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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 reviewed[1] 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 A_1 and A_2, 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 A_1- and A_2-receptor agonists are now available. Newer antagonist ligands include a large number of 8-substituted xanthine derivatives, some of them over 10000-fold more potent than the parent compound 2, as well as numerous classes of non-xanthine heterocyclic compounds[8] 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 approved 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 solutions[11] to delay the onset of ischemic contractions, and as a cardioprotectant in postischemic

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Table I. Purinoceptor Subtypes

subtype	potency[a] agonists	potency[a] antagonists	location
		P_1 (Adenosine) Receptors	
A_1	CPA > R-PIA > NECA ≫ MPEA > CGS 21680	CPX > XAC > CPT > 8-PT	hippocampus, adipocytes, atrioventicular 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 tubercule
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}	2-MeSADP > ADP	ATP, AMP	platelet
P_{2u} "nucleotide receptor"	UTP = ATP > ADP > 2-MeSATP		hepatocytes, bovine aorta smooth muscle, Ehrlich ascites tumor cells, HL-60 cells, rat renal mesangial cells, neutrophils, fibroblasts
P_{2x}	α,β-MeATP > ATP	suramin	bladder, vas deferens, ear artery
P_{2y}	2-MeSATP > ATP = ADP > UTP	reactive blue 2	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 P_1 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-(methyloxy)phenyl]aminoadenosine] is more active than N^6-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 P_2 receptors see ref 167.

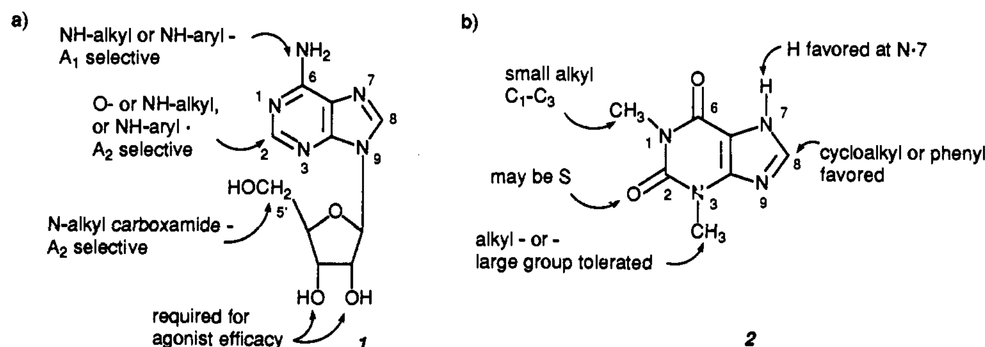

Figure 1. The structures of adenosine (1) and theophylline (2), showing the effects of structural modifications at various sites on receptor binding.

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

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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 N^6-, 2-, and 5'-positions.[6] Among these are N^6-cyclohexyladenosine (CHA, **6**), (*R*)-N^6-(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 A_1, 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 A_3[20] 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]

N^6-substituted analogues of adenosine have generally proven to be A_1-receptor selective, with N^6-cyclopentyladenosine (CPA, **3**) and CHA (**6**) being 400–800-fold selective.[6] N^6-bicycloalkyladenosines are even more A_1 selective with N^6-*endo*-norborn-2-yladenosine (*S*-ENBA, **7**) being 4700-fold selective for the A_1 receptor.[6] Combined substitutions at the N^6- and 2-positions have yielded 2-chloro-CPA (CCPA, **4**) which is 1500-fold A_1 selective.[26,164] A computer-generated model of the N^6 region of the A_1 receptor (Figure 2) has been shown to accurately predict the affinities of a number of N^6-substituted adenosines.[27]

The 5'-substituted adenosine analogue, NECA (**30**), has been extensively used to define tissue responses mediated by A_2 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 A_1 and A_2 receptors.[18] Ascribing effects elicited by NECA to A_2 receptor-mediated processes can only be validated if such effects are not seen with equivalent doses/concentrations of A_1 selective ligands such as CPA **3** or CHA **6**. In the seminal A_2-receptor binding assay developed by Bruns and co-workers,[18] the A_1 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]

A_2-selective adenosine agonists have been developed more recently. Although most N^6-substituted adenosine derivatives are A_1 selective,[29] a series of N^6-(2,2-diphenylethyl)-substituted adenosine analogues includes potent and A_2-selective compounds like CI 936 (**15**) and DPMA (**16**, N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine), the racemate of which is 30-fold selective for the A_2 receptor.[30] The design of this series was aided by a computer-generated model (Figure 2) of the N^6 region of the A_2 receptor.[31]

The 2-(arylamino)adenosine analogue, CV 1808 (**23**)[32] (Table II) while only moderately potent ($K_i \sim 100$ nM) at A_2 receptors has a modest 5-fold selectivity versus the A_1 receptor.[18] Evaluation of 2-position modifications of **30** led to the identification of CGS 21680 (**31**)[33] which is

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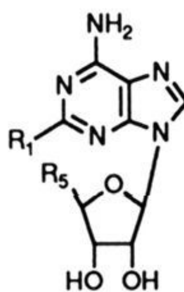
Table II. Structures of Adenosine Agonists and Their Affinities at A_1 and A_2 Adenosine Receptors[a]

NHR$_2$; R$_1$; HOCH$_2$; HO OH

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	S, H		0.3	1390	4600
8	OH		7.0	4920	700
9	H, CH_3, CH_2		1.2	124	100
10	H, CH_3, CH_2, I, OH		0.94[b]	-	-
11			4.6	663	140
12	$-CH_2-$, I, NH_2		0.7[b]	-	-
13	$-(CH_2)_2-$		4.1	-	-
14	$-(CH_2)_2-$, I, NH_2		2.0[b]	-	-
15	CH_2, CH		6.8	25	3.7
16	CH_3, CH_2, CH, OCH_3, OCH_3		142	4.4	0.31
17	CH_2, CH		5.2	4.9	0.94
18	$-C_6H_4-CH_2CONH-C_6H_4-CH_2CO-NH(CH_2)_2NH_2$		0.85	210	250
19	$-C_6H_4-CH_2CONH-C_6H_4-CH_2CO-NH(CH_2)_2NHCO(CH_2)_{16}CH_3$		0.22	8400	38000
20	$-C_6H_4-CH_2CONH-C_6H_4-CH_2CO-NH(CH_2)_2NHCSNH-C_6H_4-NCS$		0.47	191	410
21	$-C_6H_4-CH_2CONH-C_6H_4-CH_2CO-NH(CH_2)_2NH-FITC$[c]		7.1	-	-

Table II (Continued)



compd	R_1	K_i A_1	K_i A_2	A_2/A_1
	$R_5 = CH_2OH$			
22	Cl	9.3	63	6.8
23	NH–C_6H_5 (phenyl)	560	119	0.21
24	$NH(CH_2)_2$–cyclohexyl	12000	22	0.0018
25	$NH(CH_2)_2$–cyclohexenyl	2700	13	0.0048
26	$O(CH_2)_2$–cyclohexyl	1500	22	0.014
27	$O(CH_2)_2$–C_6H_4–CH_3	48	11	0.22
28	$C{\equiv}C-(CH_2)_3CH_3$	147	4.1	0.028
29	$C{\equiv}C-(CH_2)_5CH_3$	211	12	0.057
	$R_5 = CONHCH_2CH_3$			
30	H	6.3	10.3	1.6
31	$NH(CH_2)_2$–C_6H_4–$(CH_2)_2COOH$	2600	15	0.0058
32	$NH(CH_2)_2$–C_6H_4–$(CH_2)_2CONH(CH_2)_2NH_2$	240	5.7	0.024
33	$NH(CH_2)_2$–C_6H_4–$(CH_2)_2CONH(CH_2)_2NHCOCH_2$–$C_6H_3$(I)–$NH_2$	–	1.0[d]	–
34	$NH(CH_2)_2$–C_6H_4–$(CH_2)_2CONH(CH_2)_2NHCSNH$–$C_6H_4$–NCS	280	7.1[e]	0.025

[a] Unless noted, K_i values for binding experiments (using [^{3}H]CHA or [^{3}H]PIA at A_1 or [^{3}H]NECA at A_2, 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 [^{3}H]PIA. [e] Versus [^{125}I]PAPA-APEC in bovine striatum. [f] R_1 = H, unless noted.

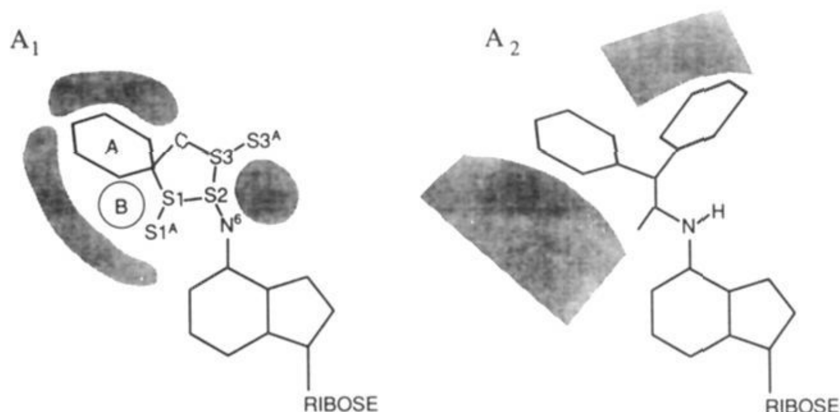


Figure 2. Computer-generated models of the N[6] regions of adenosine A_1 and A_2 receptors. For the A_1 model, areas are indicated where hydrophobic substituents may lead to enhanced affinity. These are designated S1, S1[A], S2, S3, S3[A], A(ryl), B(ulk), and C(ycloalkyl). In both models, shaded areas indicate the receptor boundaries (adapted from ref 27 and 31).

140-fold selective for the A_2 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 A_2 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 [^{3}H]CGS 21680[36] was instrumental to the identification of the cloned A_2 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

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(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 receptor[37] in this functional model of adenosine receptor selectivity. The 2-aralkoxyadenosine 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 tissue[39] 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 modest[41] 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 classification[42] 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 μM) and antagonist activity at the human VC13 cell A_{2b} receptor (K_i = 8.2 μM).

Few purine ring modifications can be tolerated, but 1-deazaadenosines retain high affinity. Thus, 1-deaza-2-chloro-N^6-cyclopentyladenosine is a potent and A_1-selective agonist.[44]

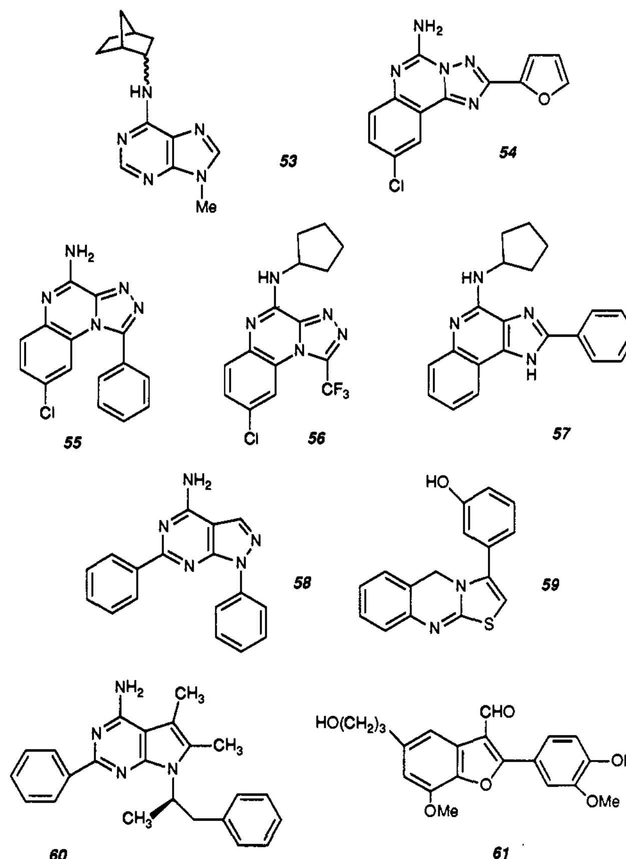

Figure 3. The structures of non-xanthine adenosine antagonists. (See text and Table III for description.)

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

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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'*]dipyrazoles	1,7-dihydro-3,5,8-trimethylbenzo[1,2-*c*:5,4-*c'*]dipyrazole[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-carbolines	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-6*H*-imidazo[4,5-*e*][1,4]diazepine-5,8-dione[157]
imidazo[4,5-*f*]quinazoline-7,9-diones	prox-benzotheophylline[59]
imidazo[4,5-*g*]quinazoline-6,8-diones	lin-benzotheophylline[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-9*H*-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**)[48]
xanthines	caffeine (**33**), theophylline (**2**), CPX (**39**),[58] PACPX (**48**),[18] XAC (**50**)[62]
xanthines, benzo-separated	(see imidazo[4,5]quinazolinediones)
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 N^6-substituted 9-methyladenines,[51] including N-0861 **53** [(±)-N^6-*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 binding[53] 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 μM; A_2, 25 μM).[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-

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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
			Xanthines				
2 theophylline	Me	Me	H		8500	25000	3.0[18]
35 caffeine	Me	Me	H	Me	29000	48000	1.7[18]
36 DMPX	$CH\equiv CCH_2$	Me	H	Me	22000	9600	0.44[60]
37 CHC[i]	Me	Me	cyclohexyl	Me	2.0[c]	0.19[d]	0.095[155]
38 CPT	Me	Me	cyclopentyl		11	1400	130[18]
39 CPX	Pr	Pr	cyclopentyl		0.46	340	740[56]
40 KFM19	Pr	Pr	O		10.5	1510	144[67]
41	Pr	Pr	$CH_2NHC(O)CH_3$		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	$(CH_2)_2$, I, NH_2	cyclopentyl		0.23	2000	8700[55]
45	Pr	Pr	H, CH_3		6.9	160	23[70]
46 8-PT	Me	Me	phenyl		86	850	9.9[18]
47 8-PST	Me	Me	p-(SO_3H)phenyl		2600	1500	5.8[18,64]
48 PACPX	Pr	Pr	H_2N, Cl		2.5	92	37[18]
49 XCC	Pr	Pr	OCH_2COOH		58	2200	57[62]
50 XAC	Pr	Pr	$OCH_2CONH(CH_2)_2NH_2$		1.2	60	50[62]
51 I-PAPA-XAC	Pr	Pr	$OCH_2CONH(CH_2)_2NHCOCH_2CH_2$, I, NH_2		0.1[f]	-	ref 108
52 m-DITC-XAC	Pr	Pr	$OCH_2CONH(CH_2)_2NHCSNH$, NCS		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[46]
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[50]

[a] K_i or IC_{50} values in nM, displacement of [^{3}H]PIA or [^{3}H]CHA in rat brain cortical membranes, unless indicated otherwise. [b] Displacement of [^{3}H]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 IC_{50} value for displacement of [^{3}H]CHA in guinea pig forebrain membranes. [f] K_d in bovine brain cortical membranes. [g] In bovine brain. [h] R_7 = 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 A_2 affinity to some extent,[60] no xanthine (or non-xanthine) antagonists with appreciable potency and selectivity at A_2 receptors have yet been reported. Thus the lack of potent, selective A_2-receptor antagonists remains

a major obstacle in the characterization of the function of adenosine A_2 receptors.

Phenyl or cycloalkyl substitutions in the 8-position may yield highly potent and, in many cases, also highly A_1-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 A_1 selective than CPX in guinea pig forebrain.[65] 8-[*trans*-4-(Acetamidomethyl)cyclohexyl]-1,3-dipropylxanthine (**41**) has surprisingly high potency at A_2 receptors, unlike other cycloalkylxanthines (K_i for antagonism of adenylate cyclase activity in rat adipocytes (A_1, 8 nM) or human platelets (A_2, 20 nM)).[66] KFM 19 (**40**, (±)-8-(3-oxocyclopentyl)-1,3-dipropylxanthine) is a potent A_1-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, A_1 = 1.3 nM; A_2 = 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 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 IC_{50} of 3 nM at the A_2 receptor.[46] CGS 15943 while under development as a potential antiasthmatic was found to be a potent skin irritant and was discontinued.

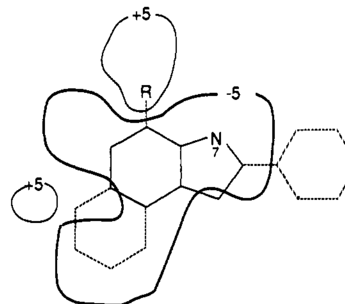

Figure 4. Computer-generated model of the antagonist binding site of the adenosine A_1 receptor. Indicated is the molecular electrostatic potential (at the +5 and −5 kcal/mol level) in the plane of the 6:5-fused heterocycle that is common to xanthines and many non-xanthine adenosine antagonists. R (corresponds to the N^6 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).

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 A_2-receptor-selective adenosine antagonists, such as CP 66713 (**55**) and some highly A_1-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 A_1 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**) (A_1, 10 nM; A_2, 450 nM).[49] A second computer-generated model of the antagonist binding site of the adenosine receptor assumes that N^6 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-

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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 A_2 selective, but this may be altered by various substitutions. Whereas in the triazoloquinoxaline series substitution at the exocyclic amino group may impart very high A_1 affinity and selectivity (e.g. CP-68,247 **56**),[48] analogous substitution in the triazoloquinazolines often diminishes rather than enhances A_1 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 A_1 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 A_1 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**),[62,83] 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 A_2-receptor activation can influence A_1-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" A_2-receptor agonists have the potential to increase the activity of A_1 ligands by increasing their access to the brain.

Dipyridamole (**63**) (Figure 5), mioflazine, 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 A_1 receptors.[75,76] They also potentiate the inhibitory effects of the purine in adenylate cyclase[76] and electrophysiological paradigms.[83] By analogy with the benzodiazepines at the benzodiazepine–GABA-A receptor complex[84] and various modulators of the *N*-methyl-D-aspartate receptor complex,[85] it has been postulated that an

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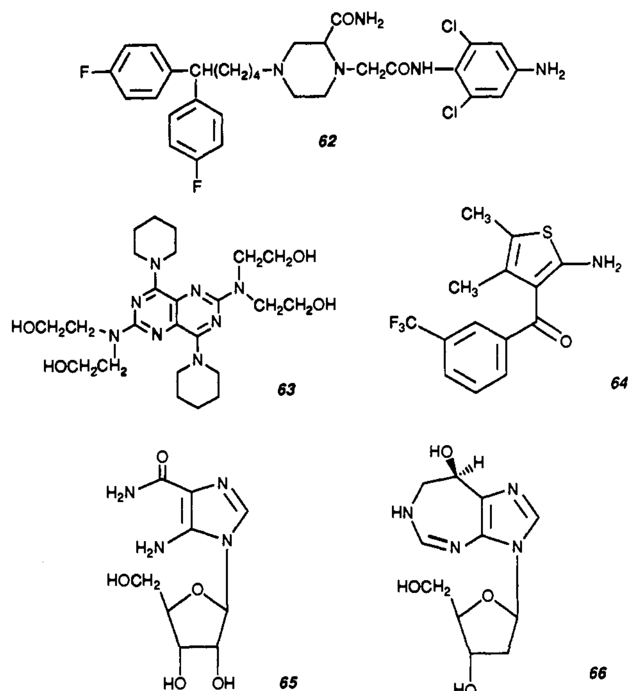

Figure 5. Agents for indirect modulation of adenosine function through transport (62 and 63) or metabolic processes (65 and 66), or at an allosteric site on the A_1 receptor (64). See text for description.

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 ischemia[86] 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 thought to be via the AICA riboside formed due to methotrexate inhibition of AICA riboside transformylase.[90]

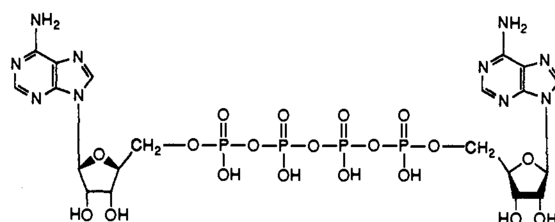

Figure 6. Structure of Ap4A.

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", Ap_4A (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 concensus 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 agent[99] and in the treatment of shock[100] have been suggested.

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Second Messenger Systems

The effects of adenosine on cell function were initially described in terms of agonist actions on cAMP production, A_1-receptor activation leading to adenylate cyclase inhibition and A_2-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 formation[103] 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 agent[2] 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 A_1 receptor was labeled by photoaffinity crosslinking using the ligand [^{125}I]APNEA (**14**, Table II) in combination with the cross-linker SANPAH[105] and by converting the amine of [^{125}I]APNEA, in advance, to a photolabile azido group, yielding [^{125}I]AZPNEA. In both cases, a protein of molecular weight 36 000 was labeled. The azide derived from N^6-(4-amino-3-iodobenzyl)adenosine (**12**, [^{125}I]ABA)[106] and 2-azido-N^6-[2-(*p*-hydroxyphenyl)-1-methylethyl]adenosine ([^{125}I]AHPIA)[107] (similar to **10**, except that $R_1 = N_3$) have also been used to photoaffinity label the A_1 receptor with similar results. Photoaffinity labeling the A_1 receptor with an antagonist ligand, (see also ref 55) [^{125}I]PAPA-XAC (**51**, Table IV), gave a molecular weight of 38 000.[108]

Photoaffinity labeling of bovine brain A_1 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 A_1 receptors compared to the antagonist-occupied A_1 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 A_2 receptor in bovine striatum was affinity labeled using the agonist [^{125}I]PAPA-APEC (**33**) and found to be a single glycoprotein of molecular weight 45 000.[35] The A_2 receptor in human striatum,[110] rat PC12 cells,[111] and frog erythrocytes[111] have molecular weights in the 44 000–47 000 range, while that in the DDT_1MF-2 (Syrian hamster) cell line[112] has a molecular weight 42 000. Furthermore human and rabbit striatal A_2 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 A_1-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 A_2-adenosine receptors irreversibly.[165]

Purification to apparent homogeneity of cortical A_1 receptors from rat[114] and bovine[115] brain have been achieved

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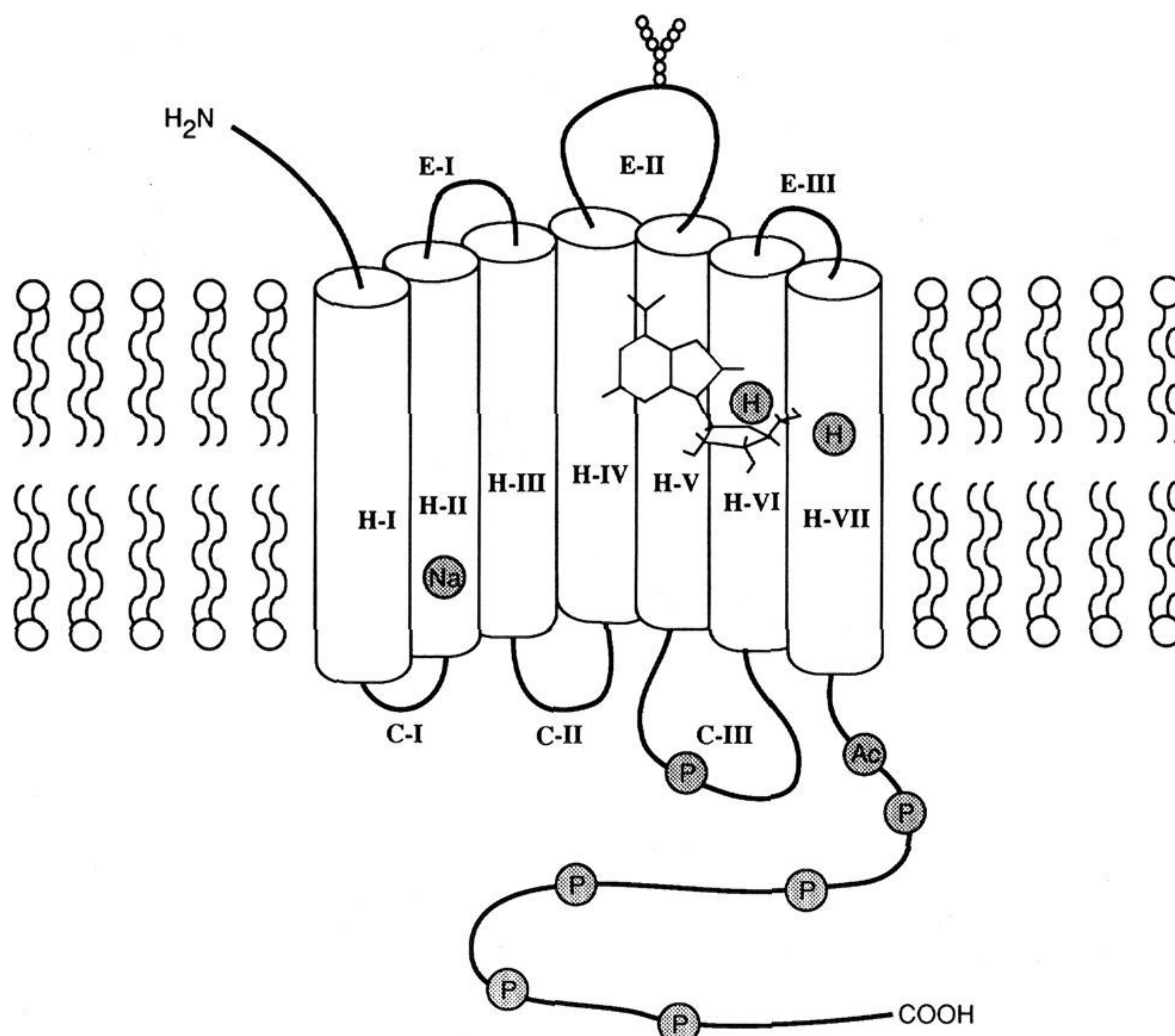

Figure 7. A proposed model (**121**) for adenosine receptors deduced from the primary sequences, showing features common to both A_1 and A_2 receptors, including the seven transmembrane helices typical of G-protein-coupled receptors (I–VII), three extracellular loops (EI–III), three cytoplasmic loops (CI–III), two histidinyl 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 cytoplasmic 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: Asn^{159} for canine A_1 receptors and Asn^{145} and Asn^{154} 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, caseine 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 Cys^{309}) which is of potential interest given the presence of a similar palmitoyl group in the β_2 adrenergic receptor[122] and the

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visual pigment rhodopsin[123] is required for interaction with G-proteins. Both A_1 and A_2 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 A_1, Asp[55]; canine A_2, Asp[52]). Two different histidine residues have been implicated in ligand binding to both A_1[125] and A_2[165] 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 A_1 His[251] and His[278]).[121] Site-directed mutagenesis and chemical modification studies together with recombinant DNA techniques[5] 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 disorders[128] 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 anxiety[129] 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 A_2 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 ischemia[134] 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 A_1-receptor-selective antagonist, is apparently devoid of these side effects[67] 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 A_1 antagonists have protective effects in various models of renal failure. KW-3902 (**43**) is an effective diuretic at doses as low as 10 $\mu g/kg$.[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 pentoxyfylline 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] Pentoxyfylline has also been reported

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(139) Scrip, Pentoxyfylline 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 A_2-receptor activation[91] and also affect the interactions between B and T cells.[141] The arthritic process may also involve a purinergic component[142] 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 P_2 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 P_1-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 studies[147] with CGS 21680 (**31**) and CGS 22989 (**25**) have shown that the hypotensive actions of these A_2 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 A_2-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 autocoids,[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 cascade[149] where ATP, en route to adenosine

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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 A_3 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 defined[42] 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 ligands 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 A_2-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 reported[170] on GR 79236, an orally active A_1-receptor selective agonist targeted for the treatment of type II diabetes.

The Pzy receptor has been expressed from guinea pig brain mRNA.[171]

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