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Adenosine A_3 receptors: novel ligands and paradoxical effects

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Abstract

The physiological role of the adenosine A_3 receptor is being investigated using newly synthesized, selective ligands. Recently, in addition to agonists, selective antagonists have been developed that belong to three distinct, non-purine chemical classes: flavonoids, 1,4-dihydropyridine derivatives (e.g. MRS1191, which is 1300-fold selective for human adenosine A_3 vs A_1/A_{2A} receptors, with a K_i value of 31 nM) and the triazoloquinazolines (e.g. MRS1220, which has a K_i value of 0.65 nM). The A_3 receptor has proven enigmatic in terms of antagonist ligand specificity, coupling to second messengers, and biological effects in the CNS, inflammatory system and cardiovascular system. A_3 receptors are also potentially involved in apoptosis. It appears that intense, acute activation of A_3 receptors acts as a lethal input to cells, while low concentrations of A_3 receptor agonists protect against apoptosis. Here, **Kenneth Jacobson** describes how A_3 receptor agonists might be useful in treating inflammatory conditions, possibly through their inhibition of tumour necrosis factor α (TNF-α) release, which has been shown in macrophages. A_3 receptor antagonists might be useful in treating asthma or acute brain ischaemia. Recently, the versatility of A_3 receptor agonists, administered either before or during ischaemia, in eliciting potent cardioprotection has been shown.

Adenosine has been shown to be a critical modulator of a vast array of physiological functions, through activation of one or more of the four known receptor subtypes: A_1, A_{2A}, A_{2B} and A_3 (Refs 1, 2). The adenosine A_1 and A_{2A} receptors, pharmacologically well characterized through the use of selective ligands, generally have a protective role, i.e. in decreasing energy demand and increasing energy supply, respectively, under conditions of stress. Relatively recently identified through cloning[1,2], the A_3 receptor (Fig. 1) has provided a new challenge to medicinal chemists in search of selective ligands[3] and to pharmacologists in defining its role *in vivo*. With the recent availability of selective agonists and antagonists, both protective and lethal effects of A_3 receptor activation have been discovered.

In humans, A_3 receptors are found in the lungs, liver, heart and kidneys, with a lower density being found in the brain and testes[2]. A_3 receptors are found in both neurones[4] and astrocytes[5]. Although the density of A_3 receptors in the brain is possibly too low for mapping using either autoradiography with a high-affinity agonist, [^{125}I]N^6-(4-aminobenzyl)-adenosine-5′-*N*-methyluronamide {[^{125}I]I-AB-MECA} (Fig. 2)[6,7], or *in situ* hybridization[8], a role for this subtype in the CNS has been proposed[9,10].

The A_3 receptors are under scrutiny in relation to potential therapeutic approaches for treating inflammatory and neurodegenerative diseases, asthma and cardiac ischaemia[10–17]. A_3 receptor ligands are protective in cerebral ischaemia models in gerbils[10]. In the heart, both A_1 and A_3 receptor agonists appear to protect cardiac myocytes[14–17], but the latter do not cause the hypotension and hypothermia associated with agonists for the other adenosine receptors. Several years ago, a commentary by Beaven *et al.*[11] suggested, on the basis of studies on a rat basophilic cell line, that a then hypothetical A_3 receptor antagonist could be

a useful anti-asthmatic drug. Indeed, eosinophils are the only cells in which native human A_3 receptors have been characterized using radioligand binding[18]. The occurrence of A_3 receptors on these cells is consistent with the proposed relevance of this subtype to asthma, in which eosinophils may be activated.

Unique pharmacological properties within the adenosine receptor family

The concentration of endogenous adenosine required for half-occupancy of A_1 and A_{2A} receptors is in the range of 10^{-8} to 10^{-7} M (Refs 1, 9), 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 has been estimated to be 10^{-6} M (Ref. 9). Thus, activation of this subtype may require a relatively high concentration of adenosine, such as would occur during hypoxic stress and other cellular damage. Therefore, the pathophysiological role of the A_3 receptor might be very different from the role of the A_1 and A_{2A} subtypes, in that it would act as an endogenous regulator under conditions of more severe challenge.

The low affinity of xanthines, the classic antagonists of the A_1, A_{2A} and A_{2B} subtypes, at rat A_3 receptors is striking[1,2,9]. At human, dog and sheep A_3 receptors[19–21], 8-phenyl-substituted xanthines bearing acidic groups are of higher potency as antagonists; however, none have been found to be highly selective. These differences in xanthine affinity among species and the relatively low degree of homology between human and rat receptor sequences (72%) have raised the hypothesis of two potential subtypes of A_3 receptors, although this remains unsubstantiated. The dramatic species differences in antagonist affinity and in pharmacological responses make the extrapolation of studies of A_3 receptors in rodents to the potential treatment of human disease more challenging. In general, one must be be cautious in comparing responses between species in which tissue and cellular expression of A_3 receptors might be different.

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 (Refs 1, 22) and D (Ref. 23) and to inhibit adenylate cyclase[1]. In HL-60 cells, activation of A_3 receptors results in the influx of Ca^{2+} and its release from intracellular stores[24]. In addition, in the RBL-2H3 basophilic cell line, the potency of adenosine receptor agonists in raising $[Ca^{2+}]_i$ but not IP_1 levels parallels A_3 receptor affinity[25]. In the human eye, A_3 receptors also regulate chloride channels of non-pigmented ciliary epithelial cells [M. M. Civan *et al.*, in abstracts from the *XIII International Congress of Eye Research* (in press)]. There is a strikingly large potency differential among various functional activities of A_3 selective agonists (Table 1), i.e. the same agonists might act functionally in the low nanomolar range (consistent with their affinity in competitive binding assays) for some functional responses, while 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 receptors, i.e. spanning ≥4 orders of magnitude, is unusual. The role of spare receptors in this phenomenon has not been explored.

Rat A_3 receptors can interact with $G_{i\alpha2}$, $G_{i\alpha3}$ and to a lesser extent G_q (Ref. 33). Agonist-induced desensitization of recombinant human A_3 receptors occurs within 20 min, and this is associated with specific down-regulation of $G_{i\alpha3}$ and β subunits. The mechanism of desensitization involves phosphorylation of the C-terminal segment of the receptor by G protein receptor-coupled kinases (GRKs), such as GRK 2, 3 and 5 (Ref. 34).

Selective agonists and antagonists

To obtain high potency agonists that selectively activate A_3 receptors, modifications at two sites in the adenosine structure, the N^6- and 5′-positions, are required[9]. N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyl-uronamide (IB-MECA; Fig. 2) was the first highly potent and selective A_3 agonist, both *in vitro*, in species as diverse as human[31], dog[21] and chick[16], and *in vivo*[9,10]. It is approximately 50-fold selective in binding assays for rat A_3 vs either A_1 or A_{2A} receptors. The radio-ligand [^{125}I]I-AB-MECA (Fig. 2) is widely used as a high-affinity radioligand for A_3 receptors[6], although it is not as selective as IB-MECA (Ref. 7). [^{125}I]I-AB-MECA bound to cloned human A_3 receptors expressed in HEK293 cells with a K_d value of 0.59 nM (Ref. 35), but also bound to other subtypes in autoradiographic studies[7]. Substitution at the 2-position of adenosine in combination with modifications at the N^6- and 5′-positions further enhanced A_3 affinity and selectivity. Thus, 2-chloro-N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide (2Cl-IB-MECA; Fig. 2)[9] displayed a K_i value of 0.33 nM at A_3 receptors and is 2500- and 1400-fold selective for rat A_3 vs A_1 and A_{2A} receptors, respectively.

A_3 receptor antagonists, which have been introduced only recently[35–38], were previously hypothesized[10,11] to act as potential anti-asthmatic[1], anti-inflammatory or cerebroprotective agents. Selective antagonists are needed, especially as most of the effects of high concentrations of A_3 agonists (Table 1) have not been ascribed unequivocally to activation of A_3 receptors. Attempts to find leads for selective xanthine-based antagonists were unproductive[9,20]. An approach, suggested through molecular modelling, to increase potency of xanthines in A_3 receptor binding by forming the 7-riboside derivative, did indeed enhance subtype selectivity.[9] Thus, 1,3-dibutylxanthine-7-riboside-5′-*N*-methylcarboxamide (DBXRM; Fig. 2) is 140-fold selective in binding to rat A_3 vs A_1 receptors. However, as the affinity increased at A_3 receptors, so did the agonist efficacy, and DBXRM proved to be a full agonist at recombinant rat A_3 receptors.

Because xanthines tended to bind only weakly to A_3 receptors, an alternative strategy of screening diverse molecules in chemical libraries for leads was adopted in the design of selective A_3 receptor antagonists. Once the structural principles of A_3 receptor selectivity were discovered in these novel antagonist classes, the leads could be optimized through iterative cycles of chemical synthesis and pharmacological testing. Promising leads for A_3 receptor antagonists appeared among non-xanthine heterocycles (Fig. 3). For example, 1,4-dihydropyridines, known as potent blockers of L-type Ca^{2+} channels and used widely in treating coronary heart disease, were found to bind to human adenosine A_3 receptors. Common dihydropyridine drugs typically bound either non-selectively (for example, nifedipine, with a K_i value of 8.3 μM) or in some cases with selectivity for the A_3 vs other adenosine receptor subtypes (for example, S-niguldipine, with a K_i value of 2.8 μM)[36].

We have used the 1,4-dihydropyridine core as a template, in which it has been possible to select for affinity at adenosine receptors and completely deselect for affinity at L-type Ca^{2+} channels (K_i <100 μM), principally through introduction of a 6-phenyl group. At human A_{2B} receptors, such 1,4-dihydropyridines are similarly inactive[4]. For example, a trisubstituted 1,4-dihydro-6-phenylpyridine analogue, MRS1191 (Fig. 3)[36], has been found to inhibit radioligand binding at the human A_3 receptor with a K_i value of 31 nM, while the same derivative was nearly inactive in binding at A_1 and A_{2A} receptor sites (i.e. >1300-fold selective). Even in the rat, MRS1191 was selective pharmacologically in various paradigms; it bound with 28-fold higher affinity for A_3 (K_i value of 1.42 μM) vs A_1 receptors, and at a 10 μM concentration it antagonized only the A_3 subtype in the CA1 region of the hippocampus[4,36]. Furthermore, MRS1191 antagonized the effects of the A_3 receptor-selective agonist IB-MECA on inhibition of adenylate cyclase via recombinant human or rat

A_3 receptors[31]. In chick ventricular myocyte cultures, MRS1191 antagonized the anti-ischaemic effects of Cl-IB-MECA (Ref. 16). Thus, dihydropyridine derivatives, such as MRS1191, appear to be useful as A_3 receptor antagonists across species, although there is still a need for high-affinity antagonists of rat A_3 receptors.

The structure–activity relationships of analogues of MRS1191, containing both subtle and drastic structural changes at various positions of the dihydropyridine ring (its 3- and 5-acyl substituents, the 4- and 6-aryl/alkyl substituents and the 2-methyl group), have been investigated systematically[36]. Substitutions of a 5-benzyl ester group provide the greatest versatility for achieving >30000-fold human A_3 receptor selectivity and nanomolar potency. Affinity and selectivity for the human A_3 receptor within this series was optimal in MRS1334 (Fig. 3), which has a K_i value of 2.7 nM (Ref. 36). These racemic dihydropyridines await optical resolution; however, side-by-side comparison of previously known dihydropyridine enantiomers shows that the stereoselectivity at A_3 receptors favours the R-isomer, the opposite of the stereoselectivity at L-type Ca^{2+} channels.

Naturally occurring phenolic derivatives (e.g. the flavones and flavonols) provided another structural lead for development of A_3 receptor antagonists[35]. The affinity of common phytochemicals at adenosine receptors suggests that a wide range of natural substances in the human diet might potentially antagonize the effects of endogenous adenosine, including those mediated via the A_3 subtype. The flavonoid class has been chemically optimized in the form of MRS1067 (Ref. 35), which is 200-fold selective for human A_3 vs A_1 adenosine receptors. Other high-affinity A_3 receptor-selective antagonists that have been reported recently include a triazolonaphthyridine (L249313; Fig. 3)[38], thiazolopyrimidine (L268605; Fig. 3)[38] and a derivative of the triazoloquinazoline CGS15943 (MRS1220; Fig. 3)[37]. L249313 binds to human A_1 and A_{2A} receptors with K_i values of 6.6 and 1.25 μM, respectively. The K_i value at rat A_3 receptors is 33 μM. Although not as selective as MRS1191, MRS1220 is the antagonist with the highest affinity (K_i 0.65 nM) for human A_3 receptors reported yet. MRS1220 is not A_3-selective in the rat, further emphasizing the species differences in antagonist affinity.

Binding of MRS1067, MRS1191 and MRS1220 at human A_3 receptors was shown to be competitive by Scatchard analysis versus binding of [^{125}I]I-AB-MECA (Ref. 31). Antagonism was demonstrated in functional assays consisting of agonist-induced inhibition of adenylate cyclase and the stimulation of binding of [^{35}S]GTP-γ-S to the associated G proteins. MRS1220 and MRS1191, with K_B values of 1.7 and 92 nM, respectively, were highly selective for human A_3 receptor vs human A_1 receptor-mediated effects on adenylate cyclase.

Protective versus lethal effects of A_3 receptor activation

The varied effects of A_3 receptor agonists, *in vitro* and *in vivo*, appear 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.

Nanomolar concentrations of selective agonists tend to protect cells, while micromolar concentrations are often toxic (Table 1)[24]. In certain cultured cell lines, antagonists alone are toxic[39]. The fact that A_3 receptor antagonists representing three diverse chemical classes evoked the common biological effect of apoptosis (programmed cell death, see below) suggests that a tonic state of activation of the A_3 receptor might exist, and that this possible low level of receptor activation has a protective role. If a tonic A_3 receptor activation does exist, the apoptotic effects of A_3 receptor antagonists might simply be explained on the basis of a block of a protective action induced by endogenous adenosine. To explain how very

high doses of agonist alone might 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.

Central nervous system

The first cytoprotective effects of an A_3 receptor agonist were shown following its chronic administration in gerbils in a model of stroke. In an *in vivo* gerbil model of global ischaemia, the acute administration of IB-MECA during ischaemia exacerbated histological and functional damage, clearly worsening the post-occlusive outcome[10]. However, chronic pre-administration of the same agent over several weeks had a highly neuroprotective, post-ischaemic effect, in which the agonist was highly cerebroprotective, preserved microtubule-associated protein 2 (MAP2) immunoreactivity, and depressed NO synthase. In primary astroglial cell cultures, nanomolar concentrations of selective A_3 receptor agonists caused protection against cell death and induced differentiation, while high concentrations increased cell death[32]. In human ADF cells of astroglial lineage, 100 nM Cl-IB-MECA caused a marked reorganization of the cytoskeleton, with appearance of stress fibres and numerous cell protrusions (which became enriched in the anti-apoptotic protein Bcl-x_L), accompanied by induction of the expression of Rho, a small GTP-binding protein[5]. A high concentration of Cl-IB-MECA (≥10 μM) was lethal to cultured rat cerebellar granule neurons, and the toxic effects of glutamate were also augmented[27]. In preliminary experiments, acute adminstration of the selective A_3 receptor antagonist MRS1191 proved to be cerebroprotective in the gerbil global ischaemia model[40].

Several possible explanations for the damaging effects of acute A_3 activation during ischaemia have been offered. These include the detrimental effects seen on cerebral bood flow[10] or the release of a cytotoxic agent. Alternatively, the effects might be via neuronal A_3 receptors. In general, high concentrations of IB-MECA and Cl-IB-MECA directly cause influx of Ca^{2+} (Refs 24, 25); however, this may not be relevant to potent *in vivo* effects. In addition, examples of cross-talk between the A_3 receptor subtype and other adenosine receptors are being discovered. For example, acute activation of presynaptic hippocampal A_3 receptors antagonizes the action of metabotropic glutamate receptors, thus resulting in enhanced glutamate release[41]. Dunwiddie *et al.*[4] found that A_3 activation counteracts the protective effects of A_1 receptor activation at the hippocampal synapse, i.e. the depression of excitatory transmission (EPSPs) elicited by A_1 agonists is blunted by selective A_3 agonists. In contrast, Mogul and coworkers[27] have shown that A_3 receptor activation increases cellular excitability in these neurones through a pathway independent of A_1 receptors. Activation of A_3 receptors in isolated CA3 pyramidal neurones from guinea-pig hippocampus by a low concentration of a selective agonist was also found to potentiate a Ca^{2+} current through a cAMP-dependent protein kinase (PKA)-dependent/protein kinase C (PKC)-independent mechanism[27].

The immune system and inflammation

In a variety of human cell lines of the immune system, A_3 agonists at high concentrations often prove lethal (Table 1). Apoptosis, with characteristic DNA fragmentation, has been shown to occur in human leukaemia HL-60 cells, MCF-7 breast cancer cells and in human peripheral blood eosinophils in response to high concentrations (≥10 μM) of A_3 receptor-selective agonists[24,39]. A mediator of apoptosis, bak (pro-apoptotic Bcl-2 homology protein), is upregulated under these conditions[39]. The protective effects of A_3 receptor activation might involve cytokines. Sajjadi *et al.*[30] have shown that micromolar concentrations of the agonist IB-MECA in U937 cells inhibit the release of tumour necrosis

factor α (TNF-α), which in turn might induce apoptosis. Clarification of the need for such high doses of agonists, thousands of times 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. As discussed above, A_3 receptor antagonists of diverse structures alone cause apoptotic cell death[39], and cells may be rescued by subcytotoxic concentrations of selective A_3 receptor agonists.

Walker *et al.*[12] postulated a role for A_3 receptors in lung inflammation, as adenosine leads to exaggerated airway narrowing in individuals with inflammatory airway disorders. Evidence was found that in humans A_3 receptor gene expression is localized to inflammatory cells (eosinophils, but not mast cells) and that gene expression is upregulated in airway inflammation. Cl-IB-MECA inhibited eosinophil chemotaxic responses to PAF, RANTES or LTB, without affecting adhesion receptors CD18 and selectin, or assembly of F-actin. This effect was blocked by the selective A_3 antagonist L249313 (Ref. 13). Based on this effect, it is not known whether an A_3 agonist or antagonist would be more useful in treating asthma, as, theoretically, eosinophil activation could either augment (via migration to site) or counteract (via migration away from site) inflammation. However, other experiments suggest that an antagonist might be more useful. For example, Meade *et al.* found that in the BDE-strain rat model of airway disease, A_3 receptor agonists induced bronchospasm via mast-type cells[42]. Although aerosol challenge of antigen-immunized rabbits with the non-selective agonist N^6-[2-(4-aminophenylethyl)-adenosine] (APNEA) did not elicit dose-dependent changes in either airway resistance or dynamic compliance[43], Ali *et al.* found that the agonists IB-MECA and Cl-IB-MECA caused bronchoconstriction[44].

Selective activation of A_3 receptors appears to inhibit human neutrophil degranulation, suggesting the anti-inflammatory potential of A_3 receptor agonists in neutrophil-mediated tissue injury[29].

There might be an involvement of A_3 receptors in cancer[45]. Activation of A_3 receptors reduced cytotoxic lymphocyte adhesion to tumour cells.

Cardiovascular and renal systems

The acute activation of A_3 receptors in rodents leads to hypotension, exclusively via release of histamine and other mediators from peripheral mast cells[46]. However, canine and human mast cells do not react in this manner[21]. Shepherd *et al.*[47] found that in microcirculation of the hamster cheek pouch, activation of A_3 receptors results in vasoconstriction, which also occurs through activation of mast cells.

A_3 receptors occur on ventricular, but not atrial, chick cardiac myocytes[15]. There are protective effects of A_3 receptor activation in heart cells, administered both prior to[15,17] and during[16] an ischaemic episode (Fig. 4)[17]. IB-MECA also protects against myocardial stunning in conscious rabbits[48]. 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[14,15], thus simulating the protection afforded by a brief hypoxic period, a phenomenon termed ‘preconditioning’. Activation by endogenous adenosine of both A_1 and A_3 receptors is thought to mediate preconditioning. Because the culture consisted almost exclusively of ventricular myocytes, this was not an indirect effect of activation of A_3 receptors on mast cells, as has been reported by Fozard and co-workers[46] to explain hypotensive effects *in vivo*. Thus, an A_3 agonist at low concentration is potentially useful therapeutically as a cardioprotective agent, having a more sustained duration of protection and fewer *in vivo* side-effects than other (e.g. A_1-selective agonists) adenosine agonists[14,16,49]. However, high concentrations of the same agonists were shown to be damaging, i.e. they induce apoptosis in rat cardiac myocytes[28].

Concluding remarks

By virtue of regulating programmed cell death, A_3 receptors might play a critical role in human disease states. The relation of the A_3 receptor to apoptosis suggests that both A_3 receptor agonists and antagonists might be useful for treating diseases either in which cytotoxicity is undesirable, such as neurodegeneration, or desirable, such as cancer and inflammation. A_3 receptor antagonists might also be useful in treating asthma. The acute administration of an A_3 receptor antagonist or the chronic adminstration of an A_3 receptor agonist appears to protect brain cells in a global ischaemia model, and thus are potential therapeutic approaches for preventing stroke damage. In the heart, because A_3 receptor activation protects both in a preconditioning model and during prolonged ischaemia, selective agonists might be of great clinical importance.

Chemical names

CGS15943	9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-*c*] quinazolin-5-amine
L249313	6-carboxymethyl-5,9-dihydro-9-methyl-2-phenyl-[1,2,4]-triazolo[5,1-*a*] [2,7]naphthyridine
L268605	3-(4-methoxyphenyl)-5-amino-7-oxo-thiazolo[3,2]pyrimidine
MRS1067	3,6-dichloro-2′-(isopropoxy)-4′-methylflavone
MRS1191	3-ethyl 5-benzyl 2-methyl-6-phenyl-4-phenyl-ethynyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate
MRS1220	9-chloro-2-(2-furyl)-5-phenylacetylamino [1,2,4]triazolo[1,5-*c*]quinazoline
MRS1334	3-ethyl 5-(4-nitrobenzyl) 2-methyl-6-phenyl-4-phenylethynyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate

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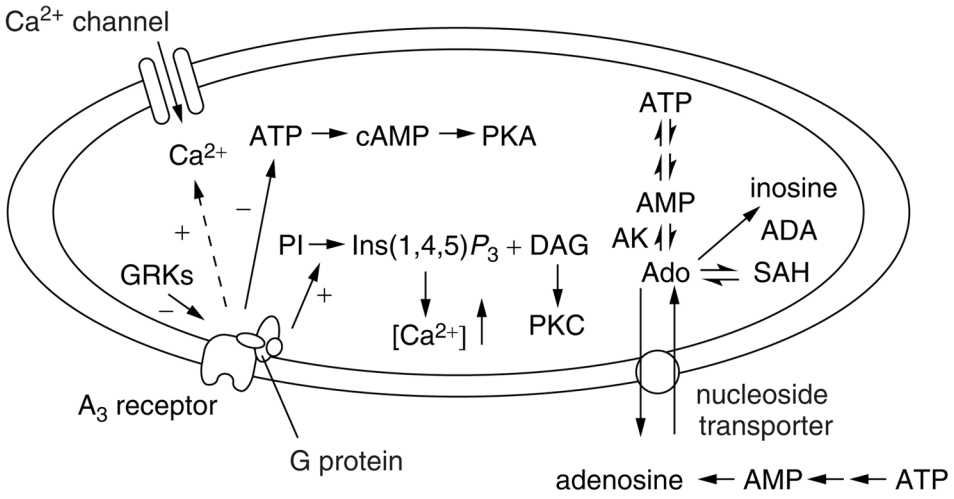

Fig. 1.

Processes of formation and degradation of adenosine in the cell and actions at A_3 adenosine receptors. The dashed line indicates activity only at micromolar concentrations of IB-MECA. ADA, adenosine deaminase; AK, adenosine kinase; GRKs, G protein-coupled receptor kinases; Ins(1,4,5)P_3, inositol (1,4,5)-trisphosphate; PI, phosphatidylinositol; PKA, cAMP-dependent protein kinase; SAH, *S*-adenosyl homocysteine.

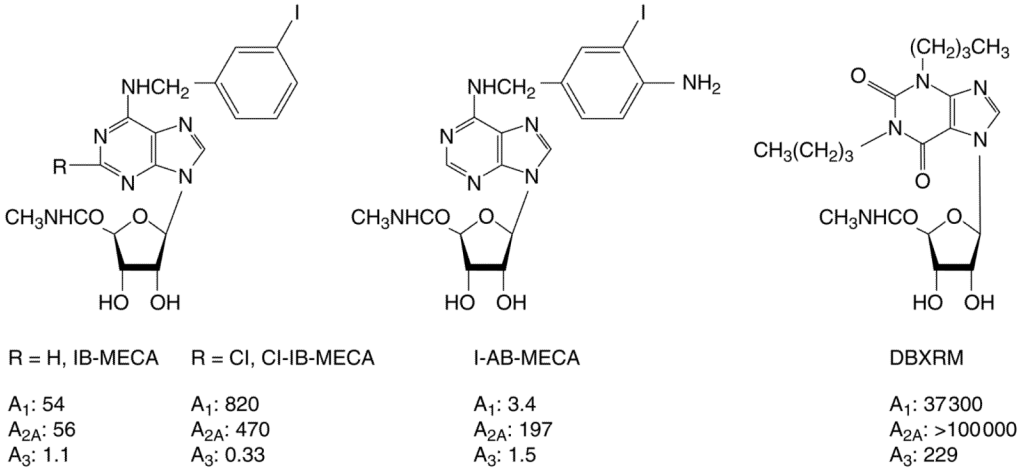

Fig. 2.

Structures of highly potent adenosine A_3 receptor agonists. Abbreviations used in the text and receptor binding affinities at rat $A_1/A_{2A}/A_3$ receptors (nM) are indicated. Cl-IB-MECA, chloro-N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide; DBXRM, 1,3-dibutylxanthine-7-riboside-5′-*N*-methyl-carboxamide; I-AB-MECA, [^{125}I]N^6-(4-aminobenzyl)-adenosine-5′-*N*-methyluronamide; IB-MECA, N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide.

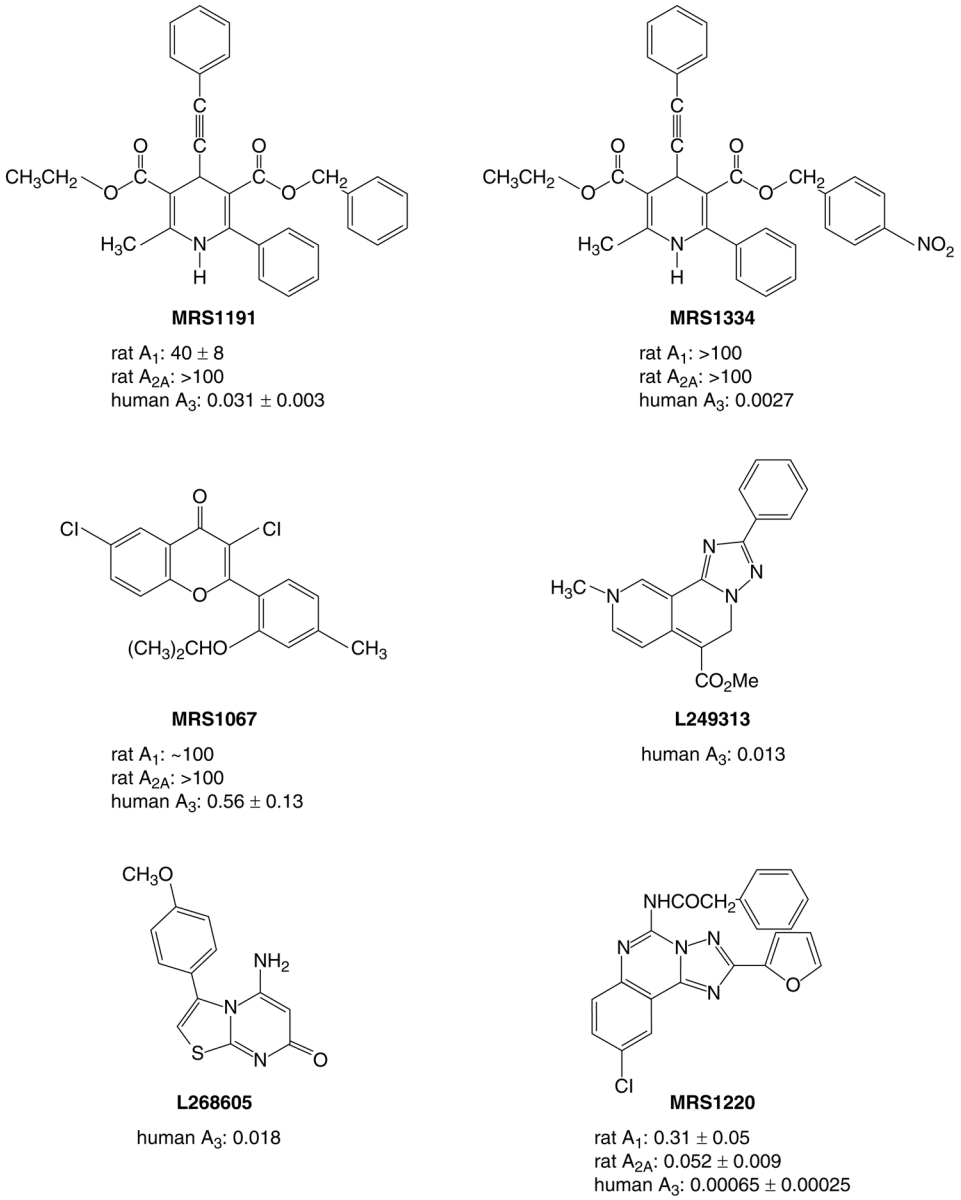

Fig. 3.

Structures of selective A_3 adenosine receptor antagonists. Abbreviations used in the text and receptor binding affinities (μM) are indicated.

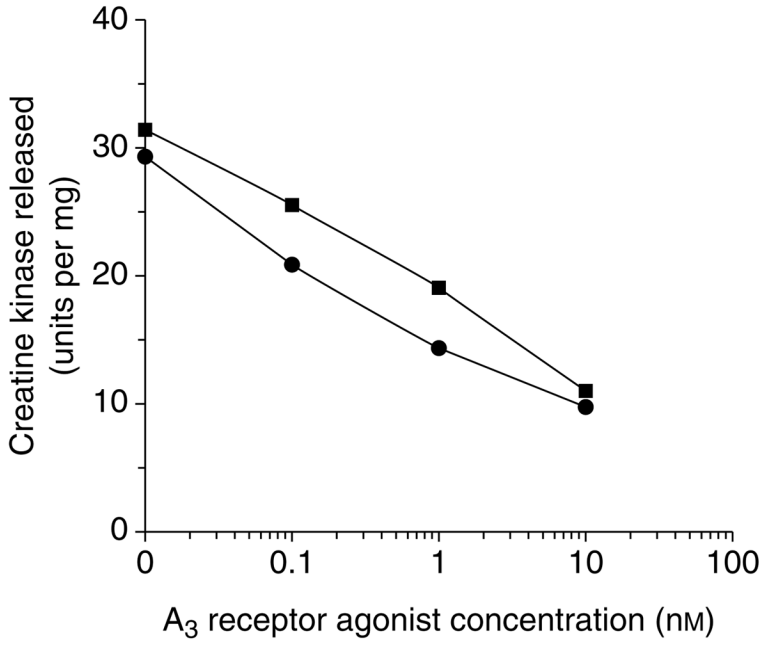

Fig. 4.

Cardioprotection elicited by the selective A_3 adenosine receptor agonists, N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide (IB-MECA) and Cl-IB-MECA, during prolonged ischaemia (modified from Ref. 16). Cardiac ventricular myocytes were cultured from chick embryos 14 days *in ovo*, and cell injury was induced by a 90 min exposure of the culture to hypoxia with glucose deprivation. Release of creatine kinase into the medium was directly proportional to the degree of cell injury. Curves shown were measured in the presence of 1 μM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (selective A_1 adenosine receptor antagonist), which had no effect on the cardioprotection observed. The selective A_3 adenosine receptor antagonist MRS1191 antagonized the cardio-protection provided by 10 nM Cl-IB-MECA, with an IC_{50} of ~10 nM. A_3 adenosine agonists were also cardioprotective following a brief exposure, prior to ischaemia (Ref. 15). Squares, IB-MECA; circles, Cl-IB-MECA.

Table 1

Functional effects of adenosine A_3 receptor agonists

Site	Action	Agonist	Approximate EC_{50} (nM)	Refs
Primary tissue/culture				
Rat hippocampal slices[a]	PLC activation	Cl-IB-MECA	50	22
	PLC activation	IB-MECA	180	22
Rat hippocampal slices[a]	Inhibition of A_1 effects	Cl-IB-MECA	100–1000	4
Rat hippocampal slices	Potentiation of Ca^{2+} current	APNEA	20	27
Rat cerebellar granule cells	Death			
	Increased cAMP	Cl-IB-MECA	15 000	26
Chick ventricular myocytes[a]	Inhibition of cAMP	Cl-IB-MECA	0.6	15
	Stimulation of PLD	Cl-IB-MECA	2	
	Preconditioning	Cl-IB-MECA	8	
	Protection during prolonged hypoxia	Cl-IB-MECA	0.2	16
Rat cardiac myocytes	Apoptosis	IB-MECA	≥10 000	28
Human eosinophils	Apoptosis			
	Elevation of Ca^{2+}	Cl-IB-MECA	≥10 000	18
	Inhibition of chemotaxis	Cl-IB-MECA	0.1	13
Neutrophils	Degranulation	IB-MECA	1	29
Cell lines				
Rat RBL (mast) cells[a]	Elevation of Ca^{2+}	Cl-IB-MECA	70	25
		IB-MECA	110	25
Human leukaemia (HL-60)	Apoptosis			
	Elevation of Ca^{2+}	Cl-IB-MECA	≥10 000	24
		IB-MECA	≥10 000	24
	Protection against apoptosis	Cl-IB-MECA	10	24
Human U937 macrophage[a]	Inhibition of TNF-α	IB-MECA	3000	30
		Cl-IB-MECA	3600	31
Human ADF (astroglial) cells[a]	Changes in cytoskeleton, Bcl-X_L, Rho	Cl-IB-MECA	100	5
Recombinant receptors				
Rat A_3–CHO cells[a]	Inhibition of cAMP	Cl-IB-MECA	70	9
	Inhibition of cAMP	IB-MECA	90	9
Human A_3–CHO cells[a]	Inhibition of cAMP	IB-MECA	59	31
	Inhibition of growth	Cl-IB-MECA	≥10000	32

[a]Activity was antagonized either by a nonselective or selective adenosine A3 receptor antagonist. APNEA, N^6-[2-(4-aminophenylethyl)adenosine], a nonselective agonist; IB-MECA, N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide; PLC, phospholipase C; PLD, phospholipase D; TNF-α, tumour necrosis factor α.