Pharmacology. The evaluation of compounds was carried out by using procedures described in part 1 of this series.[1]

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Registry No. **2a**, 107114-98-7; **2a**·HCl, 107114-83-0; **2b**, 107114-84-1; **2c**, 107114-99-8; **2c**·HCl, 107114-85-2; **2d**, 107114-86-3; **2e**, 107114-87-4; **2f**, 107114-88-5; **2g**, 107114-89-6; **2h**, 107114-90-9; **2i**, 107114-91-0; **3**, 107115-00-4; **4**, 107114-92-1; **5**, 107114-93-2; **6**, 107114-94-3; **7**, 107114-95-4; **8** (R^1 = H), 10445-00-8; **9** (R^1 = H), 107114-96-5; **9** (R^1 = CH_3), 107114-97-6; NH_2CN-Na, 17292-62-5; CH_3NH_2, 74-89-5; $(Me)_2N(CH_2)_3NH_2$, 109-55-7; $BuNH_2$, 109-73-9; $PhCH_2NH_2$, 100-46-9; $MeO(CH_2)_3NH_2$, 5332-73-0; cyanogen bromide, 506-68-3; ethylenediamine, 107-15-3; 1,2-diamino-2-methylpropane, 811-93-8; 1-(2-amino-2-methylpropyl)-2-[5-[2-(trifluoromethyl)phenyl]-1,3,4-thiadiazol-2-yl]-thiourea, 107115-01-5; guanidine hydrochloride, 50-01-1.

N^6-Phenyladenosines: Pronounced Effect of Phenyl Substituents on Affinity for A_2 Adenosine Receptors

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A number of N^6-phenyladenosines with various substitutions on the phenyl ring have been synthesized and tested for their affinities toward brain A_1 and A_2 adenosine receptors. Compounds with meta substituents, such as (*m*-hydroxy- and *m*-iodophenyl)adenosine, were found to have high A_1 selectivity. Meta substitution caused a selective decrease in the affinity of these compounds for A_2 receptors. The results suggest that, in contrast to what is commonly held, certain N^6-substituents have pronounced effects on affinity for brain A_2 adenosine receptors. Thus, brain A_2 receptors may have a well-defined region that recognizes N^6-substitutions.

Adenosine causes a number of physiological effects that are mediated by specific adenosine receptors termed A_1 (or R_i) and A_2 (or R_a) receptors.[1] In many cells, including neuronal cells, the activation of A_1 receptors causes the inhibition of adenylate cyclase activitiy, while the activation of A_2 receptors causes an increase in adenylate cyclase activity.[1-3] The two types of receptors differ in their selectivity toward various adenosine analogues (see Chart I for structures). For example, the potencies of N^6-(phenylisopropyl)adenosine (PIA) and cyclohexyladenosine (CHA) to inhibit adenylate cyclase (via A_1 receptors) are greater than that of 5′-(*N*-ethylcarbamoyl)adenosine (NECA), while the potency of NECA to stimulate adenylate cyclase (via A_2 receptors) is greater than that of PIA and CHA.[1-3]. The same A_2/A_1 selectivity is seen in ligand-binding assays conducted with PIA and CHA, although under these conditions NECA exhibits nearly equal affinity for A_1 and A_2 receptors[2-4] (and see Table I). These and other results have led to the hypothesis that A_1 receptors have a specialized region that interacts with the N^6 domain of the adenosine molecule, while A_2 receptors have a specific domain that interacts with 5′-substituents on the ribose ring.[5-7,16] However, both A_1 and A_2 receptors show selectivity for the two stereoisomers of PIA. While the stereoselectivity at A_1 receptors is greater than that at A_2 receptors,[2-4] the fact that A_2 receptors can discriminate between the *R* and *S* enantiomers of PIA suggests that the A_2 receptors also have specialized regions to interact with the N^6 domain. Recent studies have shown that apparent A_2 receptors in coronary artery recognize N^6-substitutions.[5-7] On the basis of these results, it was concluded that the coronary artery adenosine receptor might be a "hybrid" A_1/A_2 receptor.[5]

N^6-Phenyladenosine (Chart I) is a potent A_1 agonist with a moderate A_2/A_1 selectivity (Table II).[1] In this paper, we report on the synthesis of a number of phenyl-substituted analogues of this compound and their affinities for A_1 and A_2 receptors in brain preparations. Meta, but not para, substitution led to selective decreases in the affinities of these compounds for A_2 receptors. The results suggest that brain A_2 receptors, like those in coronary artery,[5-7] have a well-defined region to interact with the

Table I. Physical–Chemical Characteristics of Various N^6-Phenyladenosines

no.	phenyl substit	mp,[a] °C	(formula) anal.
1	H	199[b]	
2	*m*-OH	256–257	($C_{16}H_{17}N_5O_5$) C, H, N; C[c]
3	*m*-I	205–206	($C_{16}H_{16}N_5O_4I$) C, H, N; N[d]
4	*m*-C_2H_5	192–194	($C_{18}H_{21}N_5O_4$) C, H, N
5	*m*-NH_2	202–204	($C_{16}H_{18}N_6O_4$) C, H, N
6	*m*-$B(OH)_2$	280	($C_{16}H_{18}N_5O_6B$) C, H, N
7	*m*-F	207-208	($C_{16}H_{16}N_5O_4F$) C, H, N
8	*p*-F	231–232	($C_{16}H_{16}N_5O_4F$) C, H, N
9	*p*-I	220–221	($C_{16}H_{16}N_5O_4I$) C, H, N, I; N[e]
10	*p*-CH_3	214–216	($C_{17}H_{19}N_5O_4$) C, H, N; C[f]
11	*p*-C_2H_5	194–196	($C_{18}H_{21}N_5O_4$) C, H, N
12	*p*-OCH_3	207-208	($C_{17}H_{19}N_5O_5$) C, H, N

[a] Uncorrected. [b] Reference 8. [c] C: calcd, 53.48; found, 53.00. [d] N: calcd, 14.92; found, 14.04. [e] N: calcd, 14.92; found, 14.34. [f] C: calcd, 57.14; found, 56.68.

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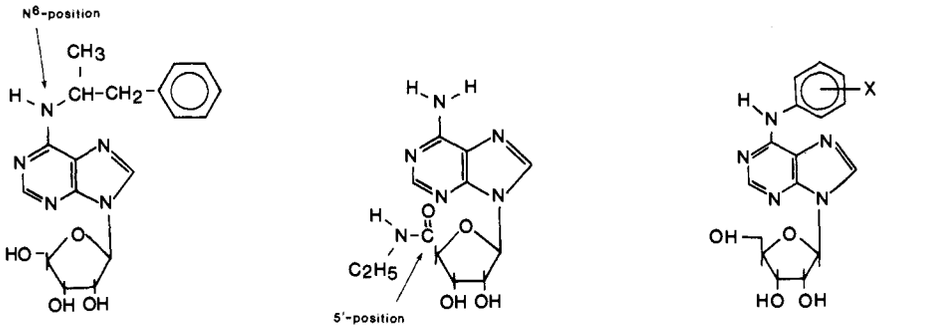
Chart I



N^6-Phenylisopropyladenosine (PIA) | Adenosine 5'-Ethylcarboxamide (NECA) | N^6-Phenyladenosine (X = H)

Table II. In Vitro Affinities of Various N^6-Phenyladenosines for Brain A_1 and A_2 Adenosine Receptors

		A_1 receptors		A_2 receptors		
no.	phenyl substit	IC_{50},[a] nM	n_H	IC_{50}, nM	n_H	selectivity ratio A_2/A_1[b]
1	H	4.6 ± 0.2	0.94 ± 0.01	660 ± 120	0.80 ± 0.04	140
2	*m*-OH	11.1 ± 0.3	0.90 ± 0.03	7640 ± 1040	0.72 ± 0.03	690
3	*m*-I	19.0 ± 1.4	0.89 ± 0.01	11900 ± 1000	0.93 ± 0.03	620
4	*m*-C_2H_5	25.3 ± 2.1	0.79 ± 0.02	10200 ± 800	0.82 ± 0.05	403
5	*m*-NH_2	45.1 ± 1.7	1.00 ± 0.06	10100 ± 1100	0.92 ± 0.03	225
6	*m*-$B(OH)_2$	42.1 ± 5.7	0.84 ± 0.02	9300 ± 900	0.95 ± 0.04	220
7	*m*-F	10.3 ± 0.5	0.90 ± 0.05	1600 ± 200	0.80 ± 0.02	155
8	*p*-F	13.1 ± 0.4	0.87 ± 0.04	860 ± 180	0.79 ± 0.01	65
9	*p*-I	8.5 ± 1.5	0.90 ± 0.01	1280 ± 100	0.82 ± 0.03	150
10	*p*-CH_3	8.5 ± 0.2	0.91 ± 0.02	920 ± 120	0.90 ± 0.06	110
11	*p*-C_2H_5	12.8 ± 0.6	0.76 ± 0.01	960 ± 20	0.86 ± 0.01	75
12	*p*-OCH_3	6.4 ± 0.6	0.93 ± 0.02	1760 ± 210	0.87 ± 0.05	275
CHA		2.5 ± 0.8	0.80 ± 0.05	510 ± 70	0.78 ± 0.02	204
NECA		17.0 ± 3.3	0.85 ± 0.06	25 ± 3.5	0.90 ± 0.03	1.5

[a] IC_{50} and n_H values were obtained from radioligand binding studies using [^{125}I]ABA for A_1 receptors and [^{3}H]NECA for A_2 receptors as described in the Methods section. All values shown are the mean ± SEM with $N = 3$. [b] Selectivity ratio = IC_{50} A_2/IC_{50} A_1.

N^6 domain of the adenosine molecule.

Chemistry. All compounds were prepared by coupling appropriately substituted anilines with 6-chloropurine ribonucleoside in absolute ethanol, in the presence of $CaCO_3$ as described by Fleysher et al.,[8] except compound **6**, which was made in methanol. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, IL. [^{125}I]Iodo(4-aminobenzyl)adenosine ([^{125}I]ABA) was prepared from (4-aminobenzyl)adenosine as described by Linden et al.[9]

Ligand-Binding Studies. Crude membrane preparations of newborn chick cerebellum and rat striatum were prepared as previously described.[10] The cerebellar membranes were used as the source of A_1 receptors, while the striatal membranes were used as the source of A_2 receptors.

Ligand binding to A_1 receptors in cerebellar membranes was performed essentially as described previously[11] in a reaction volume of 50 μL containing the following: histidine, 10 mM, pH 7.4; $MgSO_4$, 4 mM; EDTA, 4 mM; [^{125}I]ABA, 0.6–0.7 nM (which is the approximate K_d; Green, R., unpublished observation); adenosine deaminase, 1 unit/mL; digitonin, 0.03%; membrane protein, 20–30 μg; and competing ligand. Reactions were incubated for 40 min at 37 °C to attain equilibrium. Bound and free ligand were separated by vacuum filtration with Whatman GF/A filters presoaked in 0.3% polyethylenimine for 2 h.[12]

Binding of [^{3}H]NECA to A_2 receptors in striatal membranes was performed by using similar methods with the following modifications: the reaction mixture (100 μL) contained 20 nM [^{3}H]NECA (approximate K_d for A_2 receptors),[3] 30 nM N^6-cyclopentyladenosine (CPA), and 150–200 μg of membrane protein and was incubated at 37 °C for 15 min. The CPA was used to block [^{3}H]NECA binding to A_1 receptors, thus allowing the measurement of [^{3}H]NECA binding to A_2 receptors.[13] With the exception of NECA, all competing nucleosides reduced radioligand binding to the same extent as 100 μM *R*-PIA. NECA reduced [^{3}H]NECA binding to striatal membranes to a slightly greater extent than *R*-PIA as previously reported.[3] IC_{50} values and Hill coefficients (n_H) were determined from least-squares analyses of Hill plots.

Results and Discussion

Table I lists the physical-chemical data on various N^6-phenyladenosines that we have synthesized. Table II lists the IC_{50} and n_H values obtained for these compounds in ligand-binding studies of brain A_1 and A_2 receptors. Since most Hill coefficients were close to 1 and K_d concentrations of radioligands were used, the IC_{50} values obtained are direct reflections of the affinities of these compounds at A_1 and A_2 receptors.[14] Compound 1 had high affinity for A_1 receptors and an approximately 100-fold lower affinity for A_2 receptors (Table II). The introduction

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of a *m*-hydroxy group, compound **2**, resulted in a 2–3-fold lower affinity for A_1 receptors, but a more than 10-fold lower affinity for A_2 receptors. The A_2/A_1 selectivity ratio (IC_{50} A_2/IC_{50} A_1) of 690 obtained with compound **2** makes it nearly as selective for A_1 receptors as CPA.[13] The *m*-iodo analogue, compound **3**, showed similar A_1 selectivity. Since the affinities of compounds **2** and **3** at A_1 receptors were much less affected than those at A_2 receptors, it appeared that the affinity of phenyladenosines for A_2 receptors was selectively decreased by meta substitution. Results with three other meta-substituted analogues, compounds **4–6**, tend to support this conclusion as these compounds had much lower affinity for A_2 than for A_1 receptors. Although the meta substitution of the phenyladenosines resulted in dramatic decreases in affinity for A_2 receptors, para substitution caused much less attenuation in A_2 affinity (compounds **9–12**). Except for the *p*-methoxy analogue, compound **12**, the A_2 selectivity ratios for these compounds were not much different than for the parent compound **1**. The decrease in A_2 affinity caused by meta substitution appears to be due to steric factors as the introduction of a small substituent like fluoro, compound **7**, did not cause a substantial decrease in A_2 affinity compared to either its *para*-isomer, compound **8**, or the unsubstituted analogue, compound **1** (Table II). The present results show that the introduction of a bulky group at the meta position of N^6-phenyladenosine causes a selective decrease in A_2 affinity. A simple explanation for this observation could be that the rat brain A_2 receptor, like that in the dog coronary artery,[5-7] has a specialized region to interact with the N^6 domain of the adenosine molecule and the bulky meta substituents on compound **1** do not fit into this region.

Recently,[7,15] a number of phenyl-substituted N^6-phenyladenosines were evaluated for their abilities to increase coronary blood flow via a putative A_2 receptor. The meta-substituted analogues were found to have a lower potency than their para-substituted counterparts in increasing blood flow; furthermore, a compound with a relatively bulky meta substituent, the *m*-methyl analogue, was less potent than a compound with a smaller meta substituent, the *m*-fluoro compound. These results suggest that the deleterious effect of a bulky meta substituent on A_2 affinity observed in our in vitro assay with rat brain adenosine receptors is also seen in vivo with dog coronary artery adenosine receptors.

In conclusion, we have shown that substituents on the phenyl ring of N^6-phenyladenosine have marked effects on the selectivity of these compounds for A_1 and A_2 receptors. Substitutions with *m*-hydroxyl and *m*-iodo groups cause minimal change in affinities at A_1 receptors but lead to drastic reductions in the affinities for A_2 receptors. Thus, these meta-substituted phenyladenosines are highly selective for A_1 receptors. These compounds may be useful in future studies concerned with characterization of the properties of adenosine receptors.

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Book Reviews

Neuromethods: Series 1: Neurochemistry. Edited by Alan A. Boulton and Glen B. Baker. Humana Press, Clifton, New Jersey.

The objective of Neuromethods, a new handbook series, is to provide a comprehensive guide for establishing laboratory methods of interest to the neuroscientist. The neurochemistry series is edited by Alan A. Boulton of the University of Saskatchewan and Glen B. Baker of the University of Alberta with additional editors for most volumes. Series 1, entitled Neurochemistry, will be issued in at least 11 volumes. Vol. 1, General Techniques, Vol. 2, Amines and their Metabolites, and Volume 3, Amino Acids, have already been published. Vol. 4 (Receptor Binding Methods) and Vol. 5 (Enzymes) are scheduled for publication in 1986. Future volumes, covering a range of topics (peptides, lipids, physicochemical techniques, carbohydrates, nucleosides, and nucleotides, brain electrolytes, water spaces, and proteins) are planned. Hopefully the editors are considering the preparation of volumes on immunology and molecular biology techniques. Each chapter is formatted to cover an introduction to techniques, application of the techniques, detailed experimental procedures, trouble shooting and pitfalls, technique variations, and appropriate literature citations. Many chapters contain a wealth of detail: information rarely mentioned in manuscripts that can be critical for establishing a novel technique successfully. The most efficient way of acquiring a new laboratory procedure is to watch somebody else do it and then repeat the technique in their presence. This is often impractical. A handbook that describes procedures in detail and provides extensive background material can substitute for, and occasionally be an improvement on, hands-on technique acquisition. If the first three volumes of the series are indicators of overall quality, the series will be an important source for neuroscientists.

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Neuromethods. Volume 1. General Neurochemical Techniques. Edited by Alan A. Boulton and Glen B. Baker. Humana Press, Clifton, New Jersey. 1986. xix + 555 pp. 16 × 24 cm. ISBN 0-89603-075-X. $64.50.

The inaugural volume of this new methodological series consists of 14 chapters. The overall theme of the volume is to introduce a variety of approaches to central nervous system (CNS) research including techniques for brain dissection, electrophysiological recording, lesioning, histochemical mapping, and so on. Chapter 1, written by M. Palkovits, outlines techniques for microdissection of individual brain nuclei and areas. It includes justification for the technique, potential applications, detailed descriptions of the methods, and a valuable list of references for stereotaxic maps of avian and mammalian brains. The next chapter (A. Pellegrino de Iraldi and G. Rodriguez de Lores) contains procedures for preparing subcellular fractions, synaptosomes, subsynaptic fractions, etc. It is clearly written and provides an extensive bibliography. Past successes and potential uses of tissue slice preparations are described by P. Lipton (Chapter 3). CNS cell and tissue culture techniques, a rapidly expanding but technically difficult field, are outlined in Chapter 4 (L. Hertz, B. H. J. Ju-