nicotinic acid 2-amidinohydrazide, 4427-16-1; 2-aminoisonicotinic acid 2-amidinohydrazide, 77314-44-4; 2-(*N*-ethylamino)isonicotinic acid 2-amidinohydrazide, 77314-48-8; 2-methylisonicotinic acid, 4021-11-8; 2-ethylisonicotinic acid, 3376-96-3; 2-propylisonicotinic acid, 57663-82-8; 2-(*N*-butylamino)isonicotinic acid, 77314-78-4; 2-(*N*-isopropylamino)isonicotinic acid, 77314-51-3; 2-pyrrolidinoisonicotinic acid, 98088-04-1; 2-chloroisonicotinic acid hydrazide, 58481-04-2; 2-chloroisonicotinic acid 2-amidinohydrazide, 77314-43-3; 2-chloroisonicotinonitrile, 33252-30-1; 2-(4-pyridyl)-2,2-diethoxyethylamine, 74209-44-2; 2-(2-methyl-4-pyridyl)-2,2-diethoxyethylamine dihydrochloride, 98088-03-0; 2-(*N*-ethylamino)-4-glycylpyridine dihydrochloride, 98088-02-9.

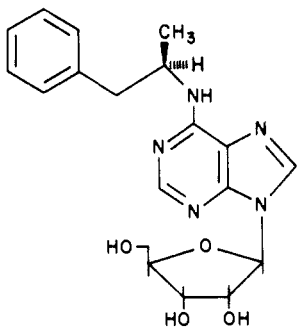
Dog Coronary Artery Adenosine Receptor: Structure of the N^6-Alkyl Subregion

Shozo Kusachi,† Robert D. Thompson,† William J. Bugni,† Nobuyuki Yamada,† and R. A. Olsson*†,‡

Suncoast Cardiovascular Research Laboratory, Departments of Internal Medicine and Biochemistry, University of South Florida College of Medicine, Tampa, Florida 33612. *Received March 14, 1985*

The moderately potent and stereoselective coronary vasoactivity of N^6-[1-phenyl-2(*R*)-propyl]adenosine (1) is the basis for the present study that maps the N^6 region of the coronary artery adenosine receptor by means of the structure–coronary vasoactivity relationships of 81 analogues of 1 in the open-thorax dog. Stereoselectivity is a general property of N^6-substituted adenosines that have a chiral center adjacent to N^6. The activity ratio of 1 to its *S* diastereomer is 10, the result of the positive interaction with the receptor of the propyl C-3 group of the *R* diastereomer in combination with the steric hindrance exerted by this group of the *S* diastereomer. Replacing the benzyl moiety of 1 by an ethyl, phenyl, phenethyl, or naphthyl group lowers potency of the *R* diastereomer and, accordingly, the *R*/*S* ratio. Propyl C-1 of 1 interacts with a receptor region large enough to accommodate three methylene residues and the propyl C-3 residue with a separate region large enough to accommodate two. The receptor subregion that interacts with the propyl C-1 of 1 is more tolerant of bulk and of polar substituents than the subregion that interacts with propyl C-3. Evidence bearing on the possible contribution of N^6 to activity, e.g. through hydrogen bonding, is ambiguous. These results support a provisional model of the N^6-alkyl subregion.

A large body of evidence indicates that the adenylate cyclase stimulatory (A2 or R_a) type of purinergic receptor mediates the coronary vasoactivity of adenosine.[1] One line of evidence is the order of coronary vasodilator potency of adenosine receptor-selective analogues in the open-chest dog: *N*-ethyladenosine-5′-uronamide > *N*-cyclopropyladenosine-5′-uronamide ≫ 2-chloroadenosine > N^6-[1-phenyl-2(*R*)-propyl]adenosine (1) > adenosine > N^6-[1-phenyl-2(*S*)-propyl]adenosine (**2**).[2] Although such an order of potency is unusual for an A2 receptor in that 1 is more active than adenosine, it does have precedent in tracheal smooth muscle[3] and in the adenylate cyclase A2 receptors of guinea pig thyroid epithelium[4] and rat aorta smooth muscle cells.[5] While the preeminent vasoactivity of the *N*-alkyladenosine-5′-uronamides implies that the ribose domain of the A2 receptor contains the "ethyl pocket" that seems to be characteristic of A2 receptors,[6,7] the potencies of 2-chloroadenosine and 1 suggest the existence of additional regions of specialized structure in the purine domain.



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A previous survey of 128 adenosine analogues as agonists at the A2 receptor of a transformed human fibroblast line[7] included 18 purine ribosides modified at C-6. Although the number of N^6-alkyladenosines was too limited to define structure–activity rules, the potency of these analogues showed that an N^6-alkyl substituent makes a modest contribution to activity.

The stereoselective coronary vasoactivity of 1[2,8] is the basis for the hypothesis tested in this study, namely, that the coronary A2 receptor contains a specialized N^6 region. Deriving the structure–activity rules of the N^6-substituted adenosines constitutes one test of this hypothesis. The experimental design, i.e. the selection of analogues for testing, is based on a model of the N^6 receptor region[9] derived from the structure of 1. This model (Figure 1) posits that each of several chemical attributes of the purine C6 substituent could contribute to potency: 1, the absolute configuration at the propyl C-2 chiral center; 2, the size and hydrophobicity of propyl C-1 and C-3; 3, the chemistry of N^6, especially its potential to act as either a donor or acceptor in hydrogen bonding; 4, the length of the alkyl chain separating the phenyl moiety from N^6; and 5, the chemical attributes of the phenyl moiety e.g. aromaticity, planarity, size, and its angulation and torsion relative to the axis of the propyl C-1 to C-2 bond. For convenience we consider the N^6 region of the coronary A2 receptor as composed of alkyl and phenyl subregions. The experiments described here concern only the structure of the alkyl subregion and thus deal with attributes 1–3 of the model depicted in Figure 1.

† Department of Internal Medicine.
‡ Department of Biochemistry.

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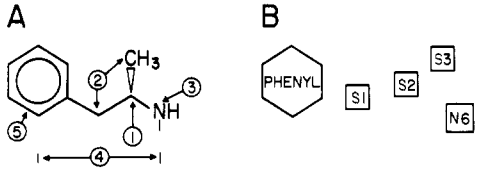

Figure 1. Model of the N^6 region of the coronary adenosine receptor. A. Structure of the C-6 substituent of 1, showing chemical attributes that could be determinants of coronary vasoactivity. See text for discussion. B. Hypothetical model of the N^6 receptor region, envisioned as consisting of subregions complementary to the C-6 substituent of 1.

The coronary vasoactivity of an adenosine analogue in the beating, blood-perfused heart only indirectly reflects the occupancy of coronary receptors. A chain of incompletely understood events couples receptor occupancy to coronary smooth muscle relaxation, so that inferences about receptor structure must explicitly assume that the degree of coupling, i.e. efficacy, does not vary greatly from one analogue to another. Ideally, one should study receptor occupancy in purified cell membrane preparations, an experimental approach that has proved useful in mapping brain A1 receptors.[10] Despite some progress in this direction,[11,12] preparations of smooth muscle membranes of coronary resistance vessels are not available at this time. The low-affinity A2 receptor of human placenta[13] is a possible surrogate for the coronary adenosine receptor. Our preliminary studies showed that [^{3}H]-*N*-ethyladenosine-5′-uronamide binds only weakly to this receptor, $K_D > 1$ μM, and N^6-alkyladenosines did not compete with the radioligand for binding sites. Accordingly, this system does not predict coronary vasoactivity.

Using the blood-perfused in situ heart to assay coronary vasoactivity complicates the interpretation of structure–activity correlations. The close arterial infusion of these nucleosides minimizes systemic effects such as bradycardia or hypotension, which could indirectly influence coronary vasomotion. The nonspecific binding of the analogues to plasma proteins or cell membranes could seriously affect their rate of penetration to the receptor in the cardiac interstitium and, more importantly, the relationship between the calculated and the true concentration of analogue at the coronary receptor. Basing potency estimates on the steady-state hemodynamic responses to continuous, constant rate infusion of analogue tends to minimize such pharmacokinetic variables. In addition, the present experiments included studies defining the quantitative relationship between hydrophobicity and binding to protein. Our interpretations of apparent potency are thus able to explicitly take into account between-analogue differences in the capacity for nonspecific binding.

Despite these limitations, a receptor model deduced from structure–activity correlations can serve as the focus for future research comparing the different types of purinergic receptors and as a guide to drug development.[14] The value of such a model depends as much on the content of structural details testable by future experimentation as on its ability to account for the observed potencies of adenosine analogues. Testing such predictions and comparing receptor subtypes seem a reasonable approach to identifying new adenosine analogues having greater potency or selectivity for the coronary receptor.

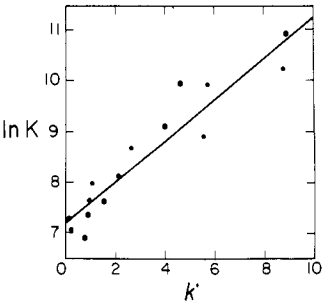

Figure 2. Dependence of the binding of adenosine analogues to albumin upon the hydrophobicity of the N^6 substituent. The dissociation constant of the analogue albumin complex, K, serves as a measure of binding affinity, and k', which is related to the retention time on a reversed-phase HPLC column, is an index of hydrophobicity. The least-squares regression equation describing this relationship is $\ln K = 7.18 \pm 0.17 + 0.41 \pm 0.04k'$, $r^2 = 0.894$.

Results and Discussion

Chemistry. The synthesis of the adenosine analogues, many of which are already known, employed published methods. Table I lists novel analogues and their properties. Analogues whose N^6 substituents contain a chiral center adjacent to N^6 exhibit interesting properties related to the absolute configuration at this center. Without exception, the *R* diastereomers have higher melting points and lower hydrophobicity indices than the *S* diastereomers and they crystallize readily. We have not found conditions under which the *S* diastereomers crystallize; accordingly, all those reported here were obtained as chromatographically and analytically pure but amorphous powders.

Hydrophobicity and Protein Binding. The binding of adenosine and its analogues to plasma albumin[15] importantly influences comparisons of apparent potency. In vitro experiments assessing this source of ambiguity show that a hydrophobicity index, k',[16] predicts the extent of binding to albumin. Table II compares the hydrophobicity indices of representative N^6-alkyladenosines with the dissociation constant of the analogue albumin complex, K, and Figure 2 shows that ln K varies directly with k'. Such a result means that the hydrophobicity of the N^6-alkyl substituent contributes part of the free energy for binding to albumin. The slope of the regression of ln K on k', equivalent to a change in free energy of −240 cal/mol per unit k', suggests that the binding of analogues to albumin in the vascular compartment could have a significant influence on apparent potency. Accordingly, the interpretations that follow either compare analogues of similar k' or at least attempt to account for the effect of differences in protein binding.

Acidic Dissociation Constants of N^6-Modified Adenosines. Table III shows that the pK_as of representative analogues do not differ significantly from that of adenosine. Such a result means that except for N^6-(2-aminoethyl)adenosine, which is known to be protonated at physiological pH, the ionization of these analogues does not influence their apparent activity.

Structure–Activity Relationships. The assays of coronary vasoactivity used 61 dogs. During the control periods prior to the estimation of the EC_{50} of adenosine, heart rate averaged (mean ± SEM) 104 ± 6 beats/min,

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Table I. Characteristics of Novel C-6-Modified Purine Ribosides

no.	purine C-6 substituent, formula (fw)	method	yield, %	mp, °C	$[\alpha]^{25}_D$	UV data λ_{max}, nM	UV data $10^{-3}\epsilon$
3	[(*R*)-1-phenylethyl]amino,[a] $C_{18}H_{21}N_5O_4$ (371.40)	A	77	173–174	−92.0	269	20.8
4	[(*S*)-1-phenylethyl]amino,[a] $C_{18}H_{21}N_5O_4$ (371.40)	A	67	93–95	−29.0	269	20.4
5	[(*R*)-4-phenyl-2-butyl]amino, $C_{20}H_{25}N_5O_4$ (399.45)	A	62	141–142	−82.2	269	17.3
6	[(*S*)-4-phenyl-2-butyl]amino, $C_{20}H_{25}N_5O_4$ (399.45)	A	59	89–90	−17.4	270	17.2
7	[(*R*)-1-(1-naphthyl)ethyl]amino, $C_{22}H_{23}N_5O_4$ (421.46)	A	82	277–278	−93.5	271	11.4
						280	13.2
8	[(*S*)-1-(1-naphthyl)ethyl]amino, $C_{22}H_{23}N_5O_4$ (421.46)	A	68	132–133	+88.7	271	11.5
						281	13.0
9	[(*R*)-2-butyl]amino, $C_{14}H_{21}N_5O_4$ (323.35)	B	81	104–105	−93.8	269	29.6
10	[(*S*)-2-butyl]amino, $C_{14}H_{21}N_5O_4$ (323.35)	B	86	95–98	−38.0	270	19.4
11	[*trans*-(1*R*,2*S*)-2-phenylcyclopropyl]amino,[a] $C_{19}H_{21}N_5O_4 \cdot 0.75H_2O$ (396.92)	A	77	150–151	−94.0	271	19.7
12	[*trans*-(1*S*,2*R*)-2-phenylcyclopropyl]amino,[a] $C_{19}H_{21}N_5O_4 \cdot 0.25H_2O$ (387.91)	A	85	168–169	+92.3	271	19.3
14	2-phenyl-2-propyl, $C_{19}H_{23}N_5O_4 \cdot 0.25H_2O$ (389.93)	A	75	199–201		270	18.9
15	(2-methyl-1-phenyl-2-propyl)amino, $C_{20}H_{25}N_5O_4$ (399.45)	A	82	185–186		272	18.0
16	(2-methyl-4-phenyl-2-butyl)amino, $C_{21}H_{27}N_5O_4$ (413.48)	A	77	88–90		274	16.8
18	(2-methyl-2-butyl)amino, $C_{15}H_{23}N_5O_4$ (337.38)	B	62	96–100		270	19.0
19	1-methylcyclopentyl, $C_{16}H_{23}N_5O_4 \cdot 0.25H_2O$ (353.89)	A	63	91–93		270	18.2
20	1-methylcyclohexyl, $C_{17}H_{25}N_5O_4$ (363.42)	A	70	96–98		271	17.5
23	(2-methyl-2-phenyl)hydrazino, $C_{17}H_{20}N_6O_4$ (372.39)	A	27	127–129		263	17.7
33	[(*R*)-2-pentyl]amino, $C_{15}H_{23}N_5O_4$ (337.38)	A	74	89–92		270	18.8
34	[(*R*)-2-hexyl]amino, $C_{16}H_{25}N_5O_4 \cdot 0.5H_2O$ (360.42)	A	27	116–119		240	18.8
35	[(*R*)-2-heptyl]amino, $C_{17}H_{27}N_5O_4 \cdot 0.25H_2O$ (369.94)	A	63	130–132		270	19.0
36	[(*R*)-2-octyl]amino, $C_{18}H_{29}N_5O_4$ (379.46)	A	35	111–113		270	19.0
37	(3-pentyl)amino, $C_{15}H_{23}N_5O_4 \cdot 0.25H_2O$ (341.88)	A	70	99–100		268	18.8
38	(4-heptyl)amino, $C_{17}H_{27}N_5O_4$ (365.44)	A	78	112–113		269	18.9
42	(3,3-dimethyl-1-butyl)amino, $C_{16}H_{25}N_5O_4 \cdot 0.33H_2O$ (357.42)	A	65	98–101		268	19.2
44	(2,4-dimethyl-3-pentyl)amino, $C_{17}H_{27}N_5O_4$ (365.44)	A	63	99–102		270	19.8
45	(diphenylmethyl)amino, $C_{23}H_{23}N_5O_4$ (433.47)	A	81	107–109		270	19.3
46	1,3-diphenyl-2-propyl, $C_{25}H_{27}N_5O_4 \cdot 0.5H_2O$ (470.54)	A	46	158–160		271	18.5
48	cyclobutylamine, $C_{14}H_{19}N_5O_4$ (321.34)	A	73	121–123		269	19.0
56	[6,6-dimethylbicyclo[3.1.1]hept-2-yl]amino, $C_{20}H_{29}N_5O_4$ (403.48)	A	75	181–183		268	16.7
57	(*exo*-2-norbornanyl)amino, $C_{17}H_{23}N_5O_4$ (361.40)	A	56	122–124		270	19.4
58	(*endo*-2-norbornanyl)amino, $C_{17}H_{23}N_5O_4$ (361.40)	A	63	132–134		270	19.6
66	[(*R*)-2-phenylpropyl]amino, $C_{19}H_{23}N_5O_4 \cdot 0.5H_2O$ (394.44)	A	63	93–95	−5.0	270	18.3
67	(*S*)-2-phenylpropylamine, $C_{19}H_{23}N_5O_4$ (385.43)	A	67	128–129	−115.8	271	18.7
68	[(*R,S*)-2-phenylbutyl]amino, $C_{20}H_{25}N_5O_4$ (399.45)	A	71	96–100		270	18.4
72	[(*S*)-1-hydroxy-2-propyl]amino, $C_{13}H_{19}N_5O_5 \cdot 0.5H_2O$ (334.34)	B	71	151–154	−49.4	268	18.1
73	[(*R*)-1-hydroxy-2-butyl]amino,[a] $C_{14}H_{21}N_5O_5$ (339.36)	B	77	96–100	−11.5	268	18.6
74	[(*S*)-1-hydroxy-2-butyl]amino,[a] $C_{14}H_{21}N_5O_5$ (339.36)	B	74	214–215	−76.5	267	18.7
79	*N*-methyl-*N*-[(*R*)-1-phenylethyl]amino, $C_{19}H_{23}N_5O_4$ (385.43)	A	52	93–95		281	17.1
80	*N*-methyl-*N*-(2-phenylethyl)amino, $C_{19}H_{23}N_5O_4$ (385.43)	A	74	94–96		281	18.0
82	2-phenethoxy, $C_{18}H_{20}N_4O_5$ (372.38)	*a*	40	152–153		253	12.1

[a] The literature reports the composition of a racemic mixture. [b] Synthesis described in the Experimental Section.

Table II. Binding of Representative N^6-Alkyladenosines to Bovine Serum Albumin

no.	N^6 substituent	k'[a]	analogue bound, mol/g BSA	$10^{-3}K$, M^{-1}	$-\Delta F°$, kcal/mol
64	2-hydroxyethyl	0.14	0.53 ± 0.02	1.44	4.1
32	2-propyl	0.23	0.41 ± 0.02	1.12	4.3
9	(*R*)-2-butyl	0.89	0.36 ± 0.04	1.01	4.1
10	(*S*)-2-butyl	0.96	0.53 ± 0.02	1.47	4.3
40	2-methyl-1-propyl	1.00	0.74 ± 0.05	2.02	4.5
29	1-butyl	1.07	1.06 ± 0.03	2.89	4.7
37	3-pentyl	1.64	0.78 ± 0.03	2.12	4.5
50	cyclohexyl	2.12	1.30 ± 0.07	3.57	4.8
58	*endo*-2-norbornanyl	2.64	2.09 ± 0.02	5.69	5.1
51	cycloheptyl	4.04	3.40 ± 0.02	9.25	5.4
53	cyclohexylmethyl	4.67	7.10 ± 0.01	19.3	5.8
44	2,4-dimethyl-3-pentyl	5.52	2.97 ± 0.07	7.08	5.3
38	4-heptyl	5.57	7.10 ± 0.02	19.3	5.8
52	cyclooctyl	8.80	10.1 ± 0.02	27.5	6.0
55	cyclohexylethyl	8.92	19.3 ± 0.50	52.5	6.4

[a] Abbreviations: k', hydrophobicity index as defined in the text; K, dissociation constant of analogue BSA complex; BSA, bovine serum albumin; $-\Delta F$, standard free energy. Analogue bound is the mean ±SEM of four estimates, two at each of two BSA concentrations.

Table III. pK_a of Representative N^6-Substituted Adenosines

no.	N^6 substituent	pK_a
	H (adenosine)	3.6
1	(*R*)-1-phenyl-2-propyl	3.4
3	(*R*)-1-phenethyl	3.3
9	(*R*)-2-butyl	3.7
13	2-phenethyl	3.6
37	3-pentyl	3.3
79	*N*-methyl-2-phenethyl	3.0
80	*N*-methyl-pheneth-1(*R*)-yl	3.2

mean arterial pressure 97 ± 3 mmHg, coronary flow rate 96 ± 5 mL/min per 100 g, arterial blood P_{O_2} 144 ± 5 mmHg, P_{CO_2} 39 ± 1 mmHg, and pH 4.39 ± 0.01. Coronary flow rate at the peak of the active hyperemia response following a 30-s coronary occlusion averaged 480 ± 27% of basal rate, and the coronary flow rate during maximum vasodilation by adenosine was 493 ± 34% of control. The EC_{50} of adenosine averaged 1.20 ± 0.15 μM.

Every analogue having a molar potency ratio vs. adenosine (MPR) greater than 0.05 raised coronary flow to at least 75% of maximum, suggesting that all were full agonists. The slopes of the regressions of logit (coronary conductance) on log [nucleoside] of the active analogues averaged 7.39 ± 0.46, not significantly different from the average of the adenosine controls, 6.93 ± 0.84, evidence that adenosine and these analogues acted at a common receptor. Generally, the intracoronary administration of adenosine or one of the analogues had no effect on heart

Table IV. Coronary Vasoactivity and Hydrophobicity of N^6-Modified Adenosines: Stereoselectivity

no.	N^6 substituent	MPR[a]	R/S	k'
1	(*R*)-1-phenyl-2-propyl	4.3 ± 0.7 (5)[b]	10	2.57
2	(*S*)-1-phenyl-2-propyl	0.41 ± 0.06 (5)[b]		2.61
3	(*R*)-1-phenethyl	0.52	2.6	1.64
4	(*S*)-1-phenethyl	0.20		1.89
5	(*R*)-4-phenyl-2-butyl	0.19	3.8	6.11
6	(*S*)-4-phenyl-2-butyl	0.05		6.47
7	(*R*)-1-(1-naphthyl)ethyl	0.23	2.1	5.98
8	(*S*)-1-(1-naphthyl)ethyl	0.11		6.32
9	(*R*)-2-butyl	0.88 ± 0.031 (4)	0.34	0.89
10	(*S*)-2-butyl	2.6 ± 0.031 (4)		0.96
11	(1*R*,2*S*)-*trans*-2-phenylcyclopropyl[c]	0.70	2.3	2.36
12	(1*S*,2*R*)-*trans*-2-phenylcyclopropyl[c]	0.31		2.43

[a] Abbreviations: MPR, molar potency ratio; k', hydrophobicity index (see the Experimental Section). Number of experiments in parentheses. [b] From ref 2. [c] Absolute configuration of (phenylcyclopropyl)amine according to: *J. Med. Chem.* **1972**, *15*, 1187.

rate or blood pressure. N^6-(Dicyclopropylmethyl)adenosine (**43**) was the sole exception, causing concentration-dependent bradycardia and hypotension in both animals in which it was tested.

The analogues listed in Table IV test the hypothesis that the stereoselectivity of **1** and **2** is a general attribute of analogues whose N^6 substituent contains a chiral center adjacent to N^6. One alkyl substituent on the chiral carbon of analogues **1–10** is a methyl group; the other is either a phenyl, benzyl, phenethyl, naphthyl, or ethyl group. Cyclopropane C-3 of **11** and **12** corresponds to propyl C-3 of **1** and **2** such that the absolute configuration at the C-1 chiral center of **11** resembles that of **1** and **12** and that of **2**. Among analogues **1–8** and **11** and **12**, the *R* diastereomer is the most potent of each pair. The R/S activity ratio of **1** and **2** was 10, which is the highest such ratio of any pair of diastereomers in this set. The lower ratios of the other analogues in part reflects the lower activity of the *R* diastereomers relative to that of **1**.

The N^6-(2-butyl)adenosines, **9** and **10**, are exceptions to an $R > S$ rule; the *S* diastereomer is about 3 times more potent than the *R*. Such a result is still consistent with the idea that chiral substituents adopt a common absolute configuration in order to fit into the receptor. Further, it is evidence that the S-1 and S-3 receptor subregions differ in their affinity for alkyl groups. The butyl group of these analogues can rotate around the axis of the butyl C-2 to N^6 bond such that the ethyl moiety of the *S* diastereomer can occupy the same position in space as the methyl group of the *R* diastereomer, and vice versa. It is possible that the interaction of the ethyl moiety of **10** with the S-3 receptor subregion is stronger than that of the ethyl moiety of **9** with the S-1 receptor subregion (see below). Thus, the stereoselectivity of **1–12** supports a superimposition rule, namely that analogues having secondary alkyl N^6 substituents will fit into the receptor in such a way that the carbon attached to N^6 and the carbons on either side of it will be in a conformation superimposable on propyl C-1, C-2, and C-3 of **1**.

Further exploration of the mechanism of the stereoselectivity of the analogues **1** and **2** employed N^6-(2-phenethyl)adenosine (**13**), three N^6-(phenyl-*tert*-alkyl)-adenosines, **14–16**, two N^6-*tert*-alkyladenosines, **17** and **18**, and two N^6-*tert*-cycloalkyladenosines, **19** and **20** (Table V). One model envisions stereoselectivity as the consequence of a positive interaction of propyl C-3 of **1** with the S-3 subregion of the receptor. This model predicts—and experiment shows—that **1** is more active than **13**, which lacks such a group. The N^6-*tert*-alkyladenosines discriminate between two mechanisms for the low activity of **2**. If the activity of **2** were due simply to propyl C-3 being situated such that it does not interact with the receptor, one would expect **2** to be as active as **13**. Further, the N^6-*tert*-alkyladenosines, **14–18**, each of which has a methyl group superimposable on propyl C-3 of **1**, should be as active as their *sec*-alkyl congeners. On the other hand, if propyl C-3 of **2** were sterically hindering, **13** will be more potent than **2** and the *tert*-alkyladenosines will be less active than their *sec*-alkyl congeners. Experiment bears out the latter prediction; the N^6-*tert*-alkyladenosines have either no or at best only marginal coronary vasoactivity. A third model posits that **1** and **2** bind to the receptor equally well but propyl C-3 of **2** prevents the conformational change that presumably occurs in the receptor during activation. This model predicts that **2** will inhibit the vasoactivity of **1**, but experiment shows that such is not the case; **2** has no effect on the action of **1**.

Table V. Coronary Vasoactivity and Hydrophobicity of N^6-Modified Adenosines: Mechanism of Stereoselectivity

no.	N^6 substituent	MPR	*sec*-alkyl analogues MPR[a]	k'
13	2-phenethyl	2.0		1.77
14	2-phenyl-2-propyl	7% at 27 μM[b]	**3**, 0.52	4.45
15	2-methyl-1-phenyl-2-propyl	0.011	**1**, 4.3	7.43
16	2-methyl-4-phenyl-2-butyl	4% at 50 μM	**5**, 0.19	18.7
17	2-methyl-2-propyl	7% at 35 μM	**32**, 0.71	2.44
18	2-methyl-2-butyl	12% at 47 μM	**9**, 0.88	4.28
19	1-methylcyclopentyl	0.033	**49**, 1.8	5.02
20	1-methylcyclohexyl	5% at 46 μM	**50**, 1.6	9.29
	equimolar mixture of 1 and 2 vs. 1 alone	1.5 ± 0.1 (3)		

[a] Abbreviations as in Table IV. [b] Maximum increase in coronary flow, percent, and analogue concentration, μM.

The experiments summarized in Tables IV and V indicate that a combination of three mechanisms best explains the stereoselectivity of analogue **1**. The R/S ratio reflects the fact that the benzyl moiety of **1** is optimum for activity, and the positive interaction of propyl C-3 with the S-3 receptor subregion further promotes activity of the *R* diastereomer. The low activity of the *S* diastereomer owes to steric hindrance. The prominent steric hindrance means that the S-2 and S-3 receptor subregions have very limited bulk tolerance.

The contribution of propyl C-3 to the vasoactivity of **1** prompted a systematic examination of the influence of each of the alkyl carbons on activity. Table VI lists the analogues used to test this idea. N^6-Methyladenosine (**21**) lacks the entire N^6 substituent of **1** except propyl C-2 and is 20 times less potent than adenosine. Possible explanations for such a profound loss of activity include the hydrophobicity of this methyl group and, because it is larger than a hydrogen atom of the unmodified amino group of adenosine, steric hindrance.

Analogues **22–26**, the N and O isosteres of **21** and some of their higher alkyl homologues, test the hypothesis that only hydrophobic substituents can occupy S-2 receptor subregion. Both 6-hydrazinopurine riboside (**22**) and N^6-hydroxyadenosine (**24**) are as active as **21**. Moreover, 6-(2-methyl-2-phenylhydrazino)purine riboside (**23**) is nearly 3 times as active as its carbon isostere **3**. N^6-(Benzyloxy)adenosine (**26**) retains one-fourth of the activity of **13**. Such results suggest that the S-2 receptor subregion is indifferent to the polarity of atoms that occupy it and that the low activity of **21**, **22**, and **24** may be due to highly localized steric hindrance that can be overcome by larger N^6 substituents whose additional carbons

Table VI. Coronary Vasoactivity and Hydrophobicity of Adenosine Analogues: N^6-Alkyl Substituents

no.	N^6 substituent	MPR[a]	k'
21	methyl	0.05	0.07
22	amino[b]	0.046	0.14
23	(2-methyl-2-phenyl)amino[c]	1.8	0.93
24	hydroxyl	0.16	0.16
25	*o*-methyloxy	0.12 ± 0.03 (3)	0.16
26	*o*-benzyloxy	0.42	1.04
27	ethyl	0.15	0.29
28	1-propyl	0.64	0.57
29	1-butyl	0.38	1.59
30	1-pentyl	0.36	3.08
31	1-hexyl	0.20	5.88
32	2-propyl	0.71	0.54
33	(*R,S*)-2-pentyl	1.10	2.38
34	(*R,S*)-2-hexyl	0.56	4.46
35	(*R,S*)-2-heptyl	0.31	8.54
36	(*R,S*)-2-octyl	0.052	16.88
37	3-pentyl	4.0 ± 1.0 (4)	1.64
38	4-heptyl	0.81	5.57
39	cyclopropylmethyl	0.58	0.71
40	2-methyl-1-propyl	0.75	1.00
41	2,2-dimethyl-1-propyl	0.064	2.50
42	3,3-dimethyl-1-butyl	0.084	4.71
43	dicyclopropylmethyl	1.5	2.68
44	2,4-dimethyl-3-pentyl	0.75	1.00
45	diphenylmethyl	27% at 61 μM	6.11
46	1,3-diphenyl-2-propyl	0.33	14.83

[a] Abbreviations as in Table IV. [b] I.e., 6-hydrazinopurine riboside. [c] I.e., 6-(2-methyl-2-phenylhydrazino)purine riboside.

Table VII. Coronary Vasoactivity and Hydrophobicity of Adenosine Analogues: N^6-Cycloalkyl Substituents

no.	N^6 substituent	MPR[a]	k'
47	cyclopropyl	0.38	0.29
48	cyclobutyl	1.6	0.71
49	cyclopentyl	1.8 ± 0.46 (4)	1.29
50	cyclohexyl	1.6 ± 0.20 (5)[b]	2.12
51	cycloheptyl	0.18	4.04
52	cyclooctyl	12% at 37 μM	8.80
53	cyclohexylmethyl	0.14	4.67
54	2-cyclohexylethyl	0.04	8.92
55	(*R,S*)-1-cyclohexyl-2-propyl	0.15	16.63
56	[6,6-dimethylbicyclo[3.3.1]hept-2-yl]-methyl	8% at 37 μM	21.34
57	*exo*-2-norbornanyl	0.54	2.68
58	*endo*-2-norbornanyl	0.55	2.64
59	7-norbornanyl	0.39	3.52
60	1-adamantanyl	0 at 27 μM	19.6
61	2-adamantanyl	0 at 18 μM	12.2

[a] Abbreviations as in Table IV. Numbers in parentheses refer to number of estimates. [b] From ref 2.

interact with continuous portions of the alkyl subregion.

The unexpected potency of **23** suggests that other 6-(alkylhydrazino)purine ribosides might also be more potent than their carbon isosteres. Accordingly, we tried to prepare purine ribosides having as C-6 substituents 2-benzyl-2-methylhydrazine, 2,2-dimethylhydrazine, and 1-aminopiperidine. Such nucleosides would be nitrogen isosteres of **1**, **32**, and **50**. In each instance the reaction of 6-chloropurine riboside with the hydrazine appeared to yield product, but these nucleosides decomposed during purification.

The structure–activity relationships of analogues **9**, **10**, **21**, and **27–46** probe the structures of the S-1 and S-3 receptor subregions. Although N^6 methylation greatly reduces activity, the additional carbons of **27** and **28** restore activity toward that of adenosine. Presumably, such an additive effect on vasoactivity reflects the interaction of both carbons with either the S-1 or S-3 subregion. Since the position of an N^6 substituent is not fixed, these *n*-alkyl groups do not give information about which receptor subregion may be involved. Still larger *n*-alkyl substituents, those of the *n*-butyl, *n*-pentyl and *n*-hexyl congeners **29–31**, are progressively more hydrophobic and less potent than **28**, perhaps as much a reflection of their binding to plasma albumin as the possible bulk intolerance of the alkyl subregion.

Adenosines having a secondary alkyl N^6 substituent, **9**, **10**, and **32–38**, support several inferences. First, each carbon able to occupy the S-1 receptor subregion augments the effect of a carbon occupying the S-3 subregion. Second, N^6-[2(*R,S*)-pentyl]adenosine (**33**) is more potent than **9**, which implies that the S-1 subregion is large enough to accommodate a three-carbon alkyl fragment. However, the fact that N^6-(4-heptyl)adenosine (**38**) is only one-fifth as active as **37** suggests that the S-3 subregion may only be large enough to accommodate a two-carbon fragment. The activities of the *R* and *S* diastereomers of N^6-(3-hexyl)-adenosine, which has both an ethyl and a propyl group attached to the chiral carbon, would permit a definitive test of the receptor model, which predicts the activities of the two diastereomers will be $R > S$. Our inability to resolve the enantiomers of 3-hexylamine has thus far frustrated such a test. Third, the affinity of the S-3 receptor subregion for a two-carbon fragment is greater than that of the S-1 subregion. Thus, N^6-[2(*S*)-butyl]adenosine (**10**) is over 3 times more potent than N^6-propyladenosine (**32**), whereas N^6-[2(*R*)-butyl]adenosine (**9**) is equipotent with **32**. A similar comparison of the two N^6-(2-butyl)-adenosines with N^6-(3-pentyl)adenosine (**37**) shows that adding a carbon to butyl C-1 of the *R* diastereomer to enhance its interaction with the S-2 subregion raises activity over 4-fold. However, a similar chain lengthening in **10** to enhance interaction with the S-1 subregion of the receptor raises activity only 1.5-fold.

An important result arising from the structure–activity correlations of the N^6-alkyladenosines is the support gained for a carbon additivity rule: The S-1 and S-3 subregions of the receptor can accommodate, respectively, three- and two-carbon alkyl moieties; each of these carbons contributes additively to vascoactivity. We will later invoke this rule when interpreting the activities of nucleosides probing the kinds of chemical forces that govern binding to the S-1 and S-3 receptor subregions.

Analogues **39–44**, which have branched-chain alkyl N^6 substituents, refine the estimates of the dimensions of the S-1 and S-3 receptor regions by providing information about their widths. N^6-(Cyclopropylmethyl)adenosine (**39**) and N^6-(2-methyl-1-propyl)adenosine (**40**) are analogues of N^6-(1-propyl)adenosine (**28**) in which propyl C-2 and C-3 are part of, respectively, a cyclopropyl or an isopropyl group. Analogues **28**, **39**, and **40** are equipotent; such a result suggests that the additional carbon introduced by cyclization or chain branching does not interact with the receptor. However, N^6-(2,2-dimethyl-1-propyl)adenosine (**41**) and N^6-(3,3-dimethyl-1-butyl)adenosine (**42**) have bulkier alkyl groups and only marginal coronary vasoactivity, which suggests that the methyl groups are sterically hindering. N^6-(Dicyclopropylmethyl)adenosine (**43**) and N^6-(2,4-dimethyl-3-pentyl)adenosine (**44**) are less potent than N^6-(3-pentyl)adenosine, likewise evidence of limited bulk tolerance in the S-1 and S-3 subregions. The still larger phenyl group of N^6-(diphenylmethyl)adenosine (**45**) abolishes activity. Interestingly, N^6-(1,3-diphenyl-2-propyl)adenosine (**46**) is moderately active. Apparently, separating the phenyl group from propyl C-2 by a single methylene residue is sufficient to relieve the impingement of the phenyl group on the S-3 subregion.

N^6-Cycloalkyladenosines **47–61**, which have semirigid N^6 substituents, probe the more distal portions of the S-1

Table VIII. Effect of Hydrophobicity of N^6 Substituent on Coronary Vasoactivity of N^6-Alkyladenosines

no.	N^6 substituent	atom/group	receptor subregion	MPR[a]	k'
	Group A: Analogue of N^1-propyladenosine				
28	1-propyl	CH_2CH_3	C-1 or C-3	0.64	0.57
62	2-propenyl	$CH{=}CH_2$	C-1 or C-3	0.23	0.36
63	2-propynyl	$C{\equiv}CH$	C-1 or C-3	0.23	0.21
64	2-hydroxyethyl	CH_2CH_2OH	C-1 or C-3	0.14	0.14
65	2-aminoethyl	$CH_2CH_2NH_2$	C-1 or C-3	0.072	0.11
	Group B: Analogues of N^6-(2-phenethyl)adenosine				
13	2-phenethyl	H	C-1	2.0	1.77
66	(*R*)-2-phenylpropyl	CH_3	C-1	2.4	2.89
67	(*S*)-2-phenylpropyl	CH_3	C-1	3.0	3.00
68	(*R*,*S*)-2-phenylbutyl	C_2H_5	C-1	3.3	5.89
69	(*R*,*S*)-2-hydroxy-2-phenethyl	OH	C-1	1.4	0.79
	Group C: Analogues of **1**				
1	(*R*)-1-phenyl-2-propyl	H	C-1	4.3	2.57
70	(1*R*,2*R*)-1-hydroxy-1-phenyl-2-propyl	OH	C-1	1.8	0.96
71	(1*S*,2*R*)-1-hydroxy-1-phenyl-2-propyl	OH	C-1	2.9	1.32
	Group D: Analogues of N^6-[(*S*)-2-butyl]adenosine				
10	(*S*)-2-butyl	CH_3	C-3	2.6	0.96
72	(*S*)-1-hydroxy-2-propyl	OH	C-3	0.098	0.28
	Group E: Analogues of N^6-(3-pentyl)adenosine				
37	3-pentyl	CH_3	C-1 or C-3	4.0	1.64
73	(*R*)-1-hydroxy-2-butyl	CH_2OH	C-1	0.77	0.40
74	(*S*)-1-hydroxy-2-butyl	CH_2OH	C-3	0.12	0.52

[a] Abbreviations as in Table IV.

and S-3 subregions (Table VII). Owing to the flexibility of alkyl chains, carbons such as butyl C-4 of **9** and **10** or pentyl C-1 or C-5 of **37** may lie either cis or trans to N^6. In **47** and **48**, however, cyclization fixes these carbons trans to N^6. The cyclobutyl, cyclopentyl, and cyclohexyl analogues **48–50** are the most potent of the N^6-cycloalkyladenosines. The lower activity of **51** and **52** may reflect steric hindrance exerted by the distal cycloalkyl carbons or, possibly, binding to plasma albumin, since they are substantially more hydrophobic than **48–50**. The three cyclohexylalkyl analogues **53–55** have only marginal coronary vasoactivity and N^6-[[6,6-dimethylbicyclo[3.1.1]-hept-2-yl]methyl]adenosine (**56**) is inactive. The modest activity of the N^6-norbornanyladenosines, **57–59**, and the inactivity of the adamantanyl analogues, **60** and **61**, imply that these even more rigid substituents encounter steric hindrance during receptor occupancy or are themselves hindering during receptor activation. Alternatively, **56** and the N^6-adamantanyladenosines are extremely hydrophobic; it is thus possible that binding to plasma proteins prevented these analogues from reaching the receptor.

The usefulness of the carbon additivity rule in the interpretation of the structure–activity relationships of the N^6-alkyladenosines urged experiments to examine its physical basis. Since the interaction of a receptor and ligand is a chemical reaction, the problem is one of identifying the molecular attributes that contribute to the free energy needed to drive the reaction. Hydrophobicity is a prominent characteristic of alkanes; accordingly, we tested the hypothesis that vasoactivity will vary concordantly with the hydrophobicity of the N^6 substituent. Structure–activity correlations based on the nucleosides listed in Table VIII support this hypothesis. Analogues **62–65** of group A differ from N^6-(1-propyl)adenosine (**28**) according to the polarity of the N^6-alkyl substituent, as reflected in the hydrophobicity indices, k'. In this subset, coronary vasoactivity varies directly with hydrophobicity.

Table IX. Chemistry of N^6

no.	C-6 substituent	MPR[a]	k'
75	H	0.023	ND[b]
76	Cl	0.023	ND
77	CH_3	0.007	ND
78	$N(CH_3)_2$	0.001	ND
79	$N(CH_3)CH(CH_3)C_6H_5$	0.018	4.04
80	$N(CH_3)C_2H_4C_6H_5$	0.043 ± 0.025 (3)	3.79
81	OCH_3	0.004	0.16
82	$OC_2H_4C_6H_5$	9% at 107 μM	2.36

[a] Abbreviations as in Table IV. [b] ND = not determined.

Because the alkyl substituents of the analogues in group A can interact with either the S-1 or S-3 subregion of the receptor, it was necessary to resort to alternative strategies to evaluate differences in hydrophobicity between the two regions. The first stratagem exploits the interaction of a phenyl moiety with the phenyl receptor region to orient alkyl substituents to interact with the S-1 receptor subregion (groups B and C). The second stratagem employs the superimposition rule to achieve this orientation (groups D and E). Analogues of group B show that methylenes interacting with the S-1 subregion increase activity and that a hydroxyl group decreases activity. Similarly, the addition of a hydroxyl group oriented to interact with the S-1 subregion reduces the vasoactivity of **1** (group C). The small degree of stereoselectivity exhibited by **66** and **67** and also by **70** and **71** suggests that the S-1 subregion is fairly capacious. The limited information available (analogues **72** and **74** of groups D and E) suggests that the S-3 subregion may be less tolerant of polar substituents than the S-1 subregion.

The coronary vasoactivity of adenosine depends absolutely on the 6-amino group.[17] Chemical attributes of this functional group that could contribute to activity include its polarity, its effect on the electronic structure of the purine moiety, and its participation in hydrogen bonding as either a donor or an acceptor. Table IX lists analogues that probe these factors. The purine C-6 substituents of analogues **75–77** cannot participate in hydrogen bonding, and all three are essentially inactive. However, these substituents also alter the electronic structure of the purine base, which could as well explain the lack of activity. The N^6 substituents of **78–80** do not greatly alter the electronic structure of the purine but do abolish the ability to donate to a hydrogen bond. Although inability to hydrogen bond seems a plausible explanation for the low activity of the N^6-dialkyladenosines, steric hindrance exerted by the *N*-methyl group is equally plausible. Analogues **81** and **82** are oxygen isosteres of N^6-methyl- and N^6-(2-phenethyl)adenosine, **21** and **13**. Like **78–80**, they can act as hydrogen-bond acceptors, but like **75–77**, the C-6 substituent greatly alters the electronic structure of the purine. They, too, are inactive. Consequently, the manner by which N^6 contributes to vasoactivity is uncertain.

Receptor Model. The structure–activity correlations described above support a provisional three-dimensional model of the N^6 region of the coronary adenosine receptor (Figure 3). Like an earlier model,[9] this model proceeds from the structure of the N^6 substituent of **1** and includes appropriate modifications consistent with the results of the present study. The four atoms of the alkylamine moiety of **1** are depicted as lying in a Y-shaped groove; the

(17) Olsson, R. A.; Khouri, E. M.; Bedynek, J. L., Jr.; McLean, J. *Circ. Res.* **1979**, *45*, 468.

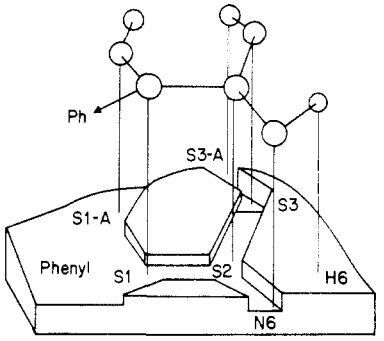

Figure 3. Provisional model of the alkyl portion of the N^6 region of the coronary adenosine receptor deduced from the structure–activity relationships developed in this study. See text for additional discussion.

S-2 subregion is at the intersection and the N^6, S-1, and S-3 subregions are at the extremities of the arms. In accordance with the evidence for stereoselectivity developed in the present study, the arm of the Y joining the S-2 and S-3 subregions is inclined relative to the plane described by the N^6, S-1, and S-2 subregions. The evidence supporting the earlier model did not include observations on the vasoactivity of the N^6-*sec*-alkyl- and N^6-cycloalkyladenosines. This new evidence suggests extensions of the S-1 and S-3 subregions, here designated S1-A and S3-A, in directions trans to N^6. The S-1/S-1A subregion is represented as being capacious in keeping with the evidence for little stereoselectivity (analogues **66**, **67**, **70**, and **71**) and the ability to accommodate three carbons (analogues **33** and **68**). The model depicts a hydrogen-bond acceptor adjacent to the N^6 subregion, recognizing that the evidence on this point is ambiguous. Available evidence suggests the S-3/S-3A subregion is hydrophobic and the S-1/S-1A region is less so. Although the S-1A and phenyl regions are depicted as separate, it is possible that they are parts of the same, perhaps aromatic, site. The location and chemical attributes of the phenyl subregion will be addressed in a subsequent report.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241MC spectropolarimeter. MHW Laboratories, Phoenix, AZ, performed the elemental analyses, which agreed within ±0.4% of the calculated composition. Characterization of previously reported compounds included ^{1}H NMR and UV spectra, melting points, and estimates of purity by reversed-phase HPLC. Characterization of novel nucleosides additionally included analysis for C, H, and N. Amines not commercially available were prepared by $LiAlH_4$ reduction of the appropriate amide or nitrile,[18] by the Ritter reaction,[19] or in the case of optically active amines by an asymmetric synthesis.[20]

6-Chloropurine Riboside. Modifications in the chlorination and deblocking of 2′,3′,5′-tri-*O*-acetylinosine that differed in some details from a published procedure[21] produced a somewhat larger yield and reduced contamination of the product by adenosine. A mixture of 2′,3′,5′-tri-*O*-acetylinosine (25 g, 63.4 mmol), $SOCl_2$ (18.4 mL, 253.6 mmol), and DMF (9.8 mL, 126.8 mmol) in 250 mL of dry $CHCl_3$ was refluxed for 5 h, cooled, and poured into 400 mL of vigorously stirred ice/saturated $NaHCO_3$. The addition of solid $NaHCO_3$ completed neutralization. The organic layer was washed with water, dried ($MgSO_4$), and evaporated to a syrup. After one coevaporation with dry CH_3OH, the residue was taken up in 70 mL of dry CH_3OH and cooled to 10 °C in an ice bath. Three additions of 0.1 g of Na metal at hourly intervals followed by an additional 2 h of cooling and stirring completed deblocking. The crude product was filtered off, washed with H_2O, heated at 60 °C in 100 mL of CH_3OH, filtered, and washed with CH_3OH to remove traces of 6-methoxypurine riboside: yield 10 g (55%) of pale yellow solid; mp 181–183 °C dec, lit.[21] mp 182–183 °C. TLC on silica gel ($CHCl_3/CH_3OH$ (4:1)) showed a single spot of R_f identical with that of a commercial sample. It is possible to scale up the chlorination as much as 4-fold without affecting yield. However, scaling up the deblocking step significantly curtails yield.

N^6-Substituted Adenosines. Method A (General Procedure).[22] A mixture of 6-chloropurine riboside (1.5 g, 5.2 mmol), 6.5 mmol of amine or amine salt, and a 3-fold excess of triethylamine in 50 mL of absolute C_2H_5OH was heated at reflux until TLC showed disappearance of starting material (6–72 h). The residue after evaporation was triturated with $(C_2H_5)_2O$ and the product crystallized from CH_3OH/H_2O or CH_3OH/ethyl acetate. If the nucleoside did not crystallize, it was first purified by low-pressure reversed-phase preparative chromatography on C18 silica. Yields ranged between 50 and 95%.

Method B. In the case of volatile amines, reaction mixtures were heated for 48 h at 80 °C in a stainless-steel bomb. Proportions of reactants and workup were the same as in method A.

Method C. This method was used to minimize racemization of product in the synthesis of **70** and **71**. A mixture of 1.5 g (4.54 mmol) 6-(methylsulfonyl)-9-(β-D-ribofuranosyl)purine[23] and 1.72 g (11.4 mmol) of (−)-norpseudoephedrine in 50 mL of absolute C_2H_5OH gave, after 3 weeks at room temperature, a clear solution. Evaporation in vacuo (bath temperature <30 °C) left a white solid that was triturated with $CHCl_3$, filtered, and washed with H_2O: yield 1 g (55%) of N^6-[(*1R,2R*)-1-hydroxy-1-phenyl-2-propyl]-adenosine[24] (**70**); $[\alpha]^{25}_D$ −104.4° (*c* 1, 95% C_2H_5OH). A similar preparation proceeding from (+)-norephedrine and employing purification by chromatography on silica gel eluted with $CHCl_3/CH_3OH$ (15:1) yielded 1.3 g (71%) of N^6-[(*1S,2R*)-1-hydroxy-1-phenyl-2-propyl]adenosine (**71**); $[\alpha]^{25}_D$ −115.0° (*c* 1, 95% C_2H_5OH).

6-(2-Phenethoxy)purine Riboside (82). Clean Na metal (0.1 g, 4.3 mmol) was reacted with 15 mL of 2-phenylethanol, 6-chloropurine riboside (1.15 g, 4 mmol) was added, and the mixture was stirred for 3 h at 80 °C. The reaction mixture was cooled, diluted with $CHCl_3$, and washed with water. The organic layer was dried ($MgSO_4$) and evaporated. Crystallization from benzene yielded 0.6 g (40%) of product, mp 151–152 °C.

Hydrophobicity Index. The retention time of a nucleoside on a reversed-phase HPLC column provided an index of hydrophobicity. Such estimates employed an Altex 110A chromatograph fitted with an 0.45 × 15 cm column of Ultrasphere ODS silica (Altex) eluted isocratically with 0.01 M $NaHPO_4$, pH 7.0, CH_3OH (4:6). The hydrophobicity index, k', related the retention time of a nucleoside, t, to the appearance time of the solvent front, t_0, by the formula $k' = (t - t_0)/t_0$.[16]

Binding of Analogues to Albumin. The method of Hummel and Dryer,[25] adapted for HPLC, served for estimates of the affinity of analogues for bovine serum albumin (BSA). Aliquots (20 μL) of solutions of BSA (0.25–2 g/dL) and 25 μM analogue in 0.1 M $NaHPO_4$, pH 7.4, were applied to an 0.45 × 25 cm column of Aquaphore OH-100 (Rainin Instruments, Woburn, MA) equilibrated with 25 μM analogue in the $NaHPO_4$ buffer. Absorbance of the effluent was monitored at 254 nm. An experimental run consisted of duplicate estimates of binding at each of two protein concentrations, usually 1 and 2 g/dL. Preliminary experiments showed that binding was proportional to the concentrations of BSA and analogue in accordance with the law of mass action and

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was indifferent to the presence of fatty acids in the BSA.

Acidic Dissociation Constant (pK_a). Spectrophotometric titrations to estimate the pK_a of representative nucleosides followed previously described procedures.[26,27]

Biological Activity. Assays of coronary vasoactivity followed a published procedure.[2,17] Each analogue was tested in two dogs, or, if the estimates of molar potency ratio vs. adenosine (MPR) differed by >20%, the analogue was assayed in additional dogs. The MPRs reported here are the arithmetic mean of two assays or the mean ±SEM of three or more assays.

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Registry No. 1, 38594-96-6; 2, 38594-97-7; 3, 97905-50-5; 4, 97905-51-6; 5, 97948-60-2; 6, 97905-52-7; 7, 97826-32-9; 8, 97826-33-0; 9, 97905-53-8; 10, 97905-54-9; 11, 97905-55-0; 12, 97905-56-1; 13, 20125-39-7; 14, 97826-34-1; 15, 97826-35-2; 16, 97826-36-3; 17, 30784-93-1; 18, 97826-37-4; 19, 97826-38-5; 20, 97826-39-6; 21, 1867-73-8; 22, 5746-27-0; 23, 97826-40-9; 24, 3414-62-8; 25, 19399-25-8; 26, 50474-52-7; 27, 14357-08-5; 28, 17270-22-3; 29, 23096-10-8; 30, 26293-51-6; 31, 15824-83-6; 32, 17270-23-4; 33, 37676-67-8; 34, 97826-41-0; 35, 97826-42-1; 36, 97826-43-2; 37, 97826-44-3; 38, 97826-45-4; 39, 36397-00-9; 40, 36031-53-5; 41, 97826-46-5; 42, 30562-03-9; 43, 57294-65-2; 44, 97826-47-6; 45, 57294-69-6; 46, 57294-70-9; 47, 97374-48-6; 48, 97374-49-7; 49, 41552-82-3; 50, 36396-99-3; 51, 41552-83-4; 52, 41552-84-5; 53, 97826-48-7; 54, 97826-49-8; 55, 57294-66-3; 56, 97826-50-1; 57, 97826-51-2; 58, 97905-57-2; 59, 97826-52-3; 60, 55582-99-5; 61, 97826-53-4; 62, 15763-12-9; 63, 67005-97-4; 64, 4338-48-1; 65, 35662-04-5; 66, 97826-54-5; 67, 97826-55-6; 68, 97826-56-7; 69, 54712-70-8; 70, 97905-58-3; 71, 20330-18-1; 72, 97826-57-8; 73, 97905-59-4; 74, 97905-60-7; 75, 550-33-4; 76, 5399-87-1; 77, 14675-48-0; 78, 2620-62-4; 79, 97826-58-9; 80, 97877-18-4; 81, 5746-29-2; 82, 97826-59-0; 2',3',5'-tri-O-acetylinosine, 3181-38-2; 6-(methylsulfonyl)-9-(β-D-ribofuranosyl)purine, 53821-41-3; (−)-norpseudoephedrine, 37577-07-4; (+)-norephedrine, 37577-28-9.

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Structure–Activity Relationships among Benextramine-Related Tetraamine Disulfides at Peripheral α-Adrenoreceptors

Piero Angeli, Livio Brasili, Egle Brancia, Dario Giardinà, Wilma Quaglia, and Carlo Melchiorre*

Department of Chemical Sciences, University of Camerino, 62032 Camerino (MC), Italy. *Received January 21, 1985*

Several N,N''-(dithiodi-2,1-ethanediyl)bis[N'-(arylmethyl)-1,6-hexanediamines] were prepared and evaluated for their blocking activity on postsynaptic α_1-adrenoreceptors in the isolated rat vas deferens. The results were compared with those obtained for benextramine (**1**). N,N''-(Dithiodi-2,1-ethanediyl)bis[N'-(pyrrol-2-ylmethyl)-1,6-hexanediamine] (pyrextramine, **29**) was the most potent among the tetraamine disulfides investigated. Thus, it was selected for further pharmacological evaluation to assess its receptor specificity. At a concentration of 10 μM it did not affect the responses elicited by 5-hydroxytryptamine and histamine in guinea pig ileum and by isoproterenol in guinea pig atria and tracheal chain. Furthermore, it was more specific than benextramine (**1**) toward the muscarinic receptor, being significantly less potent in inhibiting the carbachol-induced response in rat jejunum. These results show that pyrextramine (**29**) is an irreversible α-blocking agent that is more potent and specific than benextramine (**1**). In conclusion, **29** may be a useful tool in the elucidation and characterization of the peripheral α_1-adrenoreceptor.

Two categories of α-adrenoreceptor inhibitors are known: ligands that inhibit through the formation of a covalent bond with some components of the receptor molecule (irreversible blockade) and those that bind reversibly to the receptor, preventing access of the agonist (competitive or reversible inhibition). Among the irreversible inhibitors, β-haloalkylamines have received much attention in the past in the investigation of the α-adrenoreceptor.[1] However, this class of α-blockers is not specific, as other receptor systems are affected as well. In the late 1970s the new class of tetraamine disulfides was developed, and it was shown to be a useful tool in α-adrenergic pharmacology.[2,3] Tetraamine disulfides, exemplified by benextramine (**1**, BHC), whose main feature is a cystamine moiety carrying aminoalkyl substituents on the nitrogens, have shed new light on the active-site chemistry of the α-adrenoreceptor. It has been demonstrated that **1** irreversibly blocks α_1-adrenoreceptors in the isolated rabbit aorta,[4,5] dog aorta,[6] rat vas deferens,[4,7] rat anococcygeus muscle,[8,9] cat spleen[5], and rat and rabbit atrium.[10,11] Furthermore, it has been shown that the inhibition is time dependent and that this is consistent with covalent bond formation in the drug–receptor interaction, which is the result of a protein thiol–disulfide interchange reaction.[4,12,13] An extensive structure–activity

Chart I. General Structure of Tetraamine Disulfides (Table I)

$$[RNH(CH_2)_nNH(CH_2)_2S-]_2 \cdot 4HX$$

1 (benextramine, BHC): R = 2-MeO-benzyl, n = 6

Scheme I

$$[H_2N(CH_2)_6NH(CH_2)_2S-]_2 \xrightarrow{ArCHO,\ NaBH_4} \textbf{3-39}\ (\text{Table I})$$

2

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