# Communications to the Editor

N^6-Cycloalkyladenosines. Potent, A₁-Selective Adenosine Agonists

Sir:

Adenosine and adenosine agonists have numerous physiological effects in the nervous system, including inhibition of neurotransmitter release, anticonvulsant activity, analgesia, respiratory depression, hypothermia, and profound decreases in locomotor activity.[12] The receptors that mediate these responses have been studied indirectly through the responses that they elicit[8,14] and directly by receptor binding methods.[1,15] There are two main classes of extracellular adenosine receptors: A₁ receptors whose activation leads to inhibition of adenylate cyclase and A₂ receptors whose activation leads to stimulation of adenylate cyclase.[14] These receptors are designated R$_i$ and R$_a$, respectively, in an alternative nomenclature.[8] Although N^6-substituted adenosines, especially N^6-cyclohexyladenosine (CHA), are known to be selective A₁ agonists[1,13,15] and to possess activity as inhibitors of platelet aggregation[7] and neurotransmission,[9] structure–activity relationships for lower and higher homologues of CHA have not been explored to date. The present study reports the discovery of N^6-cyclopentyladenosine (CPA) as a potent, A₁-selective adenosine agonist, a finding that has allowed the development of an A₂ receptor binding assay. Additionally, this study reports the binding affinities for a series of N^6-cycloalkyladenosines at both A₁ and A₂ adenosine receptors.

Chemistry. All adenosine analogues were synthesized at Warner-Lambert/Parke-Davis according to standard chemical procedures[5,7,10] except 2-chloroadenosine, which was obtained from the Sigma Chemical Co. Physical properties (¹H nuclear magnetic resonance, infrared, and

N^6 –Cycloalkyladenosines

R–PIA

NECA

1–Methylisoguanosine
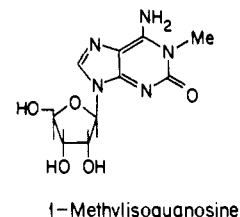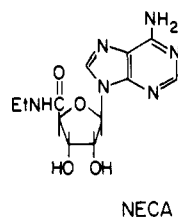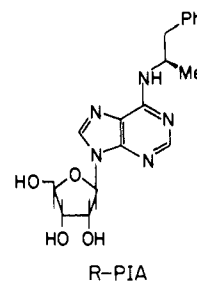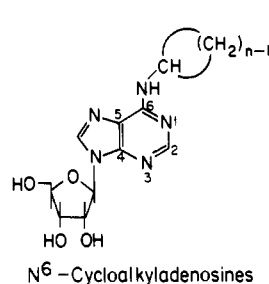
mass (low- and/or high-resolution) spectra, elemental analyses, melting points) were consistent with the chemical structures. Lipophilicity (log k') was determined by using a high-performance liquid chromatography (HPLC) correlation method.[6] Statistical analyses were performed by using the Statistical Analysis System (SAS).[11]

Receptor Binding. [³H]CHA binding to A₁ receptors was performed essentially as previously described[1] except that whole rat brain (minus brainstem and cerebellum) was used instead of guinea pig brain.

A₂ receptor binding was performed in exactly the same way as A₁ receptor binding with the following exceptions: 4 nM [³H]-1-(6-amino-9H-purin-9-yl)-1-deoxy-N-ethyl-β-D-ribofuronamide ([³H]NECA) was used as radioligand, the tissue was 5 mg of tissue wet weight of rat striatal membranes, the incubation volume was 1 mL, 10 mM MgCl₂ was added to the buffer, and all incubations contained 50 nM CPA to eliminate A₁ receptor binding. Nonspecific binding was defined as binding in the presence of 100 μM CPA. This method is a variation of the [³H]-NECA binding assay of Yeung and Green;[15] a detailed characterization of the method will be reported elsewhere.[2,3]

IC₅₀ values in A₁ and A₂ binding were calculated from eight-point curves, including total binding, nonspecific

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Table I. Affinities of N^6-Cycloalkyladenosines and Reference Agents in A_1 and A_2 Adenosine Receptor Binding Assays

	A_1 K_i,[a] nM, [³H]CHA	A_2 K_i,[a] nM, [³H]NECA	A_1 selec ratio[b]	log k'	mp,[f] °C
		N^6-Cycloalkyladenosines			
N^6-cyclopropylado[c]	319 ± 0.49	1240 ± 130	389	0.53	184–187[h]
N^6-cyclobutylado	0.777 ± 0.028	263 ± 5	338	1.22	138[h]
N^6-cyclopentylado (CPA)	0.590 ± 0.023	462 ± 15	783	1.56	g
N^6-cyclohexylado (CHA)	1.31 ± 0.009	363 ± 46	277	1.60	g
N^6-cycloheptylado	2.79 ± 0.36	1690 ± 190	606	2.40	g
N^6-cyclooctylado	4.03 ± 0.16	2450 ± 500	608	2.90	g
N^6-cyclodecylado	100 ± 11	5190 ± 50	51.9	3.62	138–140[h]
N^6-cyclododecylado	5390 ± 351	47700 ± 8500	8.85	4.52	144–145[i]
		Reference Adenosine Agonists			
NECA[d]	6.24 ± 0.45	10.6 ± 0.5	1.70		
2-chloroado	8.74 ± 0.11	63.1 ± 7.5	7.22		
1-methylisoguanosine	144 ± 3	3250 ± 92	22.6		
S-PIA[e]	47.6 ± 2.3	1810 ± 380	38.0		
R-PIA[e]	1.15 ± 0.20	124 ± 9	108		

[a] Values are means ± standard errors for three or four separate experiments for each compound. [b] A_1 selectivity ratio is the A_2 K_i divided by the A_1 K_i. [c] Adenosine is abbreviated ado. [d] 1-(6-Amino-9H-purin-9-yl)-1-deoxy-N-ethyl-β-D-ribofuronamide. [e] N^6-[(R or S)-1-Methyl-2-phenylethyl]adenosine. [f] Melting points are listed for new compounds only and are uncorrected. [g] See ref 7. [h] C, H, N: ±0.4%. [i] C, H: ±0.4%; N: +0.6%. Exact mass: ±5 ppm.

binding, and six drug concentrations that bracketed the IC_{50}.

K_i values for compounds in A_1 receptor binding were calculated from the Cheng–Prusoff equation[4] using 1.31 nM as the K_d for [³H]CHA. The K_d for [³H]CHA was calculated from the IC_{50} for unlabeled CHA of 2.31 nM. K_i values in A_2 receptor binding were calculated on the basis of K_d values of 10.6 nM for [³H]NECA and 462 nM for CPA, which in turn were calculated from IC_{50} values of 15.8 nM for NECA and 685 nM for CPA.

SAR. Affinity of the N^6-cycloalkyladenosines in A_1 receptor binding varies as a smooth function of ring size, reaching a maximum with N^6-cyclopentyladenosine (Table I). CPA is approximately twice as potent as CHA at the A_1 receptor, and with 0.59 nM affinity, CPA is the most potent adenosine agonist reported to date. For ring sizes $n = 3$–8, the adenosine analogues are all quite potent; only the two largest analogues ($n = 10$, 12) are substantially less potent than CHA. Preliminary attempts to correlate receptor binding in this series with physicochemical properties suggest a correlation with lipophilicity (log k'), as indicated by the following equations, where n is the number of compounds included in the analysis, s is the root mean square error, r^2 is the square of the correlation coefficient, F relates the variance of the null hypothesis to the correlation variance, p is the probability that a random set of data would yield a higher F value, and terms are given ± their standard errors.

$$\log (A_1\ K_i) = [-1.33\ (\pm 0.23)]\ \log k' +$$
$$[0.43\ (\pm 0.04)](\log k')^2 + [0.99\ (\pm 0.25)]$$

$$n = 8,\ s = 0.18,\ r^2 = 0.99,\ F = 185.21,\ p < 0.0001$$

$$\log (A_2\ K_i) = [-0.50\ (\pm 0.31)]\ \log k' +$$
$$[0.19\ (\pm 0.06)](\log k')^2 + [3.05\ (\pm 0.34)]$$

$$n = 8,\ s = 0.25,\ r^2 = 0.92,\ F = 28.87,\ p < 0.0018$$

Whether the correlations reflect whole molecule lipophilicity, side chain lipophilicity, size effects, or a combination of factors is currently under investigation. Finally, it is interesting to note that A_1 and A_2 binding affinities are correlated in this series, as defined by the following equation:

$$\log (A_1\ K_i) = [1.74\ (\pm 0.21)]\ \log (A_2\ K_i) - [4.72\ (\pm 0.70)]$$

$$n = 8,\ s = 0.42,\ r^2 = 0.92,\ F = 66.23,\ p < 0.0002$$

Because of its high affinity for A_1 receptors, CPA proved useful in developing the A_2 receptor binding assay used in the present study. A major problem in the use of [³H]NECA as an A_2 receptor ligand is its high affinity for A_1 receptors, so that even in favorable tissues such as rat striatum A_2 receptors account for only about half of specific binding.[15] Reference agents including CHA give shallow dose–inhibition curves with incomplete separation between A_1 and A_2 phases of receptor occupancy. In contrast, CPA shows a biphasic dose–inhibition curve with a clear plateau between the A_1 and A_2 phases.[2] For this reason, 50 nM CPA is used routinely in our A_2 receptor binding assay to eliminate the A_1 component of [³H]NECA binding. The relative affinities of NECA and R-PIA in A_1 and A_2 binding (Table I) are in good agreement with their affinities in A_1-inhibited and A_2-stimulated adenylate cyclase,[8] respectively.

CPA is the most A_1 selective of the N^6-cycloalkyladenosines (780-fold, Table I), but the cycloheptyl and cylcooctyl homologues are almost equally selective. All of the N^6-cycloalkyladenosines except the cyclodecyl and cyclododecyl homologues are more A_1 selective than the most selective reference agent, N^6-[(R)-1-methyl-2-phenylethyl]adenosine (R-PIA).

Studies exploring the biological properties of this homologous series and the use of these potent adenosine agonists as pharmacological tools are in progress.

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