

Adenosine and Its Receptors in Neurology

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Abbreviations:

ADAC :	Adenosine amine congener
AMP :	Adenosine monophosphate
AR :	Adenosine receptor
BDNF :	Brain-derived neurotrophic factor
BIIP20 :	<i>S</i> -(<i>-</i>)-8-(3-Oxocyclopentyl)-1,3-dipropyl-7 <i>H</i> -purine-2,6-dione
cAMP :	Cyclic adenosine monophosphate
CCPA :	2-Chloro- <i>N</i> 6-cyclopentyladenosine
CGS15943 :	5-Amino-9-chloro-2-(2-furyl)-1,2,4-triazolo[1,5- <i>c</i>]quinazoline
CGS21680 :	2-[4-(2-Carboxyethyl)-phenylethylamino]-5- <i>N</i> -ethyl-carboxamido-adenosine
CHA :	<i>N</i> 6-Cyclohexyladenosine
CJD :	Creutzfeldt–Jakob disease
Cl-IB-MECA :	2-Chloro- <i>N</i> 6-(3-iodobenzyl)adenosine-5- <i>N</i> -methyluronamide
CNS :	Central nervous system
CP66,713 :	4-Amino-1-phenyl[1,2,4]-triazolo[4,3- <i>a</i>]quinoxaline
CPA :	Cyclopentyl adenosine
8-CPT :	8-Cyclopentyltheophylline
CREB :	Cyclic AMP responsive element binding protein
CSC :	8-(3-Chloro styryl)caffeine
DMPX :	3,7-Dimethyl-1-propargylxanthine
DPCPX :	8-Cyclopentyl-1,3-dipropylxanthine
EAE :	Allergic encephalomyelitis
ERK1/2 :	Extracellular signal-regulated kinases 1 and 2
GABA :	Gamma-aminobutyric acid
HD :	Huntington's disease
HGPRT :	Hypoxanthine-guanine phosphoribosyltransferase
IB-MECA :	<i>N</i> 6-(3-Iodobenzyl)adenosine-5- <i>N</i> -methyluronamide
IL :	Interleukin
KFM19 :	<i>RS</i> -(<i>-</i>)-8-(3-oxocyclopentyl)-1,3-dipropyl-7 <i>H</i> -purine-2,6-dione
LNS :	Lesch–Nyhan syndrome
MAP-2 :	Microtubule-associated protein 2
MAPK :	Mitogen-activated protein kinases
MCAo :	Middle cerebral artery occlusion
MPTP :	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRS2179 :	<i>N</i> 6-Methyl-2_-deoxyadenosine-3_5_bisphosphate

MRS1706 : *N*-(4-Acetylphenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-phenoxy]acetamide

MS : Multiple sclerosis

NBTI : Nitrobenzylthioinosine

NECA : 5-*N*-Ethylcarboxamidoadenosine

NGF : Nerve growth factor

NMDA: *N*-Methyl-D-aspartate

3-NP : 3-Nitro-propionic acid.

PKC: Protein kinase C

PLC: Phospholipase C

R-PIA : *R*-Phenylisopropyladenosine

SAH : *S*-Adenosylhomocysteine

SCH58261 : 7-(2-Phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine

TNF- α : Tumor necrosis factor alpha

Trk : Tropomyosin-related kinase

ZM241385 : 4-(2-[7-Amino-2-(2-furyl)(1,2,4)-triazolo(2,3-*a*)-(1,3,5)triazin-5-yl-amino]ethyl)phenol

Introduction

Adenosine is a chemical messenger that modulates cellular activity in both the CNS and peripheral organs. There are several excellent reviews on recent experimental findings and insights into adenosine signaling in the CNS, and on adenosine as a potential therapeutic target in neurologic disorders) **(King AE et al., 2006)**.

Adenosine levels in the brain extracellular space increase dramatically during metabolically stressful conditions, such as ischemia, seizures, or trauma **(warscolat et al., 2007)**.

Adenosine, acting via different receptors, modulates excitability in the CNS, and has a role in mechanisms of seizure susceptibility, sleep induction, basal ganglia function, pain perception, cerebral blood flow, and respiration **(Trevor W. Stone et al. 2009)**.

Adenosine exerts widespread modulatory effects in the nervous system via four types of guanine nucleotide binding (G) protein-coupled receptors A_1 , A_{2A} , A_{2B} , and A_3 that trigger several signal transduction pathways. The high affinity A_1 and A_{2A} receptors are the most abundant adenosine receptors in the nervous system and the most relevant in physiologic conditions **(Cassada et al. 2002)**.

Activation of presynaptic A_1 receptors results in inhibition of release of glutamate, acetylcholine, and other neurotransmitters whereas activation of postsynaptic A_1 receptors decreases neuronal excitability and inhibits NMDA receptor mediated responses **(Varani et al. 2007)**.

Adenosine receptors are inhibited by caffeine and other methylxanthines and provide a potential target for treatment of cerebral ischemia, seizures, pain, Parkinson disease (PD), and Huntington disease **(Coelho et al. 2006)**.

The effects of adenosine offer unique therapeutic opportunities and challenges. For example, A1 receptor agonists may have therapeutic use in acute ischemic or traumatic injury, epilepsy, pain, insomnia, and neurodegenerative diseases (**Popoli et al. 2007**).

However, current A1 receptor agonists do not readily cross the blood–brain barrier and their use may be limited by their systemic effects, including cardiac depression, hypothermia, and glucose intolerance (**Chen et al. 2006**).

Whereas A2A receptor antagonists may have potential neuroprotective action in neurodegenerative disorders such as Huntington disease. (**Rodriguez et al.,2006**).

Adenosine kinase inhibitors have also been shown to exert antiseizure and neuroprotective effect in experimental models, and may potentially be useful for clinical use (**Basheer, R. et al;2004**).

Development of drugs or delivery systems that selectively affect specific components of adenosine signaling in the CNS will hopefully provide new therapeutic opportunities for a large variety of neurologic disorders (**Trevor W. Stone et al. 2009**).

Aim of the work

The aim of this work is to study the functions of adenosine and its receptors in nervous system and the possibility of using in different neurological disorders.

Chapter 1:

Adenosine metabolism and adenosine receptors.

Adenosine is a nucleoside composed of a molecule of adenine attached to a ribose sugar molecule (ribofuranose) bound via a β -N9 glycosidic bond (**Magger et al, 1990**). It plays an important role in biochemical process, such as energy transfer as adenosine tri phosphate(ATP), and adenosine diphosphate (ADP) as well as signal transduction as cyclic adenosine mono phosphate CAMP (**Revkees et al, 1995**).

Adenosine is a constitutive metabolite of all cells, involved in key pathways such as purinergic nucleic acid base synthesis, amino acid metabolism and modulation of cellular metabolic status (**Stone,1985**). Considering this homeostatic role of adenosine related to the control of cellular metabolism (**McIlwain, 1979**), adenosine has been termed as ‘local hormone’ (**Arch and New sholme, 1978**).

1.1 Adenosine as a ubiquitous homeostatic substance:

Today it is still a matter of conjecture why the main fuel ATP, the essential constituent of all living cells that allows them to work, and its metabolite adenosine are directly involved in nerve cell communication. Adenosine is ‘omnipresent’, i.e. exists in all cells, and is released from apparently all cells, including neurons and glia. Adenosine is indeed consensually recognized as a very important substance in the homeostasis of the cells of the nervous system, elegantly named by **Newby (1981)** in the early eighties a ‘retaliatory metabolite’, or according to others ‘a signal of life’ (**Engler, 1991**). However, adenosine is involved in cell death namely in the prevention or induction of apoptosis (**DiIorio et al., 2002**). While ATP may function as a neurotransmitter in some brain areas

(Edwards et al., 1992; Nieber et al., 1997; Pankratov et al., 1998; Mori et al., 2002).

Adenosine is neither stored nor released as a classical neurotransmitter since it does not accumulate in synaptic vesicles, being released from the cytoplasm into the extracellular space through a nucleoside transporter. The adenosine transporters also mediate adenosine reuptake, the direction of the transport being dependant upon the concentration gradient at both sides of the membrane **(Gu et al., 1995)**.

Since it is not exocytotically released, adenosine behaves as an extracellular signal molecule influencing synaptic transmission without itself being a neurotransmitter, i.e. modulates the activity of the nervous system at cellular level presynaptically by inhibiting or facilitating transmitter release, postsynaptically by hyperpolarising or depolarising neurones and/or exerting non-synaptic effects (e.g. on glial cells) **(Jacobson KA et al.2006))**.

Adenosine, therefore, belongs to the group of neuromodulators. In the same synapse, adenosine can exert both pre- and postsynaptic inhibitory actions, which are mediated by similar receptors (A1); interestingly, there is a differential desensitization of presynaptic A1 responses from those mediated postsynaptically. Presynaptic A1 receptors desensitize much more slowly than postsynaptic A1 receptors, a difference that could not be explained only on the basis of differences in receptor reserve and that might suggest differences in the mechanisms that regulate receptor functioning **(Wetherington and Lambert, 2002)**.

Besides its direct neuromodulatory effects, adenosine also has more indirect actions on the nervous system, demonstrated by studies on adenosine receptor–receptor interactions. Because adenosine acts in a subtle fashion to participate in these interactions, it was proposed as a fine-tuner, considering that in this way, adenosine is a partner of a very sophisticated interplay between its own

receptors and with receptors for other neurotransmitters and/or neuromodulators (**Sebastião and Ribeiro, 2000**).

1.2 ADENOSINE SIGNALING IN THE NERVOUS SYSTEM.

There is tight and dynamic regulation of adenosine levels in the nervous system. All cell types contribute to the accumulation of extracellular adenosine. Extracellular concentrations of adenosine are determined by the interplay between the activity of intracellular and extracellular enzymes involved in adenosine metabolism and the transport of adenosine across the cell membrane. Metabolic stress triggers the dephosphorylation of adenosine triphosphate (ATP) and formation of adenosine monophosphate (AMP), which is converted to adenosine by 5-nucleotidase. Adenosine is then released from cells by facilitated diffusion via nucleoside transporters, the best characterized being the equilibrative nucleoside transporters (ENT)(**Fig. 1**)(**King AE et al., 2006**).

Adenosine may also be produced by the extracellular metabolism of ATP via a cascade of ectonucleases (**Dunwiddie TV et al.2001; Ribeiro JA et al., 2002**). The primary route of adenosine metabolism is adenosine kinase (ADK), which is expressed primarily in astrocytes. The concentration of extracellular adenosine increases dramatically in the setting of metabolic stress such as hypoxia, ischemia, trauma, or seizures. (**Dunwiddie TV et al., 2001; Ribeiro JA et al., 2002; Ribeiro JA. 2005; Jacobson KA et al., 2006; Bosion D 2006**).

Transport from the astrocyte is the primary source of adenosine in the setting of ischemia or hypoxia; this correlates with increased breakdown of intracellular ATP and down regulation of ADK in the astrocyte (**Bosion D 2006**).

In contrast, extracellular conversion of synaptically released ATP is the main source of adenosine in the setting of seizures. Adenosine exerts widespread

release of adenosine as such is the main source of extracellular adenosine in metabolically stressed cells or tissues. It has also been concluded that the release of adenosine through adenosine transporters also occurs at the synaptic level (**White and MacDonald, 1990; Cunha and Sebastião, 1993; Mitchell et al., 1993; Dunwiddie and Diao, 1994; Brundage and Dunwiddie, 1996; Cunha et al., 1996**). The intracellular concentration of adenosine is estimated to be nearly 50 nM, whereas the extracellular synaptic concentration of adenosine may reach 4 mM (**Dunwiddie and Diao, 1994; Cunha, 1997**).

Thus, it is difficult to understand how the cell manages to release adenosine against its concentration gradient through non-concentrative transporters. Also, it is important to keep in mind that the synapse is a very restricted region of nerve terminals. But, the possibility exists that, even in a synaptosomal preparation, the release of adenosine as such might result from a mild stressful response of the preparation, which may mask the formation of adenosine from released adenine nucleotides (**Cunha et al., 1996**).

This source of adenosine resulting from the catabolism by ectonucleotidases of exocytotic-released ATP (**Richardson and Brown, 1987; Richardson et al., 1987; Terrian et al., 1989; White and MacDonald, 1990;**), appears to be the main source of adenosine at the synaptic level. the ecto-nucleotidase pathway is constituted by an ATPase and/or an ATP diphosphohydrolase activity, which converts ATP into AMP, and by an ecto-5'-nucleotidase activity, which then converts AMP into adenosine(**Cunha, 1997**).

1.4 Metabolic pathways of adenosine formation upon metabolic imbalance.

Adenosine is particularly well suited to be used as a trans-cellular messenger to signal metabolic imbalance. Although changes in ATP should be the initial

sensor of metabolic imbalance, ATP is hardly ever used as a control parameter. This is because the intracellular ATP concentration is so tightly controlled, that it is only allowed to change upon profound metabolic imbalance (**Doolette, 1997; Schwendel et al., 1997; Stumpe and Schrader, 1997**).

Long before marked changes in ATP concentrations occur, cells need to generate signals indicating a cellular stressful condition. Cells take advantage of the near equilibrium of adenylate kinase reaction to convert minor changes in ATP concentration into several fold changes in AMP concentrations (**Veechet al., 1979; Fell and Sauro, 1985**).

This results from the fact that the intracellular concentration of ATP is in the milimolar range, whereas the intracellular concentration of AMP is in the low nano molar range. Thus, it is AMP that play the role of control signal to adapt primary metabolism to metabolic imbalance (**Fell and Sauro, 1985**).

But it is also advantageous to rapidly signal stressful situation to neighboring cells, and AMP is not well suited for this purpose, since it cannot cross cell membranes. The existence of a substrate cycle between AMP and adenosine, with the opposite activities of 5'-nucleotidase and adenosine kinase, introduces a further implication checkpoint to regulate the formation of intracellular adenosine upon changes in the concentration of intracellular AMP (**Kroll et al., 1993; Decking et al., 1997**).

The existence of non-concentrative bi-directional adenosine (or nucleoside) transporters allows to equilibrate changes in the intracellular and extracellular adenosine concentrations (**Plage-mann and Wohlhueter, 1984**). As a whole, this enzymatic pathway allows to convert minor changes in intracellular ATP concentration into disproportional larger changes in the extracellular concentration of adenosine. Although very little is known about the metabolic control of this pathway, several reports have documented an increase in the extracellular

concentration of adenosine upon stressful metabolic challenges (**Doolette, 1997; Stumpe and Schrader, 1997; Zhu and Krnjevic, 1997**).

This provides a metabolic basis for the role of adenosine as a refraining signal of intracellular metabolism in cellular stressful situations and the term ‘retaliatory metabolite’ has been coined for this homeostatic role of adenosine, which occur in virtually all cell types (**Newby, 1984**).

1.5 Adenosine receptors.

Adenosine receptors are essentially ubiquitous, with almost all cell types expressing functional forms of at least one of the four known subtypes (A1, A2A, A2B, A3), *distributed all over the nervous system*, (**Fig 2**). Each of these subtypes has been associated with a range of actions, some of which may become over- or under expressed, over- or underactive. Such a change in activity could lead to the evidence for a possible contribution of adenosine receptors to the processes abnormalities of tissue function, which may be severe enough to lead to overt disease (**Macdonald, 1995**).

Relevant General Features of Adenosine Receptor Actions.

1.5.1 A1 Adenosine Receptors

A1 adenosine receptors occur throughout the central nervous system (CNS), with a high density in the hippocampus and neocortex (**Fig. 2**). The widespread distribution of these receptors is seen in almost all mammalian species examined, including humans (**Fastbom et al. 1986, 1987**). All cell types in the CNS possess these receptors, including both neurons and microglia (**Goodman and Snyder 1982; Lee and Reddington 1986; Rivkees et al. 1995; Fiebich et al. 1996; Svenningsson et al. 1997; Ochiishi et al. 1999**), with neuronal receptors existing