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Review

Adenosine A_{2A} receptor interactions with receptors for other neurotransmitters and neuromodulators

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Abstract

Adenosine, by activating adenosine A_{2A} receptors, seems to have a crucial function in regulating the activation of multiple receptors that affect neurotransmitter release and/or synaptic transmission, in particular receptors for neuropeptides (calcitonin gene related peptide (CGRP) and vasoactive intestinal peptide (VIP)), and NMDA receptors, metabotropic glutamate receptors, nicotinic autofacilitatory receptors, dopamine receptors and adenosine A_1 receptors. The manner in which these A_{2A} receptors are involved in interactions with the receptors for other neurotransmitters and or neuromodulators opens novel avenues for the action of this 'omnipresent' nucleoside. Either by direct receptor–receptor modulation or by post-receptor mechanisms, adenosine, in its 'obsession' to protect cells from insults, uses as many receptor systems as possible to synchronize synaptic transmission, in order to exert what seems to be the 'destiny' of this nucleoside —protection of the nervous system.

Keywords: Adenosine; Adenosine A_{2A} receptor; Receptor interaction; CGRP (calcitonin gene related peptide); VIP (vasoactive intestinal peptide); Glutamate receptor; Dopamine receptor; Nicotinic receptor; Neuroprotection

1. Introduction

The interactions between adenosine A_{2A} receptors and receptors for other neurotransmitters will be discussed. Before doing this, I will briefly review information concerning the extracellular accumulation of adenosine at a synaptic level, the characteristics of adenosine A_{2A} receptors, and the origin of adenosine that activates A_{2A} receptors.

2. Extracellular accumulation of adenosine

In recent years, fairly detailed information has been obtained on the way adenosine accumulates at the synaptic level to exert its role as a neuromodulator. For instance, in the case of the neuromuscular junction, it is generally accepted that adenosine triphosphate (ATP) is stored together with acetylcholine in cholinergic synaptic vesicles in motor nerve terminals (Dowdall et al., 1974). ATP can be co-released with acetylcholine from motor nerve terminals (Silinsky, 1975) or released independently of acetylcholine secretion (Marsal et al., 1987). When ATP is released at the neuromuscular junction, it is degraded into ADP, AMP, IMP, adenosine, inosine and hypoxanthine. Evidence that different metabolites (IMP, adenosine, inosine and hypoxanthine) are formed from AMP is provided by interfering with the ectoenzymes that form these metabolites. Thus, inhibition of the ecto-5′nucleotidase with the ADP analogue, α,β-methylene ADP (AOPCP), prevents adenosine formation (Cunha and Sebastião, 1991). The amount of adenine nucleotides, released upon electrical stimulation of innervated skeletal muscle preparations in the presence of a supramaximal concentration of tubocurarine is approximately half the amount released in the absence of tubocurarine, suggesting that half of the adenine nucleotides released come from cholinergic nerve terminals and the other half from skeletal muscle fibres (Cunha and Sebastião, 1993).

Adenosine as such also accumulates at the neuromuscular junction (Cunha and Sebastião, 1993). During nerve

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stimulation, in the presence of the ecto-5′-nucleotidase inhibitor, AOPCP, and providing that a supramaximal concentration of tubocurarine is used to block the nicotinic receptors in order to prevent muscle contractions, the amount of adenosine detected is approximately half that detected in the absence of tubocurarine. This suggests that, as with the adenine nucleotides, half of the adenosine being released comes from the nerve and the other half comes from contracting muscle fibres. In the absence of external calcium, but in the presence of tubocurarine, no adenosine is released, which is consistent with it having a neural origin. Removal of ecto-5′-nucleotidase inhibition reveals that the catabolism, at the neuromuscular junction, of the adenine nucleotides released during stimulation contributes to about 50% of the amount of endogenous adenosine that accumulates extracellularly (Cunha and Sebastião, 1993).

In the central nervous system, there is also the machinery to metabolize extracellular ATP into AMP (Cunha et al., 1992). Exogenously added AMP is catabolized into adenosine and then into inosine at rat hippocampal cholinergic nerve terminals and in hippocampus slices, as well as in cortex slices. In contrast with the neuromuscular junction, IMP formation from extracellular AMP is not detected in the central nervous system. In immunopurified cholinergic nerve terminals obtained from the rat brain cortex, AMP catabolism was not detected, whereas in immunopurified cholinergic nerve terminals of the hippocampus, the activity of ecto-5′-nucleotidase was about 50 times higher than in a crude synaptosomal fraction (Cunha et al., 1992). The explanation for this finding could reside in the fact that cholinergic nerve terminals in the hippocampus are enriched with ecto-5′-nucleotidase, whereas in the cerebral cortex ecto-5′-nucleotidase activity seems to be located preferentially outside cholinergic nerve terminals (Cunha et al., 1994c).

3. Adenosine A_{2A} receptors

Four major classes of adenosine receptors have been pharmacologically defined and cloned. These are the adenosine A_1 and A_3 receptors (see Linden, 1994), which are mostly inhibitory, and the A_{2A} and A_{2B} receptors (Daly et al., 1983), which are stimulatory. The adenosine A_{2A} receptor has high affinity (0.1–1 μM) and the A_{2B} receptor low affinity ($\approx$ 10 μM) for adenosine.

In this initial study on A_2 receptor subtypes (Daly et al., 1983), it was mentioned that A_{2A} receptors were mainly localized in the striatum, whereas A_{2B} receptors were found throughout the brain. The subdivision of A_2 receptors into A_{2A} and A_{2B} subtypes gained further support with the advent of the agonist, CGS 21680 (2-[*p*-(2-carbonyl-ethyl)-phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine) (e.g., Jarvis and Williams, 1989), and the antagonists, 5-amino-2-(2-furyl)-7phenylethyl-pyrazolo-(4,3-e)-1,2,4-triazolo(1,5c)pyrimidine (SCH58261), and 4-(2-(7-amino-2-(2-furyl)(1,2,4)triazolo(2,3-a)(1,3,5,)triazin-5-yl amino)ethyl)phenol (ZM241385), which selectively bind with high (nanomolar) affinity to A_{2A} receptors (see, e.g., Ongini et al., 1999), thus allowing a much better characterization of the A_{2A} receptor subtype.

3.1. Molecular structure of the adenosine A_{2A} receptors

Photoaffinity of the A_{2A} receptor of brain striatal membranes revealed an Mr 45,000 protein with appropriate A_{2A} receptor pharmacology (Barrington et al., 1989). Detailed models for the A_{2A} receptor have been developed (Ijzerman et al., 1994). Like other adenosine receptor subtypes, the adenosine A_{2A} receptor has seven transmembrane domains, making it a member of the large family of G-protein-coupled receptors (e.g., Linden et al., 1991). The A_{2A} receptor is a glycoprotein containing a single carbohydrate chain, which can be either of a high mannose or a complex type (Barrington et al., 1990). Aspargine residues, which are potential glycosylation sites, are located on the second extracellular loop (E-II) (see Jacobson et al., 1993). Receptor glycosylation is, however, not essential for A_{2A} ligand binding (Piersen et al., 1994). Disulfide linkages are probably involved in maintaining the structural integrity of the A_{2A} receptor, since binding of CGS 21680 to striatal A_{2A} receptors is disrupted following treatment with reactive reagents (Jacobson et al., 1992). Chemical modification of the striatal A_{2A} receptor revealed that two histidine residues present in the transmembrane domains VI and VII are important for ligand binding (Jacobson et al., 1992). The transmembrane span V, as well as the transmembrane spans VI and VII, can be specifically labelled by A_{2A} agonists. According to the three-dimensional model proposed by Ijzerman et al. (1994), the A_{2A} receptor consists of seven amphipatic α-helices, which form a pore that is rather hydrophilic compared with the hydrophobic outside of the protein. In this model, two histidine residues and two serine residues coordinate the binding of the hydrophilic purine riboside. The increase in adenosine A_{2A} receptor agonist binding to striatal A_{2A} receptors seen when the ambient pH decreases from 7.0 to 5.5 is compatible with the hypothesis that one of the histidines conserved in the adenosine receptor binding site acts as a hydrogen bond donor to the oxygen of the 2′-hydroxyl group of adenosine A_{2A} receptor agonists (Askalan and Richardson, 1994).

The adenosine A_{2A} receptor was initially cloned from the dog thyroid (Maenhaut et al., 1990) and subsequently from the human hippocampus (Furlong et al., 1992), the rat brain (Chern et al., 1992; Fink et al., 1992) and the guinea pig brain (Meng et al., 1994). The human A_{2A} adenosine receptor has been mapped to chromosome 22 (MacCollin et al., 1994; Le et al., 1996). Sequence analysis of the A_{2A} receptor clone isolated from the human

hippocampal library revealed a 93% homology with the A_{2A} receptor cloned from the dog thyroid and a 40% homology with the cloned A_1 and A_3 adenosine receptors (Furlong et al., 1992). The rat A_{2A} receptor cloned from the rat brain has 82% amino acid homology with the dog thyroid A_{2A} receptor, the variations in amino acid sequences being largely confined to the extracellular second loop and to the carboxyl terminus (Chern et al., 1992; Fink et al., 1992). Once transfected to a 293 cell line, the cloned A_{2A} receptor from the human hippocampus has high affinity for CGS 21680 (K_d 10 nM) and markedly increases cyclic AMP production (Furlong et al., 1992).

3.2. G-protein coupling of A_{2A} receptors

It is now generally accepted that the A_{2A} adenosine receptor is coupled to stimulatory guanine nucleotide binding (G_s) proteins, which positively couple the receptors to adenylate cyclase. Direct evidence for the coupling of the bovine striatal A_{2A} receptor to G_s has been obtained (Marala and Mustafa, 1993). However, guanine nucleotides, which uncouple receptors from G-proteins, have minimal effects ($\cong$ 10%) on agonist binding to A_{2A} receptors in bovine striatal membranes (Barrington et al., 1989). A moderate effect of the GTP analogue, guanylylimidodiphosphate (GppNHp), on CGS 21680 binding, i.e., a 2–5-fold increase in K_d value, was observed in rat striatal cells and PC12 cells (Hide et al., 1992). In rabbit striatal membranes, the binding of CGS 21680 was reduced by GTP analogues, but only when endogenous proteolysis was not inhibited; upon inhibition of endogenous proteolysis, the affinity of CGS 21680 binding to Gpp(NH)p in rabbit striatal membranes was markedly reduced, being similar to that observed in bovine striatal membranes without protease inhibitors (Nanoff et al., 1991). Photoaffinity with an A_{2A} receptor probe demonstrated that two proteins with Mr 47,000 and 38,000, are specifically labelled in rabbit brain membranes, and that inhibition of protease activity reduces the doublet to a single labelled protein of Mr 47,000 (Nanoff et al., 1991). In bovine striatal membranes, only a protein with Mr 45,000 is specifically labelled (Barrington et al., 1989; Nanoff et al., 1991). These observations suggest that adenosine A_{2A} receptors in different animal species are differently affected by endogenous proteases and that, at least in animal species where adenosine A_{2A} receptors are susceptible to endogenous proteolysis, agonist binding is enhanced by proteolysis. In spite of the presence of protease inhibitors, agonist A_{2A} binding to rat striatal membranes is more sensitive to guanine nucleotides than agonist A_{2A} binding to bovine striatal membranes (Mazzoni et al., 1993), which suggests that besides differences in susceptibility to endogenous proteolysis, differences in A_{2A} receptor-G-protein coupling also exist in different animal species.

Divalent cations (Mg^{2+}, Ca^{2+}) markedly increase (10–20 fold at agonist concentrations near the K_d value) A_{2A} agonist binding to rat striatal membranes. This enhancement of agonist binding cannot be entirely explained by an increase in the proportion of receptors in a high affinity form associated with G-proteins, but it is also probably due to an interaction with the receptor itself (Johansson et al., 1992). Nevertheless, modulation by guanine nucleotides of A_{2A} agonist binding to rat striatal membranes requires the presence of Mg^{2+} (Mazzoni et al., 1993). Monovalent cations decrease A_{2A} agonist binding to rat striatal membranes, an effect additive to that of guanine nucleotides, which suggests that monovalent cations do not act at the receptor-G-protein coupling level, but probably at the receptor level (Johansson et al., 1992). However, in bovine striatal membranes, where A_{2A} agonist binding is rather insensitive to guanine nucleotides, the presence of sodium ions is a prerequisite for the demonstration of a modest guanine nucleotide effect (Nanoff and Stiles, 1993), suggesting that, in this animal species, Na^+ ions decrease the tight coupling between the A_{2A} receptor and the G-protein. Accordingly, upon solubilization, the striatal bovine A_{2A} receptor reveals significant modulation of agonist binding by guanine nucleotides per se, and under these conditions the effects of sodium and guanine nucleotides are merely additive (Nanoff and Stiles, 1993).

The guanine nucleotide sensitivity of CGS 21680 is much greater in the rat cerebral cortex and hippocampus than in the rat striatum (Johansson et al., 1993; Johansson and Fredholm, 1995). Thus, besides species differences in relation to A_{2A} receptor-G-protein coupling, it appears that in the same animal species there are differences in the coupling between G-proteins and the A_{2A} receptor in different brain areas. Whether these differences represent agonist binding to two subpopulations of A_{2A} adenosine receptors that are differently coupled to G-proteins, or to different affinity states of the same receptor entity, needs to be known. In relation to this aspect, it is quite interesting that the specific binding of the A_{2A} antagonist to [^{3}H]KF178375S cerebral cortical or hippocampal membranes corresponds to 15–18% of its specific binding to striatal membranes (Nonaka et al., 1994). A similar proportion of the specific binding of CGS 21680 to cortical and hippocampal membranes has been shown to correspond to binding to typical (striatal-like) A_{2A} receptors, whereas remaining specific high-affinity binding of CGS 21680 to the hippocampus and cerebral cortex might represent binding to a hitherto unrecognized binding site (Johansson and Fredholm, 1995) or to an A_{2A} receptor slightly different from the A_{2A} striatal receptor (Cunha et al., 1996a).

3.3. Distribution of the adenosine A_{2A} receptors

The initial studies with CGS 21680 binding showed that the A_{2A} receptor subtype was confined to the caudate putamen, nucleus accumbens, olfactory tubercle and parts of the globulus pallidus (Jarvis and Williams, 1989), whereas A_2 receptors, labelled with *N*-ethylcarboxami-

doadenosine (NECA) in the presence of excess $R(-)$-N^6-(2-phenylisopropyl)adenosine (PIA), appeared to have a much more widespread distribution in the brain. This led to the idea that the A_{2B} receptor subtype was widely distributed in the brain, whereas the A_{2A} subtype was found almost solely in the basal ganglia. However, in some studies (Wan et al., 1990; James et al., 1992), a small amount of CGS 21680 specific binding was detected in the human hippocampus and cerebral cortex. Evidence for high-affinity CGS 21680 binding sites and the expression of mRNA for A_{2A} adenosine receptors in the rat hippocampus, cerebral cortex, and striatum was obtained (Cunha et al., 1994a). Whether the adenosine A_{2A} receptor in the cerebral cortex and hippocampus has different (Johansson et al., 1993; Cunha et al., 1996a) or similar (Kirk and Richardson, 1995) pharmacological characteristics to those of the striatal A_{2A} receptor is still a matter of debate. Evidence for different subtypes of adenosine A_{2A} receptors has also been obtained with the use of immunological techniques (Palmer et al., 1994a).

A_{2A} adenosine receptors, identified in the nervous system by means of specific ligand binding, functional studies, or genetic molecular techniques, have been demonstrated in the striatum and other basal ganglia structures (e.g., Wan et al., 1990; Schiffmann et al., 1991a), as well as in the hippocampus (Wan et al., 1990; Sebastião and Ribeiro, 1992; Cunha et al., 1994a), in the cerebral cortex (Wan et al., 1990; Weaver, 1993; Cunha et al., 1994b), in the nucleus tractus solitarius (Barraco et al., 1993; Castillo-Meléndez et al., 1994), in motor nerve terminals (Correia-de-Sá et al., 1991), in noradrenergic terminals of the vas deferens (Gonçalves and Queiroz, 1993), in a subset of AH/type 2 neurones of myenteric ganglia of the ileum (Christofi et al., 1994), in the retina (Blazynski and McIntosh, 1993), and in the carotid body (Weaver, 1993).

As noticed in the initial studies, the A_{2A} receptors in the rat striatum are associated primarily with intrinsic striatal neurons, probably neurons that are postsynaptic to the dopaminergic system (Alexander and Reddington, 1989), and no association was observed with dopaminergic (Wojcik and Neff, 1983) or serotonergic (Alexander and Reddington, 1989) nerve terminals. mRNA coding for the A_{2A} receptor was detected by in situ hybridization techniques in γ-aminobutyric acid (GABA)/met-enkephalin containing neurons in the striatum (Schiffmann et al., 1991b; Augood and Emson, 1994) and in the pyramidal and granular cell layers of the hippocampus (Cunha et al., 1994a). Interestingly, the distribution of A_{2A} mRNA in the hippocampus closely matches that of A_1 mRNA, although the relative proportions of A_{2A} and A_1 mRNA expressed in each area are different (Cunha et al., 1994a).

3.4. Desensitization of A_{2A} receptors

Studies with rat kidney cells (Newman and Levitzki, 1983), vascular smooth muscle cells from rat aorta (Anand-Srivastava et al., 1989), NG 108-15 cells (Kenimer and Nirenberg, 1981), DDT_1 MF-2 cells (Ramkumar et al., 1991) and rat pheochromocytoma PC12 cells (Chern et al., 1993) have shown that the adenosine A_{2A} receptor-mediated response undergoes rapid desensitization after short-term exposure to agonists.

Pretreatment of DDT_1 MF-2 cells with A_{2A} agonists causes a rapid loss ($t_{1/2} = 45$ min) of agonist stimulation of adenylate cyclase activity, with there being no change in receptor number, affinity, or mobility on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It was suggested that the tight interaction between the A_{2A} receptor and its G-protein diminishes its ability to uncouple or to be internalized upon prolonged exposure to agonists (Ramkumar et al., 1991). In PC12 cells (Chern et al., 1993), exposure to A_{2A} agonists (CGS 21680 or NECA) also significantly reduces the ability of the agonist to subsequently stimulate adenylate cyclase. Again, no changes in the number of A_{2A} binding sites, A_{2A} agonist affinity or A_{2A} receptor transcript levels were observed in PC12 cells pretreated for up to 17 h with an A_{2A} receptor agonist (CGS 21680, 1 μM). Analysis of the mechanisms underlying the A_{2A} receptor desensitization suggests that the unresponsiveness of adenylate cyclase, which occurs after short-term (30 min) exposure to the agonist, followed by down-regulation of excitatory G-protein α-subunit ($G_{s}\alpha$) levels, and the activation of phosphodiesterases, which occurs after long-term (12–20 h) agonist exposure, can both contribute to the decrease in agonist-mediated responses in PC12 cells (Chern et al., 1993; Chang et al., 1997). Chern et al. (1995) and Chang et al. (1997) also suggested that phosphorylation of type VI adenylyl cyclase could be related to inhibition of adenyl cyclase activity during A_{2A} desensitization in PC-12 cells. It was, however, reported (Saitoh et al., 1994) that exposure of PC12 cells to A_{2A} agonists, such as CGS 21680, causes a transient increase followed by a decrease in A_{2A} receptor mRNA levels. In the same study, it was observed that there was a significant decrease in A_{2A} receptor number during agonist exposure. The reason for the discrepancy between the results obtained by Chern et al. (1993) and by Saitoh et al. (1994), who used the same cell line and similar agonist concentrations and exposure time, is unknown, and further investigation could help to clarify this difference. It is worthwhile noting that the affinity of CGS 21680 for the A_{2A} receptor in the PC12 cell line used by Chern et al. (1993) is nearly 10 times lower than the affinity of this agonist for striatal A_{2A} receptors. Also, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) prevented desensitization of the A_{2A} receptor in this cell line at a concentration (100 nM) that should not markedly affect striatal A_{2A} receptors (Chern et al., 1993). Whether this PC12 cell line has A_{2A} receptors that are more similar to the second high-affinity CGS 21680 binding site found in the hippocampus and cerebral cortex (Cunha et al., 1996a) than to the striatal A_{2A} receptor remains to be studied.

A different mechanism for A_{2A} receptor desensitization was proposed after investigation of the desensitization of the canine thyroid-derived A_{2A} receptor in transfected Chinese hamster ovary (CHO) cells (Palmer et al., 1994b). In this model, short-term (30 min) A_{2A} agonist exposure causes a rapid impairment of the receptor/G-protein interaction, leading to a reduced A_{2A}-receptor mediated stimulation of adenylate cyclase activity. This is associated with a reduced affinity of the A_{2A} receptor for the agonist, receptor phosphorylation and sequestration of receptors into a light vesicle population; recovery from this short-term desensitization occurs a few minutes after removal of the agonist. Long-term (24 h) treatment with the agonist leads to receptor down-regulation, recovery from which takes several hours. This mechanism of A_{2A} receptor desensitization is quite similar to that proposed for other G_s protein-coupled receptors (e.g., Pitcher et al., 1998).

4. Origin of adenosine that activates adenosine A_{2A} receptors

Endogenous adenosine activates both A_1 and A_{2A} receptors, and these two subtypes of adenosine receptors can co-exist in the same motor nerve terminal. So, the question is: how does adenosine choose to activate A_1 or A_{2A} receptors, and under which conditions does it discriminate between these receptors? It was observed (Cunha et al., 1996b) that AMP, which originates from adenosine after being hydrolysed by ecto-5′-nucleotidase, enhances the evoked release of [^{3}H]acetylcholine from motor nerve endings. When the enzyme that causes the hydrolysis of adenosine, adenosine deaminase, was added to the bath, this excitatory effect of AMP was prevented. The same occurred when AMP was added in the presence of the ecto-5′-nucleotidase inhibitor, AOPCP. Thus, a possible interpretation of these results is that the excitatory effect of AMP is a consequence of its metabolite, adenosine, which activates excitatory receptors.

Another indication that the adenosine originating from released adenine nucleotides preferentially activates A_{2A} receptors was obtained (Cunha et al., 1996b) in investigations with the ecto-5′-nucleotidase inhibitor, α, β-methylene ADP (AOPCP). This compound reduced [^{3}H]acetylcholine release from rat motor nerve endings stimulated at a high frequency (5 Hz). Like the excitatory effect of AMP, the inhibitory effect of AOPCP was prevented by adenosine deaminase and was antagonized by A_{2A} antagonists but not by A_1 antagonists. This suggests that the inhibitory effect of AOPCP on [^{3}H]acetylcholine release is a result of its ability to prevent the formation of adenosine, which enhances [^{3}H]acetylcholine release by acting on A_{2A} receptors.

The AMP-excitatory and the AOPCP-inhibitory effects, taken together with the observation that adenosine deaminase under similar experimental conditions enhances [^{3}H]acetylcholine release from the rat motor nerve endings, tempted us to suggest that, instead of the classical view that endogenous adenosine activates A_1 receptors regardless of whether it is released as such or formed from adenine nucleotides, adenosine formed from adenine nucleotides acts preferentially on A_{2A} receptors, and adenosine released as such acts preferentially at A_1 receptors. Whether this is a consequence of a different localization of A_1 and A_{2A} receptors in relation to adenosine release sites and ecto-5′-nucleotidase localization, or whether it is a consequence of a burst-like formation of adenosine from released adenine nucleotides (see James and Richardson, 1993), leading to adenosine concentrations high enough to activate A_{2A} receptors, awaits further investigation. An indication that burst-like adenosine formation from released adenine nucleotides might be, at least in part, responsible for its preferential action on A_{2A} receptors is the finding that when motor nerve endings are stimulated at low frequency, i.e., under conditions where smaller amounts of adenine nucleotides are released, AOPCP enhances (Ribeiro and Sebastião, 1987; Redman and Silinsky, 1994) rather than inhibits acetylcholine release.

5. Interactions between A_{2A} receptors and other neurotransmitter receptors

5.1. Adenosine A_1 receptors

Both adenosine A_1 (Sebastião et al., 1990) and A_{2A} receptors (Sebastião and Ribeiro, 1992) modulate neuronal excitability in the CA1 area of the hippocampus, and there is co-localization, as well as co-expression, of mRNA encoding for A_1 and A_{2A} receptors in the hippocampus (Cunha et al., 1994a). There is a functional interaction between these receptors, so that activation of A_{2A} receptors with low nanomolar concentrations of CGS 21680 attenuates the ability of the A_1 agonist, N^6-cyclopentyladenosine (CPA), to inhibit hippocampal excitability (Cunha et al., 1994a). This indicates that there is cross-talk between A_1-inhibitory and A_{2A}-excitatory adenosine receptors in the hippocampus, and suggests that A_1 receptor-mediated actions of endogenous adenosine may be attenuated if there is concomitant activation of A_{2A} receptors. Also, the consequences of A_{2A} receptor activation in the hippocampus may be exacerbated due to a decrease in tonic A_1 receptor activation by endogenous adenosine. This might be particularly important during aging, where there is an increase in A_{2A} binding sites in parallel to a decrease in A_1 binding sites in the hippocampus and cerebral cortex (Cunha et al., 1995).

Besides the A_{2A} receptor-mediated attenuation of A_1 receptor-mediated actions (Cunha et al., 1994a), A_1-mediated inhibition of A_{2A} receptor actions has been suggested (Abbracchio et al., 1992) on the grounds that striatal adenosine A_1 receptor desensitization is accompanied by a

time-dependent amplification of A_2 receptor-mediated stimulation of adenylate cyclase activity. At the presynaptic level of the neuromuscular junction, adenosine A_1 and A_{2A} receptors also interact, since A_1 inhibitory responses are enhanced in the presence of A_2-antagonists and A_{2A} excitatory responses are increased in the presence of A_1-antagonists (Correia-de-Sá and Ribeiro, 1994b).

Recently, O'Kane and Stone (1998) showed interactions between adenosine A_1 and A_2 receptor-mediated responses in the rat hippocampus in vitro. According to these authors an explanation for this interaction is that activation of the adenosine A_{2A} receptor binding site by CGS 21680 induces conformational changes in the A_1 receptor, which may decrease the affinity of the A_1 binding site. Desensitization of the A_1 binding site by the A_{2A}-selective agonist CGS 21680 has been suggested to contribute to the A_{2A} attenuation of the A_1 inhibitory effects (Dixon et al., 1997).

5.2. Dopamine D_1 receptors

After desensitization of striatal A_{2A} receptors in vivo (Porter et al., 1988), dopamine D_1 receptor-mediated stimulation of adenylate cyclase is attenuated. The maximal stimulation by adenosine receptor agonists and dopamine receptor agonists is additive (e.g., Porter et al., 1988), which indicates that A_{2A} receptors and D_1 receptors are associated with different pools of adenylate cyclase. Indeed, in situ hybridization techniques have shown that A_{2A} receptor mRNA is expressed in gabaaminobutyric acid (GABA)-(ergic)-enkephalin striatopallidal neurons (Schiffmann et al., 1991b), which contain dopamine D_2 receptors but not D_1 receptors (Gerfen et al., 1990). D_1 receptors are mainly confined to the GABAergic-substance P-dynorphin nigrostriatal neurons (Gerfen et al., 1990). It thus appears that the interactions between dopamine D_1 and adenosine A_{2A} receptors might be indirect (Morelli et al., 1994). That A_{2A} receptors can interact negatively with dopamine D_1 receptors was been proposed on the basis that acute injections of CGS 21680 completely block the contralateral turning behavior induced by the dopamine D_1 agonist, SKF 38393 in 6-hydroxylamine-lesioned rats, an effect which is even more pronounced than the ability of CGS 21680 to reduce turning induced by D_2 receptor agonists (Morelli et al., 1994). According to Morelli et al. (1994), activation of A_{2A} receptors in the striatopallidal neurons might impair dopamine D_1 responses indirectly by driving the output areas in an opposite manner with respect to the dopamine D_1-containing nigrostriatal pathway. Another possibility advanced by Porter et al. (1988) is that activation of adenosine receptors in vivo may cause disinhibition of the nigrostriatal dopaminergic neurons and consequently an increase in dopamine release, which would be responsible for the desensitization of dopamine D_1 receptors. Further work is needed to clarify the mechanisms by which adenosine A_2 and dopamine D_1 receptors interact.

5.3. Dopamine D_2 receptors

Interactions between adenosine and the dopaminergic system were initially described by Green et al. (1982). They observed that the injection of adenosine agonists into the rat neostriatum after administration of the dopamine agonist, apomorphine, induced turning behavior similar to that observed after lesioning of the dopaminergic nigrostriatal pathway. However, interest in adenosine/dopamine interactions in the basal ganglia increased after the observation by Ferré et al. (1991) that activation of A_{2A} receptors in rat striatum membranes inhibits the affinity of dopamine D_2 agonists. The binding of dopamine D_2 antagonists is not modified by A_{2A} receptor activation, suggesting an interaction at the receptor/G-protein coupling level (Ferré et al., 1991), though the mechanism appears to be different from that of GTP (see Ferré et al., 1992). Similar A_{2A}/D_2 receptor interactions were observed in membranes from a fibroblast cell line after stable co-transfection of A_{2A} and D_2 receptors cDNA (see Fuxe et al., 1998). Thus, the possible existence of A_{2A} modulatory sites on D_2 receptors can be excluded, since no interaction took place when D_2 receptors alone were present (Fuxe et al., 1998). In dopamine-denervated animals, the effect of A_{2A} agonists on D_2 agonist binding is stronger (Ferré and Fuxe, 1992). This dopamine denervation-induced increase in A_{2A}/D_2 interaction might explain the contralateral turning behavior induced by systemic administration of caffeine in unilaterally dopamine-denervated animals and the stronger turning behavior induced by caffeine injected in the denervated striatum (Herrera-Marschitz et al., 1988; Josselyn and Beninger, 1991). CGS 21680 decreases the contralateral turning behavior caused by a dopamine D_2 agonist in a 6-hydroxydopamine-lesioned rats. This indicates the existence of a negative interaction at the postsynaptic level between dopamine D_2 and adenosine A_{2A} receptors (Morelli et al., 1994).

More direct evidence for the functional relevance of the A_{2A}/D_2 interaction was provided by the observation that the ability of D_2 agonists to inhibit acetylcholine release in the striatum was reduced after activation of A_{2A} receptors (Jin et al., 1993). It is worth noting that the D_2 receptor-mediated inhibition of dopamine release is not significantly altered by activation of striatal A_{2A} receptors (Jin et al., 1993). The link between the D_2 and A_{2A} receptors is also supported by the results of in situ hybridization and morphological studies, which suggest that A_{2A} and D_2 receptors are co-localized in the striatum. As with dopamine D_2 receptors (Gerfen et al., 1990), A_{2A} receptor mRNA is strongly expressed in GABAergic-enkephalin striatopallidal neurons (Schiffmann et al., 1991b).

In the intact striatum of the rat, caffeine acting through A_{2A} receptors increases enkephalin expression and decreases substance P gene expression (Schiffmann and Vanderhaeghen, 1993). Caffeine also increases neurotensin gene expression in the subcallosal region of the caudate

putamen and in the shell sector of the nucleus accumbens, as well as cholecystokinin gene expression in the lateral caudate putamen. These changes caused by caffeine in the intact striatum are identical to those caused by dopamine depletion, and according to Schiffmann and Vanderhaeghen (1995), chronic caffeine treatment may result in dopamine depletion by the blockade of presynaptic A_{2A} receptors located in nigrostriatal terminals. In contrast, in the dopamine-depleted striatum, where enkephalin gene expression is increased, caffeine treatment decreases and therefore tends to normalize the level of enkephalin (Schiffmann and Vanderhaeghen, 1993). This action of an adenosine receptor antagonist is similar to that observed for a D_2 agonist (Gerfen et al., 1990), thus reinforcing the idea that A_{2A} receptors and D_2 receptors have opposite actions in the striatum (see Schiffmann and Vanderhaeghen, 1995). Interactions between A_{2A} receptors and dopamine D_2 receptors have also been shown in the isolated rat nodose ganglion. Neither A_1 nor A_3 receptors modify the responses to dopamine (Lawrence et al., 1997). A major mechanism (see Fuxe et al., 1998) for direct intramembrane A_{2A}/D_2 and A_1/D_1 receptor interactions may involve the formation of A_{2A}/D_2 and A_1/D_1 heterodimers, leading to allosteric changes that alter receptor affinity as well as G-protein coupling, and thus the efficacy to control the target proteins in the membranes. Fuxe et al. (1998) also speculated that multiple receptor–receptor interactions within membranes may lead to the formation of receptor clusters, which may be responsible for the storage of information, i.e., memory traces, in the membranes.

The therapeutic implications of these interactions between adenosine A_{2A} and D_2 receptors in the basal ganglia, namely, those related to movement disorders, have been a matter of recent and extensive discussions (see, e.g., Fuxe et al., 1998). Since the striopallidal neurons are essential components of the basal ganglia circuitry, D_2 receptors might therefore be important targets for antiparkinsonism drugs in the case of the dorsal striopallidal neuron, and for antischizophrenic drugs in the case of the ventral striopallidal neurons, (see Ferré, 1997; Ferré et al., 1997). It happens that selective adenosine A_{2A} receptor antagonists are active in models of Parkinson's disease, and yield encouraging results, i.e., with a low incidence of secondary effects (Kanda et al., 1998), and selective A_{2A} agonists have 'atypical' antipsychotic profiles in animal models. This is of interest considering the low probability of extrapyramidal side effects (Ferré, 1997; Rimondini et al., 1997, 1998). The relationship between the human A_{2A} receptor gene and a potential schizophrenia loci on chromosome 22q has been described by Deckert et al. (1997). The biochemical effects induced by activation of D_2 receptors are inhibited is as a consequence of A_{2A} receptor stimulation, and the effects induced by blockade of A_{2A} receptors are counteracted by the action of dopamine D_2 antagonists. Chronic treatment with the D_2 antagonist, haloperidol (Parsons et al., 1995), up-regulates A_{2A} receptors. In animal models of schizophrenia, adenosine A_{2A} receptor agonists block behaviors in a manner similar to dopamine receptor antagonists (Kafka and Corbett, 1996). There is also evidence of an imbalance between A_{2A} and D_2 receptors in favor of A_{2A} receptors in aged animals. With aging, both receptors are downregulated, with D_2 receptors being more reduced in number than A_{2A} receptors (Popoli et al., 1998).

Although Ferré et al. believe that A_{2A}/D_2 receptor–receptor interactions are essential, other authors have stressed the role of A_{2A} receptors as the major mechanism of action of adenosine agonists and antagonists, without dopamine D_2 receptors being involved (see review by Richardson et al., 1997). Alterations of D_2 receptor binding after A_{2A} receptor activation have also been observed in a mouse fibroblast cell line co-transfected with A_{2A} (dog) receptor cDNA and D_2 (D2L human) receptor cDNA (Dasgupta et al., 1996). These transfection studies apparently did not involve adenylate cyclase, thus suggesting a possible intramembrane A_{2A}/D_2 interaction (Dasgupta et al., 1996).

Besides intramembrane interactions, opposing effects of A_{2A} and D_2 receptors could also occur at the level of the second messenger and beyond. The c-*fos* expression in the dopamine-denervated striatum induced by the A_{2A} agonist, CGS 21680, is inhibited by D_2 receptor agonists (Morelli et al., 1995), suggesting that there is an antagonistic D_2–A_{2A} interaction at the adenylyl cyclase level. D_2 receptor agonists or A_{2A} receptor antagonists reduce the expression of some immediate early genes in the striatum (Svenningson et al., 1995). Similar effect of caffeine was observed in striopallidal neurons and was inhibited by antagonists of D_2 receptors.

Ferré et al. (1993) showed that the selective A_{2A} agonist, CGS 21680, in the caudate putamen inhibits a D_2 receptor agonist-induced decrease in GABA levels in the ipsilateral globus pallidus. In contrast, the striatal infusion of a low concentration of the adenosine receptor antagonist, theophylline, enhances D_2 receptor agonist-mediated effects on pallidal GABA extracellular levels (Ferré et al., 1993). When infused in the nucleus accumbens, CGS 21680 causes the same type of changes in GABAergic neurotransmission as those obtained by infusion of dopamine antagonists. Furthermore, striatal GABA release induced by D_2 receptor activation is abolished in the presence of the A_{2A} agonist, CGS 21680 (Mayfield et al., 1996). Activation of A_{2A} receptors in the striatum also decreases the D_2 receptor-mediated inhibition of acetylcholine release (Jin et al., 1993). There is evidence that blockade of muscarinic receptors partially inhibits the increase in c-*fos* expression obtained in the dopamine-denervated striatum (Morelli et al., 1995). Inhibition of a dopamine receptor agonist-mediated behavior induced by CGS 21680 has also been shown (Vellucci et al., 1993). Thus, it seems that striopallidal neuronal function is mainly

regulated by A_{2A} receptor activation at the post-synaptic level, involving direct antagonistic A_{2A}/D_2 and D_2/A_{2A} receptor–receptor interactions, as well as at the presynaptic level, through modulation of neurotransmitter release.

Dopamine depletion or blockade of dopamine receptors inhibits the motor activation caused by adenosine receptor antagonists, and adenosine receptor agonists inhibit and antagonists enhance the motor effects caused by dopamine agonists (Rimondini et al., 1997, 1998). It has also been shown that the adenosine A_{2A} agonist, CGS 21680, opposes amphetamine-induced motor activity, showing that this compound could have an atypical antipsychotic profile in animal models (Rimondini et al., 1997, 1998).

Recently, Morelli and Pinna (1998) showed that A_{2A} receptor agonists acted in limbic rather than in striatal areas, and that A_{2A} receptors appeared to participate in the induction of Fos-like immunoreactivity induced by the neuroleptic, clozapine, which suggests that adenosine A_{2A} receptors participate in the mediation of antipsychotic effects.

Another interesting aspect related to the role of A_{2A} receptors is their involvement in the modulation of central reward processes in the rat. A_{2A} receptor selective drugs diminish the rewarding impact of stimulation without producing performance deficits and A_2 antagonists do not alter reward thresholds (Baldo et al., 1998).

Chronic caffeine exposure has been shown to increase the sensitivity to adenosine of platelets from rats (Zhang and Wells, 1990) and humans (Biaggioni et al., 1991), which suggests that A_2 receptors become sensitized due to prolonged exposure to antagonists. Indirect evidence for heterologous up-regulation of A_2 adenosine and prostacyclin receptors in humans after caffeine withdrawal has been obtained, indicating that an increase in functional activity to adenosine and prostacyclin is part of the caffeine withdrawal syndrome in humans (Paul et al., 1993). Rat striatal A_{2A} adenosine receptors are upregulated after chronic caffeine ingestion (Hawkins et al., 1988), but this was not observed in a mice strain (NIH Swiss strain) which does not develop behavioral tolerance to caffeine (Shi et al., 1994).

5.4. Calcitonin gene related peptide (CGRP) receptors

The neuropeptide, calcitonin gene related peptide (CGRP), is localized in the motor nerve terminals (Rodrigo et al., 1985) and its exogenous application to the rat neuromuscular junction facilitates acetylcholine release (Correia-de-Sá and Ribeiro, 1994a). This effect depends upon the presence of endogenous adenosine, since the hydrolysis of this nucleoside by adenosine deaminase prevents the facilitatory effect of CGRP on acetylcholine release. The effect of endogenous adenosine is mediated through adenosine A_{2A} receptors, since application of the A_{2A} agonist, CGS21680, in low nanomolar concentrations, in the presence of adenosine deaminase, restores the facilitation of [^{3}H]acetylcholine release induced by CGRP. This effect of CGRP is antagonized by adenosine A_2 receptor antagonists, but not by the A_1-selective antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), which further indicates that the interaction between CGRP and adenosine is mediated by adenosine A_2 receptors and not by A_1 receptors. The CGRP effect was even enhanced in the presence of DPCPX, indicating that there is some counteraction of the A_{2A}-mediated effect by the adenosine A_1 inhibitory action on [^{3}H]acetylcholine release. This supports the idea that adenosine A_1 and A_{2A} receptors interact to regulate acetylcholine release from the rat phrenic motor nerve terminals. The CGRP facilitation of acetylcholine release is not a consequence of presynaptic nicotinic facilitation, since tubocurarine does not significantly modify the facilitation by CGS 21680 of [^{3}H]acetylcholine release.

Since both the CGRP receptor and the A_{2A} receptor increase acetylcholine release by a cyclic AMP-dependent pathway, it is possible that the positive cooperation between pre-synaptic adenosine A_{2A} and the CGRP receptors occurs at the transduction level. The enhancement caused by the adenylate cyclase activator, forskolin, of acetylcholine release from rat motor nerve terminals also depends on tonic activation of A_{2A} receptors by endogenous adenosine (Correia-de-Sá and Ribeiro, 1993). Thus, one is tempted to speculate that, as occurs with CGRP receptors, the neuromodulatory action of other receptors positively linked to adenylate cyclase might be facilitated by activation of A_{2A} receptors by endogenous adenosine. In relation to this, it is interesting that one of the first studies on interactions between excitatory adenosine receptors and other receptors in the brain (Sattin et al., 1975) reported that the α-adrenergic-mediated increase in cyclic AMP levels in the cerebral cortex requires the presence of endogenous adenosine.

In the rat hippocampus, CGRP, in a concentration that on its own is devoid of effect, increases the slope of the field excitatory post-synaptic potentials (EPSPs) when applied to hippocampus slices in the presence of the selective A_1 antagonist, DPCPX. The selective A_{2A} receptor antagonist, ZM 241385, prevented this effect of CGRP, which suggests that this excitatory effect of CGRP is dependent upon A_{2A} receptor activation by endogenous adenosine (Sebastião et al., 1998).

5.5. Vasoactive intestinal peptide (VIP) receptors

Adenosine A_{2A} receptors modulate the vasoactive intestinal peptide (VIP) neuromodulation of K^+ evoked ^{3}H-GABA release from rat hippocampus synaptosomes (Cunha-Reis et al., 1998). Also at the periphery (e.g., the rat neuromuscular junction), the facilitation of neuromuscular transmission induced by VIP depends on tonic A_{2A}-adenosine presynaptic receptor activation (Correia-de-Sá et al., 1998). This modulation of the facilitatory effects of VIP has many similarities with the way A_{2A} receptors

modulate the facilitatory effects of CGRP on transmitter release and/or synaptic transmission (see above).

Interactions between VIP and A_{2A} receptors have also been observed in hippocampus slices, and, as happens with adenosine A_{2A} interactions with CGRP, the excitatory effect of VIP on synaptic transmission is also facilitated by co-activation of adenosine A_{2A} receptors (Cunha-Reis et al., 1998).

5.6. The NMDA receptor

In isolated rat hippocampal neurons, A_1 receptor activation inhibits NMDA receptor-mediated currents (de Mendonça et al., 1995). This is of interest if one considers that endogenous adenosine might tonically activate the adenosine A_1 receptors which mediate inhibition of the NMDA responses caused by hypoxia (Canhão et al., 1994). In contrast, in the rat striatum adenosine receptors, probably of the A_{2A} subtype, inhibit the conductance of NMDA receptor channels in a subset of medium spiny neurons (Norenberg et al., 1997). Apparently, this occurs through a transduction mechanism, which involves a G-protein.

5.7. Metabotropic glutamate receptors

The increase in cyclic AMP accumulation caused by activation of metabotropic glutamate receptors in the hippocampus with 1-aminocyclopentane-1*S*,3-*R*-dicarboxylic acid (1*S*,3*R*-ACPD) is dependent upon activation of A_2 receptors by endogenous adenosine (Winder and Conn, 1993), but the A_2 receptor agonists and antagonists used by these authors do not distinguish whether this action is mediated by A_{2A} or A_{2B} receptors. Conversely, the enhancement of cyclic AMP accumulation caused by NECA in cerebral cortex slices (Alexander et al., 1992) or by 2-chloroadenosine in the hippocampus (Winder and Conn, 1993) is potentiated by the activation of metabotropic glutamate receptors. It thus appears that the cyclic AMP-mediated responses elicited by A_2 agonists can influence, and be influenced by, metabotropic glutamate receptors. Interestingly, activation of metabotropic glutamate receptors may, in turn, attenuate A_1 receptor-mediated actions (de Mendonça et al., 1995).

5.8. Nicotinic autofacilitatory receptors

Excitatory adenosine A_{2A} receptors on motor nerve terminals regulate the autofacilitation of acetylcholine release by favoring desensitization of the presynaptic facilitatory nicotinic receptors, an effect probably mediated through an adenylate cyclase/cyclic AMP-dependent mechanism (Correia-de-Sá and Ribeiro, 1994b). Thus, the presynaptic nicotinic agonist, dimethyl-4-phenylpiperazinium (DMPP), causes facilitation of acetylcholine release when used in low concentrations and inhibition when tested in high concentrations or when applied for long periods. This change of facilitation into inhibition is due to desensitization of the autofacilitatory nicotinic receptors (Wessler et al., 1987). This nicotinic desensitization is prevented if endogenous extracellular adenosine is inactivated (e.g., by adenosine deaminase) and is enhanced if A_{2A} receptors are activated or if adenylate cyclase is stimulated (Correia-de-Sá and Ribeiro, 1994b). Furthermore, blockade of A_{2A} receptors with DMPX attenuates nicotinic desensitization, whereas blockade of A_1 receptors with DPCPX might enhance desensitization of presynaptic nicotinic receptors (Correia-de-Sá and Ribeiro, 1994c). These findings suggest that A_1 and A_{2A} receptors are tonically activated by endogenous adenosine, and have opposite actions with respect to their ability to interact with nicotinic receptors.

6. Therapeutic implications

Some of the excitatory effects of adenosine and its analogues occur in several brain regions. These effects are particularly interesting in terms of potential therapeutic applications, especially when these effects are mediated by high-affinity adenosine A_{2A} receptors in regions where clear functions have been identified. This is the case of the striatum, which is affected in Parkinson's disease and Huntington's chorea. The striatal A_{2A}/D_2 receptor interactions can also provide the basis for a new treatment for schizophrenia. Inhibition of D_2 receptors in the ventral striatum seems to be associated with the antipsychotic effect of neuroleptics while inhibition of dopamine D_2 receptors in the dorsal striatum is related to their extrapyramidal side effects. Because the A_{2A}/D_2 interaction is more intense in the ventral striatum than in the dorsal striatum, A_{2A} receptors can be used as targets for antipsychotic drugs with a lower possibility of causing extrapyramidal side effects (see Ferré, 1997).

In the periphery, the interaction of A_{2A} receptors with other receptors that regulate acetylcholine release from motor nerve terminals could reveal the importance of A_{2A} receptors when deficits in acetylcholine release occur (e.g., myasthenic syndromes). The excitatory effects of A_{2A} receptors on acetylcholine release might prove useful when developing cognitive enhancers that by increasing acetylcholine release, could be of therapeutic interest in dementia (e.g., Alzheimer's disease). One can also anticipate that adenosine-related medicines that combine adenosine A_1 receptor blockade with A_{2A} receptor activation will be useful in situations where an increase in neurotransmitter release is needed. There are also situations where A_{2A} receptor activation might prove to be excitotoxic (e.g., increase in glutamate release) and, therefore, A_{2A} antagonism will be needed. Some potential therapeutics based upon adenosine A_{2A} receptors have been recently reviewed in relation to cerebroprotective effects (see Von Lubitz, 1999).

7. Conclusions

A_{2A} receptor activation enhances the release of neurotransmitters in the central and peripheral nervous system. Besides this direct excitatory action of A_{2A} receptor activation on transmitter release and synaptic transmission, the ability of A_{2A} receptors to interact with other neurotransmitter and/or neuromodulator receptors should be emphasized. Besides the A_{2A}/D_1 and A_{2A}/D_2 receptor interactions in the striatum, A_{2A}/A_1 interactions and involvement of A_{2A} receptors in the effects of neuropeptides, such as CGRP and VIP, and on NMDA receptors, glutamate metabotropic receptors, and on the desensitization of nicotinic autofacilitatory receptors have been demonstrated in studies using the rat neuromuscular junction and hippocampus. This opens the possibility that modifications of extracellular adenosine levels and/or of A_{2A} receptor activation interfere with the action of other neurotransmitters or neuromodulators, not only in the processes involved in their release, but also in their ability to activate or desensitize their own receptors. This cross-talk may result from intracellular cascade processes occurring between common transducing systems (e.g., cyclic AMP) or through G-proteins.

This enormous potential of adenosine modulation at the inter-receptor level expands the role of adenosine as a neuromodulator-homeostatic substance. This is not unexpected if we recall the physiological role of adenosine in the modulation of cell signalling.

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