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A_3 Adenosine Receptors: Protective vs. Damaging Effects Identified Using Novel Agonists and Antagonists

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Abstract

Investigation of the physiologic role of the A_3 adenosine receptor has been facilitated by the availability of selective agonists and antagonists. Selective agonists include IB-MECA and the 2-chloro derivative Cl-IB-MECA. Selective antagonists have been identified and designed with the aid of molecular modeling among various nonpurine classes of heterocycles: flavonoids, 1,4-dihydropyridine derivatives, triazoloquinazolines, isoquinolines, and a triazolonaphthyridine. The dihydropyridine 3-ethyl 5-benzyl 2-methyl-6-phenyl-4-phenylethynyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (MRS 1191) is 1,300-fold selective for human A_3 (K_i of 31 nM) vs. A_1/A_{2A} adenosine receptors and also 28-fold A_3 selective in rat tissue (K_i of 1.42 mM). 9-Chloro-2-(2-furyl)-5-phenylacetylamino[1,2,4]-triazolo[1,5-c]quinazoline (MRS 1220) is useful as an A_3 selective antagonist only in human tissue, with a K_i value of 0.65 nM. The pyridine derivative 5-propyl 2-ethyl-4-propyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate (MRS 1523) is a selective antagonist of both rat and human A_3 receptors, with K_i values of 113 and 19 nM, respectively. Paradoxical effects of A_3 agonists in the brain, heart and other tissues indicate that acute activation of A_3 receptors at greater than 10 mM concentrations acts as a lethal input to cells, whereas low, nanomolar concentrations of A_3 receptor agonists protect against apoptosis or ischemic damage. Adenosine A_3 receptor agonists, antagonists, or both, may be useful in treating inflammatory conditions.

Keywords

purines; A_3 receptors; cell viability; dihydropyridines; pyridines; molecular modeling

INTRODUCTION

Most tissues contain one or more of the four known adenosine receptor subtypes, A_1, A_{2A}, A_{2B}, and A_3 [Linden, 1994; Olah and Stiles, 1995], consistent with the ubiquitous role of adenosine in maintaining homeostasis, especially in conditions of stress or ischemia. The A_1 and A_{2A} adenosine receptors, pharmacologically well characterized mainly through the use of selective ligands, generally have a protective role, i.e., in decreasing energy demand and increasing energy supply, respectively. A_1 receptor activation inhibits the release of

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potentially damaging excitatory neurotransmitters (Fig. 1). Mice in which the A_{2A} receptors have been knocked out have been bred [Ledent et al., 1997], and observations with these viable animals point to regulatory differences in the cardiovascular and central nervous systems. A_{2B} receptors are the only subtype for which there are not yet highly selective agonists and antagonists, although SAR studies have been carried out in functional assays at this subtype [de Zwart et al., 1998]. Development of selective agonists and antagonists for the A_3 receptor has made possible pharmacologic studies of this novel receptor. By virtue of effects on apoptosis, adenosine A_3 receptors may play a critical role in human disease states, such as neurodegeneration, cancer, and inflammation. Adenosine A_3 receptor antagonists may be useful in treating asthma. The acute administration of an A_3 antagonist or the chronic administration of an A_3 agonist appears to protect brain cells in a global ischemia model and, thus, may be potential therapeutic approaches for preventing stroke damage. In the heart, because A_3 receptor activation protects both in a preconditioning model and during prolonged ischemic, selective agonists may be of great clinical importance.

The distribution of A_3 adenosine receptors is species dependent and in the human occurs in the lungs, liver, heart, kidneys, brain, and testes [Linden, 1994]. The only primary human tissue in which high density radioligand binding to A_3 adenosine receptors has been demonstrated is eosinophils, suggesting a role of this subtype in inflammatory diseases. In functional studies, A_3 adenosine receptors have been detected in the central nervous system, in both neurons [Dunwiddie et al., 1997] and astrocytes [Abbracchio et al., 1997b], although the density in the brain is low and relatively diffusely distributed. On the basis of studies that used selective agonists and antagonists, it has been proposed that modulating A_3 adenosine receptors may provide new therapeutic approaches for treating inflammatory and neurodegenerative diseases, asthma, and cardiac ischemia [Beaven et al., 1994; Liu et al., 1994; von Lubitz et al., 1994; Strickler et al., 1996; Tracey et al., 1997; Knight et al., 1997; Stambaugh et al., 1997; Walker et al., 1997]. A_3 knockout mice are also being studied [Salvatore and Jacobson, 1996], and preliminary information indicates that the homozygous knockout is nonlethal.

SELECTIVE A_3 ADENOSINE RECEPTORS AGONISTS

All of the currently synthesized adenosine agonists with moderate to high selectivity for the A_3 receptor subtype contain modifications at two sites on the adenosine structure, the N^6- and 5′-positions [Jacobson et al., 1995]. The monosubstituted N^6-(3-iodobenzyl)adenosine is only slightly A_3 selective, whereas the corresponding 5′-uronamide derivatives are more highly selective. For example, N^6-(3-iodobenzyl)-adenosine-5′-*N*- methyluronamide (IB-MECA, **1**, Fig. 2) was the first highly potent and selective A_3 agonist, both in vitro, in species as diverse as human [Jacobson et al., 1997], dog [Auchampach et al., 1997b], and chick [Stambaugh et al., 1997], and in vivo [Jacobson et al., 1995; von Lubitz et al., 1994]. It is approximately 50-fold selective in binding assays for rat A_3 vs. either rat A_1 or rat A_{2A} receptors. Substitution at the 2-position of adenosine in combination with modifications at N^6 and 5′-positions further enhanced A_3 affinity and selectivity. Thus, 2-chloro-N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide (**2**, Cl-IB-MECA) [Jacobson et al., 1995] displayed a K_i value of 0.33 nM at A_3 receptors and is selective for rat A_3 vs. A_1 and A_{2A} receptors by 2500- and 1400-fold, respectively. Although highly selective, Cl-IB-MECA at

micromolar concentrations has been shown to activate A_{2A} receptors in human neutrophils [Hannon et al., 1998]. Thus, for the range of A_3 receptor effects that have been demonstrated only at micromolar concentrations of A_3 agonists (Table 1), it is imperative to compare results with agonists of selectivity for A_1 and A_{2A} adenosine receptors and where feasible to test antagonism by A_3 receptor selective antagonists

N^6-(4-Aminobenzyl)-adenosine-5′-*N*-methyluronamide (AB-MECA, 3) was prepared as a precursor for radioiodination, such that an iodo substituent directed to the 3-position would be expected to enhance affinity at A_3 receptors. Although AB-MECA is a moderately A_3-selective agonist, the resulting radioligand [^{125}I]I-AB-MECA [Olah et al., 1994], 4, although a high-affinity (K_d of 0.59 nM) probe for A_3 receptors, is not as A_3 selective as IB-MECA [Shearman and Weaver, 1997]. Thus, in brain autoradiographic studies, [^{125}I]I-AB-MECA also bound to A_1 and A_{2A} subtypes [Shearman and Weaver, 1997].

In an attempt initially directed toward the derivatization of xanthines as A_3 receptor antagonists by forming 7-riboside derivatives, DBXRM (**5**, Fig. 2) was found to be 140-fold selective in binding to rat A_3- vs. A_1 adenosine receptors. However, DBXRM proved to be an agonist at recombinant rat A_3 receptors [Kim et al., 1994; Park et al., 1998]. A 3′-deoxy derivative of DBXRM was found to be an antagonist at A_1 and partial agonist at A_3 adenosine receptors. Thus, it is possible for the same compound to stimulate one adenosine receptor subtype (A_3) and block another subtype (A_1) within the same species [Park et al., 1998]. Full agonists, such as Cl-IB-MECA or I-AB-MECA, were more potent and effective than the partial agonist DBXRM in causing desensitization of rat A_3 receptors, as indicated by loss of [^{35}S]GTPγ[S] binding to RBL-2H3 cell membranes.

Knutsen and coworkers have developed hydroxylamino derivatives such as **6** and **7** as A_3-selective agonists [Knutsen et al., 1998]. This series emphasized the flexibility of substitution at the 5′-position with amide, chloromethyl, vinyl, ester, and isoxazole groups. Baraldi and coworkers [Baraldi et al., 1998] have developed urea derivatives such as **8** and related amides as A_3 selective agonists. IJzerman and coworkers [van Tilburg et al., 1998] have reported that substitution with a methylthio group at the 5′-position of N^6-benzyladenosine analogues results in partial agonists such as **9**.

SELECTIVE A_3 ADENOSINE RECEPTOR ANTAGONISTS

The low affinity of xanthines, the classic antagonists of A_1, A_{2A}, and A_{2B} subtypes, at rat A_3 adenosine receptors is striking [Linden, 1994; Jacobson et al., 1995; Olah and Stiles, 1995]. At human, dog, and sheep A_3 adenosine receptors [Linden et al., 1993; Salvatore et al., 1993; Auchampach et al., 1997a], certain xanthines are of intermediate potency as antagonists; however, highly A_3 selective xanthines have not yet been identified. The species differences in antagonist affinity and the low degree of homology between human and rat receptor sequences (72%) suggest the existence of two subtypes of A_3 adenosine receptors, although this needs to be further investigated.

A_3 adenosine receptor antagonists, which have been introduced only recently [Jacobson et al., 1996; Karton et al., 1996; Kim et al., 1996; Jiang et al., 1997a], were previously

hypothesized [Beaven et al., 1994; von Lubitz et al., 1994] to act as potential antiasthmatic [Olah and Stiles, 1995], anti-inflammatory, or cerebroprotective agents. The need for selective antagonists is critical, especially in light of the fact that most effects of high concentrations of A_3 agonists (Table 1) have not unequivocally been ascribed to activation of A_3 receptors. The dramatic species differences in antagonist affinity and in A_3 receptor responses makes the extrapolation of studies in rodents to the potential treatment of human disease more challenging. Thus, it is desirable to obtain antagonists that are A_3 selective across species [Li et al., 1998] for preclinical studies. Promising leads for selective antagonists for human A_3 receptors have appeared among nonxanthine heterocycles (Fig. 3), including flavonoids, 1,4-dihydropyridine derivatives, triazoloquinazolines, isoquinolines, and a triazolonaphthyridine. Our initial screening of chemically diverse substances as potential antagonists, consisted of single-point displacement of [^{125}I]I-AB-MECA binding at human A_3 receptors expressed in HEK-293 cells. 1,4-Dihydropyridines (DHPs) [Jiang et al., 1997a], which act as potent L-type calcium channel antagonists, were found to have micromolar affinity at this subtype. Common DHP drugs typically bound to various adenosine receptor subtypes either nonselectively, as for example, nifedipine, with a K_i value of 8.3 μM at A_3 receptors, or in some cases with selectivity for the A_3 vs. other adenosine receptor subtypes, as for example, *S*-niguldipine, with a K_i value of 2.8 μM. At human A_{2B} receptors, such 1,4-DHPs are essentially inactive [Dunwiddie et al., 1997]. Careful structural optimization of the 1,4-DHP core as a template for adenosine antagonists then ensued. Key features that boost affinity at adenosine (especially A_3) receptors and completely deselect for affinity at L-type Ca^{2+}-channels (K_i < 100 μM) are separation at the 4-position of the typical phenyl ring substituent by a two-carbon unsaturated chain (vinyl or acetylene) and at the 6-position substitution of methyl with a bulky phenyl group. Small cycloalkyl groups at the 6-position of 4-phenylethynyl-DHPs were also favorable for high affinity at human A_3 adenosine receptors.

Among those DHPs binding to A_3 receptors selectively and with high affinity were a trisubstituted 1,4-dihydro-6-phenylpyridine analogue, 3-ethyl 5-benzyl 2-methyl-6-phenyl-4-phenylethynyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (MRS 1191, Fig. 3, **10**) [Jiang et al., 1997]. The enhancement of affinity in MRS 1334, **11**, apparently corresponded to the presence of the electron-withdrawing *p*-nitro group on the benzyl ring. These racemic DHPs await optical resolution before radioligands may be synthesized; however, side-by-side comparison of previously known DHP enantiomers shows that the stereoselectivity at A_3 receptors favors the *R*-isomer, the opposite of the stereoselectivity at L-type calcium channels. Even in rat tissue, MRS 1191 was a moderately selective antagonist, e.g., it bound with 28-fold higher affinity for A_3 (K_i of 1.42 μM) vs. A_1 receptors [Jacobson et al., 1997]. In chick ventricular myocyte cultures [Stambaugh et al., 1997] and in the CA1 region of the rat hippocampus [Dunwiddie et al., 1997], 10 μM MRS 1191 selectively antagonized the A_3 subtype in the presence of A_1 and A_2 receptors.

Flavones and flavonols, which are naturally occurring phenolic derivatives, provided another structural lead for development of A_3 antagonists [Karton et al., 1996]. The affinity of common phytochemicals at adenosine receptors suggests that a wide range of natural substances in the human diet may potentially antagonize the effects of endogenous

adenosine, including those mediated by means of the A_3 subtype. The flavonoid class has been chemically optimized in the form of MRS 1067 (**12**) [Karton et al., 1996], which is 200-fold selective for human A_3 vs. A_1 adenosine receptors. Other high-affinity A_3-selective antagonists that have been recently reported include a triazolonaphthyridine (L-249313, **14**) [Jacobson et al., 1996], a series of isoquinoline derivatives such as VUF 8504 (**16**) [Van Muijlwijk-Koezen et al., 1998], and a derivative (MRS 1220, **17**) of the triazoloquinazoline CGS 15943, a nonselective adenosine antagonist [Kim et al., 1996]. Related to the parent compound CGS 15943 simply through acylation at the N^5-amino position with a phenylacetyl group, MRS 1220 is the antagonist of highest affinity (K_i 0.65 nM) at human A_3 receptors currently reported. In rat tissue, the selectivity of MRS1220 shifts to A_{2A} >> A_3 receptors.

Binding of MRS 1067, MRS 1191, and MRS 1220 at human A_3 receptors was shown to be competitive by Scatchard analysis vs. binding of [^{125}I]I-AB-MECA [Jacobson et al., 1997]. Antagonism was demonstrated in functional assays consisting of agonist-induced inhibition of adenylate cyclase and the stimulation of binding of [^{35}S]GTPγ[S] to the associated G-proteins. MRS 1220 and MRS 1191, with K_B values of 1.7 and 92 nM, respectively, proved to be highly selective for human A_3 receptor vs. human A_1 receptor-mediated effects on adenylate cyclase.

Recently, we have explored pyridine derivatives, prepared from 1,4-DHPs through oxidation, as A_3 receptor antagonists [Li et al., 1998]. Certain 3,5-diacyl-2,4-dialkyl-6-phenylpyridine derivatives displayed nanomolar affinity in radioligand binding at recombinant human A_3 receptors and were also considerably selective in binding to recombinant rat A_3 receptors. The 4-Pr derivative, MRS 1523, **15**, was selective and highly potent at both human and rat A_3 receptors (K_i values of 18.9 and 113 nM, respectively). Key modifications of the SAR in the pyridine series included a thioester at the 3-acyl substituents and a small alkyl group at the 4-position. As for the 4-substituted 1,4-DHPs, a 6-phenyl group was required for optimal A_3 selectivity. Unlike the DHPs, a 5-position benzyl ester in the pyridine series decreased affinity at adenosine receptors.

A general pharmacophore model for antagonist binding to the human A_3 receptor has been constructed [Moro et al., 1998b]. A combination of ab initio quantum mechanical calculations, electrostatic potential map comparison, and the steric and electrostatic alignment (SEAL) method led to a general pharmacophore map that was based on adenines, xanthines, triazoloquinazolines, flavonoids, thiazolopyridines, 6-phenyl-1,4-DHPs, and 6-phenyl-pyridines as A_3 adenosine receptor antagonists. According to the proposed pharmacophore map (Fig. 4), recognition of all antagonists at a common region inside the receptor binding site and, consequently, a common electrostatic potential profile is possible. To help interpret these results, a rhodopsin-based model of the human A_3 receptor (Fig. 5) was built, and the triazoloquinazoline reference ligand CGS 15953 (9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine) could be docked in the putative binding site. The model of the ligand-receptor complex was derived and refined by using our recently introduced *cross-docking* procedure [Moro et al., 1998a], which simulates the reorganization of the native receptor structure induced by a ligand. All other ligands could be docked according to the overlay with respect to CGS 15953 as a template, obtained through the

SEAL approach. The proposed interactions between the ligands and specific helical domains of the human A_3 receptor are shown in Figure 5. A major structural difference between the hypothetical binding sites among adenosine receptor subtypes is that the A_3 receptor does not contain the histidine residue in TM6 common to all A_1 (His251 in hA_1 receptor) and A_2 (His250 in hA_{2A} and His251 in hA_{2B} receptors) subtypes. This histidine residue has been shown to participate in both agonist and antagonist recognition to A_{2A} receptors [Jiang et al., 1997b; Kim et al., 1995]. In the A_3 receptor, this histidine in TM6 is replaced with a serine residue (Ser275 in hA_3 receptors). This replacement reduces the steric hindrance in this region of the binding cavity. In our model, substituents at the N^5-position of CGS 15943 are located close to Ser275 (TM6) (see Fig. 5). According to this model, the binding region of the receptor surrounding the α-carbon of the acetyl group of MRS1220 is not sterically restricted, and large substituents could enhance the A_3 binding affinity. In fact, we found that phenylacetyl (MRS 1220) and diphenylacetyl (MRS 1406) derivatives are among the most potent antagonists at the human A_3 receptor (K_i values of 0.65 nM and 0.59 nM, respectively). As shown in Figure 5, other important residues for the ligand binding are the following: Ser271 and Ser275 (TM7), close to the N^5-HR-position of CGS 15943; Asn250 (TM6), close to the oxygen of the furan ring of CGS 15943; Leu90 (TM3), Phe182 (TM5), and Ile186 (TM5) around the chlorophenyl moiety of CGS 15943.

BIOLOGICAL EFFECTS OF A_3 RECEPTOR AGONISTS

In general, there is a strikingly large potency differential among various functional activities of A_3 selective agonists (Table 1), i.e., the same agonists may act functionally in the low nanomolar range (consistent with their affinity in competitive binding assays) for some functional responses, whereas in other activities, even within the same species, micromolar concentrations of the agonists are needed. Although for many receptors, the measured affinity is typically lower than EC_{50} values in functional assays, the wide range of these values for A_3 adenosine receptors, i.e., spanning ≥ 4 orders of magnitude, is unusual. The role of spare receptors in this phenomenon has not been explored.

Effector mechanisms.

In addition to a unique structure-activity profile for agonists and particularly for antagonists, activation of the A_3 receptor has a characteristic second messenger profile (Fig. 1), in that it has been shown to stimulate directly phospholipases C [Abbracchio et al., 1995; Olah and Stiles, 1995] and D [Ali et al., 1996] and to inhibit adenylate cyclase [Olah and Stiles, 1995]. Rat adenosine A_3 receptors can interact with $G_{i\alpha 2}$, $G_{i\alpha 3}$, and to a lesser extent G_q [Palmer et al., 1995]. Recombinant A_3 adenosine receptors undergo agonist-induced desensitization, the mechanism of which involves phosphorylation of the C-terminal segment of the receptor by G protein receptor coupled kinases such as GRK 2, 3, and 5 [Palmer et al., 1996].

The effects of A_3 agonists on intracellular calcium are complex. In HL-60 cells, activation of A_3 receptors by ≥10 μM Cl-IB-MECA results in influx of Ca^{2+} and release from intracellular stores [Kohno et al., 1996]. Similar concentrations of IB-MECA in rat cardiac myocytes cause Ca^{2+} release in the absence of extracellular Ca^{2+} [Shneyvays et al., 1998].

In RBL-2H3 mast cells, the potency of adenosine agonists in raising $[Ca^{2+}]_i$ but not IP_1 levels parallels A_3 receptor affinity [Shin et al., 1996]. In frog A6 kidney cells, a commonly used model of the mammalian collecting duct, micromolar concentrations of Cl-IB-MECA applied to the apical membrane cause an influx of Ca^{2+} but not a release from intracellular stores [Casavola et al., 1998].

Chloride channels are also activated by the A_3 adenosine receptors. In the human eye, A_3 adenosine receptor agonists stimulate chloride channels of nonpigmented ciliary epithelial cells [Mitchell et al., 1999].

Protective versus lethal effects of A_3 receptor activation.

With the recent availability of selective agonists and antagonists, both protective and lethal effects of A_3 adenosine receptor activation have been discovered (Table 2). The concentration of endogenous adenosine required for half-occupancy of A_1 and A_{2A} adenosine receptors is in the range of 10^{-8} to 10^{-7} M [Jacobson et al., 1995; Olah and Stiles, 1995] concentrations that might be achieved in the basal, resting state of an organ. The K_i value of adenosine in binding to the rat A_3 receptor has not been determined directly, but rather estimated to be 10^{-6} M [Jacobson et al., 1995]; activation of this subtype may require a relatively high concentration of adenosine, such as would occur during hypoxic stress and other cellular damage. Thus, the pathophysiologic role of A_3 receptors may be very different from the role of A_1 and A_{2A} subtypes, in that it would act as an endogenous regulator only under conditions of more severe challenge. The varied effects of A_3 receptor agonists, in vitro and in vivo, seem to be dual and opposite, i.e., either cytoprotective or cytotoxic, depending on the level of receptor activation and the paradigm studied. The mechanisms involved in these opposite effects are not yet fully understood.

In the heart, both A_1 and A_3 adenosine receptor agonists appear to protect cardiac myocytes [Liu et al., 1994; Strickler et al., 1996; Stambaugh et al., 1997; Tracey et al., 1997; Auchampach et al., 1997b]; but the latter evoke a longer window of protection [Liang and Jacobson, 1998] and do not cause the hypotension and hypothermia associated with agonists for the other adenosine receptor s. A_3 receptors occur on ventricular but not atrial cardiac myocytes [Strickler et al., 1996]. There are protective effects of A_3 receptor activation in heart cells, administered both before [Strickler et al., 1996; Tracey et al., 1997] and during [Stambaugh et al., 1997] an ischemic episode. IB-MECA also protects against myocardial stunning in conscious rabbits [Auchampach et al., 1997b]. In cultured chick cardiac myocytes, a brief prior exposure to nanomolar concentrations of the A_3 receptor agonist Cl-IB-MECA protected cells from damage induced by subsequent hypoxia [Liu et al., 1994; Strickler et al., 1996], thus simulating the protection afforded by a brief hypoxic period, a phenomenon termed “preconditioning.” Activation by endogenous adenosine of both adenosine A_1 and A_3 receptors is thought to mediate preconditioning. The protective potential was prolonged up to 45 min after exposure to the A_3 agonist [Liang and Jacobson, 1998]. Because the culture consisted only of almost exclusively ventricular myocytes, this was not an indirect effect of activation of mast cells. Thus, an A_3 agonist at low concentration is potentially useful therapeutically as a cardioprotective agent, having fewer

in vivo side effects than other (e.g., A_1 selective) adenosine agonists [Jacobson et al., 1996; Liu et al., 1994].

Although nanomolar concentrations of selective A_3 agonists tend to protect cells, ≥10 μM concentrations are often toxic (Table 2), causing apoptosis, as in rat cardiac myocytes [Shneyvays et al., 1998]. In a variety of human cell lines of the immune system, A_3 agonists at such high concentrations often prove lethal (Table 1). Apoptosis, with the characteristic DNA fragmentation, has been shown to occur in human leukemia HL-60 cells, MCF-7 breast cancer cells, and in human blood eosinophils in response to high concentrations of A_3 selective agonists [Kohno et al., 1996; Yao et al., 1997]. A positive mediator of apoptosis, bak, is up-regulated under these conditions [Yao et al., 1997]. Clarification of the need for such high doses of agonists, thousands of fold higher than the K_i values at A_3 receptors, has awaited the introduction of selective A_3 receptor antagonists, which are now available for the human A_3 receptor.

In certain cultured cell lines, antagonists alone, representing three diverse chemical classes, caused apoptosis (programmed cell death, see below) [Yao et al., 1997], suggesting that there may exist a tonic state of low-level activation of the A_3 receptor which has a protective role. If indeed a tonic A_3 receptor activation exists, the apoptotic effects of A_3 antagonists may simply be explained on the basis of a blockade of a protective action induced by endogenous adenosine. To explain how ≥10 μM concentrations of agonist alone may induce rather than prevent apoptosis, one could propose differential activation of different second messengers by the same receptor at low and high doses (Fig. 1). Such hypotheses will require further investigation, which would be greatly aided by the development of a high-affinity antagonist radioligand for the A_3 receptor. The low density of A_3 receptors has also made study difficult.

A_3 adenosine receptor ligands have been shown to be protective in cerebral ischemia models in gerbils [von Lubitz et al., 1994]. The first cytoprotective effects of an A_3 agonist were shown after its chronic administration in gerbils in a model of stroke. In an in vivo gerbil model of global ischemia, the acute administration of IB-MECA during the ischemia exacerbated histologic and functional damage, i.e., clearly worsened the postocclusive outcome [von Lubitz et al., 1994]. However, chronic preadministration of the same agonist over several weeks had a highly neuroprotective, postischemic effect. In the same gerbil model, acute administration of the A_3 antagonist MRS1191 was protective [von Lubitz et al., 1997].

Several possible explanations for the damaging effects of acute A_3 activation during ischemia have been offered. It may involve detrimental effects observed on cerebral blood flow [von Lubitz et al., 1994] or conceivably release of a cytotoxic agent. Alternately, the effects may be by means of neuronal A_3 receptors, which may regulate other receptors. For example, acute activation of presynaptic hippocampal A_3 receptors antagonizes the action of metabotropic glutamate receptors, thus, resulting in enhanced glutamate release [Macek et al., 1998]. Dunwiddie et al. [1997] found that A_3 activation counteracts protective effects of A_1 receptor activation at the hippocampal synapse; i.e., the depression of excitatory transmission elicited by A_1 agonists is blunted by selective A_3 agonists. In contrast, Fleming

and Mogul [1997] have shown that A_3 receptor activation increases cellular excitability in these neurons through a pathway independent of A_1 receptors. Activation of A_3 receptors in isolated CA3 pyramidal neurons from the guinea pig hippocampus by a low concentration of a selective agonist was also found to potentiate a calcium current through a PKA-dependent/PKC-independent mechanism.

In primary astroglial cell cultures, effects of selective A_3 agonists are also biphasic, with 100–200 nM protecting against cell death and inducing differentiation, whereas ≥10 μM concentrations increased cell death [Abbracchio et al., 1997a]. In human ADF cells of astroglial lineage, 100 nM Cl-IB-MECA caused a marked reorganization of the cytoskeleton, with appearance of stress fibers and numerous cell protrusions (which became enriched in the antiapoptotic protein Bcl-X_L), accompanied by induction of the expression of Rho, a small GTP-binding protein possibly related to cytoskeletal changes [Abbracchio et al., 1997b]. 10 μM Cl-IB-MECA was lethal to cultured rat cerebellar granule neurons, and the toxic effects of glutamate were also augmented [Sei et al., 1997].

Several years ago, a commentary by Beaven et al. [1994] suggested that a then hypothetical A_3 receptor antagonist could be a useful antiasthmatic drug. The acute activation of A_3 receptors in rodents leads to release of histamine and other mediators from mast cells, which also results in hypotension [Fozard et al., 1996]. In microcirculation of the hamster cheek pouch, activation of A_3 receptors results in vasoconstriction, which also occurs through activation of mast cells [Shepherd et al., 1996]. Walker et al. [1997] postulated a role for A_3 receptors in lung inflammation, because adenosine leads to exaggerated airway narrowing in individuals with inflammatory airway disorders. Evidence was found that in humans the A_3 receptor gene expression is localized to inflammatory cells (eosinophils, but not mast cells) and that gene expression is up-regulated in airway inflammation. Cl-IB-MECA was found to inhibit eosinophil migration without affecting adhesion receptors CD18 and selectin or assembly of F-actin, and this effect was blocked by L-294,313 [Knight et al., 1997]. Based on this effect, it is not known whether an A_3 agonist or antagonist would be more useful in treating asthma, because eosinophil activation could theoretically either augment (by means of migration to site) or counteract (by means of migration away from site) inflammation. However, other experiments suggest the utility of an antagonist. For example, Meade et al. [1996] found that in the BDE rat model of airway disease, A_3 agonists induced bronchospasm by means of mast-type cells. Although aerosol challenge of antigen-immunized rabbits with the nonselective agonist APNEA did not elicit dose-dependent changes in either airways resistance or dynamic compliance [Ali et al., 1997], it was found that the agonists IB-MECA and Cl-IB-MECA caused bronchoconstriction. Selective activation of A_3 receptors appears to inhibit human neutrophil degranulation, suggesting the anti-inflammatory potential of A_3 adenosine agonists in neutrophil-mediated tissue injury [Bouma et al., 1997].

There may be an involvement of A_3 receptors in cancer. Activation of A_3 receptors reduced cytotoxic lymphocyte adhesion to tumor cells [Mackenzie et al., 1994].

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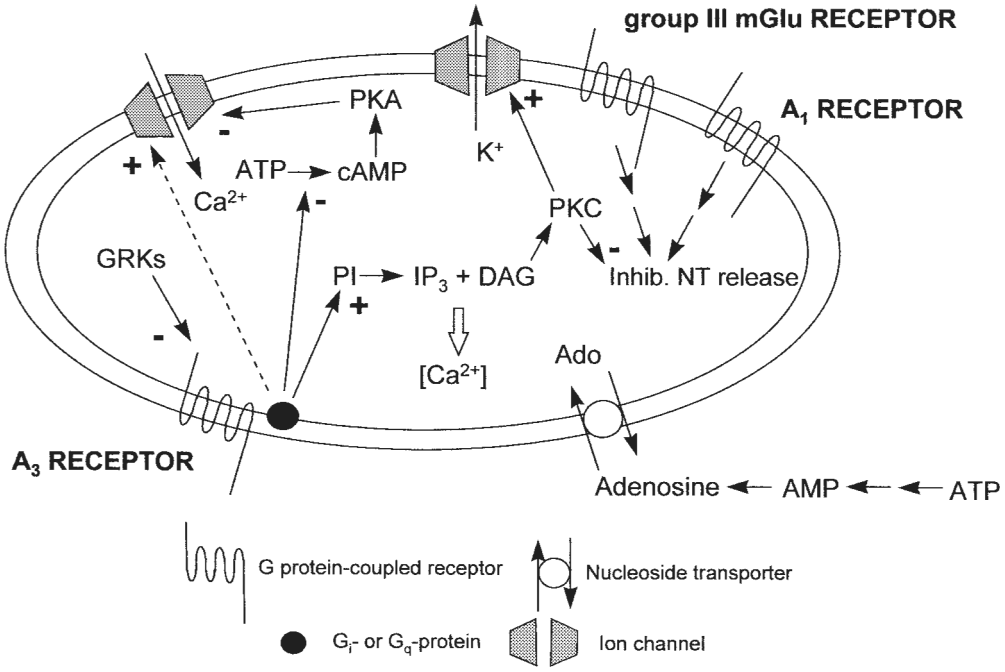

Fig. 1.

Processes resulting from activation of A_3 adenosine receptors. The dashed line indicates activity only at micromolar concentrations of IB-MECA [Kohno et al., 1996; Casavola et al., 1998]. Presynaptic hippocampal A_3 receptor activation induces inhibition of the effects of A_1 receptors [Dunwiddie et al., 1997] and a PKC-dependent inhibition of mGlu receptors [Macek et al., 1998]. In CA3 pyramidal neurons, potentiated calcium current through a protein kinase A (PKA)-dependent mechanism [Fleming and Mogul, 1997]. In cardiac myocytes, A_3 receptor activation is proposed to induce PKC-dependent activation of ATP-sensitive K^+ channels, which results in cardioprotection [Stambaugh et al., 1997]. PI, phosphatidyl inositol; PKC, protein kinase C; DAG, diacylglycerol; NT, neurotransmitter; GRKs, G-protein-coupled receptor kinases.

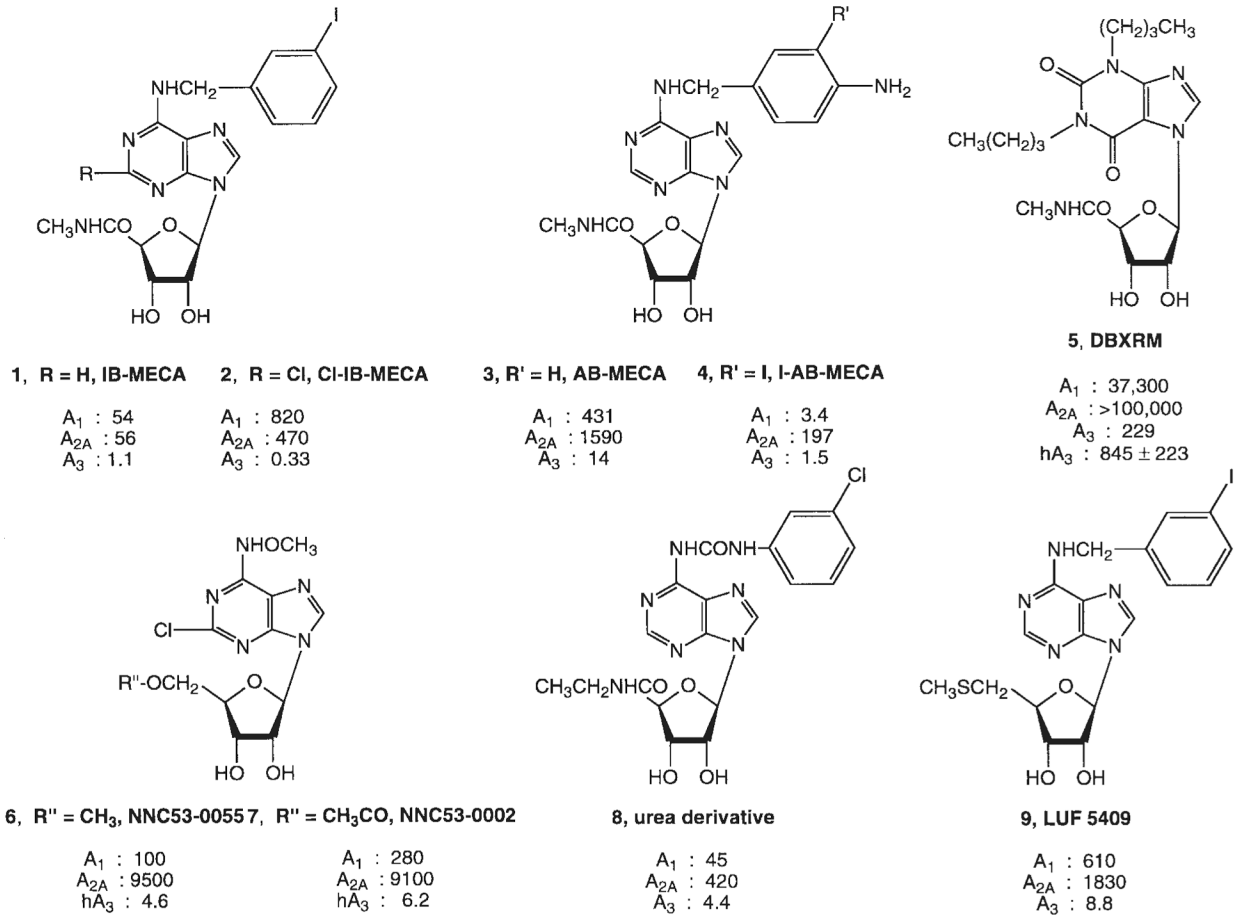

Fig. 2.

Structures of highly potent A_3 adenosine receptor agonists. K_i values in receptor binding at rat (unless indicated) $A_1/A_{2A}/A_3$ receptors (nM) are shown. h, human.

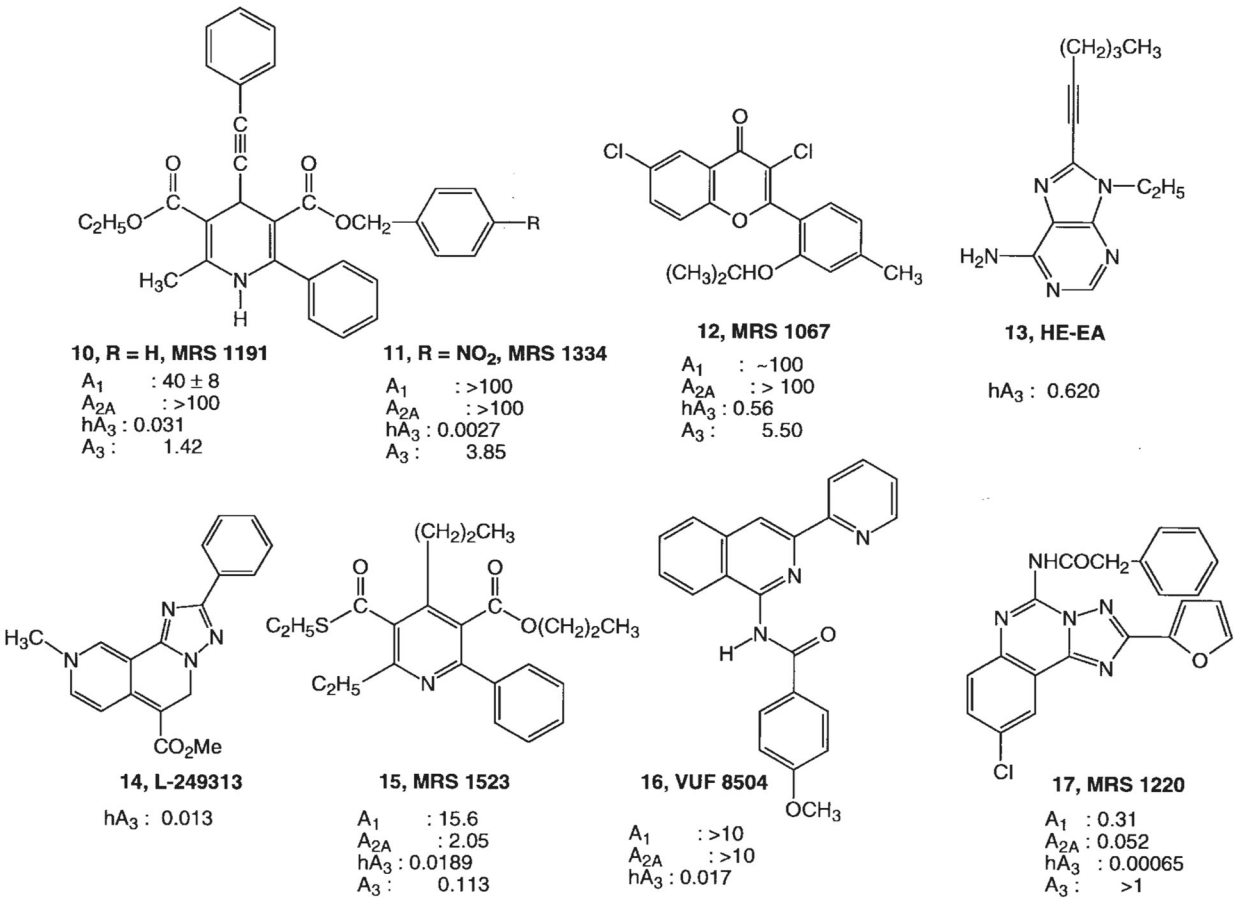

Fig. 3.

Structures of selective A_3 adenosine receptor antagonists. K_i values in receptor binding at rat (unless indicated) $A_1/A_{2A}/A_3$ receptors (micromolar) are shown. h, human.

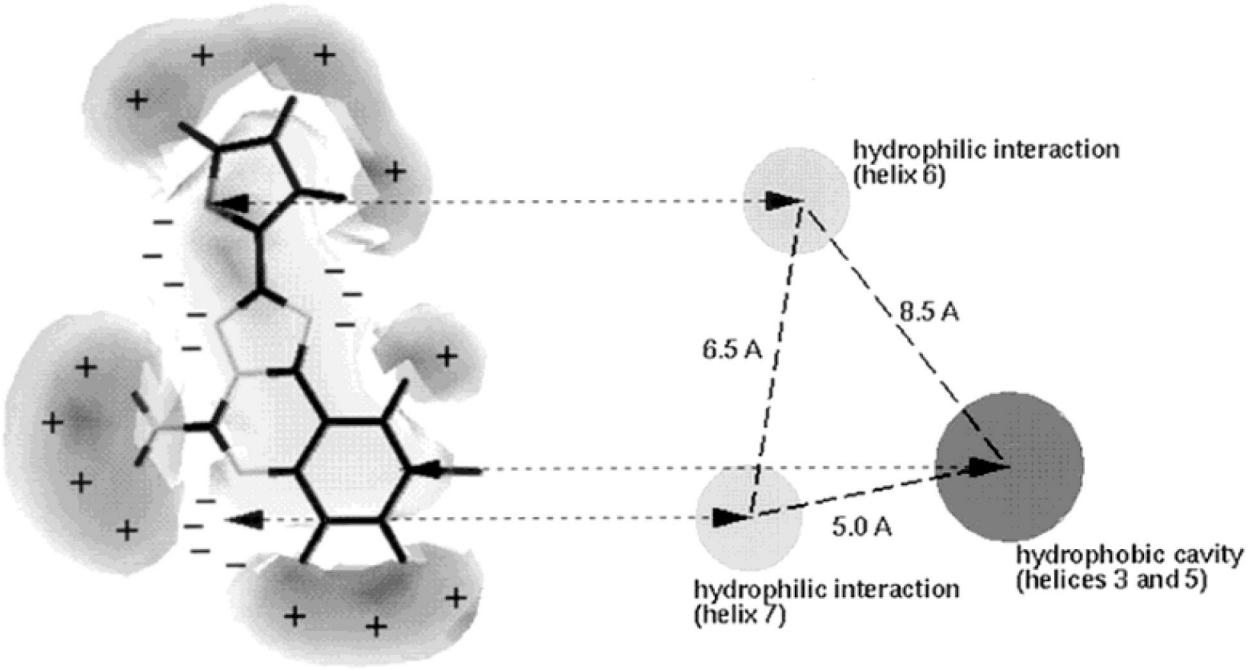

Fig. 4.

Common pharmacophore model for binding of antagonists at the human A_3-adenosine receptor. The isopotential surface of CGS 15943 (+ region = 5 kcal/mol; – region = −5 kcal/mol) and three points of interaction between transmembrane helical domains of the receptor protein and shared features of antagonist molecules (e.g., Fig. 3) are shown.

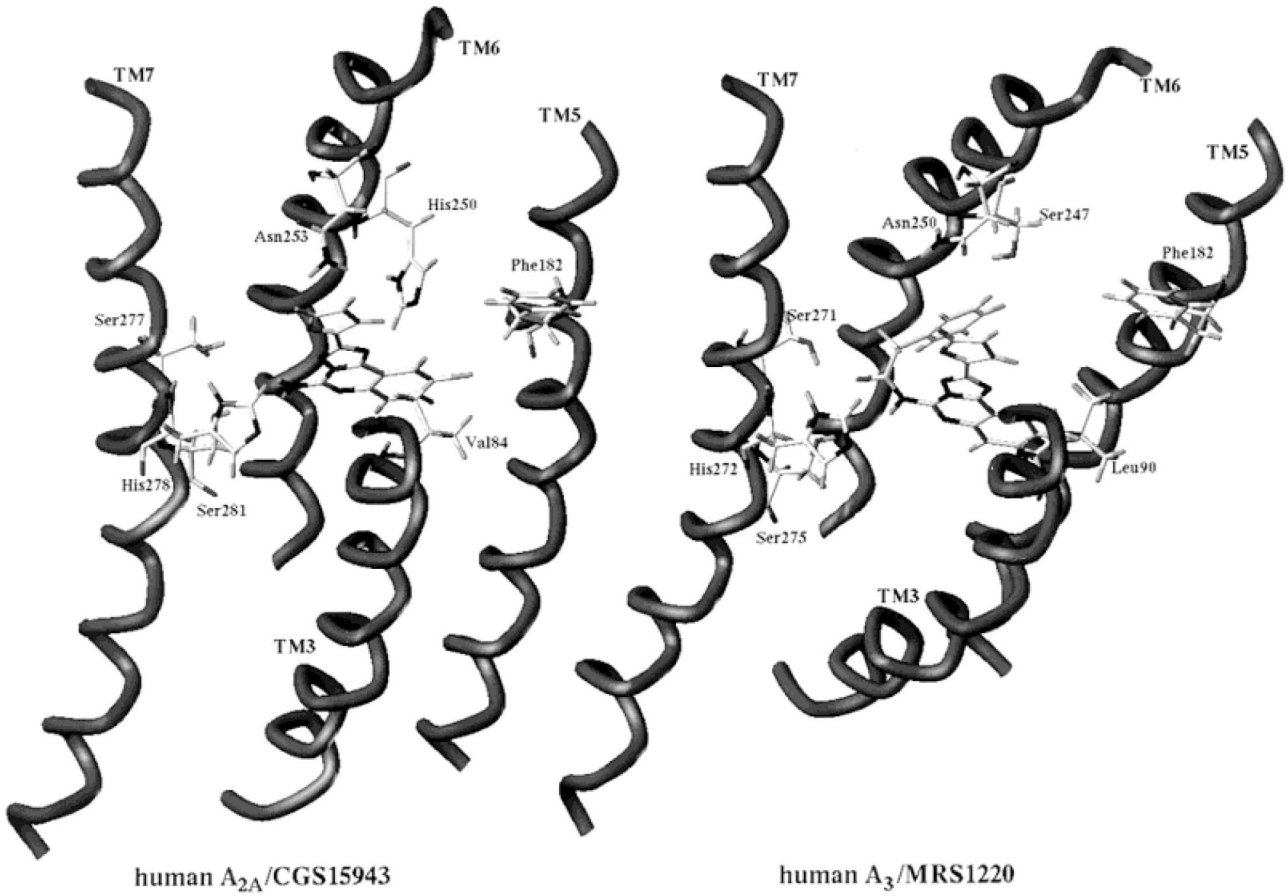

Fig. 5.

Model of the ligand (high-affinity triazoloquinazoline antagonists shown) binding site of human A_{2A} and A_3 adenosine receptors. CGS 15943 is shown bound to the A_{2A} receptor, and the corresponding N^5-phenylacetyl derivative MRS 1220 is shown bound to the A_3 receptor. According to this model, the absence of a key aromatic residue found in A_1 and A_{2A} receptors (His 250, TM6) in the A_3 receptor allows for the introduction of the added bulky phenylacetyl group of MRS 1220 (or similarly a diphenylacetyl group in MRS 1406, not shown).

TABLE 1.

Effects of Potent A_3 Receptor Agonists

Parameter	Cl-IB-MECA (~EC_{50} nM)
In vitro	
Inhibition of chemotaxis (eosinophils)	0.1
Cardioprotection	1–10
Stimulation of phospholipase C (striatum)	50
Inhibition of adenylyl cyclase	60
Astroglial cell changes (morphology, Bcl-X_L)	100
Antagonism of A_1-mediated inhibition of neurotransmission Elevation of $[Ca^{2+}]_i$	500
• RBL-2H3 basophils	70
• A6 cells (distal nephron)	2000
• HL-60 cells, eosinophils, cardiac myocytes	10,000
Necrosis (cerebellum), apoptosis (cardiac, inflamm. system), and cell growth arrest (recomb. receptor in CHO cells)	10,000
Locomotor depression	
Histamine release	
Cerebroprotection—chronic administration (NOS ⇓, MAP2 ⇑)	

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TABLE 2.

Cytoprotective vs. Lethal Effects of A_3 Receptor Agonists and Antagonists

Effect	Heart	Brain
Protective	Low concentration of agonist[a] prior to or during prolonged ischemia	In vivo chronic agonist[c] or acute antagonist,[d] low concentration of agonist in astroglial cells[e]
Lethal	High concentration of agonist[b]	In vivo acute agonist,[c] or high concentration of agonist[f] in vitro

[a] Stambaugh et al., 1997.

[b] Shainberg et al., 1998.

[c] von Lubitz et al., 1994.

[d] von Lubitz et al., 1997.

[e] Abbracchio et al., 1997b.

[f] Sei et al., 1997.

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