0031-6997/97/4904-0381$03.00/0
PHARMACOLOGICAL REVIEWS
Vol. 49, No. 4

Printed in U.S.A.

Adenosine A_{2B} Receptors

IGOR FEOKTISTOV AND ITALO BIAGGIONI[a]

Divisions of Clinical Pharmacology and Cardiology, Departments of Medicine and Pharmacology, Vanderbilt University, Nashville, Tennessee

I. Introduction

Adenosine is an endogenous nucleoside that modulates many physiological processes. Its actions are mediated by interaction with specific cell membrane receptors. Four subtypes of adenosine receptors have been cloned: A_1, A_{2A}, A_{2B}, and A_3. Significant advancement has been made in the understanding of the molecular pharmacology and physiological relevance of adenosine receptors, but our knowledge of A_{2B} receptors lags behind that of other receptor subtypes. The lack of selective pharmacological probes has hindered research in this area. Perhaps because of their lower affinity for adenosine compared with other receptors, it is often assumed that A_{2B} receptors are a low-affinity version of the A_{2A} receptor and are of lesser physiological relevance. It has been only recently that potentially important functions have been discovered for the A_{2B} receptor, prompting a renewed interest in this receptor type. It is also recently recognized that A_{2B} receptors are coupled to intracellular pathways different from those of A_{2A} receptors, a finding that may provide the basis for their distinct physiological role. A_{2B} receptors have been implicated in mast cell activation and asthma, vasodilation, regulation of cell growth, intestinal function, and modulation of neurosecretion. We try to review the recent advances made in the study of A_{2B} receptors and

[a] Address for correspondence: Italo Biaggioni, Division of Clinical Pharmacology, Vanderbilt University, Nashville, TN 37232-2195.

Dr. Feoktistov is the recipient of an American Lung Association Research Grant Award and an Asthma and Allergy Foundation of America Investigator Grant Award and is supported also by NIH grants RR00095 and R29HL55596.

Abbreviations: alloxazine, 2,4-dioxobenzo[g]pteridine; ATP, adenosine 5c-triphosphate; cAMP, adenosine 3c,5c-cyclic monophosphate; cDNA, complementary deoxyribonucleic acid; CGS 21680, 4-[(N-ethyl-5′-carbamoyladenos-2-yl)-aminoethyl]-phenylpropionic acid; CHO, Chinese hamster ovary; CPA, N^6-cyclopentyladenosine; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; DPSPX, 1,3-dipropyl-8-(p-sulfophenyl)xanthine; EC_{50}, concentration that produces half-maximal effect; enprofylline, 3-n-propylxanthine; HEL, human erythroleukemia; IB-MECA, N^6-(3-iodobenzyl)-N-methyl-5′-carbamoyladenosine; IgE, immunoglobulin E; IL-8, interleukin-8; K_B, dissociation constant of antagonist-receptor complex; L-249313, 6-carboxymethyl-5,9-dihydro-9-methyl-2-phenyl-[1,2,4]-triazolo-{5,1-a}-[2,7]-naphthyridine; L-268605, 3-(4-methoxyphenyl)-5-amino-7-oxo-thiazolo-[3,2]-pyrimidine; L-NAME, N^G-nitro-L-arginine methyl ester; mRNA, messenger ribonucleic acid; MRS 1067, 3,6-dichloro-2′-isopropyloxy-4′-methylflavone; MRS 1097, 3,5-diethyl 2-methyl, 6-phenyl-4-[2-[phenyl-(trans)-vinyl]-1,4(±)dihydropyiridine-3,5-dicarboxylate; MRS 1191, 3-ethyl 5-benzyl 2-methyl-phenylethynyl-6-phenyl-1,4(±)dihydropyridine-3,5-dicarboxylate; MRS 1222, 3,5-diethyl 2-methyl-4-[2-(4-nitrophenyl)-(*E*)-vinyl-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate; NECA, 5′-N-ethylcarboxamidoadenosine; NO, nitric oxide; R-PIA, (R)-N^6-phenylisopropyladenosine; S-PIA, (S)-N^6-phenylisopropyladenosine; SCH 58261, 5-amino-7-(phenylethyl)-2-(1-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine; WRC-0571, C^8-(N-methylisopropyl)-amino-N^6-(5′-endohydroxy)-endonorbornan-2-yl-9-methyladenin; XAC, xanthine amine congener; ZM 241385, 4-(2-[7-amino-2-)2-furyl(triazolo {2,3-a}-[1,3,5]triazin-5-ylamino]ethyl)phenol.

underscore areas in which more progress is needed. We discuss some of the characteristics of A_1, A_{2A}, and A_3 receptors only to highlight their similarities and differences with A_{2B} receptors. Recent reviews on specific adenosine receptor subtypes can be found elsewhere (Linden, 1991, 1994; Dalziel and Westfall, 1994; Fredholm, 1995; Palmer and Stiles, 1995; Sebastião and Ribeiro, 1996; Daval et al., 1996; Ongini and Fredholm, 1996).

II. Classification of Adenosine Receptors

The properties of extracellular adenosine as a protective autacoid have been known since the study of its cardiovascular effects conducted in 1929 by Drury and Szent-Györgyi (1929). The purinergic receptors that mediate the effects of adenosine were classified as P_1 receptors, whereas the receptors activated by nucleotides like adenosine 5c-triphosphate (ATP) were classified as P_2 receptors (Burnstock, 1978). Adenosine receptors were found to modulate intracellular levels of adenosine 3c,5c-cyclic monophosphate (cAMP) and were initially subdivided into A_1 and A_2 subtypes based on their ability to inhibit or stimulate adenyl cyclase, respectively (van Calker et al., 1979; Londos et al., 1980). The alternative classification of adenosine receptors as R_i and R_a (Londos et al., 1980) was replaced by the A_1 and A_2 terms (van Calker et al., 1979). The further division of A_2 receptors into two subtypes was proposed originally by Daly et al. (1983) based on the finding of high-affinity A_2 receptors in rat striatum and low-affinity A_2 receptors throughout the brain, both of which activated adenyl cyclase. The existence of subtypes of A_2 receptors was also suggested by the finding, independently reported by Elfman et al. (1984), of high-affinity A_2 receptors in cultured neuroblastoma cells and low-affinity A_2 receptors in glioma cells. These high- and low-affinity receptor subtypes were later designated as A_{2A} and A_{2B}, respectively (Bruns et al., 1986). The classification of P_1 receptors has been validated by the recent success in molecular cloning and expression of all three anticipated A_1, A_{2A}, and A_{2B} adenosine receptors and the previously unrecognized A_3 receptor (Maenhaut et al., 1990; Libert et al., 1991; Zhou et al., 1992; Rivkees and Reppert, 1992; Pierce et al., 1992). This classification has been endorsed by IUPHAR Committee on Receptor Nomenclature and Drug Classification (Fredholm et al., 1994, 1996b, 1997).

III. Molecular Characterization of A_{2B} Receptors

Adenosine A_{2B} receptors were cloned from rat hypothalamus (Rivkees and Reppert, 1992), human hippocampus (Pierce et al., 1992), and mouse mast cells (Marquardt et al., 1994), employing standard polymerase chain reaction techniques with degenerate oligonucleotide primers designed to recognize conserved regions of most G protein-coupled receptors. The human A_{2B} receptor shares 86 to 87% amino acid sequence homology with the rat and mouse A_{2B} receptors (Rivkees and Reppert, 1992; Pierce et al., 1992; Marquardt et al., 1994) and 45% amino acid sequence homology with human A_1 and A_{2A} receptors (fig. 1). As expected for closely related species, the rat and mouse A_{2B} receptors share 96% amino acid sequence homology. By comparison, the overall amino acid identity between A_1 receptors from various species is 87% (Palmer and Stiles, 1995). A_{2A} receptors share 90% of homology between species (Ongini and Fredholm, 1996), with most differences occurring in the 2nd extracellular loop and the long C-terminal domain (Palmer and Stiles, 1995). The lowest (72%) degree of identity between species is observed for A_3 receptor sequences (Palmer and Stiles, 1995). Differences in amino acid sequence of adenosine receptors between species may result in distinct pharmacological characteristics. For example, the rat A_1 receptor has a rank order of potency (R)-N^6-phenylisopropyladenosine (R-PIA) > 5′-N-ethylcarboxamidoadenosine (NECA) > (S)-N^6-phenylisopropylaolenesine (S-PIA), and the bovine A_1 receptor has a potency order R-PIA > S-PIA > NECA (Klotz et al., 1991), whereas the canine A_1 receptor binds NECA with a higher affinity than that of R-PIA (Tucker and Linden, 1993). The differences between amino acid sequences of A_3 receptors are reflected in the insensitivity of the rat A_3 receptor to antagonism by methylxanthines (Zhou et al., 1992), a phenomenon that is not observed in the human or sheep A_3 receptor (Linden et al., 1993; Salvatore et al., 1993). These interspecies differences between adenosine receptors explain why the adenosine agonist xanthine amine congener (XAC) is a selective A_1 agonist in the rat, but not in the human or rabbit (Jacobson et al., 1992; Jacobson and

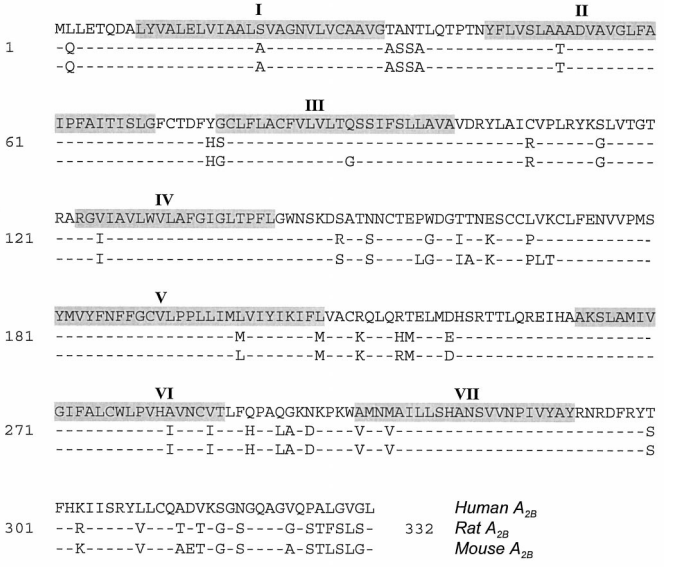

FIG. 1. Comparison of amino acid sequences of human (M97759) (Pierce et al., 1992), rat (M91466) (Stehle et al., 1992), and mouse (U05673) (Marquardt et al., 1994) A_{2B} receptors. Dashed lines indicate amino acid identity. Predicted transmembrane spanning domains are highlighted and indicated by roman numerals.

Suzuki, 1996). Few comparisons have been made between A_{2B} receptors from different species. No differences in pharmacological profiles were found between A_{2B} receptors from fibroblasts of murine and human origin (Bruns, 1981; Brackett and Daly, 1994) or between human A_{2B} receptor expressed in Chinese hamster ovary (CHO) cells and guinea pig brain A_{2B} receptors (Alexander et al., 1996).

The proposed membrane structure of A_{2B} receptors is typical of G protein–coupled receptors, with seven transmembrane domains connected by three extracellular and three intracellular loops, and flanked by an extracellular N-terminus and an intracellular C-terminus (Rivkees and Reppert, 1992; Pierce et al., 1992; Marquardt et al., 1994; fig. 2). The highest degree of identity in amino acid sequences between A_{2B} receptors of different species is found in the transmembrane domains (fig. 1). The 2nd extracellular loop of the human, mouse, and rat A_{2B} receptors contains two potential N-glycosylation sites (Rivkees and Reppert, 1992; Pierce et al., 1992; Marquardt et al., 1994). It should be noted that enzymatic treatment failed to demonstrate N-glycosylation of A_{2B} receptors in T84 epithelial cells (Puffinbarger et al., 1995). However, it is not clear whether A_{2B} receptors are glycosylated in other cells or glycosylation can alter A_{2B} function. A_{2A} receptors were found to be glycosylated in canine striatum and liver membranes (Palmer et al., 1992), but the binding characteristics of A_{2A} receptors for 4-[(N-ethyl-5′-carbamoyladenos-2-yl)-aminoethyl]-phenylpropionic acid (CGS 21680) appear to be the same in both glycosylated or unglycosylated forms of the receptor expressed in COS M6 cells (Piersen et al., 1994).

The predicted molecular mass of A_{2B} receptors is similar to that of A_1 and A_3 receptors (36–37 kDa), whereas A_{2A} receptors have a larger predicted size (45 kDa). The greater molecular mass of the A_{2A} receptor is explained by the presence of a longer intracellular C-terminus. Together with the 3rd intracellular loop, the intracellular C-terminus is thought to be involved in the coupling of A_{2A} receptors to G proteins (Palmer and Stiles, 1995). To date, no mutational analysis of A_{2B} receptor-G protein coupling has been reported. However, some parallels could be drawn from studies using chimeric A_1/A_{2A} adenosine receptors (Tucker et al., 1996; Olah, 1997). Using this approach, it has been shown that the amino terminal portion of the 3rd intracellular loop of the A_{2A} receptor determines its selective coupling with G_s (Olah, 1997). This 15-mer portion of the A_{2A} receptor shares 57% amino acid sequence homology with the A_{2B} receptor, both of which are coupled to G_s, and only 27% with the A_1 receptor, which is not coupled to G_s (fig. 3). In addition, the nature of the amino acids in the 2nd intracellular loop may indirectly modulate A_{2A} receptor coupling. In particular, lysine and glutamic acid residues in that portion of the molecule were found to be necessary for efficient A_{2A} adenosine receptor-G_s coupling (Olah, 1997). These amino acid residues are also present in the A_{2B} receptor. The long intracellular C-terminal tail of the A_{2A} receptor, which represents a major structural difference with the A_{2B}, does not appear to be involved in the determination of receptor coupling to G_s protein. The removal of the C-terminal tail of the A_{2A} receptor, or its replacement with a cytoplasmic tail of the A_1 receptor, does not impair stimulation of adenyl cyclase when these truncated or chimeric receptors are expressed in CHO cells (Tucker et al., 1996; Palmer and Stiles, 1997; Olah, 1997). The data generated from these studies, however, leave the possibility that this region can still play a role in the modulation of the coupling of A_{2A} receptors to G proteins. For example, it was suggested that the C-terminal tail confers the A_{2A} receptors' ability to couple tightly to G_s, a feature considered to be unique for this receptor subtype (Nanoff et al., 1991).

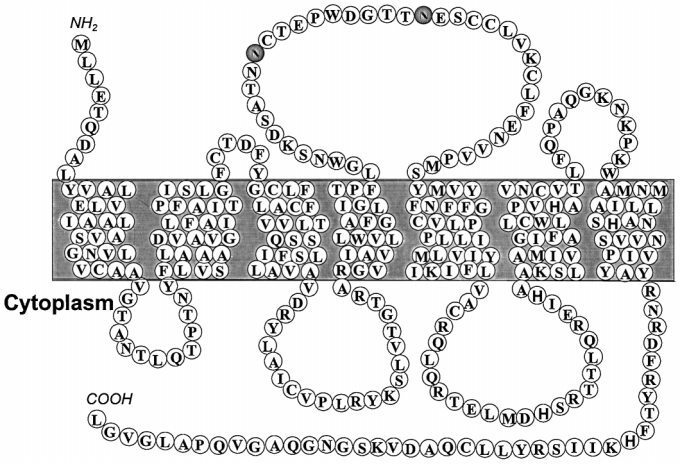

FIG. 2. Amino acid sequence of the human A_{2B} receptor. The receptor is drawn according to the seven-membrane spanning motif common to the G protein-coupled receptor superfamily. Possible sites of N-linked glycosylation on the 2nd extracellular loop are highlighted.

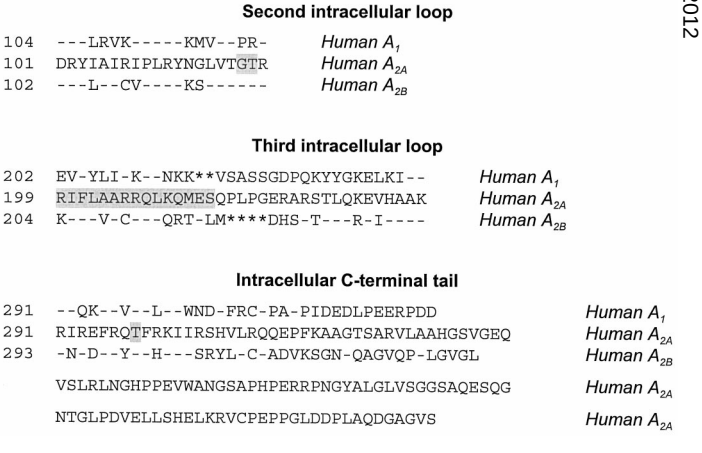

FIG. 3. Comparison of amino acid sequences of 2nd and 3rd intracellular loops and C-terminus of human A_{2A} receptors, with corresponding regions of human A_1 and A_{2B} receptors. Dashed lines indicate amino acid identity. Asterisks represent gaps in the sequence introduced to highlight amino acid homologies. The amino acid residues discussed in the text are highlighted.

Mutational studies of A_{2A} receptors revealed that a threonine residue (Thr^{298}) of the C-terminal tail of the A_{2A} receptor, located in proximity to the seventh transmembrane span (fig. 3), is essential for the development of rapid agonist-mediated desensitization (Palmer and Stiles, 1997). This amino acid residue is also present in the human A_{2B} receptor (Thr^{300}), but its role in receptor desensitization has not been explored. Although the mechanisms of desensitization are not completely identified, it is of interest that rapid desensitization of A_{2A} as well as A_{2B} receptors can be mediated by G protein-coupled receptor kinase 2 (Mundell et al., 1997). It should be noted that A_{2B} receptors can be coupled to other intracellular signaling pathways in addition to G_s and adenyl cyclase. The similarities and differences in A_{2B} and A_{2A} receptor coupling to G proteins warrant studies involving mutational analysis of A_{2B} receptors, and possibly chimeric A_{2A}/A_{2B} receptors, to better understand determinants of A_{2B}-G protein coupling.

The human A_{2B} receptor gene was mapped to chromosome 17p11.2-p12 (Jacobson et al., 1995; Townsend-Nicholson et al., 1995). A single intron interrupts the coding sequence of the human A_{2B} receptor gene in a region corresponding to the 2^{nd} intracellular loop between $Leu^{111}$and Arg^{112} (Jacobson et al., 1995). In this respect, the human A_{2B} receptor gene is similar to the other human adenosine receptor genes in that it also contains a single intron in its coding sequence (Ren and Stiles, 1994; Peterfreund et al., 1994; Murrison et al., 1996). Some G protein-coupled receptors are known to have multiple introns in the coding sequences of their corresponding genes. Alternative splicing of their primary transcripts results in heterogeneity in protein sequences, as observed with EP_3 prostanoid receptors (Neglishi et al., 1995), D_2 dopamine receptors (Giros et al., 1989), lutropin/choriogonadotropin receptors (Aatsinki et al., 1992), and fibroblast growth factor receptors 2 (Dell and Williams, 1992). The presence of only one intron within the coding region of the human A_{2B} receptor gene precludes structural variations of A_{2B} receptors by alternative splicing.

In addition to the human A_{2B} receptor gene, an A_{2B} pseudogene with 79% identity with the A_{2B} receptor complementary deoxyribonucleic acid (cDNA), has been localized to chromosome 1q32 (Jacobson et al., 1995; Townsend-Nicholson et al., 1995). When compared with the coding sequence of the A_{2B} receptor, the pseudogene contained multiple deletions, point mutations, and frame shifts and two in-frame stops (Jacobson et al., 1995). It is doubtful that with all these changes the pseudogene would encode a functional adenosine receptor. However, further studies are needed to determine whether the A_{2B} pseudogene is transcriptionally competent. For example, dopamine D_5 pseudogene transcripts can be detected in human brain tissues (Nguyen et al., 1991). The same possibility should always be considered in Northern blot analysis or in situ hybridization of A_{2B} receptor in various tissues, because the use of sequences common between the functional A_{2B} cDNA and the A_{2B} pseudogene as probes could potentially lead to misinterpretation of results.

IV. Pharmacology of A_{2B} Receptors

Highly selective and potent agonists have been designed for A_1, A_{2A}, and A_3 receptors. These compounds have been important tools in the characterization of adenosine receptors and the determination of their functions. All four subtypes, including A_{2B} receptors, have a typical order of potency for agonists (table 1; fig. 4). However, no selective agonist for A_{2B} receptors has been found so far. The adenosine analog NECA remains the most potent A_{2B} agonist (Bruns, 1981; Feoktistov and Biaggioni, 1993, 1997; Brackett and Daly, 1994), with a concentration producing a half-maximal effect (EC_{50}) for stimulation of adenyl cyclase of approximately 2 μM. It is, however, nonselective and activates other adenosine receptors with even greater affinity, with an EC_{50} in the low nanomolar (A_1 and A_{2A}) or high nanomolar (A_3) range (table 1; fig. 4). The characterization of A_{2B} receptors, therefore, often relies on the lack of effectiveness of compounds that are potent and selective agonists of other receptor types. A_{2B} receptors have been characterized by a method of exclusion, i.e., by the lack of efficacy of agonists that are specific for other receptors. The A_{2A} selective agonist CGS 21680 (Webb et al., 1992), for example, has been useful in differentiating between A_{2A} and A_{2B} adenosine receptors (Hide et al., 1992; Chern et al., 1993; Feoktistov and Biaggioni, 1995; van der Ploeg et al., 1996). Both receptors are positively coupled to adenyl cyclase and are activated by the nonselective agonist NECA. CGS 21680 is virtually ineffective on A_{2B} receptors but is as potent as NECA in activating A_{2A} receptors, with an EC_{50} in the low nanomolar range for both agonists (Jarvis et al., 1989; Nakane and Chiba, 1990; Webb et al., 1992; Hide et al., 1992; Feoktistov and Biaggioni, 1993; Alexander et al., 1996). A_{2B} receptors have also a very low affinity for the A_1 selective agonist R-PIA (Feoktistov and Biaggioni, 1993; Brackett and Daly, 1994) as well as for the A_3 selective agonist N^6-(3-iodobenzyl)-N-methyl-5′-carbamoyladenosine (IB-MECA) (Feoktistov and Biaggioni, 1997). The agonist profile NECA > R-PIA = IB-MECA > CGS 21680 was determined in human erythroleukemia (HEL) cells for A_{2B}-mediated cAMP accumulation. The difference between EC_{50} for NECA and the rest of the agonists is approximately 2 orders of magnitude. Therefore, responses elicited by NECA at concentrations in the low micromolar range (1–10 μM), but not by R-PIA, IB-MECA or CGS 21680, are characteristic of A_{2B} receptors.

Pharmacological characterization of receptors based on apparent agonist potencies, however, is far from ideal, because it depends not only on agonist binding to the receptor but also on multiple processes involved in

TABLE 1
Pharmacological characteristics of adenosine receptor subtypes

Adenosine receptor	Order of potency for agonists[a] (μM)	Selective agonists[b]	Selective antagonists[b]
A_1	R-PIA (0.001) > NECA (0.006) > IB-MECA (0.054) > CGS 21680 (2.6)	R-PIA, CPA	DPCPX, N-0861
A_{2A}	NECA (0.01) = CGS 21680 (0.015) > IB-MECA (0.056) > R-PIA (0.124)	CGS 21680, APEC	SCH 58261, ZM 241385
A_{2B}	NECA (2) > R-PIA (160) = IB-MECA (201) > CGS 21680 (1600)	None	Enprofylline
A_3	IB-MECA (0.001) > NECA (0.113) = R-PIA (0.158) > CGS 21680 (0.584)	IB-MECA, Cl-IB-MECA	MRS 1067, MRS 1097 L-249313, L-268605

[a] Data shown for rat A_1, A_{2A}, and A_3 receptors are K_i values based on radioligand binding, from van Galen et al. (1994) and Gallo-Rodriguez et al. (1994). Data shown for A_{2B} receptors are EC_{50} values for cAMP accumulation in human erythroleukemia cells from Feoktistov and Biaggioni (1993) and Feoktistov and Biaggioni (1997a).
[b] Data are derived from Feoktistov and Biaggioni (1995), Palmer and Stiles (1995), Jacobson et al. (1996), and Jacobson (1996).

FIG. 4. Chemical structure and radioligand binding data on the affinity of adenosine agonists. K_i values (nM) for rat $A_1/A_{2A}/A_{2B}/A_3$ receptors are shown, except as indicated (h, human). Numbers in brackets represent EC_{50} of adenosine agonists for cAMP accumulation in HEL cells. Data compiled from Feoktistov and Biaggioni (1993), van Galen et al. (1994), Jacobson and Suzuki (1996), and Feoktistov and Biaggioni (1997).

signal transduction. Selective antagonists are preferable for receptor subtype identification (Kenakin et al., 1992). Highly selective and potent A_{2B} antagonists are not yet available, but, whereas A_{2B} receptors have a lower affinity for agonists compared with other receptor subtypes, this is not true for antagonists. The structure-activity relationship of A_{2B} receptors for adenosine antagonists has not been completely characterized, but at least some xanthines are as potent antagonists at A_{2B} receptors as at other adenosine receptors (Feoktistov and Biaggioni, 1993; Brackett and Daly, 1994).

The antiasthmatic drug enprofylline (3-n-propylxanthine), is the most selective A_{2B} antagonist known to date. In early studies, enprofylline was found to be about 20 times more potent in blocking hippocampal A_2 receptors compared with rat fat cell A_1 receptors (Fredholm and Persson, 1982). It was initially proposed, therefore, that enprofylline can selectively block a subtype of A_2 receptors in the hippocampus (Fredholm and Persson, 1982). However, enprofylline was then found to be a poor antagonist of A_2 receptors in thymocytes (Fredholm and

Sandberg, 1983) and platelets (Ukena et al., 1985). More recently, enprofylline has also been found to have a low affinity for A_3 receptors (Linden et al., 1993). These findings led to the conclusion that enprofylline was not an adenosine receptor antagonist. These original studies need to be reinterpreted in the light of our current knowledge of adenosine receptor subtypes. It is now known that accumulation of cAMP in hippocampal slices, which was shown to be blocked by enprofylline, is mediated by A_{2B} receptors (Lupica et al., 1990), and that platelets, found to be insensitive to enprofylline, express mainly A_{2A} receptors (Feoktistov and Biaggioni, 1993; Dionisotti et al., 1996; Ledent et al., 1997). Therefore, previous contradictory results can now be explained by a selective antagonism of A_{2B} receptors by enprofylline. Indeed, it was recently demonstrated that enprofylline is equipotent to theophylline as an A_{2B} receptor antagonist in HEL cells, with a dissociation constant of antagonist-receptor complex (K_B) of 7 μM (Feoktistov and Biaggioni, 1995). An analysis of the original results in the hippocampus (Fredholm and Persson, 1982) reveals an approximate K_B of 6 μM. An identical K_i for enprofylline (7 μM) was found in CHO cells stably transfected with A_{2B} using radioligand binding with [^{3}H]1,3 diethyl-8-phenylxanthine (Robeva et al., 1996). This value also correlated well with the K_B estimated from inhibition of NECA-induced cAMP generation in a similar cell model (23 μM) (Alexander et al., 1996). Enprofylline is also an effective antagonist of A_{2B} receptors in human HMC-1 mast cells (Feoktistov and Biaggioni, 1995) and canine BR mastocytoma cells (Auchampach et al., 1996). In comparative radioligand binding studies on all four human adenosine receptors permanently expressed in CHO cells, enprofylline has been shown to be 22-fold selective for A_{2B} versus A_1, five-fold versus A_{2A}, and six-fold versus A_3 (Robeva et al., 1996). Enprofylline, therefore, can be considered a relatively selective, though not potent A_{2B} antagonist.

More potent but nonselective A_{2B} receptor antagonists have been also characterized. These compounds include 1,3-dipropyl-8-(p-sulfophenyl)xanthine (DPSPX), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), and XAC (Feoktistov and Biaggioni, 1993; Brackett and Daly, 1994). The xanthine antagonist DPSPX is 20-fold more potent at A_{2B} receptors in HEL cells (K_B = 141 nM) compared with platelet A_{2A} receptors (Feoktistov and Biaggioni, 1993). However, the affinity of A_{2B} receptors for DPSPX (Feoktistov and Biaggioni, 1993) is similar to those of sheep A_3 (Linden et al., 1993) and rat A_1 (Ukena et al., 1986) receptors. Among nonxanthine compounds, 2,4-dioxobenzo[g]pteridine (alloxazine) was reported to be nine-fold more potent as an antagonist of A_{2B} receptors in VA13 and NIH 3T3 cells compared with A_{2A} receptors in PC12 cells (Brackett and Daly, 1994; fig. 5).

A_{2B} receptors are frequently found with other adenosine receptor subtypes in the same tissue, and are even coexpressed in the same cells. Recent advances in the development of selective A_1, A_{2A}, and A_3 antagonists (table 1; fig. 5) provide a new approach to the study of A_{2B} receptors; the nonselective agonist NECA can be used in conjunction with highly selective antagonists of other adenosine receptor subtypes to selectively stimulate A_{2B} receptors. The ability to selectively block other adenosine receptors is particularly useful in situations in which they are present with A_{2B} receptors.

The first selective A_1 antagonist DPCPX was discovered by two independent groups of investigators (Martinson et al., 1987; Bruns et al., 1987) and has become the reference A_1 receptor antagonist. It is highly selective for A_1 versus A_{2A} (80- to 500- fold across species) (Jacobson et al., 1992; Robeva et al., 1996). In recent radioligand binding studies involving all four human recombinant adenosine receptors, DPCPX has been confirmed to be 20-fold selective for A_1 versus A_{2B} (Robeva et al., 1996). Selective blockade of A_1 receptors with DPCPX was successfully used to reveal functional A_{2B} receptors in tissues coexpressing both A_1 and A_{2B} receptors (Mogul et al., 1993; Murthy et al., 1995; Nicholls et al., 1996). Other compounds have been identified with even greater selectivity for the A_1 receptor; C^8-(N-methylisopropyl)-amino-N^6-(5′-endohydroxy)-endonorbornan-2-yl-9-methyladenin (WRC-0571) binds to human A_1 receptors with a K_i of 3 nM and to human A_{2B} receptors with a K_i of 19 μM. This compound, therefore, is approximately 6300-fold selective for A_1 versus A_{2B} (Robeva et al., 1996).

Among the new generation of A_{2A} antagonists, 4-(2-[7-amino-2-)2-furyl(triazolo {2,3-a}-[1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385) was reported to be 30- to 80-fold selective for A_{2A} versus A_{2B} (Poucher et al., 1995). Another antagonist, 5-amino-7-(phenylethyl)-2-(1-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261), has a high affinity (K_i = 0.7–2.2 nM) for A_{2A} receptors (Belardinelli et al., 1996; Lindström et al., 1996; Dionisotti et al., 1996; Zocchi et al., 1996a,b; Ongini et al., 1996; Ongini and Fredholm, 1996) but was found not to block NECA-induced vasorelaxation of guinea pig aorta, a process thought to be mediated by A_{2B} receptors (Zocchi et al., 1996a). The selectivity of SCH 58261 for A_{2A} versus A_{2B} has been also confirmed in a cellular system; this compound was ineffective on HEL cell A_{2B} receptors up to a concentration of 100 nM, whereas it inhibited the CGS 21680-induced cAMP accumulation in HMC-1 cell (A_{2A} receptor) with a K_B of 0.1 nM (Feoktistov and Biaggioni, 1997). SCH 58261, therefore, can be useful in the discrimination of A_{2B} function in cells also coexpressing A_{2A} receptors. This approach was applied to the study of adenosine receptors in the human mast cells HMC-1 (fig. 6). The concentration-response relationship of the nonselective adenosine agonist NECA for cAMP accumulation in these cells follows a curve with a Hill slope of 0.64 ± 0.07 best fitted to a two-site model with an apparent pD_2 of 7.69 ± 0.42 and 5.92 ± 0.21 for the high- and low-affinity sites,

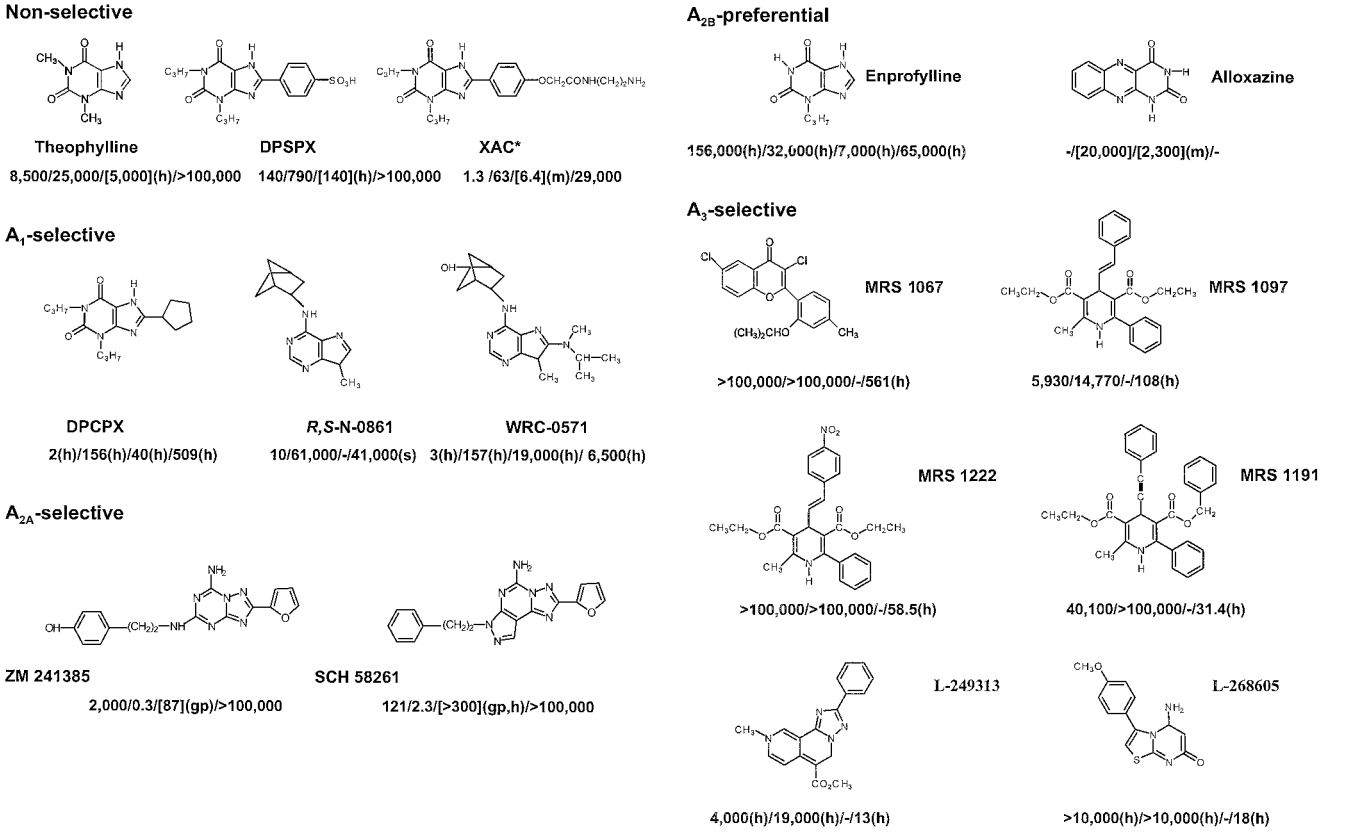

FIG. 5. Chemical structure and radioligand binding data on the affinity of adenosine antagonists. K_i values (nM) for rat $A_1/A_{2A}/A_{2B}/A_3$ receptors are shown, except as indicated (h, human; gp, guinea pig; s, sheep; m, mice). Numbers in brackets represent K_B of adenosine antagonists. Data compiled from Feoktistov and Biaggioni (1993), Brackett and Daly (1994), van Galen et al. (1994), Jacobson et al. (1996), Robeva et al. (1996), Jiang et al. (1996), van Rhee et al. (1996), Zocchi et al. (1996a), and Jacobson and Suzuki (1996). *, selective for rat, but not for human or rabbit A_1 receptors (Jacobson and Suzuki, 1996).

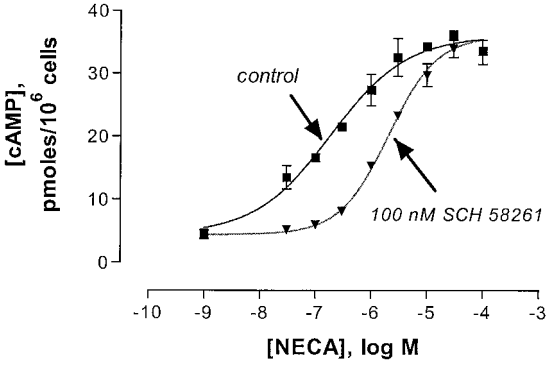

FIG. 6. Selective activation of A_{2B} receptors with NECA in HMC-1 human mast cells coexpressing A_{2A} and A_{2B} receptors, made possible by blockade of A_{2A} receptors with the selective antagonist SCH 58261 (Feoktistov and Biaggioni, 1997). See text for details.

respectively. Upon complete blockade of A_{2A} receptors with 100 nM SCH 58261, the concentration-response curve of NECA was transformed into a typical sigmoid curve with a Hill slope of 0.93 ± 0.06 and a pD_2 of 5.68 ± 0.03, consistent with activation of A_{2B} receptors. Blockade of A_{2A} receptors in the same cells with SCH 58261 did not affect NECA-induced calcium mobilization, confirming that this process is mediated solely via A_{2B} receptors (Feoktistov and Biaggioni, 1997), as it has been previously suggested on the basis of the lack of CGS 21680 effectiveness (Feoktistov and Biaggioni, 1995).

Recently, several antagonists with A_3 selectivity versus A_1 and A_{2A} receptors have been introduced. These compounds include the flavonoid derivative 3,6-dichloro-2′-isopropyloxy-4′-methylflavone (MRS 1067) and the dihydropyridine derivatives 3-ethyl 5-benzyl 2-methyl-phenylethynyl-6-phenyl-1,4(±)dihydropyridine-3,5-dicarboxylate (MRS 1191), 3,5-diethyl 2-methyl-4-[2-(4-nitrophenyl)-(*E*)-vinyl-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (MRS 1222), and 3,5-diethyl 2-methyl, 6-phenyl-4-[2-[phenyl-(trans)-vinyl]-1,4(±)dihydropyiridine-3,5-dicarboxylate (MRS 1097) (fig. 5), which are selective for the human A_3 receptor by a factor of 45- to 1700-fold, versus rat A_1 and A_{2A} receptors, as determined from radioligand binding studies (Jiang et al., 1996; Karton et al., 1996; van Rhee et al., 1996). It should be noted, however, that the highest degree of selectivity for these compounds is observed when their effects on human A_3 receptors are compared with their effects on rat A_1 and A_{2A} receptors. For example, MRS

1191 was selective for the human A_3 receptor by factor of 1300-fold, whereas for the rat A_3 receptor, the selectivity was only 11-fold versus the rat A_1 receptor (Jiang et al., 1996). Among other compounds, the triazolonaphthyridine derivative 6-carboxymethyl-5,9-dihydro-9-methyl-2-phenyl-[1,2,4]-triazolo-{5,1-a}-[2,7]-naphthyridine (L-249313) (fig. 5) is highly potent on human A_3 receptors (K_i =13 nM), but not on rat A_3 receptors (K_i = 58 μM). The thiazolopyrimidine derivative 3-(4-methoxyphenyl)-5-amino-7-oxo-thiazolo-[3,2]-pyrimidine (L-268605) was also shown to be a potent antagonist on human A_3 receptor (K_i = 18 nM). Both compounds are highly selective for the human A_3 receptor versus the human A_1 (>300-fold) and A_{2A} (>1400-fold) receptors (Jacobson et al., 1996). Unfortunately, A_{2B} receptors have not been included when characterizing the selectivity of A_3 antagonists. Additional studies of A_3 antagonists with respect to A_{2B} receptors are required to verify whether they can be useful to discriminate between A_3 and A_{2B}-mediated effects.

In summary, potent and selective agonists and antagonists are available for all adenosine receptors except for the A_{2B} subtype. The characterization of A_{2B} receptors has been based on apparent potencies of agonists selective to other adenosine receptor subtypes. The development of selective A_1, A_{2A}, and A_3 antagonists provides a new approach when used in conjunction with the nonselective agonist NECA to selectively stimulate A_{2B} receptors. This approach is particularly useful in tissues or cells expressing more than one adenosine receptor. However, much progress in this field could be achieved by the development of selective A_{2B} receptor antagonists. Because of the low affinity of this receptor for agonists, the design of selective and potent A_{2B} antagonists seems to be more promising than the development of selective agonists.

V. Distribution of A_{2B} Receptors

The generation of cDNA for A_{2B} receptors has made possible the identification of the tissue distribution of this receptor subtype. A_{2B} receptor messenger ribonucleic acid (mRNA) was originally detected in a limited number of rat tissues by Northern blot analysis, with the highest levels found in cecum, bowel, and bladder, followed by brain, spinal cord, lung, epididymis, vas deferens, and pituitary (Stehle et al., 1992). The use of more sensitive reverse transcriptase-polymerase chain reaction techniques revealed a ubiquitous distribution of A_{2B} receptors. mRNA encoding A_{2B} receptors was detected at various levels in all rat tissues studied, with the highest levels in the proximal colon and lowest in the liver (Dixon et al., 1996). In situ hybridization of A_{2B} receptors showed widespread and uniform distribution of A_{2B} mRNA throughout the brain (Stehle et al., 1992; Dixon et al., 1996). The expression of A_{2B} receptors in a variety of human and murine tissues has been confirmed by Western blotting and by immunostaining with an anti-A_{2B} receptor antibody (Puffinbarger et al., 1995).

Pharmacological identification of A_{2B} receptors, based on their low affinity and characteristic order of potency for agonists, also indicates a widespread distribution of A_{2B} receptors. In brain, functional A_{2B} receptors are found in neurons (Mogul et al., 1993; Okada et al., 1996; Kessey et al., 1997) and glial cells (van Calker et al., 1979; Elfman et al., 1984; Hösli and Hösli, 1988; Altiok et al., 1992; Peakman and Hill, 1994, 1996; Fiebich et al., 1996a). Although there is no evidence that A_{2B} receptor are present in microglia (Fiebich et al., 1996b), there is ample data that show that they are expressed in astrocytes and in different glioma cell lines (Elfman et al., 1984; Hösli and Hösli, 1988; Altiok et al., 1992; Peakman and Hill, 1994, 1996; Fiebich et al., 1996a). The expression of A_{2B} receptors in glial cells, which represent a majority of the brain cell population, can explain the original observation that slices from all brain areas examined showed an adenosine-stimulated cAMP response (Sattin and Rall, 1970; Daly, 1976).

Functional A_{2B} receptors have been found in fibroblasts (Brackett and Daly, 1994) and various vascular beds (Martin, 1992; Martin et al., 1993; Chiang et al., 1994; Martin and Potts, 1994; Haynes et al., 1995; Rubino et al., 1995; Prentice and Hourani, 1996; Dubey et al., 1996b). Contamination with these cells may also contribute to the widespread pattern of A_{2B} receptor distribution in all organs. This possibility should always be considered, especially when data from crude tissue preparations are analyzed. The presence of functional A_{2B} receptors also has been demonstrated in hematopoietic cells (Feoktistov and Biaggioni, 1993; Porzig et al., 1995), mast cells (Marquardt et al., 1994; Feoktistov and Biaggioni, 1995), myocardial cells (Liang and Haltiwanger, 1995), intestinal epithelial (Strohmeier et al., 1995) and muscle cells (Murthy et al., 1995; Nicholls et al., 1996), retinal pigment epithelium (Blazynski, 1993; Gregory et al., 1994), endothelium (Iwamoto et al., 1994), and neurosecretory cells (Casado et al., 1992; Gharib et al., 1992; Mateo et al., 1995).

Coexpression of A_{2B} receptors together with other adenosine receptors has been reported in various cell preparations and cell lines. Functionally coupled A_{2B} and A_{2A} receptors are coexpressed in rat pheochromocytoma PC12 cells (Hide et al., 1992; Chern et al., 1993; van der Ploeg et al., 1996), T-cell leukemia Jurkat cells (van der Ploeg et al., 1996), mouse bone marrow-derived mast cells (Marquardt et al., 1994), human mast HMC-1 cells (Feoktistov and Biaggioni, 1995), human aortic endothelial cells (Iwamoto et al., 1994), human umbilical vein endothelial cells (Feoktistov and Biaggioni, unpublished observations), and human neutrophil leukocytes (Fredholm et al., 1996c). mRNA encoding A_{2A}, A_{2B}, and A_3, but not A_1 receptors, have been found in rat RBL 2H3 mast cells (Ramkumar et al., 1993; Marquardt et al., 1994).

Functional A_1 receptors can also be coexpressed with A_{2A} and/or A_{2B} receptors. In most cases, a selective blockade of A_1 receptors is required to unmask functional A_{2B} receptors. This approach was successfully used in dispersed guinea pig small intestinal muscle cells (Murthy et al., 1995), in rat duodenum longitudinal muscle muscularis mucosae cells (Nicholls et al., 1996), and in guinea pig pyramidal neurons from the hippocampal CA3 region (Mogul et al., 1993). Similarly, uncoupling of A_1 receptor using pertussis toxin unmasks the presence of A_{2A} and A_{2B} receptors in ventricular myocytes (Liang and Haltiwanger, 1995). By contrast, it was not necessary to block A_1 receptors in various glial cells to observe either A_{2A} or A_{2B} receptor-mediated stimulation of adenyl cyclase (Elfman et al., 1984; Altiok et al., 1992; Peakman and Hill, 1994, 1996; Fiebich et al., 1996a). Also, the balance between A_1- and A_2-mediated responses can be modulated. For example, corticosteroid treatment of DDT_1 MF2 smooth muscle cells increased A_1 receptor number and signaling and decreased A_2 receptor signaling (Gerwins and Fredholm, 1991). A similar decrease in the A_{2B} signaling upon dexamethasone treatment was also reported in Jurkat cells (Svenningsson and Fredholm, 1997).

Coexistence of different adenosine receptor types in cells obtained from primary tissue cultures (Iwamoto et al., 1994; Peakman and Hill, 1994, 1996) may be attributed to the presence of different subpopulations of cells, each one expressing a single type of adenosine receptor. However, studies on established cell lines (Hide et al., 1992; Feoktistov and Biaggioni, 1995; van der Ploeg et al., 1996) have confirmed the coexpression of adenosine receptors in a single target cell. Moreover, studies performed on single cells have also demonstrated the presence of more than one adenosine receptor subtype (Liang and Morley, 1996; Strickler et al., 1996), including A_{2B} receptors (Liang and Haltiwanger, 1995).

Coexpression of A_{2B} and A_{2A} receptors has been demonstrated even in clonal cell lines originally used to describe prototypic A_{2A} (PC12 cells) and A_{2B} receptors (Jurkat cells) (van der Ploeg et al., 1996). These cells predominantly express A_{2A} and A_{2B} receptors, respectively, and the presence of the other receptor type was recognized only after carefully conducted studies using differential responses to a series of 2-substituted adenosine analogs (Hide et al., 1992; van der Ploeg et al., 1996). It is entirely possible, therefore, that more examples of cells coexpressing adenosine receptors may become apparent after selective adenosine antagonists are applied in the characterization of these cells.

The functional meaning of this simultaneous expression of multiple adenosine receptor subtypes in a single target cell is not known. Because A_1 and A_{2A} receptors have a higher affinity for adenosine, in many cellular systems, these receptors need to be blocked before A_{2B}-mediated effects are apparent (Mogul et al., 1993; Liang and Haltiwanger, 1995; Murthy et al., 1995; Nicholls et al., 1996; Kessey et al., 1997; Feokstistov and Biaggioni, 1997). This, however, is not always the case. Both A_1 and A_{2B} receptors are present in glial cells of rat astrocytes, and stimulation of A_{2B} receptors with the nonselective agonist NECA induces cAMP accumulation that is evident even in the presence of A_1 receptors (Elfman et al., 1984; Altiok et al., 1992; Peakman and Hill, 1994, 1996; Fiebich et al., 1996a). It is possible that the relative importance of A_{2B} receptors is greater in situations in which high interstitial levels of adenosine are reached, e.g., in tissues in which metabolic demands are increased or oxygen supply is decreased, whereas the high affinity A_1 and A_{2A} receptors may modulate cellular functions in response to lower concentrations of this autacoid. The recent recognition that in cells coexpressing other adenosine receptors, A_{2B} receptors can be coupled to distinct intracellular pathways (Feoktistov and Biaggioni, 1995), may also provide the basis for a differential physiological role.

VI. Intracellular Pathways Regulated by A_{2B} Receptors

It is generally accepted that A_{2A} and A_{2B} receptors are coupled to G_s proteins, because both activate adenyl cyclase in virtually every cell in which they are expressed. Although activation of adenyl cyclase is arguably an important signaling mechanism for A_{2A} receptors, this is not necessarily the case for A_{2B} receptors, as other intracellular signaling pathways have been found to be functionally coupled to A_{2B} receptors in addition to adenyl cyclase (fig. 7).

Recombinant rat A_{2B} receptors expressed in *Xenopus* oocytes activate calcium-dependent chloride conductance presumably by stimulation of phospholipase C (Yakel et al., 1993). Likewise, it has been proposed that A_{2B} receptors stimulate phospholipase C in mouse bone marrow-derived mast cells (Marquardt et al., 1994). Regulatory proteins of the G_q family are thought to play a role in the coupling of A_{2B} receptors to β-phospholipase C in human mast HMC-1 cells (Feoktistov and Biaggioni, 1995) and canine BR mastocytoma cells (Auchampach et al., 1996), because this process is unaffected by treatment with pertussis or cholera toxins. A_{2B} receptor-mediated stimulation of β-phospholipase C results in mobilization of intracellular calcium in HMC-1 cells and eventually promotes synthesis of interleukin-8 (IL-8) (Feoktistov and Biaggioni, 1995; fig. 7a). In contrast to A_{2B} receptors, there is no evidence that A_{2A} receptors can stimulate phospholipase C under physiological conditions, even though cotransfection of human A_{2A} receptors with murine $G\alpha_{15}$ and human $G\alpha_{16}$, but not with $G\alpha_q$, $G\alpha_{11}$ or $G\alpha_{14}$, results in A_{2A}-mediated stimulation of phospholipase C in COS-7 cells (Offermanns and Simon, 1995). However, promiscuous coupling of $G\alpha_{15}$ and $G\alpha_{16}$ has been observed when these G proteins are coexpressed with receptors which are otherwise not normally coupled to phospholipase C (Milligan et al., 1996).

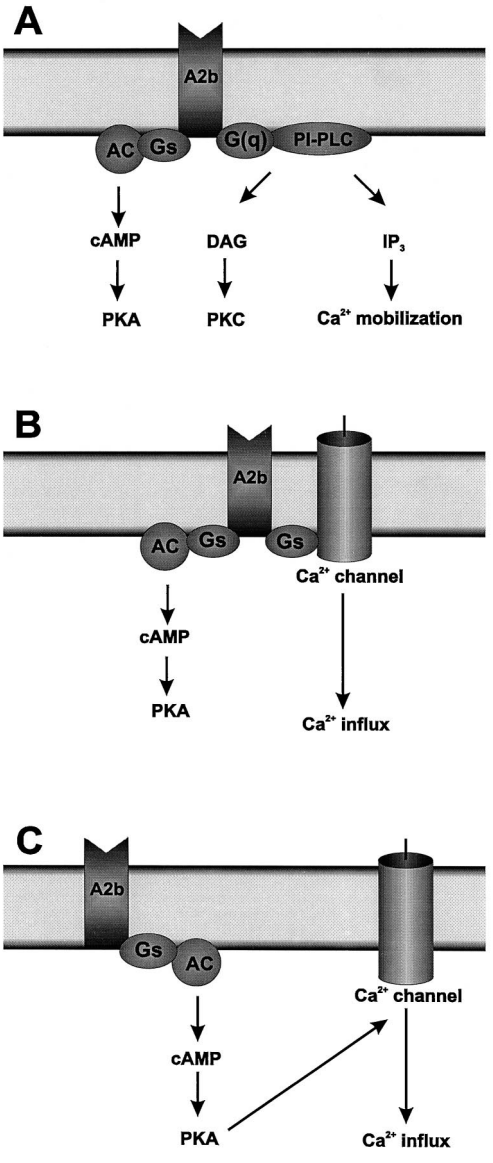

FIG. 7. Schematic representation of intracellular pathways coupled to adenosine A_{2B} receptors in various cells. A_{2B} receptors are coupled to adenyl cyclase (AC) in all cells shown. Activation of this pathway results in accumulation of cAMP and stimulation of protein kinase A (PKA). (A) A_{2B} receptors are coupled to phosphatidylinositol-specific phospholipase C (PI-PLC) via a G protein of the G_q family [$G_{(q)}$] in mast cells (Marquardt et al., 1994; Feoktistov and Biaggioni, 1995; Auchampach et al., 1996). Activation of this pathway results in increase in diacylglycerol (DAG) and inositol trisphosphate (IP_3). Diacylglycerol stimulates protein kinase C (PKC). Inositol trisphosphate activates mobilization of calcium from intracellular stores. (B) A_{2B} receptors potentiate calcium influx directly by coupling with G_s protein in HEL cells (Feoktistov et al., 1994). (C) In contrast, A_{2B} receptors potentiate calcium influx via cAMP and activation of protein kinase A in pyramidal neurons from the CA3 region of guinea pig hippocampus (Mogul et al., 1993).

Also, expression of $G\alpha_{15}$ and $G\alpha_{16}$ is limited only to a subset of hematopoietic cells (Amatruda et al., 1991; Wilkie et al., 1991).

Stimulation of A_{2B} receptors also increases intracellular calcium in HEL cells but not through a mechanism involving phospholipase C activation (fig. 7b). In contrast to the cholera toxin- and pertussis toxin-insensitive mobilization of intracellular calcium observed in HMC-1 mast cells, A_{2B} receptors facilitate calcium influx through a cholera toxin-sensitive mechanism in HEL cells. This effect was observed only when intracellular calcium levels were elevated, either by receptor-dependent (e.g., by thrombin) or -independent (e.g., thapsigargin) mechanisms. Even though this process is coupled to G_s-proteins, it is cAMP-independent. It has been suggested that αG_s, coupled to A_{2B} receptors, can directly stimulate a putative calcium channel (Feoktistov et al., 1994), as proposed for other G_s-coupled receptors (Imoto et al., 1988; Scamps et al., 1992).

Of interest, a similar mechanism has been suggested for A_{2A} receptors in fetal chicken ventricular myocardium cells. These cells coexpress A_{2A} and A_{2B} receptors, and both are positively coupled to stimulation of adenyl cyclase and myocyte contractility (Liang and Haltiwanger, 1995). Selective activation of A_{2A} receptors with CGS 21680 results in cAMP-independent calcium entry in pertussis toxin-treated cells. This effect does not involve simulation of phospholipase C and was blocked by the selective A_{2A} antagonist 8-(3-chlorostyryl)caffeine (Liang and Morley, 1996). This study did not explore the possibility that A_{2B} receptors share a common mechanism of G_s-mediated stimulation of calcium entry with A_{2A} receptors. This could be tested by using the nonspecific A_2 agonist NECA in the presence of a selective A_{2A} antagonist such as SCH 58261.

In another example of positive modulation of intracellular calcium, it has been reported that activation of A_{2B} receptors results in significant potentiation of P-type, but not N-type, calcium currents in pyramidal neurons from the CA3 region of guinea pig hippocampus. This mechanism was thought to be mediated by adenyl cyclase, because this potentiation could be inhibited by blocking the cAMP-dependent protein kinase (Mogul et al., 1993; fig. 7c).

It has recently been recognized that intracellular signaling of A_{2B} receptors can be modulated by interaction with other receptor systems (Fredholm, 1995; Fredholm et al., 1996a; fig. 8). For example, agents that increase intracellular calcium or activate protein kinase C significantly potentiate A_{2B}-mediated cAMP production in various cells (Hollingsworth et al., 1985; Norstedt and Fredholm, 1987; Fredholm et al., 1987; Norstedt et al., 1989; Kvanta et al., 1989, 1990; Altiok et al., 1992; fig. 8a). On the other hand, bradykinin-stimulated calcium entry caused inhibition of A_{2B} receptor-stimulated adenyl cyclase in astrocytoma D384 cells (fig. 8b), but direct stimulation of protein kinase C enhanced the A_{2B} response (Altiok et al., 1992; Altiok and Fredholm, 1993). The exact mechanism of the interaction between protein kinase C and A_{2B}-mediated pathways is not known, but it cannot be considered a unique feature of A_{2B} receptors. For instance, activation of thrombin-induced phos-

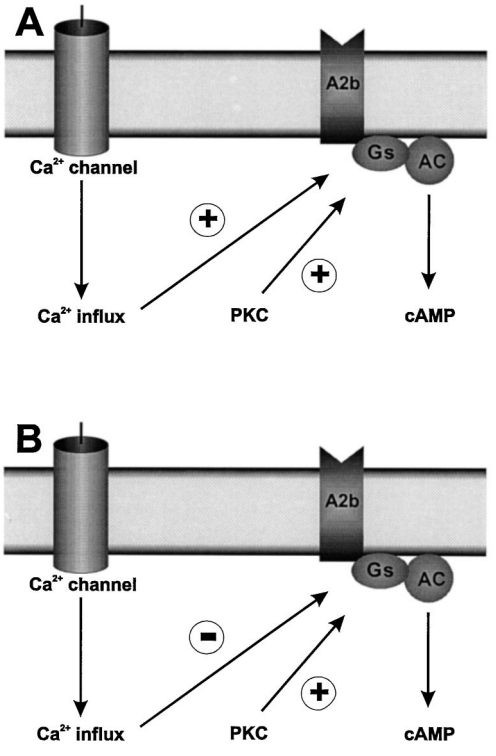

FIG. 8. Modulation of A_{2B} receptor signaling. (A) In Jurkat cells, stimulation of calcium entry and of protein kinase C (PKC) by T-cell receptor activation increases the magnitude of the cAMP response to stimulation of A_{2B} receptors (Kvanta et al., 1989; Fredholm et al., 1996a). (B) In human astrocytoma cells, D384 activation of protein kinase C is also able to enhance A_{2B} receptor-mediated cAMP accumulation, but the stimulation of calcium entry via the bradykinin B_2 receptor leads to inhibition of A_{2B} receptor-mediated cAMP accumulation (Altiok and Fredholm, 1993; Fredholm et al., 1996a).

pholipase C pathways potentiate cAMP accumulation stimulated by IP prostanoid receptors in HEL cells (Turner et al., 1992; Feoktistov et al., 1997). It has been suggested that protein kinase C does not exert its effect at the level of the receptor but rather affects the coupling of the stimulated G_s protein with adenyl cyclase (Fredholm, 1995). The synergistic interaction between the A_{2B} receptors and the calcium/protein kinase C pathway can occur further down in the signaling cascade. Thus, A_{2B} receptors greatly potentiate the phorbol 12-myristate 13-acetate-induced synthesis of IL-8 in human mast cells (Feoktistov and Biaggioni, 1995). In T-lymphocytes, the T-receptor is known to activate immediate early gene transcription, leading to the activation of the AP-1 transcription factor (Kvanta et al., 1992). A_{2B} receptors significantly potentiate this response, implying that cAMP, and calcium/protein kinase C pathways, may act in concert in the regulation of gene transcription (Kontny et al., 1992; Kvanta and Fredholm, 1994).

In summary, A_{2B} receptors are coupled to adenyl cyclase through G_s proteins in every cell studied. Current evidence suggests that the actions of A_{2B} receptors can be mediated not only by cAMP, but also by other intracellular pathways that may vary between cells. A_{2B} receptors can couple to calcium channels through G_s, but additional studies are needed to determine the type of channel involved. Similarly, it remains to be determined which member of the G_q family is responsible for A_{2B} receptor coupling to phospholipase C. It is of interest that, as far as intracellular pathways are concerned, A_{2B} receptors have as much in common with A_1 or A_3 receptors (activation of phospholipase C), as with A_{2A} receptors (activation of adenyl cyclase). It would be important to determine which domain of the A_{2B} receptor defines the differences in G protein coupling between A_{2A} and A_{2B} receptors.

VII. Physiological Functions of A_{2B} Receptors

A. Control of Vascular Tone

Adenosine-induced vasodilation has been traditionally attributed to activation of A_{2A} receptors. However, the recent finding of the presence of A_{2B} receptors in some vascular beds raised the possibility that they participate in the regulation of vascular tone. Indeed, there are vascular beds in which the nonselective agonist NECA produces profound vasodilation, but the selective A_{2A} agonist CGS 21680 has little effect, suggesting that adenosine-induced vasodilation is mediated via A_{2B} receptors (for review, see Webb et al., 1992). This phenomenon is observed in guinea pig aorta and dog saphenous vein (Hargreaves et al., 1991), and in dog coronary arteries (Balwierczak et al., 1991). This effect is not due to species differences, because both A_{2A} and A_{2B} receptors may mediate vasodilation in the same species. In guinea pig, for instance, A_{2A} receptors mediate relaxation of coronary vessels, whereas A_{2B} receptors produce vasodilation of the aorta (Martin, 1992; Martin et al., 1993). Likewise, the A_{2A} agonist CGS 21680 lowers blood pressure in the intact dog (Levens et al., 1991), presumably by inducing vasodilation, despite its lack of efficacy in the coronary arteries of this species.

The vasodilatory effects of adenosine can be accounted for by a direct relaxing action on vascular smooth muscle cells. However, recent studies have suggested that the endothelium contributes to, or is even essential for, the vasodilatory effects of intravascular adenosine. It has been shown that most of the labeled adenosine administered intra-arterially is contained within endothelial cells, and very little escapes this endothelium trap to reach the underlying vascular smooth muscle (Nees et al., 1985). Similarly, intravascular administration of adenosine linked to macromolecules, and therefore less likely to cross the endothelium, is still able to produce vasodilation (Olsson et al., 1977).

In vitro studies, however, have yielded conflicting results as to whether the vasodilatory actions of adenosine are different in vascular preparation with intact or denuded endothelium (Rubanyi and Vanhoutte, 1985; Yen et al., 1988; Falcone et al., 1993; Maekawa et al., 1994).

Evaluation of a putative endothelium-dependent vasodilation by adenosine is challenging in ring preparation, because adenosine will produce vasodilation in preparations with or without endothelium. This is particularly true when stable agonists are used, because they are not trapped by the endothelium in the way adenosine is and have more ready access to the underlying vascular smooth muscle. Other endothelium-dependent vasodilators will constrict vascular smooth muscle in the absence of endothelium (Furchgott, 1984), making their distinction easier. Conversely, adenosine-induced vasodilation could conceivably produce flow-related release of nitric oxide (NO), giving the appearance of NO-mediated vasodilation (Olsson, 1996).

Methodological difficulties notwithstanding, more fundamental differences may explain the apparent discrepancies regarding the role of the endothelium on adenosine-induced vasodilation. Given the diversity of endothelial cell types, it is possible that endothelial vasodilatory responses to adenosine vary between species, and within the same species depending on the vascular bed being studied. It is unclear to what degree endothelial A_{2A} or A_{2B} receptors may contribute to these differences. This issue will be resolved only if studies that examine the endothelium-dependency of adenosine-induced vasodilation also define the adenosine receptor subtype involved.

A_{2B} receptors have been shown in endothelial cells. Both A_{2B} and A_{2A} receptors regulate cAMP production in human aortic (Iwamoto et al., 1994) and human umbilical vein (Feoktistov and Biaggioni, unpublished observations) endothelial cells, and A_{2B} receptor mRNA has been detected in human aortic endothelial cells (Iwamoto et al., 1994). Few studies have directly examined the possible interaction between A_{2B} receptors and endothelium-derived vasodilation, and results vary depending on the vascular bed studied. A_{2B} receptors mediate vasodilation in the rat mesenteric arterial bed (Rubino et al., 1995) and in the isolated blood-perfused rat lung preparation (Haynes et al., 1995). In both cases, A_{2B}-mediated vasodilation seems to be independent of NO generation, because they were not reversed by inhibition of NO synthase by N^G-nitro-L-arginine methyl ester (L-NAME) (Haynes et al., 1995; Rubino et al., 1995). On the contrary, isolated rat renal artery rings contain A_{2B} receptors that are located exclusively on the endothelium and cause NO release and vasodilation, because this vasodilation can be blocked with L-NAME and prevented by removal of the endothelium (Martin and Potts, 1994). Similarly, A_{2B} receptors also appear to vasodilate the rabbit corpus cavernosum, and this effect is reduced by removal of the endothelium (Chiang et al., 1994).

In summary, both A_{2A} and A_{2B} receptors mediate vasodilation. The relative contribution of A_{2B} receptors to adenosine-induced vasodilation is not defined. There are also conflicting results as to the importance of NO generation in adenosine-induced and A_{2B}-induced vasodilation, but there are some examples in which the endothelium contributes to A_{2B}-mediated vasodilation. To complicate things further, in some vascular beds, adenosine-induced vasodilation is endothelium-dependent but does not appear to be mediated by NO because it is not blocked by inhibition of NO synthase, raising the possibility that other endothelial factors, such as endothelium-dependent hyperpolarizing factor, may be involved (Headrick and Berne, 1990). The precise nature of the interaction between A_{2B} receptors and endothelial cells and their role in the regulation of vascular tone are areas where more research is needed.

B. Cardiac Myocyte Contractility

Adenosine has important protective effects against ischemia in the myocardium, but these effects are largely attributed to A_1 receptors (for review, see Olsson and Pearson, 1990). It has been reported recently that myocytes isolated from fetal chick ventricles, but not from the atria, possess functional A_{2B} and A_{2A} receptors. Both receptors are capable of augmenting myocardial contractility in this model. These adenosine effects, however, become evident only after inhibitory A_1 receptor pathways are inactivated with pertussis toxin (Liang and Haltiwanger, 1995). Presence of A_2 receptors, capable of stimulating cAMP accumulation, was demonstrated in cultured adult rodent myocardial cells after A_1 receptor blockade (Romano et al., 1989; Stein et al., 1993; Xu et al., 1996). These results could be explained by a possible contamination of myocardial preparations with fibroblasts and endothelial cells expressing A_{2B} receptors. However, studies performed on single cells argue against this possibility. A positive inotropic response mediated via A_2 receptors was demonstrated in cultured rat and guinea pig ventricular myocytes (Stein et al., 1993; Xu et al., 1996; Dobson and Fenton, 1997). The role of myocardial A_2 receptors in mediating a positive inotropic effect remains a controversial issue (Olsson, 1996), and their physiological significance is unclear, given that their effects become evident only under blockade of A_1 receptors.

C. Modulation of Neurosecretion and Neurotransmission

Adenosine is in general considered to be a depressor of neurons, inhibiting neurotransmitter release and other neuronal functions (Phillis et al., 1993a) and acts as a neuroprotective against ischemia (Dragunow and Faull, 1988). Many of these inhibitory actions are mediated by A_1 receptors (Dunwiddie and Fredholm, 1989). A_2 receptors, on the other hand, have been shown to mediate excitatory actions on the nervous system (Sebastião and Ribeiro, 1996). Earlier studies did not use specific agonists or antagonists to allow a precise identification of the A_2 receptor subtype involved, and relatively little information is available for the A_{2B} receptor. More re-

cently, several excitatory actions have been linked to the A_{2A} receptor, including enhancement of the release of several neurotransmitters, including acetylcholine, the excitatory amino acids glutamate and aspartate, dopamine, and norepinephrine (for review, see Sebastião and Ribeiro, 1996). However, gene knockout mice lacking A_{2A} receptors exhibit aggressive behavior and lack the stimulant effect of caffeine (Ledent et al., 1997), suggesting that A_{2A} receptors normally exert a tonic central depressant action. This is in agreement with several observations indicating a depressant effect of A_{2A} agonists on locomotor activity (Sebastião and Ribeiro, 1996). It should be noted, however, that comparisons between molecular mechanisms of excitation and integrated physiological responses need to be done with care. For example, adenosine depresses sympathetic nerve activity and blood pressure when injected into the nucleus tractus solitarii (Tseng et al., 1988) via activation of A_{2A} receptors (Barraco et al., 1991). This apparent depressant action, however, is mediated by local stimulation of the release of the excitatory amino acid glutamate (Mosqueda-Garcia et al., 1989, 1991), mediated by A_{2A} receptors (Castillo-Melendez et al., 1994).

A_{2B} receptors are widespread in the brain, but little is known about their function. There are, however, several examples of neuroexcitatory actions. Adenosine agonists increase the release of the excitatory amino acid aspartate in rat cerebral cortex cup superfusates in vivo while depressing the release of the inhibitory amino acid GABA (Phillis et al., 1993b). The agonist profile was suggestive of an A_{2B} receptor, in that it was produced by a high concentration of N^6-cyclopentyladenosine (CPA), but not by the A_{2A} agonist CGS 21680. If confirmed, these results would suggest that A_{2B} receptors would lead to greater tissue injury if activated during ischemia, an action that is in sharp contrast to the postulated protective effects of A_1 receptors. In this same model, A_{2B} receptors also enhance basal release of acetylcholine (Phillis et al., 1993a).

Acutely isolated pyramidal neurons from the CA3 region of guinea pig hippocampus contain A_1 receptors that inhibit N-type Ca^{2+} currents. After blockade of A_1 receptors, adenosine agonists potentiate a P-type Ca^{2+} current with a pharmacological profile consistent with A_{2B} receptors, inasmuch as the A_{2A} agonist CGS 21680 had no effect (Mogul et al., 1993). Likewise, after A_1 receptor blockade, A_{2B} receptors induce long-term potentiation in the CA1 region of rat hippocampus (Kessey et al., 1997). Although these studies were not designed to explore the site of action of A_{2B} receptors, the results were consistent with a postsynaptic site of action. It should be noted that the selective A_{2A} agonist CGS 21680 was also found to facilitate long-term potentiation in the hippocampal CA1 area (de Mendonca and Ribeiro, 1994), whereas A_1 receptors inhibit long-term potentiation in this area of the brain (Arai and Lynch, 1992). The relative importance of these contrasting pathways coupled to adenosine receptor subtypes remains to be defined under physiological and pathological conditions. Similarly, A_1 receptors inhibit dopamine release in rat striatum, but selective blockade of A_1 receptors reveals a stimulatory effect of A_{2B} receptors on dopamine release (Okada et al., 1996).

Adenosine also inhibits norepinephrine release from peripheral noradrenergic nerve terminals (Wakade and Wakade, 1978). This effect is thought to be mediated by putative presynaptic A_1 receptors (Paton, 1981), but the inhibition of norepinephrine release in isolated canine pulmonary arteries was better explained by A_{2B} receptors, based on rank order of potencies of agonists (Tamaoki et al., 1997). Similarly, the pharmacological profile for adenosine-induced inhibition of neurotransmission in rabbit corpus cavernosum is consistent with that of an A_{2B} receptor (Chiang et al., 1994). On the other hand, the nonselective A_2 agonist NECA potentiated acetylcholine release evoked by electrical stimulation of the rat bronchial smooth muscle and was 100-fold more potent than the A_{2A} agonist CGS 21680 (Walday and Aas, 1991). Furthermore, this process was blocked by 10 μM enprofylline. The effect of exogenous acetylcholine was not affected by NECA. Taken together, these results provide evidence for an A_{2B} presynaptic receptor that enhances neurally mediated release of acetylcholine and thereby induces contraction of bronchial smooth muscle (Walday and Aas, 1991).

Adenosine also modulates release of catecholamines from chromaffin cells. These adrenal medullary cells are under neural control through cholinergic nicotinic receptors. No specific binding was found using selective ligands for the A_1 or A_{2A} receptors in chromaffin cells from bovine adrenal medulla, but specific binding was obtained using [^{3}H]NECA. These results were interpreted as evidence that only A_{2B} receptors are expressed in these cells (Casado et al., 1992). At high (20 μM) concentrations, the nonselective agonist NECA inhibits catecholamine release induced by nicotinic stimulation, presumably by activation of A_{2B} receptors (Mateo et al., 1995). This effect has an unusually slow time course and is seen only after 20 to 30 minutes of preincubation with NECA, and its physiological relevance is not clear.

D. Cell Growth and Gene Expression

Whereas most studies of the cardiovascular effects of adenosine have focused on its acute actions on vascular tone and adrenergic neurotransmission, recent evidence suggests that adenosine may also play a long-term modulatory role in smooth muscle growth. Exogenous adenosine was shown to inhibit rat aortic smooth muscle cell growth induced by fetal calf serum, as assessed by a decrease in thymidine incorporation and in cell number (Dubey et al., 1996b). These inhibitory effects were reversed by the A_2 receptor antagonist KF 17837, but not by the A_1 antagonist DPCPX, and were not mimicked by the A_{2A} agonist CGS 21680, suggesting that this effect is

mediated by A_{2B} receptors (Dubey et al., 1996b). Activation of adenyl cyclase is postulated as the signaling pathway involved, because this effect is mimicked by 8-bromo-cAMP. These investigators later showed that stimulation of these vascular smooth muscle cells by fetal calf serum can trigger release of endogenous adenosine, which then acts in an autocrine fashion to inhibit growth. The importance of endogenous adenosine is less certain, because inhibition of vascular smooth muscle growth by endogenous adenosine is evidenced only if adenosine deaminase is inhibited (Dubey et al., 1996a). It is postulated that this reflects a limitation of the experimental model, due to the presence of adenosine deaminase in the fetal calf serum used to stimulate vascular smooth muscle growth. If a role for endogenous adenosine is confirmed, this finding would establish a novel cardioprotective effect of adenosine, with relevance to vascular remodeling process observed in hypertension and atherosclerosis.

A_{2B} receptors can also modulate gene expression, in some cases leading to inhibition of protein synthesis. For example, stimulation of A_{2B} receptors decreases collagenase gene expression in interleukin-1–stimulated cultured fibroblast-like synoviocytes, an effect apparently mediated by cAMP elevation (Boyle et al., 1996). In contrast, A_{2B} receptors promoted the synthesis of IL-8 in HMC-1 mast cells by a cAMP-independent mechanism (Feoktistov and Biaggioni, 1995). It has been recently demonstrated that A_{2B} receptors can also induce an increase in interleukin-6 mRNA levels and protein synthesis in the human astrocytoma cell line U373 MG (Fiebich et al., 1996a). The synergistic relationship between A_{2B} receptors and T-receptors, and generally between cAMP and protein kinase C pathways in gene expression, has been discussed in detail elsewhere (Fredholm, 1995; Fredholm et al., 1996a).

E. Regulation of Intestinal Tone and Secretion

The high levels of A_{2B} receptor expression found in different parts of the intestinal tract motivated great interest in defining their function. In some studies, A_{2B} receptor mRNA expression is greatest in intestinal tissue among all organs (Stehle et al., 1992). It appears that A_{2B} receptors may be involved in modulation of intestinal tone as well as intestinal secretion. Adenosine elicits relaxation of dispersed guinea pig longitudinal muscle cells from small intestine via A_{2B} receptors coupled to adenyl cyclase but produced contraction through A_1 receptors by increasing intracellular calcium (Murthy et al., 1995). The A_{2B}-mediated relaxation was evident only after A_1 receptor blockade, raising doubts as to their importance. However, blockade of A_2 receptors potentiated A_1-mediated contraction, indicating that A_{2B} receptors do provide a restraining function against intestinal contraction. In rat duodenum, A_{2B} receptors cause relaxation of longitudinal muscle but contraction of muscularis mucosae (Nicholls et al., 1996). This is an unexpected result and the first example of an excitatory response by A_{2B} receptors in a smooth muscle preparation. This tissue is also unusual in that A_1 receptors were found to produce relaxation of duodenal longitudinal muscle (Nicholls et al., 1996). A_{2B} receptors have also been shown to relax guinea pig taenia ceci, based on the pharmacological profile of agonists (Prentice and Hourani, 1997). The functional relevance of the intestinal relaxant actions of A_{2B} receptors has not been defined. Of interest, adenosine A_{2B} receptors also mediate relaxation of other visceral smooth muscle such as rat urinary bladder (Nicholls et al., 1996) and rat vas deferens (Hourani et al., 1993).

The effect of A_{2B} receptors on epithelial secretion has received particular attention because of its potential relevance to diarrheal processes. As part of the pathophysiology of these disorders, neutrophils are recruited into intestinal crypts, where they release a soluble "neutrophil-derived secretagogue" that then activates intestinal epithelium to stimulate chloride secretion. This chloride secretion has the net effect of producing isotonic fluid secretion, an important component of diarrheal diseases. This neutrophil-derived secretagogue has recently been identified as AMP (Madara et al., 1993), which is then converted to adenosine at the epithelial cell surface by ecto-5′-nucleotidase. It is adenosine that then acts as a paracrine mediator of chloride secretion (Madara et al., 1993). It was later demonstrated that neutrophil-derived adenosine elicits chloride secretion in the intestinal epithelial cell line T84 via activation of A_{2B} receptors (Strohmeier et al., 1995), implying the possible involvement of this receptor subtype in the pathophysiology of diarrheal diseases. In contrast to these findings, A_{2B} receptors reportedly inhibit intestinal fluid secretion induced by vasoactive intestinal peptide in rat jejunum (Hancock and Coupar, 1995). These results should be interpreted with caution, because receptor characterization was done by relative potency of agonists and antagonists injected intravenously in anesthetized rats. Definite receptor characterization cannot be completed until the tissue localization of these putative A_{2B} receptors is determined and in vitro studies are performed (Hancock and Coupar, 1995). The presence of A_{2B} receptors in epithelial cells of human intestine has been demonstrated by immunohistochemistry with an anti-A_{2B} receptor antibody (Puffinbarger et al., 1995), but more studies are needed to define their role in intestinal secretion and diarrheal processes.

F. Adenosine and Asthma

Adenosine has been implicated in the pathophysiology of asthma (for review, see Church and Holgate, 1986; Feoktistov and Biaggioni, 1996), and several lines of evidence support this hypothesis. Inhaled adenosine, or its precursor AMP, provokes bronchoconstriction in asthmatic patients (Cushley et al., 1984). This effect is fairly specific for patients with asthma, and even high

concentrations of inhaled adenosine fail to produce bronchoconstriction in the majority of normal subjects. Atopic subjects appear to be more responsive to inhaled AMP than they are to methacholine (Phillips et al., 1990), suggesting that adenosine may be a better discriminator of the disease. This preferential bronchoconstrictor effect in asthmatics is also observed with intravenous administration of adenosine (Drake et al., 1994) and in isolated human bronchi (Björck et al., 1992). Dipyridamole, a drug that blocks adenosine uptake and increases its extracellular concentrations, can also produce severe bronchospasm in asthmatic patients (Eagle and Boucher, 1989). Moreover, theophylline provides a better protection against adenosine-induced bronchoconstriction than against histamine-induced bronchoconstriction (Mann and Holgate, 1985).

The mechanism by which adenosine produces bronchoconstriction has been the focus of recent interest. In particular, it would be important to define the receptor type involved. Adenosine produces a direct constrictor action on isolated guinea pig trachea with an agonist profile consistent with A_1 receptors (Ghai et al., 1987), and this process is not blocked by enprofylline (Farmer et al., 1988). In this same preparation, however, A_2 receptors were found to produce relaxation, but the receptor subtype was not identified. Based on rank order of potencies for agonists, it was found that A_1 receptors also mediate bronchoconstriction in an allergic rabbit model in vivo (Ali et al., 1994a,b), and treatment with antisense oligodeoxynucleotide targeting the adenosine A_1 receptor desensitized the allergic rabbits to subsequent challenge with either adenosine or allergen (Nyce and Metzger, 1997). Adenosine also constricts human bronchi isolated from asthmatics in vitro but not bronchi isolated from nonasthmatics (Björck et al., 1992). The contractile effect of adenosine was inhibited with 2-thio-[(1,3-dipropyl)-8-cyclopentyl]-xanthine, and this was taken as evidence of an A_1-mediated effect.

The bronchoconstriction produced by inhaled adenosine in humans appears to be mediated through mast cell activation, because it can be blocked by specific antihistamines (Phillips et al., 1987; Rafferty et al., 1987) and prevented by cromoglycate and nedocromil sodium, drugs that inhibit mast cell degranulation (Phillips et al., 1989). Furthermore, a significant rise in plasma levels of histamine is detected after AMP challenge (Phillips et al., 1990). More recently, inhaled adenosine has been shown to increase levels of histamine, PGD_2, and tryptase in bronchoalveolar lavage fluid from asthmatics but not from nonasthmatics (Polosa et al., 1995). Tryptase is a highly specific marker for mast cells (Schwartz, 1990) and provides strong evidence that these cells are activated by adenosine in vivo.

In summary, exogenous adenosine provokes asthma, potentiation of endogenous adenosine with dipyridamole also produces bronchoconstriction, and blockade of endogenous adenosine with theophylline is helpful in preventing asthma. The bronchoconstriction induced by inhaled adenosine is unique to asthmatics and not observed in nonasthmatics. Current evidence suggests that this phenomenon involves mast cell activation. It is important, therefore, to elucidate the adenosine receptor subtype that mediates this phenomenon.

G. Adenosine Receptors and Mast Cells

Marquardt et al. (1978) were the first to report that adenosine, although ineffective alone, potentiated histamine release induced by anti-immunoglobulin E (IgE), concanavalin A, compound 48/80, or the calcium ionophore A23187 in isolated rat mast cells. The mechanisms that mediate potentiation of these cells remain unclear. Stimulation of adenyl cyclase by adenosine was blocked by methylxanthines, but potentiation of histamine release was not, suggesting that these effects were mediated by different adenosine receptors (Church et al., 1986).

Because potentiation of rat peritoneal mast cells is insensitive to methylxanthines, the possibility was raised that this effect is mediated by A_3 receptors, because the rat A_3 receptor has remarkably low affinity for methylxanthines (Zhou et al., 1992). This possibility was examined in the rat basophilic leukemia cell line RBL 2H3, which has been used as a model for rat mast cells. Adenosine analogs stimulated phospholipase C, increased cytoplasmic calcium, and potentiated mediator release in these cells with a pharmacological profile consistent with A_3 receptors (Ramkumar et al., 1993). Expression of A_3 receptors in RBL 2H3 cells was confirmed by radioligand binding and detection of mRNA (Ramkumar et al., 1993). The effects mediated by A_3 receptors in RBL 2H3 were blocked by pertussis toxin, suggesting a role of G_i-derived $\beta\gamma$ subunits in the activation of β-phospholipase C. Coupling of A_3 receptors to G_{i2} and G_{i3} proteins was recently reported (Palmer and Stiles, 1995). Of interest, A_{2A} and A_{2B}, but not A_1 receptors, have also been found in RBL 2H3 cells (Marquardt et al., 1994; Ramkumar et al., 1995); however, their function has not been elucidated. It should also be noted that A_1 receptors, to our knowledge, have not been found in other mast cell types (Marquardt et al., 1994).

It is important to consider that mast cells from different species, and even from different anatomical sites within the same species, can vary substantially in their morphological and biochemical characteristics and their response to pharmacological agents. There is increasing evidence that A_{2B} receptors modulate mast cell function. Adenosine activates adenyl cyclase and protein kinase C and potentiates mediator release in mouse bone marrow-derived mast cells (Marquardt and Walker, 1990). It appears that the ability of adenosine to activate protein kinase C and thereby to augment mast cell degranulation are independent of changes in cAMP (Marquardt and Walker, 1994). Both A_{2A} and A_{2B} transcripts were detected in mouse bone marrow-derived mast cells (Mar-

quardt et al., 1994). The failure of the A_{2A}-specific agonist CGS 21680 to enhance mediator release suggests that A_{2B} receptors modulate degranulation of these mast cells (Marquardt et al., 1994).

A_{2B} receptors have been shown to activate the human mast cell line HMC-1 (Feoktistov and Biaggioni, 1995). HMC-1 cells were derived from a patient with mast cell leukemia and their neutral proteases content is similar to that of human lung mast cells. These cells coexpress A_{2A} and A_{2B} receptors, and both are coupled to adenyl cyclase through G_s proteins. However, only A_{2B} receptors activate HMC-1 cells, as indicated by stimulation of IL-8 secretion. Furthermore, this effect was not mediated by cAMP, but by coupling to phospholipase C through a cholera toxin- and pertussis toxin-insensitive G protein, presumably of the G_q family (Feoktistov and Biaggioni, 1995). A_{2B} receptors not only produced direct stimulation of HMC-1 cells, but also potentiated phorbol 12-myristate 13-acetate-stimulated secretion of IL-8 (Feoktistov and Biaggioni, 1995). The expression of A_{2B} receptors in HMC-1 cells was recently confirmed by immunoblotting and fluorescent immunostaining with a specific anti-A_{2B} antibody (Feoktistov et al., 1996). Virtually identical findings have been reported in a canine BR mastocytoma cell line. Stimulation of A_{2B}, but not A_3 receptors, directly increased β-hexosaminidase release and also potentiated A23187-induced degranulation of mast cells. Also, these effects were not blocked by pertussis toxin (Auchampach et al., 1996).

In parenchymal human lung mast cells, obtained from normal sections of surgical specimens, adenosine does not directly evoke release of histamine and LTC_4, but in micromolar concentrations it potentiates mediator release from immunologically activated cells (Peachell et al., 1991). The order of potency of adenosine analogs and the low affinity of this process suggests that the response of human lung mast cells to adenosine is mediated by A_{2B} receptors. In support for this notion, the presence of A_{2B} has been recently demonstrated in bronchoalveolar lavage mast cells by double immunostaining with specific anti-A_{2B} and antitryptase antibodies (Feoktistov et al., 1996).

Given that inhaled adenosine affects only asthmatics but has no effect in nonasthmatics, there appears to be an intrinsic difference in the way adenosine interacts with mast cells from asthmatics. The in vitro response produced by A_{2B} receptors in HMC-1 cells and in canine BR mastocytoma cells appears to mimic the in vivo responses to inhaled adenosine in asthmatics, inasmuch as adenosine provokes mast cells activation in these cell lines as it does in asthmatics. On the other hand, the in vitro response of mast cells from normal human lung to adenosine resembles the effect of A_{2B} receptors in mouse bone marrow-derived mast cells, because in both cases adenosine potentiates mast cells activation but does not evoke direct activation. The molecular mechanisms behind these differential A_{2B}-mediated responses in asthmatic versus normal mast cells, and in HMC-1 cells versus mouse bone marrow-derived mast cells, remain unknown.

Despite the direct bronchoconstricting action of A_1 receptors observed in allergic rabbits (Nyce and Metzger, 1997), the bronchoconstriction induced by inhaled adenosine in humans is better explained by an A_{2B} receptor. Adenosine-induced bronchoconstriction appears to be mediated by mast cell activation, and A_1 receptors have not been described in mast cells, whereas A_{2B} receptors are expressed in human mast cells. Moreover, enprofylline is an effective antiasthmatic and is a selective A_{2B} blocker at concentrations achieved clinically. Finally, A_{2B} receptors have been shown to potentiate neurally mediated cholinergic bronchoconstriction through an enprofylline-sensitive process (Walday and Aas, 1991). The evidence presented so far does not preclude the contribution of more than one adenosine receptor in asthma, or the possibility that nonadenosinergic mechanisms contribute to the antiasthmatic effects of enprofylline and other methylxanthines.

VIII. A_{2B} Receptors as Therapeutic Targets

The ability of adenosine to delay atrioventricular node conduction was the basis for its development as a therapeutic agent in the treatment of supraventricular arrhythmias (Belardinelli et al., 1989) and has become the drug of choice for the termination of that arrhythmia. Taking advantage of its profound vasodilatory effects, intravenous adenosine is used as a stress test in the diagnosis of myocardial ischemia (Verani et al., 1990). Adenosine was investigated as a hypotensive agent during anesthesia (Sollevi et al., 1984), where the reflex sympathetic activation induced by this agent (Biaggioni, 1992) is not observed. However, adenosine has been implicated in many other physiological and pathological processes. The biggest problem in translating this knowledge into therapeutic tools is perhaps the ubiquity of adenosine receptors, which often mediate contrasting effects. The challenge is how to develop drugs that will selectively target a receptor mediating a specific action. The ongoing development of selective agonists or antagonist represents a substantial advancement toward this goal. Nonetheless, even if specific agents can be developed for a given receptor subtype, the problem remains of selectively targeting the site of action. For example, A_1-selective agonists could be developed for their antilipolytic potential. If given systemically, however, it is possible that atrioventricular conduction delay or bradycardia may be an undesirable, and perhaps limiting effect, given that these actions are also mediated by A_1 receptors. In the development of useful therapeutic agents, therefore, care should be taken not only in the targeting of the receptor subtype, but also the site of action. This problem, of course, is not limited to adenosinergic systems and is common to others characterized by the widespread nature of their receptors.

Given that the functional role of A_{2B} receptors is only now being addressed, a discussion of potential therapeutic opportunities arising from modulation of such receptors is necessarily speculative. There are, however, some promising areas that deserve further attention. The potential role of A_{2B} receptors in asthma can be used as an example. If confirmed, this mechanism would provide a novel approach for the treatment of this condition. Asthma continues to be a substantial medical problem that affects approximately 5 to 7% of the population. Despite advances in its treatment, the prevalence of asthma, emergency department visits, hospitalizations, and mortality related to the disease all appear to be on the rise (Gergen et al., 1988; Vollmer et al., 1992; Weiss et al., 1993). Theophylline continues to be an effective treatment in the prevention of asthma attacks, more than as an acute bronchodilator (Weinberger and Hendeles, 1996), but considerable plasma levels, in the range of 20–80 μM, are needed for it to be effective. Moreover, it has many side effects, which can be attributed to its nonspecificity. Its central actions contribute to theophylline's side-effect profile and are of doubtful benefit for the treatment of asthma.

If indeed blockade of A_{2B} receptors contributes to the antiasthmatic effects of theophylline, it would be possible to develop selective antagonists for this receptor subtype. Lipophobic compounds would have the advantage of not crossing the blood-brain barrier. Specific targeting to the site of action can also be accomplished if compounds that can be administered by inhalation are developed. This proposition is not unrealistic. For example, the xanthine antagonist DPSPX is approximately 100-fold more potent than theophylline as an A_{2B} receptor antagonist. Because of a charge moiety in its molecule, this water-soluble xanthine does not penetrate cell membranes or cross the blood-brain barrier (Tofovic et al., 1991). It appears that the ionic p-sulfophenyl substituent in DPSPX may confer high A_{2B} potency. The lack of L-alkyl substituents in the enprofylline molecule renders it an ineffective antagonist of other adenosine receptor subtypes. The systematic study of the structure-activity relationship for blockade of A_{2B} receptors, considering the above-mentioned properties, could result in more potent and specific agents. Similarly, A_{2B} receptor antagonists can be developed for the treatment of diarrheal processes, if adenosine is confirmed to play a role in this process. Targeting to the site of action could be achieved with compounds that are poorly absorbed, as long as they are able to reach the intestinal crypts involved in intestinal inflammatory processes.

Development of agonists to target A_{2B} receptors, for example, to inhibit vascular smooth muscle growth, would be a greater challenge. Substantial progress would need to be made to develop an agonist potent enough to selectively activate the low-affinity A_{2B} receptor while having negligible actions at other receptors. The actions of endogenous adenosine could be enhanced with uptake inhibitors, or adenosine-regulating agents (Mullane, 1993), but this approach would activate all adenosine receptors, perhaps others even more than A_{2B} receptors. Targeting the site of action is an additional problem that would have to be resolved for any drug that had to be administered systemically. The observation that A_{2B} receptors mediate vasodilation of the rabbit corpus cavernosum (Chiang et al., 1994) raises the possibility that an agonist to this receptor type can be useful in impotence. Direct injections of adenosine into the corpus cavernosum of impotence patients produce a brief erection (Kilic et al., 1994), particularly if combined with prostaglandin E_1 (Chiang et al., 1994). The short duration of effect is clearly related to the short half-life of adenosine in humans (Moser et al., 1989). If this effect is also mediated by A_{2B} receptors in humans, it will be possible to develop stable and selective agonists that can be given locally.

IX. Concluding Remarks

Until recently, relatively little attention has been paid to A_{2B} receptors. It is instructive that contemporary reviews about adenosine only briefly mention their existence. Conclusions about the selective nature of agonists or antagonists at specific adenosine receptors have often been made without testing these compounds on A_{2B} receptors. The A_{2B} receptor was often assumed to be simply a low-affinity variant of the A_{2A} receptor. A_{2A} and A_{2B} receptors are frequently found in the same tissue, and, because both were thought to act by adenyl cyclase activation, it is easy to assume that A_{2B} receptors will be of lesser physiological significance. The lack of selective pharmacological probes with which to study this receptor subtype has been the main obstacle in defining the role of A_{2B} receptors.

Several factors can explain the increasing interest in A_{2B} receptors. The cloning of adenosine receptors validated the belief that A_{2B} receptors were distinct from A_{2A} receptors. It also revealed its widespread and distinct distribution in tissues. The development of selective agonists and antagonists for other adenosine receptor types has indirectly improved our knowledge of A_{2B} receptors, but their pharmacological characterization is still mostly done by a method of exclusion, i.e., by the lack of efficacy of agonists and antagonists that are selective at other receptors. Also, other tools now are available for the study of this receptor. The amino acid sequence of the receptor, the nucleotide sequence encoding the receptor, and genomic information are now available. This opens the door for mutational or chimeric analysis of A_{2B} receptors to understand the molecular determinants for its unique coupling to G proteins. It is also possible to determine with precision the cellular localization of A_{2B} receptors, particularly now that antibodies against this receptor have been generated.

Significant progress has been made using currently available tools. It is now clear that, in addition to adenyl

cyclase, A_{2B} receptors can also couple to other intracellular pathways, including calcium channels and phospholipase C. In that sense, A_{2B} receptors have as much in common with A_1 and A_3 receptors, as with A_{2A} receptors. The functional relevance of A_{2B} receptors is being defined, but much work is needed in this area. Given the ubiquitous nature of adenosine receptors, it is not surprising that more than one adenosine subtype is found in the same tissue, but it is now evident that they can be coexpressed in the same cell. In these situations, it would seem that the functional role of higher-affinity receptors would predominate over A_{2B} receptors. Nonetheless, A_{2B} receptors can play a role under these conditions by modulating events triggered by other receptor systems. Examples have been presented in this review of A_{2B} receptors restraining the actions of A_1 receptors, or potentiating the effects of thrombin and T-cell receptors. The relative importance of A_{2B} receptors may be greater in situations characterized by substantial increases in interstitial levels of adenosine, as occurs during ischemia in metabolically active tissues. On the other hand, there are cellular systems in which the actions of A_{2B} receptors appear to predominate. Such is the case, for example, of human mast cells, epithelial intestinal cells, and the regulation of vascular smooth muscle growth, among others.

Greater advances can now be made by the simple appreciation of the unique features of this receptor type. In the past, investigators often have failed to realize the potential involvement of A_{2B} receptors in their experimental systems. However, the study of A_{2B} receptors will be considerably improved by the introduction of specific pharmacological probes for this receptor type. Even though adenosine analogs have, in general, a poor affinity for A_{2B} receptors, this is not the case for antagonists. Therefore, development of potent and selective A_{2B} antagonists appears particularly promising and likely will further our knowledge of A_{2B} receptors. Specific antagonists will be particularly useful in defining the role of A_{2B} receptors in physiological and pathological situations. The appreciation of the potential role of A_{2B} receptors in the pathogenesis of disease processes, including asthma, vascular remodeling, and diarrhea, raises the possibility that A_{2B} receptors may become the target for future drug development.

Acknowledgments. The authors would like to thank Drs. Jack N. Wells and Lee Limbird, Department of Pharmacology, Vanderbilt University, for helpful discussions in the preparation of this manuscript. The authors would also like to thank the reviewers for their constructive suggestions.

REFERENCES

AATSINKI, J. T., PIETILA, E. M., LAKKAKORPI, J. T., AND RAJANIEMI, H. J.: Expression of the LH/CG receptor gene in rat ovarian tissue is regulated by an extensive alternative splicing of the primary transcript. Mol. Cell. Endocrinol. **84:** 127–135, 1992.

ALEXANDER, S. P. H., COOPER, J., SHINE, J., AND HILL, S. J.: Characterization of the human brain putative A_{2B} receptor expressed in Chinese hamster ovary (CHO) A_{2B4} cells. Br. J. Pharmacol. **119:** 1286–1290, 1996.

ALI, S., MUSTAFA, S. J., AND METZGER, W. J.: Adenosine-induced bronchoconstriction and contraction of airway smooth muscle from allergic rabbits with late-phase airway obstruction: evidence for an inducible adenosine A1 receptor. J. Pharmacol. Exp. Ther. **94:** 1328–1334, 1994a.

ALI, S., MUSTAFA, S. J., AND METZGER, W. J.: Adenosine receptor-mediated bronchoconstriction and bronchial hyperresponsiveness in allergic rabbit model. Am. J. Physiol. **94:** L271–L277, 1994b.

ALTIOK, N., BALMFORTH, A. J., AND FREDHOLM, B. B.: Adenosine receptor-induced cAMP changes in D384 astrocytoma cells and the effect of bradykinin thereon. Acta Physiol. Scand. **144:** 55–63, 1992.

ALTIOK, N., AND FREDHOLM, B. B.: Bradykinin inhibits cyclic AMP accumulation in D384 human astrocytoma cells via a calcium-dependent inhibition of adenylyl cyclase. Cell. Signalling **5:** 279–288, 1993.

AMATRUDA, T. T., III, STEELE, T. D., SLEPAK, V. Z., AND SIMON, M. I.: $G\alpha_{15}$, a G protein alpha subunit specifically expressed in hematopoietic cells. Proc. Natl. Acad. Sci. USA **88:** 5587–5591, 1991.

ARAI, A., AND LYNCH, G.: Factors regulating the magnitude of long-term potentiation induced by theta pattern stimulation. Brain Res. **598:** 173–184, 1992.

AUCHAMPACH, J. A., CAUGHEY, G. H., AND LINDEN, J.: A_{2B} and not A_3 adenosine receptors mediate degranulation of canine BR mastocytoma cells. (Abstract) Drug Dev. Res. **37:** 147, 1996.

BALWIERCZAK, J. L., SHARIF, R., KRULAN, C. M., FIELD, F. P., WEISS, G. B., AND MILLER, M. J.: Comparative effects of a selective adenosine A2 receptor agonist, CGS 21680, and nitroprusside in vascular smooth muscle. Eur. J. Pharmacol. **196:** 117–123, 1991.

BARRACO, R. A., EL-RIDI, M. R., ERGENE, E., AND PHILLIS, J. W.: Adenosine receptor subtypes in the brainstem mediate distinct cardiovascular response patterns. Brain Res. Bull. **26:** 59–84, 1991.

BELARDINELLI, L., LINDEN, J., AND BERNE, R. M.: The cardiac effects of adenosine. Prog. Cardiovasc. Dis. **32:** 73–97, 1989.

BELARDINELLI, L., SHRYOCK, J. C., RUBLE, J., MONOPOLI, A., DIONISOTTI, S., ONGINI, E., DENNIS, D. M., AND BAKER, S. P.: Binding of the novel nonxanthine A_{2A} adenosine receptor antagonist [^{3}H]SCH 58261 to coronary artery membranes. Circ. Res. **79:** 1153–1160, 1996.

BIAGGIONI, I.: Contrasting excitatory and inhibitory effects of adenosine in blood pressure regulation. Hypertension **20:** 457–465, 1992.

BJÖRCK, T., GUSTAFSSON, L. E., AND DAHLÉN, S. E.: Isolated bronchi from asthmatics are hyperresponsive to adenosine, which apparently acts indirectly by liberation of leukotrienes and histamine. Am. Rev. Respir. Dis. **145:** 1087–1091, 1992.

BLAZYNSKI, C.: Characterization of adenosine A_2 receptors in bovine retinal pigment epithelial membranes. Exp. Eye Res. **56:** 595–599, 1993.

BOYLE, D. L., SAJJADI, F. G., AND FIRESTEIN, G. S.: Inhibition of synoviocyte collagenase gene expression by adenosine receptor stimulation. Arthritis Rheum. **39:** 923–930, 1996.

BRACKETT, L. E., AND DALY, J. W.: Functional characterization of the A_{2b} adenosine receptor in NIH 3T3 fibroblasts. Biochem. Pharmacol. **47:** 801–814, 1994.

BRUNS, R. F.: Adenosine antagonism by purines, pteridines and benzopteridines in human fibroblasts. Biochem. Pharmacol. **30:** 325–333, 1981.

BRUNS, R. F., FERGUS, J. H., BADGER, E. W., BRISTOL, J. A., SANTAY, L. A., HARTMAN, J. D., HAYS, S. J., AND HUANG, C. C.: Binding of the A_1-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. Naunyn-Schmiedeberg's Arch. Pharmacol. **335:** 59–63, 1987.

BRUNS, R. F., LU, G. H., AND PUGSLEY, T. A.: Characterization of the A_2 adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes. Mol. Pharmacol. **29:** 331–346, 1986.

BURNSTOCK, G.: A basis for distinguishing two types of purinergic receptors. *In* Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach, ed. by R. W. Straub and L. Bolis, pp. 107–118, Raven Press, New York, 1978.

CASADO, V., CASILLAS, T., MALLOL, J., CANELA, E. I., LLUIS, C., AND FRANCO, R.: The adenosine receptors present on the plasma membrane of chromaffin cells are of the A_{2b} subtype. J. Neurochem. **59:** 425–431, 1992.

CASTILLO-MELENDEZ, M., KRSTEW, E., LAWRENCE, A. J., AND JARROTT, B.: Presynaptic adenosine A_{2a} receptors on soma and central terminals of rat vagal afferent neurons. Brain Res. **94:** 137–144, 1994.

CHERN, Y., LAI, H. L., FONG, J. C., AND LIANG, Y.: Multiple mechanisms for desensitization of A2a adenosine receptor-mediated cAMP elevation in rat pheochromocytoma PC12 cells. Mol. Pharmacol. **44:** 950–958, 1993.

CHIANG, P. H., WU, S. N., TSAI, E. M., WU, C. C., SHEN, M. R., AND HUANG, C. H.: Adenosine modulation of neurotransmission in penile erection. Br. J. Clin. Pharmacol. **38:** 357–362, 1994.

CHURCH, M. K., AND HOLGATE, S. T.: Adenosine and asthma. Trends Pharmacol. Sci. **7:** 49–50, 1986.

CHURCH, M. K., HUGHES, P. J., AND VARDEY, C. J.: Studies on the receptor mediating cyclic AMP-independent enhancement by adenosine by IgE-dependent mediator release from rat mast cells. Br. J. Pharmacol. **87:** 233–242, 1986.

CUSHLEY, M. J., TATTERSFIELD, A. E., AND HOLGATE, S. T.: Adenosine-induced bronchoconstriction in asthma. Am. Rev. Respir. Dis. **129:** 380–384, 1984.

DALY, J. W.: The nature of receptors regulating the formation of cyclic AMP in brain tissue. Life Sci. **18:** 1349–1358, 1976.

DALY, J. W., BUTTS-LAMB, P., AND PADGETT, W.: Subclasses of adenosine

receptors in the central nervous system: interaction with caffeine and related methylxanthines. Cell. Mol. Neurobiol. **3:** 69–80, 1983.
DALZIEL, H. H., AND WESTFALL, D. P.: Receptors for adenine nucleotides and nucleosides: subclassification, distribution, and molecular characterization. Pharmacol. Rev. **46:** 449–466, 1994.
DAVAL, J.-L., NICOLAS, F., AND DORIAT, J.-F.: Adenosine physiology and pharmacology: how about A_2 receptors? Pharmacol. Ther. **71:** 325–335, 1996.
DELL, K. R., AND WILLIAMS, L. T.: A novel form of fibroblast growth factor receptor 2: alternative splicing of the third immunoglobulin-like domain confers ligand binding specificity. J. Biol. Chem. **267:** 21225–21229, 1992.
DE MENDONCA, A., AND RIBEIRO, J. A.: Endogenous adenosine modulates long-term potentiation in the hippocampus. Neuroscience **62:** 385–390, 1994.
DIONISOTTI, S., FERRARA, S., MOLTA, C., ZOCCHI, C., AND ONGINI, E.: Labeling of A_{2A} adenosine receptors in human platelets by use of the new nonxanthine antagonist radioligand [^{3}H]SCH 58261. J. Pharmacol. Exp. Ther. **278:** 1209–1214, 1996.
DIXON, K. D., GUBITZ, A. K., SIRINATHSINGHJI, D. J. S., RICHARDSON, P. J., AND FREEMAN, T. C.: Tissue distribution of adenosine receptor mRNAs in the rat. Br. J. Pharmacol. **118:** 1461–1468, 1996.
DOBSON, J. G., JR., AND FENTON, R. A.: Adenosine A_2 receptor function in rat ventricular myocytes. Cardiovasc. Res. **34:** 337–347, 1997.
DRAGUNOW, M., AND FAULL, R. L. M.: Neuroprotective effects of adenosine. Trends Pharmacol. Sci. **9:** 193–194, 1988.
DRAKE, I., ROUTLEDGE, P. A., AND RICHARDS, R.: Bronchospasm induced by intravenous adenosine. Hum. & Exp. Toxicol. **13:** 263–265, 1994.
DRURY, A. W., AND SZENT-GYÖRGYI, A.: The physiological activity of adenosine compounds with a special reference to their action upon the mammalian heart. J. Physiol. **68:** 213–237, 1929.
DUBEY, R. K., GILLESPIE, D. G., MI, Z., SUZUKI, F., AND JACKSON, E. K.: Smooth muscle cell-derived adenosine inhibits cell growth. Hypertension **27:** 766–773, 1996a.
DUBEY, R. K., GILLESPIE, D. G., OSAKA, K., SUZUKI, F., AND JACKSON, E. K.: Adenosine inhibits growth of rat aortic smooth muscle cells: possible role of A_{2b} receptor. Hypertension **27:** 786–793, 1996b.
DUNWIDDIE, T. V., AND FREDHOLM, B. B.: Adenosine A_1 receptors inhibit adenylate cyclase activity and neurotransmitter release and hyperpolarize pyramidal neurons in rat hippocampus. J. Pharmacol. Exp. Ther. **249:** 31–37, 1989.
EAGLE, K. A., AND BOUCHER, C. A.: Intravenous dipyridamole infusion causes severe bronchospasm in asthmatic patients. (Editorial) Chest **95:** 258–289, 1989.
ELFMAN, L., LINDGREN, E., WALUM, E., AND FREDHOLM, B. B.: Adenosine analogues stimulate cyclic AMP-accumulation in cultured neuroblastoma and glioma cells. Acta Pharmacol. Toxicol. **55:** 297–302, 1984.
FALCONE, J. C., KUO, L., AND MEININGER, G. A.: Endothelial cell calcium increase during flow-induced dilation in isolated arterioles. Am. J. Physiol. **264:** H653–H659, 1993.
FARMER, S. G., CANNING, B. J., AND WILKINS, D. E.: Adenosine receptor-mediated contraction and relaxation of guinea-pig isolated tracheal smooth muscle: effects of adenosine antagonists. Br. J. Pharmacol. **95:** 371–378, 1988.
FEOKTISTOV, I., AND BIAGGIONI, I.: Characterization of adenosine receptors in human erythroleukemia cells: further evidence for heterogeneity of adenosine A_2 receptors. Mol. Pharmacol. **43:** 909–914, 1993.
FEOKTISTOV, I., AND BIAGGIONI, I.: Adenosine A_{2b} receptors evoke interleukin-8 secretion in human mast cells: an enprofylline-sensitive mechanism with implications for asthma. J. Clin. Invest. **96:** 1979–1986, 1995.
FEOKTISTOV, I., AND BIAGGIONI, I.: Role of adenosine in asthma. Drug Dev. Res. **39:** 333–336, 1996.
FEOKTISTOV, I., AND BIAGGIONI, I.: Pharmacological characterization of adenosine A_{2B} receptors: studies in human mast cells co-expressing A_{2A} and A_{2B} adenosine receptor subtypes. Biochem. Pharmacol., in press, 1997.
FEOKTISTOV, I., BREYER, R. M., AND BIAGGIONI, I.: Prostanoid receptor with a novel pharmacolgical profile in human erythroleukemia cells. Biochem. Pharmacol. 54: 917–926, 1997.
FEOKTISTOV, I., MURRAY, J. J., AND BIAGGIONI, I.: Positive modulation of intracellular Ca^{2+} by adenosine A_{2b}, prostacyclin and prostaglandin E_1 receptors via a cholera toxin-sensitive mechanism in human erythroleukemia cells. Mol. Pharmacol. **45:** 1160–1167, 1994.
FEOKTISTOV, I., SHELLER, J. R., AND BIAGGIONI, I.: Adenosine A_{2b} receptors in human lung mast cells as a target for antiasthmatic methylxanthines. (Abstract) FASEB J. **10:** A1232, 1996.
FIEBICH, B. L., BIBER, K., GUYFKO, K., BERGER, M., BAUER, J., AND VAN CALKER, D.: Adenosine A_{2b} receptors mediate an increase in interleukin (IL)-6 mRNA and IL-6 protein synthesis in human astroglioma cells. J. Neurochem. **66:** 1426–1431, 1996a.
FIEBICH, B. L., BIBER, K., LIEB, K., VAN CALKER, D., BERGER, M., AND GEBICKE-HAERTER, P. J.: Cycooxygenase-2 expression in rat microglia is induced by adenosine A_{2A} receptors. Glia **18:** 152–160, 1996b.
FREDHOLM, B. B.: Purinoceptors in the nervous system. Pharmacol. Toxicol. **76:** 228–239, 1995.
FREDHOLM, B. B., ABBRACCHIO, M. P., BURNSTOCK, G., DALY, J. W., HARDEN, T. K., JACOBSON, K. A., LEFF, P., AND WILLIAMS, M.: Nomenclature and classification of purinoceptors. Pharmacol. Rev. **46:** 143–156, 1994.
FREDHOLM, B. B., ABBRACCHIO, M. P., BURNSTOCK, G., DUBYAK, G. R., HARDEN, T. K., JACOBSON, K. A., SCHWABE, U., AND WILLIAMS, M.: Towards a revised nomenclature for P1 and P2 receptors. Trends Pharmacol. Sci. **18:** 79–82, 1997.
FREDHOLM, B. B., ARSLAN, G., KULL, B., KONTNY, E., AND SVENNINGSSON, P.: Adenosine (P1) receptor signaling. Drug Dev. Res. **39:** 262–268, 1996a.
FREDHOLM, B. B., BURNSTOCK, G., HARDEN, T. K., AND SPEDDING, M.: Receptor nomenclature. Drug Dev. Res. **39:** 461–466, 1996b.
FREDHOLM, B. B., LINDGREN, E., LINDSTRÖM, K., AND NORSTEDT, C.: Alpha-adrenoreceptor stimulation, but not muscarinic stimulation, increases cyclic AMP accumulation in brain slices due to protein kinase C mediated enhancement of adenosine receptor effects. Acta Physiol. Scand. **131:** 543–551, 1987.
FREDHOLM, B. B., AND PERSSON, C. G.: Xanthine derivatives as adenosine receptor antagonists. Eur. J. Pharmacol. **81:** 673–676, 1982.
FREDHOLM, B. B., AND SANDBERG, G.: Inhibition by xanthine derivatives of adenosine receptor-stimulated cyclic adenosine 3′-5′-monophosphate accumulation in rat and guinea-pig thymocytes. Br. J. Pharmacol. **80:** 639–644, 1983.
FREDHOLM, B. B., ZHANG, Y., AND VAN DER PLOEG, I.: Adenosine A_{2A} receptors mediate the inhibitory effect of adenosine on formyl-Met-Leu-Phe-stimulated respiratory burst in neutrophil leucocytes. Naunyn-Schmiedeberg's Arch. Pharmacol. **354:** 262–267, 1996c.
FURCHGOTT, R. F.: The role of endothelium in the responses of vascular smooth muscle to drugs. Ann. Rev. Pharmacol. Toxicol. **24:** 175–197, 1984.
GALLO-RODRIGUEZ, C., JI, X.-D., MELMAN, N., SIEGMAN, B. D., SANDERS, L. H., ORLINDA, J., FISCHER, B., PU, Q., OLAH, M. E., VAN GALEN, P. J. M., STILES, G. L., AND JACOBSON, K. A.: Structure-activity relationship of N^6-Benzyladenosine-5′-uronamides as A_3-selective adenosine agonists. J. Med. Chem. **37:** 636–646, 1994.
GERGEN, P. J., MULLALLY, D. I., AND EVANS, R., III.: National survey of prevalence of asthma among children in the United States, 1976 to 1980. Pediatrics **81:** 1–7, 1988.
GERWINS, P., AND FREDHOLM, B. B.: Glucocorticoid receptor activation leads to up-regulation of adenosine A_1 receptors and down-regulation of adenosine A_2 responses in DDT_1 MF-2 smooth muscle cells. Mol. Pharmacol. **40:** 149–155, 1991.
GHAI, G., ZIMMERMAN, M. B., AND HOPKINS, M. F.: Evidence for A_1 and A_2 adenosine receptors in guinea pig trachea. Life Sci. **41:** 1215–1224, 1987.
GHARIB, A., DELTON, I., LAGARDE, M., AND SARDA, N.: Evidence for adenosine A_{2b} receptor in the rat pineal gland. Eur. J. Pharmacol. **225:** 359–360, 1992.
GIROS, B., SOKOLOFF, P., MARTRES, M. P., RIOU, J. F., EMORINE, L. J., AND SCHWARTZ, J. C.: Alternative splicing directs the expression of two D_2 dopamine receptor isoforms. Nature (Lond.) **342:** 923–926, 1989.
GREGORY, C. Y., ABRAMS, T. A., AND HALL, M. O.: Stimulation of A_2 adenosine receptors inhibits the ingestion of photoreceptor outer segments by retinal pigment epithelium. Invest. Ophthalmol. Visual Sci. **35:** 819–825, 1994.
HANCOCK, D. L., AND COUPAR, I. M.: Functional characterization of the adenosine receptor mediating inhibition of intestinal secretion. Br. J. Pharmacol. **114:** 152–156, 1995.
HARGREAVES, M. B., STOGGALL, S. M., AND COLLIS, M. G.: Evidence that the adenosine receptor mediating relaxation in dog lateral saphenous vein and guinea pig aorta is of the A_{2B} subtype. (Abstract) Br. J. Pharmacol. **102:** 198P, 1991.
HAYNES, J. J., OBIKAO, B., THOMPSON, W. J., AND DOWNEY, J.: Adenosine-induced vasodilation: receptor characterization in pulmonary circulation. Am. J. Physiol. **268:** H1862–H1868, 1995.
HEADRICK, J. P., AND BERNE, R. M.: Endothelium-dependent and -independent relaxations to adenosine in guinea pig aorta. Am. J. Physiol. **259:** H62–H67, 1990.
HIDE, I., PADGETT, W. L., JACOBSON, K. A., AND DALY, J. W.: A_2 adenosine receptors from rat striatum and rat pheochromocytoma PC12 cells: characterization with radioligand binding and by activation of adenylate cyclase. Mol. Pharmacol. **41:** 352–359, 1992.
HOLLINGSWORTH, E. B., SEARS, E. B., AND DALY, J. W.: An activator of protein kinase C (phorbol-12-myristate-13-acetate) augments 2-chloradenosine-elicited accumulation of cyclic AMP in guinea pig cerebral cortical particulate preparations. FEBS Lett. **184:** 339–342, 1985.
HÖSLI, E., AND HÖSLI, L.: Autoradiographic studies on the uptake of adenosine and on binding of adenosine analogues in neurons and astrocytes of cultured rat cerebellum and spinal cord. Neuroscience **24:** 621–628, 1988.
HOURANI, S. M., NICHOLLS, J., LEE, B. S., HALFHIDE, E. J., AND KITCHEN, I.: Characterization and ontogeny of P1-purinoceptors on rat vas deferens. Br. J. Pharmacol. **108:** 754–758, 1993.
IMOTO, Y., YATANI, A., REEVES, J. P., CODINA, J., BIRNBAUMER, L., AND BROWN, A. M.: α-subunit of G_s directly activates cardiac calcium channels in lipid bilayers. Am. J. Physiol. **255:** H722–H728, 1988.
IWAMOTO, T., UMEMURA, S., TOYA, Y., UCHIBORI, T., KOGI, K., TAKAGI, N., AND ISHII, M.: Identification of adenosine A_2 receptor-cAMP system in human aortic endothelial cells. Biochem. Biophys. Res. Commun. **199:** 905–910, 1994.
JACOBSON, K. A.: Molecular diversity in the search for selective ligands for A_3-adenosine and ATP receptors. (Abstract) Drug Dev. Res. **37:** 112, 1996.
JACOBSON, K. A., AND SUZUKI, F.: Recent developments in selective agonists and antagonists acting at purine and pyrimidine receptors. Drug Dev. Res. **39:** 289–300, 1996.

JACOBSON, K. A., VAN GALEN, P. J. M., AND WILLIAMS, M.: Perspective: adenosine receptors—pharmacology, structure-activity relationships and therapeutic potential. J. Med. Chem. **35:** 407–422, 1992.
JACOBSON, M. A., CHAKRAVARTY, P. K., JOHNSON, R. J., AND NORTON, R.: Novel selective non-xanthine A_3 adenosine receptor antagonists. (Abstract) Drug Dev. Res. **37:** 131, 1996.
JACOBSON, M. A., JOHNSON, R. G., LUNEAU, C. J., AND SALVATORE, C. A.: Cloning and chromosomal localization of the human A_{2b} adenosine receptor gene (ADORA2B) and its pseudogene. Genomics **27:** 374–376, 1995.
JARVIS, M. F., SCHULZ, R., HUTCHINSON, A. J., DO, U. H., SILLIS, M. A., AND WILLIAMS, M.: [^{3}H]CGS 21680, a selective A_2 adenosine receptor agonist directly labels A2 receptors in rat brain. J. Pharmacol. Exp. Ther. **251:** 888–893, 1989.
JIANG, J. L., VAN RHEE, A. M., MELMAN, N., JI, X. D., AND JACOBSON, K. A.: 6-Phenyl-1,4-dihydropyridine derivatives as potent and selective A_3 adenosine receptor antagonists. J. Med. Chem. **39:** 4667–4675, 1996.
KARTON, Y., JIANG, J. L., JI, X. D., MELMAN, N., OLAH, M. E., STILES, G. L., AND JACOBSON, K. A.: Synthesis and biological activities of flavanoid derivatives as A_3 adenosine receptor antagonists. J. Med. Chem. **39:** 2293–2301, 1996.
KENAKIN, T. P., BOND, R. A., AND BONNER, T. I.: Definition of pharmacological receptors. Pharmacol. Rev. **44:** 351–361, 1992.
KESSEY, K., TROMMER, B. L., OVERSTREET, L. S., JI, T., AND MOGUL, D. J.: A role for adenosine A_2 receptors in the induction of long-term potentiation in the CA1 region of rat hippocampus. Brain Res. **756:** 184–190, 1997.
KILIC, S., SALIH, M., ANAFARTA, K., BALTACI, S., AND KOSAR, A.: Adenosine: a new agent in the diagnosis of impotence. Int. J. Impot. Res. **6:** 191–198, 1994.
KLOTZ, K.-N., VOGT, H., AND TAWFIC-SCHLIEPER, H.: Comparison of A_1 adenosine receptors in brain from different species by radioligand binding and photoaffinity labeling. Naunyn-Schmiedeberg's Arch. Pharmacol. **343:** 196–201, 1991.
KONTNY, E., KVANTA, A., AND FREDHOLM, B. B.: Activation of protein kinase C and elevation of cAMP interact synergistically to raise c-Fos, c-June and AP-1 activity in Jurkat cells. Eur. J. Pharmacol. **227:** 333–338, 1992.
KVANTA, A., AND FREDHOLM, B. B.: Synergistic effects between protein kinase C and cAMP on activator protein-1 activity and differentiation of PC-12 pheochromocytoma cells. J. Mol. Neurosci. **4:** 205–214, 1994.
KVANTA, A., GERWINS, P., JONDAL, M., AND FREDHOLM, B. B.: Stimulation of T-cells OKT3 antibodies increases forskolin binding and cyclic AMP accumulation. Cell Signalling **2:** 461–470, 1990.
KVANTA, A., KONTNY, E., JONDAL, M., OKRET, S., AND FREDHOLM, B. B.: Mitogen stimulation of T-cells increases c-Fos and c-Jun protein levels, AP-1 binding and AP-1 transcriptional activity. Cell Signalling **4:** 275–286, 1992.
KVANTA, A., NORSTEDT, C., JONDAL, M., AND FREDHOLM, B. B.: Activation of protein kinase C via the T-receptors potentiates cyclic AMP responses in T-cells. Naunyn-Schmiedeberg's Arch. Pharmacol. **340:** 715–717, 1989.
LEDENT, C., VAUGEOIS, J.-M., SCHIFFMANN, M. E., CANDERHAEGHEN, J.-J., COSTENTIN, J., HEATH, J. K., VASSART, G., AND PARMENTIER, M.: Aggressiveness, hypoalgesia and high blood pressure in mice lacking adenosine A_{2a} receptor. Nature (Lond.) **388:** 674–678, 1997.
LEVENS, N., BEIL, M., AND SCHULZ, R.: Intrarenal actions of the new adenosine agonist CGS 21680A, selective for the A_2 receptor. J. Pharmacol. Exp. Ther. **257:** 1013–1019, 1991.
LIANG, B. T., AND HALTIWANGER, B.: Adenosine A2a and A2b receptors in cultured fetal chick heart cells: high- and low-affinity coupling to stimulation of myocyte contractility and cAMP accumulation. Circ. Res. **76:** 242–251, 1995.
LIANG, B. T., AND MORLEY, J. F.: A new cyclic AMP-independent, G_s-mediated stimulatory mechanism via the adenosine A_{2a} receptor in the intact cardiac cell. J. Biol. Chem. **271:** 18678–18685, 1996.
LIBERT, F., SCHIFFMANN, S. N., LEFORT, A., PARMENTIER, M., GERARD, C., DUMONT, J. E., VANDERHAEGHEN, J. J., AND VASSART, G.: The orphan receptors cDNA RDC7 encodes an A_1 adenosine receptor. EMBO J. **10:** 1677–1682, 1991.
LINDEN, J.: Structure and function of A_1 adenosine receptors. FASEB J. **5:** 2668–2676, 1991.
LINDEN, J.: Cloned adenosine A_3 receptors: pharmacological properties, species differences and receptor functions. Trends Pharmacol. Sci. **15:** 298–306, 1994.
LINDEN, J., TAYLOR, H. E., ROBEVA, A. S., TUCKER, A. L., STEHLE, J. H., RIVKEES, S. A., FINK, J. S., AND REPPERT, S. M.: Molecular cloning and functional expression of a sheep A_3 adenosine receptor with widespread tissue distribution. Mol. Pharmacol. **44:** 524–532, 1993.
LINDSTRÖM, K., ONGINI, E., AND FREDHOLM, B. B.: The selective adenosine A_{2A} receptor antagonist SCH 58261 discriminates between two different binding sites for [^{3}H]-CGS 21680 in the rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. **354:** 539–541, 1996.
LONDOS, C., COOPER, D. M. F., AND WOLFF, J.: Subclasses of external adenosine receptors. Proc. Natl. Acad. Sci. USA **77:** 2551–2554, 1980.
LUPICA, C. R., CASS, W. A., ZAHNISER, N. R., AND DUNWIDDIE, T. V.: Effects of the selective adenosine A_2 receptor agonist CGS21680 on in vitro electrophysiology, cAMP formation and dopamine release in rat hippocampus and striatum. J. Pharmacol. Exp. Ther. **252:** 1134–1141, 1990.
MADARA, J. L., PATAPOFF, T. W., GILLECE-CASTRO, B., COLGAN, S. P., PARKOS, C. A., DELP, C., AND MRSNY, R. J.: 5′-adenosine monophosphate is the neutrophil-derived paracrine factor that elicits chloride secretion from T84 intestinal epithelial cell monolayers. J. Clin. Invest. **91:** 2320–2325, 1993.
MAEKAWA, K., SAITO, D., OBAYASHI, N., UCHIDA, S., AND HARAOKA, S.: Role of endothelium-derived nitric oxide and adenosine in functional myocardial hyperemia. Am. J. Physiol. **267:** H166–H173, 1994.
MAENHAUT, C., VAN SANDE, J., LIBERT, F., ABRAMOWITZ, M., PARMENTIER, M., VANDERHARGEN, J. J., DUMONT, J. E., VASSART, G., AND SCHIFFMANN, S.: RDC8 codes for an adenosine A_2 receptor with physiological constitutive activity. Biochem. Biophys. Res. Commun. **173:** 1169–1178, 1990.
MANN, J. S., AND HOLGATE, S. T.: Specific antagonism of adenosine-induced bronchoconstriction is asthma by oral theophylline. Br. J. Clin. Pharmacol. **19:** 685–692, 1985.
MARQUARDT, D. L., PARKER, C. W., AND SULLIVAN, T. J.: Potentiation of mast cell mediator release by adenosine. J. Immunol. **120:** 871–878, 1978.
MARQUARDT, D. L., AND WALKER, L. L.: Modulation of mast cell responses to adenosine by agents that alter protein kinase C activity. Biochem. Pharmacol. **39:** 1929–1934, 1990.
MARQUARDT, D. L., AND WALKER, L. L.: Inhibition of protein kinase A fails to alter mast cell adenosine responsiveness. Agents Actions **43:** 7–12, 1994.
MARQUARDT, D. L., WALKER, L. L., AND HEINEMANN, S.: Cloning of two adenosine receptor subtypes from mouse bone marrow-derived mast cells. J. Immunol. **152:** 4508–4515, 1994.
MARTIN, P. L.: Relative agonist potencies of C2-substituted analogues of adenosine: evidence of adenosine A_{2b} receptors in the guinea pig aorta. Eur. J. Pharmacol. **216:** 235–242, 1992.
MARTIN, P. L., AND POTTS, A. A.: The endothelium of the rat renal artery plays an obligatory role in A_2 adenosine receptor-mediated relaxation induced by 5′-N- ethylcarboxamidoadenosine and N^6-cyclopentyladenosine. J. Pharmacol. Exp. Ther. **270:** 893–899, 1994.
MARTIN, P. L., UEEDA, M., AND OLSSON, R. A.: 2-Phenylethoxy-9-methyladenine: an adenosine receptor antagonist that discriminates between A_2 adenosine receptors in the aorta and the coronary vessels from the guinea pig. J. Pharmacol. Exp. Ther. **265:** 248–253, 1993.
MARTINSON, E. A., JOHNSON, R. A., AND WELLS, J. N.: Potent adenosine receptor antagonists that are selective for the A_1 receptor subtype. Mol. Pharmacol. **31:** 247–252, 1987.
MATEO, J., CASTRO, E., ZWILLER, J., AUNIS, D., AND MIRAS-PORTUGAL, M. T.: 5′-(n-ethylcarboxamido)adenosine inhibits Ca^{2+} influx and activates a protein phosphatase in bovine adrenal chromaffin cells. J. Neurochem. **64:** 77–84, 1995.
MILLIGAN, G., MARSHALL, F., AND REES, S.: G_{16} as a universal G protein adapter: implications for agonist screening strategies. Trends Pharmacol. Sci. **17:** 235–237, 1996.
MOGUL, D. J., ADAMS, M. E., AND FOX, A. P.: Differential activation of adenosine receptors decreases N-type but potentiates P-type Ca^{2+} current in hippocampal CA3 neurons. Neuron **10:** 327–334, 1993.
MOSER, G. H., SCHRADER, J., AND DEUSSEN, A.: Turnover of adenosine in plasma of human and dog blood. Am. J. Physiol. **256:** C799–C806, 1989.
MOSQUEDA-GARCIA, R., APPALSAMY, M., AND ROBERTSON, D.: Mechanisms of the central cardiovascular effects of adenosine: interaction with brain L-glutamate. (Abstract) Neuroscience **15:** 1178, 1989.
MOSQUEDA-GARCIA, R., TSENG, C. J., APPALSAMY, M., BECK, C., AND ROBERTSON, D.: Cardiovascular excitatory effects of adenosine in the nucleus of the solitary tract. Hypertension **18:** 494–502, 1991.
MULLANE, K.: Acadesine: the prototype adenosine regulating agent for reducing myocardial injury. Cardiovasc. Res. **27:** 43–47, 1993.
MUNDELL, S. J., BENOVIC, J. L., AND KELLY, E.: A dominant negative mutant of G protein-coupled receptor kinase 2 selectively attenuates adenosine A_2 receptor desensitization. Mol. Pharmacol. **51:** 991–998, 1997.
MURRISON, E. M., GOODSON, S. J., EDBROOKE, M. R., AND HARRIS, C. A.: Cloning and characterization of the human adenosine A_3 receptor gene. FEBS Lett. **384:** 243–246, 1996.
MURTHY, K. S., MCHENRY, L., GRIDER, J. R., AND MAKHLOUF, G. M.: Adenosine A_1 and A_{2b} receptors coupled to distinct interactive signaling pathways in intestinal muscle cells. J. Pharmacol. Exp. Ther. **274:** 300–306, 1995.
NAKANE, T., AND CHIBA, S.: Adenosine constricts the isolated and perfused monkey coronary artery. Heart Vessels **5:** 71–75, 1990.
NANOFF, C., JACOBSON, K. A., AND STILES, G. L.: The A_2 adenosine receptor: guanine nucleotide modulation of agonist binding is enhanced by proteolysis. Mol. Pharmacol. **39:** 130–135, 1991.
NEES, S., HERZOG, V., BECKER, B. F., BOCK, M., DES ROSIERS, C., AND GERLACH, E.: The coronary endothelium: a highly active metabolic barrier for adenosine. Basic Res. Cardiol. **80:** 515–529, 1985.
NEGLISHI, M., SUGIMOTO, Y., AND ICHIKAWA, A.: Molecular mechanisms of diverse actions of prostanoid receptors. Biochim. Biophys. Acta **1259:** 109–120, 1995.
NGUYEN, T., SUNAHARA, R., MARCHESE, A., VAN TOL, H. H. M., SEEMAN, P., AND O'DOWD, B. F.: Transcription of a human dopamine D_5 pseudogene. Biochem. Biophys. Res. Commun. **181:** 16–21, 1991.
NICHOLLS, J., BROWNHILL, V. R., AND HOURANI, S. M. O.: Characterization of P1-purinoceptors on rat isolated duodenum longitudinal muscle muscularis mucosae. Br. J. Pharmacol. **117:** 170–174, 1996.
NORSTEDT, C., AND FREDHOLM, B. B.: Phorbol-12,13-dibutirate enhances the cyclic AMP accumulation in rat hippocampal slices induced by adenosine analogues. Naunyn-Schmiedeberg's Arch. Pharmacol. **335:** 136–142, 1987.

NORSTEDT, C., KVANTA, A., VAN DER PLOEG, I., AND FREDHOLM, B. B.: Dual effects of protein kinase C on receptor-stimulated cAMP accumulation in a human T-cell leukemia line. Eur. J. Pharmacol. **172:** 51–62, 1989.
NYCE, J. W., AND METZGER, W. J.: DNA antisense therapy for asthma in an animal model. Nature (Lond.) **97:** 721–725, 1997.
OFFERMANNS, S., AND SIMON, M. I.: $G\alpha_{15}$ and $G\alpha_{16}$ couple a wide variety of receptors to phospholipase C. J. Biol. Chem. **270:** 15175–15180, 1995.
OKADA, M., MIZUNO, K., AND KANEKO, S.: Adenosine A_1 and A_2 receptors modualte extracellular dopamine levels in rat striatum. Neurosci. Lett. **212:** 53–56, 1996.
OLAH, M. E.: Identification of A_{2a} adenosine receptor domain involved in selective coupling to G_s: analysis of chimeric A_1/A_{2a} adenosine receptors. J. Biol. Chem. **272:** 337–344, 1997.
OLSSON, R. A.: Adenosine receptors in the cardiovascular system. Drug Dev. Res. **39:** 301–307, 1996.
OLSSON, R. A., DAVIS, C. C., AND KHOURI, E. M.: Coronary vasoactivity of adenosine covalently linked to polylysine. Life Sci. **21:** 1343–1350, 1977.
OLSSON, R. A., AND PEARSON, J. D.: Cardiovascular purinoceptors. Pharmacol. Rev. **70:** 761–845, 1990.
ONGINI, E., DIONISOTTI, S., MORELLI, M., FERRE, S., SVENNINGSSON, P., FUXE, K., AND FREDHOLM, B. B.: Neuropharmacology of the adenosine A_{2A} receptors. Drug Dev. Res. **39:** 450–460, 1996.
ONGINI, E., AND FREDHOLM, B. B.: Pharmacology of adenosine A_{2A} receptors. Trends Pharmacol. Sci. **17:** 364–372, 1996.
PALMER, T. M., JACOBSON, K. A., AND STILES, G. L.: Immunological identification of A_2 adenosine receptors by two antipeptide antibody preparations. Mol. Pharmacol. **42:** 391–397, 1992.
PALMER, T. M., AND STILES, G. L.: Adenosine receptors. Neuropharmacology **34:** 683–694, 1995.
PALMER, T. M., AND STILES, G. L.: Identification of an A_{2a} adenosine receptor domain specifically responsible for mediating of short-term desensitization. Biochemistry **36:** 832–838, 1997.
PATON, D. M.: Structure-activity relations for presynaptic inhibition of noradrenergic and cholinergic transmission by adenosine: evidence for action on A_1 receptors. J. Auton. Pharmacol. **1:** 287–290, 1981.
PEACHELL, P. T., LICHTENSTEIN, L. M., AND SCHLEIMER, R. P.: Differential regulation of human basophil and lung mast cell function by adenosine. J. Pharmacol. Exp. Ther. **256:** 717–726, 1991.
PEAKMAN, M. C., AND HILL, S. J.: Adenosine A_{2b}-receptor-mediated cyclic AMP accumulation in primary rat astrocytes. Br. J. Pharmacol. **111:** 191–198, 1994.
PEAKMAN, M. C., AND HILL, S. J.: Adenosine A_1 receptor-mediated inhibition of cyclic AMP accumulation in type-2 but not type-1 rat astrocytes. Eur. J. Pharmacol. **306:** 281–289, 1996.
PETERFREUND, R. A., MACCOLLIN, M., GUSELLA, J., AND FINK, J. S.: Structure of the human A_{2a} adenosine receptor gene. (Abstract) Drug Dev. Res. **31:** 306, 1994.
PHILLIPS, G. D., NG, W. H., CHURCH, M. K., AND HOLGATE, S. T.: The response of plasma histamine to bronchoprovocation with methacholine, adenosine 5′-monophosphate, and allergen in atopic nonasthmatic subjects. Am. Rev. Respir. Dis. **90:** 9–13, 1990.
PHILLIPS, G. D., RAFFERTY, P., BEASLEY, R., AND HOLGATE, S. T.: Effect of oral terfenadine on the bronchoconstrictor response to inhaled histamine and adenosine 5′-monophosphate in non-atopic asthma. Thorax **87:** 939–945, 1987.
PHILLIPS, G. D., SCOTT, V. L., RICHARDS, R., AND HOLGATE, S. T.: Effect of nedrocromil sodium and sodium cromoglycate against bronchoconstriction induced by inhaled adenosine 5′-monophosphate. Eur. Respir. J. **2:** 210–217, 1989.
PHILLIS, J. W., O'REGAN, M. H., AND PERKINS, L. M.: Effect of adenosine receptor agonist on spontaneous and K^+-evoked acetylcholine release from the in vivo rat cerebral cortex. Brain Res. **605:** 293–297, 1993a.
PHILLIS, J. W., PERKINS, L. M., AND O'REGAN, M. H.: Potassium-evoked efflux of transmitter amino acids and purines from rat cerebral cortex. Brain Res. Bull. **31:** 547–552, 1993b.
PIERCE, K. D., FURLONG, T. J., SELBIE, L. A., AND SHINE, J.: Molecular cloning and expression of an adenosine A_{2b} receptor from human brain. Biochem. Biophys. Res. Commun. **187:** 86–93, 1992.
PIERSEN, C. E., TRUE, C. D., AND WELLS, J. N.: A carboxyl-terminally truncated mutant and nonglycosylated A_{2a} adenosine receptors retain ligand binding. Mol. Pharmacol. **45:** 861–870, 1994.
POLOSA, R., NG, W. H., CRIMI, N., VANCERI, C., HOLGATE, S. T., CHURCH, M. K., AND MISTRETTA, A.: Release of mast cell-derived mediators after endobronchial adenosine challenge in asthma. Am. J. Respir. Crit. Care Med. **151:** 624–629, 1995.
PORZIG, H., GUTKNECHT, R., KOSTOVA, G., AND THALMEIER, K.: G protein-coupled receptors in normal human erytroid progenitor cells. Naunyn-Schmiedeberg's Arch. Pharmacol. **353:** 11–20, 1995.
POUCHER, S. M., KEDDIE, J. R., SINGH, P., STOGGALL, S. M., CAULKETT, P. W. R., JONES, G., AND COLLIS, M. G.: The in vitro pharmacology of ZM 241385, a potent non-xanthine, A_{2a} selective adenosine receptor antagonist. Br. J. Pharmacol. **115:** 1096–1102, 1995.
PRENTICE, D. J., AND HOURANI, S. M. O.: Activation of multiple sites by adenosine analogues in the rat isolated aorta. Br. J. Pharmacol. **118:** 1509–1517, 1996.
PRENTICE, D. J., AND HOURANI, S. M. O.: Adenosine analogues relax guinea-pig taenia caeci via an adenosine A_{2B} receptor and a xanthine-resistant site. Eur. J. Pharmacol. **323:** 103–106, 1997.
PUFFINBARGER, N. K., HANSEN, K. R., RESTA, R., LAURENT, A. B., KNUDSEN, T. B., MADARA, J. L., AND THOMPSON, L. F.: Production and characterization of multiple antigenic peptide antibodies to the adenosine A_{2b} receptor. Mol. Pharmacol. **47:** 1126–1132, 1995.
RAFFERTY, P., BEASLEY, C. R., AND HOLGATE, S. T.: The contribution of histamine to immediate bronchoconstriction provoked by inhaled allergen and adenosine-5′monophosphate in atopic asthma. Am. Rev. Respir. Dis. **136:** 369–373, 1987.
RAMKUMAR, V., STILES, G. L., BEAVEN, M. A., AND ALI, H.: The A_3 adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. J. Biol. Chem. **268:** 16887–16890, 1993.
RAMKUMAR, V., WILSON, M., DHANRAJ, D. N., GETTYS, T. W., AND ALI, H.: Dexamethasone up-regulates A_3 adenosine receptors in rat basophilic leukemia (RBL-2HE) cells. J. Immunol. **154:** 5437–5443, 1995.
REN, H., AND STILES, G. L.: Characterization of the human A_1 adenosine receptor gene: evidence for alternative splicing. J. Biol. Chem. **269:** 3104–3110, 1994.
RIVKEES, S. A., AND REPPERT, S. M.: RFL9 encodes an A_{2b}-adenosine receptor. Mol. Endocrinol. **6:** 1598–1604, 1992.
ROBEVA, A. S., WOODARD, R. L., JIN, X., GAO, Z., BHATTACHARYA, S., TAYLOR, H. E., ROSIN, D. L., AND LINDEN, J.: Molecular characterization of recombinant human adenosine receptors. Drug Dev. Res. **39:** 243–252, 1996.
ROMANO, F. D., MACDONALD, S. G., AND DOBSON, J. G., JR.: Adenosine receptor coupling to adenylate cyclase of rat ventricular myocyte membrane. Am. J. Physiol. **257:** H1088–H1095, 1989.
RUBANYI, G., AND VANHOUTTE, P. M.: Endothelium removal decreases relaxations of canine coronary arteries caused by β-adrenergic agonists and adenosine. J. Cardiovasc. Pharmacol. **7:** 139–144, 1985.
RUBINO, A., RALEVIC, V., AND BURNSTOCK, G.: Contribution of P1-(A_{2b} subtype) and P2-purinoceptors to the control of vascular tone in the rate isolate mesenteric arterial bed. Br. J. Pharmacol. **115:** 648–652, 1995.
SALVATORE, C. A., JACOBSON, M. A., TAYLOR, H. E., LINDEN, J., AND JOHNSON, R. G.: Molecular cloning and characterization of the human A_3 adenosine receptor. Proc. Natl. Acad. Sci. USA **90:** 10365–10369, 1993.
SATTIN, A., AND RALL, T. W.: The effect of adenosine and adenine nucleotides on the adenosine 3′,5′-phosphate content of guinea pig cerebral cortex slices. Mol. Pharmacol. **6:** 13–23, 1970.
SCAMPS, F., RYBIN, V., PUCEAT, M., TKACHUK, V., AND VASSORT, G.: A G_s protein couples P2-purinergic stimulation to cardiac Ca channels without cyclic AMP production. J. Gen. Physiol. **100:** 675–701, 1992.
SCHWARTZ, L. B.: Tryptase, a mediator of human mast cells. J. Allergy Clin. Immunol. **86:** 594–598, 1990.
SEBASTIÃO, A. M., AND RIBEIRO, J. A.: Adenosine A_2 receptor-mediated excitatory actions on the nervous system. Prog. Neurobiol. **48:** 167–189, 1996.
SOLLEVI, A., LAGERKRANSER, M., IRESTEDT, L., GORDON, E., AND LINDQUIST, C.: Controlled hypotension with adenosine in cerebral aneurysm surgery. Anesthesiology **61:** 400–405, 1984.
STEHLE, J. H., RIVKEES, S. A., LEE, J. J., WEAVER, D. R., DEEDS, J. D., AND REPPERT, S. M.: Molecular cloning and expression of the cDNA for a novel A_2- adenosine receptor subtype. Mol. Endocrinol. **6:** 384–393, 1992.
STEIN, B., MENDE, U., NEUMANN, J., SCHMITZ, W., AND SCHOLZ, H.: Pertussis toxin unmasks stimulatory myocardial A_2-adenosine receptors on ventricular cardiomyocytes. J. Mol. Cell. Cardiol. **25:** 655–659, 1993.
STRICKLER, J., JACOBSON, K. A., AND LIANG, B. T.: Direct preconditioning of cultured chick ventricular myocytes: novel functions of cardiac adenosine A_{2a} and A_3 receptors. J. Clin. Invest. **98:** 1773–1779, 1996.
STROHMEIER, G. R., REPPERT, S. M., LENCER, W. I., AND MADARA, J. L.: The A_{2b} adenosine receptor mediates cAMP responses to adenosine receptor agonists in human intestinal epithelia. J. Biol. Chem. **270:** 2387–2394, 1995.
SVENNINGSSON, P., AND FREDHOLM, B. B.: Glucocorticoids regulate the expression of adenosine A_1 but not A_{2A} receptors in rat brain. J. Pharmacol. Exp. Ther. **280:** 1094–1101, 1997.
TAMAOKI, J., TAGAYA, E., CHIYOTANI, A., TAKEMURA, H., NAGAI, A., KONNO, K., ONUKI, T., AND NITTA, S.: Effect of adenosine on adrenergic neurotransmission and modulation by endothelium in canine pulmonary artery. Am. J. Physiol. **272:** H1100–H1105, 1997.
TOFOVIC, S. P., BRANCH, K. R., OLIVER, R. D., MAGEE, W. D., AND JACKSON, E. K.: Caffeine potentiates vasodilator-induced renin release. J. Pharmacol. Exp. Ther. **256:** 850–860, 1991.
TOWNSEND-NICHOLSON, A., BAKER, E., SUTHERLAND, G. R., AND SCHOFIELD, P. R.: Localization of the adenosine A_{2b} receptor subtype gene (ADORA2B) to chromosome 17p11.2-p12 by FISH and PCR screening of somatic cell hybrids. Genomics **25:** 605–607, 1995.
TSENG, C. J., BIAGGIONI, I., APPALSAMY, M., AND ROBERTSON, D.: Purinergic receptors in the brainstem mediate hypotension and bradycardia. Hypertension **11:** 191–197, 1988.
TUCKER, A., HOLETON, D., CHO, E. S., TAYLOR, A., AND LINDEN, J.: Chimeric canine A_1/A_{2A} adenosine receptors identify intracellular domains important for determining selective G protein coupling. (Abstract) Drug Dev. Res. **37:** 110, 1996.
TUCKER, A. L., AND LINDEN, J.: Cloned receptors and cardiovascular responses to adenosine. Cardiovasc. Res. **27:** 62–67, 1993.

TURNER, J. T., CAMDEN, J. M., KANSRA, S., SHELTON-JAMES, D., WU, H., AND HALENDA, S. P.: Potentiation of cyclic adenosine monophosphate production by thrombin in the human erythroleukemia cell line HEL. J. Pharmacol. Exp. Ther. **263:** 708–716, 1992.

UKENA, D., JACOBSON, K. A., KIRK, K. L., AND DALY, J. W.: A [^{3}H]amine congener of 1,3-dipropyl-8-phenylxanthine: a new radioligand for A_2 adenosine receptors of human platelets. FEBS Lett. **199:** 269–274, 1986.

UKENA, D., SCHIRREN, C. G., AND SCHWABE, U.: Effects of enprofylline on A_1 and A_2 adenosine receptors. Eur. J. Pharmacol. **117:** 25–33, 1985.

VAN CALKER, D., MUELLER, M., AND HAMPRECHT, B.: Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. J. Neurochem. **33:** 999–1005, 1979.

VAN DER PLOEG, I., AHLBERG, S., PARKINSON, F. E., OLSSON, R. A., AND FREDHOLM, B. B.: Functional characterization of adenosine A2 receptors in Jurkat cells and PC12 cells using adenosine receptor agonists. Naunyn-Schmiedeberg's Arch. Pharmacol. **353:** 250–260, 1996.

VAN GALEN, P. J. M., VAN BERGEN, A. H., GALLO-RODRIGUEZ, C., MELMAN, N., OLAH, M. E., IJZERMAN, A. P., STILES, G. L., AND JACOBSON, K. A.: A binding site model and structure-activity relationships for rat A_3 adenosine receptor. Mol. Pharmacol. **45:** 1101–1111, 1994.

VAN RHEE, A. M., JIANG, J. L., MELMAN, N., OLAH, M. E., STILES, G. L., AND JACOBSON, K. A.: Interaction of 1,4-dihydropyridine and pyridine derivatives with adenosine receptors: selectivity for A_3 receptors. J. Med. Chem. **39:** 2980–2989, 1996.

VERANI, M. S., MAHMARIAN, J. J., HIXSON, J. B., BOYCE, T. M., AND STAUDACHER, R. A.: Diagnosis of coronary artery disease by controlled coronary vasodilatation with adenosine and thallium-201 scintigraphy in patients unable to exercise. Circulation **82:** 80–87, 1990.

VOLLMER, W. M., BUIST, A. S., AND OSBORNE, M. L.: Twenty year trends in hospital discharges for asthma among members of a health maintenance organization. J. Clin. Epidemiol. **45:** 999–1006, 1992.

WAKADE, A. R., AND WAKADE, T. D.: Inhibition of noradrenaline release by adenosine. J. Physiol. **282:** 35–49, 1978.

WALDAY, P., AND AAS, P.: Prejunctional stimulation of cholinergic nerves in rat airway smooth muscle by an adenosine analogue. Pulm. Pharmacol. **4:** 114–119, 1991.

WEBB, R. L., SILLS, M. A., CHOVAN, J. P., BALWIERCZAK, J. L., AND FRANCIS, J. E.: CGS 21680: a potent selective adenosine A_2 receptor agonist. Cardiovasc. Drug Rev. **10:** 26–53, 1992.

WEINBERGER, M., AND HENDELES, L.: Theophylline in asthma. N. Engl. J. Med. **334:** 1380–1388, 1996.

WEISS, K. B., GERGEN, P. J., AND WAGENER, D. K.: Breathing better or wheezing worse? The changing epidemiology of asthma morbidity and mortality. Annu. Rev. Public Health **14:** 491–513, 1993.

WILKIE, T., SCHERLE, P., STRATHMANN, M. P., SLEPAK, V. Z., AND SIMON, M. I.: Characterization of G protein alpha subunits in the G_q class: expression in murine tissues and in stromal and hematopoietic cell lines. Proc. Natl. Acad. Sci. USA **88:** 10049–10053, 1991.

XU, H., STEIN, B., AND LIANG, B. T.: Characterization of a stimulatory adenosine A_{2a} receptor in adult rat ventricular myocyte. Am. J. Physiol. **270:** H1655–H1661, 1996.

YAKEL, J. L., WARREN, R. A., REPPERT, S. M., AND NORTH, R. A.: Functional expression of adenosine A_{2b} receptor in *Xenopus* oocytes. Mol. Pharmacol. **43:** 277–280, 1993.

YEN, M.-H., WU, C.-C., AND CHIOU, W.-F.: Partially endothelium-dependent vasodilator effect of adenosine in rat aorta. Hypertension **11:** 514–518, 1988.

ZHOU, Q.-Y., LI, C., OLAH, M. E., JOHNSON, R. A., STILES, G. L., AND CIVELLI, O.: Molecular cloning and characterization of an adenosine receptor: the A_3 adenosine receptor. Proc. Natl. Acad. Sci. USA **89:** 7432–7436, 1992.

ZOCCHI, C., ONGINI, E., CONTI, A., MONOPOLI, A., NEGRETTI, A., BARALDI, P. G., AND DIONISOTTI, S.: The non-xanthine heterocyclic compound SCH 58261 is a new potent and selective A_{2a} adenosine receptor antagonist. J. Pharmacol. Exp. Ther. **276:** 398–404, 1996a.

ZOCCHI, C., ONGINI, E., FERRARA, S., BARALDI, P. G., AND DIONISOTTI, S.: Binding of the radioligand [^{3}H]-SCH 58261, a new non-xanthine A_{2A} adenosine receptor antagonist, to rat striatal membranes. Br. J. Pharmacol. **117:** 1381–1386, 1996b.