] The depressive effect of adenosine in different areas of the brain was later demonstrated by Kostopoulos and Phillis (1977).[4] In 1978 Burnstock proposed the terms P_1 for nucleoside receptors like adenosine and P_2 for nucleotide receptors like ATP.[5] The adenosine receptors were divided by Van Calker et al. (1979) into A_1 and A_2 based on the stimulation or inhibition of cAMP.[6] 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.[7]

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.[8] Adenosine as a nucleoside of adenine can also be synthesized *de novo* from 5-phosphoribosyl-1-pyrophosphate and glutamine.[9]

Adenosine can be formed largely within the cytosol, especially under conditions of metabolic stress.[10] Adenosine levels in the brain are regulated by the balance of energy supply and demand.[11] There is equilibrium between the cytoplasmic concentrations of ATP, ADP and AMP, and because the ATP concentration in resting cells is much more

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.[22]

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 adenosine by ecto-nucleotidases, blocking of this enzyme was found to be without effect on the basal or evoked release of adenosine from rat hippocampal slices.[24] It seems that 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_1 for the nucleoside receptor and P_2 for nucleotide receptors.[5] The P_1 receptors are most readily characterized as sites at which xanthines act as competitive antagonists and adenosine has higher affinity than ATP.[26-27]

P_1 receptors are further subdivided into A_1 and A_2 based on the inhibition or stimulation of adenylate cyclase, respectively. Adenosine has nanomolar affinity for A_1 receptors and micromolar affinity for A_2 receptors.[6] In another study P_1 receptors were subdivided into Ri and Ra for A_1 and A_2 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_1/A_2 nomenclature which does not inherently imply any activation or inhibition for adenylate cyclase was recommended.[31] Based on [^{3}H]NECA binding, adenosine A_2 receptors are also further divided into A_{2a} (high affinity) and A_{2b} (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.[29] Some adenosine derivatives, e.g. dideoxyadenosine, are specifically active at this P-site. Inhibition of adenylyl cyclase via the P-site is most efficient when the enzyme is activated.[33]

Besides extracellular (A_1 and A_2 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_3 receptor.[34] 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_3 site which was proposed by Ribeiro and Sebastião.[35] The first A_3 receptor was based on pharmacological tools. The non-selective adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA) is equipotent to the nominally selective A_1 receptor ligands cyclohexyladenosine (CHA) and R-N^6-phenylisopropyladenosine (R-PIA), and more potent than 2-chloroadenosine. This receptor was not linked to adenylyl cyclase but involved inhibition of Ca^{2+} 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.[36]

B- Distribution

Adenosine A_1 receptor

A quantitative autoradiographic study in the human brain showed that adenosine A_1 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 [^{3}H]DPCPX in the brain also showed high levels of adenosine A_1 receptor in tissues such as the cerebellum and hippocampus and a lower density in the brain stem and hypothalamus.[38]

Adenosine A_2 receptors

With the autoradiographic study of distribution of

[^{3}H]CGS21680, an A_{2a} adenosine receptor agonist, Jarvis and Williams showed that this receptor is highly concentrated in the striatum, nucleus accumbens and olfactory tubercle of rat brain.[39] Lower levels of binding were also found in the globus pallidus. No significant amounts of specific ligand binding were observed in any other brain region. A similar result was also reported in human and rat brain by binding assay. Low binding of the A_2 agonist was found in the cerebellum and hippocampus.[40] A_{2b} adenosine receptors are distributed widely throughout brain tissue.[32]

Adenosine A_3 receptor

There are limited studies concerning adenosine A_3 receptors. There was relatively low expression of this novel receptor in the central nervous system, but high expression was observed in the testis.[34]

C- Ligands for adenosine receptors

Many agonist ligands for the adenosine A_1 receptor have been investigated over the past 30 years. These include the N^6-substituted analogues cyclohexyladenosine (CHA), cyclopentyladenosine (CPA), phenylisopropyladenosine (PIA) and 2-chloroadenosine (2-CADO).[41-43]

There has been less success in developing A_2 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_2 receptor activation. However, this ligand is non-selective and approximately equipotent at both A_1 and A_2 receptors. A recently produced compound, CGS21680, is 70-140 fold selective on A_{2a} receptors in binding assays.[44] At present for the A_3 receptor, N^6-2-(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_1: CPA>R-PIA= CHA≥ NECA> 2-CADO> S-PIA

A_{2a}: CGS21680=NECA> 2-CADO > R-PIA = CHA= CPA> S-PIA

A_{2b}: NECA> 2-CADO > R-PIA= CHA > S- PIA ≥ CGS21680

A_3: 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_1 and A_2 adenosine receptors.[9,49-50] 8-phenyl substituted xanthine molecules like 8-phenyltheophylline show more selectivity for A_1 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_1 antagonist with 18-fold selectivity. The cyclopentyl xanthine,8-cyclopentyl-1,3,-dipropylxanthine (CPX or DPCPX) has subnanomolar affinity for the A_1 receptor and is 740-fold selective. Cyclopentyltheophylline (CPT) is 130-fold selective for the A_1 receptor.[44] CPT is 40 times more soluble than DPCPX.[51]

There is no selective A_{2b} adenosine receptor ligand available. There are a few A_{2a} antagonists such as 8-(3-chlorostyryl) caffeine (CSC) which is 520-fold selective for this receptor.[52]

Recently, an A_3 receptor antagonist, 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)-1-propylxanthine (BW-A522) has been introduced which blocks adenosine A_3 receptor-mediated hypotensive responses in the rat.[53]

D- Functional effects of adenosine receptors

Generally, adenosine has depressant actions in the hippocampus,[30,54-56] cerebral cortical neurons,[3] olfactory cortex,[57-59] and different areas of the brain.[4]

The A_1 and A_2 receptors mediate somewhat different effects in most tissues. A_1 adenosine receptors have inhibitory action.[60-62] In guinea pig hippocampal slices, the highly selective adenosine A_1 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_2 receptors.[63] Sebastião 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.[65] 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,[76] GABA[77] and serotonin[78] 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.[79-81]

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

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

2-Chloroadenosine decreased calcium currents in cultured rat hippocampal pyramidal or dorsal root ganglion neurons under whole-cell voltage clamp.[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_1 agonists were of the N-type.[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.[92] In another report Schubert demonstrated that endogenous adenosine also, via A_1 receptors, inhibits calcium influx in the synaptic and pyramidal cell soma layer in the CA1 area of rat hippocampal slices.[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.[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 suppressed excitatory postsynaptic potentials (EPSP) by a presynaptic effect, without any effect on resting membrane potential.[95] Okada and Ozawa (1980) also showed a hyperpolarizing action of adenosine in guinea pig hippocampal slices.[96]

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

Besides acting on potassium, adenosine via an A_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^- channel blocker.[100]

In the absence of extracellular calcium, adenosine-evoked inhibition of pyramidal neuron excitability appears to be lost, probably through some form of desensitization[101] or resulting from the increased sodium conductances in calcium-free solution.[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.[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.[98] This potassium channel mediates the postsynaptic effects of adenosine and is sensitive to barium and coupled to a pertussis toxin-sensitive GTP binding protein.[80]

B- Cyclic AMP

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

Raising [cAMP]i 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.[98] In rat hippocampal slices, application of $PbCl_2$, 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.[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 inbibition of the adenosine effect by DPCPX, an A_1 receptor antagonist, indicated the involvement of A_1 receptors.[105]

D- Arachidonic acid

The inhibitory actions of adenosine on hippocampus responses were unaffected by a phospholipase inhibitor (p-bromophenacyl bromide), lipoxygenase inhibitor (nordihydroguaiaretic acid=NDGA) and a cyclo-oxygenase inhibitor, indomethacin.[54] The lipoxygenase inhibitor NDGA also failed to antagonize adenosine inhibition of the release of acetylcholine evoked by electrical pulses.[69]

7. Therapeutic aspects of adenosine

In the CNS, the potential therapeutic uses[9,26] of adenosine consist of: anti-convulsant,[106] anti-ischemic,[107-109] anti-Parkinson's disease,[112] antipsychotic, anxiolytic, sedative, analgesic and Alzheimer's disease.[43,113-115] The failure of existing entities and the perceived disadvantages of classical medicinal chemical approaches for adenosine receptor drugs has led a number of laboratories to produce prodrugs, and indirect adenosine agonists such as adenosine uptake blockers and allosteric enhancers.[116]

REFERENCES

1. Drury AN, Szent-Györgyi A: The influence upon the heart of a substance present in heart muscle and other tissues. J Physiol 68: XIV-XV, 1929.
2. Sattin A, Rall TW: The effect of adenosine and adenine nucleotides on the cyclic adenosine 3', 5'- phosphate content of guinea pig cerebral cortex slices. Mol Pharmacol 6:13-23, 1970.
3. Phillis JW, Kostopoulos KG, Limacher JJ: A potent depressant action of adenine derivatives on cerebral cortical neurones. Eur J Pharmacol 30: 125-129, 1975.
4. Kostopoulos GK, Phillis JW: Purinergic depression of neurons in different areas of the rat brain. Exp Neurol 55: 719-729, 1977.
5. Burnstock G: Overview, purinergic mechanisms. Ann NY Acad Sci 603: 1-17, 1990.
6. Van Calker D, Müller M, Hamprecht B: Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. J Neurochem 33: 999-1005, 1979.
7. Cushing DJ, Mustafa SJ: Historical perspectives: adenosine. In: Phillis JW (ed.), Adenosine and Adenine Nucleotides as Regulators of Cellular Function. Oxford: CRC Press, pp. 3-13, 1991.
8. Snyder SH: Adenosine as a neuromodulator. Ann Rev Neurosci 8:103-124, 1985.
9. Stone TW, Simmonds HA: Purines: Basic and Clinical Aspects. London: Kluwer Academic Press, pp. 1-7, 69-89, 1991.
10. Higgins MJ, Hosseinzadeh H, MacGregor DG, Oglivy H, Stone TW: Release and actions of adenosine in the central nervous system. Pharm World Sci 16: 62-68, 1994.
11. Meghji P: Adenosine production and metabolism. In: Stone T, (ed.), Adenosine in the Nervous System. London: Academic Press, pp. 25-42, 1991.
12. Fredholm BB: Analysis of purines. Life Sci 41: 837-840, 1987.
13. During MJ, Spencer DD: Adenosine: a potential mediator of seizure arrest and potential refractoriness. Ann Neurol 32: 618-624, 1992.
14. Fowler JC: Adenosine antagonists alter the synaptic response to *in vitro* ischemia in the rat hippocampus. Brain Res 509: 331-334, 1990.
15. Fowler JC: Glucose deprivation results in a lactate preventable increase in adenosine and depression of synaptic transmission in rat hippocampal slices. J Neurochem 60: 572-576, 1993.
16. Fowler JC: Purine release and inhibition of synaptic transmission during hypoxia and hypoglycemia in rat hippocampal slices. Neurosci Lett 157: 83-86, 1993.
17. Gribkoff VK, Bauman LA: Endogenous adenosine contributes to hypoxic synaptic depression in hippocampus from young rats. J Neurophysiol 68: 620-628, 1992.
18. Pedata F, Pazzagli M, Pepeu G: Endogenous adenosine release from hippocampal slices: excitatory amino acid agonists stimulate release, antagonists reduce the electrically-evoked release. Naunyn-Schmiedberg's Arch Pharmacol 344: 538-543, 1991.
19. Sciotti VM, Park TS, Berne RM, Van Wylen DGL: Change in extracellular adenosine during chemical or electrical brain stimulation. Brain Res 613: 16-20, 1993.
20. MacDonald WF, White TD: Nature of extrasynaptosomal accumulation of endogenous adenosine evoked by K^+ and veratridine. J Neurochem 45: 791-797, 1985.
21. Meghji P: Storage, release, uptake, and inactivation of purines. Drug Dev Res 28: 214-219, 1993.
22. Greene RW, Haas HL: Electrophysiology action of adenosine in rat and human hippocampus. In: Chan-plag V, Koler C, (eds.), The Hippocampus–New Vistas, Neurology and Neurobiology. New York: Alan Liss Inc, pp. 53-69, 1989.
23. Stone TW, Newby AC, Lloyd HGE: Adenosine release. In: Williams M (ed.), Adenosine and Adenosine Receptors. Clifton, New Jersey: Humana Press, pp. 173-223. 1990.
24. Lloyd HGE, Lindström K, Fredholm BB: Intracellular formation and release of adenosine from rat hippocampal slices evoked by electrical stimulation or energy depletion. Neurochem Int 23: 173-185, 1993.
25. Browning RA: Overview of neurotransmission: relationship to the action of antiepileptic drugs. In: Faingold CL, Formm GH (eds.), Drugs in Epilepsy: Action on Neuronal Networks Involved in Seizure Disorders. Oxford: CRS press, pp. 23-56, 1992.
26. Stone TW: Purine receptors and their pharmacological roles. In: Advances in Drug Research. London: Academic Press, pp. 291-429, 1989.
27. Stone TW: Receptors for adenosine and adenine nucleotides. Gen Pharmacol 22: 25-31, 1991.

28. Londos C, Cooper DMF, Wolff J: Subclasses of external adenosine receptors. Proc Natl Acad Sci USA 77: 2551-2554, 1980.
29. Londos C, Wolff J: Two distinct adenosine-sensitive sites on adenylate cyclase. Proc Natl Acad Sci USA 74: 5482-5486, 1977.
30. Dunwiddie TV, Hoffer BJ: Adenine nucleotides and synaptic trasmission in the *in vitro* rat hippocampus. Br J Pharm 69: 59-68, 1980.
31. Stone TW: Purine receptor classification: a discussion point. Trends Pharmacol Sci 5: 492-493, 1985.
32. Bruns RF, Lu GH, Pugsley TA: Characterization of the A_2 adenosine receptor labelled by [^{3}H]NECA in rat striatal membranes. Mol Pharmacol 29: 331-346, 1986.
33. Reithmann C, Gierschik P, Jakobs KH: Stimulation and inhibition of adenylyl cyclase. In: Roberts J, Kirk C, Venis M (eds.). Hormone Perception and Signal Transduction in Animals and Plants. Great Britain: Society for Experimental Biology, pp. 207-224, 1990.
34. Zhou Q-Y, Li C, Olah ME, Johnson RA, Stiles GL, Civelli O: Molecular cloning and characterization of an adenosine receptor: the A_3 adenosine receptor. Proc Natl Acad Sci USA 89: 7432-7436, 1992.
35. Ribeiro JA, Sebastião MA: Adenosine receptors and calcium: basis for proposing a third (A_3) adenosine receptor. Prog Neurobiol 26: 179-209, 1986.
36. Carruthers AM, Fozard JR: Adenosine A_3 receptors: two into one won't go. Trends Pharmacol Sci 14: 290-291, 1993.
37. Fastbom J, Pazos A, Probst A, Palacios JM: Adenosine A_1 receptors in the human brain: a quantitative autoradiographic study. Neurosci 22: 827-839, 1987.
38. Bisserbe JC, Pascal O, Deckert J, Mazière B: Potential use of DPCPX as probe for *in vivo* localization of brain A_1 adenosine receptors. Brain Res 599: 6-12, 1992.
39. Jarvis MF, Williams M: Direct autoradiographic localization of adenosine A_2 receptors in the rat brain using A_2-selective agonist [^{3}H]CGS 21680. Eur J Pharmacol 168: 243-246, 1989.
40. Wan W, Sutherland GR, Geiger JD: Binding of the adenosine A_2 receptor ligand [^{3}H]CGS 21680 to human and rat brain: evidence for multiple affinity sites. J Neurochem 55: 1763-1771, 1990.
41. Williams M, Brauwalder A, Erickson TJ: Evaluation of the binding of the A-1 selective adenosine radioligand, cyclopentyladenosine (CPA), to rat brain tissue. Naunyn-Schmiedberg's Arch Pharmacol 332: 179-183, 1986.
42. Williams M: Purine receptors in mammalian tissues: pharmacology and functional significance. Ann Rev Pharmacol Toxicol 27: 315-345, 1987.
43. Williams M, Cusack NJ: Neuromodulatory roles of purine nucleosides and nucleotides: their receptors, adenosine ligands. Neurotransmissions 6: 1-6, 1990.
44. Williams M: Adenosine receptor agonists and antagonists. In: Stone T (ed.), Adenosine in the Nervous System. London: Academic Press, pp. 137-171, 1991.
45. Carruthers AM, Fozard JR: Effect of pertussis toxin treatment on the putative adenosine A_3 receptor-mediated hypotensive response in the rat. Eur J Pharmacol 250: 185-188, 1993.
46. Carruthers AM, Fozard JR: Hypotensive responses to the putative adenosine A_3 receptor agonist N^6-2-(4-aminophenyl)-ethyladenosine in the rat. Drug Dev Res 30: 147-152, 1993.
47. Fozard JR, Carruthers AM: Adenosine A_3 receptors mediate hypotension in the angiotensin II-supported circulation of the pithed rat. Br J Pharmacol 109: 3-5, 1993.
48. Collis MG, Hourani SMO: Adenosine receptor subtypes. Trends Pharmacol Sci 14: 360-366, 1993.
49. Daly JW, Bruns RF, Snyder SH: Adenosine receptors in the central nervous system: relationship to the central actions of methylxanthines. Life Sci 28: 2083-2097, 1981.
50. Fredholm BB, Persson CGA: Xanthine derivatives as adenosine receptor antagonists. Eur J Pharmacol 81: 673-676, 1982.
51. Bruns RF, Fergus JH, Badger EW, Bristol JA, Santay LA, Hartman JD, Hays SJ, Huang CC: Binding of the A_1-selective adenosine antagonist 8-cyclopentyl-1, 3-dipropylxanthine to rat brain membranes. Naunyn-Schmiedberg's Arch Pharmacol 335: 59-63, 1987.
52. Jacobson KA, Nikodijevic O, Padgett WL, Gallo-rodriguez C, Maillard M, Daly JW: 8-(3-chlorostyryl) caffeine (CSC) is a selective A_2-adenosine antagonist *in vitro* and *in vivo*. FEBS 323: 141-144, 1993.
53. Fozard JR, Hannon JP: BW-A522 blocks adenosine A_3 receptor-mediated hypotensive responses in the rat. Eur J Pharmacol 252: R5-R6, 1994.
54. Dunwiddie TV, Taylor M, Lupica CR, Cass WA, Allniser NR, Fredholm BB: Adenosine modulation of excitatory transmission in the rat hippocampus. In: Imai S, Nakazawa M, (eds.), Role of Adenosine and Adenine Nucleotides in the Biological System. Amsterdam: Elsevier, pp. 631-643, 1991.
55. Lee K, Schubert P: Modulation of an inhibitory circuit by adenosine and AMP in the hippocampus. Brain Res 246: 311-314, 1982.
56. Schubert P, Mitzdorf U: Analysis and quantitative evaluation of the depressive effect of adenosine on evoked potentials in hippocampal slices. Brain Res 172: 186-190, 1979.
57. Collins GG, Anson J: Adenosine A_1 receptors mediate the inhibitory effects of exogenous adenosine in the rat olfactory cortex slice. Neuropharmacol 24: 1077-1084, 1985.
58. Motley SJ, Collins GG: Endogenous adenosine inhibits excitatory transmission in the rat olfactory cortex slice. Neuropharmacol 22: 1081-1086, 1983.
59. Scholfield CN: Depression of evoked potentials in brain slices by adenosine compounds. Br J Pharmac 63: 239-244, 1978.
60. Alzheimer C, Kargl L, Bruggencate GT: Adenosinergic inhibition in hippocampus is mediated by adenosine A_1 receptors very similar to those of peripheral tissues. Eur J Pharmacol 196: 313-317, 1991.
61. Dunwiddie TV, Basile AS, Palmer MR: Electrophysiological responses to adenosine analogues in rat hippocampus and cerebellum: evidence for mediation by adenosine receptors of the A_1 subtype. Life Sci 34: 37-47, 1984.
62. Sebastião AM, Stone TW, Ribeiro JA: The inhibitory adenosine receptor at the neuromuscular junction and hippocampus of the rat: antagonism by 1,3,8-substituted xanthines. Br J Pharmacol 101: 453-459, 1990.
63. Ameri A, Jurna I: Adenosine A_1 and non-A_1 receptors: intracellular analysis of the action of adenosine agonists and antagonists in rat hippocampal neurones. Brain Res 546: 69-78, 1991.
64. Sebastião AM, Ribeiro JA: Evidence for the presence of excitatory A_2 adenosine receptors in the rat hippocampus. Neurosci Lett 138: 41-44, 1992.

65. Janusz CA, Bruns RF, Berman RF: Functional activity of the adenosine binding enhancer, PD81,723, in the *in vitro* hippocampal slice. Brain Res 567: 181-187, 1991.
66. Janusz CA, Berman RF: Adenosine binding enhancer, PD81,723, inhibits epileptiform bursting in the hippocampal brain slice. Brain Res 619: 131-136, 1993.
67. Fastbom J, Fredholm BB: Inhibition of [^{3}H]glutamate release from rat hippocampal slices by L-phenylisopropyladenosine. Acta Physiol Scand 125: 121-123, 1985.
68. Burke SP, Nadler JV: Regulation of glutamate and aspartate release from slices of the hippocampal CA1 area: effects of adenosine and baclofen. J Neurochem 51: 1541-1551, 1988.
69. Fredholm BB: Differential sensitivity to blockade by 4-aminopyridine of presynaptic receptors regulating [^{3}H]acetylcholinerelease from rat hippocampus. J Neurochem 54: 1386-1390, 1990.
70. Jackish R, Strittmatter H, Kasakov L, Hertting G: Endogenous adenosine as amodulator of hippocampal acetylcholine release. Naunyn-Schmiedberg's Arch Pharmacol327: 319-325, 1984.
71. Kirkpatrick KA, Richardson PJ: Adenosine receptor-mediated modulation of acetylcholine release from rat striatal synaptosomes. Br J Pharmacol 110: 949-954, 1993.
72. AllgaierC, Hertting G, Kügelgen OV: Theadenosine receptor-mediated inhibition of noradrenaline release possibly involves a N-Protein and is increased by α_2- autoreceptor blockade. Br J Pharmac 90: 403-412, 1987.
73. Jonzon B, Fredholm BB: Adenosine receptor-mediated inhibition of noradrenaline release from slices of the rat hippocampus. Life Sci 35: 1971-1979, 1984.
74. Jin S, Johansson B, Fredholm BB: Effects of adenosine A_1 and A_2 receptor activation on electrically evoked dopamine and acetylcholine release from rat striatal slices. J Pharmacol Exp Ther 267: 801-808, 1993.
75. Michaelis ML, Michaelis EK, Myers SL: Adenosinemodulation of synaptosomal dopamine release. Life Sci 24: 2083-2092, 1979.
76. Hollins C, Stone TW: Adenosine inhibition of γ-aminobutyric acid release fom slices of rat cerebral cortex. Br J Pharmacol 69: 107-112, 1980.
77. Feuerstein TJ, Bär KI, Lücking CH: Activationof A_1 adenosine receptors decreases the release of serotonin in the rabbit hippocampus, but not in the caudate nucleus. Naunyn-Schmiedberg's Arch Pharmacol 338: 664-670, 1988.
78. Nagano O, FöldesFF, NakatsukaH, ReichD, Ohta Y, Sperlagh B, Vizi ES: Presynaptic A_1-purinoceptor-mediated inhibitory effects of adenosine and its stable analogues on the mouse hemidiaphragm preparation. Naunyn-Schmiedberg's Arch Pharmacol 346:197-202, 1992.
79. Lambert NA, Teyler TJ: Adenosine depresses excitatory but not fast inhibitory synaptic transmission in area CA1 of the rat hippocampus. Neurosci Lett 122: 50-52, 1991.
80. Thompson SM, Haas HL, Gähwiler BH: Comparison of the actions of adenosine at pre- and postsynaptic receptors in the rat hippocampus *in vitro*. J Physiol 451: 347-363, 1992.
81. Yoon K-W, Rothman SM: Adenosine inhibits excitatory but not inhibitory synaptic transmission in the hippocampus. J Neurosci 11: 1375-1380, 1991.
82. Sihra TS, Nichols RA: Mechanisms in the regulation of release from brain nerve terminals: current hypotheses. Neurochem Res 18: 47-58, 1993.
83. Zucker RS: Calcium and transmitter release. J Physiol (Paris) 87: 25-36, 1993.
84. Ribeiro JA, Sá-Almeida AM, Namorado JM: Adenosine and adenosine triphosphate decrease ^{45}Ca uptake by synaptosomes stimulated by potassium. Bio Pharmacol 28: 1297-1300, 1979.
85. Wu PH, Phillis JW, Thierry DL: Adenosine receptor agonists inhibit K^+-evoked calcium uptake by rat brain cortical synaptosomes. J Neurochem 39: 700-708, 1982.
86. Barr E, Daniell LC, Lesile SW: Synaptosomal calcium uptake unaltered by adenosine and 2-chloroadenosine. Biochem Pharmacol 34: 713-715, 1985.
87. GarritsenA, Ijzerman AP, Soudijn W: Adenosine-A_1 receptors are not coupled to Ca^{2+} uptake in rat brain synaptosomes. Biochem Pharmacol 38: 693-695, 1989.
88. MichaelisML, Johe KK, Moghadam B, Adams RN: Studies on the ionic mechanism for the neuromodulatory actions of adenosine in the brain. Brain Res 473: 249-260, 1988.
89. Dolphin AC, Forda SR, Scott RH: Calcium-dependent currents in cultured rat dorsal root ganglion neurones are inhibited by an adenosine analogue. J Physiol 373: 47-61, 1986.
90. Scholz KP, Miller RJ: Analysis of adenosine actions on Ca^{2+} currents and synaptic transmission in culturedrat hippocampal pyramidal neurones. J Physiol 435: 373-393, 1991.
91. Mogul DJ, Adams ME, Fox AP: Differential activation of adenosine receptors decreases N-type but potentiates P-type Ca^{2+} current in hippocampal CA3 neurons. Neuron 10: 324-327, 1993.
92. Schubert P, Heinemann U, Kolb R: Differential effects of adenosine on pre- and postsynaptic calcium fluxes. Brain Res 376: 382-386, 1986.
93. Schubert P: Physiological modulation by adenosine: selective blockade of A_1-receptors with DPCPX enhances stimulus train-evoked neuronal calcium influx in rathippocampal slices. Brain Res 458: 162-165, 1988.
94. Silinsky EM: Inhibition of transmitterrelease by adenosine: are Ca^{2+} currents depressed or are the intracellular effects of Ca^{2+} impaired? Trends Pharmacol Sci 7: 180-185, 1986.
95. Segal M: Intracellular analysis of a postsynaptic action of adenosine in the rat hippocampus. Eur J Pharmacol 79: 193-199, 1982.
96. Okada Y, OzawaS: Inhibitory action of adenosine on synaptic transmission in the hippocampus of the guinea pig *in vitro*. Eur J Pharmacol 68: 483-492, 1980.
97. Trussell LO, Jackoson MB: Adenosine-activated potassium conductance in cultured striatal neurons. Proc Natl Acad Sci USA 82: 4857-4861, 1985.
98. Trussell LO, Jackoson MB: Dependence of an adenosine-activated potassium current on a GTP-binding protein in mammalian central neurons. J Neurosci 7: 3306-3316, 1987.
99. Li H, Henry JL: Adenosine-induced hyperpolarization is depressed by glibenclamide in rat CA1 neurones. NeuroReport 3: 1113-1116, 1992.
100. Mager R, Ferroni S, Schybert P: Adenosine modulates a voltage-dependent chloride conductance in cultured hippocampal neurons. Brain Res 532: 58-62, 1990.
101. Hosseinzadeh H, Stone TW: The effectof calcium removal on the suppression by adenosine of epileptiform activity in the hippocampus: demonstration of desensitization. Br J Pharmacol 112: 316-322, 1994.
102. Hosseinzadeh H, Stone TW: Mechanism of the hippocampal

loss of adenosine sensitivity in calcium-free media. Brain Res 569: 221-225, 1994.
103. Dunlap K, Holz GG, Stanley GR: G-proteins as regulators of ion channel function. Trends Neurosci 10: 241-244, 1987.
104. Dunwiddie TV, Fredholm BB: Adenosine modulation of synaptic responses in rat hippocampus: possible role of inhibition or activation of adenylate cyclase. In: Cooper DMF, Seamon KB, (eds.), Advances in Cyclic Nucleotide and Protein Phosphorylation Reseach. New York: Raven Press, pp. 259-272, 1985.
105. Paoletti AM, Balduini W, Cattabeni F, Abbracchio MP: Adenosine modulation of histamine-stimulated phosphoinositides metabolism in rat cortical slices. Neurosci Res Communications 11: 19-26, 1992.
106. Dragunow M: Purinergic mechanisms in epilepsy. Prog Neurobiol 31: 85-108, 1988.
107. Kolvenbach R, Figge C, Godehardt E, Sandmann W: Adenosine agonists and global cerebral ischaemia. Br J Clin Pharmac 36: 134-135, 1993.
108. MoriM, Nishizaki T, Okada Y: Protective effect of adenosine on the anoxic damage of hippocampal slices. Neurosci 46: 301-307, 1992.
109. Rudolphi KA, Schubert P, Parkinson FE, Fredholm BB: Neuroprotective role of adenosine in cerebral ischaemia. Trends Pharmacol Sci 13: 439-445, 1992.
110. MacGregor DG, Stone TW: Inhibition by the adenosine analogue (R-)-N^6-phenylisopropyladenosine of kainic acid neurotoxicity in rat hippocampus after systemic administration. Br J Pharmacol 109: 316-321, 1993.
11-. MacGregor DG, Stone TW: Mediation of the neuroprotective action of R-phenylisopropyladenosine through a centrally located adenosine A_1 receptor. Br J Pharmacol 110: 470-476, 1993.
112. Lau Y-S, Mouradian MM: Protection against acute MPTP-induced dopamine depletion in mice by adenosine A_1 agonist. J Neurochem 60: 768-771, 1993.
113. Daval J-L, Nehlin A, Nicolas F: Physiological and pharmacological properties of adenosine: therapeutic implications. Life Sci 49: 1435-1453, 1991.
114. Williams M: Purine nucleosides and nucleotides as central nervous system modulators. Ann NY Acad Sci USA 603: 93-107, 1990.
115. Williams M: Purinergic drugs: opportunities in the 1990s. Drug Dev Res 28: 438-444,1993.
116. Jacobson KA, TrivediBK, Churchill PC, Williams M: Novel therapeutics acting via purine receptors. Biochem Pharmacol 41: 1399-1410, 1991.