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STRUCTURE–ACTIVITY RELATIONSHIPS FOR N^6-SUBSTITUTED ADENOSINES AT A BRAIN A_1-ADENOSINE RECEPTOR WITH A COMPARISON TO AN A_2-ADENOSINE RECEPTOR REGULATING CORONARY BLOOD FLOW

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Abstract—A series of 145 N^6-substituted adenosines have been screened as inhibitors of the binding of [^{3}H]cyclohexyladenosine to an A_1-adenosine receptor in rat brain membranes and the results compared to the potencies of these analogs in increasing coronary blood flow via activation of an A_2-adenosine receptor. The A_1 receptor shows greater stereoselectivity in the N^6 region of the receptor towards asymmetric aralkyl substituents, and shows greater bulk tolerance in the N^6 region such that it retains affinity for certain N^6-tertiary alkyladenosines and N^6-cycloalkyladenosines that are inactive at the coronary A_2 receptor. At the A_1 receptor, the most potent analogs have either aliphatic N^6-substituents with four or more methylene residues or have an N^6-halophenyl substituent. At the A_2 receptor, the most potent analogs have an N^6-phenethyl or similar heteroarylethyl substituent. Certain sets or series of analogs appear useful for identifying the subtypes of adenosine receptors involved in physiological functions.

Two classes of cell surface adenosine receptors appear to mediate many of the biological actions of adenosine and its analogs [for a review see Ref. 1]. A receptor designated either A_1 [2] or R_i [3] subserves inhibition of adenylate cyclase in fat, brain and heart cells. A receptor designated either A_2 or R_a subserves stimulation of adenylate cyclase in brain, liver, endocrine, endothelial and smooth muscle cells. At present the identification of one or the other type of adenosine receptor with a physiological function relies primarily on the rank order of potency of certain adenosine analogs. The potency order at A_1-adenosine receptors is N^6-*R*-1-phenyl-2-propyladenosine (*R*-PIA)‖ ≈ N^6-cyclohexyladenosine (CHA) > *N*-ethyl adenosine-5′-uronamide (NECA) ≥ 2-chloroadenosine > *S*-PIA. Typically, A_2-adenosine receptors exhibit a potency order NECA ≫ 2-chloroadenosine > *R*-PIA ~ CHA > *S*-PIA. An adenosine receptor mediates coronary vasodilation and has been assigned to the A_2 receptor class based on a rank order of potency of NECA ≫ 2-chloroadenosine > *R*-PIA > CHA > *S*-PIA [4].

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‖ Referred to in the literature as L-N^6-phenylisopropyladenosine, L-PIA, (−)PIA, *l*-PIA, and *R*-PIA. The present name, N^6-*R*-1-phenyl-2-propyladenosine, is the IUPAC name, but the abbreviation *R*-PIA will be used because of widespread use of the PIA abbreviation in the literature.

Structure–activity relationships of the N^6 region of this coronary A_2 receptor have been studied in detail [5–7]. A binding site for [^{3}H]-cyclohexyladenosine in brain membranes exhibits a profile of affinities for adenosine analogs consonant with an A_1 receptor, namely *R*-PIA ≈ CHA > NECA ≈ 2-chloradenosine > *S*-PIA [8]. The present study defines the structural requirements of the N^6 region of the brain A_1 receptor and contrasts them with those of the N^6 region of the coronary A_2 receptor. Like the investigation on the coronary receptor, the present study proceeds from a general model of the N^6 receptor region (Fig. 1). This model emphasizes the stereoselectivity that is such a prominent attribute of adenosine receptors and is essential to analysis of the effects of structural

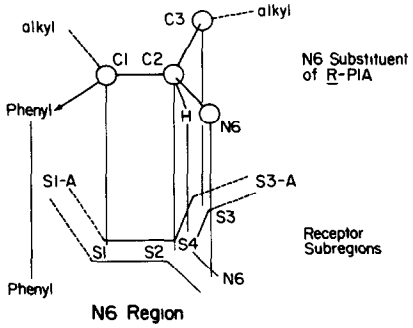

Fig. 1. Schematic representation of the N^6 region of an adenosine receptor based on stereoselective interaction with *R*-PIA and other N^6-substituted adenosines and low potency of N^6-tertiary-alkyl adenosines.

alterations. Stereoselectivity requires that the receptor recognize the absolute configuration of the N^6 substitutent, which for definition requires a minimum of a chiral carbon attached to the N^6-nitrogen with two different carbon substituents on it. In the case of *R*-PIA and *S*-PIA, the carbon propyl C2 is the chiral center and the positions of propyl C1 carbon and C3 carbon define absolute configurations. A proposed preferred binding of *R*-PIA to the adenosine receptor model is shown in Fig. 1. The receptor subregion for interaction with the propyl C2 carbon is designated as S-2 and those for the propyl C1 and C3 carbons as subregions S-1 and S-3 respectively. The receptor subregion for interaction with the proton on the chiral carbon of *R*-PIA is designated S-4. All compounds will be discussed in terms of interactions with S-1, S-2, S-3 and S-4 receptor subregions. A phenyl (aryl) subregion is also designated adjacent to the S-1 subregion. This subregion contributes markedly to activity of analogs at the A_2 receptor, but not at the A_1 receptor (see below). Structures of the N^6-substituents for many of the adenosine analogs are shown in Fig. 2.

MATERIALS AND METHODS

Materials. The synthesis and chemical properties of the N^6-adenosine analogs are described elsewhere [5, 6]. Adenosine deaminase (Type I) and N^6-methyladenosine were from the Sigma Chemical Co. (St. Louis, MO). 2-Chloroadenosine, CHA, *R*- and *S*-PIA and NECA are commercially available (Research Biochemicals Inc., Wayland, MA). [^{3}H]-N^6-Cyclohexyladenosine was from New England Nuclear (Boston, MA).

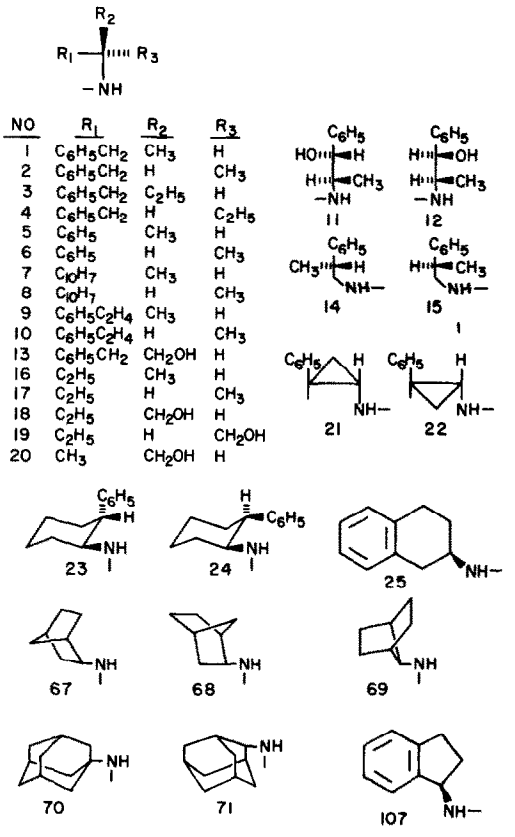

Fig. 2. Structures of the N^6-substituent of various adenosine analogs. The number of the analog is given (see Tables 2–7).

Binding assay. Inhibition of the binding of 1 nM [^{3}H]N^6-cyclohexyladenosine (CHA) to A_1-adenosine receptors in rat cerebral cortical membranes was assessed as previously described [9]. A range of concentrations of each analog was assayed in triplicate in at least two separate experiments, and an IC_{50} was estimated from manually drawn curves. The IC_{50} is related to the K_i by the equation $K_i = IC_{50}/(1 + [CHA]/K_D$ of CHA). Since the K_D for CHA binding to rat cortical membranes is 1.0 nM [9], this equation reduces to $K_i = IC_{50}/2$.

Coronary action. Dogs anesthetized with sodium thiamylal, 18 mg/kg i.v., were maintained in surgical anesthesia throughout an experiment by ventilation with O_2-enriched room air containing 0.5% halothane. Thoracotomy through the left 5th intercostal space exposed the heart for the implantation of an electromagnetic flowmeter and occlusive cuff near the origin of the left circumflex coronary artery and the transmural insertion of an intracoronary catheter for drug infusion just distal to the occluder. A catheter advanced into the aortic root via the left common carotid artery served for monitoring coronary perfusion pressure. Adjustments of minute ventilation and, when appropriate, the i.v. administration of 0.14 M $NaHCO_3$ maintained arterial blood PO_2, PCO_2 and pH in the physiological range. An assay consisted of recording the steady-state coronary flow and pressure responses to continuous intracoronary infusions of analog at rates up to those causing maximum vasodilation. Mean coronary conductance, the quotient of coronary flow divided by perfusion pressure, served as an index of coronary tone. Knowledge of the infusate concentration, rate of administration, coronary flow, and blood hematocrit allowed calculations of analog concentration in coronary plasma. Logit transformation of the conductance data and solution of the regression of logit (conductance) on log [analog] yielded an estimate of EC_{50}, the concentration of analog causing a half-maximum change in conductance. An assay of the coronary vasoactivity of adenosine preceded each analog assay and served as a check on the stability and reactivity of the experimental preparation. To account for between-dog variation in sensitivity to adenosine, results are expressed as a molar potency ratio, the quotient of the EC_{50} of adenosine divided by that of the analog (see also Refs. 5 and 6).

RESULTS

Brain and coronary adenosine receptors. The binding site(s) for [^{3}H]N^6-cyclohexyladenosine in rat cerebral cortical membranes exhibited properties expected of A_1-adenosine receptors when the relative potencies of a set of five adenosine analogs as antagonists of binding were compared (Table 1). Similarly, the coronary responses elicited by adenosine analogs exhibited properties expected of an A_2-adenosine receptor when the relative potencies of a

Table 1. Potency of adenosine analogs at a brain A_1-adenosine receptor and at a coronary A_2-adenosine receptor controlling blood flow

Analog	A_1-Adenosine receptor IC_{50}* (nM)	A_1-Adenosine receptor MPR† relative to MeAdo	A_2-Adenosine receptor MPR‡ relative to adenosine	A_2-Adenosine receptor MPR† relative to MeAdo
R-PIA	2.4 ± 0.1 (4)	50	4.3	86
CHA	1.7 ± 0.3 (3)	71	1.6	32
NECA	10.2 ± 0.6 (3)	12	150	3000
2-Chloroadenosine	11.5 ± 2.0 (3)	10	27	540
S-PIA	105 ± 15 (3)	1.1	0.41	8.2
N^6-Methyladenosine	120 ± 22 (6)	1.0	0.05	1.0

* IC_{50} values for A_1-receptors were obtained from antagonism of binding of 1 nM [^{3}H]-N^6-cyclohexyladenosine to rat cerebral cortical membranes. Values are means ± S.E.M. for two or more (N in parentheses when N > 2) separate experiments, each in triplicate.

† Molar potency ratio relative to N^6-methyladenosine (MeAdo), which is set equal to 1.0.

‡ Molar potency ratio relative to adenosine, which is set equal to 1.0. Data are from Refs. 6 and 7.

set of five adenosine analogs in increasing blood flow were compared (Table 1). The potencies of adenosine analogs as antagonists of binding of [^{3}H]-N^6-cyclohexyladenosine have been reported as IC_{50} or K_i values [8]. The potencies of adenosine analogs in enhancing coronary flow rates have been reported as molar potency ratios: a molar potency ratio is the EC_{50} value of adenosine divided by that of the adenosine analog [4–7]. A common reference compound on which to base molar potency ratios was needed to facilitate comparisons of activities of adenosine analogs at the A_1 and A_2 receptors. Adenosine itself cannot be used as a reference in the binding assay of A_1 receptors since treatment with adenosine deaminase of the brain membranes is a requisite to the assay [8]. N^6-Methyladenosine (MeAdo) appears to be a suitable reference compound since a methyl group is the simplest substituent in a series of N^6-substituted adenosines. MeAdo is at least 10-fold less active than adenosine at A_1 receptors [see Ref. 10] and about 20-fold less active than adenosine at the coronary A_2 receptors [7]. Thus, analogs with MPR values relative to MeAdo less than unity can be considered to have very low activity for both the brain A_1 and the coronary A_2 receptor. MeAdo has been reported to be a substrate, albeit poor, for adenosine deaminase [11]. However, preincubation of a range of concentrations of MeAdo with brain membranes and adenosine deaminase (10 μg/ml) for 1 hr has no effect on subsequent potency versus [^{3}H]cyclohexyladenosine in the binding assay (data not shown). Similarly, preincubations with brain membranes and adenosine deaminase, as expected, had no effect on the potency of *R*-PIA or NECA. Thus, it would appear that the presence of adenosine deaminase during binding assays will not have any significant effects on observed IC_{50} values of N^6-substituted adenosines including the reference standard MeAdo.

Stereoselectivity in the N^6-region of adenosine receptors (*Table* 2). A high degree of stereoselectivity towards *R*- and *S*-PIA is a notable characteristic of A_1 receptors [1]. A_2 receptors also exhibit significant, though less pronounced, stereoselectivity. This indicates that the three substituents on the chiral carbon of PIA, namely the 6-aminopurine riboside, the methyl and the benzyl, interact significantly with both adenosine receptors (Fig. 1). In the present study, the *R*-isomer of PIA (1) was 45-fold more potent than the *S*-isomer (2) at the A_1 receptor, and was 10-fold more potent than the *S*-isomer at the A_2 receptor (Table 2). Several other N^6-aralkyladenosines with a chiral carbon corresponding to the C-2 carbon of PIA were investigated. Replacing the methyl substituent on the C-2 chiral carbon of PIA with an ethyl group enhanced the potencies of both the *R*- and *S*-isomers at the A_1 receptor relative to PIA, but enhanced the potency only of the *R*-isomer at the A_2 receptor: the *R*-isomer (3) was 50-fold more potent than the *S*-isomer (4) at the A_1 receptor and 30-fold more potent than the *S*-isomer at the A_2 receptor. Replacing the benzyl ($C_6H_5CH_2$) substituent on the chiral carbon of PIA with phenyl (C_6H_5) decreased activity of the *R*- and *S*-isomers at both the A_1 and the A_2 receptors: the *R*-isomer (5) was 58-fold more potent than the *S*-isomer (6) at the A_1 receptor, while being 2.5-fold more potent than the *S*-isomer at the A_2 receptor. Similarly, the pair of isomers with a naphthyl substituent on the chiral carbon (7 and 8) were very selective for the A_1 receptor ($R/S = 35$), while showing little stereoselectivity for the A_2 receptor ($R/S = 2.1$) (Table 2). The presence of a hydroxyl moiety on the methyl carbon corresponding to the S-3 subregion (compound 11) reduced activity of *R*-PIA at both the A_1 and the A_2-receptor. The presence of a hydroxyl moiety on the methylene carbon corresponding to the S-1 subregion (compounds 12 and 13) reduced potency of PIA at both the A_1 and the A_2 receptor, but the potency was unaffected by the chirality at the carbon corresponding to S-1. Chirality at the carbon corresponding to S-1 also had little effect on potencies of the *R*- and *S*-2-phenyl-1-propyl isomers (compounds 14 and 15). The *R*- and *S*-isomers of N^6-4-phenyl-2-butyladenosine (9 and 10), which have a chiral carbon corresponding to S-2, exhibited only modest stereoselectivity for the A_1 (5.4-fold) and the A_2 (4-fold) receptors. These are

Table 2. Potency of N^6-aralkyl- and N^6-alkyladenosines containing a chiral center in the N^6-substituent for a brain A_1-adenosine receptor with a comparison to activity at a coronary A_2-adenosine receptor controlling blood flow

Compound No., chirality at S-2 carbon, and N^6-substituent		A_1-Receptor IC_{50} (nM)	A_1-Receptor MPR relative to MeAdo	A_2-Receptor MPR relative to MeAdo
		ARALKYL		
1. *R*	(*R*)-1-Phenyl-2-propyl	2.4 ± 0.1 (4)	50	80
2. *S*	(*S*)-1-Phenyl-2-propyl	105 ± 15 (3)	1.1	8.2
3. *R*	(*R*)-1-Phenyl-2-butyl	0.52 ± 0.04 (3)	230	150
4. *S*	(*S*)-1-Phenyl-2-butyl	26 ± 6 (3)	4.6	5
5. *R*	(*R*)-1-Phenethyl	6.7 ± 0.8 (4)	18	10
6. *S*	(*S*)-1-Phenethyl	390 ± 40 (4)	0.31	4.0
7. *R*	(*R*)-1-(1-Naphthyl)ethyl	18 ± 1	6.7	4.6
8. *S*	(*S*)-1-(1-Naphthyl)ethyl	630 ± 20	0.19	2.2
9. *R*	(*R*)-4-Phenyl-2-butyl	16 ± 3 (3)	7.5	0.19
10. *S*	(*S*)-4-Phenyl-2-butyl	85 ± 6 (3)	1.4	0.05
11. *R**	(*S*)-1-Hydroxy-3-phenyl-2-propyl	5.4 ± 0.8	22	32
12. *R*	(1*R*,2*R*)-1-Hydroxy-1-phenyl-2-propyl	9.3 ± 3.5 (3)	13	36
13. *R*	(1*S*,2*R*)-1-Hydroxy-1-phenyl-2-propyl	9.8 ± 4.0 (3)	12	58
14. —	(*R*)-2-Phenyl-1-propyl	2.8 ± 0.2 (3)	43	48
15. —	(*S*)-2-Phenyl-1-propyl	7.0 ± 0.5 (3)	17	60
		ALKYL		
16. *R*	(*R*)-2-Butyl	2.5 ± 0.3	48	18
17. *S*	(*S*)-2-Butyl	1.6 ± 0.4	75	52
18. *R*	(*R*)-1-Hydroxy-2-butyl	9.0 ± 1.0	13	2.4
19. *S*	(*S*)-1-Hydroxy-2-butyl	6.1 ± 0.9	20	15
20. *S*	(*S*)-1-Hydroxy-2-propyl	11 ± 2	11	2.0
		ARYLCYCLOALKYL		
21. *R*	(1*R*,2*S*)-2-Phenyl-1-cyclopropyl	10 ± 3 (4)	12	14
22. *S*	(1*S*,2*R*)-2-Phenyl-1-cyclopropyl	14 ± 7 (4)	8.6	6.2
23. *R*	*trans*(1*R*,2*S*)-2-Phenyl cyclohexyl	12 ± 6 (6)	10	48
24. *R*	*cis*(1*R*,2*R*)-2-Phenyl cyclohexyl	30 ± 12 (4)	4	3.8
25. *R*	(*R*)-1,2,3,4-Tetrahydro-naphth-2-yl	6.5 ± 1.5	18	2.8

* From the strict standpoint of nomenclature, this compound should be termed the *S*-isomer, but to illustrate that it is the hydroxyl analog of *R*-PIA it is called the *R*-isomer.

homologs of *R*- and *S*-PIA in which the phenyl moiety is now separated from the chiral center by two methylenes rather than one methylene in the case of PIA. Both had very low activity at the A_2 receptor.

Only one pair of N^6-alkyladenosines with a chiral carbon corresponding to the S-2 subregion was investigated: the substituents on the chiral carbon at S-2 were methyl and ethyl. The *R*-isomer (16) and *S*-isomer (17) showed little stereoselectivity, the *S*-isomer being slightly more potent than the *R*-isomer at both the A_1 and the A_2 receptor. The lack of marked stereoselectivity is not surprising in view of the similarity in size and character of the two alkyl substituents on the chiral carbon. The presence of a hydroxyl moiety on the methyl of *R*- and *S*-2-butyl isomers yields N^6-*S*-1-hydroxy-2-butyl- and N^6-*R*-1-hydroxy-2-butyl-adenosine (compounds 18 and 19) respectively. The *S*-isomer was only slightly more potent than the *R*-isomer at the A_1 receptor ($R/S = < 2$) and 6-fold more potent than the *R*-isomer at the A_2 receptor. The lower homolog, namely N^6-*S*-1-hydroxy-2-propyladenosine (compound 20) was only slightly less active than its higher homolog (compound 19) at the A_1 receptor, while being significantly less active than its higher homolog at the A_2 receptor.

Several adenosine analogs with an N^6-cycloalkyl substituent containing a chiral center at the carbon corresponding to S-2 and an aryl moiety were investigated. Interpretation of the activities of such compounds is not straightforward because of the complexity of the N^6-moiety. The two N^6-*trans*-2-phenylcyclopropyladenosines, 21 and 22, are cyclic isosteres of *R*- and *S*-PIA that were synthesized to test the generality of stereoselectivity at the coronary A_2 receptor [5]. The lack of pronounced stereoselectivity was surprising and could be due to the

fact that the carbons of the highly strained cyclopropane ring do not correspond in position to carbons of *n*-propane and, accordingly, it is possible that the cyclopropyl ring only interacts with the S-1 and S-2 subregions of the receptor. In support of this notion, 21 and 22 had about the same potency as N^6-phenethyladenosine (compound 108, Table 7) at the A_1 receptor, but were less potent than N^6-phenethyladenosine at the A_2 receptor, perhaps because of a suboptimal interaction of the phenyl ring with the phenyl subregion. The two N^6-(2-phenylcyclohexyl)adenosines can be considered as corresponding to *S*-PIA with a 3-carbon methylene bridge linking the carbon at S-1 to the carbon at S-3. Since these methylenes undoubtedly contribute significantly to activity, no comparison to *S*-PIA is warranted. The *trans*-isomer (23) was only somewhat more active than the *cis*-isomer (24) at the A_1 receptor, while being 13-fold more potent than the *cis*-isomer at the A_2 receptor. One other N^6-arylcycloalkyladenosine with a chiral carbon at S-2 was investigated, namely N^6-*R*-1,2,3,4-tetrahydronaphth-2-yladenosine (compound 25). It was potent at the A_1 receptor and weak at the A_2 receptor.

Alkyl moieties and the N^6-region of adenosine receptors (*Table* 3). The effects of N^6-alkyl substituents on potencies at adenosine receptors are relevant both to the bulk tolerance at this region and to the contributions of hydrophobic interactions between such alkyl residues and the N^6 region of the receptor. An N^6-methyl substituent causes a marked reduction in potency of adenosine at both the A_1 and the A_2 receptors (see Ref. 10; also Table 1, and compound 26 in Table 3). Thus, bulk tolerance at the S-3 subregion is very low at both classes of adenosine receptors. Substituents on the carbon (methyl) corresponding to the S-2 subregion markedly increased activity. In the simplest monosubstituted case, namely the N^6-ethyl analog (compound 27), the additional carbon residue enhanced activity relative to N^6-methyladenosine by 12-fold at the A_1 receptor and by 3-fold at the A_2 receptor. A further increase in the size of the alkyl substituent caused a further increase in activity, reaching an optimum with the *n*-propyl or *n*-butyl analogs (compounds 28 and 29) at the A_1 receptor and with the *n*-propyl at the A_2 receptor. In the simplest disubstituted case, namely the N^6-2-propyl analog (compound 43), the additional carbon residues enhanced activity relative to N^6-methyladenosine by 32-fold at the A_1 receptor and by 14-fold at the A_2 receptor. Increases in the size of the two alkyl substituents on the carbon at S-2 caused further increases in activity, reaching an optimum at the N^6-3-pentyl analog (compound 49), which was about 80-fold more potent than the parent N^6-methyl analog (compound 26) at both the A_1 and the A_2 receptors (Table 3). Three alkyl substituents on the carbon at S-2, as in the simplest case of the N^6-*tert*-butyl analog (compound 52), reduced activity by about 10-fold at the A_1 receptor with respect to the disubstituted analog (compound 43) and abolished activity at the A_2 receptor. Thus, a methyl group is tolerated poorly at the S-4 subregion of the A_1 receptor, while the S-4 subregion of the A_2 receptor cannot accept a substituent of size greater than hydrogen.

The alkyl substituents of the N^6-primary-alkyladenosines are free to rotate around N^6 such that they are able to interact with either the S-1-S1A or the S-3-S3A receptor subregions (see Fig. 1). Accordingly, one cannot unambiguously correlate activity with interaction of the alkyl moiety with one or the other subregion. While branching in the alkyl side chain is tolerated to some extent, it is not certain, because of the free rotation of the alkyl side chain, whether this reflects bulk tolerance at S-1-1A or S-3-3A subregions or both. Extensive "branching" in the primary alkyl substituent can reduce activity (compare compound 33 to compounds 32 and 28 and compound 35 to compounds 30 and 29). The N^6-cyclopropyl analog (compound 36) was quite active, which is not surprising since this small ring corresponds roughly in dimensions to an ethyl group. However, the two analogs containing a bulky cyclohexyl ring as a substituent (compounds 37 and 38) had relatively low activity. Polar moieties, such as a double bond (compound 39), a triple bond (compound 40), a hydroxyl (compound 41), or an amino group (compound 42) can reduce activity (compare to compounds 27 and 28). In the case of compound 40, steric effects might also be important, since the three carbons of the the alkynyl moiety are in a straight line unlike alkyl or alkenyl chains, which are angulated.

Analogs with secondary alkyl N^6-substituents are able to interact with both the S-1 and S-3 subregions. Simultaneous interactions at more than one subregion appear to contribute to potency, since the N^6-secondary-alkyladenosines are more active than analogs having primary alkyl groups of the same size (Table 3). Different substituents on the carbon corresponding to the S-2 subregion result in stereoselectivity as, for example, with *R*- and *S*-PIA. Several of the N^6-secondary-alkyl adenosines (44–47) are mixtures of the *R*- and *S*-diastereomers. In some of the cases, the stereoselectivity (see above) of the N^6 region may favor one of the diastereomers, although this was not the case for the *R*- and *S*-2-butyl analogs (compounds 16 and 17), presumably because of the similarity of the two substituents, methyl versus ethyl. The results with the 2-butyl analogs suggest that the interaction of the ethyl with the S-3 subregion is only slightly favored over interaction with the methyl with this subregion.

N^6-Dialkyladenosines (compounds 54–58) were inactive or nearly inactive at both the A_1 and the A_2 receptors. The lack of activity of the N^6-dialkyladenosines suggests that the S-2 subregion only can accommodate one carbon substituent on the N^6 nitrogen.

Cycloalkyl moieties and the N^6 region of adenosine receptors (*Table* 4). A series of N^6-cycloalkyladenosines were investigated (Table 4, see also Table 2, compounds 21–24). Many are analogs of open chain N^6-secondary-alkyladenosines, but the degree of conformational freedom is reduced through formation of the ring (compare compounds 43, 16, 17, 44–47 with compounds 59–64). Conformational freedom is even more constrained in the analogs whose N^6-substituents are bicyclic (compounds 67–69) or tricyclic (compounds 70 and 71). The potency of the monocyclic analogs (compounds 59–64) was

Table 3. Potency of N^6-alkyladenosines and related analogs at a brain A_1-adenosine receptor with a comparison to activity at a coronary A_2-adenosine receptor controlling blood flow

Compound No.	N^6-Substituent	A_1-Receptor IC_{50} (nM)	A_1-Receptor MPR relative to MeAdo	A_2-Receptor MPR relative to MeAdo
		MONOALKYL		
Monosubstituted at S-2				
		Straight chain		
26	Methyl	120 ± 22 (6)	1.0	1.0
27	Ethyl	9.7 ± 0.4	12	3.0
28	*n*-Propyl	3.7 ± 0.1	32	13
29	*n*-Butyl	3.8 ± 0.4	32	7.6
30	*n*-Pentyl	28 ± 7	4.3	7.2
31	*n*-Hexyl	33 ± 5	3.6	4.0
		Branched chain		
32	2-Methyl-1-propyl	2.0 ± 0.2	60	15
33	2,2-Dimethyl-1-propyl	33 ± 11	3.6	1.3
34	3-Methyl-1-butyl	26 ± 6 (4)	8.0	10
35	3,3-Dimethyl-1-butyl	110 ± 9	1.1	1.7
		Cycloalkyl containing chain		
36	Cyclopropylmethyl	1.5 ± 0.5	80	12
37	Cyclohexylmethyl	38 ± 14	3.2	1.0
38	2-Cyclohexylethyl	30 ± 6	4.0	2.8
		Polar moieties in chain*		
39	Allyl	19 ± 0	6.3	4.6
40	Propargyl	110 ± 35 (3)	1.1	4.6
41	2-Hydroxyethyl	20 ± 7	6.0	2.8
42	2-Aminoethyl	160 ± 27 (3)	0.75	1.4
Disubstituted at S-2				
		One (or two) substituent(s) methyl		
43	2-Propyl	3.7 ± 0.1	32	14
16	(*R*)-2-Butyl	2.5 ± 0.3	48	18
17	(*S*)-2-Butyl	1.6 ± 0.4	75	52
44	(*RS*)-2-Pentyl	4.2 ± 2.1	29	22
45	(*RS*)-2-Hexyl	3.2 ± 0.5	38	11
46	(*RS*)-2-Heptyl	11 ± 4	11	6.2
47	(*RS*)-2-Octyl	6.5 ± 3	18	1.0
		Both substituents larger than methyl		
48	Dicyclopropylmethyl	1.5 ± 0.3	80	31
49	3-Pentyl	1.5 ± 0.3	80	80
50	2,4-Dimethyl-3-pentyl	7.5 ± 1.6	16	33
51	4-Heptyl	6.5 ± 1.5	18	16
Trisubstituted at S-2				
52	*t*-Butyl	40 ± 2	3.0	I
53	2-Methyl-2-butyl	31 ± 4	3.9	I
		DIALKYL		
54	N^6-Dimethyl	10,000	0.012	I
55	6-Piperidinyl	12,000	0.010	I
56	N^6-Benzyl-N^6-methyl	2000	0.06	0.36
57	N^6-Methyl-N^6-(*R*)-1-phenethyl	1000	0.12	0.36
58	N^6-Methyl-N^6-2-phenethyl	4100	0.029	0.86

* See also compounds 18, 19, and 20 (Table 2).

Table 4. Potency of N^6-cycloalkyladenosines at a brain A_1-adenosine receptor with a comparison to activity at a coronary A_2-adenosine receptor controlling blood flow

Compound No. and N^6-Substituent		A_1-Receptor IC_{50} (nM)	A_1-Receptor MPR relative to MeAdo	A_2-Receptor MPR relative to MeAdo
59	Cyclopropyl	4.2 ± 0.2	29	7.6
60	Cyclobutyl	1.3 ± 0.2	92	32
61	Cyclopentyl	0.64 ± 0.06	190	36
62	Cyclohexyl	1.7 ± 0.3(3)	71	32
63	Cycloheptyl	6.3 ± 1.4	19	3.6
64	Cyclooctyl	3.4 ± 0.1	35	I
65	1-Methylcyclopentyl	3.6 ± 0.7(3)	33	I
66	1-Methylcyclohexyl	140 ± 40	0.86	I
67	Endo-2-norbornanyl	0.67 ± 0.12	180	11
68	Exo-2-norbornanyl	1.4 ± 0.4	86	11
69	7-Norbornanyl	0.95 ± 0.01	130	8.8
70	1-Adamantyl	145 ± 5	0.83	I
71	2-Adamantyl	91 ± 9	1.3	I

the same or slightly greater than the corresponding open chain compounds. In both series, optimal potency was reached at five carbons (compound 49 and 61) after which potency decreased. The decrease was much greater at the A_2 receptor, and N^6-cyclooctyladenosine (compound 64), while still relatively active at the A_1 receptor, namely 3.4-fold greater than N^6-methyladenosine, was inactive at the A_2 receptor.

An additional methyl substituent on the C-2 carbon had an unexpectedly small effect on activity of N^6-cyclopentyladenosine at the A_1 receptor. The analog (compound 65) now has a tertiary carbon on the N^6-nitrogen and, therefore, was expected to have low potency. Instead, it remained very active at the A_1 receptor and, indeed, was nearly as active as *R*-PIA. It was, as expected of a N^6-tertiary-alkyladenosine, inactive at the A_2 receptor. The corresponding methylcyclohexyl analog (compound 66) showed the expected low activity at the A_1 receptor and was inactive at the A_2 receptor.

The conformations in which the cyclobutyl, cyclohexyl, etc. rings interact with the N^6 region are unknown. These are fixed in the norbornanyl analogs (compounds 67–69) and all three of these are potent, particularly at the A_1 receptor. The endo-2-norbornanyl and 7-bornanyl isomers presumably will fit into the receptor in such a way that the cyclopentane

Table 5. Potency of N^6-phenyl adenosine analogs at a brain A_1-adenosine receptor with a comparison to activity at a coronary A_2-adenosine receptor controlling blood flow

Compound No. and N^6-Substituent		A_1-Receptor IC_{50} (nM)	A_1-Receptor MPR relative to MeAdo	A_2-Receptor MPR relative to MeAdo
72	Phenyl	6.5 ± 0.5 (3)	18	28
73	2-Fluorophenyl	4.0 ± 1.0	30	18
74	3-Fluorophenyl	1.0 ± 0.2 (3)	120	8.6
75	4-Fluorophenyl	2.0 ± 0.23 (3)	60	30
76	2-Chlorophenyl	5.5 ± 0.5	22	8.4
77	3-Chlorophenyl	3.9 ± 0.4	31	4.0
78	4-Chlorophenyl	2.2 ± 0.1	55	19
79	2-Methylphenyl	9 ± 3	13	13
80	3-Methylphenyl	27 ± 3	4.4	0.46
81	4-Methylphenyl	4.9 ± 0.1	24	27
82	2-Methoxyphenyl	85 ± 15	1.4	5.6
83	3-Methoxyphenyl	47 ± 4	2.6	5.2
84	4-Methoxyphenyl	4.6 ± 1.6	26	70

ring will interact with the N^6 region, whereas in the case of the exo-2-nornbornanyl analog the cyclohexane ring in the boat conformation will interact. Such proposed interactions would place the smallest substituent, namely hydrogen, of the C-2 carbon at the S-4 subregion. The low activity of the norbornanyl analogs at the A_2 receptor probably reflects the lower bulk tolerance of the N^6 region of that receptor. The 2-adamantyl analog (compound 71) has the cyclohexane ring fixed in the chair conformation, had low potency at the A_1 receptor, and was inactive at the A_2 receptor, but such low potency may be due to bulk effects rather than the ring conformation. The 1-adamantyl analog (compound 70) has a trisubstituted carbon at the S-2 subregion and, as expected, had low potency at the A_1 receptor and was inactive at the A_2 receptor.

Aryl moieties and the N^6 region of adenosine receptors (Tables 5–7). The stereoselectivity exhibited by various N^6-aralkyladenosine analogs for the adenosine receptors (Table 1) suggested major contributions from the interactions of the aryl moiety with the N^6 region. Comparison of the activity of N^6-alkyladenosines with the corresponding N^6-aralkyladenosines provides further evidence that interactions of aryl groups with the receptor can strongly affect potency. Three major classes of N^6-substituted analogs with an aryl moiety were investigated. These were the N^6-phenyl analogs (Table 5), the N^6-benzyl and related analogs (Table 6), and the N^6-2-phenethyl and related analogs (Table 7). Remarkably, whereas N^6-phenyladenosine (compound 72) and N^6-(2-phenylethyl)adenosine (compound 108) were very potent at adenosine receptors, N^6-benzyladenosine (compound 85) was very weak. This order of potency holds generally for the aryl ring-substituted analogs as well. The effects of various aryl substituents on the potencies of N^6-phenyl, N^6-benzyl and N^6-phenethyl analogs are summarized in Table 8.

The nature and position of ring substituents greatly influence the activity of the N^6-phenyladenosines (Table 5), and do so differently at the A_1 and A_2 receptors. At the A_2 receptor the fluorophenyl and chlorophenyl analogs were more potent than the methylphenyl and methoxyphenyl analogs and, except for the fluoro analogs, the para-substituted

Table 6. Potency of N^6-benzyladenosines and related analogs at a brain A_1-adenosine receptor with a comparison to activity at a coronary A_2-adenosine receptor controlling blood flow

	Compound No. and N^6-Substituent	A_1-Receptor IC_{50} (nM)	A_1-Receptor MPR relative to MeAdo	A_2-Receptor MPR relative to MeAdo
		BENZYL		
85	Benzyl	250 ± 40 (4)	0.48	10
86	(2-Fluorophenyl)methyl	63 ± 7	1.9	11
87	(3-Fluorophenyl)methyl	100 ± 18	1.2	16
88	(4-Fluorophenyl)methyl	112 ± 20	1.1	7.2
89	(2-Chlorophenyl)methyl	33 ± 6	3.6	24
90	(3-Chlorophenyl)methyl	90 ± 20	1.3	6.8
91	(4-Chlorophenyl)methyl	122 ± 5	1.0	0.10
92	(2-Methylphenyl)methyl	94 ± 16	1.3	36
93	(3-Methylphenyl)methyl	86 ± 19	1.4	17
94	(4-Methylphenyl)methyl	240 ± 30	0.5	0.80
95	(2-Methoxyphenyl)methyl	130 ± 20 (3)	0.92	14
96	(3-Methoxyphenyl)methyl	260 ± 50 (3)	0.46	10
97	(4-Methoxyphenyl)methyl	220 ± 24 (3)	0.55	0.8
5	(*R*)-1-Phenethyl	6.7 ± 0.8 (4)	18	10
6	(*S*)-1-Phenethyl	390 ± 40	0.31	4.0
98	2-Phenyl-2-propyl	1200 ± 250	0.11	I
		BENZYL ANALOGS		
99	(2-Pyridyl)methyl	450 ± 10 (3)	0.27	12
100	(3-Pyridyl)methyl	230 ± 8 (3)	0.52	14
101	(4-Pyridyl)methyl	140 ± 10	0.86	8.4
102	(2-Thienyl)methyl	71 ± 14 (3)	1.7	4.6
103	(3-Thienyl)methyl	90 ± 18 (3)	1.3	1.8
104	(2-Furanyl)methyl	190 ± 18	0.63	1.2
105	1-Naphthylmethyl	26 ± 6	4.6	36
106	Diphenylmethyl	280 ± 8	0.43	I
107	(*R*)-1-Indanyl	130 ± 20	0.9	4.8

Table 7. Potency of N^6-2-phenethyladenosines and related analogs at a brain A_1-adenosine receptor with a comparison to activity at a coronary A_2-adenosine receptor controlling blood flow

	Compound No. and N^6-Substituent	A_1-Receptor IC_{50} (nM)	A_1-Receptor MPR relative to MeAdo	A_2-Receptor MPR relative to MeAdo
		2-PHENETHYL		
108	2-Phenylethyl	8.1 ± 7.1 (6)	15	40
109	(2-Fluorophenyl)ethyl	11 ± 2	11	38
110	(3-Fluorophenyl)ethyl	13 ± 3	9.2	32
111	(4-Fluorophenyl)ethyl	15 ± 4	8.0	42
112	(2-Chlorophenyl)ethyl	26 ± 6 (3)	4.6	13
113	(3-Chlorophenyl)ethyl	6.4 ± 0.3 (3)	19	26
114	(4-Chlorophenyl)ethyl	12 ± 4	10	8.2
115	(2-Methylphenyl)ethyl	45 ± 1	2.7	13
116	(3-Methylphenyl)ethyl	53 ± 3	2.3	22
117	(4-Methylphenyl)ethyl	22 ± 2	5.5	9.2
118	(2-Methoxyphenyl)ethyl	71 ± 4 (3)	1.7	24
119	(3-Methoxyphenyl)ethyl	22 ± 6 (3)	5.5	26
120	(4-Methoxyphenyl)ethyl	21 ± 7 (3)	5.7	5.8
121	(4-Hydroxyphenyl)ethyl	22 ± 2	5.5	18
122	(4-Aminophenyl)ethyl	23 ± 9	5.2	18
123	(3-Trifluoromethyl phenyl)ethyl	49 ± 10	2.4	16
124	(3,4-Dimethoxyphenyl) ethyl	22 ± 4	5.5	54
125	(3,4,5-Trimethoxyphenyl) ethyl	54 ± 6	2.2	104
126	2,2-Diphenylethyl	14 ± 1	8.6	14
127	(*RS*)-2-Phenyl-1-butyl*	7.0 ± 4	17	66
128	1,3-Diphenyl-2-propyl	11 ± 2.8	11	6.6
1	(*R*)-1-Phenyl-2-propyl	2.4 ± 0.4 (4)	50	80
2	(*S*)-1-Phenyl-2-propyl	105 ± 15 (3)	1.1	8.2
129	2-Methyl-1-phenyl-2-propyl	56 ± 6 (4)	2.1	0.22
3	(*R*)-1-Phenyl-2-butyl	0.52 ± 0.04 (3)	230	150
4	(*S*)-1-Phenyl-2-butyl	26 ± 6 (3)	4.6	5
130	(*RS*)-2-Hydroxy-2-phenyl-ethyl†	34 ± 11 (3)	3.5	28
131	(*RS*)-2-Hydroxy-2-(3-hydroxyphenyl)ethyl†	45 ± 13 (3)	2.7	32
		2-PHENETHYL ANALOGS		
132	2-(2-Pyridyl)ethyl	52 ± 15	2.3	20
133	2-(3-Pyridyl)ethyl	32 ± 4	3.8	60
134	2-(4-Pyridyl)ethyl	40 ± 2	3.0	8.4
135	2-(2-Thienyl)ethyl	13 ± 2	9.2	80
136	2-(3-Thienyl)ethyl	21 ± 3	5.7	50
137	2-Benzimidazoylethyl	300 ± 57	0.40	8.0
138	2-(1-Methyl-2-pyrrolyl) ethyl	160 ± 32	0.75	13
139	2-(1-Naphthyl)ethyl	18 ± 6 (4)	6.7	2.2
140	2-(2-Naphthyl)ethyl	4.4 ± 1.5 (3)	27	2.4
		OTHER ANALOGS		
141	3-Phenylpropyl	25 ± 4 (3)	4.8	6.6
142	4-Phenylbutyl	15 ± 3 (3)	8.0	0.92
143	Cinnamyl	87 ± 4 (3)	1.4	3.0
144	2-Indanyl	13 ± 3	9.2	1.6
9	(*R*)-4-Phenyl-2-butyl	16 ± 3 (3)	7.5	0.19
10	(*S*)-4-Phenyl-2-butyl	85 ± 6 (3)	1.4	0.05
145	2-Methyl-4-phenyl-2-butyl	1450 ± 270	0.083	I

* Compare to compounds 14, and 15 (Table 2).
† Compare to compounds 11–13 (Table 2).

Table 8. Effects of aryl substituents on potency of N^6-phenyl, N^6-benzyl and N^6-2-phenethyladenosines at a brain A_1-adenosine receptor and at a coronary A_2-adenosine receptor controlling blood flow

	Effect of aryl substituent on activity*							
	Fluoro		Chloro		Methyl		Methoxy	
Adenosine analog	A_1	A_2	A_1	A_2	A_1	A_2	A_1	A_2
Ortho-substituents								
N^6-Phenyl	↑	—	—	↓	—	↓	⇊	↓
N^6-Benzyl	↑	—	↑	↑	↑	↑	↑	—
N^6-2-Phenylethyl	—	—	↓	↓	↓	↓	↓	—
Meta-substituents								
N^6-Phenyl	↑	↓	—	↓	↓	↓	↓	↓
N^6-Benzyl	↑	—	↑	—	↑	↑	—	—
N^6-2-Phenylethyl	—	—	—	—	↓	↓	↓	—
Para-substituents								
N^6-Phenyl	↑	—	↑	—	—	—	—	↑
N^6-Benzyl	↑	—	↑	⇊	—	⇊	—	⇊
N^6-2-Phenylethyl	↓	—	—	↓	↓	↓	↓	↓

* Effect on activity: ⇈ large increase (> 10-fold); ↑ increase (20- to 10-fold); — no marked effect (< 2-fold); ⇊ large decrease (> 10-fold); ↓ decrease (2- to 10-fold).

isomer was the most potent. At the coronary A_2 receptor, all of the analogs, with the exception of the meta-methyl analog, were relatively potent. The para-isomer was in all cases the most potent of the three isomers.

N^6-Benzyladenosine and various aryl-substituted benzyl analogs and heteroarylmethyl and naphthylmethyl analogs all had low activity at the A_1 receptor (Table 6). This was not the case with regard to activity at the A_2 receptor where several analogs (compounds 87, 89, 92, 93, 95, 100 and 105) were at least 14-fold *more* active than N^6-methyladenosine. Thus, although an aryl or heteroaryl ring on the carbon corresponding to the S-2 subregion is accommodated very poorly at the A_1 receptor, leading to a 2-fold reduction in potency compared to N^6-methyladenosine, the same aryl or heteroaryl ring can make a positive contribution to activity at the A_2 receptor leading to at least a 10-fold increase in potency. The most striking aryl substituent effect was the greater than 10-fold reduction in activity of N^6-benzyladenosine at the A_2 receptor conferred by a para-chloro, methyl or methoxy substituent (compounds 91, 94 and 97). This is in marked contrast to the results with N^6-phenyladenosines where the para-substituted isomer was always the most active (Table 5). The position of the substituent had little effect on activity of N^6-benzyladenosines at the A_1 receptor (Table 6).

The activities of several N^6-heteroarylmethyladenosines provide additional contrasts between the receptors. As in the case of the benzyl analogs, the N^6-pyridylmethyladenosines (compounds 99–101) were all less potent than N^6-methyladenosine at the A_1 receptor, but were more potent at the A_2 receptor. The N^6-thienylmethyladenosines (compounds 102 and 103) were somewhat more active than their pyridyl congeners at the A_1 receptor, but were less active at the A_2 receptor.

One benzyl analog (compound 106) with two aryl substituents on the carbon corresponding to S-2 subregion was investigated. The additional phenyl substituent had no further effect on the low potency of the parent N^6-benzyladenosine at the A_1 receptor, while abolishing activity at the A_2 receptor. Whether this means that the S-1 and S-3 subregions of the A_1 receptor are equally able to accommodate an aryl ring or whether there is an initial interaction at the S-1 subregion, which then permits the interaction of the S-3 subregion with the second aryl ring, is uncertain. In contrast, the A_2 receptor cannot accommodate an aryl ring simultaneously in both S-1 and S-3 subregions. The configuration of a methyl as the second substituent of the carbon at the S-2 subregion markedly influences potency at the A_1 receptor as compared to the potency of the parent N^6-benzyladenosine (compound 85). At the A_1 receptor, the *R*-isomer (compound 5) was much more potent, whereas the *S*-isomer (compound 6) was slightly less potent than the parent N^6-benzyladenosine. At the A_2 receptor, the *R*-isomer was equipotent to N^6-benzyladenosine, while the *S*-isomer was less potent. The presence of two methyls on the benzylic carbon at the S-2 subregion yielded a virtually inactive analog (compound 98) as expected from the lack of bulk tolerance at the S-4 subregion.

N^6-2-Phenethyladenosine (compound 108) was quite active at both adenosine receptors (Table 7). The activity was, however, more striking at the A_2 receptor where it and its various analogs were among the most active of the 145 N^6-substituted adenosines. Monosubstitution of the phenyl in many cases caused a reduction in potency, which was greater for the A_1 receptor than for the A_2 receptor. The di- and trimethoxyphenylethyl analogs (compounds 124 and 125) are noteworthy in that potency was decreased significantly compared to the parent N^6-2-phenylethyladenosine at the A_1 receptor, while being

further increased at the A_2 receptor. Certain of the 2-phenethyl analogs with heteroaryl rather than phenyl rings (compounds 133, 135 and 136) exhibited very high activity at the A_2 receptor, while being less active than the 2-phenethyl analog at the A_1 receptor.

An analog (compound 126) with two phenyl substituents on carbon corresponding to S-1 subregion was only 2- to 3-fold less potent than the parent N^6-2-phenethyladenosine at either of the adenosine receptors. Likewise, an analog (compound 127) with an ethyl substituent on the carbon corresponding to S-1 subregion had a potency similar to that of the parent N^6-2-phenethyladenosine. The presence of two $C_6H_5CH_2$ substituents on the carbon corresponding to S-2 (compound 128) had only a very slight effect on potency at the A_1 receptor, compared to the parent N^6-2-phenethyladenosine, and retained moderate potency at the A_2 receptor. The configuration of a methyl as the second substituent on the carbon at S-2 markedly affected potency at the A_1 and A_2 receptor compared to the potency of the parent N^6-2-phenethyladenosine. At the A_1 receptor, the *R*-isomer (*R*-PIA, compound 1) was 3-fold more potent than N^6-2-phenethyladenosine, whereas the *S*-isomer (*S*-PIA, compound 2) was 14-fold less potent. At the A_2 receptor, the *R*-isomer was 2-fold more potent than N^6-2-phenethyladenosine, whereas the *S*-isomer was 5-fold less potent. Similar effects were noted when the second substituent on the carbon at S-2 was ethyl (compounds 3 and 4).

Extension of the spacer alkyl chain between N^6 and the aryl ring resulted in a reduction of potency (compounds 141 and 142), which was greater at the A_2 receptor. In the case of the N^6-3-phenylpropyladenosine (compound 141), an additional methyl in the *R*-configuration on the carbon corresponding to the S-2 subregion increased activity at the A_1 receptor slightly (compound 9), while a methyl in the *S*-configuration decreased activity (compound 10). Both diastereomers were much less active than the parent analog at the A_2 receptor. Two methyl groups at the carbon of compound 141 corresponding to the S-2 subregion yielded an analog (compound 145) that was virtually inactive at either of the receptors.

DISCUSSION

Two types of adenosine receptor modulate the activity of adenylate cyclase, those which activate (A_2 or R_a) and those which inhibit the enzyme (A_1 or R_i). At the present time the classification of these receptors depends strongly on the empirical structure–activity relationships of a limited number of adenosine analogs. Previous experiments to refine the structure–activity rules for each kind of receptor include a study of 128 analogs at the A_2 receptor of fibroblasts in culture [12]. Studies of the A_1 receptor have mainly dealt with the receptor in rat cells or fat cell membranes [3, 13]. Such *in vitro* studies indicate clear differences between the two classes of receptor, but these differences tend to blur when the structure–activity approach is used to probe the type of adenosine receptor that mediates a particular physiological response [14]. The present study was designed to explore in detail the structure–activity relationships of N^6-substituted adenosine at a rat cerebral cortical A_1 receptor [8] and to compare these relationships with those of an A_2 receptor that mediates coronary vasodilation in dogs [6, 7]. On a fundamental level, such a comparison reveals distinct differences in the structures of the N^6 regions of the two receptors. A practical consequence of this comparison is the identification of several analogs that may complement those currently employed for classifying adenosine receptors.

There are three major differences between the structure–activity relationships of the N^6-substituted adenosines at the brain A_1 receptor and the coronary A_2 receptor. These differences are the degree of stereoselectivity of those analogs in which the carbon attached to N^6 is a chiral center, the apparent mechanism of stereoselectivity, and the contribution of an aryl group to potency. We ascribe these differences in structure–activity relationships to underlying differences in the structures of the two receptors. We recognize that the studies of the brain A_1 receptor measure only ligand affinity, whereas those of the coronary receptor reflect not only receptor occupancy but receptor activation and the cascade of subsequent events. Additionally, pharmacokinetic factors may have some influence, for such factors will differ considerably between an *in vitro* membrane binding assay and an *in vivo* assay of coronary vasoactivity. Finally, it is possible that some analogs have effects on coronary flow unrelated to "classical" adenosine receptors.

Although the interaction of agonists with receptors is a dynamic process involving conformational changes in the receptor macromolecule and, in all likelihood, concomitant conformational changes in the agonist, a static model has served as a simple first approximation for our interpretations. Although incomplete, such a model can take into account differences in bulk tolerance, stereochemical factors and hydrophobicity.

Stereoselectivity is a prominent attribute of the A_1 receptor, and the present study confirms that stereoselectivity is greater at the A_1 receptor [1]. The stereoselectivity of analogs such as *R*-PIA, expressed by the *R*/*S* activity ratio of the diastereomers, probably is due both to factors that promote the activity of the *R* diastereomer and also to factors that reduce the activity of the *S* diastereomer. The positive interaction of the propyl C3 methyl group of *R*-PIA with the S-3 subregion of the A_1 receptor clearly promotes activity. Thus, N^6-2-phenethyladenosine, which lacks such as substituent is 3-fold less active than *R*-PIA and 15-fold less active than N^6-*R*-1-phenyl-2-butyladenosine, which has an ethyl group oriented so as to interact with the S-3 subregion. Two explanations for the low activity of *S*-PIA can be proposed. The first possibility is that *S*-PIA binds preferentially to the A_1 receptor with the $C_6H_5CH_2$ moiety oriented for interaction with the S-1 subregion as proposed for *R*-PIA (see Fig. 1). In this case, the methyl moiety then will be oriented to the S-4 subregion and the favorable interaction at the S-3 subregion of the methyl in *R*-PIA will be lost. The low activity of the N^6-tertiary alkyl adenosines indicates that a methyl interaction at the S-4 subregion will markedly

reduce activity, and could thus account for the low activity of *S*-PIA and other *S*-N^6-aralkyladenosines. The second possibility is that *S*-PIA binds preferentially to A_1 receptor with the $C_6H_5CH_2$ moiety oriented for interaction with the S-3 subregion rather than the S-1 subregion as proposed for *R*-PIA (see Fig. 1). In this case, the methyl moiety will be oriented to the S-1 subregion. Loss of the favorable interactions of the methyl with S-3 subregion and of the $C_6H_5CH_2$ with the S-1 and phenyl subregion, as proposed for *R*-PIA, would account for the low activity of the *S*-diastereomer. The available evidence does not permit a choice between these two possibilities.

A similar analysis applied to the coronary A_2 receptor [5, 6] proposes binding of the phenyl group to the aryl subregion and of the propyl C-3 methyl group to the S-3 subregion to account for the activity of *R*-PIA (see Fig. 1). The N^6-*tert*-alkyladenosines are essentially inactive at the A_2 receptor. Such a result means that the S-4 region of the coronary A_2 receptor is dimensionally very restricted and, for example, will not accommodate the methyl moiety of *S*-PIA. It thus appears that steric hindrance is an important factor in stereoselectivity at the A_2 receptor.

Optimal activity at the A_1 brain receptor. Analysis of the structures of the twenty-one most potent N^6-substituted analogs at the A_1 receptor provides insights into the requirements for optimal activity (Table 9). The high potencies of the N^6-halo-phenyladenosines are exceptional and, in light of the majority of highly potent analogs, the reason for their very high activity is not clear. The most potent N^6-substituted adenosine analog at the A_1 receptor is N^6-*R*-1-phenyl-2-butyladenosine. It was some 230-fold more potent than N^6-methyladenosine, the rather weak adenosine analog chosen as the standard for comparison in the present study. It differs from *R*-PIA, a widely used, so-called "selective" A_1-adenosine receptor agonist in having an ethyl rather than a methyl attached to the carbon corresponding to the S-2 subregion. This change resulted in a 5-fold increase in potency at the A_1 receptor compared to *R*-PIA (Table 9).

Of the twenty-one most potent N^6-substituted adenosines at A_1 receptors, only six, including the very potent N^6-3-fluorophenyladenosine, contain a phenyl ring. Three of these are N^6-halophenyladenosines. Three, namely N^6-*R*-1-phenyl-2-butyladenosine, *R*-PIA, and N^6-*R*-2-phenyl-1-propyladenosine, are in essence N^6-2-phenethyladenosines. In these cases, the potencies of the very active aliphatic parent nucleosides, N^6-*S*-2-butyladenosine, N^6-isopropyladenosine and N^6-*n*-propyladenosine, were increased only modestly (1.4- to 3-fold) by the introduction of the phenyl group. Other simple 2-phenethyl analogs were not particularly potent at the A_1 receptor (Table 7), suggesting that the high potencies of these three compounds are probably due primarily to the butyl or propyl aliphatic moieties. Indeed, of the twenty-one most active N^6-substituted analogs at the A_1 receptor, fourteen contain only hydrophobic aliphatic residues. The most potent is N^6-cyclopentyladenosine, with five methylene residues. It was 190-fold more potent than N^6-methyladenosine at the A_1 receptor. The three norbornanyl analogs are among the eight most potent N^6-analogs and contain many methylene residues. Other potent N^6-cycloalkyl analogs include the N^6-cyclobutyl analog, which was 92-fold more potent than N^6-methyladenosine, and the widely used N^6-cyclohexyl

Table 9. Rank order of the twenty-one most potent N^6-substituted adenosine analogs at brain A_1-adenosine receptors and the twenty-one most potent at coronary A_2-adenosine receptors [*N*-ethylcarboxamidoadenosine (NECA) and 2-chloroadenosine listed for comparison]

Analog or N^6-Substituent	MPR relative to MeAdo
A_1-RECEPTOR	
R-1-Phenyl-2-butyl	230
Cyclopentyl-	190
Endo-2-norbornanyl-	180
7-Norbornanyl	130
3-Fluorophenyl-	120
Cyclobutyl	92
Exo-2-norbornanyl	86
3-Pentyl	80
Cyclopropylmethyl	80
Dicyclopropylmethyl	80
S-2-Butyl	75
Cyclohexyl	71
Isobutyl	60
4-Fluorophenyl	60
4-Chlorophenyl	55
R-1-Phenyl-2-propyl (*R*-PIA)	50
R-2-Butyl	48
Cycloheptyl	46
(*R*)-2-Phenyl-1-propyl	43
Cyclooctyl	35
1-Methylcyclopentyl	33
NECA	12
2-Chloroadenosine	10
A_2-RECEPTOR	
NECA	3000
2-Chloroadenosine	540
R-1-Phenyl-2-butyl	150
2-(3,4,5-Trimethoxyphenyl)ethyl	104
R-1-Phenyl-2-propyl (*R*-PIA)	80
3-Pentyl	80
2-(2-Thienyl)ethyl	80
4-Methoxyphenyl	70
RS-2-Phenyl-1-butyl	66
2-(3-Pyridyl)ethyl	60
S-2-Phenyl-1-propyl	60
1*S*,2*R*-1-Hydroxy-1-phenyl-2-propyl	58
2-(3,4-Dimethoxyphenyl)ethyl	54
S-2-Butyl	52
2-(3-Thienyl)ethyl	50
R-2-Phenyl-1-propyl	48
(2-Methylphenyl)methyl	48
trans(1*R*,2*S*)-2-Phenylcyclohexyl	48
2-(4-Fluorophenyl)ethyl	42
2-Phenylethyl	40
2-(2-Fluorophenyl)ethyl	38
1*R*,2*S*-1-Hydroxy-1-phenyl-2-propyl	36
Cyclopentyl	36

analog, which was 71-fold more potent than N^6-methyladenosine. Three of the other highly potent N^6-substituted adenosines can be considered open chain analogs of the N^6-cycloalkyladenosines. These are the 3-pentyl and *S*- and *R*-2-butyl analogs. Other very potent N^6-alkyladenosines contain compact hydrophobic moieties such as cyclopropylmethyl, dicyclopropylmethyl and isobutyl. Seventeen of the most potent N^6-substituted adenosines do contain three or more aliphatic carbons. It would appear that such hydrophobic residues with branching at the carbon corresponding to S-2 carbon confer high potency at the A_1 receptor. The only exceptions among the twenty-one are the four N^6-halophenyl analogs, which have no aliphatic residues. The high activity of these four compounds for the A_1 receptor (35- to 230-fold greater than *S*-PIA) is apparently conferred by the presence of the ortho-, meta-, or para-fluoro substituent or by the para-chloro substituent, since the parent N^6-phenyladenosine was only 18-fold more potent than N^6-methyladenosine.

The twenty-one most potent N^6-substituted adenosines displayed an 11-fold range of potency at the A_1 receptor. NECA and 2-chloroadenosine had much *lower* potencies at the A_1 receptor than the least potent of these twenty-one N^6-substituted analogs (Table 9).

Optimal activity at the coronary A_2 receptor. Analysis of the structures of the twenty-one most potent N^6-substituted analogs at the A_2 receptor provides insights into the requirements for optimal activity and emphasizes differences between these requirements at the brain A_1 and the coronary A_2 receptors. N^6-*R*-1-Phenyl-2-butyladenosine is the most potent of these N^6-substituted adenosines at the A_2 receptor (Table 9). It was 150-fold more potent than N^6-methyladenosine. It seems likely that its high activity at the A_2 receptor is due in large measure to the 2-phenethyl moiety with lesser contributions from the aliphatic residues. Indeed, of the twenty-one most potent N^6-substituted adenosines, sixteen contain a 2-phenylethyl moiety or a similar heteroarylethyl moiety (2-pyridylethyl, 2-thienylethyl). Two other potent analogs, namely the N^6-4-methylphenyl- and the N^6-(2-methylphenyl)methyladenosines contain an aryl ring but without the ethylene "spacer" of the 2-phenylethyl analogs. The three exceptions without an aryl ring are the 3-pentyl, *S*-2-butyl and cyclopentyl analogs. Thus, high activity in the absence of an aryl ring obtains at the A_2 receptor only when the alkyl portion of the N^6-substituent contains 4 or 5 carbons.

The twenty-one most potent N^6-substituted adenosine analogs exhibited a 4-fold range of potencies at the A_2 receptor. NECA and 2-chloroadenosine had much *higher* potencies at the A_2 receptor than even the most potent of the twenty-one N^6-substituted analogs (Table 9).

Analogs and series or pairs of analogs for definition of adenosine receptors. Adenosine analogs *specific* for either A_1- or A_2-adenosine receptors would provide a clear approach for defining the nature of the receptors involved in the various physiological functions modulated by adenosine. Certain N^6-substituted adenosines were discovered in the present study that are essentially inactive at the coronary A_2-adenosine receptor, but retain activity at the brain A_1-adenosine receptor and thus may represent specific A_1 receptor agonists. These include the N^6-tertiary alkyladenosines, such as the N^6-methylcyclopentyl analog, and the N^6-cyclooctyl analog. They represent potentially useful tools for definition of the involvement of A_1-adenosine receptor in physiological phenomenon since all three have no activity at the A_2 receptor used in the present comparison.

In lieu of *specific* receptor agonists, the most productive strategy for the delineation of the type of adenosine receptor involved in a physiological response is to search for series or pairs of analogs which exhibit the widest possible differences in potency at A_1- and A_2-adenosine receptors. NECA, 2-chloroadenosine, *R*-PIA and *S*-PIA have been considered such a series, but in practice have not proven completely satisfactory [14]. The present study delineates some possible further pairs and series of analogs for definition of adenosine receptor type (Table 10). N^6-Cyclopentyladenosine is included as one of the most active N^6-alkyladenosines at both the A_1 and the A_2 receptors. The tertiary amine analog N^6-methylcyclopentyladenosine retains high activity at the A_1 receptor, but is inactive at the coronary A_2 receptor. N^6-Cyclohexyladenosine and N^6-cyclooctyladenosine are another potentially useful pair. The former is active at both adenosine receptors, while the latter is inactive at the coronary A_2 receptor. *R*-PIA and *S*-PIA are included because of extensive use, although their relative selectivity at A_1- and A_2-adenosine receptors has not proven entirely satisfactory. The tertiary amine analog (1-phenyl-2-methyl-2-propyladenosine) should complement *R*- and *S*-PIA for definition of receptor subclass. It is slightly more active than *S*-PIA at the brain A_1 receptor, while being many fold less active than *S*-PIA at the coronary A_2 receptor. The *R* and *S* diastereomers of N^6-1-phenyl-2-butyladenosine show marked stereoselectivity at both the A_1 and the A_2 receptor and are included because of the very high activity of the *R* isomer at both receptors. The *R*-1-phenethyl and *S*-1-phenethyl analogs appear much more satisfactory than *R*- and *S*-PIA for definition of receptor subclass, showing a 62-fold selectivity at the A_1 receptor and only a 2.6-fold selectivity at the A_2 receptor.

These and the other pairs or series of potentially useful analogs (Table 10) have been selected not only as possible discriminators of the two subclasses of adenosine receptors, but so as to include pairs or series of compounds with similar physicochemical properties. Further investigation in other systems will be required to confirm the validity of conclusions reached based *only* on data with the brain A_1 receptor and the coronary A_2 receptor. Recent results with the N^6-substituted analogs and adenosine receptor-dependent responses in ileum and vas deferens [7] show a very strong correspondence to the present results with the same N^6-substituted analogs and the brain A_1 receptor (Tables 2–7).

Summary. The present comparison provides further insights into differences between examples of each of the two major proposed classes of adenosine receptors, namely an A_1 receptor, which at least in

Table 10. N^6-Substituted adenosine analogs that are potentially useful alone or in pairs or series for definition of the class of receptor involved in physiological functions of adenosine

N^6-Substituent	MPR relative to MeAdo A_1-Receptor	A_2-Receptor
Cyclopentyl	190	36
Methylcyclopentyl	33	I
Cyclohexyl	71	32
Cyclooctyl	35	I
R-1-Phenyl-2-propyl (*R*-PIA)	50	80
S-1-Phenyl-2-propyl (*S*-PIA)	1.1	8.2
1-Phenyl-2-methyl-2-propyl	2.1	0.22
R-1-Phenethyl	18	10
S-1-Phenethyl	0.31	4.0
R-1-Phenyl-2-butyl	230	150
S-1-Phenyl-2-butyl	4.6	5
2-Methyl-1-propyl(isobutyl)	60	15
3-Pentyl	80	80
3-Fluorophenyl	120	8.6
4-Fluorophenyl	60	30
Phenyl	18	28
Benzyl	0.48	10
2-Phenethyl	15	40
4-Phenylbutyl	8.0	0.92
(3-Chlorophenyl)methyl	1.3	6.8
(4-Chlorophenyl)methyl	1.0	0.10
2-(3-Pyridyl)ethyl	3.8	60
2-(4-Pyridyl)ethyl	3.0	8.4

some cells subserves an inhibitory input to adenylate cyclase, and an A_2 receptor, which at least in some cells subserves a stimulatory input to adenylate cyclase. Further series or pairs of analogs for definition of the nature of receptors involved in physiological responses to adenosine are proposed. Interpretation of the results with such analogs must, however, be tempered by a lack of knowledge in different physiological systems as to the ability of various adenosine analogs to serve as full or only partial agonists and by a cognizance of the fact that the pharmacokinetics may differ widely as structures are varied. Certain analogs that retain moderate activity at the A_1 receptor, while being inactive at the A_2 receptor, should prove useful in research on adenosine receptors. As yet no such selective A_2-adenosine receptor agonists have been discovered, nor does the present data provide any insights as to structural alterations that might provide selective A_2-adenosine receptor agonists. The present model is, of course, only presented as a basis for discussion and a starting point for detailed analysis of excluded volumes for agonist–macromolecule interactions [15], for further structural and conformational analysis of adenosine agonists [16], and for design of further agonists with more constraints in degrees of conformational freedom relevant to this static model.

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