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MOLECULAR APPROACH TO ADENOSINE RECEPTORS: Receptor-Mediated Mechanisms of Tissue Protection

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■ **Abstract** Adenosine accumulation during ischemia and inflammation protects tissues from injury. In ischemic tissues adenosine accumulates due to inhibition of adenosine kinase, and in inflamed tissues adenosine is formed from adenine nucleotides that are released from many cells including platelets, mast cells, nerves, and endothelium. Nucleotides are rapidly converted to adenosine by a family of ecto-nucleotidases including CD39 and CD73. Activation of A_1 and possibly A_3 adenosine receptors (ARs) protects heart and other tissues by preconditioning through a pathway including protein kinase C and mitochondrial K_{ATP} channels. Activation of A_{2A} receptors limits reperfusion injury by inhibiting inflammatory processes in neutrophils, platelets, macrophages and T cells. Adenosine produces proinflammatory responses mediated by receptors that vary among species; A_3 and A_{2B} receptors mediate degranulation of rodent and human or canine mast cells, respectively. Novel adenosine receptor subtype–selective ligands have recently been developed. These include MRS1754 (A_{2B} blocker), MRS1220 (A_3 blocker), MRE 3008F20 (human A_3 blocker), MRS1523 (rat A_3 blocker), and ATL146e (A_{2A} agonist). These new pharmacologic tools will help investigators to sort out how adenosine protects tissues from injury and to identify new therapeutic agents that hold promise for the treatment of inflammatory and ischemic diseases.

BACKGROUND

Adenosine is a primordial signaling molecule that has evolved to modulate physiological responses in all mammalian tissues. Due to the breadth of its effects, it is not possible to summarize all of the new developments in our understanding of adenosine receptor physiology even within the past year. This review is confined to recent insights in the understanding of receptor regulation and signaling and the description of significant new pharmacological tools. I focus on new information about how activation or inhibition of adenosine receptors may limit ischemic or

inflammatory tissue injury, an area of particularly interesting mechanistic and therapeutic importance.

ADENOSINE RECEPTORS: Physical and G Protein Coupling Characteristics

Receptor-mediated effects of adenosine are mediated by four G protein–coupled receptors designated A_1, A_{2A}, A_{2B}, and A_3 (Table 1). All four receptors are *N*-linked glycoproteins, and all but A_{2A} have sites for palmitoylation near the carboxyl terminus (1–4). Glycosylation has no effect on the affinity of ligands for receptors and may be involved in targeting newly formed receptors to the cell surface. In view of emerging evidence that certain G protein–coupled receptors may form homo- or heterodimers, it is possible that hydrophilic glycosylation of adenosine receptors, and G protein–coupled receptors in general, may inhibit dimerization reactions driven by hydrophobic interactions. All of the adenosine receptors can be readily deglycosylated upon incubation with *N*-glycosidase F. One practical application of deglycosylation is its use to distinguish between specific and nonspecific antibody binding to putative receptors detected by western blotting.

Figure 1 illustrates the use for western blotting of two different anti-A_{2A} receptor antibodies. Both antibodies specifically detect overexpressed recombinant A_{2A} receptors, but only one of the antibodies is able to detect low levels of receptors found on tissues. Furthermore, nonreceptor immunoreactivity is found in proteins that have nearly the same molecular mass as the receptor. This illustrates two points about the use of antireceptor antibodies: (*a*) Detection of overexpressed recombinant receptors is not necessarily predictive of the ability of an antibody to detect endogenous receptors that are expressed at a much lower levels and (*b*) deglycosylation is an important control to check for specificity when doing western

TABLE 1 Properties of adenosine receptor subtypes

Adenosine receptor subtype		Genbank accession number	Amino acids	G Protein coupling	Chromosomal location	References
A_1	Human	S45235	326	i,o	1q32.1	(48)
	Mouse	U05671	326			(49)
A_{2A}	Human	S46950	412	s,olf	22q11.2	(50)
	Mouse	U05672	410			(49)
A_{2B}	Human	X68487	332	s,q	17p11.2–12	(51)
	Mouse	U05673	332			(49)
A_3	Human	L22607	318	i,o	1p13.3	(52)
	Mouse	L20331	319			(53)

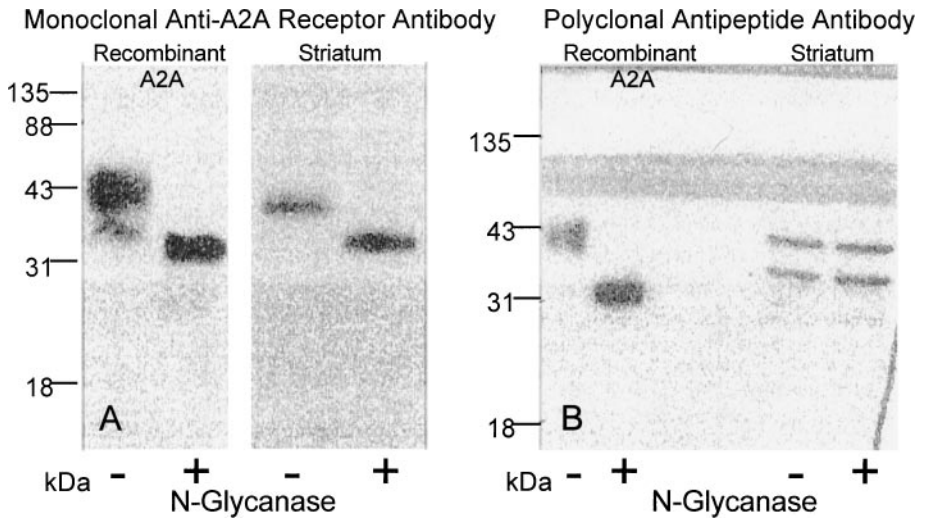

Figure 1 Western blots showing specific and nonspecific binding of anti-A_{2A} receptor antibodies. Treatment of adenosine receptors with *N*-glycosidase F produces a characteristic decrease in their molecular mass. (*A*) Specific immunoreactivity of overexpressed recombinant and striatal A_{2A} receptors with a monoclonal antibody. (*B*) Specific and nonspecific immunoreactivity of a commercially available polyclonal antibody. The absence of a shift in molecular mass by glycosidase F probably is indicative of nonspecific immunoreactivity. (Adapted from Reference 43.)

blotting. The use of poorly characterized antireceptor antisera may have resulted in several instances of erroneous conclusions about adenosine receptor expression, distribution, and regulation. Anti-A_{2A} receptor antibodies have been used to detect receptors selectively in areas where they are highly expressed, such as striatum (5), but not convincingly in endogenous tissues in which the receptor density is lower. The best control for immunohistochemical experiments is tissue derived from mice in which specific receptor genes have been deleted. These considerations indicate that for the purpose of quantifying adenosine receptors, radioligand binding still is generally preferable to immunohistochemistry or western blotting.

ADENOSINE METABOLISM

It has long been known that hypoxia, ischemia, or inflammation all stimulate local adenosine production. Because the endothelium is a barrier to adenosine, adenosine formed within the lumen of blood vessels may be derived in large part from nucleotides released from platelets or endothelial cells. By contrast, interstitial adenosine may produce vasodilation predominantly by acting on A_{2A} receptors on vascular smooth muscle cells that are particularly accessible to interstitial nucleoside. The source of this adenosine is likely ischemic parenchymal cells or

nucleotides derived from nerves or interstitial mast cells. It has recently been shown in the heart that hypoxia-induced inhibition of adenosine kinase amplifies small changes in free myocardial AMP into a major rise in adenosine. This mechanism plays an important role in causing high sensitivity of the myocardium and other tissues to impaired oxygenation (6). The concentration of endogenous adenosine acting at the receptor level during an ischemic episode was estimated to be 30 μM in rat hippocampal slices, based on the ability of the selective A_1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (CPX), to reverse the effects of ischemia (7). Adenosine also can be derived from adenine nucleotides released from many cell types by mechanisms that are not yet clearly understood (8). Substantial amounts of adenosine may be formed from the breakdown of adenine nucleotides that are present in the granules of autonomic nerves, platelets, and mast cells. Adenine nucleotides are rapidly converted to adenosine by a family of ecto-ATP/ADPases including CD39 (NTPDase 1) and ecto-5′ nucleotidases including CD73 (Figure 2). The expression of CD39 on the endothelial cell surface may be regulated because palmitoylation targets the enzyme to caveolae (9). This in turn may regulate the rate of ADP conversion to adenosine.

Inosine, formed by adenosine deamination, accumulates to even higher levels (>100 μM) than adenosine in ischemic tissues. Inosine has been found to activate rat and guinea pig A_3 receptors with Ki values in the range of 15–25 μM (10). In contrast to its effects to activate rodent A_3 receptors, inosine is a weak partial agonist of the human A_3 receptor (J Linden, unpublished).

A_1 RECEPTORS

A_1 adenosine receptors signal through Gi/o pathways and inhibit adenylyl cyclase, activate K^+ channels, or inhibit Ca^{2+} channels in various cells. A_1 receptors also can stimulate Ca^{2+} mobilization via a pertussis toxin–sensitive pathway through activation of PLCβ with G protein $\beta\gamma$ subunits (11). This signaling pathway appears to be synergistic with receptors that activate PLC via Gq, possibly including P2Y receptors and A_{2B} receptors. A_1 receptors couple preferentially to G proteins containing $\gamma 2$ or $\gamma 3$ over subunits containing $\gamma 1$ (12). This preference has been shown to be mediated by the prenylation state of the γ-subunit. G proteins containing geranyl geranylated γ subunits (including $\gamma 2$ and $\gamma 3$) interact more effectively with A_1 receptors then do G proteins containing farnesylated γ subunits (13). It has recently been shown that PKC can phosphorylate the $\gamma 12$ subunit of heterotrimeric G proteins, resulting in increased G protein affinity for A_1 receptors (14).

Much recent work has focused on A_1 receptor–PKC signaling cascades. The A_1 agonist, N^6-cyclopentyladenosine (CPA), has been recently recognized as a facilitator of insulin-stimulated leptin release through a pathway involving protein kinase C (15). Activation of A_1 adenosine receptors increases nucleoside efflux from DDT1 MF-2 cells by a PKC-dependent inhibition of adenosine kinase activity (16).

A_1 receptors and the heterotrimeric G protein, Go, are abundant in the brain. Recently, GRIN1, a probable regulator of neurite growth, has been identified as an effector of Go (17). This suggests a possible newly identified function of A_1

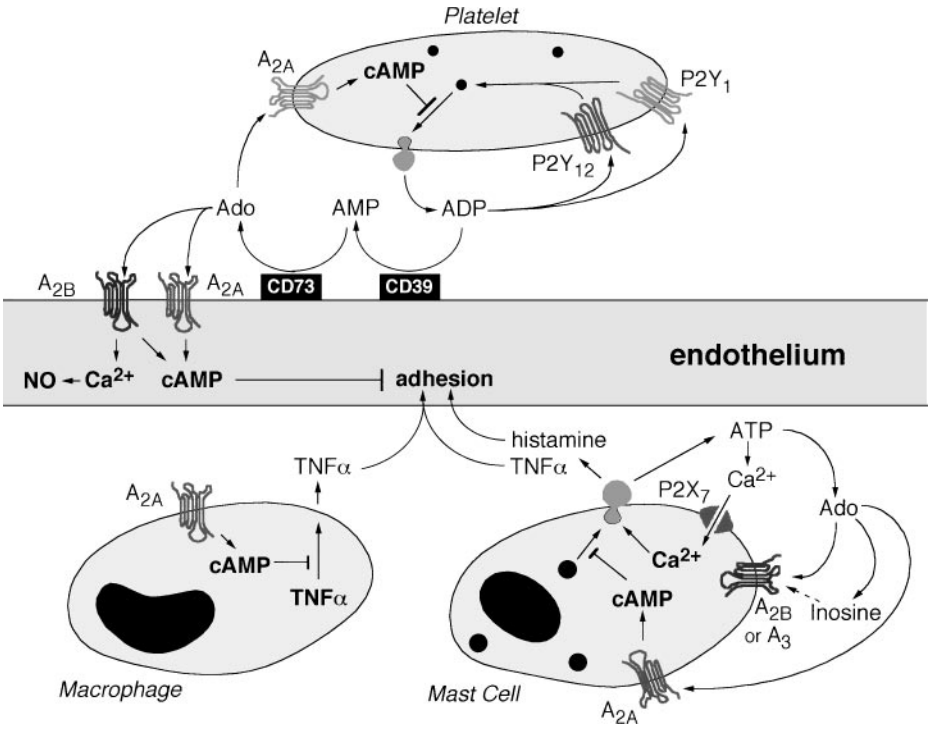

Figure 2 Purinergic regulation of inflammation. ADP derived from activated platelets exerts a pro-aggregatory effect on platelets through cell surface P2Y receptors, including a newly identified P2Y12 receptor (9a), that is countered by ecto-nucleotidases that degrade ADP and produce adenosine (ado) that activates anti-aggregatory A_{2A} receptors. Activation of A_{2A} receptors also reduces histamine and cytokine release from certain mast cells and macrophages and inhibits the expression of adhesion molecules on endothelium. A_3 receptors regulate rodent mast cells and A_{2B} receptors regulate human and canine mast cells. Inosine activates rodent, but not human, A_3 receptors. A_{2B} receptors are dually coupled to adenylyl cyclase via Gs and to Ca^{2+} via Gq.

receptors to regulate neurite growth in the central nervous system through a Go/GRIN1 pathway.

Palmitoylation of A_1 receptors has no effect on receptor-effector coupling, receptor downregulation, or receptor interactions with G proteins. However, A_1 palmitoylation may divert new synthesized receptors from a pathway leading to rapid receptor degradation (1).

A_{2A} RECEPTORS

A_{2A} receptors are most highly expressed in intermediate spiney neurons of the striatum. A_{2A} receptors are known to activate Gs, but receptors in striatum may interact predominantly with G_{olf} (first identified in the olfactory epithelium) because

G_{olf} is much more highly expressed in the striatum than Gs (18). Functionally, activation of A_{2A} receptors opposes the action of D2 dopamine receptors in spiney neurons, and antagonists of A_{2A} receptors are being investigated for possible use in Parkinson's disease (19)

Activation of A_{2A} receptors causes vasodilatation of coronary arteries and, to a variable extent, other blood vessels. A_{2A} receptors also are found on bone marrow–derived cells including neutrophils, monocytes, macrophages, platelets, and mast cells. Activation of A_{2A} receptors produces a constellation of responses that in general can be classified as anti-inflammatory (20, 21).

A_{2B} RECEPTORS

A_{2B} receptors have long been known to couple to Gs. Recent studies indicate that A_{2B} receptors also couple to Gq and produce Ca^{2+} mobilization and mitogen-activated protein kinase (MAPK) activation (3, 4). Ca^{2+} mobilization is not limited to cells that overexpress A_{2B} receptors because the endogenous A_{2B} receptors of HEK-293 cells produce a robust A_{2B}-mediated Ca^{2+} mobilization (22). In HEK-293 cells the agonist, *N*-ethylcarboxamido-adenosine (NECA) is equipotent in elevating cyclic AMP and stimulating MAPK activation. The protein kinase A inhibitor, H89, blocks forskolin but not NECA-stimulated MAPK activation in HEK cells, suggesting that the Gq pathway contributes to MAPK activation through a pathway including MEK and Ras (22). A_{2B} receptors on vascular endothelial cells may contribute to an NO-dependent component of vasodilation mediated by Ca^{2+}-dependent NO synthase activation (Figure 2).

A_3 RECEPTORS

A_3 receptors appear to signal through Gi in much the same way as A_1 receptors. Unlike A_1 receptors, depalmitoylation of A_3 receptors renders them susceptible to phosphorylation by G protein–coupled receptor kinases (GIRKs) (23). This in turn leads to rapid phosphorylation and desensitization of A_3 receptors that does not occur in the case of A_1 receptors (2, 24).

ADENOSINE AND TISSUE PROTECTION

Adenosine protects tissues from hypoxic or ischemic damage through multiple receptor subtypes. The activation of A_1 and possibly A_3 (25) receptors produces preconditioning to protect the heart and other tissues from subsequent ischemic injury. Adenosine has been postulated to trigger preconditioning by increasing mitochondrial K-ATP channel activity through a pathway including PKC (26). A late phase of preconditioning in response to A_1 receptor activation in the rabbit

heart appears to be mediated in part by the induction of manganese superoxide dismutase (27). In contrast to preconditioning, agonists of A_{2A} receptors can protect tissues from ischemia/reperfusion damage when added during the reperfusion period. The agonist CGS21680, which is highly selective for A_{2A} over A_1 and A_{2B} receptors, was found to attenuate reperfusion injury in the dog heart (28). This effect is correlated with an inhibition of neutrophil accumulation, superoxide generation, and coronary endothelial adherence, suggesting that reduced inflammation may be responsible for protecting the heart during reperfusion injury. A new agonist, ATL146e, which is >50 times more potent at human A_{2A} receptors than CGS21680, has recently been found to produce profound protection of the rabbit lung (29) and rat kidney (30) from reperfusion injury at concentrations that are well below the threshold to produce hemodynamic responses. Protection from reperfusion injury is accompanied not only by reduced neutrophil accumulation in ischemic renal microvessels, but also by reduced expression of the adhesion molecules, P-selectin, and ICAM-1 on the reperfused vascular endothelium (31). A_{2A} agonists also protect isolated crystalloid-perfused rabbit heart from ischemia/reperfusion injury. The effect is manifest as a significant reduction in contracture development during reperfusion. These data imply a role for A_{2A} receptors on cardiomyocytes or tissue-resident inflammatory cells in A_{2A} receptor–mediated cardioprotection (32).

Activation of A_{2A} ARs on human monocytes inhibits, by a cyclic AMP-dependent mechanism, the secretion of IL-12, a proinflammatory cytokine and a major inducer of Th1 responses (33). Through this mechanism, adenosine released in excess during inflammatory and ischemic conditions, or tissue injury, may contribute to selective suppression of Th1 responses and cellular immunity.

It is thought that activation of T lymphocytes is required for neutrophil recruitment during ischemia reperfusion injury in the liver. The subacute phase of ischemia/reperfusion injury in the liver is absent in athymic nude mice but can be restored by adoptive transfer of $CD4^+$ T-cells (34). Inhibition of T-cell activation may contribute to the protection of tissues from ischemia/reperfusion injury because inhibitory A_{2A} receptors are found on $CD4^+$ T cells (35).

ADENOSINE RECEPTORS ON MAST CELLS

Aerosolized adenosine has the effect of causing mast-cell-dependent bronchoconstriction in asthmatic subjects but bronchodilation in nonasthmatics (36, 37). 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, is also therapeutically efficacious in the treatment of asthma and was thought to act through a non–adenosine receptor–mediated mechanism owing to its low affinity at A_1 and A_{2A} receptors (37). The A_3 adenosine receptor was initially implicated as the receptor subtype that facilitates the degranulation of

rat RBL 2H3 mast-like cells (38) and perivascular mast cells of the hamster cheek pouch (10). There is also evidence of mast cell degranulation when agonists of A_3 ARs are administered to rats or mice (39, 40). In contrast, the A_{2B} AR has been implicated as the receptor subtype that facilitates the release of allergic mediators from canine BR and human HMC-1 mastocytoma cells (41, 42). A role for A_{2B} ARs in human asthma is also suggested by the efficacy of enprofylline, which at therapeutic concentrations of 20–50 μM only blocks the A_{2B} AR subtype (4). In sum, the literature indicates that the release of allergic mediators in some mast cells is mediated by A_3 ARs and in other cells is mediated by A_{2B} ARs. This may result from species differences, with rodent (rat, guinea pig, and mouse) and canine or human mast cells responding primarily to A_3 or A_{2B} adenosine receptor stimulation, respectively.

ADENOSINE RECEPTOR PHARMACOLOGY

Potent and selective agonists and antagonists of adenosine receptor subtypes developed recently (Figure 3) have been valuable for further defining the physiological effects of the various adenosine receptor subtypes. However, it is not always appreciated that the selectivity of these compounds is limited. For example, at concentrations above 1 μM, CGS21680 is not a selective A_{2A} agonist, IB-MECA is not a selective A_3 agonist, and CPX is not a selective A_1 antagonist (Table 2).

TABLE 2 Antagonist binding to human adenosine receptor subtypes, (Ki, nM)

	A_1	A_{2A}	A_{2B}	A_3	References
A_1 selective					
CPX	2	156	40	509	(53)
WRC0571	3	157	19,000	6,500	(53)
A_{2A} selective					
ZM241385	536	1.4	31	269	(4)
SCH58261	287	0.6	5,011	>10,000	(54)
A_{2B}-selective					
MRS1754	403	503	1.97	570	(46)
Enprofylline[a]	44,000	32,000	6,300	158,000	(4)
A_3 selective					
MRS1220	305	52	—	0.65	(55)
MRS1523 (rat)	15,600	2,050	—	519[b] (113)	(29)
MRE300F20	1,100	140	2,100	0.29	(56)

[a]It is notable that plasma concentration of enprofylline achieved in the treatment of asthma is 20–50 μM, sufficient to block human A_{2B} receptors but not other adenosine receptor subtypes.

[b]A K_I of 519 ± 86 nM ($N = 6$) of MRS1523 for the rat A_3 receptors determined in the author's laboratory is somewhat higher than the K_I reported previously.

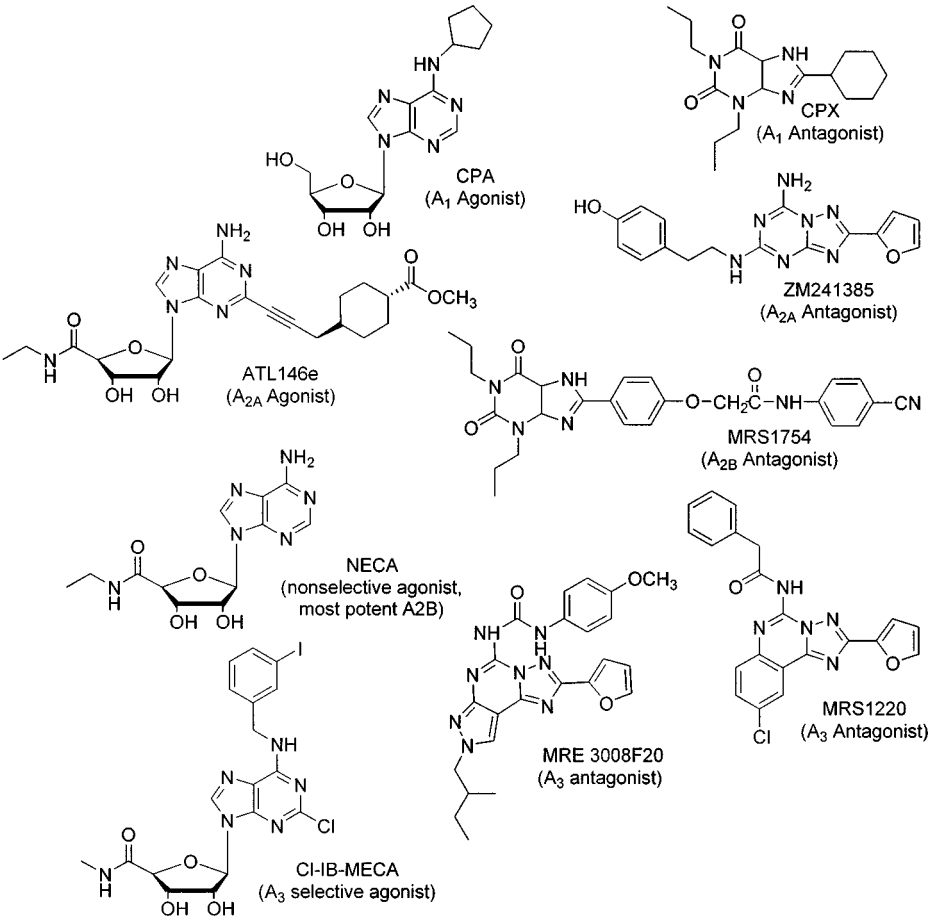

Figure 3 Selective agonists and antagonists of adenosine receptor subtypes.

The use of agonists to determine the receptor subtype that mediates a particular response is particularly problematic because agonists bind with two different affinity states of G protein–coupled adenosine receptors, resulting in wide differences in reported binding affinities, depending on the source of receptors (recombinant receptors tend to be uncoupled from G proteins) and the use of agonist or antagonist radioligands (antagonists detect more uncoupled receptors). Moreover, the ED_{50} of agonists in functional responses is highly variable, depending on the extent of receptor reserve (43). The judicious use of new selective antagonists is simplifying the process of identifying which adenosine receptor subtypes mediate various physiological responses. It is important to note, however, that there are substantial species differences in the affinity of these compounds. For example, the widely used A_1 selective antagonist, CPX binds with >10 times lower affinity

to canine than to human or rat A_1 receptors (44) and is fairly potent as an antagonist of human A_{2A} receptors (Table 2). Compounds that block human A_3 ARs generally are weak antagonists of rodent A_3 receptors, including the new selective antagonist of human A_3 receptors, MRE 3008-F20 (45). MRS1523 is the only compound reported to be moderately selective as an antagonist of the rat A_3 receptor (Table 2). Recently, the first potent and selective antagonists of A_{2B} adenosine receptors have been described. These compounds, exemplified by MRS1754 are anilide derivatives of 8-phenylxanthines (46). It has recently been suggested that selective inhibitors of A_{2B} receptors may be useful for the treatment of asthma and other allergic diseases (4, 42, 46).

SUMMARY AND FUTURE DIRECTIONS

Because adenosine is a metabolic breakdown product of ATP, adenosine receptors may have evolved in part to protect tissues from various injurious stimuli. As summarized in this review, activation of A_1 and A_3 receptors ellicit protective responses in various tissues by several processes collectively referred to as preconditioning. Activation of A_{2A} receptors produces a constellation of effects on various inflammatory cells types that can attenuate injury due to ischemia/reperfusion or inflammation. The roles played by A_{2B} and A_3 receptors in inflammation are at the present time somewhat confusing and controversial and apparently vary among species. Recent developments using pure reconstituted components have begun to refine our understanding of specific G protein subunits that participate in various adenosine receptor signaling cascades. Advances in medicinal chemistry have led to the generation of new potent and selective receptor-subtype-selective agonists and antagonists, particularly for the A_{2B} and A_3 subtypes. These and additional selective agonists and antagonists that are currently under development will provide investigators in the adenosine field with important new tools to understand the receptors and cell-types that contribute to the various effects of adenosine. It is also becoming increasingly apparent that highly selective agonists or antaognists of adenosine receptor subtypes have great potential as therapeutic agents for the treatment of various inflammatory or ischemic diseases.

LITERATURE CITED

1. Gao Z, Ni Y, Szabo G, Linden J. 1999. Palmitoylation of the recombinant human A1 adenosine receptor: enhanced proteolysis of palmitoylation-deficient mutant receptors. *Biochem. J.* 342:387–95
2. Gao Z, Robeva AS, Linden J. 1999. Purification of A1 adenosine receptor-G-protein complexes: effects of receptor down-regulation and phosphorylation on coupling. *Biochem. J.* 338:729–36
3. Linden J, Auchampach JA, Jin X, Figler RA. 1998. The structure and function of A1 and A2B adenosine receptors. *Life Sci.* 62:1519–24

4. Linden J, Thai T, Figler H, Jin X, Robeva AS. 1999. Characterization of human A(2B) adenosine receptors: radioligand binding, western blotting, and coupling to G(q) in human embryonic kidney 293 cells and HMC-1 mast cells. *Mol. Pharmacol.* 56:705–13
5. Rosin DL, Robeva A, Woodard RL, Guyenet PG, Linden J. 1998. Immunohistochemical localization of adenosine A2A receptors in the rat central nervous system. *J. Comp. Neurol.* 401:163–86
6. Decking UKM, Schlieper G, Kroll K, Schrader J. 1997. Hypoxia-induced inhibition of adenosine kinase potentiates cardiac adenosine release. *Circ. Res.* 81:154–64
7. Latini S, Bordoni F, Pedata F, Corradetti R. 1999. Extracellular adenosine concentrations during in vitro ischaemia in rat hippocampal slices. *Br. J. Pharmacol.* 127:729–39
8. Harden TK, Lazarowski ER. 1999. Release of ATP and UTP from astrocytoma cells. *Prog. Brain Res.* 120:135–43
9. Koziak K, Kaczmarek E, Kittel A, Sevigny J, Blusztajn JK, et al. 2000. Palmitoylation targets CD39/endothelial ATP diphosphohydrolase to caveolae. *J. Biol. Chem.* 275:2057–62

9a. Hollopeter G, Jantzen H-M, Vincent D, Li G, England L, et al. 2001. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* 409:202–7

10. Jin X, Shepherd RK, Duling BR, Linden J. 1997. Inosine binds to A3 adenosine receptors and stimulates mast cell degranulation. *J. Clin. Invest.* 100:2849–57
11. Dickenson JM, Hill SJ. 1998. Involvement of g-protein beta-gamma subunits in coupling the adenosine a(1) receptor to phospholipase c in transfected cho cells. *Eur. J. Pharmacol.* 355:85–93
12. Figler RA, Lindorfer MA, Graber SG, Garrison JC, Linden J. 1997. Reconstitution of bovine A_1 adenosine receptors and G proteins in phospholipid vesicles: betagamma-subunit composition influences guanine nucleotide exchange and agonist binding. *Biochemistry* 36:16288–99
13. Yasuda H, Lindorfer MA, Woodfork KA, Fletcher JE, Garrison JC. 1996. Role of the prenyl group on the G protein gamma subunit in coupling trimeric G proteins to A1 adenosine receptors. *J. Biol. Chem.* 271:18588–95
14. Lindorfer MA, Myung CS, Savino Y, Yasuda H, Khazan R, Garrison JC. 1998. Differential activity of the G protein beta5 gamma2 subunit at receptors and effectors. *J. Biol. Chem.* 273:34429–36
15. Cheng JT, Liu IM, Chi TC, Shinozuka K, Lu FH, et al. 2000. Role of adenosine in insulin-stimulated release of leptin from isolated white adipocytes of Wistar rats. *Diabetes* 49:20–24
16. Sinclair CJ, Shepel PN, Geiger JD, Parkinson FE. 2000. Stimulation of nucleoside efflux and inhibition of adenosine kinase by A1 adenosine receptor activation. *Biochem. Pharmacol.* 59:477–83
17. Chen LT, Gilman AG, Kozasa T. 1999. A candidate target for G protein action in brain. *J. Biol. Chem.* 274:26931–38
18. Herve D, Levi-Strauss M, Marey-Semper I, Verney C, Tassin JP, et al. 1993. G(olf) and Gs in rat basal ganglia: possible involvement of G(olf) in the coupling of dopamine D1 receptor with adenylyl cyclase. *J. Neurosci.* 13:2237–48
19. Aoyama S, Kase H, Borrelli E. 2000. Rescue of locomotor impairment in dopamine D2 receptor-deficient mice by an adenosine A2A receptor antagonist. *J. Neurosci.* 20:5848–52
20. Cronstein BN. 1994. Adenosine, an endogenous anti-inflammatory agent. *J. Appl. Physiol.* 76:5–13
21. Sullivan GW, Linden J. 1998. Role of A_{2A} adenosine receptors in inflammation. *Drug Dev. Res.* 45:103–12
22. Gao Z, Chen T, Weber MJ, Linden J. 1999. A_{2B} adenosine and $P2Y_2$ receptors stimulate mitogen-activated protein kinase in human embryonic kidney-293 cells:

cross-talk between cyclic AMP and protein kinase C pathways. *J. Biol. Chem.* 274:5972–80
23. Palmer TM, Stiles GL. 2000. Identification of threonine residues controlling the agonist-dependent phosphorylation and desensitization of the rat A(3) adenosine receptor. *Mol. Pharmacol.* 57:539–45
24. Palmer TM, Benovic JL, Stiles GL. 1996. Molecular basis for subtype-specific desensitization of inhibitory molecular adenosine receptors—analysis of a chimeric A(1)-A(3) adenosine receptor. *J. Biol. Chem.* 271:15272–78
25. Liang BT, Jacobson KA. 1998. A physiological role of the adenosine A3 receptor: sustained cardioprotection. *Proc. Natl. Acad. Sci. USA* 95:6995–99
26. Sato T, Sasaki N, O'Rourke B, Marban E. 2000. Adenosine primes the opening of mitochondrial ATP-sensitive potassium channels: a key step in ischemic preconditioning? *Circulation* 102:800–5
27. Dana A, Jonassen AK, Yamashita N, Yellon DM. 2000. Adenosine A(1) receptor activation induces delayed preconditioning in rats mediated by manganese superoxide dismutase. *Circulation* 101:2841–48
28. Jordan JE, Zhao ZQ, Sato H, Taft S, Vinten-Johansen J. 1997. Adenosine A2 receptor activation attenuates reperfusion injury by inhibiting neutrophil accumulation, superoxide generation and coronary endothelial adherence. *J. Pharmacol. Exp. Ther.* 280:301–9
29. Ross SC, Tribble CG, Linden J, Gangemi JJ, Lanpher BC, et al. 1999. Selective adenosine-A(2A) activation reduces lung reperfusion injury following transplantation. *J. Heart Lung Transplant.* 18:994–1002
30. Okusa MD, Linden J, Macdonald T, Huang LP. 1999. Selective A(2A) adenosine receptor activation reduces ischemia-reperfusion injury in rat kidney. *Am. J. Physiol. Renal Fluid Electrolyte Physiol.* 277:F404–12
31. Okusa MD, Linden J, Huang L, Rieger JM, MacDonald TL, Huynh LP. 2000. A2A-adenosine receptor mediated inhibition of ischemia reperfusion injury and neutrophil adhesion in rat and mouse kidneys. *Am. J. Physiol. Renal Fluid Electrolyte Physiol.* 279:F809–18
32. Cargnoni A, Ceconi C, Boraso A, Bernocchi P, Monopoli A, et al. 1999. Role of A2A receptor in the modulation of myocardial reperfusion damage. *J. Cardiovasc. Pharmacol.* 33:883–93
33. Link AA, Kino T, Worth JA, McGuire JL, Crane ML, et al. 2000. Ligand-activation of the adenosine A2a receptors inhibits IL-12 production by human monocytes. *J. Immunol.* 164:436–42
34. Fan C, Zwacka RM, Engelhardt JF. 1999. Therapeutic approaches for ischemia/reperfusion injury in the liver. *J. Mol. Med.* 77:577–92
35. Koshiba M, Rosin DL, Hayashi N, Linden J, Sitkovsky MV. 1999. Patterns of A2A extracellular adenosine receptor expression in different functional subsets of human peripheral T cells. Flow cytometry studies with anti-A2A receptor monoclonal antibodies. *Mol. Pharmacol.* 55:614–24
36. Cushley MJ, Tattersfield AE, Holgate ST. 1983. Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. *Br. J. Clin. Pharmaco.* 15:161–65
37. Vilsvik JS, Persson CG, Amundsen T, Brenna E, Naustdal T, et al. 1990. Comparison between theophylline and an adenosine non-blocking xanthine in acute asthma. *Eur. Respir. J.* 3:27–32
38. Ramkumar V, Stiles GL, Beaven MA, Ali H. 1993. The A3 adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J. Biol. Chem.* 268:16887–90
39. Fozard JR, Pfannkuche HJ, Schuurman HJ. 1996. Mast cell degranulation following adenosine A(3) receptor activation in rats. *Eur. J. Pharmacol.* 298:293–97
40. Tilley SL, Wagoner VA, Salvatore CA,

Jacobson MA, Koller BH. 2000. Adenosine and inosine increase cutaneous vasopermeability by activating A(3) receptors on mast cells. *J. Clin. Invest.* 105:361–67

41. Auchampach JA, Jin J, Wan TC, Caughey GH, Linden J. 1997. Canine mast cell adenosine receptors: cloning and expression of the A3 receptors and evidence that degranulation is mediated by the A2B receptor. *Mol. Pharmacol.* 52:846–60
42. Feoktistov I, Biaggioni I. 1995. Adenosine A_{2b} receptors evoke interleukin-8 secretion in human mast cells: an enprofylline-sensitive mechanism with implications for asthma. *J. Clin. Invest.* 96:1979–86
43. Shryock JC, Snowdy S, Baraldi PG, Cacciari B, Spalluto G, et al. 1998. A(2A)-Adenosine receptor reserve for coronary vasodilation. *Circulation* 98:711–18
44. Tucker AL, Robeva AS, Taylor HE, Holeton D, Bockner M, et al. 1994. A_1 adenosine receptors: two amino acids are responsible for species differences in ligand recognition. *J. Biol. Chem.* 269:27900–6
45. Baraldi PG, Cacciari B, Romagnoli R, Varani K, Merighi S, et al. 2000. Synthesis and preliminary biological evaluation of [H-3]-MRE 3008-F20: the first high affinity radioligand antagonist for the human A(3) adenosine receptors. *Bioorg. Med. Chem. Lett.* 10:209–11
46. Kim Y-C, Ji X-D, Melman N, Linden J, Jacobson KA. 2000. Anilide derivatives of an 8-phenylxanthiine carboxylic congener are highly potent and selective antagonists at human A2B adenosine receptors. *J. Med. Chem.* 43:1165–72
47. Libert F, Van Sande J, Lefort A, Czernilofsky A, Dumont JE, et al. 1992. Cloning and functional characterization of a human A1 adenosine receptor. *Biochem. Biophys. Res. Commun.* 187:919–26
48. Marquardt DL, Walker LL, Heinemann S. 1994. Cloning of two adenosine receptor subtypes from mouse bone marrow-derived mast cells. *J. Immunol.* 152:4508–15
49. Furlong TJ, Pierce KD, Selbie LA, Shine J. 1992. Molecular characterization of a human brain adenosine A_2 receptor. *Mol. Brain Res.* 15:62–66
50. Jacobson MA, Johnson RG, Luneau CJ, Salvatore CA. 1995. Cloning and chromosomal localization of the human A_{2b} adenosine receptor gene (ADORA2B) and its pseudogene. *Genomics* 27:374–76
51. Salvatore CA, Jacobson MA, Taylor HE, Linden J, Johnson RG. 1993. Molecular cloning and characterization of the human A3 adenosine receptor. *Proc. Natl. Acad. Sci. USA* 90:10365–69
52. Wilkie TM, Chen Y, Gilbert DJ, Moore KJ, Yu L, et al. 1993. Identification, chromosomal location, and genome organization of mammalian G-protein-coupled receptors. *Genomics* 18:175–84
53. Robeva AS, Woodard RL, Jin XW, Gao ZH, Bhattacharya S, et al. 1996. Molecular characterization of recombinant human adenosine receptors. *Drug Dev. Res.* 39:243–52
54. Ongini E, Dionisotti S, Gessi S, Irenius E, Fredholm BB. 1999. Comparison of CGS15943, ZM241385, and SCH58261 as antagonists at human adenosine receptors. *Naunyl-Schmiedegergs Arch. Pharmacol.* 359:7–10
55. Li AH, Moro S, Melman N, Ji XD, Jacobson KA. 1998. Structure-activity relationships and molecular modeling of 3,5-diacyl-2,4-dialkylpyridine derivatives as selective a(3) adenosine receptor antagonists. *J. Med. Chem.* 41:3186–201
56. Varani K, Merighi S, Gessi S, Klotz KN, Leung E, et al. 2000. [H-3]MRE 3008F20: a novel antagonist radioligand for the pharmacological and biochemical characterization of human A(3) adenosine receptors. *Mol. Pharmacol.* 57:968–75