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Perspective

Adenosine Receptors: Pharmacology, Structure-Activity Relationships, and Therapeutic Potential

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Introduction

In the 10 years since Daly reviewed¹ the potential of adenosine receptors as drug targets, considerable advances have been made in the area of purinergic receptor related research such that there is little doubt remaining that adenosine, as well as adenosine 5'-triphosphate (ATP) and related nucleotides, functions as both neurohumoral agents and autacoids regulating the process of cell to cell communication.²

The techniques of molecular pharmacology have been extensively used to delineate purinergic receptor function, resulting in the identification of several receptor subclasses that subserve discrete physiological functions (Table I).² And more recently, the two major classes of adenosine receptors, the A₁ and A₂, have been cloned,^{3,4} offering the potential to model the receptor-ligand interaction from the receptor side.⁵

On the ligand front, structure-activity relationships (SAR) studies (Figure 1) for derivatives of adenosine (1), as agonists, and of theophylline (2), as antagonists, have revealed selective agents,⁶⁻⁸ and potent and selective A₁- and A₂-receptor agonists are now available. Newer antagonist ligands include a large number of 8-substituted xanthine derivatives, some of them over 10 000-fold more potent than the parent compound 2, as well as numerous classes of non-xanthine heterocyclic compounds⁸ described in further detail below.

The exceptional progress in the preclinical area, both chemical and biological, has not however been paralleled in the clinic. Very few adenosine agonists and antagonists have entered clinical trials and none of these, to the authors' knowledge, have been successful.⁷ The only ap-

proved compound known to produce its therapeutic actions via a direct interaction with adenosine receptors is adenosine itself, used for the treatment of supraventricular tachycardia (SVT),⁹ a use designated by the U.S. Food and Drug Administration in their coveted 1A category, indicating a drug for major unmet medical need. Additional potential uses for adenosine include cardiac imaging,¹⁰ in cardioplegic solutions¹¹ to delay the onset of ischemic contractions, and as a cardioprotectant in postischemic

- (1) Daly, J. W. Adenosine receptors: targets for future drugs. *J. Med. Chem.* 1982, 25, 197-207.
- (2) Williams, M. Adenosine: the prototypic neuromodulator. *Neurochem. Intern.* 1989, 14, 249-264.
- (3) Libert, F.; Schiffmann, S.; Lefort, A.; Parmentier, M.; Gerard, C.; Dumont, J. E.; Vanderhaegen, J. J.; Vassart, G. The orphan receptor cDNA RDC7 encodes an A₁ adenosine receptor. *EMBO J.* 1991, 10, 1677-1682.
- (4) Maenhaut, C.; van Sande, S. J.; Libert, F.; Abramowicz, M.; Parmentier, M.; Vanderhaegen, J. J.; Dumont, J. E.; Vassart, G.; Schiffmann, S. RDC8 codes for an adenosine A₂ receptor with physiological constitutive activity. *Biochem. Biophys. Res. Commun.* 1990, 173, 1169-1178.
- (5) Hollenberg, M. D. Receptor triggering and receptor regulation: Structure-activity relationship from the receptor's point of view. *J. Med. Chem.* 1990, 33, 1275-81.
- (6) Trivedi, B. K.; Bridges, A. J.; Bruns, R. F. Structure-activity relationships of adenosine A₁ and A₂ receptors. In *Adenosine and Adenosine Receptors*; Williams, M., Ed.; Humana: Clifton, NJ, 1990; pp 57-103.
- (7) Jacobson, K. A.; Trivedi, B. K.; Churchill, P. C.; Williams, M. Novel therapeutics acting via purine receptors. *Biochem. Pharmacol.* 1991, 41, 1399-1410.
- (8) Williams, M. Adenosine antagonists as therapeutic agents. *Med. Res. Rev.* 1989, 9, 219-43.
- (9) Pantely, G. A.; Bristow, J. D. Adenosine. Renewed interest in an old drug. *Circulation* 1990, 82, 1854-1856.
- (10) Verani, M. S.; Mahmanian, J. J.; Hixson, J. B.; Boyce, T. M.; Staudacher, R. A. Diagnosis of coronary artery disease by controlled coronary vasodilation with adenosine and thallium-201 in patients unable to exercise. *Circulation* 1990, 82, 80-87.

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Table I. Purinoceptor Subtypes

Table 1. P ₁ Receptor Subtypes			
subtype	potency ^a		location
	agonists	antagonists	
P ₁ (Adenosine) Receptors			
A ₁	CPA > R-PIA > NECA >> MPEA > CGS 21680	CPX > XAC > CPT > 8-PT	hippocampus, adipocytes, atrioventricular node
A _{1a}	R-PIA > NECA > S-PIA >> MeAdo > CV 1808 >> CV 1674	XAC = PD 113,297 > XCC	rat brain ^b
A _{1b}	R-PIA > NECA >> S-PIA > CV 1808 >> MeAdo > CV 1674	XAC ≥ XCC > PD 113,297	guinea pig ileum ^b
A _{2a} "high affinity"	APEC ≈ CGS21680 ≈ CGS 22492 ≈ NECA >> CPA ≈ CV1674 ^c > N ⁶ -MeADO	CGS15943 > XAC ≈ PD115,199 > CPX > XCC	striatum, platelets, neutrophils, coronary vasculature, olfactory tubercle
A _{2b} "low affinity"	NECA >> CGS21680 ≈ N ⁶ -MeADO > CV1674 ^c	XCC ≈ XAC >> CPX, 5'MTA	brain
A ₃			brain
P ₂ (ATP) Receptors			
P _{2t} P _{2u} "nucleotide receptor"	2-MeSADP > ADP UTP = ATP > ADP > 2-MeSATP	ATP, AMP	platelet hepatocytes, bovine aorta smooth muscle, Ehrlich ascites tumor cells, HL-60 cells, rat renal mesangial cells, neutrophils, fibroblasts
P _{2x} P _{2y}	α,β-MeATP > ATP 2-MeSATP > ATP = ADP > UTP	suramin reactive blue 2	bladder, vas deferens, ear artery taenia coli, endothelium, turkey erythrocytes
P _{2z}	ATP ⁻⁴ > ATP	DIDS ^d	mast cells, lymphocytes
P ₃ Receptors			
P ₃	UTP, ATP, APPCP	8-PST	prejunctional-rat caudal artery, vas deferens

^a For structures of P₁ ligands refer to text and Tables II and IV. ^b Receptors also defined on basis of affinity/potency of ligands. See ref 19 for further details. ^c CV1674 [2-[4-(methoxy)phenyl]aminoadenosine] is more active than N⁶-methyladenosine at A_{2b} receptors and is inactive at A_{2b} receptors.¹⁸ ^d DIDS—4,4'-diisothiocyano-2,2'-stilbenedisulfonate.^{150,151} For details of SAR of P₂ receptors see ref 167.

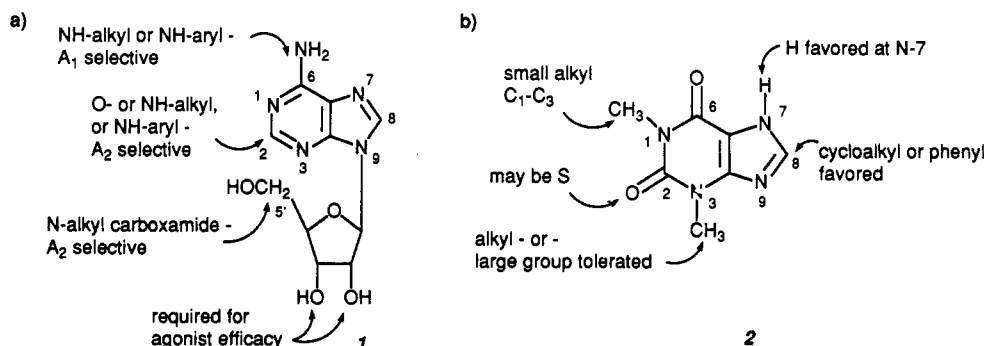


Figure 1. The structures of adenosine (1) and theophylline (2), showing the effects of structural modifications at various sites on receptor binding.

reperfusion.¹² While caffeine and theophylline represent prototypic, albeit weak, adenosine antagonists, second generation forms of these compounds with improved antagonist activity for use as cardiotonics, cognition enhancers or antiasthmatics have not been forthcoming despite considerable chemical effort.⁸

The reasons for the limited progress in adenosine therapeutics are several-fold and include the ubiquity of action of adenosine (and ATP) on a variety of diverse tissue systems, a paucity of receptor selective ligands that are orally bioavailable and soluble, lack of knowledge of disease states involving a purinergic etiology, and probably

most importantly, a failure to target adenosine agents in terms of unmet therapeutic need.¹³ Thus, agonists have been routinely targeted toward hypertension, an area where these agents have probable CNS and renal side effects and compare unfavorably with the many excellent and efficacious antihypertensive agents currently available in the clinic.¹³

In the present perspective, advances in knowledge related to adenosine function at the molecular level will be reviewed together with information on the structure-activity relationships for a number of pharmacophore series interacting with adenosine receptors. Therapeutic areas where improved adenosine ligands may represent potentially important therapeutic agents will also be indicated.

Adenosine Receptor Ligands

Development of Adenosine Agonists. Adenosine 1 has been extensively used as a probe for the study of

- (11) Lasley, R. D.; Rhee, J. W.; Van Wylen, D. G. L.; Mentzer, R. M., Jr. Adenosine A₁ receptor mediated protection of the globally ischemic isolated rat heart. *J. Mol. Cell. Cardiol.* 1990, 22, 39–47.
- (12) Pitarys, C. J., II; Virmani, R.; Vildibill, H. D.; Jackson, E. K.; Forman, M. B. Reduction of myocardial reperfusion injury by intravenous adenosine administered during the early reperfusion period. *Circulation* 1991, 83, 237–247.

- (13) Williams, M. Purinergic pharmaceuticals for the 1990s. *Nucleosides Nucleotides* 1991, 10, 1087–1089.

adenosine systems in mammalian tissues since the initial reports on the cardiovascular actions of the purine some 60 years ago.¹⁴ The metabolic lability of adenosine precluded its use as an antihypertensive agent in the early 1930s,^{15,16} but more stable analogues have been synthesized (Table II), focusing primarily on modifications of the N⁶-, 2-, and 5'-positions.⁶ Among these are N⁶-cyclohexyladenosine (CHA, 6), (R)-N⁶-(2-phenyl-1-methylethyl)-adenosine (R-PIA, 9), 2-chloroadenosine (2-CADO, 22) and N-ethyladenosine-5'-uronamide (NECA, 30). The availability of these agents provided the tools by which adenosine receptors have been classified into A₁, A_{2a}, and A_{2b} subtypes, on the basis of the pharmacology of radioligand binding^{17,18} (Table I). A_{1a}, A_{1b},¹⁹ and A₃²⁰ subtypes have also been proposed. Well-documented species differences in adenosine receptors^{21,22} have, however, provided a spurious basis for adenosine receptor classification,²³ which can only serve to complicate nomenclature issues. Structure-efficacy relationships for adenosine receptor ligands have not been well defined, especially in regard to partial agonist activity.^{24,25}

N⁶-substituted analogues of adenosine have generally proven to be A₁-receptor selective, with N⁶-cyclopentyladenosine (CPA, 3) and CHA (6) being 400–800-fold selective.⁶ N⁶-bicycloalkyladenosines are even more A₁ selective with N⁶-endo-norborn-2-yladenosine (S-ENBA, 7) being 4700-fold selective for the A₁ receptor.⁶ Combined substitutions at the N⁶- and 2-positions have yielded 2-chloro-CPA (CCPA, 4) which is 1500-fold A₁ selective.^{26,164} A computer-generated model of the N⁶ region of the A₁

receptor (Figure 2) has been shown to accurately predict the affinities of a number of N⁶-substituted adenosines.²⁷

The 5'-substituted adenosine analogue, NECA (30), has been extensively used to define tissue responses mediated by A₂ receptor activation.^{2,7} This analogue is however nonselective in its interactions with adenosine receptors, being approximately equipotent ($K_i \approx 10$ nM) at both A₁ and A₂ receptors.¹⁸ Ascribing effects elicited by NECA to A₂ receptor-mediated processes can only be validated if such effects are not seen with equivalent doses/concentrations of A₁ selective ligands such as CPA 3 or CHA 6. In the seminal A₂-receptor binding assay developed by Bruns and co-workers,¹⁸ the A₁ component of the binding profile of NECA was eliminated by the use of 50 nM CPA.

Other ribose modifications, especially at the 2' and 3' positions are generally not well tolerated at the binding site: 2' substitution abolishes affinity altogether, and an unsubstituted 3'-hydroxyl group is required for high efficacy.^{6,28}

A₂-selective adenosine agonists have been developed more recently. Although most N⁶-substituted adenosine derivatives are A₁ selective,²⁹ a series of N⁶-(2,2-diphenylethyl)-substituted adenosine analogues includes potent and A₂-selective compounds like CI 936 (15) and DPMA (16, N⁶-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine), the racemate of which is 30-fold selective for the A₂ receptor.³⁰ The design of this series was aided by a computer-generated model (Figure 2) of the N⁶ region of the A₂ receptor.³¹

The 2-(arylamino)adenosine analogue, CV 1808 (23)³² (Table II) while only moderately potent ($K_i \sim 100$ nM) at A₂ receptors has a modest 5-fold selectivity versus the A₁ receptor.¹⁸ Evaluation of 2-position modifications of 30 led to the identification of CGS 21680 (31)³³ which is

- (14) Drury, A. N.; Szent-Györgyi, A. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *J. Physiol. (Lond)* 1929, 68, 213–237.
- (15) Honey, R. M.; Ritchie, W. T.; Thompson, W. A. R. The action of adenosine upon the human heart. *Quart. J. Med.* 1930, 23, 485–490.
- (16) Olsson, R. A.; Pearson, J. D. Cardiovascular purinoceptors. *Physiol. Rev.* 1990, 70, 761–845.
- (17) Hamprecht, B.; Van Calcar, D. *Trends Pharmacol. Sci.* 1985, 6, 153–154.
- (18) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. Characterization of the A₂ adenosine receptor labeled by [³H]NECA in rat striatal membranes. *Mol. Pharmacol.* 1986, 29, 331–346.
- (19) Gustafsson, L. E.; Wiklund, C. U.; Wiklund, N. P.; Stenius, L. Subclassification of neuronal adenosine receptors. In *Purines in Cellular Signaling. Targets for New Drugs*; Jacobson, K. A., Daly, J. W., Manganiello, V., Eds.; Springer Verlag: New York, 1990; pp 200–205.
- (20) Ribeiro, J. A.; Sebastião, A. M. Adenosine receptors and calcium: basis for proposing a third (A₃) adenosine receptor. *Prog. Neurobiol.* 1986, 26, 179–209.
- (21) Ferkany, J. W.; Valentine, H. L.; Stone, G. A.; Williams, M. Adenosine A₁ receptors in mammalian brain: species differences in their interactions with agonists and antagonists. *Drug Dev. Res.* 1986, 9, 85–93.
- (22) Stone, G. A.; Jarvis, M. F.; Sills, M. A.; Weeks, B.; Snowhill, E. W.; Williams, M. Species differences in high-affinity adenosine A₂ binding sites in striatal membranes from mammalian brain. *Drug Dev. Res.* 1988, 15, 31–46.
- (23) Weiner, H. L.; Craddock-Royal, B.; Maaynai, S. Tentative subclassification of the adenosine A₁ (AD A₁) receptor in mammalian hippocampus. *Abstr. Soc. Neurosci.* 1990, 16, 33.8.
- (24) Bazil, C. W.; Minneman, K. P. An investigation of the low intrinsic activity of adenosine and its analogs at low affinity (A₂) adenosine receptors in rat cerebral cortex. *J. Neurochem.* 1986, 47, 547–553.
- (25) Lohse, M.; Klotz, K. N.; Schwabe, U. Mechanism of A₂ adenosine receptor activation. I. Blockade of A₂ adenosine receptors by photoaffinity labeling. *Mol. Pharmacol.* 1991, 39, 517–523.

- (26) Lohse, M. J.; Klotz, K. N.; Schwabe, U.; Cristalli, G.; Vittori, S.; Grifantini, M. 2-Chloro-N⁶-cyclopentyladenosine: a highly selective agonist at A₁ adenosine receptors. *Naunyn Schmiedeberg's Arch. Pharmacol.* 1988, 337, 687–689.
- (27) van Galen, P. J. M.; Leusen, F. J. J.; IJzerman, A. P.; Soudijn, W. Mapping the N⁶-region of the adenosine A₁ receptor with computer graphics. *Eur. J. Pharmacol.-Mol. Pharmacol. Sect.* 1989, 172, 19–27.
- (28) Taylor, M. D.; Moos, W. H.; Hamilton, H. W.; Szotek, D. S.; Patt, W. C.; Badger, E. W.; Bristol, J. A.; Bruns, R. F.; Heffner, T. G.; Mertz, T. E. Ribose-modified adenosine analogues as adenosine receptor agonists. *J. Med. Chem.* 1986, 29, 346–53.
- (29) Daly, J. W.; Padgett, W.; Thompson, R. D.; Kusachi, S.; Bugni, W. J.; Olsson, R. A. Structure-activity relationships for N⁶-substituted adenosines at a brain A₁-adenosine receptor with a comparison to an A₂-adenosine receptor regulating coronary blood flow. *Biochem. Pharmacol.* 1986, 35, 2467–2481.
- (30) Trivedi, B. K. Structure-activity relationships for adenosine agonists. In *Purines in Cellular Signaling. Targets for New Drugs*; Jacobson, K. A., Daly, J. W., Manganiello, V., Eds.; Springer Verlag: New York, 1990; pp 136–145.
- (31) Ortwin, D. F.; Bridges, A. J.; Humblet, C.; Trivedi, B. K. Adenosine agonists. Characterization of the N⁶-region of the adenosine A₂ receptor via molecular modeling techniques. In *Purines in Cellular Signaling. Targets for New Drugs*; Jacobson, K. A., Daly, J. W., Manganiello, V., Eds.; Springer Verlag: New York, 1990; pp 152–157.
- (32) Kawazoe, K.; Matsumoto, M.; Tanabe, S.; Fujiwara, M.; Yanagimoto, M.; Hirata, M.; Kakiuchi, K. Coronary and cardiohemodynamic effects of 2-phenyl-aminoadenosine (CV1808) in anaesthetized dogs and cats. *Arzneim. Forsch.* 1980, 30, 1083–1087.
- (33) Hutchison, A. J.; Williams, M.; de Jesus, R.; Yokoyama, R.; Oei, H. H.; Ghai, G. R.; Webb, R. L.; Zoganas, H. C.; Stone, G. A.; Jarvis, M. F. 2-(Arylalkylamino)adenosin-5'-uronamides: a new class of highly selective adenosine A₂ receptor ligands. *J. Med. Chem.* 1990, 33, 1919–1924.

Table II. Structures of Adenosine Agonists and Their Affinities at A₁ and A₂ Adenosine Receptors^a

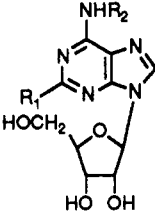



















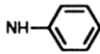
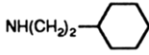
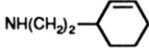
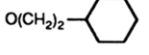
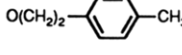
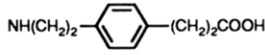
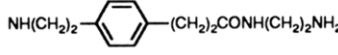


					
compd	R ₂	R ₁ ^f	K _i		A ₂ /A ₁
			A ₁	A ₂	
3			0.59	462	780
4		Cl	0.6	950	1500
5		NH ₂	8.3	6100	730
6			1.3	514	400
7			0.3	1390	4600
8			7.0	4920	700
9			1.2	124	100
10			0.94 ^b	—	—
11			4.6	663	140
12			0.7 ^b	—	—
13			4.1	—	—
14			2.0 ^b	—	—
15			6.8	25	3.7
16			142	4.4	0.31
17			5.2	4.9	0.94
18			0.85	210	250
19			0.22	8400	38000
20			0.47	191	410
21			7.1	—	—

Table II (Continued)

compd	R ₁	R ₅	K _i		A ₂ /A ₁
			A ₁	A ₂	
R ₅ = CH ₂ OH					
22	Cl		9.3	63	6.8
23			560	119	0.21
24			12000	22	0.0018
25			2700	13	0.0048
26			1500	22	0.014
27			48	11	0.22
28	C≡C—(CH ₂) ₃ CH ₃		147	4.1	0.028
29	C≡C—(CH ₂) ₅ CH ₃		211	12	0.057
R ₅ = CONHCH ₂ CH ₃					
30	H		6.3	10.3	1.6
31			2600	15	0.0058
32			240	5.7	0.024
33			—	1.0 ^d	—
34			280	7.1 ^e	0.025

^a Unless noted, K_i values for binding experiments (using [³H]CHA or [³H]PIA at A₁ or [³H]NECA at A₂, in rat brain unless indicated) are given in nM; data is from refs 6, 18, 29, 30, 33, 34, 43, and 167. ^b K_d value for radioligand binding to rat brain membranes. ^c FITC = fluorescein isothiocyanate, which forms a thiourea linkage. ^d Versus [³H]PIA. ^e Versus [¹²⁵I]PAPA-APEC in bovine striatum. ^f R₁ = H, unless noted.

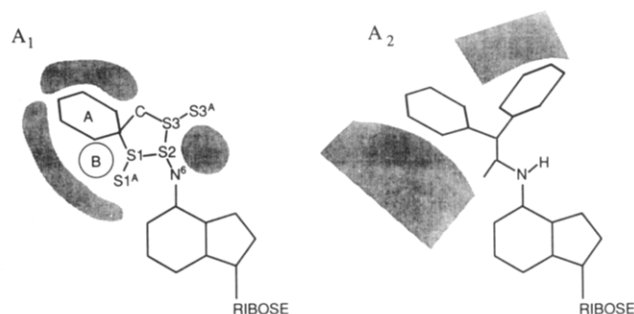


Figure 2. Computer-generated models of the N⁶ regions of adenosine A₁ and A₂ receptors. For the A₁ model, areas are indicated where hydrophobic substituents may lead to enhanced affinity. These are designated S1, S1^A, S2, S3, S3^A, A(ryl), B(ulk), and C(cycloalkyl). In both models, shaded areas indicate the receptor boundaries (adapted from ref 27 and 31).

140-fold selective for the A₂ receptor, with a K_i value of 21 nM. CGS 22492 2-[(cyclohexylethyl)amino]adenosine (24) and the 2-cyclohexenyl analogue, CGS 22989 (25), two monosubstituted adenosine derivatives related to CV 1808, are 530- and 210-fold selective, respectively, for the A₂ receptor with K_i values in the range of 13–22 nM.³⁴ CGS

21680 has been derivatized as iodo-PAPA-APEC (33, Table II) and used as a probe to explore the A_{2a} receptor (see below).³⁵ The availability of [³H]CGS 21680³⁶ was instrumental to the identification of the cloned A₂ receptor.⁴

Further structural modifications at the 2-position led to development of the 2-alkoxyadenosines^{37,38} and the 2-alkynyladenosines.³⁹ 2-(2-Cyclohexylethoxy)adenosine

- (34) Francis, J. E.; Webb, R. L.; Ghai, G. R.; Hutchison, A. J.; Moskal, M. A.; de Jesus, R.; Yokoyama, R.; Rovinski, S. L.; Contrado, N.; Dotson, R.; Barclay, B.; Stone, G. A.; Jarvis, M. F. Highly selective adenosine A₂ receptor agonists in a series of N-alkylated 2-aminoadenosines. *J. Med. Chem.* 1991, 34, 2570–2579.
- (35) Barrington, W. W.; Jacobson, K. A.; Hutchison, A. J.; Williams, M.; Stiles, G. L. Identification of the A₂ adenosine receptor binding subunit by photoaffinity crosslinking. *Proc. Natl. Acad. Sci. U.S.A.* 1989, 86, 6572–6576.
- (36) Jarvis, M. F.; Schulz, R.; Hutchison, A. J.; Do, U. H.; Sills, M. A.; Williams, M. [³H] CGS 21680, a selective A₂-adenosine receptor agonist ligand directly labels A₂-receptors in rat brain. *J. Pharmacol. Exp. Ther.* 1989, 251, 888–893.
- (37) Ueda, M.; Thompson, R. D.; Arroyo, L. H.; Olsson, R. A. 2-Alkoxyadenosines: potent and selective agonists at the coronary artery A₂ adenosine receptor. *J. Med. Chem.* 1991, 34, 1334–1339.

(26) (CHEA; Table II) has an EC_{50} value of 1 nM at the A_2 receptor in heart modulating coronary vasodilation, resulting in an 8700-fold selectivity for the A_2 receptor³⁷ in this functional model of adenosine receptor selectivity. The 2-alkoxyadenosine derivative 2-[2-(4-methylphenyl)ethoxy]adenosine (27) (MPEA) is 44 000-fold selective for the coronary A_2 receptor with an EC_{50} value of 190 pM.³⁸ Evaluation of these 2-alkoxy compounds in binding assays in rat brain tissue³⁹ indicated that while both are active at the A_2 receptor (MPEA, K_i = 11 nM; CHEA, K_i = 22 nM), MPEA is only 5-fold A_2 selective while CHEA is 73-fold selective, a marked contrast to the 8700- and 44 000-fold selectivity for the A_2 receptor seen in the guinea pig tissue models.³⁷⁻³⁹ These results do not appear to be species dependent since affinity in guinea pig tissues is very comparable with that seen in rat brain binding assays.

The 2-alkynyl derivatives, 2-hexynyladenosine (2-HNA, 28) and 2-octynyladenosine (2-ONA; YT-146, 29), are potent A_2 agonists (K_i values 4 and 12 nM) with 36- and 17-fold selectivity, respectively, for the A_2 receptor in binding assays.⁴⁰ In the spontaneously hypertensive rat (SHR), 2-HNA and 2-ONA are 390- and 260-fold selective for the A_2 receptor, mediating blood pressure lowering. Like 2-HNA and 2-ONA, the differences in selectivity between binding and functional test procedures for CGS 21680 are more modest⁴¹ than those reported for the alkoxyadenosines.^{37,38} Furthermore, the A_1 -selective agonist, CPA is considerably less selective in these functional assays than has been reported by numerous laboratories using binding assays. There is thus a considerable need for caution in comparing in vitro binding assay activity with classical functional paradigms. The latter determine efficacy as well as activity and depend in large part on the choice of tissue used, an issue that has caused concern within the context of receptor classification⁴² and requires further study in regard to the delineation of adenosine receptor function. This issue is typified by recent biochemical data on 5'-methylthioadenosine (MTA),⁴³ a potent A_1 -receptor agonist (EC_{50} = 90 nM), with weak partial agonist activity at the PC12 A_{2a} receptor (EC_{50} = 8.9 μ M) and antagonist activity at the human VC13 cell A_{2b} receptor (K_i = 8.2 μ M).

Few purine ring modifications can be tolerated, but 1-deazaadenosines retain high affinity. Thus, 1-deaza-2-chloro- N^8 -cyclopentyladenosine is a potent and A_1 -selective agonist.⁴⁴

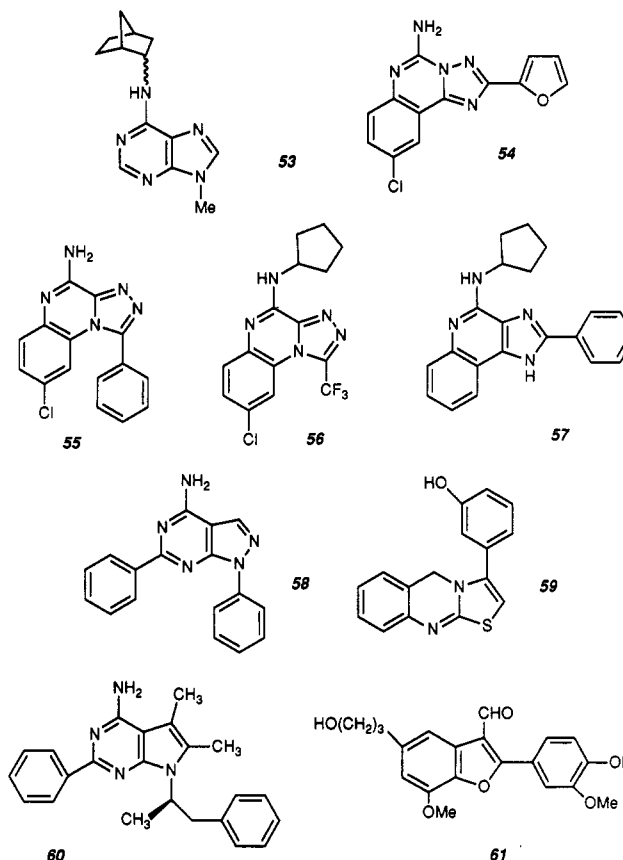


Figure 3. The structures of non-xanthine adenosine antagonists. (See text and Table III for description.)

Development of Adenosine Antagonists. Structures reported as adenosine receptor antagonists are shown in Table III. The prototypic adenosine receptor antagonists were the xanthines, theophylline (2), and caffeine (35).⁴⁵ Since then a multitude of xanthines has been synthesized and studied as antagonists at A_1 and A_2 receptors.⁸ Numerous structurally diverse non-xanthine antagonists (Figure 3) have also been identified during the last decade, many of which have only poor to moderate affinity and are not well defined in terms of SAR. Three classes of related heterocycles comprise more active entities and are termed the "tricyclic" non-xanthine antagonists. These include the triazoloquinazolines,⁴⁶ the triazoloquinoxalines,^{47,48} and the imidazoquinolines.⁴⁹ Other potent and A_1 -selective antagonists have been derived from

- (38) Ueeda, M.; Thompson, R. D.; Arroyo, L. H.; Olsson, R. A. 2-Aralkoxyadenosines: potent and selective agonists at the coronary artery A_2 adenosine receptor. *J. Med. Chem.* 1991, 34, 1340-1344.
- (39) Ueeda, M.; Thompson, R. D.; Padgett, W. L.; Secunda, S.; Daly, J. W.; Olsson, R. A. Cardiovascular actions of adenosines, but not adenosine receptors, differ in rat and guinea pig. *Life Sci.* 1991, 49, 1351-1358.
- (40) Abiru, T.; Yamaguchi, T.; Watanabe, Y.; Kogi, K.; Aihara, K.; Matsuda, A. The antihypertensive effect of 2-alkynyladenosines and their selective affinity for adenosine A_2 -receptors. *Eur. J. Pharmacol.* 1991, 196, 69-76.
- (41) Hutchison, A. J.; Webb, R. L.; Oei, H. H.; Ghai, G. R.; Zimmerman, M. B.; Williams, M. CGS 21680C, an A_2 selective adenosine agonist with selective hypotensive activity. *J. Pharmacol. Exp. Ther.* 1989, 251, 47-55.
- (42) Black, J. W. Should we be concerned about the state of hormone receptor classification? In *Perspectives on Receptor Classification*; Black, J. W., Jenkinson, D. H., Gerskowitch, V. P., Eds.; Liss: New York, 1987; pp 11-15.
- (43) Daly, J. W.; Padgett, W. L. Agonist activity of 2- and 5'-substituted adenosine analogs and their N^8 -cycloalkyl derivatives at A_1 - and A_2 -adenosine receptors coupled to adenylate cyclase. *Biochem. Pharmacol.* 1992, in press.

- (44) Cristalli, G.; Franchetti, P.; Grifantini, M.; Vittori, S.; Klotz, K. N.; Lohse, M. J. Adenosine receptor agonists: synthesis and biological evaluation of 1-deaza analogues of adenosine derivatives. *J. Med. Chem.* 1988, 31, 1179-1183.
- (45) Sattin, A.; Rall, T. W. The effect of adenosine and adenine nucleotides on the cyclic adenosine 3',5'-phosphate content of guinea pig cerebral slices. *Mol. Pharmacol.* 1970, 6, 13-23.
- (46) Francis, J. E.; Cash, W. D.; Psychoyos, S.; Ghai, G.; Wenk, P.; Friedmann, R. C.; Atkins, C.; Warren, V.; Furness, P.; Hyun, J. L.; et al. Structure-activity profile of a series of novel triazoloquinazoline adenosine antagonists. *J. Med. Chem.* 1988, 31, 1014-20.
- (47) Trivedi, B. K.; Bruns, R. F. [1,2,4]-Triazolo[4,3-a]-quinoxalin-4-amines: a new class of A_1 receptor selective adenosine antagonists. *J. Med. Chem.* 1988, 31, 1011-1014.
- (48) Sarges, R.; Howard, H. R.; Browne, R. G.; Lebel, L. A.; Seymour, P. A.; Koe, B. K. 4-Amino[1,2,4]triazolo[4,3-a]-quinoxalines. A novel class of potent adenosine receptor antagonists and potential rapid-onset antidepressants. *J. Med. Chem.* 1990, 33, 2240-2254.

Table III. Classes of Adenosine Receptor Antagonists

chemical class	example
adenines	N-0861 (53), ⁵² N ⁶ -butyl-8-phenyladenine ⁶¹
adenosines, ribose-modified	5'-deoxy-5'-methylthioadenosine ¹⁵²
barbiturates	DMBB ¹⁵³
benzimidazoles	1-methyl-2-phenylimidazole ¹⁵⁴
benzo[1,2-c:5,4-c']dipyrzoles	1,7-dihydro-3,5,8-trimethylbenzo[1,2-c:5,4-c']dipyrzole ¹⁵⁵
benzo[b]furanes	5-(3-hydroxypropyl)-7-methoxy-2-(3'-methoxy-4'-hydroxyphenyl)-3-benzo[b]-furancarbaldehyde ⁵³
benzo[g]pteridine-2,4-diones	alloxazine ⁵⁷
β -carboline	β -carboline-3-ethylcarboxylate ¹⁵⁴
7-deazadenines	(see pyrrolo[2,3-d]pyrimidines)
dibenz[b,f]azepines	carbamazepine ¹⁵⁶
imidazo[1,2-a]pyrazines	SC-12 ¹⁵⁴
imidazo[4,5-b]pyridines	sulmazole ¹⁵⁴
imidazo[4,5-c]quinolines	CPPIQA (55) ⁴⁹
imidazo[4,5-e][1,4]diazepine-5,8-diones	4,7-dipropyl-1-benzyl-4,5-tetrahydro-6H-imidazo[4,5-e][1,4]diazepine-5,8-dione ¹⁵⁷
imidazo[4,5-f]quinazoline-7,9-diones	prox-benothophylline ⁵⁹
imidazo[4,5-g]quinazoline-6,8-diones	lin-benothophylline ⁵⁹
imidazolidines	DPI ¹⁵⁴
pteridine-2,4-diones	lumazine ⁵⁷
pyrazolo[3,4-b]pyridines	cartazolate, ethazolate, tracazolate ¹⁵⁸
pyrazolo[3,4-d]pyrimidines	DJB-KK, ¹⁵⁹ APPP (57) ⁶¹
pyrazolo[4,3-d]pyrimidines	5-(2-amino-4-chlorophenyl)-1,3-dimethylpyrazolo[4,3-d]pyrimidin-7-one ¹⁶⁰
pyrazolo[4,3-c]quinolines	CGS 8216 ⁶⁹
pyrimidines	amiloride ¹⁶¹
pyrimido[4,5-b](tetrahydro)indoles	4-amino-9-phenyl-9H-pyrimido[4,5-b]indole ⁵⁰
pyrrolo[2,3-d]pyrimidines (7-deazaadenines)	ADPEP (55) ⁵⁰
quinazolines	ADQZ ⁶¹
thiazolo[2,3-b]quinazolines	HTQZ (58) ⁶¹
thiazolo[4,5-d]pyrimidine-5,7-diones	4,6-dimethyl-2-phenyl-4,5,6,7-tetrahydrothiazolo[4,5-d]pyrimidine-5,7-dione ¹⁵⁴
thiazolo[5,4-d]pyrimidine-5,7-diones	DJB-W ¹⁵⁹
[1,2,4]triazolo[4,3-b]pyridazines	CL 218872 ¹⁵⁴
[1,2,4]triazolo[1,5-c]quinazolines	CGS 15943 (51) ⁴⁶
[1,2,4]triazolo[4,3-a]quinoxalines	CP 66713 (53), CP 68247 (54) ⁴⁶
xanthines	caffeine (33), theophylline (2), CPX (39), ⁵⁸ PACPX (48), ¹⁸ XAC (50) ⁶²
xanthines, benzo-separated	(see imidazo[4,5]quinazolinodiones)
xanthines, mesoionic	anhydro-6,8-di-n-propyl-5-hydroxy-7-oxothiazolo[3,2-a]pyrimidinium hydroxide ¹⁵⁴
xanthine-7-ribosides	1,3-dibutylxanthine-7-riboside ¹⁶²

adenine and include the 2-phenyl-7-deazaadenines such as ADPEP (60) (A_1 , 4.7 nM; A_2 , 3710 nM)⁵⁰ and the N⁶-substituted 9-methyladenines,⁵¹ including N-0861 53 [(\pm)-N⁶-endo-norbornyl-9-methyladenine (A_1 , 10 nM; A_2 , 6100 nM in bovine brain)].⁵² The structures and binding activity of some relevant compounds are shown in Figure 2 and Table IV.

In general, for high affinity at adenosine receptors the following criteria have to be fulfilled: adenosine receptor antagonists are (i) flat, (ii) aromatic or π -electron rich, (iii) nitrogen-containing heterocycles, often 6:5-fused. Hydrophobic substituents may greatly enhance affinity, whereas hydrophilic substituents are usually not tolerated, which renders many of the high-affinity antagonists quite insoluble in water. One notable exception to the general pattern is the naturally occurring benzo[b]furan (61) (containing an O rather than an N 6:5-fused heterocycle), which has an affinity of 17 nM in bovine A_1 binding⁵³ and may provide an important new lead to further non-

xanthine, non-nitrogen-containing adenosine receptor antagonists.

The SAR for xanthines at adenosine receptors is summarized in Figure 1b. Theophylline (1,3-dimethylxanthine) has only moderate affinity and is essentially nonselective (A_1 , 8.5 μ M; A_2 , 25 μ M).¹⁸ Increasing chain length at positions 1 and 3 increases affinity. 1,3-Dipropyl substitution is optimal, resulting in a 19-fold increase in affinity at A_1 receptors.⁵⁴ In the 3-position, the A_1 receptor has considerably more bulk tolerance than the A_2 receptor. For example, BW-A844U (1-propyl-3-(4-amino-3-iodophenethyl)-8-cyclopentylxanthine, 44) is 8700-fold A_1 selective, whereas the parent compound, CPX (1,3-dipropyl-8-cyclopentylxanthine, 39) is equipotent yet only 740-fold selective.^{55,56} Substitutions at the 7-position are usually not favorable^{57,58} while 9-substitutions are detri-

(49) van Galen, P. J. M.; Nissen, P.; van Wijngaarden, I.; IJzerman, A. P.; Soudijn, W. 1H-imidazo[4,5-c]quinolin-4-amine: novel non-xanthine adenosine antagonists. *J. Med. Chem.* 1991, 34, 1202-1206.

(50) Müller, C. E.; Hide, I.; Daly, J. W.; Rothenhäusler, K.; Eger, K. 7-Deaza-2-phenyladenines: structure-activity relationships of potent A_1 selective adenosine receptor antagonists. *J. Med. Chem.* 1990, 33, 2822-2828.

(51) Ukena, D.; Padgett, W. L.; Hong, O.; Daly, J. W.; Daly, D. T.; Olsson, R. A. N⁶-substituted 9-methyladenines: a new class of adenosine receptor antagonists. *FEBS Lett.* 1987, 215, 203-208.

(52) May, J. M.; Martin, P. L.; Miller, J. R. N-0861: A selective A_1 -adenosine receptor antagonist. *FASEB J.* 1991, 5, 1572.

(53) Yang, Z.; Hon, P. M.; Chui, K. Y.; Xu, Z. L.; Chang, H. M.; Lee, C. M.; Cui, Y. X.; Wong, H.; Poon, C. D.; Fung, B. M. Naturally occurring benzofuran: isolation, structure elucidation and total synthesis of 5-(3-Hydroxypropyl)-7-methoxy-2-(3'-methoxy-4'-hydroxyphenyl)-3-Benzo[b]furancarbaldehyde, a novel adenosine- A_1 receptor ligand isolated from *Salvia Miltiorrhiza bunge* (Danshen). *Tetrahedron Lett.* 1991, 32, 2061-2064.

(54) Daly, J. W. Adenosine agonists and antagonists. In *Purines in Cellular Signaling. Targets for New Drugs*; Jacobson, K. A., Daly, J. W., Manganiello, V., Eds.; Springer Verlag: New York, 1990; pp 3-12.

(55) Patel, A.; Craig, R. H.; Daluge, S. M.; Linden, J. ¹²⁵I-BW-A844U, an antagonist radioligand with high affinity and selectivity for adenosine A_1 receptors, and ¹²⁵I-azido-BW-A844U, a photoaffinity label. *Mol. Pharmacol.* 1988, 33, 585-591.

Table IV. Affinities of Some Relevant Adenosine Antagonists

compd	R ₁	R ₃	R ₈	R ₇ ^h	A ₁ , nM ^a	A ₂ , nM ^b	A ₂ /A ₁
2 theophylline	Me	Me	Xanthines		8500	25000	3.0 ¹⁸
35 caffeine	Me	Me	H	Me	29000	48000	1.7 ¹⁸
36 DMPX	CH≡CCH ₂	Me	H	Me	22000	9600	0.44 ⁶⁰
37 CHC ⁱ	Me	Me	cyclohexyl	Me	2.0 ^c	0.19 ^d	0.095 ¹⁸⁶
38 CPT	Me	Me	cyclopentyl		11	1400	130 ¹⁸
39 CPX	Pr	Pr	cyclopentyl		0.46	340	740 ⁶⁶
40 KFM19	Pr	Pr			10.5	1510	144 ⁶⁷
41	Pr	Pr			8 ^c	20 ^d	2.5 ⁶⁶
42 KF15372	Pr	Pr			3 ^e	430	140 ⁶⁵
43 KW-3902	Pr	Pr			1.3	380	290 ⁶⁸
44 I-BW-A844U	Pr		cyclopentyl		0.23	2000	8700 ⁶⁵
45	Pr	Pr			6.9	160	23 ⁷⁰
46 8-PT	Me	Me	phenyl		86	850	9.9 ¹⁸
47 8-PST	Me	Me	p-(SO ₃ H)phenyl		2600	1500	5.8 ^{18,64}
48 PACPX	Pr	Pr			2.5	92	37 ¹⁸
49 XCC	Pr	Pr			58	2200	57 ⁶²
50 XAC	Pr	Pr			1.2	60	50 ⁶²
51 I-PAPA-XAC	Pr	Pr			0.1 ^f	-	ref 108
52 m-DITC-XAC	Pr	Pr			2.4	343	144 ¹¹³
Non-Xanthines (see Figure 2 for structures)							
53 N-0861					10 ^g	6100 ^g	610 ⁵²
54 CGS 15943					21	3.3	0.16 ⁴⁸
55 CP 66713					270	21	0.078 ⁴⁸
56 CP 68247					28	>100000	>3000 ⁴⁸
57 CPPIQA					10	450	45 ⁴⁵
58 APPP					23	35	1.5 ⁶¹
59 HTQZ					3100	120	0.04 ⁶¹
60 ADPEP					4.7	3700	790 ⁶⁰

^a K_i or IC₅₀ values in nM, displacement of [³H]PIA or [³H]CHA in rat brain cortical membranes, unless indicated otherwise. ^b Displacement of [³H]NECA in rat brain striatal membranes (in the presence of 50 nM CPA), unless indicated otherwise. ^c K_i for antagonism of adenylate cyclase inhibition in rat adipocytes. ^d K_i for antagonism of adenylate cyclase activation in human platelets. ^e K_i or IC₅₀ value for displacement of [³H]CHA in guinea pig forebrain membranes. ^f K_d in bovine brain cortical membranes. ^g In bovine brain. ^h R₇ = H, unless noted. ⁱ Not selective in adenylate cyclase assays.

mental to affinity.^{57,59} Although certain alkyl modifications at the 1-, 3-, and 7-positions (e.g. DMPX, 36) may favor A₂ affinity to some extent,⁶⁰ no xanthine (or non-

xanthine) antagonists with appreciable potency and selectivity at A₂ receptors have yet been reported. Thus the lack of potent, selective A₂-receptor antagonists remains

a major obstacle in the characterization of the function of adenosine A₂ receptors.

Phenyl or cycloalkyl substitutions in the 8-position may yield highly potent and, in many cases, also highly A₁-selective antagonists including PACPX (48),^{8,18} CPX (39),⁶¹ and XAC (50).^{62,63} These compounds have proven valuable as radioligands and/or pharmacological tools, and 8-(*p*-sulfophenyl)theophylline (8-PST, 47)⁶⁴ is a useful peripheral acting antagonist. Some interesting newer 8-substituted xanthines include 8-(dicyclopropylmethyl)-1,3-dipropylxanthine (KF15372, 42) which is even more potent and A₁ selective than CPX in guinea pig forebrain.⁶⁵ 8-[*trans*-4-(Acetamidomethyl)cyclohexyl]-1,3-dipropylxanthine (41) has surprisingly high potency at A₂ receptors, unlike other cycloalkylxanthines (K_i for antagonism of adenylate cyclase activity in rat adipocytes (A₁, 8 nM) or human platelets (A₂, 20 nM)).⁶⁶ KFM 19 (40, (±)-8-(3-oxocyclopentyl)-1,3-dipropylxanthine) is a potent A₁-selective compound with sufficient aqueous solubility to display good bioavailability. It is currently under development as a potential cognition enhancer.⁶⁷ KW 3902 (43, 8-noradamant-3-yl-1,3-dipropylxanthine, A₁ = 1.3 nM; A₂ = 380 nM) has potent diuretic and renal protective activity.⁶⁸

One of the first non-xanthine adenosine receptor antagonists identified was CGS 8216,⁶⁹ a pyrazoloquinoline

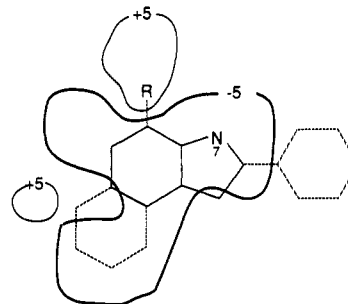


Figure 4. Computer-generated model of the antagonist binding site of the adenosine A₁ receptor. Indicated is the molecular electrostatic potential (at the +5 and -5 kcal/mol level) in the plane of the 6:5-fused heterocycle that is common to xanthines and many non-xanthine adenosine antagonists. R (corresponds to the N⁶ position of adenosine) and dotted lines indicate regions where hydrophobic substitution may enhance affinity. N7 is thought to act as a hydrogen bond acceptor (adapted from ref 49).

whose predominant activity was at the benzodiazepine receptor. Subsequent SAR work on this novel heterocycle led to the identification of the triazoloquinazoline, CGS 15943 (54), a potent adenosine receptor antagonist with 7-fold selectivity and an IC₅₀ of 3 nM at the A₂ receptor.⁴⁶ CGS 15943 while under development as a potential antiasthmatic was found to be a potent skin irritant and was discontinued.

Another series of tricyclic antagonists, the triazoloquinoxalines,⁴⁸ which for a time were in clinical trials as antidepressants, were later found to be adenosine antagonists, including, in dependence of the ring substitutions, both A₂-receptor-selective adenosine antagonists, such as CP 66713 (55) and some highly A₁-receptor-selective antagonists, such as CP 68247 (56).⁴⁸

A third series of tricyclic non-xanthine antagonists was developed on the basis of a computer model (Figure 4) for the steric, electrostatic, and hydrophobic features of A₁ receptor antagonists.⁴⁹ This model, which assumes that xanthines bind to the receptor backward, i.e. the purine ring of xanthines overlaps the purine ring of adenosine but is upside down, identifies regions with a distinct pattern of negative and positive electrostatic potential in antagonists, as well as regions where hydrophobic substituents may greatly enhance affinity. Furthermore, it is hypothesized that a nitrogen atom at the position corresponding to N7 in adenosine may act as a hydrogen-bond acceptor. A series of imidazoquinolines, designed and synthesized to test this model, resulted in some potent adenosine antagonists, including CPPIQA (57) (A₁, 10 nM; A₂, 450 nM).⁴⁹ A second computer-generated model of the antagonist binding site of the adenosine receptor assumes that N⁶ substituents of agonists and C8 substituents of xanthine antagonists bind to the same region of the receptor.⁷⁰ This model has correctly predicted the receptors' preference for the *R*-isomer of 8-(2-phenyl-1-methyl-

- (56) Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hartman, J. D.; Hays, S. J.; Huang, C. C. Binding of the A₁-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1987, 335, 59-63.
- (57) Bruns, R. F. Adenosine antagonism by purines, pteridines and benzopteridines in human fibroblasts. *Biochem. Pharmacol.* 1981, 30, 325-333.
- (58) Schwabe, U.; Ukena, D.; Lohse, M. J. Xanthine derivatives as antagonists at A₁ and A₂ adenosine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1985, 330, 212-221.
- (59) Schneller, S. W.; Ibay, A. C.; Christ, W. J.; Bruns, R. F. Linear and proximal benzo-separated alkylated xanthines as adenosine-receptor antagonists. *J. Med. Chem.* 1989, 32, 2247-2254.
- (60) Daly, J. W.; Padgett, W. L.; Shamim, M. T. Analogues of caffeine and theophylline: effect of structural alterations on affinity at adenosine receptors. *J. Med. Chem.* 1986, 29, 1305-1308.
- (61) Bruns, R. F.; Davis, R. E.; Ninteman, F. W.; Poschel, B. P. H.; Wiley, J. N.; Heffner, T. G. Adenosine antagonists as pharmacological tools. In *Adenosine and Adenine Nucleotides. Physiology and Pharmacology*; Paton, D. M., Ed.; Taylor & Francis: London, 1988; pp 39-49.
- (62) Jacobson, K. A.; Kirk, K. L.; Padgett, W. L.; Daly, J. W. Functionalized congeners of 1,3-dialkylxanthines: preparation of analogues with high affinity for adenosine receptors. *J. Med. Chem.* 1985, 28, 1334-1340.
- (63) Jacobson, K. A.; Kirk, K. L.; Padgett, W. L.; Daly, J. W. A functionalized congener approach to adenosine receptor antagonists: amino acid conjugates of 1,3-dipropylxanthine. *Mol. Pharmacol.* 1986, 29, 26-133.
- (64) Daly, J. W.; Padgett, W.; Shamim, M. T.; Butts, L. P.; Waters, J. 1,3-Dialkyl-8-(*p*-sulfophenyl)-xanthines: potent water-soluble antagonists for A₁- and A₂-adenosine receptors. *J. Med. Chem.* 1985, 28, 487-492.
- (65) Shimada, J.; Suzuki, F.; Nonaka, H.; Karasawa, A.; Mizumoto, H.; Ohno, T.; Kubo, K.; Ishii, A. 8-(Dicyclopropylmethyl)-1,3-dipropylxanthine: a potent and selective adenosine A₁ antagonist with renal protective and diuretic activities. *J. Med. Chem.* 1991, 34, 466-469.
- (66) Katsushima, T.; Nieves, L.; Wells, J. N. Structure-activity relationships of 8-cycloalkyl-1,3-dipropylxanthines as antagonists of adenosine receptors. *J. Med. Chem.* 1990, 33, 1906-1910.
- (67) Schingnitz, G.; Küfner-Mühl, U.; Ensinger, H.; Lehr, E.; Kuhn, F. J. Selective A₁-antagonists for treatment of cognitive deficits. *Nucleosides Nucleotides* 1991, 10, 1067-1076.

- (68) Suzuki, F.; Shimada, J.; Mizumoto, H.; Nonaka, H.; Ishii, A.; Karasawa, A.; Kubo, K. KW-3902: A potent and selective adenosine A₁ antagonist with renal protective and diuretic activity. *Book of Abstracts*, National Meeting of the American Chemical Society, New York City, NY, Aug 25-30; American Chemical Society: Washington, DC, 1991; Abstract MEDI112.
- (69) Czernik, A. J.; Petrack, B.; Kalinsky, H. J.; Psychoyos, S.; Cash, W. D.; Tsai, C.; Rinehart, R. K.; Granat, F. R.; Lovell, R. A.; Brundish, D. E.; Wade, R. CGS 8216: receptor binding characteristics of a potent benzodiazepine antagonist. *Life Sci.* 1982, 30, 363-372.

ethyl)xanthines, e.g. 45, analogous to its preference for R-PIA. Further studies will be required to reconcile these two seemingly incompatible models.

Similarities in the shape and charge distribution of the tricyclic antagonists suggest a common binding mode, although the SAR's differ to some extent. In each case, the basic ring structure is nonselective or slightly A₂ selective, but this may be altered by various substitutions. Whereas in the triazoloquinoxaline series substitution at the exocyclic amino group may impart very high A₁ affinity and selectivity (e.g. CP-68,247 56),⁴⁸ analogous substitution in the triazoloquinazolines often diminishes rather than enhances A₁ affinity.⁴⁷ On the other hand, the 2-furyl group is essential for the high affinity of CGS 15943,⁴⁶ but analogous substitution in the triazoloquinoxaline series is not feasible because of the presence of a tertiary nitrogen atom in the corresponding position. Interestingly, in the imidazoquinoline series substituents at *both* positions can enhance A₁ affinity considerably, although the effects are not necessarily additive.⁴⁹

This lack of additivity has been shown to occur in a number of cases in both adenosine agonists and antagonists. Explanations for this phenomenon include (i) induction of a conformational change in the receptor by one substituent, thereby altering the binding environment for the second substituent, (ii) dissimilar or even multiple binding modes for similar compounds, (iii) direct interactions between nearby sites, and (iv) a "loose fit" concept, which assumes that the heterocyclic antagonist pharmacophore is amply accommodated by the receptor; high affinity would then be achieved by a substituent that anchors the heterocycle to the receptor, at the same time hampering the optimal orientation for a substituent at another site.⁴⁹ The latter explanation agrees well with the seemingly endless array of structural variations of heterocycles that the receptor accepts as antagonists.

Solubility has been a major issue with both xanthine and non-xanthine heterocycle antagonists of the adenosine receptor and has led to anomalous biological results as in the case of CP-66,713 (55).⁴⁸ While 8-phenyl substitution in the xanthine pharmacophore increases receptor blocking activity, it also markedly decreases solubility. While 8-phenyltheophylline (46) is 100-fold more active at the A₁ receptor than theophylline (2), it is some 6000-fold less soluble.⁷¹ Addition of charged side chains to the 8-phenyl substituent, as in the case of 8-PST (47),⁶⁴ XCC (49), and XAC (50),^{62,63} or the substitution of a cyclopentyl for the phenyl group can improve solubility, as for cyclopentyltheophylline (CPT, 38).⁶¹ Bruns, in developing a ratio concept relating solubility to receptor affinity,⁷¹ has proposed that the greater the ratio, the more optimal the compound.

Indirect Modulation of Adenosine Function

In addition to the design of ligands that directly interact with adenosine receptors, the actions of adenosine may also be potentiated via inhibition of uptake,⁷²⁻⁷⁴ by allosteric

modulation of receptor function,^{75,76} or by compounds that act to enhance the free levels of adenosine.⁷⁷ A potential permissive role wherein A₂-receptor activation can influence A₁-mediated responses has also been postulated.^{78,79} The precise mechanism for this effect is unknown as there appears to be no clear SAR for the observed effects.⁷⁹ It is noteworthy, however, in regard to the CNS effects of adenosine agonists, that agents that increase cAMP also have the potential to increase blood-brain barrier permeability.⁸⁰ Thus "classical" A₂-receptor agonists have the potential to increase the activity of A₁ ligands by increasing their access to the brain.

Dipyridamole (63) (Figure 5), miflozine, and its analogue, R 75231 (62), are adenosine transport inhibitors that have clinical utility as coronary vasodilators and hypnotic agents.^{81,82} PD 81,723 (64) and related 3-benzoylthiophenes are selective enhancers of the binding of adenosine to A₁ receptors.^{75,76} They also potentiate the inhibitory effects of the purine in adenylate cyclase⁷⁶ and electrophysiological paradigms.⁸³ By analogy with the benzodiazepines at the benzodiazepine-GABA-A receptor complex⁸⁴ and various modulators of the N-methyl-D-aspartate receptor complex,⁸⁵ it has been postulated that an

- (70) Peet, N. P.; Lentz, N. L.; Meng, E. C.; Dudley, M. W.; Ogden, A. M.; Demeter, D. A.; Weintraub, H. J.; Bey, P. A novel synthesis of xanthines: support for a new binding mode for xanthines with respect to adenosine at adenosine receptors. *J. Med. Chem.* 1990, 33, 3127-3130.
- (71) Bruns, R. F.; Fergus, J. H. Solubilities of adenosine antagonists determined by radioreceptor assay. *J. Pharm. Pharmacol.* 1989, 41, 590-594.
- (72) Plegemann, P. G. W.; Wohlhüter, R. M. Permeation of nucleosides, nucleic acid bases and nucleotides in animal cells. *Curr. Top. Membr. Trans.* 1980, 14, 225-330.
- (73) Jarvis, S. M. Kinetic and molecular properties of nucleoside transporters in animal cells. In *Topics and Perspectives in Adenosine Research*; Gerlach, E., Becker, B. F., Eds.; Springer Verlag: Berlin, 1987; pp 102-117.
- (74) Wiley, J. S.; Brocklebank, A. M.; Snook, M. B.; Jamieson, G. P.; Sawyer, W. H.; Craik, J. D.; Cass, C. E.; Robins, M. J.; McAdam, D. P.; Paterson, A. R. A new fluorescent probe for the equilibrative inhibitor-sensitive nucleoside transporter. 5'-S-(2-aminoethyl)-N6-(4-nitrobenzyl)-5'-thioadenosine (SAENTA)-chi 2-fluorescein. *Biochem. J.* 1991, 273, 667-672.
- (75) Bruns, R. F.; Fergus, J. H. Allosteric enhancement of adenosine A₁ receptor binding and function by 2-amino-3-benzoylthiophenes. *Mol. Pharmacol.* 1990, 38, 939-949.
- (76) Bruns, R. F.; Fergus, J. H.; Coughenour, L. L.; Courtland, G. G.; Pugsley, T. A.; Dodd, J. H.; Tinney, F. J. Structure-activity relationships for enhancement of adenosine A₁ receptor binding by 2-amino-3-benzoylthiophenes. *Mol. Pharmacol.* 1990, 38, 950-958.
- (77) Gruber, H. A.; Hoffer, M. E.; McAllister, D. R.; Laikind, P. K.; Lane, T. A.; Schmid-Schoenbein, G. W.; Engler, R. L. Increased adenosine concentration in blood from ischemic myocardium by AICA riboside. Effects on flow, granulocytes, and injury. *Circulation* 1989, 80, 1400-1411.
- (78) Barraco, R. A.; El-Ridi, M. R.; Parizon, M. The adenosine analog 5'-N-ethylcarboxamido adenosine, exerts mixed agonist actions of cardiorespiratory parameters in the intact but not decerebrate rat following microinjections into the nucleus tractus solitarius. *Brain Res.* 1990, 530, 54-72.
- (79) Nikodijević, O.; Sarges, R.; Daly, J. W.; Jacobson, K. A. Behavioral effects of A₁- and A₂-selective adenosine agonists and antagonists; evidence for synergism and antagonism. *J. Pharmacol. Exp. Ther.* 1991, 259, 286-294.
- (80) Rubin, L.; Porter, S.; Horner, H.; Yednock, T. Blood-brain barrier model. *Patent Pub.* 1991, WO91/05038.
- (81) IJzerman, A. P.; Thedinga, K. H.; Custers, A. F. C. M.; Hoos, B.; Van Belle, H. Inhibition of nucleoside transport by a new series of compounds related to lidoflazine and miflozine. *Eur. J. Pharmacol.* 1989, 172, 273-281.
- (82) Pirovano, I. M.; Van Belle, H.; IJzerman, A. P. Inhibition of nucleoside uptake in human erythrocytes by a new series of compounds related to lidoflazine and miflozine. *Eur. J. Pharmacol.* 1990, 189, 419-422.
- (83) Janusz, C. A.; Bruns, R. F.; Berman, R. F. Functional activity of the adenosine binding enhancer, PD 81,723, in the in vitro hippocampal slice. *Brain Res.* 1992, in press.
- (84) Izquierdo, I.; Medina, J. H. GABA_A receptor modulation of memory: the role of endogenous benzodiazepines. *Trends Pharmacol. Sci.* 1991, 12, 260-265.
- (85) Young, A. B.; Fagg, G. E. Excitatory amino acid receptors in the brain: membrane binding and receptor autoradiographic approaches. *Trends Pharmacol. Sci.* 1990, 11, 126-133.

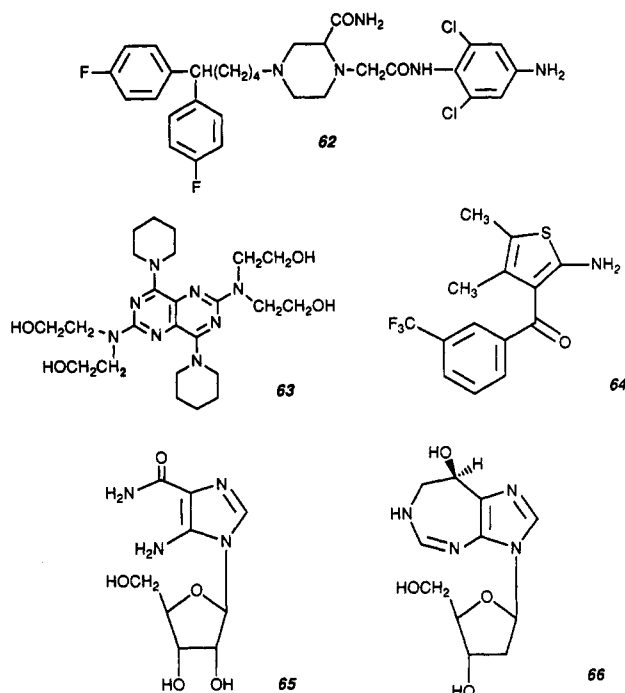


Figure 5. Agents for indirect modulation of adenosine function through transport (62 and 63) or metabolic processes (65 and 66), or at an allosteric site on the A_1 receptor (64). See text for description.

adenosine binding enhancer would have therapeutic potential with fewer side effects than administered agonists, in that it amplifies the action(s) of endogenous, situationally generated adenosine.⁷⁷ AICA riboside (acadesine, 65) is the prototypic adenosine "site and event specific" potentiator which is in phase III clinical trials for cardiac ischemia⁸⁶ with additional indications in type II diabetes. An orally active analogue, GP-1-468-3, is also under development.⁸⁷ Adenosine deaminase inhibitors like deoxycoformycin (66)⁸⁸ may also have therapeutic potential in a manner similar to AICA riboside although the in vivo efficacy of such agents requires considerable improvement.⁸⁹

The anti-inflammatory actions of the anticancer agent, methotrexate, have been tentatively related to its ability to elevate endogenous extracellular adenosine levels,⁹⁰ resulting in a putative reduction in neutrophil free radical formation presumably due to A_2 -receptor activation.⁹¹ The molecular target for the actions of methotrexate is

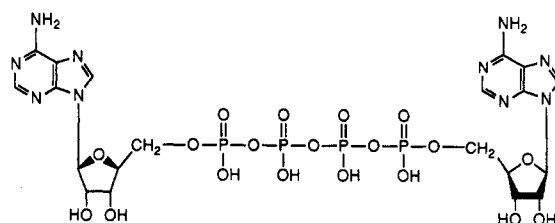


Figure 6. Structure of Ap4A.

thought to be via the AICA riboside formed due to methotrexate inhibition of AICA riboside transformylase.⁹⁰

ATP Receptor Ligands

Progress in the related area of purine nucleotide neurotransmission, specifically P_2 -receptor targets, has been hampered by the lack of selective antagonists, a lack of availability of those agonists generally accepted as efficacious, and the lack of general binding assays. ATP receptors can be classified into four major subclasses (Table II) termed P_{2x} , P_{2y} , P_{2t} , and P_{2z} .⁹² The P_{2t} receptor is actually an ADP rather than ATP receptor. Furthermore, a UTP (uridine triphosphate) receptor, distinct from the adenine nucleotide receptors already described, has been termed P_{2u} or nucleotide receptor.^{93,94} A P_3 receptor has also been postulated.^{95,96} Preliminary evidence suggests the existence of a "dipurinergic" cell surface recognition site for the "alarmone", Ap₄A (Figure 6), a dinucleotide tetraphosphate.⁹⁷

The concept that ATP, an intimate component of cellular energy as well as the other energy-rich nucleotides, could function as a neuromodulatory substance was not widely accepted until very recently. ATP is the principal agent thought to be responsible for the phenomenon of "nonadrenergic, noncholinergic" (NANC) neurotransmission.⁹⁸

Current consensus would support a role for ATP as a neuroeffector agent although the physiological and pathophysiological function has yet to be determined. Roles as an anticancer agent⁹⁹ and in the treatment of shock¹⁰⁰ have been suggested.

- (86) Gensia's ischemia therapy, Arasine, in Phase III. *Scrip* 1991, 1630, 23.
- (87) Marion Merrell Dow to evaluate Gensia ARA compound. *Scrip* 1991, 1643, 12.
- (88) Agarwal, R. P. Adenosine deaminase. Measurement of activity and use of inhibitors. In *Methods In Pharmacology*. Vol. 6. *Methods Used in Adenosine Research*; Paton, D. M., Ed.; Plenum: New York, 1985; pp 109-125.
- (89) Phillis, J. W.; Walter, G. A.; Simpson, R. E. Brain adenosine and transmitter amino acid release from the ischemic rat cerebral cortex: effects of the adenosine deaminase inhibitor deoxycoformycin. *J. Neurochem.* 1991, 56, 644-650.
- (90) Cronstein, B. N.; Eberle, M. A.; Gruber, H. E.; Levin, R. I. Methotrexate inhibits neutrophil function by stimulating adenosine release from connective tissue cells. *Proc. Natl. Acad. Sci. U.S.A.* 1991, 88, 2441-2445.
- (91) Cronstein, B. N.; Daguma, L.; Nichols, D.; Hutchison, A. J.; Williams, M. The adenosine/neutrophil paradox resolved: human neutrophils possess both A_1 and A_2 receptors that promote chemotaxis and inhibit O_2^- generation, respectively. *J. Clin. Invest.* 1990, 85, 1150-1157.

- (92) Gordon, J. Extracellular ATP: effects, sources, and fate. *Biochem. J.* 1986, 233, 309-319.
- (93) O'Connor, S. E.; Dainty, I. A.; Leff, P. Further subclassification of ATP receptors based on agonist studies. *Trends Pharmacol. Sci.* 1991, 12, 137-141.
- (94) Dubyak, G. R. Signal Transduction by P_2 -Purinergic Receptors for Extracellular ATP. *Am. J. Respir. Cell. Mol. Biol.* 1991, 4, 295-300.
- (95) Forsyth, K. M.; Bjur, R. A.; Westfall, D. P. Nucleotide modulation of norepinephrine release from sympathetic nerves in the rat vas deferens. *J. Pharmacol. Exp. Ther.* 1991, 256, 821-826.
- (96) Westfall, D. P.; Shinozuka, K.; Forsyth, K. M.; Bjur, R. A. Modulation of norepinephrine release by ATP and adenosine. In *Purines in Cellular Signaling. Targets for New Drugs*; Jacobson, K. A., Daly, J. W., Manganiello, V., Eds.; Springer Verlag: New York, 1990; pp 260-265.
- (97) Hilderman, R. H.; Martin, M.; Zimmerman, J. K.; Pivorun, E. B. Identification of a unique membrane receptor for adenosine 5', 5''-P₁P₄-tetraphosphate. *J. Biol. Chem.* 1991, 266, 6915-6918.
- (98) Burnstock, G. Purinergic mechanisms. *Ann. N.Y. Acad. Sci.* 1990, 603, 1-17.
- (99) Rapaport, E. Mechanisms of anticancer activities of adenine nucleotides in tumor-bearing hosts. *Ann. N.Y. Acad. Sci.* 1990, 603, 142-150.
- (100) Chaudry, I. H. Use of ATP following shock and ischemia. *Ann. N.Y. Acad. Sci.* 1990, 603, 130-141.

Second Messenger Systems

The effects of adenosine on cell function were initially described in terms of agonist actions on cAMP production, A₁-receptor activation leading to adenylate cyclase inhibition and A₂-receptor activation leading to adenylate cyclase stimulation. Subsequently, multiple second messenger systems for adenosine have been identified including stimulation of phosphatidylinositol (PI) turnover, potassium and calcium channel activation, and cyclic GMP formation.^{101,102} The latter effect may occur via modulation of nitric oxide formation¹⁰³ although this is somewhat controversial.¹⁰⁴ All of these second messenger systems have the potential to elicit multiple effects within the cell leading to complex effects on cell responsiveness. Adenosine acts both pre- and postsynaptically to alter cell excitability and to suppress the release of a diverse number of neuromodulators, such as excitatory amino acids, acetylcholine, dopamine, and norepinephrine. Thus, adenosine acts as paracrine effector agent² with the ability to antagonize the effects of many stimulatory neurotransmitters by inhibiting their release. The presynaptic actions of adenosine appear to predominate and may be more reflective of the actions observed with exogenously introduced adenosine agonists.

Receptor Structural Models

Chemical Approaches. Adenosine receptors have been affinity labeled using agonist and antagonist probes, often containing high specific radioactivity, carrier-free iodine-125 to facilitate identification of the labeled receptor. Two approaches have been used (i) indirect photoaffinity crosslinking, in which a radiolabeled receptor ligand containing a chemically reactive group, i.e. aryl amine, is first bound to the receptor and then exposed to a bifunctional reagent, such as SANPAH (*N*-succinimidyl 6-(4'-azido-2'-nitrophenylamino)hexanoate) designed both to acylate amines and generate a reactive nitrene; and (ii) direct photoaffinity labeling, where the ligand is preactivated for photolysis, leading to reaction with the receptor, that affords a higher percentage of available receptor being labeled.

The A₁ receptor was labeled by photoaffinity crosslinking using the ligand [¹²⁵I]APNEA (14, Table II) in combination with the cross-linker SANPAH¹⁰⁵ and by converting the amine of [¹²⁵I]APNEA, in advance, to a photolabile azido group, yielding [¹²⁵I]AZPNEA. In both cases, a protein of molecular weight 36 000 was labeled. The azide derived from N⁶-(4-amino-3-iodobenzyl)-adenosine (12, [¹²⁵I]ABA)¹⁰⁶ and 2-azido-N⁶-[2-(*p*-

hydroxyphenyl)-1-methylethyl]adenosine ([¹²⁵I]AHPIA)¹⁰⁷ (similar to 10, except that R₁ = N₃) have also been used to photoaffinity label the A₁ receptor with similar results. Photoaffinity labeling the A₁ receptor with an antagonist ligand, (see also ref 55) [¹²⁵I]PAPA-XAC (51, Table IV), gave a molecular weight of 38 000.¹⁰⁸

Photoaffinity labeling of bovine brain A₁ receptors, using azido-derivatized agonists (AZPNEA) and antagonists (preformed PAPA-XAC-SANPAH and azido-PAPA-XAC) in parallel, followed by partial peptide mapping identified identical peptide fragments when proteolysis was performed following photolabeling and denaturation.¹⁰⁹ When ligands were first bound to the receptor in membranes, followed by limited proteolysis and irradiation, distinct and different peptide fragments were obtained providing evidence for different conformational states for agonist-occupied A₁ receptors compared to the antagonist-occupied A₁ receptors. These differences probably relate to the ability of an agonist to initiate a transmembrane signal, whereas an antagonist binds to the receptor without producing an effect.

The A₂ receptor in bovine striatum was affinity labeled using the agonist [¹²⁵I]PAPA-APEC (33) and found to be a single glycoprotein of molecular weight 45 000.³⁵ The A₂ receptor in human striatum,¹¹⁰ rat PC12 cells,¹¹¹ and frog erythrocytes¹¹¹ have molecular weights in the 44 000–47 000 range, while that in the DDT₁MF-2 (Syrian hamster) cell line¹¹² has a molecular weight 42 000. Furthermore human and rabbit striatal A₂ receptors were found to undergo proteolytic cleavage,^{35,110} resulting in fragments of MW 37 000 and 38 000, respectively.

Results similar to those obtained with photoaffinity labels are found with chemical cross-linking agents. These studies use ADAC (18), APEC (32), and XAC (50), coupled to bifunctional alkylating and acylating cross-linking reagents, such as *m*- or *p*-phenylene diisothiocyanate (DITC) to provide a chemically reactive isothiocyanate group (NCS) on the ligand. For example, the A₁-receptor protein is specifically labeled by DITC-XAC with MW 38 000.¹¹³ In preliminary studies, the *m*- and *p*-DITC (34) conjugates of APEC appear to inhibit A₂-adenosine receptors irreversibly.¹⁶⁵

Purification to apparent homogeneity of cortical A₁ receptors from rat¹¹⁴ and bovine¹¹⁵ brain have been achieved

- (101) Fredholm, B. B.; Dunwiddie, T. V. How does adenosine inhibit transmitter release? *Trends Pharmacol. Sci.* 1988, 9, 130–134.
- (102) Cooper, D. M. F.; Caldwell, K. K. Signal transduction mechanisms for adenosine. In *Adenosine and Adenosine Receptors*; Williams, M., Ed.; Humana: Clifton, NJ, 1990; pp 105–141.
- (103) Burnstock, G. Dual control of local blood flow by purines. *Ann. N.Y. Acad. Sci.* 1990, 603, 31–45.
- (104) Linden, J.; Prater, M. R.; Sullivan, G. W.; Johns, R. A.; Patel, A. Contamination of adenosine deaminase by superoxide dismutase. Stabilization of endothelium-derived relaxing factor. *Biochem. Pharmacol.* 1991, 41, 273–279.
- (105) Stiles, G. A.; Daly, D. T.; Olsson, R. A. The A₂ adenosine receptor. Identification of the binding subunit by photoaffinity crosslinking. *J. Biol. Chem.* 1985, 260, 10806–10811.
- (106) Choca, J. I.; Kwatra, M. M.; Hosey, M. M.; Green, R. D. Specific photoaffinity labelling of inhibitory adenosine receptors. *Biochem. Biophys. Res. Commun.* 1985, 131, 115–121.

- (107) Lohse, M. J.; Klotz, K. N.; Schwabe, U. Agonist photoaffinity labeling of A₁ adenosine receptors: persistent activation reveals spare receptors. *Mol. Pharmacol.* 1986, 30, 403–409.
- (108) Stiles, G. L.; Jacobson, K. A. A new high affinity, iodinated adenosine receptor antagonist as a radioligand/photoaffinity crosslinking probe. *Mol. Pharmacol.* 1987, 32, 184–188.
- (109) Barrington, W. W.; Jacobson, K. A.; Stiles, G. L. Demonstration of distinct agonist and antagonist conformations of the A₁ adenosine receptor. *J. Biol. Chem.* 1989, 264, 3157–3164.
- (110) Ji, X. D.; Stiles, G. L.; van Galen, P. J. M.; Jacobson, K. A. Characterization of human striatal A₂-adenosine receptors using radioligand binding and photoaffinity labeling. *J. Recept. Res.* 1992, in press.
- (111) Nanoff, C.; Jacobson, K. A.; Stiles, G. L. The A₂ Adenosine receptor—guanine nucleotide modulation of agonist binding is enhanced by proteolysis. *Mol. Pharmacol.* 1991, 39, 130–135.
- (112) Ramkumar, V.; Barrington, W. W.; Jacobson, K. A.; Stiles, G. L. Demonstration of both A₁ and A₂ adenosine receptors in DDT₁MF-2 smooth muscle cells. *Mol. Pharmacol.* 1990, 37, 149–156.
- (113) Stiles, G. L.; Jacobson, K. A. High affinity acylating antagonists for the A₁ adenosine receptor: identification of binding subunit. *Mol. Pharmacol.* 1988, 34, 724–728.
- (114) Nakata, H. Purification of A₁ adenosine receptor from rat brain membranes. *J. Biol. Chem.* 1989, 264, 16545–16551.

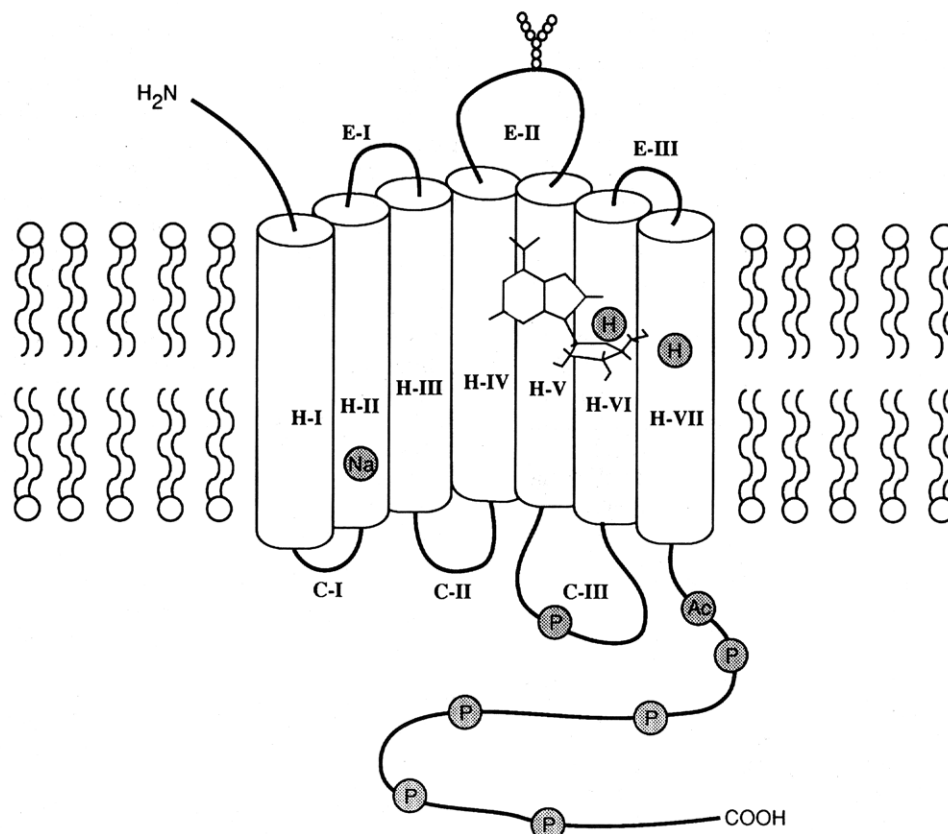


Figure 7. A proposed model (121) for adenosine receptors deduced from the primary sequences, showing features common to both A_1 and A_2 receptors, including the seven transmembrane helices typical of G-protein-coupled receptors (I–VII), three extracellular loops (E-I–III), three cytoplasmic loops (C-I–III), two histidyl residues (H) (possibly involved in ligand binding) a sodium binding site (Na), and sites for phosphorylation of serine and threonine residues (P) and glycosylation. Phosphorylation sites on the C-terminus apply to the A_2 -sequence only. A site for acylation (Ac) applies to A_1 receptors only. The C-terminal sequences (beyond H-7) are approximately 34 and 119 residues in length for canine A_1 and A_2 receptors, respectively.

through affinity chromatography with agarose-coupled XAC.

Molecular Biology Approaches. Molecular modeling approaches concerning the mode of interaction of ligands with the adenosine receptor have of necessity focused on the ligand SAR (Figures 2 and 4), in the absence of knowledge regarding receptor structure. However, the recent cloning and sequencing of canine,³ rat,^{116,117} and bovine (G. Stiles, personal communication) A_1 receptors and canine^{3,4} A_2 receptors has now yielded valuable information on some of the structural features of the receptors. A schematical model is shown in Figure 7.

The adenosine receptor sequences conform with the seven transmembrane domain topology commonly predicted for G-protein-coupled receptors, with the amino terminus on the extracellular and the carboxy terminus on the cytoplasmic side of the membrane. The seven membrane spanning regions (designated H-I to -VII) likely consist of right-handed α -helices that are interconnected by three extracellular loops (E-I to -III) and three cyto-

plasmic loops (C-I to -III). Although adenosine receptors are known to be glycosylated,^{118–120} there are no potential glycosylation sites present near the amino terminus, in contrast to other G-protein-coupled receptors. Putative glycosylation sites have been identified on E-II: Asn¹⁵⁹ for canine A_1 receptors and Asn¹⁴⁵ and Asn¹⁵⁴ for canine A_2 receptors.¹²¹ Cytoplasmic domains contain multiple serine and threonine residues that are potential substrates for phosphorylation by protein kinase A, protein kinase C, casein kinase 2, and β -adrenoceptor kinase,¹²¹ which may be relevant to receptor desensitization mechanisms. Cytoplasmic domains may also be involved in G-protein interactions. The A_1 receptor contains a potential site for palmitoylation in the carboxy terminus (canine A_1 Cys³⁰⁹) which is of potential interest given the presence of a similar palmitoyl group in the β_2 adrenergic receptor¹²² and the

- (115) Olah, M. E.; Jacobson, K. A.; Stiles, G. L. Purification and characterization of bovine cerebral cortex A_1 adenosine receptor. *Arch. Biochem. Biophys.* **1990**, *283*, 440–446.
- (116) Mahan, L. C.; McVittie, L. D.; Smyk-Randall, E. M.; Nakata, H.; Monsma, F. J.; Gerfen, C. R.; Sibley, D. R. Cloning and expression of an A_1 adenosine receptor from rat brain. *Mol. Pharmacol.* **1991**, *40*, 1–7.
- (117) Reppert, S. M.; Weaver, D. R.; Stehle, J. H.; Rivkees, S. A. Molecular cloning of a rat A_1 -adenosine receptor that is widely expressed in brain and spinal cord. *Mol. Endocrinol.* **1991**, *5*, 1037–1048.

- (118) Klotz, K. N.; Lohse, M. J. The glycoprotein nature of A_1 adenosine receptors. *Biochem. Biophys. Res. Commun.* **1986**, *140*, 406–413.
- (119) Stiles, G. L. Photoaffinity cross-linked A_1 adenosine receptor-binding subunits. Homologous glycoprotein expression by different tissues. *J. Biol. Chem.* **1986**, *261*, 10839–10843.
- (120) Barrington, W. W.; Jacobson, K. A.; Stiles, G. L. Glycoprotein nature of the A_2 -adenosine receptor binding subunit. *Mol. Pharmacol.* **1990**, *38*, 177–183.
- (121) van Galen, P. J. M.; Stiles, G. L.; Michaels, G. S.; Jacobson, K. A. Adenosine A_1 and A_2 receptors: structure function relationships. *Med. Res. Rev.* **1992**, in press.
- (122) O'Dowd, B. F.; Hnatowich, M.; Caron, M. G.; Lefkowitz, R. J.; Bouvier, M. Palmitoylation of the human β -adrenergic receptor. Mutation of Cys³⁴¹ in the carboxyl tail leads to an uncoupled nonpalmitoylated form of the receptor. *J. Biol. Chem.* **1989**, *264*, 7564–7569.

visual pigment rhodopsin¹²³ is required for interaction with G-proteins. Both A₁ and A₂ receptors, which are known to be regulated by Na⁺,¹²⁴ contain putative sites for interaction with Na⁺ at the cytoplasmic site of H-II (canine A₁, Asp⁵⁶; canine A₂, Asp⁵²). Two different histidine residues have been implicated in ligand binding to both A₁¹²⁵ and A₂¹⁶⁵ receptors, and a putative mode of interaction of histidine residues with adenosine receptor agonists and antagonists has been proposed.¹²⁶ Likely candidates for these interactions are two conserved histidine residues in H-VI and H-VII (canine A₁ His²⁶¹ and His²⁷⁸).¹²¹ Site-directed mutagenesis and chemical modification studies together with recombinant DNA techniques⁵ will aid in understanding the nature of the ligand binding site(s), the potential role of glycosylation, phosphorylation, acylation, palmitoylation, and regulation by Na⁺ in the physiological aspects of receptor function, as well as in establishing which domains are important in interacting with G-proteins.

Therapeutic Targets and Future Aspects

Considerable effort has been expended in defining more precisely a physiological role for adenosine receptor related processes in the cardiovascular system.^{13,16} A broader based albeit somewhat more circumstantial effort has focused on the role(s) of the purine nucleoside in the central nervous system.¹²⁷ In the latter system, such studies have frequently suffered from a highly reductionistic approach, wherein the effects of adenosine agonists and/or antagonists have been studied with only a single endpoint, biochemical or behavioral, and often only a single compound. In light of the largely theoretical basis for the etiology of many of the drug classes used to treat CNS disorders¹²⁸ and the multiple actions of exogenously applied adenosine agonists or antagonists, the results obtained in such limited studies have tended to confuse rather than delineate the role of adenosine (and ATP) in the pathophysiology of both psychiatric and neurological disorders.

Adenosine has been implicated in the mechanisms of drugs effective in schizophrenia, depression, epilepsy, cognition, and anxiety¹²⁹ reflecting both pre- and postsynaptic effects on neuronal function, the former via inhibition of transmitter release.² Excitatory neurotransmission process are more sensitive to the inhibitory effects of the purine than inhibitory ones.¹⁶⁶ Purinergic mechanisms

may also be involved in processes related to pain, ischemia, and regulation of cerebral blood flow and substance abuse.^{129,130} CI 936 (15), a potent A₂ receptor agonist, was profiled preclinically as an antipsychotic agent producing its actions via modulation of striatal dopamine systems.¹³¹ The compound entered clinical trials but was withdrawn.⁷ It is unknown whether clinical efficacy was observed.

Adenosine is an effective antiepileptic agent.¹³² Its endogenous production as the result of the ischemic episodes accompanying epileptic fits has led to the proposal that the purine functions as an endogenous anticonvulsant agent.¹³³ Adenosine agonists prolong survival and improve cellular morphology, in particular in the hippocampus, in animal models of cerebral ischemia¹³⁴ and represent one of the numerous experimental approaches to stroke therapy.¹³⁵

Antagonists are central stimulants as evidenced by caffeine being the most widely used nonprescription/nonillicit drug currently in use.⁸ Attempts to develop xanthine antagonists as cognition enhancers or for use in senility have not been successful due to side effect liability as proconvulsants, cardiotonics, or diuretics. The CPX analogue KFM 19 (40), an A₁-receptor-selective antagonist, is apparently devoid of these side effects⁶⁷ and is being developed as a cognition enhancer with potential in Alzheimer's disease. CPX can activate chloride flux in cell culture, suggesting a potential use in the treatment of cystic fibrosis.¹⁶⁸

Selective A₁ antagonists have protective effects in various models of renal failure. KW-3902 (43) is an effective diuretic at doses as low as 10 µg/kg.^{65,68} Targeting of adenosine antagonists to the kidney using a prodrug approach offers a potential approach to avoiding side effects.¹³⁶ Similarly systems for the delivery of adenosine analogues to the brain are under development.¹³⁷

Various xanthines have been shown at high concentration (1 mM) to increase nerve growth factor (NGF) production in cell culture systems.¹³⁸ Interestingly, Hoechst's pentoxifylline at similar concentrations is being used opportunistically in the treatment of AIDS-related infections because of its ability to modulate TNF (tumor necrosis factor) formation.¹³⁹ Pentoxifylline has also been reported

- (123) Ovchinnikov, Y. A.; Abdulaev, N. G.; Bogachuk, A. S. Two adjacent cysteine residues in the C-terminal cytoplasmic fragment of bovine rhodopsin are palmitylated. *FEBS Lett.* 1988, 230, 1-5.
- (124) Stiles, G. L. A₁ adenosine receptor-G protein coupling in bovine brain membranes: effects of guanine nucleotides, salt, and solubilization. *J. Neurochem.* 1988, 51, 1592-1598.
- (125) Klotz, K. N.; Lohse, M. J.; Schwabe, U. Chemical modification of A₁ adenosine receptors in rat brain membranes. Evidence for histidine in different domains of the ligand binding site. *J. Biol. Chem.* 1988, 263, 17522-17526.
- (126) van de Wenden, E. M.; van Galen, P. J. M.; IJzerman, A. P.; Soudijn, W. A model for the hydrogen bonding interactions between adenosine receptor ligands and histidyl residues in the adenosine A₁ receptor binding site, based on AM1 calculations. *J. Comput. Chem. (Theochem)* 1991, 231, 175-184.
- (127) Jarvis, M. F.; Williams, M. Adenosine in central nervous system function. In *Adenosine and Adenosine Receptors*; Williams, M., Ed.; Humana Press: Clifton, NJ, 1990; pp 423-474.
- (128) Williams, M. Challenges in the search for CNS therapeutics in the 1990s. *Curr. Opin. Therap. Patent* 1991, 1, 693-723.
- (129) Williams, M. Purine nucleosides and nucleotides as central nervous system modulators. *Ann. N.Y. Acad. Sci.* 1990, 603, 93-107.

- (130) Diamond, I.; Gordon, A. Use of adenosine agonists and antagonists in the treatment of alcohol abuse. Eur. Patent Application 0 431 758 A2, 1991.
- (131) Heffner, T. G.; Wiley, J. N.; Williams, A. E.; Bruns, R. F.; Coughenour, L. J.; Downs, D. A. Comparison of the behavioral effects of adenosine agonists and dopamine antagonists in mice. *Psychopharmacology* 1989, 98, 31-37.
- (132) Dunwiddie, T. V.; Worth, T. Sedative and anticonvulsant actions of adenosine analogs in mouse and rat. *J. Pharmacol. Exp. Ther.* 1982, 220, 70-76.
- (133) Dragunow, M.; Goddard, G. V.; Lavery, R. Is adenosine an endogenous anticonvulsant? *Epilepsia* 1985, 26, 480-487.
- (134) Evans, M. C.; Swan, J. H.; Meldrum, B. S. An adenosine analogue, 2-chloroadenosine protects against long term development of ischemic cell loss in the rat hippocampus. *Neurosci. Lett.* 1987, 83, 287-292.
- (135) Jacobsen, E. J.; McCall, J. M. Acute ischemic and traumatic injury to the CNS. *Ann. Rep. Med. Chem.* 1990, 25, 31-40.
- (136) Barone, S.; Churchill, P. C.; Jacobson, K. A. Adenosine receptor prodrugs. Towards kidney selective dialkylxanthines. *J. Pharmacol. Exp. Ther.* 1989, 250, 79-85.
- (137) Anderson, W.; Pop, E.; Lee, S.-K.; Bhargava, M.; Bodor, N.; Brewster, M. Brain-targeting chemical delivery systems for adenosine depresses locomotor behavior in rats. *Med. Chem. Res.* 1991, 1, 74-79.
- (138) Shinoda, I.; Furukawa, Y.; Furukawa, S. Stimulation of nerve growth factor synthesis/secretion by propentofylline in cultured mouse astroglial cells. *Biochem. Pharmacol.* 1990, 39, 1813-1816.
- (139) Scrip, Pentoxifylline for AIDS? *Scripta* 1610, 1991, 27.

to inhibit lymphocyte interleukin-2 receptor expression.¹⁴⁰ The effects of this xanthine on superoxide anion production appear to occur independently of adenosine receptor antagonism.¹⁶⁹

Adenosine is implicated in events related to both inflammation and the immune response.¹⁴¹ It is known to modulate neutrophil function via A₂-receptor activation⁹¹ and also affect the interactions between B and T cells.¹⁴¹ The arthritic process may also involve a purinergic component¹⁴² although it is unclear which receptor subtype is involved. Adenosine levels in synovial fluid are reduced in rheumatoid arthritis.¹⁴³

The purine also has effects on the pituitary-adrenocortical axis,^{144,145} increasing the release of a number of hormones, including dopamine, as part of a stress-related response.

Data on the potential therapeutic applications of compounds interacting with the various classes of P₂ receptor is yet in its early stages due to a paucity of suitable ligands and their limited availability. The effectiveness of antagonists at these receptors is also somewhat controversial as is the selectivity of the limited number of agonists. Nonetheless, knowledge related to the extracellular actions of ATP and other nucleotides is increasing. ATP can function as a growth factor,¹⁴⁶ acting to modulate the cytotoxic actions of TNF, an effect that appears to involve a permissive role related to P₁-receptor activation. Most recently, aerosolized ATP or UTP, in conjunction with the sodium channel blocker, amiloride, has been reported to stimulate chloride secretion in cystic fibrosis patients. The effect probably involves interactions with nucleotide receptors.^{163,168} Clinical efficacy remains to be shown.

Recent studies¹⁴⁷ with CGS 21680 (31) and CGS 22989 (25) have shown that the hypotensive actions of these A₂ receptor agonists can be attenuated following a 2-week chronic administration regimen using osmotic pumps, an effect paralleled by a 25–32% decrease in brain A₂-receptor number. Whether this response can be generalized to all adenosine agonists and is reflective of the situation that

might occur with a repeated po administration as opposed to steady-state administration remains to be determined.

In reviewing the various effects of adenosine in order to prioritize realistic options related to therapeutic targeting, it is important to recognize the limitations of purinergic therapy due to the ubiquitous nature of adenosine (and ATP) effects. Adenosine antagonists effective as cognition enhancers have potential use in the elderly population, a group that would not be especially tolerant of the cardiac stimulant or renal properties of such compounds. Similarly, the use of adenosine agonists as hypotensive agents may be anticipated to cause direct effects on the renin angiotensin system as well as elicit sedation via central mechanisms. The acceptance of such side effects in adenosine receptor ligands is dependent on the level of improvement ascribable to the use of a purinergic agent and the degree of unmet medical need. Thus agents that act to mimic (agonists) or potentiate (AICA riboside, uptake inhibitors) adenosine are targeted toward acute, life threatening situations such as SVT, reperfusion injury, stroke, and, perhaps, epilepsy. The use of such agents for the potential treatment of hypertension in light of the large number of highly efficacious and essentially side effect free agents currently available, consequently invites ridicule in the absence of any additional beneficial actions.¹³ The development of adenosine antagonists as selective cognition enhancers may be feasible should agents such as KFM 19 (40) prove to be selective for the CNS.

Yet the potential involvement of adenosine agonists and antagonists in inflammation, immunoregulation, and neuroendocrine function, areas in which pathophysiological mechanisms and mediators are just beginning to be understood and for which medications (like theophylline and β -receptor agonists in asthma) leave much to be desired, represents an important new arena for the study of purinergic mechanisms. The potential role of adenosine, and its related nucleotides, as paracrine homeostatic modulatory entities or as autotoxins,¹⁴⁸ may therefore also be reflected in the nature of the systems in which these agents act both physiologically and pathophysiologically, systems where malfunction is discrete and as global as the availability of the paracrine effector itself. An additional layer of complexity may also be reflected in the nature of the purinergic cascade¹⁴⁹ where ATP, en route to adenosine

- (140) Rao, K. M. K.; Currie, M. S.; McCachren, S. S.; Cohen, H. J. Pentoxifylline and other methylxanthines inhibit interleukin-2 receptor expression in human lymphocytes. *Cell. Immunol.* 1991, 135, 314–325.
- (141) Polmar, S. H.; Fernandez-Mejia, C.; Birch, R. E. Adenosine receptors: immunologic aspects. In *Adenosine Receptors*; Cooper, D. M. F., Londos, C., Eds.; Liss: New York, 1988; pp 97–112.
- (142) Green, P. G.; Basbaum, A. I.; Helms, C.; Levine, J. D. Purinergic regulation of bradykinin-induced plasma extravasation and adjuvant-induced arthritis in the rat. *Proc. Natl. Acad. Sci. U.S.A.* 1991, 88, 4162–4165.
- (143) Herbert, K. E.; Bhusate, L. L.; Scott, D. L.; Perett, D. Purine metabolism in arthritis: 1. Synovial fluid adenosine concentrations are low in rheumatoid arthritis. *Intern. J. Purine Pyrimidine Res.* 1991, 2, 31–34.
- (144) Scaccianoce, S.; Navarra, D.; Di Sciallo, A.; Angelucci, L.; Endrocz, E. Adenosine and pituitary-adrenocortical axis activity in the rat. *Neuroendocrinology* 1989, 50, 464–468.
- (145) Schettini, G.; Landolfi, E.; Meucci, O.; Floria, T.; Grimaldi, M.; Ventra, C.; Marino, A. Adenosine and its analogue (–)-N⁶-phenylisopropyladenosine modulate anterior pituitary adenylate cyclase activity and prolactin secretion in the rat. *J. Mol. Endocrinol.* 1990, 5, 69–76.
- (146) Kinzer, D.; Lehmann, V. Extracellular ATP and adenosine modulate tumor necrosis factor-induced lysis of L929 cells in the presence of actinomycin D. *J. Immunol.* 1991, 146, 2708–2711.
- (147) Sills, M. A.; Webb, R. L.; Hummel, H.; Francis, J. E.; Stone, G. A. Chronic administration of the adenosine A₂-selective agonists, CGS 21680C and CGS 22989, produces receptor down-regulation and tolerance to their hypotensive effects. *Pharmacologist* 1991, 33, 176.

- (148) Engler, R. L. Adenosine: the signal of life? *Circulation* 1991, 84, 951–954.
- (149) Williams, M. Purinergic receptors as drug targets. *Drug News Persp.* 1990, 4, 5–12.
- (150) McMillian, M. K.; Soltoff, S. P.; Cantley, L. C.; Talamo, B. R. Extracellular ATP increases free cytosolic calcium in rat parotid acinar cells. *Biochem. J.* 1988, 255, 291–300.
- (151) Soltoff, S. P.; McMillian, M. K.; Talamo, B. R. Coomassie brilliant blue G is a more potent antagonist of P2 purinergic responses than reactive blue 2 (Cibacron Blue 3GA) in rat parotid acinar cells. *Biochem. Biophys. Res. Commun.* 1989, 165, 1279–1285.
- (152) Bruns, R. F. Adenosine receptor activation in human fibroblasts: nucleoside agonists and antagonists. *Can. J. Physiol. Pharmacol.* 1980, 58, 673–691.
- (153) Lohse, M. J.; Böser, S.; Klotz, K. N.; Schwabe, U. Affinities of barbiturates for the GABA-receptor complex and A₁ adenosine receptors: a possible explanation of their excitatory effects. *Naunyn Schmiedeberg's Arch. Pharmacol.* 1987, 336, 211–217.
- (154) Daly, J. W.; Hong, O.; Padgett, W. L.; Shamim, M. T.; Jacobson, K. A.; Ukena, D. Non-xanthine heterocycles: activity as antagonists of A₁- and A₂-adenosine receptors. *Biochem. Pharmacol.* 1988, 37, 655–664.
- (155) Peet, N. P.; Dickerson, G. A.; Abdallah, A. H.; Daly, J. W.; Ukena, D. Benzo[1,2-c:5,4-c']dipyrzoles: non-xanthine adenosine antagonists. *J. Med. Chem.* 1988, 31, 2034–2039.

via successive dephosphorylation steps produces products with discrete receptor selectivity and functionality.

While consensus related to the potential existence of A_{1a}, A_{1b}, and A₃ receptors has yet to be obtained, it appears probable, based on experience from other transmitter systems that receptor subtypes represent the key to developing selective drugs and to understanding basic receptor functionality. Whether present data supporting the existence of adenosine receptor subtypes relates to the uniqueness of the experimental situations in which they were defined⁴² or is reflective of more generalized nuances of adenosine-elicited responses requires a more systematic evaluation of the effects of a number of agonists and antagonists in related systems and/or classification of receptors through cloning. As in the serotonin receptor field in the late 1970s, to which the adenosine area may loosely be compared, the major breakthroughs both from the functional and clinical viewpoint will be the selective lig-

ands for the receptor subclasses. It is highly noteworthy that it took a decade and a considerable aggregate effort both within the pharmaceutical industry and in academia targeted toward A₂-receptor-selective agonists to develop CGS 21680 (31), CGS 22492 (24), CHEA (26), and MPEA (27), from CV1808. The momentum that has been attained will hopefully result in a better understanding of the role of adenosine as the "signal of life"¹⁴⁸ in tissue function and result in new classes of therapeutic agents, that acting via purinergic receptors, will effectively treat the disease challenges of the 21st century.

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Note Added in Proof: Glaxo has recently reported¹⁷⁰ on GR 79236, an orally active A₁-receptor selective agonist targeted for the treatment of type II diabetes.

The P₂ receptor has been expressed from guinea pig brain mRNA.¹⁷¹

- (156) Skerrit, J. H.; Davies, L. P.; Johnston, G. A. R. A purinergic component in the anticonvulsant action of carbamazepine? *Eur. J. Pharmacol.* 1982, 82, 195-197.
- (157) Daly, J. W.; Hide, I.; Bridson, P. K. Imidazodiazepinediones: a new class of adenosine receptor antagonists. *J. Med. Chem.* 1990, 33, 2818-2821.
- (158) Psychoyos, S.; Ford, C. J.; Phillips, M. A. Inhibition by etazolate (SQ 20009) and cartazolate (SQ 65396) of adenosine-stimulated [³H]cAMP formation in 2-[³H]adenine-prelabeled vesicles prepared from guinea pig cerebral cortex. *Biochem. Pharmacol.* 1982, 31, 1441-1442.
- (159) Davies, L. P.; Chow, S. C.; Skerrit, J. H.; Brown, D. J.; Johnston, G. A. R. Pyrazolo[3,4-d]pyrimidines as adenosine antagonists. *Life Sci.* 1984, 34, 2117-2128.
- (160) Hamilton, H. W.; Ortwine, D. F.; Worth, D. F.; Bristol, J. A. Synthesis and structure-activity relationships of pyrazolo-[4,3-d]pyrimidin-7-ones as adenosine receptor antagonists. *J. Med. Chem.* 1987, 30, 91-96.
- (161) Garritsen, A.; IJzerman, A. P.; Beukers, M. W.; Cragoe, E. J.; Soudijn, W. Interaction of amiloride and its analogues with adenosine A₁ receptors in calf brain. *Biochem. Pharmacol.* 1990, 40, 827-834.
- (162) van Galen, P. J. M.; IJzerman, A. P.; Soudijn, W. Xanthine-7-ribosides as adenosine receptor antagonists. *Nucleoside Nucleotide* 1990, 9, 275-291.
- (163) Knowles, M.; Lane, L. C.; Boucher, R. C. Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. *New Eng. J. Med.* 1991, 325, 533-538.
- (164) Thompson, R. D.; Secunda, S.; Daly, J. W.; Olsson, R. A. Activity of N⁶-substituted 2-chloroadenosines at A₁ and A₂-adenosine receptors. *J. Med. Chem.* 1991, 34, 3388-3390.
- (165) Jacobson, K. A.; Stiles, G. L.; Ji, X. D. *J. Biol. Chem.* Chemical modification and irreversible inhibition of striated A_{2a}-adenosine receptors. *Mol. Pharmacol.* 1992, submitted.
- (166) Yoon, K.-W.; Rothman, S. M. Adenosine inhibits excitatory but not inhibitory synaptic transmission in the hippocampus. *J. Neurosci.* 1991, 11, 1375-1380.
- (167) Jacobson, K. A. Adenosine (P₁) and ATP (P₂) receptors. *Comp. Med. Chem.* 1990, 3, 601-642.
- (168) Eidelman, O.; Guay-Broder, C.; van Galen, P. J. M.; Jacobson, K. A.; Fox, C.; Turner, R. J.; Cabantchile, Z. I.; Pollard, H. B. A₁-Adenosine antagonist drugs activate chloride efflux from cystic fibrosis cells. *Proc. Natl. Acad. Sci. U.S.A.* 1992, in press.
- (169) Thiel, M.; Bardenheuer, H.; Pösch, G.; Madel, C.; Peter, K. Pentoxifylline does not act via adenosine receptors in the inhibition of the superoxide anion production of human polymorphonuclear leukocytes. *Biochem. Biophys. Res. Commun.* 1991, 180, 53-58.
- (170) Sykes, R. Comments. *F.D.C. Reports* 1991, 53 (50), 20.
- (171) Honore, E.; Fournier, F.; Collin, T.; Nargeot, T.; Guilbault, P. Functional expression of P₂ purinoceptor in *Xenopus* oocyte injected with brain mRNA. *Pflüger Arch.* 1991, 418, 447-452.