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International Union of Pharmacology. XXV. Nomenclature and Classification of Adenosine Receptors

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Abstract

Four adenosine receptors have been cloned and characterized from several mammalian species. The receptors are named adenosine A₁, A_{2A}, A_{2B}, and A₃. The A_{2A} and A_{2B} receptors preferably interact with members of the G_s family of G proteins and the A₁ and A₃ receptors with G_{i/o} proteins. However, other G protein interactions have also been described. Adenosine is the preferred endogenous agonist at all these receptors, but inosine can also activate the A₃ receptor. The levels of adenosine seen under basal conditions are sufficient to cause some activation of all the receptors, at least where they are abundantly expressed. Adenosine levels during, e.g., ischemia can activate all receptors even when expressed in low abundance. Accordingly, experiments with receptor antagonists and mice with targeted disruption of adenosine A₁, A_{2A}, and A₃ expression reveal roles for these receptors under physiological and particularly pathophysiological conditions. There are pharmacological tools that can be used to classify A₁, A_{2A}, and A₃ receptors but few drugs that interact selectively with A_{2B} receptors. Testable models of the interaction of these drugs with their receptors have been generated by site-directed mutagenesis and homology-based modelling. Both agonists and antagonists are being developed as potential drugs.

I. Introduction

The nomenclature and classification of adenosine receptors has been covered in two publications by members of a previous NC-IUPHAR subcommittee, which was devoted to “purinoceptors” (Fredholm et al., 1994a, 1997). However, these two publications were

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progress reports and were not official documents of the NC-IUPHAR. They dealt with both adenosine receptors and P₂ receptors. As a result of this work, separate subcommittees were set up for adenosine receptors, P_{2X} receptors, and P_{2Y} receptors. The present review will therefore cover adenosine receptors only. The previous publications contain the historical background and this will not be recapitulated.

The term adenosine receptor is used to denote this group of receptors. First, use of the name adenosine follows the recommendation of NC-IUPHAR that receptors be named after the preferred endogenous agonist. Second, and as discussed in the previous publications, the concept of adenosine receptors precedes the later concept of purinoceptors (P₁ and P₂) (Burnstock, 1978) by several years. The discovery by Drury and Szent-Györgyi (1929) that adenosine can influence several bodily functions inspired much research interest; the pronounced cardiovascular effects of adenosine were particularly well investigated. Several adenosine analogs were synthesized, and examination of the dose-response relationships suggested the presence of specific adenosine receptors (Cobbin et al., 1974). The essentially competitive nature of the antagonism by methylxanthines, including caffeine and theophylline, of adenosine effects in the heart (De Gubareff and Sleator, 1965) and in the brain (Sattin and Rall, 1970) also supported the idea of adenosine receptors. Finally, as the term P₂ receptor has been superseded by the names P_{2X} and P_{2Y} there is little room for the term P₁ except when referring specifically to older publications.

There are four different adenosine receptors, denoted A₁, A_{2A}, A_{2B}, and A₃ (Table 1). This terminology is well established and is coherent with the principles of receptor nomenclature adopted by NC-IUPHAR. Although the primary basis for adenosine receptor nomenclature is structural, historical reasons also played a role. As discussed previously (Fredholm et al., 1994a), careful pharmacological analysis first identified two subforms—A₁ and A₂ (van Calker et al., 1979; Londos et al., 1980a). Later pharmacological studies revealed that the A₂ receptors, coupled to adenylyl cyclase, were heterogenous, necessitating subdivision into A_{2A} and A_{2B}.

II. Molecular Basis for Receptor Nomenclature

Once the adenosine A₁ receptor was defined using binding assays, several attempts were made to purify the receptor. Despite considerable progress by several groups the receptor was never sufficiently pure to allow sequencing. Instead, the cloning of the first adenosine receptors was serendipitous. Four novel members of the G protein-coupled receptor family were cloned from a canine thyroid library (Libert et al., 1989). Of these, one turned out to be the adenosine A_{2A} receptor (Maenhaut et al., 1990), and another the adenosine A₁ receptor (Libert et al., 1991). Once these first sequences were obtained the same receptors were soon cloned from other mammals including human (Furlong et al., 1992; Libert et al., 1992; Townsend-Nicholson and Shine, 1992; Ren and Stiles, 1995; Deckert et al., 1996; Peterfreund et al., 1996). In addition, the adenosine A_{2B} receptor was cloned (Stehle et al., 1992; Jacobson et al., 1995). More surprisingly, a fourth adenosine receptor, denoted A₃, was cloned, first as an orphan (Meyerhof et al., 1991), later as a bona fide methylxanthine-insensitive adenosine receptor in rat (Zhou et al., 1992), a xanthine-sensitive receptor in sheep (Linden et al., 1993), and a partially xanthine-sensitive receptor in humans (Sajjadi

and Firestein, 1993; Salvatore et al., 1993; Linden, 1994). Thus, a family of four adenosine receptors has been cloned from several mammalian and nonmammalian species (see below). The current nomenclature is summarized in Table 1.

III. Formation and Levels of the Endogenous Agonist Adenosine

Adenosine is the main agonist at this receptor class, and this is the reason for the name. In addition, the adenosine metabolite inosine can activate at least some of the receptors (Jin et al., 1997; Fredholm et al., 2001), and there may be circumstances under which inosine provides a larger activation than adenosine, but this remains to be proven.

When given in very high amounts, adenosine can affect intracellular nucleotide pools and even provide a source of metabolizable energy. In addition, it was reported very recently that the human growth hormone secretagogue receptor (GHS-R) also accepts adenosine as a highly potent endogenous agonist, in addition to the endogenous peptide GHS-R agonist, ghrelin (Smith et al., 2000; Tullin et al., 2000). However, most effects of adenosine are due to activation of adenosine receptors.

Before we describe the activation of adenosine receptors under physiological conditions and hence the actions of antagonists, the mechanisms regulating levels of extracellular adenosine must be briefly presented. Under normal conditions, adenosine is continuously formed intracellularly as well as extracellularly. The intracellular production is mediated either by an intracellular 5'-nucleotidase, which dephosphorylates AMP (Schubert et al., 1979; Zimmermann et al., 1998), or by hydrolysis of *S*-adenosyl-homocysteine (Broch and Ueland, 1980). Adenosine generated intracellularly is transported into the extracellular space mainly via specific bi-directional transporters through facilitated diffusion that efficiently evens out the intra- and extracellular levels of adenosine. In some tissues (e.g., kidney brush-border membranes) there is a concentrative nucleoside transport protein capable of maintaining high adenosine concentrations against a concentration gradient. These transport proteins have been cloned and were termed ENT1 and ENT2 (for the equilibrative transport proteins) and CNT1 and CNT2 (for the concentrative types) (e.g., Williams and Jarvis, 1991; Anderson et al., 1996; Baldwin et al., 1999). When the activity of transporters is decreased, e.g., by drugs or by reducing temperature, extracellular biologically active levels of adenosine increase (Dunwiddie and Diao, 2000). In view of the fact that several of the transporters are equilibrative, this might seem to be a paradox. However, as discussed previously (e.g., Fredholm et al., 1994b), it must be remembered that in tissue, some cells are net producers of adenosine, and in these, intracellular levels rise whereas most cells are net eliminators of the nucleoside.

The dephosphorylation of extracellular AMP to adenosine, mediated by ecto-5'-nucleotidase, is the last step in the enzymatic chain that catalyzes the breakdown of extracellular adenine nucleotides, such as ATP, to adenosine. Ectonucleotidases include ectonucleoside triphosphate diphosphohydrolases, including CD39, which can hydrolyze ATP or ADP, ectonucleotide pyrophosphatase/phosphodiesterases, alkaline phosphatases and 5'-nucleotidases such as CD73 (Zimmermann, 2000). These enzymes are essential for the nerve activity-dependent production of adenosine from released ATP under physiological

conditions (Dunwiddie et al., 1997a; Zimmermann et al., 1998). The entire catalytic pathway is complete in a few hundred milliseconds, and the rate-limiting step seems to be the dephosphorylation of AMP to adenosine by ecto-5'-nucleotidase (Dunwiddie et al., 1997a). Recent data provide evidence for the presence of soluble 5'-nucleotidases of unknown structure that are released together with ATP from stimulated sympathetic nerve endings and participate in the extracellular hydrolysis of ATP to adenosine (Todorov et al., 1997). In striatum, local application of a 5'-nucleotidase inhibitor dose dependently decreases the normal levels of adenosine and thereby emphasizes the relevance of this enzyme in vivo (Delaney and Geiger, 1998). Nonetheless, there is good evidence that intracellular formation of adenosine is at least as important as adenosine formation from breakdown of extracellular ATP (Lloyd et al., 1993; Lloyd and Fredholm, 1995). Intracellular formation predominantly occurs as a consequence of activity of intracellular 5'-nucleotidases, of which two forms, cN-I and cN-II, have been cloned (Sala-Newby et al., 1999). These two enzymes may play different roles—cN-I breaking down AMP to adenosine and cN-II breaking down IMP and GMP to inosine and guanosine, respectively (Sala-Newby et al., 2000).

When adenosine levels in the extracellular space are high, adenosine is transported into cells by means of transporters. It is then phosphorylated to AMP by adenosine kinase (K_m = approximately 100 nM; Sychala et al., 1996) or degraded to inosine by adenosine deaminase, a process with a severalfold lower affinity (K_m = 20–100 μ M; Arch and Newsholme, 1978; Lloyd and Fredholm, 1995). In the heart, and probably also other tissues, hypoxia-induced inhibition of adenosine kinase amplifies small changes in free myocardial AMP, resulting in a major rise in adenosine (Decking et al., 1997). Adenosine deaminase, but not adenosine kinase, is also present in the extracellular space (Lloyd and Fredholm, 1995).

Adenosine can also be released into the extracellular space after application of specific neurotransmitter ligands. Glutamatergic agonists, such as NMDA or kainate, dose dependently increase adenosine levels (Carswell et al., 1997; Delaney et al., 1998). Activation of NMDA receptors seems to release adenosine itself rather than a precursor (Manzoni et al., 1994; Harvey and Lacey, 1997). Dopamine D₁ receptors enhance adenosine release via an NMDA receptor-dependent increase in extracellular adenosine levels (Harvey and Lacey, 1997), but dopamine depletion causes no significant changes in the extracellular levels of striatal adenosine as measured by in vivo microdialysis (Ballarin et al., 1987). Thus, dopaminergic input may be important to transiently elevate adenosine but not so important in maintaining a basal level of the nucleoside. Nitric oxide can also control basal levels of endogenous adenosine in vivo (Fischer et al., 1995; Delaney et al., 1998) as well as in vitro (Fallahi et al., 1996).

Another potential source of extracellular adenosine is cAMP, which can be released from neurons and converted by extracellular phosphodiesterases into AMP and thereafter by an ecto-5'-nucleotidase to adenosine. Functional evidence for a relevant role of this pathway has been obtained in the ventral tegmental area and hippocampus (Bonci and Williams, 1996; Brundege et al., 1997; Dunwiddie et al., 1997a,b). However, to provide a physiologically important adenosine release, it seems that multiple cells must release cAMP over a prolonged period (Brundege et al., 1997).

Levels of adenosine in the rodent and cat brain have been determined by different methods including freeze-blowing (Winn et al., 1981), high-energy focused microwave irradiation (Delaney et al., 1998; Delaney and Geiger, 1998) and microdialysis (Zetterström et al., 1982; Porkka-Heiskanen et al., 1997) and have been estimated to be approximately 30 to 300 nM. These levels are sufficient to cause activation of adenosine A₁ and A_{2A} receptors.

The levels of adenosine, at least in the basal forebrain, striatum, hippocampus, and thalamus, are higher during wakefulness than sleep (Huston et al., 1996; Porkka-Heiskanen et al., 1997). The highest levels of adenosine in hippocampus were estimated during the hours before rats entered into a sleep-like behavior, suggesting that adenosine has sleep-promoting properties (Huston et al., 1996). Moreover, extracellular adenosine levels increased 2-fold in the basal forebrain of the cat after 4 h of handling to ensure prolonged wakefulness (Porkka-Heiskanen et al., 1997).

As is well known, levels of adenosine increase, up to 100-fold, as a result of oxidative stress and ischemia (Rudolphi et al., 1992a; Latini et al., 1999). Excitatory amino acid-mediated release of adenosine is certainly involved; however, of greater importance is probably the fact that whenever intracellular levels of adenine nucleotides fall as a result of excessive energy use, the intracellular levels of adenosine will rise dramatically (Rudolphi et al., 1992a). For example, following hypoxia (Zetterström et al., 1982), ischemia (Berne et al., 1974), or electrical stimulation (Pull and McIlwain, 1972), there is a decrease of intracellular ATP, accompanied by an accumulation of 5'-AMP and subsequently adenosine. The nucleoside is thereafter transported into the extracellular space via the above-mentioned transporters (Jonzon and Fredholm, 1985; Fredholm et al., 1994b). An elegant illustration of the capacity of these transporters to move adenosine from the intra- to the extracellular space was provided by loading a high concentration of adenosine into a single hippocampal CA 1 neuron and shortly thereafter, identifying in the same cell an inhibition of the excitatory postsynaptic potential mediated by extracellular adenosine (Brundege and Dunwiddie, 1996). Furthermore, when the intracellular level of adenosine is very high, adenosine simply diffuses out of cells. Direct release of intracellular adenine nucleotides, such as ATP, that is thereafter converted extracellularly by ecto-ATPase and ecto-ATP-diphosphohydrolase (ecto-apyrase) to AMP and dephosphorylated by ecto-5'-nucleotidase to adenosine, should also be considered (Rudolphi et al., 1992a; Zimmermann et al., 1998).

IV. Structure

By now all four adenosine receptors have been cloned from rat, mouse, and human (the structural information is available e.g., via GPCRDB www.gpcr.org/7tm/). In addition, A₁ receptors are cloned from dog, cow, rabbit, guinea pig, and chick; the A_{2A} receptor from dog and guinea pig; the A_{2B} receptor from chick; and the A₃ receptor from dog, sheep, rabbit, and chick. As seen from the dendrogram in Fig. 1, there is a close similarity between receptors of the same subtype, at least among mammals. The largest variability is seen for the A₃ receptor for which there is almost a 30% difference at the amino acid level between human and rat. This difference is in fact larger than that between human and chick A₁ receptors.

The four adenosine receptor subtypes are asparagine-linked glycoproteins and all but the A_{2A} have sites for palmitoylation near the carboxyl terminus (Linden, 2001). Depalmitoylation of A₃ (but not A₁) receptors renders them susceptible to phosphorylation by G protein-coupled receptor kinases (GRKs), which in turn results in rapid phosphorylation and desensitization (Palmer and Stiles, 2000).

It has long been known that A₁ and A₃ receptors couple to G_{i/o} and that A_{2A} and A_{2B} receptors couple to G_s (see Section IX.). Experiments with chimeric A₁/A_{2A} receptors indicate that structural elements in both the third intracellular loop and the carboxyl terminus influence coupling of A₁ receptors to G_i, whereas elements in the third intracellular loop but not the carboxyl terminus contribute to A_{2A} receptor coupling to G_s (Tucker et al., 2000). Reconstitution experiments have revealed that the coupling of A₁ receptors is influenced by the composition, prenylation state (Yasuda et al., 1996) and phosphorylation state (Yasuda et al., 1998) of G protein γ -subunits. A_{2A} receptors vary in their affinity for G_s proteins containing various types of β -subunits and interact most avidly with G proteins containing $\beta 4$ (McIntire et al., 2001).

V. Gene Structure

The genomic structure appears to be similar for all the human adenosine receptors. There is a single intron that interrupts the coding sequence in a region corresponding to the second intracellular loop (Ren and Stiles, 1994; Fredholm et al., 2000; Olah and Stiles, 2000). The best studied receptor is the A₁ receptor. Already when the structure of the A₁ receptor was first reported, the presence of two major transcripts was noted. It was originally thought that they might represent alternative splicing, and more recent data have yielded additional information (Ren and Stiles, 1994, 1995). Transcripts containing three exons, called exons 4, 5, and 6 were found in all tissues expressing the receptor, whereas transcripts containing exons 3, 5, and 6 are in addition found in tissues such as brain, testis, and kidney, which express high levels of the receptor. There are two promoters, a proximal one denoted promoter A, and a distal one denoted promoter B, which are about 600 base pairs apart. Promoter B and exon 1B are part of an intron when promoter A is active (Ren and Stiles, 1995). Both promoters were suggested to have nontraditional TATA boxes.

Reporter assay studies in DDT₁ MF-2 cells show that 500 base pairs of promoter A contained essential elements for A₁ receptor expression, and mice expressing promoter A driving the β -galactosidase reporter gene confirmed this (Rivkees et al., 1999b). Furthermore, this promoter contained binding sites for GATA and for Nkx2.5, which factors individually drive promoter activity and also act synergistically (Rivkees et al., 1999b). Promoter B has been shown to be activated by, among other things, glucocorticoids (Ren and Stiles, 1999). It is known that glucocorticoids can stimulate the expression of A₁ receptors in DDT₁ MF-2 cells (Gerwins and Fredholm, 1991) and in brain (Svenningsson and Fredholm, 1997). The exact reason for this is unknown, since neither promoter contains a canonical glucocorticoid response element (Ren and Stiles, 1999), but interactions with e.g., SRE-2 elements and AP-1 sites may be involved. The magnitude of the glucocorticoid effect that could be shown using reporter constructs was much higher when promoter B acted alone

than when both promoters were present and active (Ren and Stiles, 1999). In DDT₁ MF-2 cells and in brain, promoter A appears important (Rivkees et al., 1999b).

The cloning of much of chromosome 22, where the A_{2A} receptor is located (MacCollin et al., 1994), suggested a two exon structure (Fredholm et al., 2000), which is similar to that reported for the rat A_{2A} receptor (Chu et al., 1996; Peterfreund et al., 1996). By comparing the human sequence data with data from rodents, putative regulatory elements were identified, including AP-1, NF 1 and AP-4 elements (Fredholm et al., 2000). The A_{2A} receptor shows one hybridizing transcript in most tissues examined (Maenhaut et al., 1990; Stehle et al., 1992; Peterfreund et al., 1996). However, examination of RNA isolated from PC12 cells suggested two different start sites (Chu et al., 1996). The expression of A_{2A} receptor can be stimulated by protein kinase C (Peterfreund et al., 1997) and hypoxia (Kobayashi et al., 1998). We do not know the transcription factors involved in either case. It should also be mentioned that the human adenosine A_{2A} receptor is polymorphic. In particular, a (silent) T1083C mutation occurs in various populations, more frequently in caucasians than in Asians (Deckert et al., 1996; Le et al., 1996; Soma et al., 1998).

Analysis of the A_{2B} receptor gene, localized on chromosome 17 in man, reveals a similar overall structure as for the other adenosine receptors. The rat A_{2B} receptor shows two hybridizing transcripts of 1.8 and 2.2 kb, where the latter is the dominant one (Stehle et al., 1992). This could, in analogy with the above, suggest the presence of multiple promoters, but so far this has not been studied to our knowledge.

The mouse A₃ receptor appears to have two exons with coding sequences of 354 and 1135 base pairs separated by an intron of about 2.3 kb (Zhao et al., 1999). Several putative transcription factor-binding sites could be detected in the mouse gene (Zhao et al., 1999), but surprisingly, few of these are matched by similar elements in the human gene. This could mean that the truly important sites have not been identified, or else that the expression of the receptor is regulated very differently in the two species. The fact that the distribution of A₃ receptors in humans and rodents is very different might indicate the latter. The human A₃ receptor shows two transcripts: the most abundant is approximately 2 kb in size, and the much less abundant one is about 5 kb (Atkinson et al., 1997). There are several possible explanations for this, one of which being a similarity with the A₁ receptor gene.

VI. Binding Sites As Revealed by Site-Directed Mutagenesis

Adenosine receptors, like the other G protein-coupled receptors (GPCR), are integral membrane proteins. Such macromolecules are not easily amenable to crystallization and, hence, to precise structure elucidation through X-ray diffraction. However, substantial progress has been made over the last decade or so in unraveling the three-dimensional architecture of two related membrane-bound proteins, i.e., bacteriorhodopsin and (mammalian) rhodopsin. The pivotal suggestion that the GPCR family bears structural homology to these two proteins has been an impetus to our current understanding of receptor structure. Bacteriorhodopsin, a proton pump present in the cell wall of *Halobacterium halobium*, and rhodopsin, itself a G protein-coupled receptor, are of similar size and share many characteristics between themselves and with other mammalian G protein-coupled

receptors. They both have the typical seven-transmembrane α -helical architecture and bind retinal, their endogenous ligand, in the cavity formed by the barrel-like arrangement of the seven transmembrane domains. On the other hand there is little sequence (i.e., amino acid) homology between the two proteins and an almost total lack of homology between bacteriorhodopsin and G protein-coupled receptors. Hibert and coworkers were the first to realize and analyze in depth the opportunities and pitfalls of using the atomic coordinates of bacteriorhodopsin, at that time available at low resolution only (Henderson et al., 1990), and later, of rhodopsin, to construct putative receptor models (Hibert et al., 1991; Hoflack et al., 1994). In subsequent years ever greater resolution and accuracy were achieved (Kimura et al., 1997; Unger et al., 1997), eventually resulting in the elucidation of the structures of bacteriorhodopsin (Fig. 2A) and rhodopsin (Fig. 2B) at 1.55 and 2.8 Å, respectively (Luecke et al., 1999; Palczewski et al., 2000).

It is obvious that so-called homology modeling, i.e., the construction of a three-dimensional model of a given protein (e.g., one of the adenosine receptor subtypes) on the basis of an experimentally determined structure of another related protein (e.g., bacteriorhodopsin or rhodopsin) can only generate highly speculative models. This is particularly true when it comes to apparent differences between the macromolecules under study, for instance in their ligand binding sites. Nevertheless, a number of receptor models have been developed on the basis of either bacteriorhodopsin or rhodopsin (at various degrees of resolution). Thus, Baldwin combined structural information on rhodopsin with a sequence analysis of other GPCRs to suggest a probable arrangement (including “borders”) of the seven α -helices (Baldwin et al., 1997). This template structure was used to provide models for all G protein-coupled receptors in an automated fashion, which can be easily retrieved from the internet (for information on G protein-coupled receptors, including these three-dimensional models, see the GPCR Database <http://www.gpcr.org/7tm/>). Although met with skepticism, such receptor models have been useful in clarifying the putative molecular basis of receptor-ligand recognition, in particular when combined with and adjusted to available pharmacological and structure-activity relationship data.

In the adenosine receptor field, the first receptor model targeted the adenosine A₁ receptor (IJzerman et al., 1992) based on the sequence of the canine orphan receptor RDC7, later identified as an A₁ receptor (Libert et al., 1989, 1991), and the low resolution structure of bacteriorhodopsin (Henderson et al., 1990). It was found that the pore formed by the seven amphipathic α -helices was characterized by a rather distinct partition between hydrophobic and hydrophilic regions. Chemical modification of histidine residues in the receptor, of which two are present in the transmembrane domains, one in helix VI and one in helix VII, strongly affects ligand binding. This provided the basis for docking the potent and A₁-selective agonist N⁶-cyclopentyladenosine into this cavity. A similar model, again based on the bacteriorhodopsin structure and the two histidine residues, was developed for the rat adenosine A_{2A} receptor (IJzerman et al., 1994).

Later, mutation studies indicated these and other amino acid residues as being important for either agonist or antagonist binding or both (see next four paragraphs). This led to further but similar models for instance for the human A_{2A} receptor, based on the low resolution structure of rhodopsin (Kim et al., 1995). As can be inferred from Fig. 2, A and

B, the two rhodopsin proteins are similar but certainly not identical in their transmembrane organization (at the time of the first receptor models, there was no structural information on the extracellular and intracellular domains). Studies of an increasing number of point mutations (often conceived and selected from the receptor models) led to further insight in the ligand binding site, giving rise to some refinement of the existing models. In Fig. 3 a snapshot of the most recently published adenosine A₁ receptor model highlights the six residues in helices III and VII probably involved in agonist binding (Rivkees et al., 1999a). With the recent structure elucidation of rhodopsin (Fig. 2B), earlier results that were somewhat anomalous can be rationalized. For instance, Jacobson and coworkers identified glutamate residues in the second extracellular loop as being important for either direct or indirect ligand recognition (Kim et al., 1996). In rhodopsin, part of this domain folds deeply into the center of the macromolecule, the site where ligand binding in both rhodopsin and adenosine receptors is thought to take place. This arrangement causes the second extracellular loop to be in extensive contact with both the extracellular regions and the ligand binding site. The proteolytic analysis of A₁ receptors photoaffinity-labeled with a xanthine antagonist indicates that the site of alkylation is in TM3 (Kennedy et al., 1996).

Extensive mutagenesis, consisting of single amino acid replacement (typically Ala scanning), has been carried out for both A₁ and A_{2A} receptors (Tables 2 and 3), and to a lesser extent for A_{2B} receptors (Table 4). The most essential interactions required for recognition of agonist and/or antagonist occur in TMs 3, 5, 6, and 7. Two His residues (6.52 and 7.43) are conserved among most of the adenosine receptor subtypes, with the exception of the A₃ receptor, which lacks His at 6.52. These His residues are important for ligand recognition (Olah et al., 1992; Kim et al., 1995; Gao et al., 2000). Mutation of His at 6.52 to Leu in the A₁ receptor selectively weakened binding of an antagonist, whereas at 7.43 the same mutation impaired both agonist and antagonist binding (Olah et al., 1992). When either of these residues in the A_{2A} receptor was mutated to Ala, there was a dramatic loss of affinity for both agonist and antagonist (Kim et al., 1995). Some substitutions by amino acids having aromatic and other side chains partially restored function of the binding site. At the A_{2A} receptor, substitution at 7.43 with Tyr selectively reduced agonist affinity (Gao et al., 2000). The hydrophilic residue at 7.42 (Thr in A₁ and Ser in A_{2A} receptors; also see Fig. 3) when mutated to Ala caused a significant loss of affinity for agonists while having little effect on antagonist binding (Townsend-Nicholson and Schofield, 1994; Kim et al., 1995). The hydrophobic residue at 7.35 in the A₁ receptor (Ile in bovine and Met in canine) appeared to correlate with the species-related differences in agonist pharmacology (Tucker et al., 1994). Mutation of an Asn at 7.36 of the A_{2B} receptor to Tyr, the homologous residue at the other subtypes, selectively enhanced the affinity of 2-substituted agonists (Beukers et al., 2000).

TM3 in the A_{2A} receptor contains a sequence of four hydrophilic amino acids, of which the first two (Thr at 3.36 and Gln at 3.37) when mutated had major effects on ligand affinity (Jiang et al., 1996). An enhancement of affinity for N⁶-substituted adenosine derivatives (140-fold for IB-MECA) in the Gln to Ala mutant appeared to correlate inversely with size of the amino acid side chain. At the A₁ receptor, mutation of the identical residues at 3.36 and 3.37 (Fig. 2) to Ala impaired agonist binding and caused a structure-dependent reduction of antagonist affinity (Rivkees et al., 1999a).

Modulatory residues have also been found in TM1, including a Glu residue (1.39) in both A₁ and A_{2A} receptors, which affects agonist binding selectively (Barbhaiya et al., 1996; IJzerman et al., 1996). A Gly to Thr mutation in TM1 (1.37) of the A₁ receptor increases agonist affinity (Rivkees et al., 1999a). At the A_{2B} receptor, mutation at the same position had no functional consequences (Beukers et al., 2000). A conserved Asp residue in TM2 (2.50) is the site of binding of Na⁺, which regulates agonist affinity (Barbhaiya et al., 1996). No mutations of TM4 in any of the adenosine receptors have yet been found to affect ligand binding.

Negatively charged residues in the second extracellular loop of the A_{2A} receptor have been found to be required for binding of both agonist and antagonist (Kim et al., 1996). Among nine native Cys residues of the human A₁ receptor, only a single pair, Cys80 and Cys169, were found to be essential for ligand binding (Scholl and Wells, 2000). This pair corresponds to Cys residues conserved among rhodopsin-like GPCRs, which form a disulfide bridge required for the structural integrity of the receptor.

VII. Distribution

It is important to study the distribution of receptors, because this will tell us where agonists and antagonists given to the intact organism can act. Furthermore, in general, the higher the number of receptors the more potent and/or efficacious will be the agonist. Thus, the rather low levels of endogenous adenosine present under basal physiological conditions have the potential of activating receptors where they are abundant, but not where they are sparse (Kenakin, 1993, 1995; Svenningsson et al., 1999c; Kull et al., 2000b; Fredholm et al., 2001).

There is much information on the distribution of the A₁ and A_{2A} receptors because good pharmacological tools including radioligands (see below) are available. There are also several studies that have used antibodies to localize adenosine A₁ receptors in brain (Swanson et al., 1995; Saura et al., 1998; Middlekauff et al., 1998) and A_{2A} receptors in striatum (Rosin et al., 1998; Hettlinger et al., 2001), carotid body (Gauda et al., 2000), and T cells (Koshiba et al., 1999). In the case of the A_{2B} and A₃ receptors, the data are less impressive. Here one tends to rely on data on the expression of the corresponding mRNA. Some of this information is summarized in Table 5. The results presented there clearly show that there is much left to examine regarding the distribution of especially A_{2B} and A₃ receptors. Furthermore, it is likely that a better understanding of the transcriptional regulation (see above) will be of considerable help in understanding the spatio-temporal aspects of adenosine receptor distribution.

Receptor protein and the corresponding message are often colocalized but there are important differences. For example, in several regions of the central nervous system, receptor binding and expression of transcript do not exactly match (Johansson et al., 1993a), and the two are differently regulated by e.g., long term antagonist treatment (Johansson et al., 1993a) and during development (Ådén et al., 2000, 2001). Much of the differential distribution can probably be explained by the fact that a substantial number of adenosine A₁ receptors are present at nerve terminals. A similar explanation probably underlies the observations that A_{2A} receptors are present in globus pallidus, despite the fact that

A_{2A} receptor mRNA cannot be detected there (Svenningsson et al., 1997, 1999c). These receptors are probably located at the terminals of the striatopallidal GABAergic neurons (Rosin et al., 1998; Svenningsson et al., 1999b; Linden, 2001).

Besides regulation at the level of gene transcription, targeting of the receptor protein to different locations within the cell is crucial. This important aspect of receptor distribution is just starting to be explored in the case of adenosine receptors. Recently it was found that in MDCK cells (a canine kidney cell line) the adenosine A₁ receptor is targeted to the apical surface, whereas the α -adrenoceptor, which is also G_i coupled, is directed to the basolateral surface (Saunders et al., 1996). The distribution is highly dependent on the third intracellular loop and/or the carboxyl-terminal segment of the receptor as judged by the distribution of receptor chimeras (Saunders et al., 1998). Interestingly, the apical distribution of adenosine A₁ receptors was disrupted by agents that interfere with microtubules, whereas the basolateral distribution of α adrenergic receptors was not (Saunders and Limbird, 1997). Thus, G protein-coupled receptors appear to use several different targeting mechanisms. This is also borne out by the fact that distribution of these receptors in the kidney epithelial cell line does not predict distribution in neurons (Wozniak and Limbird, 1998).

VIII. Classification of Adenosine Receptors Using Pharmacological Tools

Since adenosine receptors have been studied for a long time there are several useful pharmacological tools (Table 6). The structures of some typical drugs used in receptor classification are shown in Fig. 4. Data on their binding to human and rat receptors are presented in Tables 7 and 8. These tables contain information on some of the most widely used compounds, but a large number of other compounds have also been examined over the years using more or less selective functional assay systems (see Section XI.). Here we will just comment on a few points of more general interest not contained in the tables.

Ideally, agonists and antagonists should differ in potency by at least two orders of magnitude at different receptors to be really useful in receptor classification. It is apparent from Tables 7 and 8 that this is rarely the case for any of the compounds often used in classifying adenosine receptors. Nevertheless, with a judicious use of agonists and antagonists at A₁, A_{2A}, and A₃ receptors in in vitro experiments, strong conclusions can be drawn. The situation is less fortunate in vivo as the pharmacokinetics of these compounds have not been studied extensively. It has been found, however, that the A₁-selective agonist CPA has a terminal half-life in conscious rats of 6 min in blood (Mathôt et al., 1993), whereas the half-life of the A₃-selective agonist CI-IB-MECA is significantly longer, i.e., 62 min (Van Schaick et al., 1996). The relatively short half-life of CPA may be due to its substantial uptake into erythrocytes (Pavan and IJzerman, 1998).

In the case of human, rat, and mouse A₁ receptors the full agonist CCPA (and to a somewhat lesser extent CPA) and the antagonist DPCPX are quite useful. A minor problem with DPCPX is that it also interacts with appreciable affinity with A_{2B} receptors. This does not appear to be a major problem, however, since binding of DPCPX is virtually eliminated in animals lacking the A₁ receptor (Johansson et al., 2001). For the adenosine A₁ receptor, partial agonists are available as well. Careful chemical manipulation of the CPA molecule

yielded such compounds, for instance by substitution at the C8-position (Roelen et al., 1996) or by modification of the ribose 5'-substituent (van der Wenden et al., 1998). These compounds showed tissue selectivity *in vivo*, exploiting differences in receptor density, and in the efficiency of receptor coupling to further signal transduction (Mathot et al., 1995; van Schaick et al., 1998).

Another interesting class of compounds acting on adenosine A₁ receptors are the so-called allosteric enhancers. The prototype here is PD81723 (Bruns and Fergus, 1990), which has been shown by various research groups to (allosterically) increase agonist binding and effect (e.g., Linden, 1997). In recent years, analogs of PD81723 have been synthesized that show similar effects (van der Klein et al., 1999; Baraldi et al., 2000b).

NECA was long considered to be a selective adenosine A₂ receptor agonist, but as seen from the tables this view can no longer be upheld. Based on evidence that 2-substitution of NECA increased selectivity, CGS 21680 was developed as an A_{2A} receptor-selective agonist (Hutchison et al., 1989). However, in humans it is less potent and less selective than in rats (Kull et al., 1999). Indeed, there are clear-cut differences in the order of potency of agonists, but not antagonists, between human and rat A_{2A} receptors (Kull et al., 1999). There is an additional problem with CGS 21680 as a tool; it also binds to sites unrelated to A_{2A} receptors (Johansson et al., 1993b; Johansson and Fredholm, 1995; Cunha et al., 1996; Lindström et al., 1996). This means that at least in organs or cells with few A_{2A} receptors, effects of CGS 21680 must be viewed with skepticism. ATL146e was recently developed as a new A_{2A} agonist that is over 50 times more potent than CGS 21680 at the human receptor (Rieger et al., 2001). It has strong *in vivo* effects on e.g., reperfusion injury in the rabbit lung (Ross et al., 1999) and the rat kidney (Okusa et al., 1999), and reduces expression of adhesion molecules on the reperfused vascular endothelium (Okusa et al., 2000). There are several useful A_{2A} receptor antagonists. The most selective so far is SCH 58261. The structurally related ZM 241385 is more readily available (Poucher et al., 1995), but shows appreciable affinity to A_{2B} receptors (Ongini et al., 1999).

The adenosine A_{2B} receptor has low affinity for most agonists. For the other receptors, agonists with potency in the low nanomolar range are available, but in the case of A_{2B} receptors the most potent agonists have affinities only marginally below 1 μ M. Furthermore, selectivity is negligible. The situation is somewhat more favorable in the case of antagonists, where some potent and relatively selective antagonists have been found (Kim et al., 2000).

The most recently discovered adenosine receptor—the A₃ receptor—is notably insensitive to several xanthines. Hence, most A₃ antagonists have a nonxanthine structure, including dihydropyridines, pyridines, and flavonoids (Baraldi et al., 2000a). Isoquinoline and quinazoline derivatives constitute another class of highly selective human A₃ receptor antagonists. VUF8504 (4-methoxy-*N*-[2-(2-pyridinyl)quinazolin-4-yl]benzamide) was the first representative of this class, with a *K_i* value of 17 nM (van Muijlwijk-Koezen et al., 1998). Later, VUF5574 (*N*-(2-methoxyphenyl)-*N*-(2-(3-pyridyl)quinazolin-4-yl)urea) proved even more potent, with a *K_i* value of 4 nM, being at least 2500-fold selective versus A₁ and A_{2A} receptors (van Muijlwijk-Koezen et al., 2000). One of the most selective

compounds (for human A₃ receptors) is MRE-3008-F20, which is also a useful antagonist radioligand at human A₃ receptors (Varani et al., 2000).

Species differences in the affinity of adenosine receptor ligands, especially antagonists, have been noted. For example, 8-phenylxanthines such as XAC that are selective for A₁ receptors in the rat are less selective in the human, due to a decrease in the A₁ affinity and a concomitant rise in the A_{2A} receptor affinity. As expected from the structural data (see above), species differences in pharmacology are most marked for ligands at the A₃ receptor. In general, the affinity at A₃ receptors of most xanthines and other classes of antagonists is highly species-dependent, and the affinity at human receptors is typically >100-fold greater than that at rat receptors. While MRS 1523 is an A₃ antagonist of broad applicability to various species, both MRS 1220 and MRE-3008-F20 are extremely potent in binding to the human but not rat A₃ receptors and should be used cautiously in nonprimate species. In the rat, MRS 1220 is selective for the A_{2A} receptor. In contrast, the affinity of the A₃ agonist Cl-IB-MECA typically does not vary beyond an order of magnitude between species examined. Nevertheless, one must caution against receptor classification based on pharmacological tools alone, especially in nonmammalian species where the receptors have not been cloned and characterized. Despite the fact that there is much interest in mouse adenosine receptors owing to the development of several mouse strains with targeted deletions, the information on mouse adenosine receptor pharmacology is deficient. In one study potencies of selected agonists and antagonists were virtually identical at mouse and rat A₁ receptors (Maemoto et al., 1997).

IX. Signaling

A. G Protein Coupling

The adenosine A₁ and A₂ receptors were initially subdivided on the basis of their inhibiting and stimulating adenylyl cyclase, respectively (van Calker et al., 1979; Londos et al., 1980a). Indeed, A₁ and A₂ receptors are coupled to G_i and G_s proteins, respectively (Table 9). The A₃ receptor is also G_i coupled. In addition there is some evidence that the adenosine receptors may signal via other G proteins (Tables 1 and 9). However, much of the data on coupling to other G proteins are from transfection experiments and it is not known if such coupling is physiologically important. Recently, evidence was presented that the A_{2A} receptor may be coupled to different G proteins in different areas (Kull et al., 2000a). In most peripheral tissues, the receptor subtype is coupled to G_s. However, in striatum, where the brain A_{2A} receptors are enriched, G_s is very sparse. Here the dominant G protein of the class is instead G_{oif}. Indeed, immunoprecipitation experiments show that A_{2A} receptors and G_{oif} are associated with each other in the medium-sized spiny neurons of striatum.

One adenosine receptor may also be coupled to more than one G protein. This is common after transfection. Furthermore, endogenous A_{2B} receptors of HEK 293 cells, human HMC-1 mast cells and canine BR mast cells are dually coupled to G_s and G_q (Auchampach et al., 1997; Linden et al., 1999).

B. Second Messengers and Signals

After activation of the G proteins, enzymes and ion channels are affected as can be predicted from what is known about G protein signaling (see Tables 1 and 9). Thus, A₁ receptors mediate inhibition of adenylyl cyclase, activation of several types of K⁺-channels (probably via β, γ -subunits), inactivation of N-, P-, and Q-type Ca²⁺ channels, activation of phospholipase C β , etc. The same appears to be true for A₃ receptors. In CHO cells transfected with the human A₃ adenosine receptor both adenylyl cyclase inhibition and a Ca²⁺ signal are mediated via a G_{i/o}-dependent pathway (Klotz et al., 2000). Given that many of the steps in the signaling cascade involve signal amplification, it is not surprising that the position of the dose-response curve for agonists will depend on which particular effect is measured. For example, it was recently found that the so called receptor reserve in DDT₁ MF-2 cells appears very different depending on whether G protein activation or cAMP accumulation is measured (Baker et al., 2000). Both A_{2A} and A_{2B} receptors stimulate the formation of cAMP, but other actions, including mobilization of intracellular calcium, have also been described. Actions of adenosine A_{2A} receptors on neutrophil leukocytes are due in part to cAMP (Fredholm et al., 1996; Fredholm, 1997; Sullivan et al., 2001), but cAMP-independent effects of A_{2A} receptor activation in these cells have also been suggested (Cronstein, 1994).

C. Adenosine Receptor-Mediated Changes in Cell Proliferation and in Mitogen-Activated Protein Kinase Activation

It was shown more than 15 years ago that adenosine A₁ and A_{2B} receptors could regulate proliferation and differentiation in vascular smooth muscle cells (Jonzon et al., 1985). Since then several examples of such effects have been described, and they may be related to changes in mitogen-activated protein kinases (MAPK), which play an essential role in processes such as cell differentiation, survival, proliferation, and death. The family of MAPK consists of the extracellular regulated kinases (ERK) such as ERK1/2, and the stress-activated protein kinases (SAPK), such as p38 and jun-N-terminal kinase (JNK). These kinases, usually activated via receptor tyrosine kinases (Seger and Krebs, 1995), have also been shown to be activated by G protein-coupled receptors.

Adenosine A₁ receptors transiently expressed in COS-7 cells can activate ERK1/2 via β, γ -subunits released from pertussis toxin-sensitive G proteins G_{i/o} (Faure et al., 1994). Studies in CHO cells stably expressing the human A₁ receptor later showed that the activation of ERK1/2 by A₁ receptors is time- and dose-dependent (Schulte and Fredholm, 2000) and sensitive to the phosphoinositol-3-kinase inhibitors wortmannin and LY 294002 (Dickenson et al., 1998). Although speculative, this may imply a receptor tyrosine kinase transactivation as described for the epidermal growth factor (Daub et al., 1997).

Activation of A_{2A} receptors also increases MAPK activity. Adenosine agonists exerting mitogenic effects on human endothelial cells via the adenosine A_{2A} receptor activate ERK1/2 using the cAMP-ras-MEK1 pathway (Sexl et al., 1997). However, the signaling pathways used by the A_{2A} receptor seem to vary with the cellular background and the signaling machinery that the cell possesses. Thus, the A_{2A} receptor-mediated ERK1/2 activation in CHO cells is dependent on G_s-cAMP-PKA-*rap1*-p68 B-*raf*-MEK1. On the

other hand, the A_{2A} receptor-mediated activation in HEK 293 cells involves PKC, ras, and sos, but not G_s, cAMP, or PKA, even though cAMP levels do rise in a G_s-dependent manner (Seidel et al., 1999).

A_{2A} receptor activation may not only stimulate, but also inhibit ERK phosphorylation. Activation of guinea pig A_{2A} receptors expressed in CHO cells inhibited thrombin-induced ERK1/2 activation (Hirano et al., 1996). This inhibition was cAMP- and wortmannin-sensitive, implying that the nonselective adenosine analog NECA affects the two distinct pathways leading from the thrombin receptor to MAPK. In PC12 cells, activation of endogenously expressed A_{2A} receptors inhibits nerve growth factor (NGF)-induced ERK1/2 phosphorylation (Arslan et al., 1997), even though activation of these receptors alone (i.e., in the absence of NGF) can lead to an activation (Arslan and Fredholm, 2000).

The adenosine A_{2B} receptor is the only subtype that so far has been shown to activate not only ERK1/2 but also JNK and p38. In human mast cells (HMC), adenosine receptor activation leads to a time- and dose-dependent activation of ERK1/2 with a maximal degree of phosphorylation at 5 min, whereas p38 and JNK show a different kinetic profile with maximal phosphorylation at 1 and 10 to 15 min, respectively (Feoktistov et al., 1999). Adenosine A_{2B} receptor-mediated activation of MAPK is relevant for IL-8 secretion and consequently for mast cell activation. In untransfected HEK 293 cells, a cascade depending on G_{q/11}, PLC, genistein-insensitive tyrosine kinases, ras, B-raf, and MEK1/2 has been delineated (Gao et al., 1999). NECA concentrations used in these studies of endogenous HEK 293 A_{2B} receptors revealed the same potency in activating ERK and adenylyl cyclase (EC₅₀ values in the micromolar range) whereas results from another study in transfected cells (Schulte and Fredholm, 2000) show a nearly 100-fold higher potency of both NECA and adenosine in inducing ERK1/2 phosphorylation than in inducing cAMP production. The EC₅₀ value for ERK1/2 phosphorylation in transfected CHO lies in the nanomolar range, whereas cAMP production is half-maximally activated around 1 to 5 μM NECA. Thus, a G protein-coupled receptor can have substantially different potencies on different signaling pathways in the same cellular system.

It was recently reported that, in addition to binding adenosine and adenosine analogs, the A_{2B} receptor in complex with another protein, DCC (deleted in colorectal cancer), may bind netrin-1, a protein that is involved in controlling axon elongation, and that netrin effects depend on the presence of the A_{2B} receptor (Corset et al., 2000). These results have, however, recently been contested (Stein et al., 2001). Nevertheless, it seems possible that the A_{2B} receptors play very important roles in cell proliferation and/or differentiation. These effects may not only be stimulatory, however. In vascular smooth muscle cells, activation of A_{2B} receptors strongly decreases the mitogenic effects of different growth factors (Jonzon et al., 1985; Dubey et al., 1996, 1998), probably secondary to a blockade of MAP kinases (Dubey et al., 2000) stimulated by these growth factors.

The adenosine A₃ receptor has been suggested to activate ERK1/2 in human fetal astrocytes (Neary et al., 1998). A recent study, indeed, shows a clear activation of ERK1/2 via the human A₃ receptor expressed in CHO cells (Schulte and Fredholm, 2000). Both NECA and the endogenous agonist adenosine lead to a time- and dose-dependent increase in ERK1/2

phosphorylation already at concentrations as low as 10 to 30 nM. The A₃ receptor agonists CI-IB-MECA and IB-MECA have been reported to potently inhibit and less potently to activate apoptosis in various cells (Abbracchio et al., 1997). In RBL-2H3 mast-like cells, CI-IB-MECA potently blocks UV irradiation-induced apoptosis by a process that correlates with protein kinase B phosphorylation and is blocked by pertussis toxin and wortmannin (Gao et al., 2001).

Thus, the adenosine receptor-mediated activation of MAPK is similar to that encountered in the remainder of the field of GPCR-mediated MAPK activation (Gutkind, 1998; Sugden and Clerk, 1998; Luttrell et al., 1999). The common feature of all adenosine receptors, however, is the positive coupling to ERK1/2 even though the classical cAMP/PKA pathway is both activated (A₂) and inhibited (A_{1/3}). Depending on the cellular background the required signaling elements vary widely, although activation of one of the small GTP-binding proteins p21^{ras} and rap1 is essential.

D. Interactions with Other Receptor Systems

Adenosine is believed to play modulatory roles in a variety of tissues and physiological circumstances. Adenosine is, as discussed above, not primarily released in a transmitter- or hormone-like fashion, but instead it appears to be formed by groups of cells as part of a response, e.g., to challenges in energy metabolism. It can also be formed by breakdown of ATP released by cells either in a regulated fashion or in response to massive trauma. Adenosine is therefore likely to act in concert with several other messengers (transmitters, hormones, growth factors, autacoids). It is outside the scope of this type of review to delineate all such interactions and a few examples will have to suffice.

Adenosine can, as noted above, activate phospholipase C via adenosine A₁ receptors and a G_i-dependent mechanism. Interestingly, adenosine acts synergistically with nucleotides, such as ATP or UTP (Gerwins and Fredholm, 1992a), histamine (Dickenson and Hill, 1993), or with bradykinin (Gerwins and Fredholm, 1992b). ATP, histamine, and bradykinin instead act via G_{q/11}. The interaction was believed to involve β, γ -subunits released from G_i proteins by A₁ receptors, and $\alpha_{q/11}$ -subunits derived via the other receptor, somehow acting synergistically at the level of PLC. The involvement of β, γ -subunits has been confirmed (Dickenson and Hill, 1998). More recently, good evidence was provided that the mechanism involved exchange of β, γ -subunits between the two G protein pathways (Quitterer and Lohse, 1999), but other mechanisms cannot be excluded. It was also recently shown that activation of PLC by G_i-coupled receptors requires that the enzyme is already activated (Chan et al., 2000), e.g., by ATP. Given that ATP is rapidly broken down to adenosine and that ATP and adenosine act synergistically, we may be dealing with a biologically quite important mechanism.

In the brain there is evidence of important interactions between adenosine A₁, dopamine D₁, and NMDA receptors. First, it is known that activation of NMDA receptors can increase the release of adenosine (Hoehn et al., 1990; Pedata et al., 1991; Chen et al., 1992; Manzoni et al., 1994; Delaney et al., 1998; Delaney and Geiger, 1998). Second, it is well known that adenosine, by activating presynaptic receptors, can decrease the release of glutamate, and thereby the activation of NMDA receptors (Fredholm and Hedqvist, 1980; Fredholm

and Dunwiddie, 1988; Poli et al., 1991; Dunwiddie and Fredholm, 1997). In addition, activation of adenosine A₁ receptors reduces the NMDA currents by a postsynaptic action (de Mendonca and Ribeiro, 1993; de Mendonca et al., 1995) and reduces toxicity (Finn et al., 1991). A third interaction is that dopamine D₁ and NMDA receptors act synergistically in the brain, e.g., on the expression of immediate early genes (Berretta et al., 1992) and several long-term effects of dopamine D₁ activation can be blocked by antagonizing NMDA receptors (Wolf et al., 1994; Konradi et al., 1996). However, this synergistic interaction differs between regions (Keefe and Gerfen, 1996). Part of this enhancement may depend on the ability of dopamine D₁ agonists to phosphorylate DARPP-32, which in turn acts to decrease the dephosphorylation of NMDA receptors, leading to their remaining in an active conformation (Blank et al., 1997). Activation of NMDA receptors conversely leads to decreased DARPP-32 phosphorylation (Halpain et al., 1990). A fourth interaction is between dopamine D₁ receptors and adenosine A₁ receptors, which have been shown to act antagonistically (Ferré et al., 1994b, 1998; Popoli et al., 1996). All these interactions may be functionally connected (Harvey and Lacey, 1997). Thus, dopamine D₁ activation has been shown to reduce release of glutamate by enhancing NMDA activity and thereby release of adenosine, which is the agent that acts at presynaptic A₁ receptors.

Adenosine A_{2A} receptors are highly enriched in the basal ganglia of several species including human. There they are present in highest abundance on a subset of the GABAergic output neurons, namely those that project to the globus pallidus (Schiffmann et al., 1991; Fink et al., 1992; Svenningsson et al., 1997; Rosin et al., 1998). These neurons also express dopamine D₂ receptors and it is abundantly clear that the two receptors interact in binding assays (Ferré et al., 1991), on signal transduction (Kull et al., 1999), and behaviorally (Fink et al., 1992; Fredholm et al., 1999; Svenningsson et al., 1999b). It appears that one major role of dopamine in the striatum is to suppress signaling via A_{2A} receptors (Svenningsson et al., 1998b, 1999a; Chen et al., 2001). Given that antagonists of D₂ receptors are used in the treatment of schizophrenia, there are interesting implications for the pathophysiology of that disease. Adenosine A_{2A} and dopamine D₂ receptors also are colocalized on arterial chemoreceptors of the carotid body (Gauda et al., 2000). Carotid sinus nerve activity is augmented by adenosine binding to adenosine A_{2A} receptors and attenuated by dopamine binding to dopamine D₂ receptors.

Adenosine A_{2B} receptors are present on many cells, although in low abundance. It has been repeatedly found that signaling via the A_{2B} receptor is strongly influenced by concurrent signaling via other receptors that affect PLC-Ca²⁺-PKC. However, the type of interaction varies from cell to cell. In brain slices the cAMP accumulation mediated by activation of A_{2B} receptors is markedly enhanced by drugs that stimulate PKC (Hollingsworth et al., 1985; Fredholm et al., 1987b; Nordstedt and Fredholm, 1987). Since cAMP accumulation in brain slices predominantly reflects events in glial cells, it seems likely that this is the target cell for the interaction. In glial cells the positive effect of PKC activation can, however, be counteracted by an inhibitory effect of strong increases in intracellular calcium (Altiok et al., 1992; Altiok and Fredholm, 1992, 1993; Balmforth et al., 1992). A similar effect is seen in lymphocytes (Fredholm et al., 1987a; Nordstedt et al., 1987, 1989; Kvanta et al., 1989). In these cells it could be shown that the stimulatory effect of PKC activation predominantly occurs not at the receptor itself but at a signaling step after the receptor.

Numerous other examples of interaction between adenosine receptors and other receptors have been reported, and such interactions are likely to be the rule rather than the exception. This has important consequences. For example, if two receptors act synergistically then blockade of either receptor will virtually abolish the response. This can easily be erroneously interpreted as evidence that the receptor that is blocked is solely responsible for the response studied. Another important case should also be mentioned. Sometimes more than one adenosine receptor is expressed on a single cell. The resultant response will therefore be a mixed one and consequently pharmacology may be quite atypical.

X. Receptor Regulation

Exposure of any GPCR to agonists for shorter or longer times generally leads to the attenuation of the agonist response (for recent reviews, see Krupnick and Benovic, 1998; Lefkowitz, 1998; Pitcher et al., 1998; Ferguson, 2001). Adenosine receptors are no exception (Olah and Stiles, 2000). However, the magnitude of this response and the mechanisms involved seem to vary between the adenosine receptor subtypes. For example, there are major differences between the two $G_{i/o}$ coupled receptors, A_1 and A_3 , when stably transfected into CHO cells (Palmer et al., 1996). Whereas the A_1 receptor appears to desensitize slowly and incompletely, the A_3 receptor desensitizes very rapidly. This difference may be due to differences in the ability of G protein receptor kinases to phosphorylate the two receptors. However, there may be additional factors to consider. Thus in DDT₁ MF-2 cells, which constitutively express A_1 receptors, desensitization is slow when cyclase responses are studied (Ramkumar et al., 1991), but have been reported to be rapid when, instead, phospholipase C activation is examined (Ciruela et al., 1997). In this case, phosphorylation mediated via other kinases might be involved. The receptor has also been reported to internalize within half an hour of agonist stimulation together with adenosine deaminase (Saura et al., 1996). However, many questions remain to be solved in the case of receptor regulation in DDT₁ MF-2 cells, and not all reports are consistent (Olah and Stiles, 2000).

Recently, it was found that agonist treatment can rapidly translocate adenosine A_1 receptors out of caveolae, providing another type of explanation for altered receptor signaling (Lasley et al., 2000). In addition, using still other types of cells or examining the situation in vivo, several groups have described a more or less rapid decrease in receptor number following agonist administration (Green, 1987; Longabaugh et al., 1989; Green et al., 1990; Fernandez et al., 1996; Hettinger et al., 1998). These changes are not accompanied by changes in the mRNA. Furthermore, antagonist treatment has long been known to up-regulate adenosine A_1 receptors (Fredholm, 1982), even though other adenosine receptors are unaffected. Thus, we still do not understand the factors that are important in regulating adenosine A_1 and A_3 receptors under physiological and pathophysiological conditions.

Relatively little is known about desensitization of A_{2B} receptors, except that desensitization does occur in different cell lines (Mundell and Kelly, 1998; Peters et al., 1998; Haynes et al., 1999). The mechanism may involve G protein receptor kinases. A_{2A} receptors coupled to G_s proteins are believed to desensitize rapidly (Ramkumar et al., 1991; Chern et al., 1993, 1995; Palmer and Stiles, 1997). Several mechanisms, including phosphorylation of

Thr298 via receptor kinases, and phosphorylation via cAMP-dependent kinases have been implicated. Nevertheless, there is good evidence that under in vivo conditions A_{2A} receptors on leukocytes can be tonically activated by endogenous adenosine (Cronstein, 1994; Fredholm, 1997). Similarly, there is excellent evidence that A_{2A} receptors on striatopallidal neurons are tonically activated by endogenous adenosine (Svenningsson et al., 1995, 1999a, 2000). Conversely, when tonic adenosine A_{2A} receptor activation is unopposed by dopamine for a long period of time, the response to agonist stimulation decreases (Zahniser et al., 2000), even though significant effects remain (Chen et al., 2001). This is not due to any change in the receptor number, however. Thus, desensitization of adenosine A_{2A} receptors, via whatever mechanism, may be important mainly under experimental or pathological situations.

XI. Assay Systems

Adenosine receptors and their ligands may be investigated in binding and functional assays. The first useful adenosine analogs *R*-PIA and NECA were developed using assays in intact animals. Specifically, blood pressure, heart rate, and body temperature were some of the readouts (Vapaatalo et al., 1975; Raberger, 1979). Studies using changes in cAMP in fat cells, liver, Leydig cells, brain homogenates, and cultured brain cells were instrumental in the definition of A₁ (R_i) and A₂ (R_s) receptors (van Calker et al., 1978, 1979; Londos et al., 1980b). Binding assays became available in the early 1980s, but functional assays continued to play an important role. For example, studies on coronary blood flow in dogs were used to screen and develop a large number of compounds acting at A_{2A} receptors (Daly et al., 1986). Even today several functional assays remain very useful, not least because they examine receptors associated with the natural signaling pathways. For example, A₁ receptor-mediated inhibition of adenylyl cyclase can be studied in fat cells (Fredholm, 1978; Londos et al., 1978; Longabaugh et al., 1989) whereas platelet membranes is a preferred system for stimulation of adenylyl cyclase via A_{2A} adenosine receptors (Klotz et al., 1985). A_{2B} receptors have been examined using fibroblasts (Bruns, 1980, 1981) and rat aorta (Collis and Brown, 1983; Poucher et al., 1995).

For a long time rat brain membranes were the standard system for examining binding to A₁ (whole brain; Bruns et al., 1980; Schwabe and Trost, 1980) and A_{2A} receptors (striatum; Bruns et al., 1986). Now cell lines stably transfected with the receptor subtype of interest have become the standard systems for the characterization of ligands, as they have several major advantages (Klotz et al., 1998). Transfected cells allow for the comparative characterization of receptor subtypes from different species including humans. With the expression of single receptor subtypes, cross-reaction of nonselective ligands with receptors not under investigation does not occur. In addition, receptors like the A₃ subtype that occur naturally only at low expression levels become available for in vitro studies. Cellular models with transfected receptors may also be used for the characterization of effector coupling. All the typical second messenger responses for the adenosine receptor subtypes have been identified in CHO cells stably transfected with the human subtypes. However, these results may not represent the physiological situation because the receptor density and the availability of G proteins and other components necessary for signal generation may be different in artificial cellular models.

XII. Physiological Roles—Therapeutic Potential

Recently, genetically modified mice have been generated that provide insights into the physiology and pathophysiology of the different adenosine receptors. The adenosine A_{2A} receptor was first knocked out. The group in Brussels that first cloned the adenosine receptors also generated the first knock-out mice, in which the first coding exon of the A_{2A} receptor was targeted (Ledent et al., 1997). Later a group in Boston targeted the second exon (Chen et al., 1999). Using these mice has shown that A_{2A} receptors play a role in mediating pain via peripheral sites, inhibiting platelet aggregation and regulating blood pressure (Ledent et al., 1997). A_{2A} receptors are also critically important for the motor stimulant effects of caffeine (Ledent et al., 1997; El Yacoubi et al., 2000). It has also been demonstrated that A_{2A} receptors contribute to ischemic brain damage in adult mice (Chen et al., 1999).

Mice with a targeted disruption of the A₃ receptor show a decreased effect of adenosine analogs on mast cell degranulation (Salvatore et al., 2000) and a consequent decrease in vascular permeability (Tilley et al., 2000). These animals also, surprisingly, show increased cardiovascular effects of administered adenosine (Zhao et al., 2000). Given the species differences in A₃ receptor distribution and pharmacology it is, however, not clear that the roles of this receptor are similar in humans.

Mice with a targeted disruption of A₁ receptors have also been generated (Johansson et al., 2001). These mice show increased anxiety and are hyperalgesic, indicating a role for A₁ receptors in mediating endogenous antinociception. In these animals, the effect of adenosine on excitatory neurotransmission is totally eliminated (Dunwiddie et al., 2000), and the neuronal response to hypoxia is markedly altered. They also lack tubuloglomerular feedback and have elevated renin levels (Brown et al., 2001; Sun et al., 2001). Interestingly, all these mice show essentially normal viability and fertility.

Adenosine protects tissues from ischemic damage in e.g., brain and heart through multiple receptor subtypes (Lasley et al., 1990; Marangos, 1990; Rudolphi et al., 1992a,b; Auchampach and Gross, 1993; Deckert and Gleiter, 1994; Fredholm, 1996). A₁ and possibly A₃ receptor activation produces preconditioning to protect the heart and other tissues from subsequent ischemic injury. Rapid preconditioning is mediated by a pathway including PKC and increased mitochondrial K-ATP channel activation (Sato et al., 2000). A late phase of preconditioning due to A₁ receptor activation in the rabbit heart is mediated in part by the induction of manganese superoxide dismutase (Dana et al., 2000).

Fishman and coworkers demonstrated that low doses of adenosine cause a strong inhibition of lymphoma cell proliferation. A similar effect was seen with low doses of IB-MECA, a somewhat selective agonist for A₃ receptors, whereas no such action was observed with the selective A₁ receptor agonist CCPA (Fishman et al., 2000b). The same research group showed that adenosine also acts as a chemoprotective agent by stimulating granulocyte-colony-stimulating factor production. In this case, a combined action via both A₁ and A₃ receptors appeared causal (Fishman et al., 2000a).

Activation of A_{2A} receptors protects tissues from injury by reducing inflammation during reperfusion following ischemia. CGS 21680 inhibits neutrophil accumulation and protects the heart from reperfusion injury (Jordan et al., 1997). However, similar cardiac protection has recently been attributed to A₃ receptor activation (Jordan et al., 1999).

The localization of A_{2A} receptors to the dopamine rich areas of the brain and the behavioral effects of methylxanthines suggested that antagonists at A_{2A} receptors might be useful as adjuvants to dopaminergic drugs in Parkinson's disease (Fredholm et al., 1976; Ongini and Fredholm, 1996; Ferré et al., 1997; Svenningsson et al., 1999b; Impagnatiello et al., 2000) as well as schizophrenia (Ferré et al., 1994a; Rimondini et al., 1997). This idea has gained additional support by the demonstration that the adenosine A_{2A} receptor exerts effects that are at least to some extent independent of dopamine acting at D₂ receptors (Svenningsson et al., 1995, 1998a, 1999a, 2000; Chen et al., 2001). The effect of an A_{2A} receptor antagonist is synergistic with dopamine receptor agonists in primate models of Parkinson's disease (Kanda et al., 2000). Rapid tolerance develops to the motor stimulant effects of caffeine, but no such tolerance is seen following long-term treatment with selective adenosine A_{2A} receptor antagonists (Halldner et al., 2000; Popoli et al., 2000; Pinna et al., 2001).

There is evidence that IgE antibodies and mast cells play a central role in the symptoms and pathology of asthma. Aerosolized adenosine has the effect of causing mast cell-dependent bronchoconstriction in asthmatic subjects, but causes bronchodilation in nonasthmatics (Cushley et al., 1983; Vilsvik et al., 1990). Moreover, the nonselective adenosine receptor antagonist theophylline is widely used as an antiasthmatic drug although its mechanism of action is uncertain. A related xanthine, enprofylline (3-propylxanthine) (Robeva et al., 1996; Jacobson et al., 1999), is also therapeutically efficacious in the treatment of asthma, and was thought to act through a nonadenosine receptor-mediated mechanism due to its low affinity at A₁ and A_{2A} receptors.

Recently, attention has shifted to the A_{2B} and A₃ receptor subtypes found on mast cells that, when activated, facilitate antigen-mediated mast cell degranulation. The adenosine A₃ receptor was initially implicated as the receptor subtype that triggers the degranulation of rat RBL 2H3 mast-like cells (Ramkumar et al., 1993) and perivascular mast cells of the hamster cheek pouch (Jin et al., 1997). There is also evidence of mast cell degranulation when agonists of A₃ receptors are administered to rats or mice (Fozard et al., 1996; Tilley et al., 2000). In contrast, the A_{2B} receptor has been implicated as the receptor subtype that facilitates the release of allergic mediators from canine and human HMC-1 mastocytoma cells (Feoktistov and Biaggioni, 1995; Auchampach et al., 1997). A role for A_{2B} receptors in human asthma is also suggested by the efficacy of enprofylline, which at therapeutic concentrations of 20 to 50 μ M, only blocks the A_{2B} receptor subtype (Fredholm and Persson, 1982; Linden et al., 1999). In sum, the literature indicates that the release of allergic mediators from mast cells is mediated by A₃ receptors and/or A_{2B} receptors. This may result from tissue difference and/or species differences, with rodent (rat, guinea pig, and mouse) responding primarily to A₃, and canine or human mast cells mainly to A_{2B} adenosine receptor stimulation.

It is remarkable that despite intensive efforts, relatively few adenosine receptor ligands have made it into clinical trials. Adenosine itself is being marketed in several countries, for two purposes. Adenocard (i.v.) restores normal heart rhythm in patients with abnormally rapid heartbeats originating in the upper chambers of the heart, so-called paroxysmal supraventricular tachycardia. Adenoscan (i.v.) is indicated as an adjunct to thallium cardiac imaging in the evaluation of coronary artery disease in patients unable to exercise adequately. In the 1970s, metabolically stable adenosine receptor agonists were tested clinically as antihypertensives. In the last decade GR79236 (*N*-[(1*S*, *trans*)-2-hydroxycyclopentyl]adenosine) has been tested in human volunteers for various indications such as adjuvant therapy in insulin resistance (type II diabetes) and, more recently, in primary headache (P. P. A. Humphrey, personal communication).

With respect to antagonists, it can be argued that caffeine, the most widely used psychotropic substance, has its main action via adenosine receptors. The same might hold true for theophylline and enprofylline, both used in the treatment of asthma. Currently, selective adenosine A₁ receptor antagonists are undergoing clinical trials. A recently concluded Phase II trial with BG9719 ((*S*)-1,3-dipropyl-8-[2-(5,6-epoxynorbonyl)]xanthine) was successful in treating acute renal failure in patients with congestive heart failure. This was seen as a critical factor for future development of a second-generation product, BG 9928. The A_{2A} adenosine antagonist KW6002 (1,3-diethyl-8-(3,4-dimethoxystyryl)-7-ethylxanthine) is undergoing clinical trials as an anti-Parkinson agent (F. Suzuki, personal communication). Other compounds of this class are also under development. Thus, adenosine receptors remain an attractive target for drug development.

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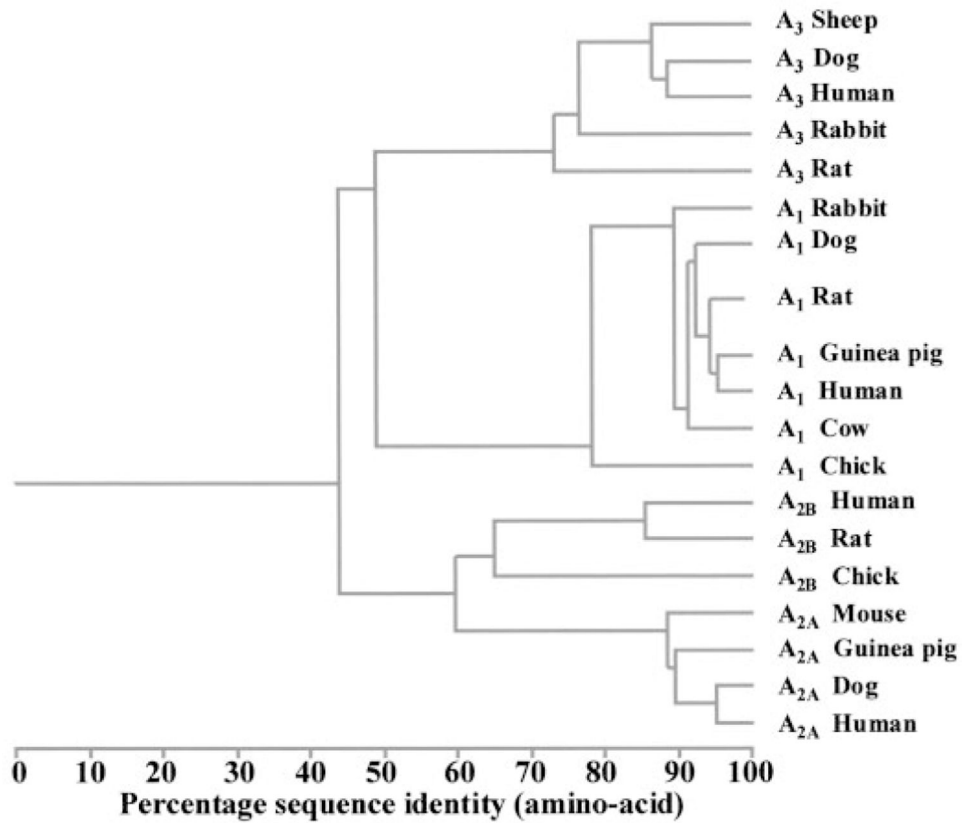


FIG. 1.

Dendrogram showing sequence similarity between cloned adenosine receptors.

The figure is slightly redrawn from that available at http://www.gpcr.org/7tm/seq/007_001/001_007_001.TREE.html. This phylogenetic tree was automatically calculated by WHAT IF based on a neighbor-joining algorithm.

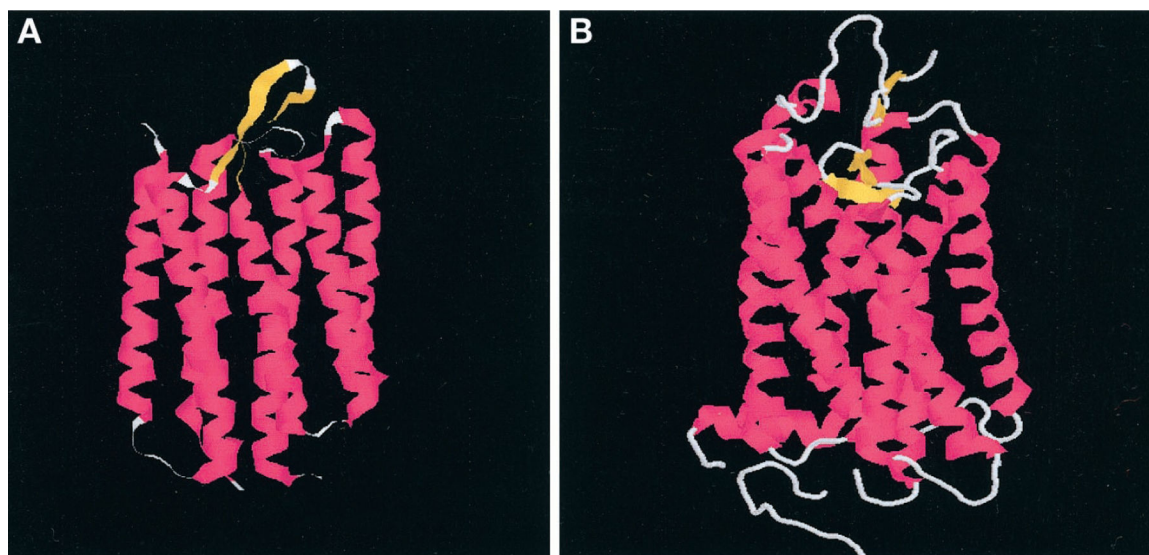


FIG. 2. Protein backbone representations of the structures of bacteriorhodopsin (A) and rhodopsin (B) as retrieved from the Brookhaven Protein Data Bank (1C3W and 1F88, respectively).

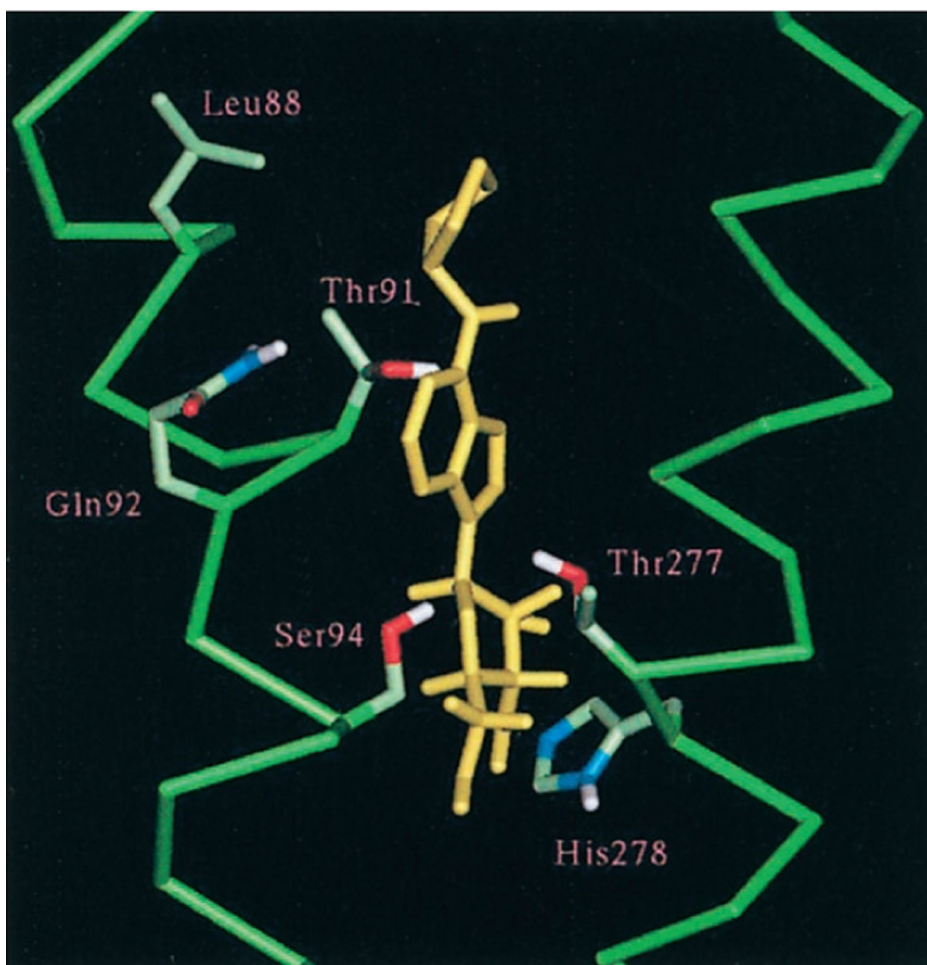


FIG. 3. Computer modeling of CPA (yellow), human A₁ adenosine receptor interactions. Only helices III (left) and VII (right) are shown (both in green) with relevant residues (half-bond colors).

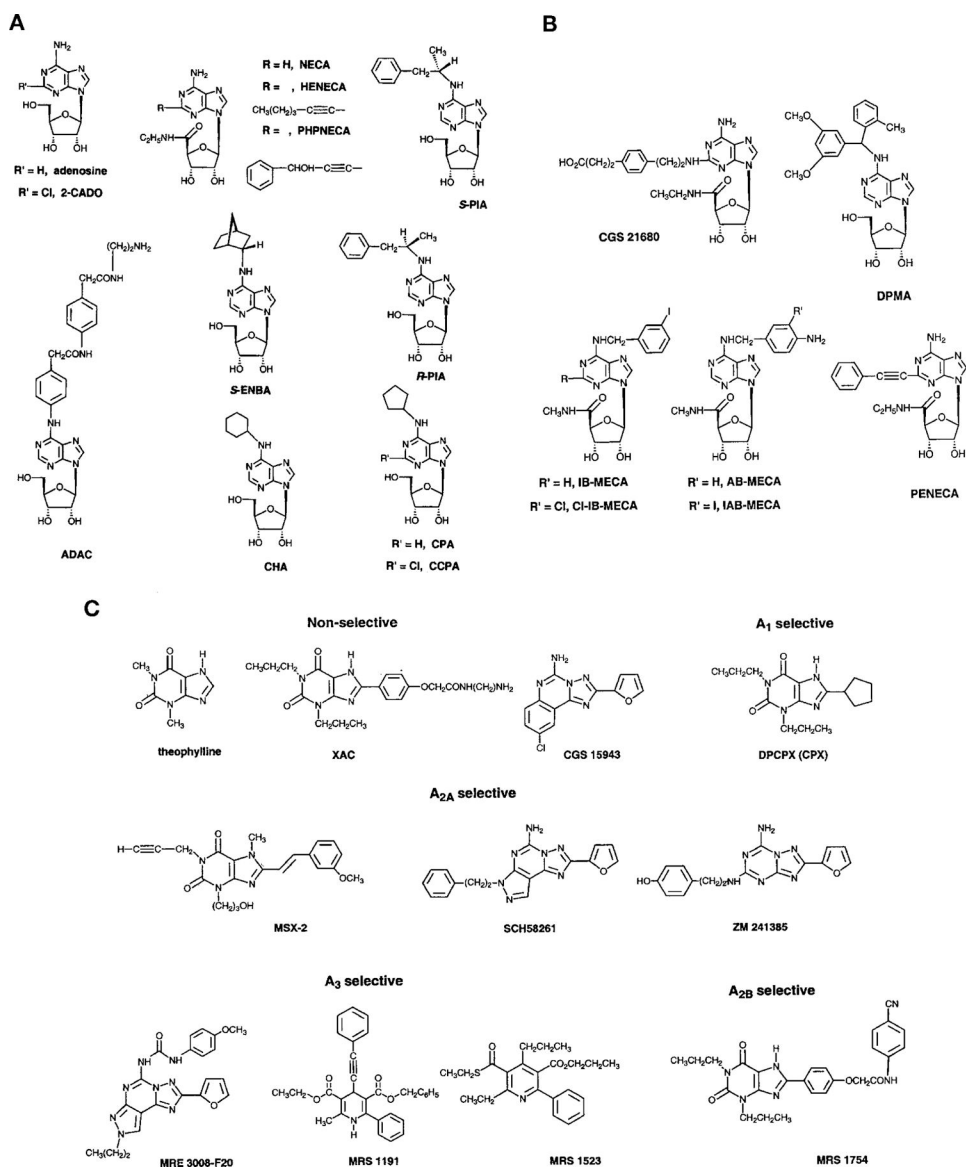


FIG. 4. Structures of reference compounds used to classify adenosine receptors. Panel A, structure of some non-selective and A₁ receptor-selective adenosine analogs; panel B, structure of adenosine analogs used to classify A_{2A} and A₃ receptors; panel C, structure of selected adenosine receptor antagonists.

TABLE 1

Current adenosine receptor nomenclature

| Receptor | A ₁ | A _{2A} | A _{2B} | A ₃ |
|------------------------|---|---|--|--|
| Receptor Code | 2.1:ADO: R _i | 2.1:ADO: A _{2A} , R _s | 2.1:ADO: A _{2B} , R _s | 2.1:ADO: |
| Previous names | | | | |
| Structural information | 7TM; human 326 aa, P30542, chr 1q32.1, rat 326 aa, P25099, mouse 326 aa | 7TM; human 410 aa, P29274, chr 22g11.2, rat 409 aa, P30543, mouse 409 aa, UO5672 | 7TM; human 328 aa, P29275, chr 17p11.2-12; rat 332 aa, P29276 mouse 332 aa, UO5673 | 7TM; human 318 aa, P33765, chr 1p21-13, rat 320 aa P28647 (see comments), mouse 320 aa, AF069778 |
| Selective agonists | CPA, CCPA, CHA | CGS 21680, HENECA, CV-1808, CV-1674, ATL146e | None | CI-IB-MECA |
| Selective antagonists | DPCPX ^a 8-cyclopentyl-theophylline, WRC0571 | selective: SCH 58261 ^{b,c} moderately selective: ZM241385, ^a KF 17387, CSC | MRS1754, ^d enprofylline, alloxazine (historical) | MRS 1220, ^e MRE 3008-F20, ^e MRS 1191; MRS 1523 |
| Tissue functions | Bradycardia; inhibition of lipolysis; reduced glomerular filtration; tubero-glomerular feedback, antinociception; reduction of sympathetic and parasympathetic activity; presynaptic inhibition; neuronal hyperpolarization; ischemic preconditioning | Regulation of sensorimotor integration in basal ganglia; inhibition of platelet aggregation and polymorphonuclear leukocytes; vasodilatation, protection against ischemic damage, stimulation of sensory nerve activity | Relaxation of smooth muscle in vasculature and intestine; inhibition of monocyte and macrophage function, stimulation of mast cell mediator release (some species) | Enhancement of mediator release from mast cells (some species). Preconditioning (some species) |
| Phenotypes | Tissue functions above confirmed in knockout mouse | Tissue functions above confirmed in knockout mouse | | Tissue functions above confirmed in knockout mouse |
| Comments | Also cloned from dog, ^f cow, ^g rabbit ^{i,j} | Also cloned from e.g. dog, ^h guinea pig. ^k Variations in structure among humans | | Alternative splicing in rat can yield product with 337 aa. ^l Also cloned from sheep ^m 317 aa, rabbit ⁿ 320 aa |

^aDPCPX and ZM241385 also have nanomolar affinity for the adenosine A_{2B} receptor.

^bDionisotti et al., 1997.

^cOngini et al., 1999.

^dKim et al., 2000.

^eLi et al., 1999; Baraldi et al., 2000b.

^fLibert et al., 1989, 1991.

^gTucker et al., 1992.

^hMaenhaut et al., 1990.

- i Bhattacharya et al., 1993.
- j Hill et al., 1997.
- k Meng et al., 1994.
- l Sajjadi et al., 1996.
- m Linden et al., 1993.

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TABLE 2

Mutational analysis of the adenosine A₁ with respect to ligand binding

| Mutation ^a | Species | Helix/position ^b | Effect on Ligand Binding and Signal Transduction | Reference |
|-----------------------|---------|-----------------------------|--|------------------------|
| G14T | H | 1.37 | Increased agonist affinity | Rivkees et al., 1999a |
| E16A/Q | H | 1.39 | Agonist affinity reduced 4- to 40-fold; little change antagonist affinity | Barbhaiya et al., 1996 |
| P25L | H | 1.48 | Modest reduction of agonist affinity | Rivkees et al., 1999a |
| I31C | H | 1.54 | No changes in radioligand binding | Rivkees et al., 1999a |
| C46A/S | H | 2.41 | No changes in radioligand binding | Scholl and Wells, 2000 |
| S50A | H | 2.45 | No changes in radioligand binding | Barbhaiya et al., 1996 |
| D55A | H | 2.50 | Increase in agonist affinity with no change in antagonist affinity; disrupted regulation of agonist binding by sodium ions | Barbhaiya et al., 1996 |
| L65F | H | 2.60 | No changes in radioligand binding | Rivkees et al., 1999a |
| C80A/S | H | 3.25 | No detectable radioligand binding | Scholl and Wells, 2000 |
| M82F | H | 3.27 | No changes in radioligand binding | Rivkees et al., 1999a |
| C85A | H | 3.30 | No changes in radioligand binding | Scholl and Wells, 2000 |
| C85S | H | | Agonist affinity reduced 4- to 13-fold; no change in antagonist affinity | Scholl and Wells, 2000 |
| P86F | H | 3.31 | Substantial reduction of agonist binding | Rivkees et al., 1999a |
| V87A | H | 3.32 | No change in ligand affinity | Rivkees et al., 1999a |
| L88A | H | 3.33 | Substantial reduction of agonist binding, but also of N-0840 antagonist binding | Rivkees et al., 1999a |
| T91A | H | 3.36 | Substantial reduction of agonist binding, but also of N-0840 antagonist binding | Rivkees et al., 1999a |
| Q92A | H | 3.37 | Substantial reduction of agonist binding, but also of N-0840 antagonist binding | Rivkees et al., 1999a |
| S93A | H | 3.38 | No changes in radioligand binding | Barbhaiya et al., 1996 |
| S94A | H | 3.39 | No detectable agonist or antagonist binding | Barbhaiya et al., 1996 |
| S94T | | | Minor changes in ligand binding | Barbhaiya et al., 1996 |
| A125K | H | 4.43 | No changes in radioligand binding | Rivkees et al., 1999a |
| C131A/S | H | 4.49 | No changes in radioligand binding | Scholl and Wells, 2000 |
| S135A | H | 4.53 | No changes in radioligand binding | Barbhaiya et al., 1996 |
| T141A | H | 4.59 | No changes in radioligand binding | Barbhaiya et al., 1996 |
| F144L | H | 4.62 | No changes in radioligand binding | Rivkees et al., 1999a |
| C169A/S | H | | No detectable radioligand binding | Scholl and Wells, 2000 |
| H251L | B | 6.52 | Antagonist affinity reduced 4-fold; no change in agonist affinity | Olah et al., 1992 |

| Mutation ^a | Species | Helix/position ^b | Effect on Ligand Binding and Signal Transduction | Reference |
|-----------------------|---------|-----------------------------|--|--|
| C255A/S | H | 6.56 | No changes in radioligand binding | Scholl and Wells, 2000 |
| C260A/S | H | | No changes in radioligand binding | Scholl and Wells, 2000 |
| C263A/S | H | | No changes in radioligand binding | Scholl and Wells, 2000 |
| I270M M270I | B, C | 7.35 | Amino acid in position 270 contributes to canine/bovine A ₁ AR binding selectivity | Tucker et al., 1994 |
| T277A | H | 7.42 | 400-fold decrease in affinity of NECA with modest changes in affinity for R-PIA and S-PIA (intact cells); substantial decrease in affinity of all agonists (membranes); no change in antagonist affinity | Townsend-Nicholson and Schofield, 1994; Dalpiaz et al., 1998 |
| T277S | | | Modest decrease in agonist affinity; no change in antagonist affinity; nature of residue in position 277 also involved in canine/bovine A ₁ AR binding specificity | Tucker et al., 1994; Townsend-Nicholson and Schofield, 1994 |
| H278L | B | 7.43 | Negligible agonist and antagonist binding | Olah et al., 1992 |
| C309A/S | H | | No changes in radioligand binding | Scholl and Wells, 2000 |

The results were obtained from site-directed mutagenesis studies of the A1 AR (species; H, human; B, bovine; C, canine).

^a Amino acids are represented in single-letter code with position number shown. The first amino acid is that of the wild-type receptor, with the second residue being that used for substitution.

^b Position on helix, using notation of van Rhee and Jacobson (1996).

TABLE 3

Mutational analysis of the human A_{2A} AR with respect to ligand binding

| Mutation ^a | Helix/Position ^b | Effect on Ligand Binding and Signal Transduction | Reference |
|-----------------------|-----------------------------|--|----------------------|
| E13Q | 1.39 | Slight reduction of agonist, but not antagonist, affinity | Uzerman et al., 1996 |
| T88A/S/R | 3.36 | Substantial decrease in agonist, but not antagonist, affinity | Jiang et al., 1996 |
| Q89A | 3.37 | Slight increase in agonist and antagonist affinity | Jiang et al., 1996 |
| Q89N/S/L | | Marginal changes in ligand binding | |
| Q89H/R | | Antagonist binding affected | |
| S90A | 3.38 | Marginal changes in ligand binding | Jiang et al., 1996 |
| S91A | 3.39 | Marginal changes in ligand binding | Jiang et al., 1996 |
| E151A/Q/D | | Loss of agonist and antagonist radioligand binding | |
| E169A | | ~1000-fold decrease in agonist potency | Kim et al., 1996 |
| E169A | | Gain in N ⁶ -substituted agonist affinity | Kim et al., 1996 |
| D170K | | No change in (radio)ligand binding | Kim et al., 1996 |
| P173R | | | |
| F180A | 5.41 | Minor changes in ligand binding | Kim et al., 1995 |
| N181S | 5.42 | Modest reduction of agonist binding | Kim et al., 1995 |
| F182A | 5.43 | Loss of agonist and antagonist radioligand binding | Kim et al., 1995 |
| F182Y | | Modest reduction of agonist binding | Kim et al., 1995 |
| F182W | | Modest reduction of agonist binding | Kim et al., 1995 |
| H250A | 6.52 | Loss of agonist and antagonist radioligand binding; no agonist activity in functional assays | Kim et al., 1995 |
| H250F/Y | | Modest reduction of agonist binding; no effect on antagonist binding | Kim et al., 1995 |
| N253A | 6.55 | Loss of agonist and antagonist radioligand binding | Kim et al., 1995 |
| C254A | 6.56 | Minor changes in ligand binding | Kim et al., 1995 |
| F257A | 6.59 | Loss of agonist and antagonist radioligand binding | Kim et al., 1995 |
| C262G | 6.64 | No change in radioligand binding | Kim et al., 1996 |
| I274A | 7.39 | Loss of agonist and antagonist radioligand binding; ~30-fold decrease in agonist potency | Kim et al., 1995 |
| S277A | 7.42 | Substantial decrease in agonist affinity and potency, but antagonist radioligand binding not altered | Kim et al., 1995 |
| S277T/C/N | | Marginal changes in ligand binding | Jiang et al., 1996 |
| H278A | 7.43 | Loss of agonist and antagonist radioligand binding; ~300-fold decrease in agonist potency | Kim et al., 1995 |
| H278Y | | Modest reduction of agonist binding; no effect on antagonist binding | Gao et al., 2000 |

| Mutation ^a | Helix/Position ^b | Effect on Ligand Binding and Signal Transduction | Reference |
|-----------------------|-----------------------------|--|------------------|
| S281A | 7,46 | Loss of agonist and antagonist radioligand binding; no agonist activity in functional assays | Kim et al., 1995 |
| S281T | | Enhanced affinity for most agonists | Kim et al., 1995 |
| S281N | | Marginal changes in ligand binding | Gao et al., 2000 |

^a Amino acids are represented in single-letter code with position number shown. The first amino acid is that of the wild-type receptor, with the second and further residues those used for substitution.

^b Position on helix, using notation of van Rhee and Jacobson (1996).

TABLE 4

Mutational analysis of the human A₂B AR with respect to ligand binding

| Mutation ^a | Helix/Position ^b | Effect on Ligand Binding and Signal Transduction | Reference |
|-----------------------|-----------------------------|--|----------------------|
| V11I | 1.36 | No changes in (radio)ligand binding | Beukers et al., 2000 |
| A12T | 1.37 | No changes in (radio)ligand binding | Beukers et al., 2000 |
| L58V | 2.55 | No changes in (radio)ligand binding | Beukers et al., 2000 |
| F59L | 2.56 | No specific radioligand binding and no cAMP production | Beukers et al., 2000 |
| N273Y | 7.36 | Up to 60-fold increase in affinity of 2-substituted adenosines | Beukers et al., 2000 |

^aAmino acids are represented in single-letter code with position number shown. The first amino acid is that of the wild-type receptor, and the second is the one used for substitution, based on the corresponding amino acid in the human A₂A receptor.

^bPosition on helix, using notation of van Rhee and Jacobson (1996).

TABLE 5

Summary of the distribution of adenosine receptors

Data where there is correspondence between mRNA distribution and receptor distribution are given in bold. Data based mainly or exclusively on distribution of mRNA are given in ordinary letters. Ambiguous data (including major species differences) are given in italics.

| A ₁ Receptor | A _{2A} Receptor | A _{2B} Receptor | A ₃ Receptor |
|--|--|--|--|
| <p>High expression Brain (cortex, cerebellum, hippocampus). Dorsal horn of spinal cord. Eye, adrenal gland, atria</p> <p>Intermediate levels Other brain regions. Skeletal muscle, liver, kidney, adipose tissue, salivary glands, esophagus, colon, antrum, testis</p> <p>Low levels Lung (but probably higher in bronchi), pancreas</p> | <p>High expression Spleen, thymus, leukocytes (both lymphocytes and granulocytes), blood platelets. Striatopallidal GABAergic neurons (in caudate-putamen, nucleus accumbens, tuberculum olfactorium), olfactory bulb</p> <p>Intermediate levels Heart, lung, blood vessels</p> <p>Low levels Other brain regions</p> | <p>High expression Cecum, colon, bladder</p> <p>Intermediate levels Lung, blood vessels, eye, median eminence, mast cells</p> <p>Low levels Adipose tissue, adrenal gland, brain, kidney, liver, ovary, pituitary gland</p> | <p>High expression <i>Testis (rat), mast cells (rat)</i></p> <p>Intermediate levels <i>Cerebellum (human?), hippocampus (human?), lung, spleen (sheep), pineal</i></p> <p>Low levels Thyroid, most of brain, adrenal gland, spleen (<i>human</i>), liver, kidney, heart, intestine, testis (<i>human</i>)</p> |

TABLE 6

Pharmacological tools used for classification of adenosine receptors

| Abbreviations | Chemical Names |
|---------------|--|
| AB-MECA | 4-aminobenzyl-5'- <i>N</i> -methylcarboxamidoadenosine |
| ATL146e | 4-[[3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9 <i>H</i> -purin-2-yl]-prop-2-ynyl]-cyclohexanecarboxylic acid methyl ester |
| BW-A1433 | 1,3-dipropyl-8-(4-acrylate)phenylxanthine |
| CGS 15943 | 5-amino-9-chloro-2-(2-furyl)-[1,2,4]-triazolo[1,5- <i>c</i>]quinoxaline |
| CGS 21680 | 2-[<i>p</i> -(2-carbonyl-ethyl)-phenylethylamino]-5'- <i>N</i> -ethylcarboxamidoadenosine |
| CP 66713 | 4-amino-8-chloro-1-phenyl-[1,2,4]-triazolo-[4,3- <i>a</i>] quinoxaline |
| CPA | <i>N</i> ⁶ -cyclopentyladenosine |
| CPX | 8-cyclopentyl-1,3-dipropylxanthine (see also DPCPX) |
| CSC | 8-(3-chlorostyryl)caffeine |
| CV-1808 | 2-phenylaminoadenosine |
| DPCPX | 1,3-dipropyl-8-cyclopentylxanthine |
| HENECA | 2-hex-1-ynyl-5'- <i>N</i> -ethylcarboxamidoadenosine |
| I-ABA | <i>N</i> ⁶ -(4-amino-3-iodobenzyl)adenosine |
| I-ABOPX | 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propylxanthine |
| IB-MECA | <i>N</i> ⁶ -(3-iodobenzyl)adenosine-5'- <i>N</i> -methyluronamide |
| KF 17837 | 1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine |
| KW 6002 | (<i>E</i>)-8-[2-(3,4-dimethoxyphenyl)ethenyl]-1,3-dietyl-3,7-dihydro-7-methyl-1 <i>H</i> -purine-2,6-dione |
| MRE 3008-F20 | 5-[[[(4-methoxyphenyl)amino]carbonylamino-8-ethyl-2-(2-furyl)-pyrazolo[4,3- <i>e</i>][1,2,4-triazolo[1,5- <i>c</i>]pyrimidine |
| MRS 1191 | 3-ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate |
| MRS 1220 | 9-chloro-2-(2-furanyl)-5-[(phenylacetyl)amino][1,2,4]-triazolo[1,5- <i>c</i>]quinoxaline |
| MRS 1523 | 2,3-diethyl-4,5-dipropyl-6-phenylpyridine-3-thiocarboxylate-5-carboxylate |
| MRS 1754 | <i>N</i> -(4-cyano-phenyl)-2-[4-(2,6-dioxo-1,3-dipropyl-2,3,4,5,6,7-hexahydro-1 <i>H</i> -purin-8-yl)-phenoxy]acetamide |
| NECA | 5'- <i>N</i> -ethyl-carboxamidoadenosine |
| PAPA-APEC | 2-(4-[2-[(4-aminophenyl)methyl]carbonyl]ethyl]phenyl)ethylamino-5'- <i>N</i> -ethylcarboxamidoadenosine |
| PD 81723 | (2-amino-4,5-dimethyl-3-thienyl)-[3-(trifluoromethyl)-phenyl]-methanone |
| <i>R</i> -PIA | <i>R</i> - <i>N</i> ⁶ -(phenylisopropyl)-adenosine |
| SCH 58261 | 5-amino-2-(2-furyl)-7-phenylethyl-pyrazolo[4,3- <i>e</i>][1,2,4-triazolo[1,5- <i>c</i>]pyrimidine |
| <i>S</i> -PIA | <i>S</i> - <i>N</i> ⁶ -(phenylisopropyl)-adenosine |

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| Abbreviations | Chemical Names |
|---------------|--|
| VUF-8504 | 4-methoxy-N-[2-(2-pyridiny)quinazolin-4-yl]benzamide |
| XAC | xanthine amine congener; 8-[4-[[[(2-aminoethy)amino]-carbonyl]oxy]phenyl]-1,3-dipropylxanthine |
| ZM241385 | 4-(2-[7-amino-2-[2-furyl]-1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]ethy)phenol |

TABLE 7

Binding affinity of agonists and antagonists at human adenosine receptor subtypes (K_i values with 95% confidence intervals or ± S.E.M. in parentheses)

| | A ₁ | A _{2A} | A _{2B} | A ₃ | Reference |
|----------------------------------|---------------------|---------------------|-------------------|-----------------|-----------|
| Agonists with unmodified ribose | | | | | |
| R-PIA | 2.0 (1–4.2) | 860 (480–1,540) | | 16 (9.3–29) | <u>a</u> |
| | | | 33,700 (±6,800) | | <u>c</u> |
| | | | 3,800 (±1,700) | | <u>e</u> |
| S-PIA | 75 (42–134) | 7,800 (7,000–8,640) | | 45 (33–61) | <u>a</u> |
| | | | 62,800 (±21,600) | | <u>c</u> |
| CPA | 2.3 (1.5–3.4) | 790 (470–1,360) | | 43 (30–61) | <u>a</u> |
| | | | 34,400 (±11,100) | | <u>c</u> |
| | | | 21,000 (±4,300) | | <u>e</u> |
| CCPA | 0.8 (0.55–1.25) | 2,300 (2,000–2,700) | | 42 (32–56) | <u>a</u> |
| | | | 40,100 (±16,400) | | <u>c</u> |
| 2-Cl-adenosine | 1.39 (1.28–1.51) | 180 (150–220) | | 19 (14–28) | <u>i</u> |
| Agonists with 5'-modified ribose | | | | | |
| NECA | 14 (6.4–29) | 20* (12–35) | | 6.2* (3.5–11) | <u>a</u> |
| | | | 330 (±60) | | <u>c</u> |
| | | | 360 (±120) | | <u>e</u> |
| CGS 21680 | 290 (230–360) | 27 (12–59) | | 67 (50–90) | <u>a</u> |
| | | | 361,000 (±21,000) | | <u>c</u> |
| HENECA | 60 (50–72) | 6.4 (3.8–11) | | 2.4 (2.0–2.9) | <u>b</u> |
| PENECA | 560 (480–650) | 620 (300–1,300) | | 6.2 (5.1–7.5) | <u>b</u> |
| PHPNECA | 2.7 (1.7–7.1) | 3.1 (2.4–3.9) | | 0.42 (0.17–1.0) | <u>b</u> |
| AB-MECA | 1,500 (1,300–1,800) | 3,600 (2,700–4,700) | | 22 (20–24) | <u>a</u> |

| | A ₁ | A _{2A} | A _{2B} | A ₃ | Reference |
|----------------------------|----------------------|---------------------|-----------------|-------------------------|-----------|
| IB-MECA | 3.7 (1.8–7.7) | 2,500 (1,900–3,400) | | 1.2 (0.96–1.5) | <i>a</i> |
| IAB-MECA | 8.5 (4.8–15) | 470 (300–740) | 54,000 (±5,600) | 0.64 (0.58–0.70) | <i>c</i> |
| CI-IB-MECA | 115 (114–116) | 2,100 (1,700–2,500) | | 11 (9.4–13) | <i>a</i> |
| Antagonists: xanthines | | | | | |
| DPCPX | 3.9* (3.5–4.2) | 129 (35–260) | | 4,000 (2,600–6,000) | <i>a</i> |
| XAC | 29 (24–35) | 1 (0.58–1.7) | | 92 (64–130) | <i>a</i> |
| MRS1754 | 400 (±190) | 500 (±11) | | 570 (±180) | <i>f</i> |
| MSX-2 | 2,500 (1,700–3,600) | 5.0 (±0.6) | | >10,000 | <i>h</i> |
| Theophylline | 6,800 (4,100–11,000) | 1,700 (1,000–2,900) | | 86,000 (74,000–101,000) | <i>a</i> |
| Antagonists: non-xanthines | | | | | |
| CGS 15943 | 3.5 (1.7–7.3) | 4.2 (2.6–6.6) | | 51 (43–60) | <i>a</i> |
| SCH 58261 | 290 (210–410) | 0.6 (0.5–0.7) | 16 (±3.6) | >10,000 | <i>e</i> |
| ZM 241385 | 260 (190–390) | 0.8 (0.7–1.0) | | >10,000 | <i>d</i> |
| MRE 3008F20 | 1,100 (±1,100) | 140 (±15) | 2,100 (±300) | 0.29 (±0.03) | <i>c</i> |
| | | | | | <i>g</i> |

* Values are from saturation experiments.

^a Klotz et al., 1998, transfected CHO cells; radioligands [³H]CCPA (A1), [³H]NECA (A2A and A3).

^b Klotz et al., 1999, transfected CHO cells; radioligands [³H]CCPA (A1), [³H]NECA (A2A and A3).

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- ^cLinden et al., 1999, transfected HEK 293 cells; radioligand ^{125}I -ABOPX.
- ^dOngini et al., 1999, transfected CHO cells (A1 and A2A) or HEK 293 cells (A2A and A3); radioligands [^3H]DPCPX (A1), [^3H]SCH 58261 (A2A), ^{125}I -AB-MECA (A3).
- ^eJi and Jacobson, 1999, transfected HEK 293 cells; radioligand [^3H]ZM 241685.
- ^fKim et al., 2000, transfected HEK 293 cells; radioligands ^{125}I -ABA (A1), ^{125}I -ZM 241685 (A2A), ^{125}I -ABOPX (A2B), ^{125}I -AB-MECA (A3).
- ^gVarani et al., 2000, transfected CHO cells; radioligands [^3H]DPCPX (A1 and A2B), [^3H]SCH 58261 (A2A), ^{125}I -AB-MECA (A3).
- ^hSauer et al., 2000, transfected CHO cells; radioligand [^3H]CHA (A1), [^3H]CGS 21680 (A2A), [^3H]NECA (A3).
- ⁱK.-N. Klotz and S. Kachler, unpublished, transfected CHO cells; radioligand [^3H]NECA.

TABLE 8

Binding affinity of agonists and antagonists at rat adenosine receptor subtypes (K_i values with 95% confidence intervals or \pm S.E.M. in parentheses)

| | A ₁ | A _{2A} | A ₃ | Reference |
|----------------------------------|--------------------|---------------------|----------------------|-----------|
| Agonists with unmodified ribose | | | | |
| R-PIA | 1.2 (\pm 0.16) | 124 (\pm 9) | | <i>a</i> |
| | 1.3 (1.1–1.6) | 730 (690–770) | | <i>b</i> |
| | 0.51 | | | <i>c</i> |
| S-PIA | 49 (\pm 2.4) | 1,800 (\pm 380) | | <i>a</i> |
| | 29 | | | <i>f</i> |
| CPA | 0.59 (\pm 0.02) | 460 (\pm 15) | 220 (\pm 43) | <i>d</i> |
| | 0.8 (0.6–1.0) | 2,000 (1,400–2,900) | 160 (\pm 52) | <i>e</i> |
| CCPA | 0.4 (0.2–0.7) | 3,900 (2,500–4,700) | 920 (\pm 310) | <i>a</i> |
| | 0.23 | | 240 (\pm 36) | <i>b</i> |
| ADAC | 0.85 (\pm 0.35) | | | <i>c</i> |
| 2-Cl-adenosine | 9.3 (\pm 0.58) | 63 (\pm 7.5) | 240 (\pm 71) | <i>e</i> |
| DPMA | 140 (\pm 23) | 4.4 (\pm 0.2) | 1,900 (\pm 900) | <i>w</i> |
| (5)-ENBA | 0.3 (\pm 0.02) | 1,400 (\pm 150) | 3,600 (\pm 1,700) | <i>a</i> |
| | | | | <i>e</i> |
| Agonists with 5'-modified ribose | | | | |
| | | | | <i>u</i> |
| | | | | <i>v</i> |

| | A ₁ | A _{2A} | A ₃ | Reference |
|------------------------|-------------------|-----------------|----------------|-----------|
| NECA | 6.3 (±0.52) | 10 (±0.5) | | <i>a</i> |
| | 11 (7.0–17) | 22 (20–25) | | <i>f</i> |
| | 3.7 | | | <i>c</i> |
| | | | 260 (±36) | <i>d</i> |
| CGS 21680 | 3100 (±470) | 22 (±4.3) | | <i>g</i> |
| | | | 580 (±32) | <i>e</i> |
| HENECA | 136 (95–195) | 3.8 (1.5–9.9) | | <i>f</i> |
| PENECA | 698 (611–798) | 120 (112–128) | | <i>h</i> |
| PHPNECA | 2.5 (2.2–2.9) | 0.9 (0.7–1.3) | | <i>i</i> |
| AB-MECA | 430 (±45) | 1,600 (±180) | 14 (±3) | <i>j</i> |
| IB-MECA | 54 (±5) | 56 (±8) | 1.1 (±0.3) | <i>j</i> |
| IAB-MECA | 18 (±5) | 200 (±84) | 1.3 (±0.18) | <i>j</i> |
| CI-IB-MECA | 820 (±570) | 470 (±370) | 0.33 (±0.08) | <i>k</i> |
| Antagonists: xanthines | | | | |
| DPCPX | 0.3 | 340 | | <i>l</i> |
| | 0.18* (0.16–0.20) | | | <i>l</i> |
| | | | >10,000 | <i>e</i> |
| XAC | 2.8 (1.9–4.2) | | | <i>m</i> |
| | 3.5 | 24 | | <i>l</i> |
| | 4 | 50 | | <i>n</i> |
| | | | >100,000 | <i>o</i> |
| MRS1754 | 17 (±3.6) | 610 (±290) | | <i>o</i> |
| MSX-2 | 900 (±10) | 8 (±3) | | <i>p</i> |
| Theophylline | 11,000 | 32,000 | | <i>b</i> |

| | A ₁ | A _{2A} | A ₃ | Reference |
|----------------------------|---------------------|------------------|--------------------------|--------------|
| Antagonists: non-xanthines | | | | |
| CGS 15943 | 21 (±3.5) | 3.3 (±1.8) | >100,000 | ^g |
| | 22 | 3 | | ⁿ |
| | 6.4 (6.2–6.6) | 1.2 (1.1–1.3) | | ^r |
| SCH 58261 | 120 (100–140) | 2.3 (2.0–2.7) | >100,000 | ^o |
| ZM 241385 | 2,000 (1,500–2,700) | 0.30 (0.10–0.95) | 150,000 (87,000–210,000) | ^s |
| MRE 3008F20 | >10,000 | 2,000 (±220) | >10,000 | ^t |

* Data from saturation experiments; where confidence limits are missing, they are within 0.15 log units.

^aBruns et al., 1986; brain membranes (A1), striatum (A2A); radioligands [³H]CHA (A1), [³H]NECA (A2A).

^bLohse et al., 1988; brain membranes (A1), striatum (A2A); radioligands [³H]PIA (A1), [³H]NECA (A2A).

^cKlotz et al., 1991; brain membranes; radioligand [³H]DPCPX.

^dOlah et al., 1994; transfected CHO cells; radioligand ¹²⁵I-AB-MECA.

^evan Galen et al., 1994; transfected CHO cells; radioligand ¹²⁵I-APNEA.

^fCristalli et al., 1992; brain membranes (A1), striatum (A2A); radioligands [³H]DPCPX (A1), [³H]NECA (A2A).

^gHutchison et al., 1989; rat brain; radioligands [³H]CHA (A1), [³H]NECA (A2A). Given are IC₅₀ values; based on the experimental conditions K₁ values should be about 0.5–0.7 and 0.9 times the IC₅₀ values for A1 and A2A, respectively.

^hCristalli et al., 1995; brain membranes (A1), striatum (A2A); radioligands [³H]CHA (A1), [³H]CGS 21680 (A2A).

ⁱCamaioni et al., 1997; brain membranes (A1), striatum (A2A); radioligands [³H]CHA (A1), [³H]CGS 21680 (A2A).

^jGallo-Rodriguez et al., 1994; rat brain (A1), rat striatum (A2A), transfected CHO cells (A3); radioligand [³H]PIA (A1), [³H]CGS 21680 (A2A), ¹²⁵I-AB-MECA (A3).

^kKim et al., 1994; rat brain (A1), rat striatum (A2A), transfected CHO cells (A3); radioligand [³H]PIA (A1), [³H]CGS 21680 (A2A), ¹²⁵I-AB-MECA (A3).

^lLohse et al., 1987; brain membranes (A1), striatum (A2A); radioligands [³H]PIA (A1), [³H]NECA (A2A).

- ^mJacobson et al., 1986; rat brain, radioligand [³H]XAC.
- ⁿJarvis et al., 1989; rat brain; radioligands [³H]CHA (A1), [³H]CGS 21680 (A2A).
- ^oKim et al., 2000; transfected HEK 293 cells (A1), rat striatum (A2A); radioligands [³H]PIA (A1), [³H]CGS 21680 (A2A).
- ^pSauer et al., 2000; brain cortex (A1), striatum (A2A); radioligand [³H]CHA (A1), [³H]CGS 21680 (A2A).
- ^qWilliams et al., 1987; brain (A1), striatum (A2A); radioligands [³H]CHA (A1), [³H]NECA (A2A). Given are IC₅₀ values; based on the experimental conditions K_i values should be about 0.5–0.7 and 0.9 times the IC₅₀ values for A1 and A2A, respectively.
- ^rBaraldi et al., 1994; rat brain (A1), rat striatum (A2A); radioligand [³H]CHA (A1), [³H]CGS 21680 (A2A).
- ^sPoucher et al., 1995; brain cortex (A1), PC 12 cells (A2A), transfected CHO cells (A3); radioligands [³H]PIA (A1), [³H]NECA (A2A), [¹²⁵I]-AB-MECA (A3). Given are IC₅₀ values; based on the experimental conditions K_i values should be about 0.1, 0.2, and 0.5 times the IC₅₀ values for A1, A2A, and A3, respectively.
- ^tVarani et al., 2000; brain cortex (A1), striatum (A2A), transfected CHO cells (A3); radioligands [³H]DPCPX (A1), [³H]SCH 58261 (A2A), [¹²⁵I]-AB-MECA (A3).
- ^uBridges et al., 1988; brain (A1), striatum (A2A); radioligand [³H]CHA (A1), [³H]NECA (A2A); DPMA or PD 125944 is compound 16 in this paper.
- ^vTrivedi et al., 1989; brain (A1), striatum (A2A); radioligand [³H]CHA (A1), [³H]NECA (A2A).
- ^wJacobson et al., 1987; brain (A1), radioligand [³H]PIA (A1).

TABLE 9

G protein coupling of the four adenosine receptor subtypes

| Adenosine Receptor Subtype | G Protein | Effects of G Protein Coupling | Cellular System | Reference |
|----------------------------|---|--|--|---|
| A ₁ | G _{11/12/3} | ↓ cAMP ↑ IP3/DAG (PLC) ↑ Arachidonate (PLA2) ↑ PE(OH) (PLD) | General, CHO cells ^a DDT ₁ MF-2 | Freissmuth et al., 1991; Akbar et al., 1994; Freund et al., 1994; Jockers et al., 1994; Gerwins and Fredholm, 1995a,b |
| A _{2A} | G ₆ G _s G _{o1f} | ↑ cAMP ↑ cAMP | General | Jockers et al., 1994; Matovcik et al., 1994 Olah, 1997 Kull et al., 2000a; Corvol et al., 2001 |
| A _{2B} | G _{15/16} G _s | ↑ IP ₃ ↑ cAMP | COS-7 ^a General | Offermanns and Simon, 1995 Pierce et al., 1992 |
| A ₃ | G _{q/11} G _{12,3} G _{q/11} | ↑ IP3/DAG (PLC) ↓ cAMP ↑ IP3/DAG (PLC) | HMC-1, HEK 293 ^a General, CHO cells ^a CHO cells ^a | Gao et al., 1999; Linden et al., 1999 ^b Palmer et al., 1995 Palmer et al., 1995 |

^aReceptor transfected cell system.^bReferences propose G_{q/11} coupling without direct evidence for G_{q/11} activation.