Adenosine A_{2A} receptors: localization and function

Nicola Simola and Jadwiga Wardas

Abstract

Adenosine is an endogenous purine nucleoside present in all mammalian tissues, that originates from the breakdown of ATP. By binding to its four receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3), adenosine regulates several important physiological functions at both the central and peripheral levels. Therefore, ligands for the different adenosine receptors are increasingly attracting attention as new potential drugs to be used in the treatment of several diseases.

This chapter is aimed at providing an overview of adenosine metabolism, adenosine receptors localization, and their signal transduction pathways. Particular attention will be paid to the biochemistry and pharmacology of A_{2A} receptors, since antagonists of these receptors have emerged as promising new drugs for the treatment of Parkinson's disease. The interactions of A_{2A} receptors with other non-adenosinergic receptors, and the effects of the pharmacological manipulation of A_{2A} receptors on different body organs will be discussed, together with the usefulness of A_{2A} receptor antagonists for the treatment of Parkinson's disease and the potential adverse effects of these drugs.

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1. Introduction

The concept of purinergic neurotransmission was first introduced by Burnstock in 1972 and subsequently adenosine 5' triphosphate (ATP) was shown to act either as a transmitter or a co-transmitter in most nerves in both the peripheral and central nervous system (CNS) (Abbracchio and Burnstock, 1998, Abbracchio et al., 2008, Burnstock, 1972, 2013). At present, it is known that ATP acts as a fast excitatory neurotransmitter or neuromodulator, and has potent long-term trophic role in cell proliferation, growth, and development, as well as in disease and cytotoxicity (Abbracchio and Burnstock 1998, Abbracchio et al., 2008, Burnstock, 2013).

ATP and other nucleotides are stored in secretory and synaptic vesicles, and exocytotic vesicular release of ATP from neurons and astrocytes is well established (Pankratov et al., 2006, 2007, Bowser and Khakh, 2007, Abbracchio et al., 2008, Burnstock, 2013). Evidences also exist indicating additional mechanisms of nucleotide release, including ATP-cassette transporters, connexin or pannexin hemichannels, plasmalemmal voltage-dependent anion channels and the ATP-sensitive P2X7 receptors (Abbracchio et al., 2008, Burnstock, 2013). After release, ATP and other nucleotides undergo rapid enzymatic degradation to adenosine by ectonucleotidases (Bonan, 2012; Kovacs et al., 2013; Yegutkin, 2008; Zimmermann, 2006).

2. Adenosine metabolism

Adenosine**,** an endogenous purine ribonucleoside present in all mammalian tissues, modulates a variety of important synaptic processes and signaling pathways, and regulates the functions of several neurotransmitters in the CNS. Adenosine is considered a neuromodulator rather than a neurotransmitter, since it is not stored in synaptic vesicles, and is not released from nerve terminals during exocytosis. . Adenosine affects neural activity through multiple mechanisms; presynaptically by controlling neurotransmitter release, postsynaptically by hyperpolaryzing or depolarizing neurons, and non-synaptically mainly via regulatory effects on glial cells (Boison et al., 2010; Dare et al., 2007; Fredholm et al., 2005). Although adenosine is generally known to be produced by the ectoenzymatic breakdown of ATP, there might be a subpopulation of neurons and/or astrocytes that release adenosine directly in an activity-dependent manner (Wall and Dale, 2007).

It is well established that adenosine may be formed in the CNS either intracelullarly after degradation of ATP to cyclic-adenosine monophosphate (cAMP) and 5'-AMP, and then transported by nucleotide transporters to the synapse, or extracellularly from nucleotides released into the synapse (Fig. 1). Thus, the formation of adenosine is dependent on the availability of oxygen and

energetic compounds, as well as on the rate of synthesis and degradation of ATP, released from both neuronal and glial cells. However, the major source of synaptic adenosine is the release of ATP from astrocytes, either vesicular (Pasqual et al., 2005) or via secretion through hemichannels (Kang et al., 2008; Kawamura et al., 2010). Moreover, adenosine can be directly released through nucleoside transporters from astrocytes due to augmentation of intracellular adenosine in response to a variety of physiological and pathological stimuli (e.g. increased cellular activity, hypoxia/hypoglycemia, ischemia), and may function as nonsynaptic signaling molecule that diffuses far away from the site of origin and tonically influences neurotransmission, inflammation, and immune responses, as described below (Bours et al., 2006; Dare et al., 2007; Geiger and Fyda, 1991; Sperlagh and Vizi, 2011).

2.1 Intracellular formation of adenosine

The process of adenosine monophosphate (AMP) hydrolysis involves 5'-nucleotidase, which belongs to the family of enzymes called ectonucleotidases (Fig. 1, Kovacs et al., 2013; Yegutkin, 2008). Seven types of 5'-nucleotidases have been cloned, characterized, and demonstrated in various tissues including brain tissue (Kovacs et al, 2013; Hunsucker et al., 2005). This pathway of adenosine formation from the catabolism of cytosolic ATP seems to represent a very sensitive signal of increased metabolic rate or metabolic stress (Latini and Pedata, 2001).

Another intracellular source of adenosine may be the hydrolysis of S-adenosylhomocysteine (SAH) by SAH hydrolase (Fig.1), an enzyme present in brain areas such as the neocortex, hippocampus, and cerebellum (Latini and Pedata 2001). However, this pathway is not strictly dependent upon the energetic state of the cells, and it does not significantly contribute to adenosine production in the brain under either physiological or ischemic conditions (Latini and Pedata 2001).

2.2 Extracellular adenosine formation

The extracellular nucleotide and nucleoside levels in the synaptic cleft are controlled by a cascade of enzymes, belonging to the family of ectonucleotidases. There are four major families of ectonucleotidases, namely ectonucleoside triphosphate diphospohydrolases (E-NTPDases), ectonucleotide pyrophosphatase/phosphodiesterases (E-NPPs), alkaline phosphatases, and ecto-5'-nucleotidase (ecto-5'-NT) (Bonan 2012, Kovacs et al., 2013, Yegutkin, 2008; Zimmerman, 2006).

The first step of ATP inactivation is mediated by the family of E-NTPDases, which are able to hydrolyse ATP and adenosine diphosphate (ADP) to AMP (Zimmermann, 2006). Moreover, ATP can be dephosphorylated by E-NPPs and alkaline phosphatases which, similarly to E-

NTPDases, have widespread distribution in the CNS (Wang and Guidotti, 1998; Zimmermann, 2006). The next step of extracellular inactivation is the hydrolysis of AMP to adenosine and phosphate by the ecto-5'-NT, also known as CD73 (Fig.1), which is attached via a GPI anchor to the extracellular membrane. Ecto-5'-NT, which is the rate-limiting step in the formation of adenosine (Sperlagh, 1996, Sperlagh and Vizi, 2007), is also widely expressed in the brain (e.g. in hippocampal and striatal nerve terminals), and it is predominantly associated to glial cells (Cunha et al., 1992; Hunsucker et al., 2005; James and Richardson, 1993; Kovacs et al., 2013; Schoen et al., 1987).

Another way of extracellular adenosine formation may be from the cAMP or 5'-AMP released into the synapse. Both these nucleotides are responsible for the slow change in the adenosine concentration; cAMP can be released through non-specific energy-dependent transporters and then, when in the synapse, it can first be converted to 5'-AMP by ecto-phosphodiesterases and then to adenosine by ecto-5'-NT. Another possibility also exists that the cAMP can be converted to 5'-AMP inside the cell and then 5'-AMP can be released into the synapse, becoming a source of adenosine (e.g. after the NMDA stimulation in cortical sections) (Latini and Pedata, 2001; Sperlagh and Vizi, 2011).

The process of extracellular adenosine formation is very fast, and occurs within seconds (Dunwiddie et al., 1997). Adenosine is normally present in a concentration between 30-300 nM, but under hypoxia or ischemia conditions adenosine concentrations in hippocampus can reach 20-30 μM (Dunwiddie et al., 1997; Latini et al., 1999). It seems that *in vivo* a large part of adenosine present in the synapse, under basal conditions comes from the extracellular metabolism of nucleotides (Latini and Pedata, 2001; Sperlagh and Vizi 2011). In contrast, numerous studies have suggested that in conditions of hypoxia or ischemia adenosine is mainly formed intracellularly and released to the synapse by transportes (Latini and Pedata, 2001; Sperlagh and Vizi, 2011).

2.3 Nucleoside Transporters

The level of extracellular adenosine is regulated by the process of bidirectional transport of nucleosides, which allows for rapid exchange between extra and intracellular levels of adenosine. In contrast to conventional neurotransmitters, the reuptake of adenosine does not depend on energy-driven transporter-mediated systems. This transport is driven by chemical gradients and by unidirectional concentrative processes, driven by sodium electrochemical gradient (Dos Santos-Rodrigues et al., 2014; Parkinson et al., 2011). There are two functionally distinct types of nucleoside transporters:

1) equilibrative nucleoside transporters (ENT), which predominate in the CNS, and carry both purine and pyrimidine nucleosides in both directions across cell membranes depending on their concentration gradient. Four types of ENT transporters have been characterized: ENT1-2-3-4; type 1 and 2 appear to be present in all cell types, including neurons and glia (Baldwin et al., 2004; King et al., 2006; Dos Santos-Rodrigues et al., 2014; Parkinson et al., 2011).

2) concentrative nucleosides transporters (CNT, sodium-dependent) which mediate the influx of nucleosides under the force of transmembrane sodium gradient (Dos Santos-Rodrigues et al., 2014; Latini and Pedata, 2001; Parkinson et al., 2011). Five subtypes of these transporters have been identified, and two types of CNT were cloned and detected in the rat brain, which are mainly present in the posterior hypothalamus, superior colliculus, brainstem, striatum, hippocampus, cerebellum and cortex (Anderson et al., 1996; Dos Santos-Rodrigues et al., 2014; Latini and Pedata, 2001; Parkinson et al., 2011).

Since the ENT transporters, which seem to dominate in the CNS, are bi-directional, they can not only increase the flow of adenosine into the cell when its extracellular level exceeds its intracellular one, but they may mediate the efflux of adenosine from the cell, when its intracellular level increases. On the other hand, when the Na^+ gradient is reversed, also the concentrative nucleoside transporters can release adenosine from the cell (Dos Santos-Rodrigues et al., 2014; Latini and Pedata, 2001; Parkinson et al., 2011).

2.4 Adenosine inactivation

Extracellular adenosine is primarily inactivated by uptake across the neuronal cell membrane, followed by either intracellular phosphorylaton to AMP by adenosine kinase (AKA), or to a lesser degree deamination to inosine by adenosine deaminase (ADA) (Fig. 1).

ADA is a cytosolic enzyme present in many neurons in the brain, but its highest activity is seen in neurons of the basal hypothalamus; ADA can also be expressed extracellularly in various tissues (Desrosiers et al., 2007; Yegutkin, 2008). In addition to the enzymatic function, ADA catalyses the irreversible deamination of adenosine to inosine. ADA can also exist in a form associated with the adenosine A_1 receptor, so called ektoADA, which can act as a positive modulator of the adenosine binding and signalling function (Ciruela et al., 1996; Ruiz et al., 2000). Moreover, inosine can be then metabolized to hypoxanthine and finally to urate by xanthine oxidase (Morelli et al., 2010).

AKA is part of the cycle between adenosine and AMP, which enables the cell to rapidly respond to changes in the concentration of adenosine. AKA can be expressed in both the cytoplasm

(short isoform) and the nuclei (long isoform) of astrocytes or neurons, and phosphorylates adenosine to AMP (Boison, 2013). In the adult brain the expression of AKA is largely restricted to astrocytes, with the exception of neurons in the olfactory bulb, which maintain high levels of AKA expression (Boison, 2013).

Several lines of evidence indicate that under basal conditions astrocytic AKA is the main regulator of extracellular adenosine, by driving adenosine influx into astrocytes via bi-directional nucleoside transporters (Boison et al., 2010). In contrast, deamination by ADA prevails under conditions in which adenosine levels become excessive (e.g. due to pathologic activity such as ischemia or hypoxia) (Latini and Pedata, 2001).

Another possible metabolic pathway of adenosine is a reversible reaction catalysed by SAH hydrolase, leading to the formation of SAH and L-homocysteine; however, it represents only a minor pathway of adenosine degradation in physiological conditions, as the level of L-homocysteine and SAH in the brain is very low (Fig. 1) (Gharib et al,., 1982; Reddington and Pusch, 1983).

Once present in the extracellular space, adenosine may diffuse far away and influence its receptors (Abbracchio and Burnstock, 1998; Abbracchio et al., 2008; Burnstock, 1976, Fredholm et al., 2001, 2011; Ribeiro et al., 2002).

3. Adenosine receptors

Currently, four subtypes of adenosine receptor (A_1, A_{2A}, A_{2B}, and A_3), which belong to the family of G protein-coupled receptors (GPCR), have been cloned and characterized (Table 1) (for recent review see Chen et al., 2014; Fredholm et al., 2000, 2001, 2011). It has been estimated that under physiological conditions, extracellular levels of adenosine in the rodent CNS (nM range) are sufficient to stimulate both the higher affinity A_1 and A_{2A} receptors. Under pathological conditions, such as hypoxia/ischemia and seizures, adenosine rises markedly to concentrations that can stimulate both the lower affinity A_3 and A_{2B} receptors.

3.1 Signal transduction

The main intracellular signalling pathways involve the formation of cAMP, with A_1 and A_3 receptors causing (through Gi and Go proteins) inhibition of adenylate cyclase (AC) and decreased cAMP production, which lead to reduction of protein kinase A (PKA) activity and cyclic AMP response element binding protein (CREB) phosphorylation. On the other hand, stimulation of A_{2A}

and A_{2B} receptors activates AC through Gs/olf proteins, resulting in activation of PKA and phosphorylation of CREB (Table 1, Fig 2) (Cunha, 2001; Fredholm et al, 2001, 2011).

Another downstream target of PKA activation induced by stimulation of A_{2A} receptors besides CREB is the dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), which is abundantly expressed in striatal projection neurons. Activation of A_{2A} receptors increases the phosphorylation of DARPP-32 protein at the threonine residue 34 (Thr34), which converts this protein into a potent inhibitor of protein phosphatase-1 (PP-1) (Fig. 2) (Fredholm et al., 2007; Svenningsson et al., 2000, 2004). In turn, blockade of A_{2A} receptors decreases the effect of D_2 receptor blockade on DARPP-32 phosphorylation at Thr34 and, at the same time, increases the phosphorylation of this protein at the threonine residue 75, which converts DARPP-32 into an inhibitor of PKA (Fredholm et al., 2007; Svenningsson et al., 2000, 2004). Thus, DARPP-32 has the unique property of being a dual-function protein, acting as an inhibitor of either PP-1 or of PKA.

Other mechanisms, such as voltage-sensitive Ca^{2+} channels (types Q, N, and P), K^+ channels and phospholipase C, are also involved in signal transduction by each of the adenosine receptors (Table 1; Fig. 2) (Dunwiddie and Masino, 2001; Ralevic and Burnstock, 1998; Fredholm et al., 2001, 2011). Additionally, the involvement of mitogen-activated protein kinase (MAPK) pathway in cells of the Chinese hamster ovary (CHO) and COS-7 fibroblast-like cells was also shown (Dickenson et al., 1998; Schulte and Fredholm, 2000, 2003).

3.2 Adenosine A_1, A_{2B} and A_3 receptors localization

The inhibitory A_1 receptors, which are expressed on both neurons and glial cells, are the most abundant adenosine receptors in many regions of the brain. These receptors are localized both pre- and postsynaptically. The highest expression of A_1 receptors has been found in the cortex, striatum, thalamus, cerebellum and hippocampus (Table 1) (Fastbom et al., 1987; Fredholm et al., 2005; Ochiishi et al., 1999; Schindler et al., 2001; Sebastiao and Ribeiro, 2009b). Moreover, the A_1 receptor mRNA is also present in basal ganglia (BG) structures, including the striatum, globus pallidus, and subthalamic nucleus (Dixon et al., 1996). These receptors are also present on astrocytes, oligodendrocytes, and microglia (Biber et al., 1997; Dare et al., 2007; Gebicke-Haerter et al., 1996; Othman et al., 2003). In the striatum, adenosine A_1 receptors are present in both direct and indirect GABAergic efferent neurons, as well as in cholinergic interneurons (Alexander and Reddington, 1989; Ferre et al., 1996; Rivkees et al., 1995). Moreover, presynaptic A_1 receptors are present on glutamatergic cortico-striatal and dopaminergic nigro-striatal afferents but also on nerve

terminals in the globus pallidus, substantia nigra and hippocampus, where they modulate the release of neurotransmitters, such as glutamate, acetylcholine, serotonin and GABA (Cunha et al., 2001; Fastbom et al., 1987; Rebola et al., 2003).

Adenosine A_{2B} receptors are mainly present in peripheral organs such as the bowel, bladder, lung, vas deferens, but can also be found in the spinal cord and brain (Feoktistov and Biaggioni, 1997; Pierce et al., 1992; Ralevic and Burnstock, 1998). In the brain, A_{2B} receptors are present in hippocampal CA1 and CA3 neurons, in the hypothalamic, thalamic, and striatal neurons; low levels of these receptors are also expressed on glial cells (Table 1) (Dare et al., 2007; Feoktistov and Biaggioni, 1997; Fredholm et al., 2001; Pierce et al., 1992; Ralevic and Burnstock, 1998).

The distribution and physiological functions A_3 receptors in the brain are still unclear, although these receptors are widely distributed in peripheral organs (mainly in the testis and lung) (Dixon et al., 1996; Rivkees et al., 2000; Shearman and Weaver, 1997). A relatively low level of A_3 receptors and their mRNA was detected in the hippocampus, cortex, cerebellum and striatum with cellular localization in neurons, astrocytes, and microglia (Table 1) (Brand et al., 2001; Dare et al., 2007; Dixon et al., 1996; Fredholm et al., 2011; Hammarberg et al., 2003; Wittendorp et al., 2004).

3.3 Adenosine A_{2A} receptors and their localization in the brain

In contrast to the widespread distribution of A_1 in the CNS, the A_{2A} receptors are highly abundant in the striatum and nucleus accumbens. Moreover, positron emission tomography (PET) studies in humans showed that A_{2A} receptors, similarly what observed in rodents, are concentrated in the caudate-putamen and nucleus accumbens (Brooks et al., 2008). However, studies performed with more sensitive techniques have demonstrated the presence of A_{2A} receptors and corresponding mRNAs, albeit at lower level of expression, in several other brain areas, such as the hippocampus, cerebral cortex, extended amygdala, thalamic nuclei, and substantia nigra (Cunha et al., 1994; Dixon et al., 1996; Jarvis and Williams, 1989; Rebola et al., 2005; Rosin et al., 1998, 2003; Svenningsson et al., 1998, 1999). It is noteworthy that A_{2A} receptors are also present on glial cells, and that about 3% of their total number are located on striatal astrocytes (Dare et al., 2007; Hettinger et al., 2001; Matos et al., 2012, 2013; Rosin et al., 2003).

In the striatum, A_{2A} receptors are homogeneously distributed throughout the lateral and medial parts and display dense labelling of the neuropil (Rosin et al., 1998, 2003). These receptors are mainly localized postsynaptically in the GABAergic medium spiny neurons of the indirect pathway projecting to the globus pallidus external segment (GPe). These neurons also express a high density of dopamine D_2 receptors and enkephalin (Augood et al., 1994; Fink et al., 1992;

Rebola et al., 2005; Rosin et al., 2003; Schiffmann et al., 1991, 2007; Svenningsson et al., 1998). Neurons of the direct striato-nigral pathway, which selectively express dopamine D_1 receptors and the peptide dynorphin, do not contain a significant level of A_{2A} receptors (Schiffmann et al., 1991). Morphologically, A_{2A} receptors in the striatum predominate in dendrites and dendritic spines and are expressed to a lesser extent in axons and axon terminals of recurrent collaterals projecting back to the striatum or from the cortical areas (Rebola et al., 2005).

The A_{2A} receptors in the striatum are also localized presynaptically on glutamatergic terminals that contact medium-sized spiny neurons of the GABAergic direct striato-nigral pathway (Rodrigues et al., 2005; Rosin et al., 2003, Quiroz et al., 2009), where they heteromerize with A_1 receptors and regulate the release of glutamate (Ciruela et al., 2006, Quiroz et al., 2009). Such a co-expression of adenosine A_{2A} and A_1 receptor mRNAs was also found on the glutamatergic nerve terminals in the hippocampus (Rebola et al., 2005), which may control glutamate release. Moreover, A_{2A} receptors located on GABAergic collateral axons may modulate in an inhibitory way the GABA release from medium-sized spiny projection neurons, likely relieving a GABA-mediated inhibition of these neurons (Mori et al., 1996). In turn, A_{2A} receptors located on striatal cholinergic nerve terminals modulate the acetylcholine (Ach) release (Brown et al., 1990; Kurokawa et al., 1994, 1996). A_{2A} receptor agonists enhance, and A_{2A} receptor antagonists reduce the Ach release *in vivo* (Kurokawa et al., 1996), an effect modulated by the dopaminergic transmission (Kurokawa et al., 1996).

Regarding the nucleus accumbens (the so-called ventral striatum), A_{2A} receptors follow the same pattern of distribution as the dopamine D_2 receptors, and the shell of the nucleus accumbens displays a density of adenosine A_{2A} receptors about 40% lower than that in the dorsal striatum (Rosin et al., 2003). A distinction between the dorsal and ventral striatum has already been suggested by others. The dorsal part seems to be the most important for the control of dopamine-mediated motor behavior (Joel and Weiner, 2000; Groenewegen 2007, Voorn et al., 2004). On the other hand, the so-called "ventral striatum", which comprises the nucleus accumbens, the ventromedial part of the striatum, and the olfactory tubercle, is a region connected with limbic structures, and seems to be strongly associated with emotional and motivational aspects of behavior (Joel and Weiner, 2000; Groenewegen 2007, Voorn et al., 2004).

4. Homo- and heteromeric complexes formed by adenosine A_{2A} receptors

A growing body of evidence indicates that A_{2A} receptors, like many other GPCR not only form homodimers and heterodimers with A_1 receptors, but also interact with other non-

adenosinergic receptors (Fredholm et al., 2007; Ferre et al., 2011, Sebastiao and Ribeiro, 2009a,b). Such heteromers are presently regarded as a molecular basis for the known direct and indirect (via adapter proteins) intramembrane receptor/receptor interactions. The most well-known heterodimeric interactions involve A_{2A} and dopamine D_2 receptors (see chapter 4).

Direct evidence for A_{2A}/D_2 heteromers in addition to A_{2A} homomeric complexes within the plasma membrane came from fluorescent and bioluminescent resonance energy transfer (FRET and BRET) analyses (Canals et al., 2003). Such a heteromer represents one of the possible molecular mechanisms for the functional antagonism between A_{2A}/D_2 receptors, demonstrated earlier at different levels, including the receptor and second messenger systems (Fig. 2) (Ferre et al., 2007, 2011; Fuxe et al., 2003; Morelli et al., 1995; Sebastiao and Ribeiro, 2009a,b; Svenningsson et al., 2000).

Moreover, heterodimerization between A_{2A} and metabotropic glutamate mGlu5 receptors has been detected in glutamatergic striatal terminals *in vivo*, and in striatal neurons by *in vitro* studies, and has been suggested to play a role in striatal plasticity and in modulation of the activity of striatopallidal neurons (Ferre et al., 2002; Rodrigues et al., 2005). Differently from what observed for A_{2A} and dopamine D_2 receptors, which interact in an opposite functional way, the A_{2A}/mGlu5 receptor interaction may account for the synergism found after combined agonist or antagonists treatments, demonstrated at both the biochemical and behavioural levels. (Fig. 2) (Popoli et al., 2001; Ferre et al., 2002; Nishi et al., 2003). A molecular mechanism underlying this functional interaction may be due to the fact that co-activation of mGlu5 and A_{2A} receptors by agonists synergistically increases phosphorylation of DARPP-32 (Nishi et al., 2003). This potentiation of A_{2A}/DARPP-32 signaling by mGlu5 receptors seems to results from the ability of mGlu5 to enhance the A_{2A}-mediated cAMP formation in an extracellular signal-regulated kinase (ERK1/2)-dependent manner. Since A_{2A}, D_2 and mGlu5 receptors are found together in the dendritic spines of the indirect striato-pallidal GABA pathway, the interactions between them may have a major role in controlling these striatal output neurons. In addition, presynaptic interactions between A_{2A} and mGlu5 receptors on striatal glutamatergic nerve terminals may also contribute to the described interaction by synergistic regulation of glutamate release (Rodrigues et al. 2005).

A further interaction was reported between A_{2A} and cannabinoid CB_1 receptors, which may also form heteromeric complexes and in this way A_{2A} activation facilitates CB_1 receptor signaling in the striatum (Fig. 2) (Carriba et al., 2007; Ferre et al., 2010; Sebastiao and Ribeiro, 2009a). Accordingly, blockade of A_{2A} receptors was found to counteract the motor depressant effects

produced by intrastriatal administration of CB_1 receptor agonists (Carriba et al., 2007; Ferre et al., 2010).

Recently a new possibility of receptor heteromultimers has been proposed. Thus, using a sequential resonance energy transfer (SRET) and bimolecular fluorescence complementation plus BRET, evidence for A_{2A}-CB_1-D_2 and A_{2A}-D_2-mGlu5 receptor heteromers in transfected cells has been obtained (Cabello et al., 2009; Carriba et al., 2008). Such interactions at both pre- and postsynaptic levels play an important role in the control of neurotransmitters and signaling in different brain structures, and provide selective targets for drug development in many disorders of the CNS. However, it has to be mentioned that recently Pinna et al., (2014) showed that the interactions between A_{2A}, CB_1, and D_2 receptors may be disrupted by L-DOPA administration in hemiparkinsonian rats, which could question the relevance of receptor heteromultimers to the therapy of motor dysfunctions in Parkinson's disease (PD).

5. Physiological functions of adenosine and adenosine A_{2A} receptors

Adenosine receptors regulate several important physiological functions at both the central and peripheral levels. However, the specific influence of each receptor subtype on these functions may vary, due to differences in both receptor distribution in the various body organs and affinity for endogenous adenosine, as described above. Remarkably, adenosine A_{2A} receptors have recently attracted a great deal of attention as potential drug targets for different pathological conditions. The remainder of this chapter will summarize the most well-characterized biological functions of adenosine A_{2A} receptors. The effects mediated by adenosine A_{2A} receptors that are more relevant to the pathological features of PD will be extensively discussed in other chapters of this book.

5.1 Central effects of adenosine A_{2A} receptors

A major branch of the research on adenosine A_{2A} receptors focuses on the modulation of motor behavior, based on the fact that these receptors are highly enriched in the striatum, a key nucleus of the BG circuitry (Fig. 3), where they are almost exclusively located on the GABAergic neurons of the striato-pallidal (or indirect) pathway that project to the GPe (Hettinger et al. 2001). At this level, adenosine A_{2A} receptors can interact in an opposite way with dopamine D_2 receptors (Svenningsson et al., 1999), so that the stimulation of A_{2A} receptors depresses the D_2 receptors-dependent signalling (Ferré et al. 1997; Diaz-Cabiale et al., 2001). In line with this, and considering that dopamine D_2 receptors crucially regulate movement execution, stimulation of adenosine A_{2A}

receptors results in motor depressant effects, while blockade of these receptors stimulates movement (Hauber and Münkle, 1997; Ferré et al., 1997). Importantly, and notwithstanding their almost exclusive expression on the striato-pallidal neurons, A_{2A} receptors, by acting on BG loops, can as well influence the effects mediated by dopamine D_1 receptors, located on GABAergic neurons belonging to the striato-nigral (or direct) pathway, which also play a crucial role in motor control (Ferré et al. 1997; Le Moine et al., 1997). Taken together, these findings justify the intensive study of adenosine A_{2A} receptor antagonists as new drugs for the treatment of the motor deficits featuring PD (see Chapters 2, 9, 14).

Besides motor control, adenosine A_{2A} receptors regulate important non-motor central functions. Studies with caffeine, a non-selective A_1/A_{2A} adenosine receptor antagonist, have clearly demonstrated that the adenosine system is involved in the regulation of attention and motivation. Data obtained from both experimental animals and humans indicate that caffeine augments alertness and wakefulness, reduces the perception of fatigue, and delays the need for sleep (Fredholm et al., 1999; Snel and Lorist, 2011). Interestingly, additional studies in experimental animals have demonstrated that adenosine A_{2A} receptors play a critical role in caffeine-induced arousal and increased alertness (Higgins et al., 2007; Lazarus et al., 2011). Moreover, caffeine improves the performance in memory tasks in both experimental animals and humans, and similar effects have been described for selective adenosine A_{2A} receptor antagonists in experimental animals (Prediger et al., 2005; Kadowaki Horita et al., 2013, see also Chapter 8), although others failed to observe beneficial effects of A_{2A} receptor antagonists on memory (O'Neill and Brown, 2007). Furthermore, A_{2A} receptors play a crucial role in reward, motivation, and perception of stimuli, and both caffeine and selective A_{2A} receptor antagonists facilitate these phenomena (Fredholm et al., 1999; Higgins et al., 2007; Mott et al., 2009). In line with this, other studies have demonstrated that adenosine A_{2A} receptors may influence the effects of psychostimulant drugs of abuse, such as cocaine, methamphetamine and nicotine (Cauli et al., 2003; Justinova et al., 2009; Kobayashi et al., 2010; Simola et al., 2006; Wells et al., 2012).

Adenosine A_{2A} receptors have also been implicated in depression, as suggested by the beneficial effects of either genetic deletion or pharmacological blockade of these receptors in animal models of this pathology (El Yacoubi et al., 2001; Yamada et al., 2014). Another crucial function which appears to be regulated by adenosine A_{2A} receptors is epileptogenesis, as indicated by the experimental and clinical evidences showing that caffeine and theophylline, another non-selective adenosine receptor antagonist, may induce and/or aggravate seizures (Boison, 2011).

However, the precise role of adenosine A_{2A} receptors in epileptogenesis is still debated, as studies in experimental animals have demonstrated that these receptors can have either facilitatory or inhibitory effects on seizures, depending on the experimental model utilized (Ates et al., 2004; Souza et al., 2013; Tchekalarova et al., 2010). In addition, adenosine A_{2A} receptors can modulate nociception, and either blockade or genetic deletion of these receptors has been shown to increase the pain threshold in experimental models (Hussey et al., 2007; Ledent et al., 1997), likely by an action on central nociceptive pathways. It has to be mentioned that adenosine A_{2A} receptors can also be found in peripheral nerves, where their stimulation decreases the pain threshold, likely by facilitating the transmission at the level of the primary afferent pathways (Khasar et al., 1995).

Regulation of neuron homeostasis and survival is another major function of adenosine A_{2A} receptors, that can be observed at the central level. A number of studies in experimental models of neurodegenerative diseases, such as Alzheimer's disease, Huntington's disease (HD), and PD (Espinosa et al., 2013; Popoli et al., 2008; Schwarzschild et al., 2003), cerebral ischemia (Chen and Pedata, 2008), and spinal cord trauma (Cassada et al, 2002) have consistently demonstrated that genetic and/or pharmacological manipulation of A_{2A} receptors may counteract the neurodegeneration and neuroinflammation associated with these conditions. However, it has to be remarked that A_{2A} receptors may differently influence these processes according to the specific experimental model used. Thus, A_{2A} receptor blockade has consistently been shown to attenuate neuronal death and inflammatory damage in models of cerebral ischemia and neurodegenerative diseases. Conversely, stimulation, rather than blockade, of A_{2A} receptors affords neuroprotection in experimental models of spinal trauma. Furthermore, evidences also exist suggesting that stimulation of A_{2A} receptors may protect neurons in models of HD (Popoli et al., 2008). It has been hypothesized that adenosine A_{2A} receptors may modulate neuronal homeostasis by attenuating either glutamate-induced excitotoxicity or glial activation (or both), two mechanisms that are known to play a crucial role in neurodegenerative and neuroinflammatory phenomena (Halliday and Stevens, 2011; Milanese et al., 2009).

5.2 Peripheral effects of adenosine A_{2A} receptors

In addition to the protective effects elicited in the CNS, studies in experimental animals have indicated that adenosine A_{2A} receptors can modulate inflammation and tissue damage in different peripheral organs, including the heart, kidney, lung, and intestine, as observed in several *in vitro* and *in vivo* models of inflammatory diseases. The modulation of inflammatory responses by

A_{2A} receptors can be explained considering that many cells of the immune system, such as basophils, lymphocytes, mast cells, monocytes, and neutrophils express A_{2A} receptors, and that these receptors profoundly influence the function of immune cells (Haskó et al., 2007; Hershfield, 2005; Revan et al., 1996). Among these functions regulated by A_{2A} receptors are the induction of pro-inflammatory mediators (Sullivan et al., 2001; Pouliot et al., 2002), activation of T cells (Sevigny et al., 2007), mast cell migration (Duffy et al., 2007), and monocyte secretion (Link et al., 2000). Pro-inflammatory effects are usually observed following the stimulation of A_{2A} receptors, although these receptors have complex effects on inflammation, and data also exist showing that blockade of A_{2A} receptors may attenuate inflammation in peripheral organs (Katebi et al., 2008).

Besides their effects on inflammation, A_{2A} receptors can modulate other important functions of peripheral organs. Adenosine A_{2A} receptors regulate several aspects of cardiovascular physiology, although some of these effects are ascribable to either the cross-talk between A_{2A} and other adenosine receptor subtypes, or to extracardiac A_{2A} receptors (Headrick et al., 2013). Stimulation of A_{2A} receptors has been reported to enhance the contractility of cardiomyocytes, to elicit a positive inotropic action (Dobson and Fenton, 1997), and to promote dilation of different vessels, including the coronary arteries (Belardinelli et al., 1998; Rump et al., 1999; Sato et al., 2005). Remarkably, the A_{2A} receptor agonist regadenoson is currently the most used vasodilators in the U.S.A. (Ghimire et al., 2013). Adenosine A_{2A} receptors have also been suggested to participate in angiogenesis by promoting the generation of vascular endothelial growth factor (VEGF) (Adair et al., 2005), in atherosclerosis, by inhibiting the formation of foam-cells (Bingham et al., 2010), and in cardioprotection during ischemia, owing to their ability to modulate cell infiltration and inflammatory responses (Glover et al., 2005). Adenosine A_{2A} receptors are also expressed at the level of the intestine, where they may influence some aspects of enteric function, such as contractility and secretion, although inconsistent results have been reported (Fornai et al., 2009; Storr et al. 2002; Tomaru et al., 1995). However, the most well-characterized effect of A_{2A} receptors at this level is the modulation of intestinal inflammation, and a marked up-regulation of high-affinity A_{2A} receptors has been observed in experimental colitis (Antonioli et al., 2006, 2008). Importantly, independent studies have shown that stimulation of A_{2A} receptors attenuates inflammatory responses in the colon (Antonioli et al., 2010; Odashima et al., 2005; Rahimian et al., 2010), although it has to be acknowledged that others failed to observe this effect (Selmeczy et al., 2007). Adenosine A_{2A} receptors have also been shown to modulate inflammation and tissue damage in the lung, as demonstrated by several preclinical studies (Eckle et al., 2009; Trevethick et al., 2008; Wilson et al., 2009). This effect may be particularly relevant to diseases such as chronic

obstructive pulmonary disease (COPD) and asthma, both of which recognize a major inflammatory mechanisms, as well as to acute lung trauma. Agonists of A_{2A} receptors have indeed been demonstrated effective in preclinical models of these diseases (Bonneau et al., 2006; Fozard et al., 2002; LaPar et al., 2011), and are currently under clinical evaluation, though with inconsistent results (Salgado Garcia et al., 2014; Trevethick et al., 2008) Adenosine A_{2A} receptors also regulate kidney physiology, by modulating the dilation of efferent arterioles, renal blood flow, and glomerular filtration rate (Levens et al., 1991; Al Mashhadi et al., 2009; Carlström et al., 2011), as well as by influencing renal inflammation (Awad et al., 2006; Garcia et al., 2011; Okusa et al., 1999). Finally, adenosine A_{2A} receptors have been suggested to participate in other physiopathological functions, such as ocular hemodinamics, protection from retinal ischemic damage (Zhong et al., 2013), wound healing (Katebi et al., 2008; Squadrito et al., 2014), inflammation in experimental models of arthritis (Mazzon et al., 2011), and tumor growth (Khalan et al., 2012; Montinaro et al., 2013).

As described above, adenosine A_{2A} receptors regulate several physiological functions at both the central and peripheral level. Therefore, in the clinical perspective of chronic utilization of drugs binding A_{2A} receptors, these effects should be taken into great consideration. However, it is noteworthy that instances of side effects, in particular at the cardiovascular level, have usually been observed with A_{2A} receptor agonists. On the other hand, A_{2A} receptor antagonists, that show the most promising antiparkinsonian potential among adenosinergic ligands, appear generally well-tolerated, as confirmed by clinical trials (LeWitt et al., 2008; Mizuno et al., 2013).

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Figure Legends

Fig.1. Adenosine synthesis and metabolic pathways. Adenosine is formed both *intracelullarly* from 5'-AMP by the cytosolic 5'-NT, and *extracelullarly* by the metabolism of nucleotides (ATP, ADP, AMP) released from the cell through the action of ecto-5'-nucleotidase. Another intracellular source of adenosine may be the hydrolysis of SAH by SAH hydrolase. Hence, adenosine formation depends on ATP breakdown and synthesis. Extracellular adenosine is primarily inactivated by uptake through the transporters (ENT), which are mainly bidirectional, followed by either phosphorylaton to AMP by AKA (under physiological conditions), or, to a lesser degree, deamination to inosine by ADA. Another possible catabolic pathway of adenosine, though of minor significance, is a reversible reaction catalysed by SAH hydrolase, leading to formation of SAH from adenosine and L-homocysteine *(for more details see the text, point 2. and Abbracchio et al., 2009; Burnstock 2013; Latini and Pedata, 2001; Sperlagh and Vizi, 2011).*

Abbreviations: ADA, adenosine deaminase; AKA, adenosine kinase; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine 5'-triphosphate; A_1, A_{2A}, A_{2B} and A_3 – adenosine receptors; ecto-5'-NT, ecto-5'-nucleotidase; ENT, nucleoside transporter; 5'-NT, 5'-nucleotidase; SAH, S-adenosylhomocysteine;

Fig. 2. Functional interactions between dopamine D_2, adenosine A_{2A}, cannabinoid CB_1 and metabotropic glutamate mGlu5 receptors in striato-pallidal neurons. At the intramembrane level adenosine A_{2A} receptors interact antagonistically with D_2 and CB_1 receptors. These receptors also exert an opposite effect at the AC level and AC-regulated downstream molecules, such as PKA, DARPP-32, CREB-P, and early genes. MGlu5 and A_{2A} receptors act synergistically to counteract the D_2 dopamine receptor signaling in striato-pallidal neurons. Synergistic interactions exist between A_{2A} and mGlu5 receptors at the level of early genes expression (e.g. c-fos), MAP kinases and phosphorylation of DARPP-32 protein;

dashed arrows, inhibitory effect; **'+'**, stimulation; **'–'**, inhibition.

Abbreviations: AC, adenylyl cyclase; Ca^{2+}, calcium ions; CaMK II/IV, calcium/calmodulin-dependent protein kinase type II/IV; cAMP, cyclic AMP; CREB, cAMP response element-binding protein; DARPP-32, dopamine- and cAMP-regulated phosphoprotein; DARPP-32-P (Thr75) and DARPP-32-P (Thr34), DARPP32-phopshorylated at threonine residues 75 and 34, respectively; Gi, Go, inhibitory G proteins; Gq, Gs, Golf, stimulatory G proteins; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PP-1, protein phosphatase-1; PP-2, protein phosphatase-2.

Fig. 3. Schematic representation of Basal Ganglia circuitry and cellular localization of A_{2A} receptors in the striatum. The picture shows the two major striatal GABAergic output pathways. A_{2A} receptors are almost selectively enriched in GABAergic neurons that express dopamine D_2 receptors and project to the GPe (striato-pallidal neurons). By contrast, GABAergic neurons projecting directly to the GPi and express D_1 receptors (striato-nigral neurons), display scarce levels of A_{2A} receptors. DA depletion in the striatum that features PD, results in a reduced stimulation of

both dopamine D_1 and D_2 receptors, leading to a disinhibition of GABAergic striato-pallidal neurons, and a reduced stimulation of GABAergic striato-nigral neurons and to a reduction of the inhibitory control on the GPi. The disinhibition of GPe neurons amplifies the excitatory Glu transmission of the STN. The resulting imbalance between the activity of the two main striatal efferent pathways, leads to a marked increase of the inhibitory output from the GPi, and to an excessive inhibition of Th-Cortex neurons, resulting in reduced movement performance. Blockade of A_{2A} receptors in PD mitigates the overactivity of striato-pallidal and STN-GPi neurons, restoring some balance between the activity of the indirect and direct pathways.

Abbreviations: DA, dopamine; GABA, γ-aminobutyric acid; GPe, globus pallidus *pars externa*; GPi, globus pallidus *pars interna*; PD, Parkinson's disease; STN, subthalamic nucleus; Th, thalamus