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Chapter 13. A₃ Adenosine Receptors

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Introduction

Extracellular adenosine (1) is involved in many cytoprotective functions of the body, including conditioning the heart against ischemia, counteracting the damaging effects of excitotoxicity and seizure activity in the brain, and suppressing an excessive immune and inflammatory response (1). The possibilities of therapeutic intervention based on modulation of adenosine receptors and adenosine levels are numerous. There are four subtypes of adenosine receptors; of which the A₃ receptor was most recently identified as a result of its cloning from various species (2). The A₃ receptor is activated endogenously by higher concentrations of adenosine than are required for activation of the A₁ /A_{2A} receptors. The effector mechanisms are inhibition of adenylate cyclase and stimulation of phospholipase C (1,3). Both protective (usually at nM concentrations) and damaging effects (usually at μ M concentrations) of A₃ agonists have been studied (3). The receptor is distributed at low, diffuse levels in the brain and in the human periphery where it is present in lungs, liver, heart, and immune cells such as eosinophils (2). Therapeutic interests related to A₃ receptors are: anti-inflammatory (possibly through depression of TNF- α levels) (4), cardioprotective (5) cerebroprotective (6), anticancer (7), and antiglaucoma (8). A mouse line lacking the A₃ receptor demonstrated that this is a non-lethal mutation that has inflammatory, cardiovascular, and behavioral consequences (9–11). The A₃-modulated release of histamine from mast cells may be specific to rodent species (12).

1

2

3

A₃ RECEPTOR STRUCTURE: LIGAND BINDING SITE, ALLOSTERIC MODULATION

Mutagenesis was recently carried out on the human A₃ receptor, a 7TM receptor, to locate a putative ligand binding site (13). In some cases, different amino acid residues are associated with agonist or with antagonist binding. Movement of a conserved Trp in TM6 has been proposed as an important step in receptor activation. In a rhodopsin-based docking model of the human A₃ receptor, this residue rotates characteristically upon *in silico* binding of agonists but not antagonists. This may be linked to a required rotation of TM6 during the receptor activation process (13).

Allosteric Modulators

Pyridinylisoquinolines (VUF5455, **2**) and imidazoquinolines (DU124183, **3**) were shown to positively modulate A₃ binding and/or action of agonists, although both substances retain antagonistic properties as well (14,15). These derivatives had no effects at the A₁/A_{2A} receptor subtypes. Compound **3** augmented the maximal efficacy of an A₃-agonist, in addition to decreasing the agonist dissociation rate from the receptor.

A₃ RECEPTOR AGONISTS

Nearly all of the known adenosine A₃-agonists are derivatives of adenosine (3). NECA (4) is a nonselective, but highly potent agonist, which was used for the initial characterization of the A₃ receptor and has been used as a less-than-optimal radioligand for the A₃ receptor (2).

Adenosine 5',N⁶-Substitutions

Certain substitution at the N⁶-position (such as the 3-iodobenzyl group) provides A₃ selectivity, although in some cases N⁶-substitution of adenosine 5'-OH derivatives with large groups (e.g., substituted benzyl groups or large cycloalkyl rings) may reduce maximal efficacy to produce partial agonist activity at the A₃-receptor. This reduction of efficacy may be overcome by combining the N⁶-substitution with 5'-uronamide substitution, as in NECA. For example, benzyl-NECA (**5**, slightly selective) and IB-MECA (**6**, ~50-fold selective versus A₁/A_{2A} receptors) are full agonists, while the corresponding 5'-OH nucleosides display roughly half of the maximal efficacy (13,16). IB-MECA (**6**) is currently in clinical trials for cancer treatment, due to its reported cytostatic effect on tumors (7). The radioiodinated ligand I-AB-MECA (**7**) is used widely for screening of new ligands of the A₃ receptor at which it is potent (K_d = 0.59 nM) but not highly selective (3). The efficacy at A₃ receptors is more easily diminished upon structural modification than at the other subtypes. Rigidification of the ribose ring moiety (5'-uronamides) also reduces A₃ efficacy. A small N⁶ substitution (e.g., methyl, methoxy, or ethyl) provides high affinity at the human but not the rat A₃ receptor (17). Several A₃ selective agonists, NNC21-0238 (**8**, K_i = 20 nM) and NNC53-0055 (**9**, K_i = 4.6 nM) containing N⁶-methoxy groups and various substitutions at the 5'-position have been reported (18). Acylation of the N⁶-position (urea derivative **10**) has also been shown to enhance agonist potency at the A₃ receptor (19). The efficacy-promoting effects of the 5'-uronamide group are sufficiently strong to allow substitution of

the adenine moiety with a 1,3-dibutylxanthine group, and thus DBXRM (11) is a selective and moderately potent, full A₃-agonist (3).

4 R = H

6 R = H

7 R = NH₂

Adenosine 2-Substitutions

A 2-chloro substitution generally improves the potency at A_3 receptors (20). For example, CI-IB-MECA (12) is a highly selective, full agonist with K_i value = 0.33 nM at the rat A_3 receptor. In some cases, chain elongation at the 2-position has enhanced A_3 receptor affinity, such as N^6 -Me-PE-NECA (13) (21).

Ribose Substitutions of Adenosine

While the 5'-hydroxyl group of the ribose ring may be replaced, the 2' and 3'-hydroxyl groups are generally required for affinity and/or the ability to fully activate the receptor; i.e., achieve full efficacy (22). The 3'-hydroxyl may be replaced with 3'-amino which will maintain agonist affinity only in combination with the appropriate N^6 -substitution. For example, CP608039 (14) is intended for use in peri-operative cardioprotection (23). The 3'-amino group, which is mainly charged at physiological pH, also enhances water-solubility. 3'-Amino-3'-deoxy analogues are useful for the design of neoligands in relation to the concept of neoreceptors which is a means of engineering the binding site of a 7TM receptor for unique recognition of synthetic ligands (24). A ribose substitution that has enhanced A_3 selectivity in a general manner is the replacement with the (N)-methanocarba ring system (15 – 17) which is superior to simple carbocyclics, such as the nonselective agonist AMP579 (18) (25, 26). The (N)-methanocarba, 5'-uronamides (e.g., MRS 1898, **17**) are full agonists, while the 5'-OH analogues (MRS 1743 (15) and MRS 1760 (16)) are low efficacy partial agonists (27). Another ring substitution that has been utilized in A_3 selective agonists is the 4'-methyl group (28).

A_3 RECEPTOR ANTAGONISTS

Nonpurines

Leads for novel A_3 antagonists have been obtained from compounds known to inhibit adenosine receptors and from library screening. The triazoloquinazoline derivative CGS15943 (19), previously known as a nonselective adenosine antagonist at the human subtypes, served as template for related nonpurine heterocyclic antagonists of the human A_3 receptor, due to the observation of its high affinity ($K_i \sim 10$ nM). Acylation of the N^5 -amino group of **19** led to MRS 1220 (20) which is potent ($K_i = 0.65$ nM) and selective for human, but not rat A_3 receptors (29). In a related series of heterocyclic derivatives, the

pyrazolotriazolopyrimidine derivative MRE3008-F20 (21) was found to be a highly selective antagonist at the human A₃ receptor and was radiolabeled to provide a hydrophobic, but useful radiotracer with a K_d = 0.80 nM (30). Another analogue (22) in the same series displayed a K_i = 0.16 nM at the A₃ receptor (31). A pyridinium moiety (23) was introduced to enhance water-solubility in this series of A₃ antagonists (32).

24 **25** **26**

27, R = H
28, R = NO₂

The earliest reported A₃ antagonists were the result of library screening in which novel heterocyclic antagonists derivatives were identified (3,33,34): L-249313 (**24**, K_i = 13 nM) and L-268605 (**25**, K_i = 18 nM), flavonoids (MRS1067, **26**, K_i = 560 nM), and dihydropyridines (27, 28). The dihydropyridines are typically antagonists of L-type Ca²⁺ channels; however, the addition of extended (arylkene or arylalkyne) groups at the 4-position and phenyl substituents at the 6-position combined to eliminate recognition at the Ca²⁺ channels. Chiral resolution of MRS1191 (27) was carried out to identify the more active isomer, although the racemic 4-nitro derivative MRS1334 (28) was the most potent in this series (K_i = 2.7 nM).

29 **30**

A diverse group of A₃ antagonists that has not yet been explored through SAR analysis was discovered by *in silico* methodology leading to structurally diverse hits (29 – 35) (35). The finding that dihydropyridines could be crafted into A₃-selective antagonists provided the lead for a series of pyridine derivatives that selectively blocked A₃ receptors (36). MRS 1523 (32) is noteworthy as a moderately potent A₃ antagonist at both human and rat receptors (K_i = 18.9 and 113 nM, respectively). Other novel heterocyclic, nonpurine-derived A₃ antagonists include a quinazoline VUF5574 (33, K_i = 4 nM), thiazole LUF5417 (34, K_i = 4 nM, nonselective), and an isoquinoline VUF8507 (35, K_i = 17 nM) (37,38). A series of N-[4-arylthiazol-2-yl]acetamides (36) was discovered to be A₃ antagonists through serendipity and then optimized structurally (39).

Purines and their derivatives

Adenine derivatives, including 9-ribosides (**37**, $K_i = 650$ nM), have been shown to be selective A_3 antagonists (40). Other nucleoside-derived antagonists include the spiro lactam derivative MRS1292 (**38**), CCPA (**39**, also a potent A_1 agonist), and DPMA (**40**, also a moderately selective A_{2A} agonist) (13,17).

Xanthines, such as caffeine and theophylline, are the classical adenosine antagonists, however, they are typically of much lower affinity at the A_3 receptors than at the $A_1 / A_{2A} / A_{2B}$ subtypes (1–3). This is a species-dependent phenomenon with affinity at the human A_3 receptor exceeding that at the rat A_3 receptor. Nevertheless, a recent report indicated the feasibility of designing A_3 -selective xanthines through cyclization between the 7- and 8-positions leading to pyridopurine-2,4-dione derivatives (41) (41).

The SAR of 2-phenylimidazolepurin-5-ones as water-soluble derivatives of xanthines has been explored (42, 43). PSB-11 (**42**, $K_i = 2.3$ nM) is highly potent and has been used as a radioligand with low nonspecific binding. The corresponding 2,3,5-trichloro derivative PSB-10 (**43**) is of even higher affinity and selectivity ($K_i = 0.43$ nM). Several classes of extended xanthine structures (44, 45) were reported as A_3 antagonists (44,45). Triazolopurines, triazoloquinazolines, and pyrazolotriazolo-pyrimidine derivatives (46, 47) have been reported as A_3 antagonists that are bioavailable *in vivo* when applied topically to the eye for use in the treatment of glaucoma (46,47). OT-7999 (**47**) displayed a $K = 0.95$ nM at the human A_3 receptor and >10,000-fold selectivity in comparison to the three other subtypes.

It has been suggested that in *in vivo* studies A₃ receptors are involved in a number of disorders of the central nervous and the cardiovascular systems, as well as in the inflammatory processes. The selective A₃ receptor agonist IB-MECA (6) has been demonstrated to induce behavioral depressant effects in mice (48). Chronic treatment with IB-MECA resulted in improved postischemic cerebral blood circulation, survival, and neuronal preservation, while the opposite effects were observed when IB-MECA was given acutely (49). A₃ receptor activation induced late preconditioning against infarction in conscious rabbits (50). In rats, CI-IB-MECA (12) resulted in a short-lasting hypotension as a result of histamine release (51). The A₃ receptors are also involved in vasoconstriction *in vivo* (52). A₃ receptor activation may be involved both pro- and anti-inflammatory responses in mice (2). Stimulation of A₃ receptors both enhanced degranulation *in vitro* and directly produced degranulation of rat mast cells *in vivo* (53,54). A₃ receptor agonists inhibited murine macrophage tumor necrosis factor- α production both *in vitro* and *in vivo* (55,56). Mucociliary transport in rabbit trachea *in vivo* was enhanced by IB-MECA but not by an A₁ or A_{2A} agonist. Also, the effect of IB-MECA was blocked by the A₃ antagonist MRS1220 (20), but not by an A₁ or A_{2A} antagonist (57). Selective A₃ receptor antagonists, including

OT-7999 (47), have been reported to reduce intraocular pressure in both mice and monkeys (58,59).

Conclusions

Highly selective agonists and antagonists of the A₃ receptor have now been reported. The adenosine receptors are now a mature field within medicinal chemistry. The further structural refinement of these selective agents and the discovery of novel agents are anticipated to provide new substances for therapeutic use.

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