formed was filtered with suction, washed with water, and dried. The crude product was then dissolved in 3 L of hot acetonitrile, and the resulting solution was filtered over HYFLO-Supercel and the filtrate was concentrated at 60-70 °C under a water-jet vacuum until crystallization began. The title compound was obtained in the form of brownish crystals: mp >250 °C; IR (KBr) 1355, 1695, and 1720 cm^{-1}; NMR (250 MHz, DMSO-d_6) δ 2.69-3.05 (m, 5 H), 7.42 (d, 2 H), 8.23 (d, 2 H), 10.94 (s, 1 H). Anal. ($C_{12}H_{10}N_2O_4$) C, H, N.

3-Alkyl-1-(4-nitrophenyl)-3-azabicyclo[3.1.1]heptane-2,4-diones 13c-e,i. Method C. Fifteen millimoles of isobutyl iodide, *n*-pentyl iodide, *n*-heptyl bromide, or benzyl bromide was added to a mixture of 2.46 g (10 mmol) of **13a** and 0.36 g (15 mmol) of sodium hydride in 25 mL of DMF. The reaction mixture was then stirred for 2.5 h and evaporated. The residue was dissolved in ethyl acetate, washed with water, dried, and crystallized after evaporation of the solvent.

5-Methyl-1-(4-nitrophenyl)-3-*n*-propyl-3-azabicyclo-[3.1.1]heptane-2,4-dione (14b). Method D. While stirring, a solution of 23.0 g (76 mmol) of **11b** and 0.22 g (1.0 mmol) of 2,6-di-*tert*-butyl-*p*-cresol in 2.3 L of acetone was irradiated for 3 h with a UV lamp (Philips 125 HPK), which was immersed in the reaction solution in a double-walled, water-cooled Pyrex glass shaft. After concentration by evaporation, the residue was recrystallized from methylene chloride/diisopropyl ether mixture, yielding **14b** in the form of white crystals: mp 128.5-129.5 °C; IR ($CHCl_3$) 1350, 1685, 1745 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 0.93 (t, 3 H), 1.45 (s, 3 H), 1.54-1.72 (m, 2 H), 2.48-2.59 (m, 2 H), 2.82-2.94 (m, 2 H), 3.72 (t, 2 H), 7.30 (d, 2 H), 8.26 (d, 2 H). Anal. ($C_{16}H_{18}N_2O_4$) C, H, N.

3-Alkyl-1-(4-aminophenyl)-3-azabicyclo[3.1.1]heptane-2,4-diones 2a-i,k, 16b, 17b. Method E. A 2-5% alcoholic, ethyl acetate, or 2-methoxyethanol solution of the nitro derivatives **13a-i,k**, **14b**, or **15b** was hydrogenated at normal pressure in the presence of 5% Pd/C. After removal of the catalyst, the solvent was distilled off under vacuum, giving the title products.

Enzyme Preparation and Assay Procedures. The aromatase enzyme was prepared and its activity checked with [1,2-^{3}H]androstenedione as substrate according to the method of Thompson.[1] The assays for inhibition of estrogen biosynthesis in comparison to the synthesis of progesterone in hamster ovaries[25] and corticosterone and aldosterone in rat adrenal[26] tissues were performed exactly as previously described.

In Vivo Aromatase Assay. The effectiveness of compounds as aromatase inhibitors in vivo was tested in immature rats treated with human chorionic gonadotropin and androstenedione and determined from the inhibition of the uterotropic effect according to the method of Steele.[7]

DMBA-Mammary Carcinoma Test. Female Sprague-Dawley rats bearing one to three DMBA-induced mammary tumors of about 1-1.2 mm in diameter were randomized and divided into treatment and control groups of 15 animals each. **1h** and AG were suspended in 0.5% sterile solution of CMC with 20% 1,2-propylene glycol. The total daily dose of the test compounds was given orally in two administrations at 8.00 a.m. and 3.00 p.m. for 42 consecutive days. The body weight, the number of tumors, and the tumor size were determined once weekly until the end of experiment.[27]

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2-Alkoxyadenosines: Potent and Selective Agonists at the Coronary Artery A_2 Adenosine Receptor

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A Langendorff guinea pig heart preparation served for the assay of agonist activity of a series of 24 2-alkoxyadenosines at the A_1 and A_2 adenosine receptors of, respectively, the atrioventricular node (conduction block) and coronary arteries (vasodilation). Activities are low at the A_1 receptor and do not show a clear relationship to the size or hydrophobicity of the C-2 substituent. All the analogues are more potent at the A_2 receptor, activity varying directly with the size and hydrophobicity of the alkyl group. The most potent analogue in this series, 2-(2-cyclohexylethoxy)adenosine, has an EC_{50} of 1 nM for coronary vasodilation and is 8700-fold selective for the A_2 receptor.

Exocyclic substituents on the adenine base profoundly alter the affinity of adenosine for its receptors. N^6-substituted adenosines such as N^6-cyclopentyladenosine[1,2] are potent and very selective agonists at the A_1 adenosine receptor (A_1AR).[3,4] Certain other N-6 substituents containing aryl groups confer selectivity for the A_2AR.[5,6] The availability of large numbers of N^6-substituted adenosines has aided the development of models of the N-6 region of the A_1AR[3,7] and also of the A_2AR.[8,9] In part because the synthesis routes to adenosines substituted at C-2 are more difficult than those at N-6, the information about the C-2 regions of the two types of adenosine receptor is much less detailed. Several early studies examined a number of 2-substituted adenosines as agonists at the A_2AR of the platelet[10,11] and the coronary artery.[12-15] However, most

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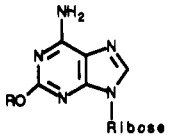
Table I. Physical and Analytical Data for Alkoxyadenosines 3a–x



no	R	formula	anal.	mp, °C	% yield[a]	HPLC[b]	UV: λ_{max} (ϵ)
3a	CH_3	$C_{11}H_{15}N_5O_5$	C, H, N	192	46	50	254 sh (11 100), 268 (14 500)
3b	C_2H_5	$C_{12}H_{17}N_5O_5$	C, H, N	197	66	60	254 sh (9 700), 268 (12 600)
3c	C_3H_7	$C_{13}H_{19}N_5O_5$	C, H, N	201–202	53	40	254 sh (10 200), 268 (12 700)
3d	C_4H_9	$C_{14}H_{21}N_5O_5$	C, H, N	180	45	40	254 sh (9 600), 268 (12 500)
3e	C_5H_{11}	$C_{15}H_{23}N_5O_5$	C, H, N	96–99	34	50–65	253 sh (7 400), 268 (9 900)
3f	C_6H_{13}	$C_{16}H_{25}N_5O_5$	C, H, N	87–90	24	50–75	255 sh (8 700), 268 (11 100)
3g	$(CH_3)_2CH$	$C_{13}H_{19}N_5O_5$	C, H, N	115–117	2	40	254 sh (9 500), 268 (12 200)
3h	$(CH_3)_2CHCH_2$	$C_{14}H_{21}N_5O_5$	C, H, N	114	45	40	254 sh (9 200), 268 (11 400)
3i	$(CH_3)_2CH(CH_2)_2$	$C_{15}H_{23}N_5O_5$	C, H, N	98–100	31	50–70	255 sh (10 100), 268 (12 800)
3j	$(CH_3)_2CH(CH_2)_3$	$C_{16}H_{25}N_5O_5$	C, H, N	102–104	39	50–70	255 sh (9 200), 268 (11 900)
3k	c-C_6H_{11}	$C_{16}H_{23}N_5O_5$	C, H, N	147	30	40	255 sh (10 500), 268 (12 700)
3l	$C_6H_{11}(CH_2)$	$C_{17}H_{25}N_5O_5$	C, H, N	163–165	13	70–80	254 sh (8 600), 268 (11 100)
3m	$C_6H_{11}(CH_2)_2$	$C_{18}H_{27}N_5O_5$	C, H, N	185–187	23	30–50	254 sh (10 200), 268 (13 100)
3n	$C_6H_{11}(CH_2)_3$	$C_{19}H_{29}N_5O_5$	C, H, N	161–163	26	60–80	255 sh (9 600), 268 (12 400)
3o	$C_6H_{11}(CH_2)_4$	$C_{20}H_{31}N_5O_5$	C, H, N	191	38	65–80	255 sh (8 500), 268 (10 300)
3p	$C_2H_5(CH_3)CH$	$C_{14}H_{21}N_5O_5$	C, H, N	141	46	45	255 sh (9 000), 269 (11 500)
3q	c-C_5H_9	$C_{15}H_{21}N_5O_5$	C, H, N	147–150	20	42	255 sh (9 100), 268 (11 500)
3r	c-$C_5H_9(CH_2)_2$	$C_{17}H_{25}N_5O_5$	C, H, N	100–103	16	30–55	255 sh (9 300), 268 (11 800)
3s	ENB-$(CH_2)_2$[c]	$C_{19}H_{27}N_5O_5$	C, H, N	185–187	15	60–80	255 sh (8 900), 267 (11 300)
3t	DBH$(CH_2)_2$[d]	$C_{21}H_{29}N_5O_5$	C, H, N	142	30	60–80	255 sh (9 800), 268 (12 200)
3u	$(C_2H_5)_2CHCH_2$	$C_{16}H_{25}N_5O_5$	C, H, N	93–95	23	50–65	253 sh (9 300), 268 (11 700)
3v	$(C_2H_5)_2CH(CH_2)_2$	$C_{17}H_{27}N_5O_5$	C, H, N	97	13	50–65	253 sh (8 900), 267 (11 300)
3w	$CH_3C{\equiv}C(CH_2)_2$	$C_{15}H_{19}N_5O_5$	C, H, N	185–186	16	30–50	254 sh (9 400), 267 (11 900)
3x	$HO(CH_2)_2$	$C_{12}H_{17}N_5O_6$	C, H, N	187	36	5–25	252 sh (8 800), 267 (11 700)

[a] Overall yield from 1. [b] Concentration of methanol in water used as eluant in purification of 3a–x by preparative HPLC. Single numbers indicate composition for isocratic elution, two numbers are the beginning and ending concentrations in gradient elutions. [c] ENB is *endo*-2-norbornanyl. [d] DBH is (1*R*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl.

of these studies antedated the discovery that there are two distinct types of adenosine receptors[16,17] and so do not address the question of selectivity for one receptor or the other. Subsequent work showed that 2-(phenylamino)-adenosine (CV-1808), a potent coronary vasodilator,[14] is only 5–20-fold selective for the A_2AR.[4,18] Accordingly, the development of CGS 21,680, a 2-(aralkylamino)adenosin-5′-uronamide that is highly selective for the A_2AR,[19,20] is a significant advance.

This report concerns the synthesis and bioassays of the A_1AR and A_2AR agonist potencies of a series of 2-alkoxyadenosines. Limited information suggesting that 2-alkoxyadenosines are more potent coronary vasodilators than the 2-alkylamino congeners[14] and the observation that isoguanosine is a more potent coronary vasodilator than 2-aminoadenosine[21] are the basis for linking the alkyl substituent to adenosine through an oxygen atom.

Results and Discussion

Chemistry. Marumoto et al.[14] prepared 2-alkoxyadenosines by displacing the chloro group of 2′,3′-*O*-(ethoxymethylidine)-2-chloroadenosine (1) with an alkoxide. The reaction of metallic Na with an excess of an alcohol generated the alkoxide, the excess alcohol serving as the solvent for the subsequent reaction with the chloro nucleoside. Because the expense of some alcohols precludes using them as solvents, we developed a more general method, namely, generating the alkoxides by adding *n*-butyllithium to a solution of a 5% molar excess of the alcohol in dry 1,2-diethoxyethane. Adding thoroughly dried 2′,3′-*O*-(ethoxymethylidine)-2-chloroadenosine (1; or the isopropylidine ketal) and heating at reflux for 5–7 days usually generated the blocked 2-alkoxyadenosine (2) in satisfactory yield. In some instances adding anhydrous DMF accelerated the reaction by bringing the reactants into solution. The lithium *sec*-alkoxides displace the chloro group of 1 poorly. However, tritylation of the 5′-OH group restores yields to acceptable levels. The alkoxides of three heterocyclic alcohols containing nitrogen as the heteratom, namely, 4-(2-hydroxyethyl)piperidine, 1-(2-hydroxyethyl)piperidine, and 1-(2-hydroxyethyl)pyrrolidine, failed to displace the chloro group of 1.

Blocking the 2′- and 3′-OH groups is essential to prevent a major side reaction, the formation of a 2→2′ polymer.[14] Cleavage of the N-9 to C-1′ glycosylic bond during deblocking is an important problem that accounts for many of the low yields of 3 reported in Table III. The lability of this bond in the 2-alkoxyadenosines probably underlies the choice[14] of the ethoxymethylidine group, which is

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Table II. Cardiac and Coronary Activities of Adenosine Analogues

analogue[a]	n	-log EC_{50}, M stim-QRS[b]	-log EC_{50}, M coronary[c]	A_1/A_2[d]
adenosine	14	5.47 ± 0.07	7.29 ± 0.06	80 ± 11
isoguanosine[e]	4	5.94 ± 0.13	7.10 ± 0.04	18 ± 6
2-CADO	5	6.72 ± 0.09	7.60 ± 0.10	9.1 ± 2.6
NECA	6	7.20 ± 0.07	8.56 ± 0.02	25 ± 5
R-PIA	4	7.47 ± 0.03	7.88 ± 0.03	2.6 ± 0.24
CPA[f]	5	7.90 ± 0.07	7.20 ± 0.09	0.3 ± 0.1
S-ENBA[g]	4	8.27 ± 0.12	7.08 ± 0.02	0.074 ± 0.018
CV-1808[h]	4	4.90 ± 0.09	7.74 ± 0.09	770 ± 220
DPMA[i]	4	5.25 ± 0.05	9.35 ± 0.05	13000 ± 1700
CGS 21,680[j]	5	4.61 ± 0.05	9.13 ± 0.04	33000 ± 2300

[a] Abbreviations and trivial names are isoguanosine, 2-oxoadenosine; 2-CADO, 2-chloroadenosine; NECA, *N*-ethyladenosine-5′-ribofuranuronamide; *R*-PIA, N^6-(1-phenyl-2(*R*)-propyl)-adenosine; CPA, N^6-cyclopentyladenosine; *S*-ENBA, N^6-[(1*R*,2*S*,4*S*)-*endo*-2-norbornanyl]adenosine; CV-1808, 2-(phenylamino)adenosine; DMPA, N^6-[2′-(3,5-dimethoxyphenyl)-2′-(2-methylphenyl)ethyl]adenosine; and CGS-21,680, *N*-ethyl-2-[[2-[(4-carboxyethyl)phenyl]ethyl]amino]adenosine-5′-ribofuranuronamide. [b] Stimulus–QRS interval prolongation, mediated by an A_1AR. [c] Coronary vasodilation, mediated by an A_2AR. [d] Ratio of EC_{50} stim-QRS/EC_{50} coronary. [e] Davol, J. *J. Am. Chem. Soc.* **1951**, *73*, 3174–3176. [f] Reference 1. [g] Trivedi, B. K.; Bridges, A. J.; Patt, W. C.; Priebe, S. R.; Bruns, R. F. *J. Med. Chem.* **1989**, *32*, 8–11. [h] Reference 14. [i] Reference 5. [j] Reference 18.

significantly more acid-labile than the isopropylidine group,[22] to block the ribose hydroxyl groups. Refluxing in 50% acetic acid and then in ammonia satisfactorily deblocked the ethoxymethylidine nucleosides. The best method for deblocking isopropylidine nucleosides is boiling in a solution of 2% formic acid and 50% acetic acid in water, *with close monitoring by HPLC*. Appreciable cleavage of the glycosylic bond does not occur until deblocking is essentially (>95%) complete. Neutralization at this time with $NaHCO_3$ gives good yields of product.

Preparative reverse-phase HPLC, usually employing elution by gradients of methanol in water, proved to be an efficient way to purify the 2-alkoxyadenosines. Because the hydrophobicity of a 2-alkoxyadenosine is often similar to that of the parent alcohol, it is necessary to separate the alcohol from the blocked nucleoside before the deblocking step. Flash chromatography on silica gel using chloroform–methanol efficiently separates the alcohol from the blocked nucleoside.

Table I lists the properties of the 2-alkoxyadenosines.

Cardiovascular Activity. The isolated Langendorff guinea pig heart preparation permitted the simultaneous assay of agonist potency at the A_1AR of the atrioventricular node[22] and at the A_2AR in the coronary artery.[24] This bioassay preparation differs from those used by other investigators in two respects, (a) the species is guinea pig rather than the rat[25,26] or rabbit[27] and (b) AV node conduction velocity rather than SA node firing rate, that is, heart rate, is the variable reflecting the A_1AR agonist activity. Because the cardiac electrophysiological effects of adenosine are strongly species dependent,[28] characterizing the responsiveness of the guinea pig heart seemed worthwhile. Table II summarizes the actions of adenosine and some analogues used in the classification of adenosine receptors. In accord with the observations of others, adenosine, 2-chloroadenosine, and NECA are rather potent but unselective agonists. Surprisingly, *R*-PIA, the archetypical A_1AR agonist,[16,17] and CPA, one of the most selective A_1AR agonists identified by radioligand and bioassay studies,[3,4] also appear to be unselective. *S*-ENBA, the most potent and selective A_1AR agonist identified to date by radioligand binding assays,[29] is only 14-fold selective for the A_1AR. On the other hand, our observations confirm those of others indicating that three analogues, CV-1808,[4] DPMA,[5] and CGS 21,680,[19] are potent and selective A_2AR agonists.

The A_1/A_2 activity ratios found in the present study tend to be higher than those of rat hearts.[19,26] Several factors, perhaps acting in concert, probably influence apparent selectivity. First, there are species differences in the affinity of these receptors for agonist ligands. For example, the affinity of the A_1 receptor of calf brain for *R*-PIA is 30-fold higher than that of the receptor in guinea pig brain; the affinity of the rat brain receptor is intermediate.[30] Evidence from the rat suggests that the A_1 receptors in different organs have similar structures.[31] Accordingly, the A_1 receptor of the guinea pig AV node, like that of the brain, may have a relatively low affinity for agonists. Second, the density of A_1 receptors in cardiac tissue is low, perhaps only a few percent of that of muscarinic receptors,[32] and the biological actions of A_1 receptor agonists are likewise smaller than those of cholinergic agonists.[33] In tissues where receptor density is low, the full expression of a biological effect may require occupancy of most of the receptors in the population. Conversely, spare receptors could contribute to the high A_2AR activity of some of these analogues. Hamilton et al.[26] showed that the affinity of adenosine analogues, measured as the K_i of the displacement of [^{3}H]NECA from the A_{2a} receptor of rat striatal membranes, is an order of magnitude higher than the EC_{50} of coronary vasodilation. One explanation for such an observation is that the biological response requires the activation of only a fraction of the receptor population. Additionally, the choice of the indices of activity can influence selectivity as measured by bioassay. In the guinea pig the AV node is more sensitive than the SA node to the electrophysiological effects of adenosine.[28] Accordingly, using the prolongation of AV conduction, expressed as the EC_{50} of prolongation of the stimulus–QRS interval of the electrocardiogram, as an index of potency at the A_1AR yields a higher estimate of potency than using slowing of atrial rate and, consequently, lowers the A_1/A_2 selectivity ratio. Pharmacodynamic factors, too, can influence apparent selectivity. The binding of adenosine and its analogues to plasma albumin,[33] which in blood-perfused hearts limits the availability of these ligands at tissue receptors,[1] was not a problem in the Langendorff preparations used here. However, a difference in the accessibility of adenosine and its analogues to the A_1 and A_2

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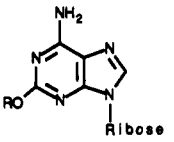
Table III. Cardiac and Coronary Activity of 2-Alkoxyadenosines **3a–x**



			−log EC_{50}, M			
no	R	n[a]	stim–QRS[a]	coronary[a]	A_1/A_2	k'
3a	CH_3	2	5.03 ± 0.02	6.16 ± 0.05	14 ± 2.5	0.12
3b	C_2H_5	2	4.10 ± 0.10	6.44 ± 0.11	240 ± 110	0.19
3c	C_3H_7	4	3.83 ± 0.14	7.28 ± 0.08	3900 ± 2000	0.34
3d	C_4H_9	4	4.43 ± 0.05	7.28 ± 0.08	760 ± 140	0.60
3e	C_5H_{11}	4	4.34 ± 0.03	7.94 ± 0.05	4200 ± 600	1.12
3f	C_6H_{13}	4	4.86 ± 0.08	8.42 ± 0.09	3800 ± 700	1.92
3g	$(CH_3)_2CH$	2	3.65 ± 0.05	5.86 ± 0.12	160 ± 26	0.27
3h	$(CH_3)_2CHCH_2$	3	3.97 ± 0.17	7.15 ± 0.03	1700 ± 480	0.56
3i	$(CH_3)_2CH(CH_2)_2$	4	4.18 ± 0.08	7.61 ± 0.08	2800 ± 500	1.47
3j	$(CH_3)_2CH(CH_2)_3$	3	4.64 ± 0.06	8.45 ± 0.03	6600 ± 1000	2.71
3k	c-C_6H_{11}	4	4.00 ± 0.07	6.18 ± 0.06	150 ± 6	0.88
3l	c-$C_6H_{11}CH_2$	2	4.30 ± 0.10	6.75 ± 0.05	300 ± 100	1.91
3m	c-$C_6H_{11}(CH_2)_2$	5	5.06 ± 0.02	8.99 ± 0.05	8700 ± 1100	3.34
3n	c-$C_6H_{11}(CH_2)_3$	4	5.43 ± 0.11	8.67 ± 0.12	2000 ± 510	5.96
3o	c-$C_6H_{11}(CH_2)_4$	4	5.58 ± 0.06	8.71 ± 0.08	1500 ± 350	10.84
3p	$C_2H_5(CH_3)CH$	2	3.05 ± 0.05	5.45 ± 0.02	250 ± 40	0.46
3q	c-C_5H_9	4	4.10 ± 0.09	7.04 ± 0.04	890 ± 120	0.58
3r	c-$C_5H_9(CH_2)_2$	4	4.85 ± 0.06	8.41 ± 0.07	3900 ± 1000	1.96
3s	ENB-$(CH_2)_2$[b]	4	5.06 ± 0.06	8.35 ± 0.07	2200 ± 190	3.64
3t	DBH-$(CH_2)_2$[b]	4	5.35 ± 0.16	8.24 ± 0.13	1200 ± 500	6.11
3u	$(C_2H_5)_2CHCH_2$	2	4.45 ± 0.05	6.54 ± 0.16	140 ± 60	0.99
3v	$(C_2H_5)_2CH(CH_2)_2$	4	4.80 ± 0.06	7.80 ± 0.03	1100 ± 180	2.47
3w	$CH_3C{\equiv}C(CH_2)_2$	4	4.20 ± 0.08	7.56 ± 0.17	2800 ± 890	0.38
3x	$HO(CH_2)_2$	4	5.66 ± 0.08	7.18 ± 006	33 ± 4	0.13

[a] Defined as in footnotes *b* and *c* to Table II. [b] ENB and DBH are defined in footnote *c* to Table I.

receptors is an important influence. Evidence that A_2 receptors on the surface of coronary endothelial cells mediate the vasoactivity of exogenous adenosine (Ueeda et al., submitted) suggests that pharmacodynamic factors have little effect on the estimates of A_2 agonist activity, and the concentration of agonist at the receptor is the same as that in the perfusate. By contrast, access to the A_1 receptors in the AV node is restricted; agonist must reach these receptors by diffusing out of the vascular compartment and is susceptible to cellular uptake and unspecific binding, which tend to lower the effective concentration at the nodal receptors. Although administering the analogues by continuous infusion and making measurements during the stable phase of the response reduces the impact of tissue uptake, the concentration of agonist at the receptor is possibly lower than at the endothelial A_2 receptor.

Table III summarizes the results of the assays of A_1AR and A_2AR activity of nucleosides that constitute three congeneric series, *n*-alkyl (**3a–f**), isoalkyl (**3g–j**), and c-hexylalkyl (**3k–o**). Information from a miscellaneous group of nucleosides (**3p–x**) supplements that provided by **3a–o**. All the analogues are full agonists at both receptors and each of the analogues is substantially more potent at the A_2AR. The unselective antagonist 8-(*p*-sulfophenyl)-theophylline competitively antagonized the activity of representative analogues at both receptors (data not shown). In all three series the EC_{50} of stimulus–QRS interval prolongation tends to vary, sometimes randomly, over the relatively narrow range between 10^{-4} and 10^{-6} M; for this reason, the EC_{50} for coronary vasodilation is the major determinant of the A_1/A_2 selectivity ratio. Thus, analogues that are weak A_2AR agonists are also unselective, for example, **3a**, **3g**, **3l**, and **3r**.

Among the 2-*n*-alkoxyadenosines (**3a–f**), coronary vasoactivity increases in direct proportion to alkyl chain length. In agreement with earlier reports,[13,14] the methoxy and ethoxy analogues are weak agonists, but the potency of the propyl and butyl analogues is substantially higher and the EC_{50} of hexyl analogue **3f** is higher still, in the low nanomolar range. None of these analogues is a strong A_1AR agonist; the EC_{50} of prolongation of the stimulus–QRS interval varies between 9 and 150 μM, independently of alkyl chain length. As a consequence, the A_1/A_2 selectivity ratio increases with alkyl chain length, those of **3c**, **3e**, and **3f** approximating 4000.

Isoalkoxyadenosines (**3g–j**) show the same dependence of coronary vasoactivity on alkyl chain length as exhibited in the *n*-alkyl series. The most potent coronary vasodilator in this series, 2-(4-methylpentoxy)adenosine (**3j**), has an EC_{50} of 3 nM. Activity at the A_1AR is low and only weakly related to the size of the alkyl substituent. Consequently, selectivity varies directly with A_2AR activity; the A_1/A_2 selectivity ratio of **3j** is 6400.

The structure–activity relationships of 2-(cyclohexylalkoxy)adenosines (**3k–o**) differ slightly from those of the other series. At the A_1AR, the potency of 2-(cyclohexyloxy)adenosine is 2 orders of magnitude lower than that of adenosine, but among the cyclohexylalkoxy analogues (**3l–o**), potency increases as a function of alkyl chain length; 2-(cyclohexylbutoxy)adenosine (**3o**) is as potent an A_1AR agonist as adenosine. Potency at the A_2AR is also related to alkyl chain length, but is maximum when the number of methylene residues is two and remains high at longer alkyl chain lengths. Among all 2-alkoxyadenosines, 2-(cyclohexylethoxy)adenosine (**3m**) is the most potent and also the most selective A_2AR agonist, the EC_{50} of coronary vasodilation and the A_1/A_2 activity ratio being 1 nM and 8400, respectively. Although the potency of **3n** and **3o** at the A_2AR are nearly as high as that of **3m**, the enhancement of A_1AR activity through chain lengthening lowers the A_1/A_2 selectivity ratio.

The study by Marumoto et al.[14] is the largest previous survey of the coronary vasoactivity of the 2-alkoxyadenosines. Those investigators found that the coronary

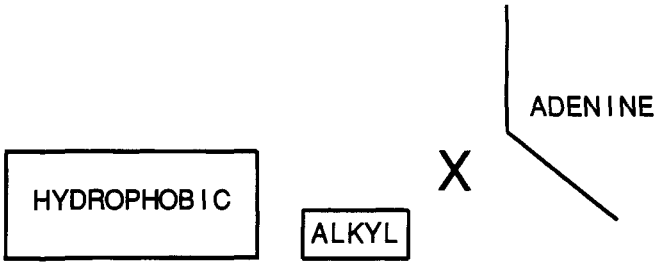


Figure 1. Model of the C-2 region of the guinea pig coronary artery A_2AR, deduced from the structure–activity relationships of the 2-alkoxyadenosines.

vasoactivity of the 2-*n*-alkoxyadenosines increased as a function of chain length up to the propoxy analogue and then decreased, the activity of the pentyl analogue being only 8% that of the propyl. The present study comes to a somewhat different conclusion, probably because the bioassay preparations differ significantly. The present study shows that the coronary vasoactivity of the *n*-alkoxyadenosines increases with chain length over the entire range up to hexyl. A fundamental difference between the experiment preparations might explain the differences between the two studies; whereas Marumoto et al. studied blood-perfused hearts, the present observations come from buffer-perfused hearts. Adenosine analogues bind to plasma proteins, particularly albumin, with an affinity that varies directly with hydrophobicity.[1,32] It is possible that the enhanced binding of adenosine with large hydrophobic residues to blood proteins reduced the bioavailability of agonist and, hence, its concentration at the receptor. Such an effect, which tends to underestimate the true potency of an analogue, increases with hydrophobicity.

A model of the A_2 adenosine receptor[9,35] advanced to account for a lack of additive effects of C-2 and N-6 substituents that confer A_2 agonist activity envisions a hydrophobic C-2 "pocket" accessible to aralkyl substituents on N-6 and also C-2. The structure–activity relationship on analogues **3a–o** further refine that model by providing some information about the dimensions of this pocket and its distance from the part of the receptor that accomodates the adenine base. Figure 1 is a general model of the C-2 region of the A_2AR that consists of three subregions, each complementary to a portion of the C-2 substituents of **3a–o**. Subregion X accommodates the atom linking the C-2 substituent to the purine. In the present study oxygen is the link, but adenosines having –NH–, –S–, and $-CH_2-$ linkages also show A_2AR agonist activity.[14] A comparison of the effects of linkage groups on biological activity is beyond the scope of the present study. A *hydrophobic* subregion as least as large as cyclohexane is a prominent feature of the C-2 region. Agonist potency is proportional to the number of methylene (or methyl) residues able to interact with the hydrophobic subregion. The interposition between the X and hydrophobic subregions of an *alkyl* subregion able to accommodate an ethyl group recognizes a feature common to all the potent agonists in this series, namely, an alkyl chain containing at least two methylene residues. The low activity of the methoxy and ethoxy analogues **3a** and **3b** suggests that the interaction of a substituent solely with the alkyl subregion impedes the binding of adenosine to the A_2AR. The interaction of still larger substituents with the hydrophobic subregion overcomes the negative influence of the alkyl subregion. The N^6-alkyladenosines show a similar biphasic influence of

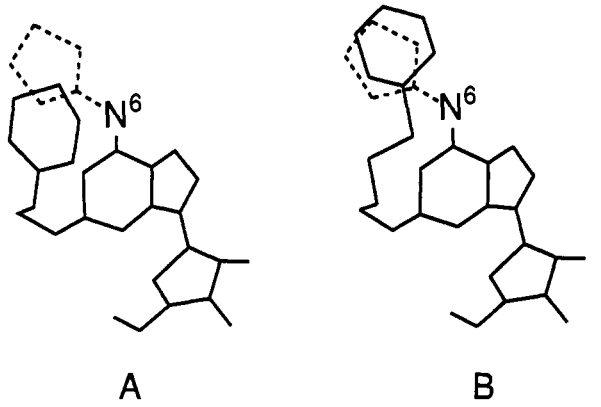


Figure 2. Possible explanation for the dependence of the A_1AR agonist activity of the 2-isoalkoxyadenosines and 2-cyclohexylalkoxyadenosines on the length of the alkyl chain. The structures shown here represent A, 2-(cyclohexylethoxy)adenosine, and B, 2-(cyclohexylbutoxy)adenosine. The dashed lines represent the portion of the N-6 region of the A_1AR that recognizes N^6-cycloalkyl groups. Note that the flexibility of the 2-alkoxy substituents permits the cycloalkyl moiety of A to partially and that of B to more extensively interact with the N-6 region.

chain length on agonist potency.[1,3]

The structure–activity relationships of the 2-alkoxyadenosines suggest that the A_1AR also contains a C-2 region, but its structure differs from that of the A_2AR, the C-2 region of the A_1AR appears to contain X and alkyl subregions that accommodate the linkage atom and interact negatively with up to two methylene residues, respectively. The evidence for a hydrophobic subregion is less convincing. Most of the 2-alkoxyadenosines are far weaker agonists than adenosine itself. Among the *n*-alkyl congeners, activity seems to be independent of chain length. The potency of the isoalkyl and cyclohexylalkyl congeners does increase as a function of chain length, but this does not necessarily reflect the interaction of these groups with the C-2 region. Studies of molecular models (Figure 2) show that such large substituents can interact with the N-6 receptor region, where isoalkyl and cycloalkyl groups strongly promote agonist activity.[3] Thus, the C-2 region of the A_1AR may consist only of an X and an alkyl subregion.

Analogues **3p–x** are intended to further test and refine the receptor model. The comparison of two *n*-alkyl analogues, **3b** and **3c**, with two *sec*-alkyl analogues having major alkyl chains of the same length, **3g** and **3p**, respectively, probes lateral bulk tolerance within the alkyl subregion. Potency rankings **3b** > **3g** and **3c** > **3p** suggest that there is limited lateral bulk tolerance in this subregion. That cyclopentyl analogue **3q** is both narrower and more potent than cyclohexyl congener **3k** further supports the notion of limited bulk tolerance.

Analogues **3r–x** probe the hydrophobic nature of the C-2 region. All contain the ethyl residue that appears to position substituents for optimum interaction with the receptor. Cyclopentyl analogue **3r** is less potent than cyclohexyl congener **3m**, which contains one more methylene residue and thus is more hydrophobic. The bicycloalkyl groups of analogues **3s** and **3t** are larger than cyclohexane and, additionally, are rigid and not planar. Their potency, only 5-fold less than that of **3m**, which has a flexible cycloalkyl moiety, suggests that the hydrophobic region is not flat. Analogues **3u** and **3v** are homologues of **3h** and **3i**, containing ethyl instead of methyl groups distal to the branching point. The coronary vasoactivity ranking **3v** > **3u** further supports the idea that the number of methylene/methyl groups able to interact with the

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hydrophobic region determines potency at the A_2AR.

The acetylenic bond of **3w** renders it more polar than its *n*-alkylisostere **3e**. Perhaps for this reason **3w** is 2.4-fold less potent than **3e**. Alternatively, the acetylenic bond is rigid and also reorients the terminal methyl group, attributes that could sterically hinder interaction with the receptor. One cannot discriminate between the two possibilities but in any event, the small loss of activity means that neither is very important. Matsuda[36] has reported that 2-alkynyladenosines are selective A_2 receptor agonists. The 2800-fold selectivity of **3w** for the A_2 receptor corroborates that report. Analogue **3x** is an oxygen isostere of **3c** and, consequently, is much more polar. However, it is approximately as active as the *n*-alkyl isostere.

In summary, the structure–activity relationships of the 2-alkoxyadenosines show that a large alkyl or cycloalkyl substituent linked to C-2 through an ethoxy group is optimum for coronary vasoactivity. Secondary alkyl substituents greatly reduce activity, probably through steric hindrance. The 2-alkoxyadenosines are very weak agonists at the A_1 receptors mediating AV block.

Experimental Section

S-ENBA and DPMA were purchased from Research Biochemicals, Inc., Natick, MA. Other analogues used to characterize the isolated heart preparation were synthesized as described in the literature. The alcohols for the synthesis of **3a–x** were either available commercially or were prepared from the corresponding acyl chlorides by reduction with $LiAlH_4$. Flash chromatography on 40–60 μm silica gel eluted with 2% CH_3OH in $CHCl_3$ served for the purification of the 2-substituted (ethoxymethylidine) and isopropylidineadenosines. The purification of final product employed a Rainin Autoprep fitted with a 1 × 25 cm column of C-18 silica, eluted with CH_3OH–water in either the isocratic or gradient mode. Analytical reverse-phase HPLC showed that product accounted for ≥99% of the UV-absorbing material in samples submitted for bioassay. Melting points are uncorrected. A Varian EM 360L spectrometer yielded proton NMR spectra of nucleoside solutions in DMSO-d_6 which were consistent with the assigned structures. MHW Laboratories, Tucson, AZ, performed the elemental analyses, which agreed within 0.4% of the theoretical compositions.

Preparation of 2-Alkoxyadenosines from Primary Alcohols. 2-(1-Pentoxy)adenosine [6-Amino-2-(1-pentoxy)-9-β-D-ribofuranosyl-9*H*-purine, 3e]. A solution of 1-pentanol (3.7 mL, 33.6 mmol) in 70 mL of dry 1,2-dimethoxyethane was cooled to 10 °C in an ice bath. To this solution was added 1.6 M *n*-butyllithium (19.9 mL, 31.9 mmol), the solution was stirred for 15 min, and 2-chloro-2′,3′-*O*-(ethoxymethylidene)adenosine (3.0 g, 8.4 mmol) was added in one portion. The resulting mixture was refluxed for 5 days, at which time HPLC showed that less than 5% of the starting material was present. The solvents were removed in vacuo and a solution of the residue in 70 mL of water was extracted with ethyl acetate (4 × 50 mL). The combined extracts were dried over $MgSO_4$ and evaporated in vacuo to a dark syrup for purification by flash chromatography. Fractions containing product were concentrated and dissolved in methanol–water. Acetic acid (15 mL) was added and the solution refluxed until TLC showed that the nucleoside was completely deblocked. The solution was adjusted to pH 9 with concentrated ammonia and was boiled for 15 min. The solvents were removed in vacuo, and the product was purified by preparative reverse-phase HPLC, eluting product with a linear gradient of 50 → 62% methanol in water to afford 1.0 g (34%) of a white solid.

Preparation of 2-Alkoxyadenosines from Secondary Alcohols. 2-(2-Butoxy)adenosine [6-Amino-2-(2-butoxy)-9-β-D-ribofuranosyl-9*H*-purine, 3p]. This compound was prepared in the same way as **5** except that 2-chloro-5-*O*-(4,4′,4″-trimethoxytrityl)-2′,3′-*O*-isopropylideneadenosine (5.7 g, 8.4 mmol) served as the starting material instead of 2-chloro-2′,3′-*O*-(ethoxymethylidene)adenosine. Deblocking was achieved by boiling in a mixture of 1 mL of formic acid in 50 mL of acetic acid–water (1:1) with monitoring by HPLC, until deblocking was 98% complete. Neutralization ($NaHCO_3$) and purification by reverse-phase HPLC yielded 1.5 g (45%) of a white solid.

Bioassay. A Langendorff guinea pig heart preparation paced at 260 beats/min via the left atrium served for assays of A_1AR and A_2AR agonist activity. The perfusion buffer consisted of (mM) NaCl (120), $NaHCO_3$ (27), KCl (3.7), KH_2PO_4 (1.3), $MgSO_4$ (0.64), $CaCl_2$ (1.3), pyruvate (2), and glucose (5). The buffer was saturated with 95% O_2–5% CO_2, equilibrated at 37 °C in a heat exchanger and delivered at a pressure equivalent to 55 mmHg. Continuous drainage of the left ventricle by means of a catheter inserted across the mitral valve insured that this cardiac chamber did no external work. An electrode in the right ventricle monitored the electrocardiogram. Timed collections of cardiac effluent in a graduated cylinder measured total coronary flow during the steady-state phase of the flow responses to analogue administration, which was also monitored by an in-line electromagnetic flowmeter in the aortic perfusion cannula. The rate of nucleoside infusion was increased stepwise until the appearance of second degree heart block. The quotient of the rated of nucleoside infusion (mol/min) divided by coronary flow rate (L/min) equals agonist concentration in the perfusate. The EC_{50} of prolongation of the stimulus–QRS interval, the concentration of agonist needed to prolong the interval by 50% of the maximum response,[23] reflects activity at the A_1AR. Logit transformation of the coronary flow data and solution of the regression of logit (coronary flow) on log [analogue] for logit = 0 yielded an estimate of EC_{50} of coronary vasodilation, an index of A_2AR activity. Table II reports the mean ± SEM of the −log EC_{50} values from assays in four or more hearts. The quotient of the EC_{50} of stimulus–QRS prolongation divided by the EC_{50} of coronary vasodilation provided an index of selectivity. Values of the index <1 indicate selectivity for the A_1AR and values >1 selectivity for the A_2AR. Table III reports the mean and SEM of the A_1/A_2 ratios of individual experiments.

Hydrophobicity Index, *k′*. The retention of nucleosides on a reverse-phase HPLC column is a useful measure of relative hydrophobicity.[37] Measurements of the retention time of a solute, t, and the transit time of the solvent, t_o, served for calculation of a hydrophobicity index, k', by the formula $k' = (t - t_o)/t_o$. The present measurements employed a column of C-18 silica eluted with a mixture of 0.01 M $KHPO_4$, pH 7.0, and CH_3OH (65:35, v/v).

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Registry No. 1, 56720-43-5; **3a**, 24723-77-1; **3b**, 50447-10-4; **3c**, 50257-83-5; **3d**, 50257-84-6; **3e**, 50257-85-7; **3f**, 50257-95-9; **3g**, 50257-94-8; **3h**, 131933-14-7; **3i**, 131933-15-8; **3j**, 131973-26-7; **3k**, 131933-16-9; **3l**, 131933-17-0; **3m**, 131933-18-1; **3n**, 131933-19-2; **3o**, 131933-20-5; **3p**, 131933-21-6; **3q**, 131933-22-7; **3r**, 131933-23-8; **3s**, 131933-24-9; **3t**, 131933-25-0; **3u**, 131933-26-1; **3v**, 131933-27-2; **3w**, 131933-28-3; **3x**, 50257-89-1; 2-chloro-5′-*O*-(4,4′,4″-trimethoxytrityl)-2′,3′-*O*-isopropylideneadenosine, 131973-27-8.

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