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

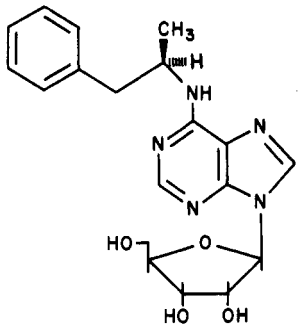
Shozo Kusachi, Robert D. Thompson, Nobuyuki Yamada, Daniel T. Daly, and R. A. Olsson*†

Suncoast Cardiovascular Research Laboratory, Departments of Internal Medicine and Biochemistry, University of South Florida College of Medicine, Tampa, Florida 33612. *Received August 5, 1985*

Previous structure–coronary vasoactivity correlations of the N^6-alkyladenosine analogues of N^6-[(*R*)-1-phenyl-2-propyl]adenosine, 1, support the hypothesis that the coronary artery A2 adenosine receptor contains an N^6 region of specialized structure. The part of this receptor region that binds the 2-propyl moiety of 1 determines stereoselectivity and contributes to coronary vasoactivity. The present study uses 92 adenosine analogues containing an aryl group in the N^6 substituent to test the hypothesis that the N^6 receptor region contains an aryl subregion that binds the phenyl moiety of 1 and thereby contributes to its coronary vasoactivity. N^6-Aralkyladenosines are often more potent than their alkyl congeners. Two methylene residues seem to provide optimum separation of the aryl group from N^6. Among adenosines with semirigid N^6 substituents, N^6-[(1*R*,2*S*)-*trans*-2-phenylcyclohexyl]adenosine was uniquely active, evidence that when 1 occupies the receptor, the axis of the propyl C-1 to phenyl C-1 bond is nearly in the plane described by N^6 and propyl C-1 and C-2. The torsion angle around this bond is unknown. Replacing the phenyl group of N^6-2-phenethyladenosine with a thienyl or a 3-pyridyl group raises activity. The structure–activity relationships of the N^6-(arylethyl)-, the N^6-(arylmethyl)-, and the N^6-phenyladenosines differ strinkingly from each other. Taken together, such results support the idea that the N^6 region of the dog coronary artery A2 adenosine receptor includes an aryl subregion.

Most studies of the structure–activity relationships of the N^6-substituted adenosine concern the A1 adenosine receptors of brain,[1-3] fat cells,[4] atrial myocardium,[5] vas deferens,[6] and ileum,[6] at which several of the N^6-substituted adenosines are potent agonists. Although there have been extensive structure–activity analyses of adenosine analogues at the A2 receptors of coronary artery,[7-9] of a transformed fibroblast line,[10] and of platelets,[11,12] these studies in sum constitute a broad survey that does not particularly emphasize adenosines substituted at N^6. This report and its antecedent[13] focus on the influence of an N^6 substituent on the coronary vasoactivity of adenosine.

The previous report in this series[13] describes the structure–coronary vasoactivity relationships of the N^6-alkyladenosine analogues of N^6-[(*R*)-1-phenyl-2-propyl]-adenosine, 1, at the A2 adenosine receptor of dog coronary artery. Those studies examined a hypothetical model that envisions the structure of the N^6 region of this A2 receptor as complementary to that of the N^6 substituent of 1. The results of those studies supported the idea[9] that the coronary receptor contains an N^6 region of specialized structure and provided an explanation for the stereoselectivity of 1 as well as some information about the shape and chemical attributes of the subregion interacting with the propyl moiety of 1, i.e., attributes 1–3 of the general receptor model shown in Figure 1.



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The studies described below test the hypothesis that the N^6 region of the coronary A2 adenosine receptor contains an aryl subregion that binds the phenyl group of 1 and thereby augments the contribution of the N^6-alkyl moiety to vasoactivity. Such structure–coronary vasoactivity relationships of the N^6-aryl- and the N^6-aralkyladenosines have two major aims. The first is to define the geometrical relationship of the aryl subregion to the rest of the receptor. The second is to identify the factors governing the interaction of the phenyl group of 1 with this region. These are listed in Figure 1 as attributes 4 and 5.

Because the assays of coronary vasoactivity employ an in situ blood-perfused heart preparation, pharmacokinetic factors such as the nonspecific binding of adenosine and its analogues to blood proteins or to cells could importantly influence apparent potency. We have previously shown that there is a quantative relationship between an index of hydrophobicity, namely, the retention of a nucleoside on a reverse-phase HPLC column and the association constant of its complex with serum albumin.[13] Such a hydrophobicity index is a very useful aid to the interpretation of the apparent potency of adenosine analogues in the blood-perfused heart. The present report includes such information as well as estimates of the pK_a of selected analogues.

Results and Discussion

Chemistry. A well-known method, the reaction of 6-

†Address for correspondence: R. A. Olsson, M.D., Department of Internal Medicine, Box 19, USF College of Medicine, 12901 N. 30th Street, Tampa, Florida 33612.

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Table I. Characteristics of Novel N^6-Modified Adenosines

no.	N^6 substituent formula (fw)	anal.	yield, %	mp, °C	$[\alpha]^{25}_D$	UV λ_{max}	UV $10^3\epsilon$
4	3-phenylpropyl, $C_{19}H_{23}N_5O_4$ (385.43)	C, H, N	78	129–130		269	18.3
5	4-phenylbutyl, $C_{20}H_{25}N_5O_4$ (399.45)	C, H, N	93	124–125		269	18.0
9	2,3-dihydro-1*H*-inden-2-yl, $C_{19}H_{21}N_5O_4$ 0.25 H_2O (387.91)	C, H, N	52	166–168		271	21.0
11	(*R*)-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-6-yl, $C_{21}H_{25}N_5O_4$ (411.46)	C, H, N	60	217–219	−11.8	271	20.0
12	(1*R*,2*R*)-*cis*-2-phenylcyclohexyl, $C_{22}H_{27}N_5O_4$·0.5CH_3OH (441.50)	C, H, N	56	109–112	−223.4	270	18.0
13	(1*R*,2*S*)-*trans*-2-phenylcyclohexyl, $C_{22}H_{27}N_5O_4$·0.25CH_3OH (433.50)	C, H, N	62	127–130	−91.7	270	18.0
14	2-(2-fluorophenyl)ethyl, $C_{18}H_{20}FN_5O_4$ (389.39)	C, H, N	81	159–160		271	18.4
15	2-(3-fluorophenyl)ethyl, $C_{18}H_{20}FN_5O_4$ (389.39)	C, H, N	74	133–134		271	17.8
16	2-(4-fluorophenyl)ethyl, $C_{18}H_{20}FN_5O_4$ (389.39)	C, H, N	96	190–191		271	19.5
17	2-(2-chlorophenyl)ethyl, $C_{18}H_{20}ClN_5O_4$ (405.84)	C, H, N	78	150–151		270	17.5
18	2-(3-chlorophenyl)ethyl, $C_{18}H_{20}ClN_5O_4$ (405.84)	C, H, N	57	128–130		270	18.1
19	2-(4-chlorophenyl)ethyl, $C_{18}H_{20}ClN_5O_4$ (405.84)	C, H, N	85	220–221		271	17.7
20	2-(2-methylphenyl)ethyl, $C_{19}H_{23}N_5O_4$ (385.43)	C, H, N	65	166–167		270	18.7
21	2-(3-methylphenyl)ethyl, $C_{19}H_{23}N_5O_4$ (385.43)	C, H, N	60	133–136		270	19.1
22	2-(4-methylphenyl)ethyl, $C_{19}H_{23}N_5O_4$ (385.43)	C, H, N	69	193–194		271	18.7
23	2-(2-methoxyphenyl)ethyl, $C_{19}H_{23}N_5O_5$ (401.43)	C, H, N	79	145–146		271	18.0
24	2-(3-methoxyphenyl)ethyl, $C_{19}H_{23}N_5O_5$ (401.43)	C, H, N	64	110–111		270	19.5
25	2-(4-methoxyphenyl)ethyl, $C_{19}H_{23}N_5O_4$ (401.43)	C, H, N	86	195–196		271	18.7
27	2-[3,4-(methylenedioxy)phenyl]ethyl, $C_{19}H_{21}N_5O_6$ (415.41)	C, H, N	70	177–179		271	18.9
28	2-(2,4,5-trimethoxyphenyl)ethyl, $C_{21}H_{27}N_5O_7$ (461.47)	C, H, N	75	144–145		272	17.8
31	2-([3-(trifluoromethyl)phenyl]ethyl, $C_{19}H_{20}F_3N_5O_4$ (439.40)	C, H, N	74	150–151		270	18.8
32	2,2-diphenylethyl, $C_{24}H_{25}N_5O_4$·0.5H_2O (456.51)	C, H, N	78	107–109		270	18.5
34	2-(1-methyl-2-pyrrolyl)ethyl, $C_{17}H_{22}N_6O_4$ (374.40)	C, H, N	62	105–106		270	18.4
35	2-(2-pyridyl)ethyl, $C_{17}H_{20}N_6O_4$·0.25H_2O (376.89)	C, H, N	67	124–126		269	20.9
36	2-(3-pyridyl)ethyl, $C_{17}H_{20}N_6O_4$ (372.39)	C, H, N	82	165–166		270	20.1
37	2-(4-pyridyl)ethyl, $C_{17}H_{20}N_6O_4$ (372.39)	C, H, N	56	169–170		269	21.0
38	2-(2-thienyl)ethyl, $C_{16}H_{19}N_5O_4S$·0.5H_2O (386.43)	C, H, N, S	51	153–154		270	18.5
39	2-(3-thienyl)ethyl, $C_{16}H_{19}N_5O_4S$ (377.42)	C, H, N, S	76	152–153		270	18.3
41	2-(2-benzimidazolyl)ethyl, $C_{19}H_{21}N_7O_4$·1.5H_2O (438.44)	C, H, N	65	150–153		273	36.1
42	2-(3-quinolinoyl)ethyl, $C_{19}H_{18}N_6O_4$·H_2O (412.41)	C, H, N	58	250(dec)		283	22.1
43	2-(1-naphthyl)ethyl, $C_{22}H_{23}N_5O_4$ (421.46)	C, H, N	82	181–182		270	19.8
44	2-(2-naphthyl)ethyl, $C_{22}H_{23}N_5O_4$ (421.46)	C, H, N	71	145–146		270	19.1
45	3-(3,4-dimethoxyphenyl)propyl, $C_{21}H_{26}N_5O_6$ (444.47)	C, H, N	65	137–139		272	16.1
46	3-(3,4,5-trimethoxyphenyl)propyl, $C_{22}H_{29}N_5O_7$ (475.50)	C, H, N	74	116–117		271	14.1
47	(2-fluorophenyl)methyl, $C_{17}H_{18}FN_5O_4$ (375.36)	C, H, N	76	181–182		270	19.5
48	(3-fluorophenyl)methyl, $C_{17}H_{18}FN_5O_4$ (375.36)	C, H, N	69	143–145		270	19.8
59	(2,4-dichlorophenyl)methyl, $C_{17}H_{17}Cl_2N_5O_4$ (426.26)	C, H, N, Cl	75	187–189		269	22.7
60	(2,5-dichlorophenyl)methyl, $C_{17}H_{17}Cl_2N_5O_4$ (426.26)	C, H, N, Cl	63	196–198		269	22.3
61	(2,6-dichlorophenyl)methyl, $C_{17}H_{17}Cl_2N_5O_4$ (426.26)	C, H, N, Cl	74	212–214		270	21.9
62	(3,4-dichlorophenyl)methyl, $C_{17}H_{17}Cl_2N_5O_4$ (426.26)	C, H, N, Cl	83	173–175		269	22.0
63	(3,5-dichlorophenyl)methyl, $C_{17}H_{17}Cl_2N_5O_4$	C, H, N, Cl	69	196–198		269	21.9
64	(*R*)-2,3-dihydro-1*H*-inden-1-yl, $C_{19}H_{21}N_5O_4$·0.5H_2O (392.42)	C, H, N	40	146–148	−34	272	23.9
68	4-pyridylmethyl, $C_{16}H_{18}N_6O_4$·0.5H_2O (367.37)	C, H, N	54	118–120		268	21.4
70	3-thienylmethyl, $C_{15}H_{17}N_5O_4S$ (363.40)	C, H, N, S	79	163–164		270	21.0
92	3,4,5-trimethoxyphenyl, $C_{19}H_{23}N_5O_7$ (433.42)	C, H, N	67	204–205		297	19.9

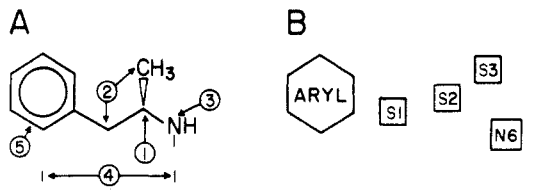

Figure 1. Hypothetical model of the N^6 region of the dog coronary artery A2 adenosine receptor. (A) Structure of the C-6 substituent of 1 showing chemical features that could be determinants of coronary vasoactivity, namely: (1) absolute configuration at the propyl C-2 chiral center, (2) the size and hydrophobicity of propyl C-1 and C-3, (3) the chemistry of N^6, particularly its potential for hydrogen bonding, (4) the length of the alkyl chain separating the phenyl group from N^6, and (5) the chemical attributes of the phenyl group. A previous report[13] examines factors 1–3, the present report concerns factors 4 and 5. (B) Topography of the N^6 region, depicted as consisting of aryl, S-1, S-2, S-3, and N-6 subregions complementary to the structure of the N^6 substituent of 1.

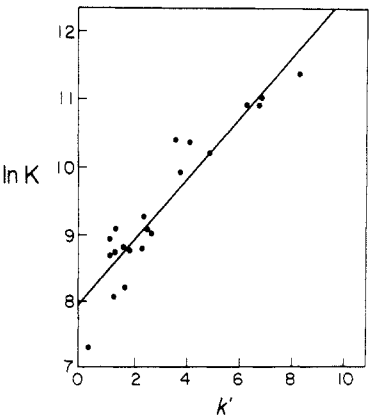

Figure 2. Influence of the hydrophobicity of the N^6 substituent on the binding of N^6-phenyl- and N^6-aralkyladenosines to bovine serum albumin. The dissociation constant of the analogue–albumin complex, K, serves as a measure of binding affinity and k', which is related to the retention time of the analogue on a reverse-phase HPLC column, is an index of hydrophobicity. The least-squares linear regression equation describing the relationship between K and k' is $\ln K = 7.96 \pm 0.15 + 0.45 \pm 0.039k'$, $r^2 = 0.87$.

chloropurine ribonucleoside with the appropriate amine in the presence of an acid acceptor,[14] furnished all the adenosine analogues required for this study. A number of the analogues are novel; Table I lists these nucleosides and their properties.

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Table II. Relationship between Affinity for Albumin and Hydrophobicity Index, k', of N^6-Aryl- and N^6-Aralkyladenosines

no.	N^6 substituent	k'[a]	analogue bound, mol/g	K, M^{-1}	ln K	$-\Delta F°$, kcal
66	2-pyridylmethyl	0.32	0.55 ± 0.10	1495	7.31	4.2
69	2-thienylmethyl	1.04	2.71 ± 0.08	7390	8.91	5.2
70	3-thienylmethyl	1.07	2.15 ± 0.16	5850	8.67	5.1
72	phenyl	1.29	1.16 ± 0.05	3155	8.06	4.7
39	2-(3-thienyl)ethyl	1.32	2.18 ± 0.03	5915	8.69	5.1
38	2-(2-thienyl)ethyl	1.36	3.19 ± 0.06	8675	9.07	5.3
	(*R*)-1-phenethyl[b]	1.64	2.44 ± 0.09	6640	8.80	5.2
75	4-fluorophenyl	1.68	1.31 ± 0.14	3565	8.18	4.8
	(*S*)-1-phenethyl[b]	1.89	2.32 ± 0.04	6310	8.75	5.1
74	3-fluorophenyl	2.36	2.34 ± 0.10	6365	8.76	5.1
16	2-(4-fluorophenyl)ethyl	2.39	3.79 ± 0.04	10310	9.24	5.4
1	(*R*)-1-phenyl-2-propyl	2.57	3.09 ± 0.11	8405	9.04	5.3
	(*S*)-1-phenyl-2-propyl[b]	2.61	3.13 ± 0.03	8515	9.05	5.3
	2-phenethoxy[b]	2.75	2.96 ± 0.04	8050	8.99	5.3
17	2-(2-chlorophenyl)ethyl	3.64	11.9 ± 0.12	32370	10.38	6.1
	N^6-methyl-N^6-2-phenethyl[b]	3.79	7.3 ± 0.09	19855	9.90	5.8
18	2-(3-chlorophenyl)ethyl	4.14	11.7 ± 0.09	31825	10.37	6.1
10	(*R*)-1,2,3,4-tetrahydronaphth-2-yl	4.89	9.7 ± 0.08	26385	10.18	6.0
	(*S*)-1-(1-naphthyl)ethyl[b]	6.32	19.7 ± 0.29	53585	10.89	6.4
43	2-(1-naphthyl)ethyl	6.82	19.7 ± 0.30	53585	10.89	6.4
6	4-phenylbutyl	6.93	22.2 ± 0.06	60385	11.01	6.4
32	2,2-diphenylethyl	8.29	30.8 ± 0.24	83775	11.34	6.6

[a] Abbreviations are k', hydrophobicity index; K, association constant; and $-\Delta F°$, free energy of binding. [b] From ref 13.

Hydrophobicity Index and Protein Binding. As in the case of the N^6-alkyladenosines,[13] a hydrophobicity index based on the retention of an analogue on a reverse-phase HPLC column[15] predicted the extent to which it associated in vitro with albumin (Table II). The association constant of the analogue albumin complex, K, was an exponential function of the hydrophobicity index, k' (Figure 2). The line describing the least-squares regression of ln K on k' of the N^6-aryl- and the N^6-aralkyladenosines had essentially the same slope as the corresponding regression of N^6-alkyladenosines.[1] However, the aryladenosine regression had a significantly greater intercept on the ln K axis, 7.96 ± 0.15 (SEM) vs. 7.18 ± 0.17 for the alkyladenosines, $p < 0.02$. Such a result means that the binding affinity of the N^6-aryladenosines, like the N^6-alkyladenosines, varies concordantly with hydrophobicity. The difference in ordinate intercept, 0.78 ln K, indicates that the aryl substituent contributes an additional 450 cal/mol to the free energy of binding to albumin.

Acidic Association Constant. The pK_as of representative phenyl-substituted N^6-phenyl-, N^6-(phenylmethyl)-, and N^6-phenethyladenosines are compiled in Tables V–VII. Although the presence and length of an alkyl chain interposed between the phenyl group and N^6 affected pK_a, neither the type of ring substituent nor its ring position seemed important. The average pK_a of the phenyl analogues was (group mean ± SEM) 2.73 ± 0.06, that of the phenylmethyl analogues 3.21 ± 0.04, and that of the phenethyl analogues 3.34 ± 0.03, essentially that of adenosine itself, 3.3–3.6.[13,16–18] Such results mean, first of all, that none in this particular set of analogues is protonated at physiological pH. Second, the phenyl group of the N^6-phenyladenosines seems to act as an electron sink, competing with the π-deficient pyrimidine ring for the electrons of N^6, indirectly lowering electron density at N^1 and, thereby, pK_a. The interpolation of one or two methylene groups between the phenyl group and N^6 diminishes the electron-withdrawing effect of the phenyl group, restoring pK_a to that of adenosine. Regression analysis shows that in this subset of 31 nucleosides, the coronary vasoactivity is independent of pK_a ($p > 0.5$), a finding incompatible with the idea that electron density at N^1 is a primary determinant of the affinity of adenosine and its analogues for adenosine receptors.[19] Finally, at least in the case of the benzyl and phenethyl analogues, biological activity is most likely related to the chemical attributes of the aryl group that affect its interaction with the receptor rather than to remote effects on the electronic structure of the purine base.

Structure–Coronary Vasoactivity Correlations. The assays of the coronary vasoactivity of the analogues used 57 dogs. During the control periods prior to the infusion of adenosine or one of the analogues, the hemodynamic status of these animals was (group mean ± SEM): heart rate 117 ± 2 beats/min, coronary perfusion pressure 101 ± 2 mmHg, coronary blood flow rate 86 ± 3 mL/min per 100 g, and coronary flow rate at the peak of a hyperemic response to a 30-s coronary occlusion 455 ± 23% of control. The EC50 of adenosine averaged 1.2 ± 0.1 μM and a supramaximum dose increased coronary blood flow rate by 475 ± 28% of control. The slopes of log–logit transforms of the dose–response curves of the analogues were indistinguishable from those of the paired adenosine control curve ($p > 0.7$), suggesting that adenosine and its analogues acted at a common receptor. Because adenosine and its analogues were infused directly into a coronary artery branch, nucleoside administration usually did not alter heart rate or blood pressure. The sole exception was N^6-[2-(3,4,5-trimethoxyphenyl)ethyl]adenosine (**29**), which caused profound and prolonged hypotension at doses above the EC50 of coronary vasoactivity.

One test of the hypothesis that the coronary A2 adenosine receptor contains an N^6-phenyl subregion is a comparison of the vasoactivity of the N^6-aralkyladenosines with their N^6-alkyl congeners. The null hypothesis predicts that the two kinds of nucleoside will be equipotent. The comparisons summarized in Table III reject the null hypothesis. N^6-(Phenylmethyl)adenosine, **2**, is 10 times more potent than its alkyl congener, N^6-methyladenosine. Heteroaryl or ring-substituted phenyl groups profoundly

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Table III. Contribution of Phenyl (or Aryl) N^6 Substituents to Coronary Vasoactivity

no.	N^6 substituent	MPR[a]	aralkyl/alkyl potency ratio
	CH_3	0.05[b]	
2	$CH_2C_6H_5$	0.52	10
47–58	$CH_2C_6H_4X$	0.005–1.8	0.1–36
66–71	CH_2(heteroaryl)	0.06–0.69	1.2–14
	C_2H_5	0.15[b]	
3	$C_2H_4C_6H_5$	2.0	13
14–25	$C_2H_4C_6H_4X$	0.29–2.1	2–14
33–42	C_2H_4(heteroaryl)	0.4–4	2.7–27
	C_2H_4OH	0.14[b]	
	$C_2H_3(OH)C_6H_5$	1.4[b]	10
	n-C_3H_7	0.7[b]	
	$C_2H_3(CH_3)C_6H_5$	2.4 (*R*), 3.2 (*S*)[b]	3.4–4.6
	i-C_3H_7	0.7[b]	
1	(*R*)-PIA	4.3[b]	6

[a] MPR, molar potency ratio vs. adenosine. [b] From ref 13.

Table IV. Location of N^6-Phenyl Subregion

no.	N^6 substituent	MPR[a]	k'
2	phenylmethyl	0.52	1.21
3	2-phenethyl	2.0	1.77
4	3-phenylpropyl	0.33	2.75
5	3-phenyl-2-propenyl	0.32	2.36
6	4-phenylbutyl	0.046	6.93
7	(*R*)-1-phenethyl	0.52[b]	1.64
1	(*R*)-1-phenyl-2-propyl	4.3[b]	2.57
8	(*R*)-4-phenyl-2-butyl	0.19[b]	6.11
9	2,3-dihydro-1*H*-inden-2-yl	0.081	2.75
10	(*R*)-1,2,3,4-tetrahydronaphth-2-yl	0.14	4.89
11	(*R*)-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-6-yl	0.05	9.07
12	(1*R*,2*R*)-*cis*-2-phenylcyclohexyl	0.19	2.00
13	(1*R*,2*S*)-*trans*-2-phenylcyclohexyl	2.4	1.96

[a] Abbreviations are MPR, molar potency ratio vs. adenosine, and k', hydrophobicity index. [b] From ref 13.

influence the activity an an N^6-(arylmethyl)adenosine. The molar potency ratios (MPRs) of such analogues range between that of N^6-[(4-chlorophenyl)methyl]adenosine, **52**, which is 10 times less potent than N^6-methyladenosine, to those of N^6-[(2-methylphenyl)methyl]adenosine, **58**, or of N^6-[(2,5-dichlorophenyl)methyl]adenosine, **60**, which are 36 times more potent. The N^6-(arylethyl)- and N^6-(arylpropyl)adenosines are with few exceptions more potent than their alkyl congeners, the difference being as much as 27-fold. Moreover, the variations in the activities among the N^6-(arylmethyl)- and N^6-(arylethyl)adenosines are not random; rather, both classes of nucleoside appear to follow definite structure–activity rules (see below).

Location of the N^6-Aryl Subregion. Defining the geometrical relationship of the N^6-aryl subregion to the remainder of the N^6 region is a second test of the existence of this hypothetical region. Table IV lists the analogues used for this test. The activities of the N^6-(phenylalkyl)adenosines **2–8** provide information about the distance between the phenyl subregion and the N^6 subregion, a convenient reference point. A potency order **3** > **2** > **4** = **5** > **6** among the *n*-alkyl analogues and a potency order **1** > **7** > **8** among the *sec*-alkyl nucleosides indicate that the distance between the N^6 and phenyl subregions is approximately that of two methylene residues, ~3 Å.

The semirigid arylcycloalkyl N^6 substituents of analogues **9–13** further define the location of the N^6-aryl subregion. In keeping with the evidence provided by analogues **2–8**, two methylene residues separate the aryl moiety from N^6 of these analogues and, in accordance with the superimposition rule,[13] the chiral center adjacent to N^6 of analogues **10–13** has an *R* absolute configuration. N^6-[(1*R*,2*S*)-*trans*-2-phenylcyclohexyl]adenosine, **13**, is uniquely vasoactive, being 13 times more potent than its 1*R*,2*R* cis diastereomer **12** and at least 17 times more potent than any of the other analogues in this set. Such a result means that the phenyl group of **13** can fit into the aryl subregion but the aryl moieties of **9–12** cannot. If the phenyl group of **13** occupies a unique volume of space, the position of that volume of space defines the location of the aryl subregion. Studies of molecular models show that there is indeed one pseudorotational conformation of the cyclohexyl moiety of **13** which places the phenyl group in a position inaccessible to the aryl groups of **9–12** (Figure 3). In this conformation the axis of the cyclohexyl C-2 to phenyl C-1 bond is very nearly in the plane defined by N^6 and cyclohexyl C-1 and C-2. When the N^6 substituent of **1** is superimposed on this conformer of **13**, the phenyl group is trans to N^6. Unfortunately, this analysis does not completely define the position of the N^6-aryl subregion. The phenyl group of **13** is capable of relatively unhindered rotation around the axis of the cyclohexyl C-2 to phenyl C-1 bond. Accordingly, **13** cannot define the torsional angle around this bond, and the inclination of the plane of the aryl subregion with respect to the reference plane formed by the N-6, S-1, and S-2 subregions remains indeterminate.

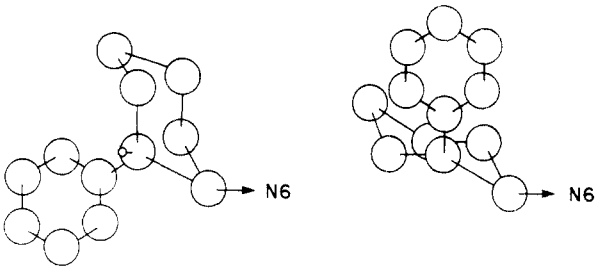

Figure 3. N^6 substituent of N^6-[(1*R*,2*S*)-*trans*-2-phenylcyclohexyl]adenosine, **20**, showing the two chair conformations that constitute extremes of the pseudorotational cycle of the cyclohexane moiety. Note how interconversion between the two conformations markedly changes the position of the phenyl moiety. Studies of molecular models of analogues **16–20** show that the phenyl group of the conformer shown at the left occupies a volume of space inaccessible to the phenyl groups of **16–19**.

Chemical Attributes of the N^6-Aryl Subregion. The N^6-(arylethyl)adenosines listed in Table V serve as probes of such chemical properties of the N^6-aryl subregion as its size and polarity. The aryl substituents include, besides ring-substituted phenyl groups, naphthyl and also heteroaryl groups. The aryl groups range in size from the five-membered imidazole ring of **33** to the 10-carbon naphthyl groups such as those of **43** and **44**. The resonance energies of the aryl substituents, an index of their aromatic character, varies concordantly with size between the 17 kcal/mole of imidazole to the 61 kcal/mole of naphthalene. A few are ionized at physiological pH, e.g., **33** and **35–37**, but most are not. All are planar. Polarity, as measured by the hydrophobicity index, spans 60-fold range, indicating that within this group of nucleosides the extent of nonspecific binding to blood and tissue proteins varies greatly. Although the heterogeneity of physical properties limits interpretation somewhat, certain deductions are still possible.

The aryl subregion appears to be relatively insensitive to the size of an aryl substituent or its exocytic substituents. Coronary vasoactivity tends to be greatest for analogues whose substituents are phenyl, pyridyl, or thienyl, then tends to fall as the size of the substituent increases further. Although such a result raises the possibility that the aryl subregion may not be large enough to accommodate the bigger aryl groups, such is probably not the case.

Table V. N^6-(2-Arylethyl)adenosines

no.	N^6-ethyl C-2 substituent	MPR[a]	k'	pK_a	θ, deg	μ, D
3	phenyl	2.0	1.77	3.57 ± 0.10	143	0.22
		A. Ring-Substituted Phenyl				
14	2-fluorophenyl	1.9 ± 0.38 (4)	2.36	3.19 ± 0.09		
15	3-fluorophenyl	1.6 ± 0.15 (3)	2.36			
16	4-fluorophenyl	2.1 ± 0.40 (4)	2.39	3.32 ± 0.03		
17	2-chlorophenyl	0.67	3.64	3.27 ± 0.05	74	1.98
18	3-chlorophenyl	1.3	4.14		124	2.40
19	4-chlorophenyl	0.41	4.07	3.23 ± 0.01	180	2.24
20	2-methylphenyl	0.67	4.52	3.32 ± 0.04	132	0.30
21	3-methylphenyl	1.1	4.64		77	0.26
22	4-methylphenyl	0.46 ± 0.02 (3)	4.76	3.40 ± 0.09	12	0.08
23	2-methoxyphenyl	1.2	2.57	3.56 ± 0.13	163	1.42
24	3-methoxyphenyl	1.3	2.00		58	1.55
25	4-methoxyphenyl	0.29	1.93	3.49 ± 0.10	10	1.41
26	3,4-dimethoxyphenyl	2.7 ± 0.9 (3)	1.45		25	1.35
27	3,4-(methylenedioxy)phenyl	0.42	2.52		141	0.70
28	2,4,5-trimethoxyphenyl	0.40	2.63		29	1.41
29	3,4,5-trimethoxyphenyl	5.2 ± 2.5 (4)	1.54		7	3.30
30	4-hydroxyphenyl	0.90	0.43		46	1.73
31	3-(trifluoromethyl)phenyl	0.82	6.68			
32	diphenyl	0.71	8.29			
		B. Non-Phenyl Substituents				
33	4(5)-imidazolyl	0.42	0.14			
34	2-(1-methylpyrroloyl)	0.64	0.86		66	1.78
35	2-pyridyl	1.0	0.39	<1	66	1.84
36	3-pyridyl	3.0	0.38	<1	127	2.08
37	4-pyridyl	0.42	0.32	<1	177	2.43
38	2-thienyl	4.0	1.36		61	1.15
39	3-thienyl	2.5	1.32		144	1.60
40	3-indoloyl	0.43	1.39		87	1.90
41	2-benzimidazolyl	0.40	1.45		67	4.02
42	3-quinolinoyl	1.2	3.64		89	2.19
43	1-naphthyl	0.11	6.82		104	0.22
44	2-naphthyl	0.12	6.29		165	0.21
		C. Phenylpropyl				
4	phenyl	0.33	2.75			
45	3,4-dimethoxyphenyl	0.14	2.05			
46	3,4,5-trimethoxyphenyl	0.056	2.05			

[a] Molar potency ratio vs. adenosine. Data are means of duplicate estimates or mean ± SEM of >2 estimates, with number of estimates in parentheses. Other abbreviations are k', hydrophobicity index; pK_a, acidic dissociation constant; θ, dipole direction as defined in the Experimental Section; and μ, dipole strength in Debye.

The low activities of the two naphthyl analogues **43** and **44** could be a consequence of their hydrophobicity and nonspecific binding to protein. N^6-[2-(3-quinolinyl)-ethyl]adenosine, **42**, has an aryl substituent that is just as large as those of **43** and **44**. That **42** is much more polar and 10 times more active than the naphthyladenosines supports the idea that polarity makes it better able to penetrate to the receptor.

Several N^6-(2-arylethyl)adenosines are stronger coronary vasodilators than N^6-2-phenethyladenosine, **3**, which has an MPR of 2.0. The aryl groups conferring such activity (compound number and MPR in parentheses) are as follows: 3,4,5-trimethoxyphenyl (**29**, 5.2), 2-thienyl (**38**, 4.0), 3-pyridyl (**36**, 3.0), 3,4-dimethoxyphenyl (**26**, 2.7), and 3-thienyl (**39**, 2.5). The three isomers of N^6-[2-(fluorophenyl)ethyl]adenosine, **14–16**, are equipotent with **3**, but other aryl groups diminish coronary vasoactivity by as much as 20-fold. Regioselective vasoactivity characterizes the nucleosides whose phenyl substituents are chloro (**17–19**), methyl (**20–22**), or methoxy (**23–25**), the rank order of potency being meta > ortho > para. Although steric hindrance, particularly in the para position, could explain such a result (and is consistent with the lack of regioselectivity of the much smaller fluoro substituent), it is more likely that the electronic effects of these substituents underlie regioselectivity. The dimethoxy and trimethoxy analogues **26** and **28** have even more steric crowding in the para position but are 9 and 18 times more potent than **25**. Further, the N^6-(2-pyridylethyl)adenosines

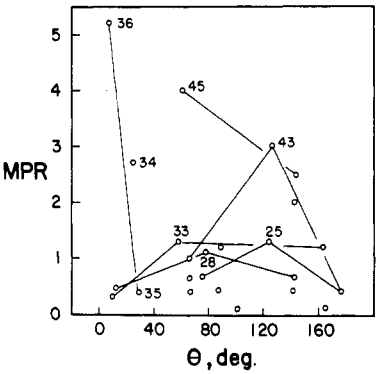

Figure 4. Relationship between coronary vasoactivity, as MPR vs. adenosine, and the direction of aryl dipole moment, θ, among the N^6-(arylethyl)adenosines. Solid lines join data from positional isomers, one member of which is designated by number in Table V. See text for additional discussion.

35–37 show an even more pronounced meta > ortho > para potency order than the ring-substituted phenyl analogues, yet they lack exocyclic substituents altogether.

There appears to be some correlation between coronary vasoactivity and aryl dipole moment, which is a global parameter of electronic structure. Table V lists MO calculations of dipole strength and direction for all N^6-(2-arylethyl)adenosines. Overall, the correlation is poor (Figure 4), but such a result could at least in part reflect the confounding effect of significant differences in the

Table VI. N^6-(Arylmethyl)adenosines

no.	N^6-methyl substituent	MPR[a]	k'	pK_a
2	phenyl	0.52	1.21	3.07 ± 0.10
	A. Ring-Substituted Phenyl			
47	2-fluorophenyl	0.57	1.57	3.08 ± 0.09
48	3-fluorophenyl	0.08 ± 0.17 (4)	1.90	
49	4-fluorophenyl	0.36 ± 0.07 (3)	1.57	3.32 ± 0.18
50	2-chlorophenyl	1.2	3.12	3.06 ± 0.04
51	3-chlorophenyl	0.34	3.36	
52	4-chlorophenyl	0.005	3.44	3.61 ± 0.06
53	2-methoxyphenyl	0.70	2.31	3.30 ± 0.06
54	3-methoxyphenyl	0.50	1.54	
55	4-methoxyphenyl	0.040	1.66	3.06 ± 0.06
56	2-methylphenyl	1.8	2.76	3.22 ± 0.06
57	3-methylphenyl	0.85	2.92	
58	4-methylphenyl	0.04	3.04	3.19 ± 0.09
59	2,4-dichlorophenyl	0.05	8.86	
60	2,5-dichlorophenyl	1.8	5.97	
61	2,6-dichlorophenyl	0.024	5.50	
62	3,4-dichlorophenyl	0.28	7.12	
63	3,5-dichlorophenyl	0.35	9.37	
	B. Non-Phenyl Substituents			
64	(*R*)-2,3-dihydro-1*H*-inden-1-yl	0.24	3.81	
65	1-naphthyl	1.8	6.27	
66	2-pyridyl	0.62	0.32	
67	3-pyridyl	0.69	0.29	
68	4-pyridyl	0.42 ± 0.16 (4)	0.21	
69	2-thienyl	0.23	1.04	
70	3-thienyl	0.091	1.07	
71	2-furyl	0.06	0.85	

[a] Abbreviations and notation as in Table V.

Table VII. N^6-Phenyladenosines

no.	N^6 substituent	MPR[a]	k'	pK_a
72	phenyl	1.4	1.29	2.73 ± 0.07
73	2-fluorophenyl	0.91	1.48	2.39 ± 0.09
74	3-fluorophenyl	0.43	2.36	2.66 ± 0.08
75	4-fluorophenyl	1.5	1.68	2.65 ± 0.06
76	2-chlorophenyl	0.42	2.36	2.76 ± 0.14
77	3-chlorophenyl	0.20	5.05	2.61 ± 0.15
78	4-chlorophenyl	0.94	4.96	2.46 ± 0.02
79	2-methylphenyl	0.65	1.40	2.98 ± 0.04
80	3-methylphenyl	0.023	3.28	2.79 ± 0.05
81	4-methylphenyl	1.3	3.28	2.96 ± 0.05
82	2,3-dimethylphenyl	0.13	2.58	
83	2,4-dimethylphenyl	0.48	3.15	
84	2,5-dimethylphenyl	0.22	4.75	
85	2,6-dimethylphenyl	22% @ 17 μM[b]	1.52	
86	3,4-dimethylphenyl	0.043	5.98	
87	3,5-dimethylphenyl	4% @ 12 μM[b]	6.43	
88	2-methoxyphenyl	0.28	1.93	2.97 ± 0.04
89	3-methoxyphenyl	0.26	1.32	2.59 ± 0.05
90	4-methoxyphenyl	3.5	1.36	2.95 ± 0.03
91	3,4-dimethoxyphenyl	0.04	2.10	
92	3,4,5-trimethoxyphenyl	28% @ 28 μM[b]	1.54	2.80 ± 0.003

[a] Abbreviations and notation as in Table V. [b] Maximum increase in coronary flow, percent of control, and nucleoside concentration in coronary plasma.

hydrophobicity of these nucleosides and, accordingly, the extent to which they penetrate tissue and reach the receptor. Confining the comparisons of potency to sets of isomers reduces the problem of analogue distribution. Such comparison show that the small differences between isomers in dipole strength account for the much larger differences in coronary vasoactivity. However, the more active isomers in each set tend to have dipole moments that are oriented transversely to, or in the direction of, the axis extending from the center of the aryl group to the atom attached to ethyl C-2.

Table V also includes two analogues of N^6-(3-phenylpropyl)adenosine, **4**. Just as **4** is less potent than **3**, so its 3,4-dimethoxy- and 3,4,5-trimethoxyphenyl analogues **45** and **46** are weaker than the corresponding phenethyl analogues **26** and **29**.

N^6-(Arylmethyl)adenosines. As shown in Table IV, the single methylene group of an N^6-(arylmethyl)adenosine does not position the aryl group for optimum interaction with the receptor. However, because the aryl groups of the analogues of N^6-(phenylmethyl)adenosine interact with a different part of the receptor, they can provide information about the receptor that complements that from the N^6-(arylethyl)adenosines. Table VI summarizes data on the coronary vasoactivity, hydrophobicity, and acidic dissociation constant of the N^6-(arylmethyl)adenosines.

Like their arylethyl congeners, the N^6-(arylmethyl)-adenosines exhibit regioselective coronary vasoactivity, but the rank order of potency differs, being ortho > meta > para. Further, this potency order appears to reflect steric hindrance by the para substituent rather than the influences of the electronic structure of the aryl substituent. Thus, the chloro, methoxy, and methyl nucleosides **52**, **55**, and **58** have in common a bulky para substituent and extremely low coronary vasoactivity. Such is not the case for the para fluoro and 4-pyridyl analogues **49** and **69**, respectively, which have either a small or no exocyclic aryl substituent and coronary vasoactivity that is only moderately lower than that of the most active of their isomers.

All of the ortho-substituted N^6-(phenylmethyl)-adenosines are more potent than **2**, particularly the chloro and methyl analogues **50** and **56**. Since chloro and methyl groups are rather hydrophobic, the exceptional activities of these nucleosides may represent the interaction of these exocyclic substituents with the S-1/S-1A portions of the alkyl subregion. Studies with molecular models show that the S-1 subregion is one of the loci accessible to an ortho-substituted phenylmethyl substituent. Previous work[13] shows that the S-1 subregion has an affinity for hydrophobic groups but can tolerate a certain degree of polarity such as that of a benzene ring.

The coronary vasoactivities of the three isomers of N^6-[(chlorophenyl)methyl]adenosine vary over a 240-fold range between the para isomer **52**, which is very nearly inactive, and the ortho isomer **50**, which is over twice as active as N^6-benzyladenosine, **2**. Such a pronounced influence of ring position on vasoactivity urged tests of the N^6-[(dichlorophenyl)methyl]adenosines to see whether the effects of ring position might be additive. The activities of the monosubstituted analogues predict that, whereas an ortho substituent should augment the contribution to activity of a substituent elsewhere on the ring, a para substituent should exert the opposite effect. Since the potency of N^6-[(3-chlorophenyl)methyl]adenosine, **51**, is not greatly different from that of the unsubstituted benzyladenosine, **2**, the effect of a meta substituent on activity could well be neutral. Five of the isomers of N^6-[(dichlorophenyl)methyl]adenosine, **59–63**, were available to test this possibility. The most active analogue in this subset, N^6-[(2,5-dichlorophenyl)methyl]adenosine, **60**, is more potent than either **50** or **51**, evidence that the ortho substituent augmented the contribution of the meta substituent. However, such an effect of an ortho substituent is not consitent; the 2,6-isomer **61** is the least active of all the [(dichlorophenyl)methyl]adenosines studied. It appears that a second substituent in either the ortho or meta position ameliorates the profoundly negative influence of a para substituent on vasoactivity.

The [(dichlorophenyl)methyl]adenosines are important in another respect, for they illustrate the need to account for differences in hydrophobicity when interpreting estimates of apparent potency. The hydrophobicity index of

the most hydrophobic of the dichlorobenzyl analogues is nearly 4 units higher than that of the chlorobenzyl analogues. Among the dichlorobenzyl analogues, k' of the least and most hydrophobic likewise differ by almost 4 units. According to the data developed in Table II and Figure 2, a difference in k' of 4 units means that the dissociation constants of the analogue albumin complexes differ by a factor of e^4, or 50-fold. To the extent that k' reflects the tendency of ana analogue to remain in the vascular compartment bound to albumin or to bind unspecifically in the tissue, for example, by incorporation into the lipid bilayer of cell membranes, the concentration of ligand available for interaction with a receptor varies inversely with k'. Since **60** and **61** have roughly similar hydrophobicity indices, a comparison of their potencies is appropriate. The other analogues are more hydrophobic, and so it is possible that their true affinities for the receptor are higher than the estimates of coronary vasoactivity would suggest. Likewise, the affinity for the receptor of N^6-[(2,5-dichlorophenyl)methyl]adenosine, **60**, $k' = 6.97$, may be much greater than that of N^6-[(2-methylphenyl)-methyl]adenosine, **56**, $k' = 1.66$, rather than equal, as their MPRs would suggest.

N^6-[(*R*)-2,3-dihydro-1*H*-inden-1-yl]adenosine, **64**, is a semirigid cyclic analogue of **2** but is much less potent. The substantial coronary vasoactivity of N^6-[1-naphthylmethyl]adenosine, **65**, confirms the original report[20] that this analogue is a powerful, long-acting coronary vasodilator. It is uncertain whether the activity of **65** owes to the interaction of naphthyl C-8 with the S-1 alkyl subregion in much the same way as the exocyclic substituents of **50** and **56** might do. Alternatively, studies of molecular models show that the β ring of **65** is almost exactly superimposable on the phenyl group of **3**.

Unlike the arylethyl analogues, replacing the phenyl group of N^6-(phenylmethyl)adenosine with an aromatic heterocycle (analogues **66–71**) does not enhance coronary vasoactivity.

N^6-Phenyladenosines. Considering N^6-phenyladenosine, **72**, as a member of the congeneric series **2–6**, gives rise to a peculiar bimodal rank order of potency, namely, **72** > **2** < **3** > **4** > **6**. Studies of molecular models show that the phenyl groups of **3** and **72** occupy volumes of space that overlap only minimally. Accordingly, we do not consider **72** to be a member of the congeneric series. However, the structure–activity relationships of the N^6-phenyladenosines are of interest because **72** has substantial coronary vasoactivity and, further, has the potential to provide information about receptor structure that complements that provided by (arylethyl)adenosines.

The singly substituted N^6-phenyladenosines **73–81** and **88–90** show strongly regioselective coronary vasoactivity that is characterized by a potency order that is para > ortho > meta and that is independent of the type of ring substituent. Indeed, N^6-(4-methoxyphenyl)adenosine, **90**, which has an MPR vs. adenosine of 3.5, is among the most potent of the N^6-modified adenosines at the coronary receptor. Model studies show that the 4-methoxy group of **90** can occupy a volume of space adjacent to that occupied by phenyl C-2 and C-3 of **3**. Perhaps the relatively high potencies of ortho- and meta-substituted N^6-2-phenethyladenosines and the para-substituted N^6-phenyladenosines owe to the interaction of these exocyclic groups with a common chemical feature of the receptor. Unfortunately, available information does not hint at what the attributes of this part of the receptor might be.

(20) Jahn, W. *Arzniem.-Forsch.* **1969**, *19*, 701.

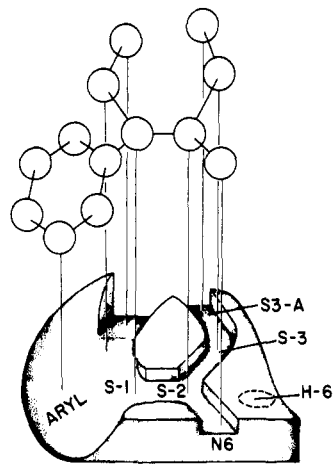

Figure 5. Provisional model of the N^6 region of the dog coronary artery A2 adenosine receptor as deduced from the structure–activity relationships of N^6-substituted adenosines. The structure above the model represents the N^6 substituent of N^6-[(3*R*,4*S*)-4-phenyl-3-hexyl]adenosine, a nucleoside that the structure–activity studies predict will fit optimally into the N^6 receptor region. See text for additional discussion.

The coronary vasoactivities of two sets of N^6-phenyladenosines having two or more phenyl ring substituents show that regioselectivity tends to be additive. All six isomers of N^6-(dimethylphenyl)adenosine, **82–87**, constitute the first set. The activities of the monosubstituted analogues **79–81** predict that analogue **83**, by having a para and an ortho but not a meta substituent, should be the most potent. N^6-(3,5-Dimethylphenyl)adenosine, which has two meta substituents, should be the least active. The other analogues should be intermediate in potency. Such is the case. The methoxy analogues **90–92** constitute the second set, which shows how the high potency of **90** is greatly reduced by one meta substituent and essentially abolished by two. The mechanism by which a meta substituent reduces activity is uncertain.

Receptor Model. The structure–activity relationships that emerge from this study support the idea that the N^6 region of the dog coronary artery A2 adenosine receptor contains an aryl subregion. However, the limitations of structure–activity correlates as an experimental approach are such that information about the topography of the aryl region and its geometrical relationship to the rest of the receptor is incomplete. Figure 5 is a provisional model of the N^6 region of this receptor that is consistent with the evidence developed here. The vasoactivities of **1** and **2–18** show that the distance between the aryl and N^6 subregions is equivalent to two methylene residues or ca. 3 Å. Semirigid analogues **9–13** partially define the geometric relationship of the aryl subregion to the rest of the receptor. The extraordinary vasoactivity of N^6-[(1*R*,2*S*-*trans*-2-phenylcyclohexyl]adenosine, **13**, relative to the potencies of **9–12** suggests that when **13** occupies the receptor, the axis of the cyclohexyl C-2 to phenyl C-1 bond is very nearly in the plane defined by the N-6, S-1, and S-2 subregions of the receptor. However, the available evidence does not identify the torsional angle around this bond. Accordingly the depiction in Figure 5 of the plane of the aryl subregion as inclined to the plane defined by the N-6, S-1, and S-2 subregions is purely illustrative.

Experimental Section

Melting points were estimated on a Thomas-Hoover apparatus and are uncorrected. A Perkin-Elmer 241 MC spectropolarimeter served for estimates of $[\alpha]_D$. ^1H NMR spectra of samples dissolved in $CDCl_3$ or Me_2SO-d_6 were recorded at 60 mHz and resonances reported as chemical shifts (δ, ppm) from the $(CH_3)_4Si$ internal

standard. MHW Laboratories, Tucson, AZ, performed elemental analyses, which agreed with calculated composition to within ±0.4%. The characterization of known nucleosides included melting point, UV and ^{1}H NMR spectra, and an estimate of purity by reverse-phase HPLC. Characterization of novel nucleosides induced, in addition, analysis for C, H, N and, when appropriate, Cl and S. Product accounted for >99% of the UV absorbance in samples of analogues assayed for coronary vasoactivity. The methods for estimating pK_a, for the hydrophobicity index of an analogue, and for the binding of analogues to serum albumin in vitro have been described.[13]

Assay of Coronary Vasoactivity.[8,9,13] Dogs anesthetized with sodium thiamylal, 18 mg/kg iv, and maintained in surgical anesthesia by ventilation with 0.5–1% halothane underwent left thoracotomy for the implantation of an electromagnetic flowmeter and occlusive cuff near the origin of a major epicardial branch of the left coronary artery and the transmural insertion of an intracoronary drug infusion catheter just distal to the occluder. Measurements of coronary flow perfusion pressure during the steady-state responses to direct, constant-rate intracoronary infusions of adenosine or an analogue served for the construction of cumulative log dose logit conductance curves, which yielded estimates of EC50, the nucleoside concentration in coronary plasma that causes a half-maximum increase in coronary conductance. To account for between-dog differences in coronary responsiveness, the activity of an analogue is referred to that of adenosine by means of a molar potency ratio, the quotient of the EC50 of adenosine divided by that of the analogue. 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 arithmetic mean of two assays or the mean ± SEM of 3 or more assays. Statistical analyses, including analysis of variance for repeated measures, employed conventional methods.[21]

Dipole Moments of N^6-Aryl Groups. These calculations applied a MNDO program (QCMP 002 version of QCPE 353) to calculate the minimum-energy conformation of a series of arylethanes that served as models of the N^6 substituents of the N^6-(2-arylethyl)adenosines. The atomic coordinates resulting from such calculations served as the input for calculations of the strength, μ, and direction, θ, of the aryl dipole moment by means of a CNDO/INDO program (QCMP 001 version of QCPE 240). Both programs were obtained from the Quantum Chemistry Program Exchange, University of Indiana, Bloomington, IN, and were run on a AT&T 6300 minicomputer augmented by 640K of ROM and a floating point processor. The direction of the aryl dipole, θ, is measured counterclockwise in degrees from a reference line originating at the geometric center of the aryl group and extending through the atom to which the ethyl group is attached, e.g., C-1 of benzene.

Synthesis of N^6-Substituted Adenosines. The reaction of 6-chloropurine ribonucleoside with the appropriate amine in the presence of $(C_2H_5)_3N$ as an acid acceptor[14] yielded all the N^6-substituted adenosines reported here. Workup consisted of evaporation of the reaction mixture and crystallization of product from CH_3OH/H_2O or C_2H_5OH/ethyl acetate. Nucleosides that did not crystallize were purified by preparative LPLC on 2.5 × 50 cm columns of octadecylsilyl silica eluted with CH_3OH/H_2O. Preliminary HPLC runs identified the ratio of CH_3OH and H_2O that gave optimum purification. This ratio usually ranged between 2:3 and 3:2, v/v. Analogues thus purified often crystallized.

Almost all of the amines needed for nucleoside synthesis were commercially available or were prepared by the $LiAlH_4$ reduction of the corresponding amide or nitrile.[22] The 1*R*,2*R* cis and 1*R*,2*S* trans isomers of 2-phenylcyclohexylamine were synthesized as described by Verbit and Price.[23] A method for the asymmetric synthesis of optically active amines from ketones[24] that employed (*R*)-(+)-1-phenethylamine and either 2,3-dihydro-1*H*-inden-1-one, 1,2,3,4-dihydro-2*H*-naphthalen-2-one, or 6,7,8,9-tetrahydro-5*H*-benzocyclohepten-6-one[25-27] furnished the *R* enantiomers of the amines needed to prepare, respectively, analogues **64**, **10**, and **11**. The amines used to prepare analogues **28**, **45**, and **46** were gifts of Dr. John W. Daly, NIADDK, NIH.

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