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EFFECTS OF VARIOUS 2-AMINO-6-ALKYLDITHIOPURINES ON BRAIN SPECIFIC [^{3}H]DIAZEPAM BINDING

SHAN-CHING SUNG*† and MINEO SANEYOSHI‡
†Division of Neurological Sciences, University of British Columbia, Vancouver, Canada, and
‡Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

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Abstract—Various derivatives of 2-amino-6-methylthiopurine with substituents at the 6-position of purine were tested for their abilities to displace [^{3}H]diazepam binding to rat brain membranes. The potency was dependent on the carbon chain-length in the 6-position of purine. Among the derivatives tested, 2-amino-6-*n*-pentyldithiopurine had the highest potency, with a K_i value of 0.92 μM.

The discovery of high affinity, saturable and stereospecific binding of benzodiazepines in the central nervous system [1, 2] has provided a basis upon which the diverse actions of the benzodiazepines may be explained. Both physiological and pharmacological evidence support the hypothesis that [^{3}H]benzodiazepine binding sites in the central nervous system mediate the various actions of the benzodiazepines.

Since various findings have suggested purines as putative endogenous ligands for benzodiazepine receptors [3, 4], we have studied recently the effect of synthetic purines and purine nucleosides on [^{3}H]diazepam binding with rat brain membranes [5]. Among these analogs, 6-methylthioguanine was found to be most potent. To obtain further information about the effects of substituents at the 6-position of 2-amino purine derivatives, we have further tested various derivatives of 6-methylthioguanine (2-amino-6-methylthiopurine) for their abilities to displace [^{3}H]diazepam binding to rat brain membranes.

Several investigators have demonstrated fundamental differences in the modulation of benzodiazepine binding sites when a β-carboline such as [^{3}H]β-carboline-3-carboxylic acid ethyl ester ([^{3}H]β-CCE) binds to these sites compared to the characteristics of [^{3}H]benzodiazepine as a radioligand. A lack of enhancement, i.e. no increase in apparent affinity, of [^{3}H]β-CCE binding by γ-aminobutyric acid (GABA) was one of the differences [6]. Ehlert *et al.* [7] also observed that GABA enhanced the binding of [^{3}H]flunitrazepam to the various receptor types whereas no significant effect of GABA on the binding of [^{3}H]propyl-β-carboline-3-carboxylate was detected.

An *in vitro* test which discriminates between benzodiazepine "agonists" and "antagonists" has been developed recently by exploiting the apparent differences in modulation of benzodiazepine receptor by these classes of compounds. Using this test Skolnick *et al.* [8] recently reported that, in the presence of 10 μM GABA, the potency of benzodiazepine agonists in displacing [^{3}H]β-CCE binding was increased significantly. In contrast, the potency of benzodiazepine antagonists was not altered by GABA.

In this study, we showed that a number of alkyldithiopurines were capable of inhibiting benzodiazepine binding and that the abilities of these compounds to inhibit [^{3}H]β-CCE binding were not altered by GABA.

MATERIALS AND METHODS

Male Wistar rats (200–250 g) were obtained from the Animal Unit, University of British Columbia.

The animals were decapitated, and well-washed membrane fragments were prepared from cerebral cortex. Tissues were routinely homogenized in 20 vol. of ice-cold 0.32 M sucrose with a Teflon–glass homogenizer and then rehomogenized in a Polytron (setting 6, 30 sec), followed by centrifugation at 16,000 rpm (30,900 *g*) for 30 min in a Sorvall RC-5B refrigerated centrifuge at 0–4°. The precipitate obtained was suspended in an equal volume of 0.05 M Tris–HCl buffer, pH 7.4, and recentrifuged. For routine assay of [^{3}H]diazepam binding, twice washed membrane preparations were used but, for the experiments described in Table 2, five times washed membranes were used. Washed membranes were suspended in 0.05 M Tris–HCl buffer, pH 7.4, before use for binding assay.

Specific binding of [^{3}H]diazepam to rat cerebral cortical membranes was determined as described previously [5].

Methyl-[^{3}H]diazepam (sp. act. 87.6 Ci/nmole) was purchased from New England Nuclear and β-[6-^{3}H]carboline-3-carboxylic acid ethyl ester was from Amersham. Unlabeled diazepam was a gift from Hoffman–La Roche, Vandreuil, Quebec. Various 2-amino-6-alkyldithiopurines used in the present study were synthesized according to the procedures reported previously [9, 10].

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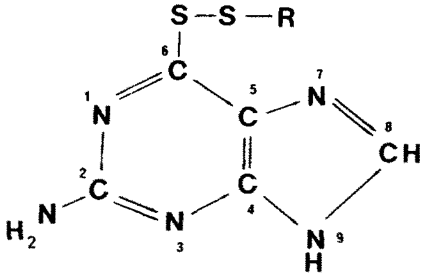
* Send correspondence to: Dr. S. C. Sung, Division of Neurological Sciences, University of British Columbia, 2255 Westbrook Mall, Vancouver, B.C., Canada V6T 1W5.



R: alkyl group

Fig. 1. Structure of 2-amino-6-alkyldithiopurine.

Table 1. Inhibition of [^{3}H]diazepam binding to rat cerebral cortical membranes by 2-amino-6-alkyldithiopurine*

2-Amino-6-alkyldithiopurine derivatives	IC$_{50}$ (μM)	K_i (μM)
Alkyl group:		
Benzyl	20 ± 3	7.3
n-Butyl	3 ± 0.4	1.1
n-Pentyl	2.5 ± 0.3	0.92
n-Hexyl	3.5 ± 0.4	1.3
n-Heptyl	4 ± 0.3	1.5
n-Octyl	8 ± 1	2.9
n-Decyl	†	
n-Tetradecyl	‡	

* IC$_{50}$ Values were assessed graphically at four to five different concentrations of the purine derivatives in triplicate and expressed as means ± S.D. K_i values were calculated according to the formula: K_i = IC$_{50}$/(1 + S/K_D). The mean IC$_{50}$ value of 2-amino-6-methylpurine was 35 ± 3.7 μM [4].

† Sixteen percent inhibition at 30 μM.

‡ Fifteen percent inhibition at 37.5 μM.

RESULTS

Inhibition of [^{3}H]*diazepam binding by various 2-amino-6-alkyldithiopurines.* The abilities of purine derivatives to replace specifically bound [^{3}H]diazepam were tested by incubating them in the standard binding mixture; the results are shown in Table 1. The potency of 2-amino-6-benzyldithiopurine (K_i value 7.3 μM) was somewhat higher than that of 2-amino-6-methylthiopurine (K_i value 16 μM). When the benzyl group was replaced by an alkyl group, the potency increased significantly. The potency to displace [^{3}H]diazepam binding was dependent on the carbon chain-length in the 6-position: the *n*-pentyl derivative seemed to show the highest potency with a K_i value of 0.92 μM. However, the potency decreased greatly with *n*-decyl and *n*-tetradecyl derivatives.

Effect of GABA on the inhibition of [^{3}H]*β-CCE binding by a benzodiazepine agonist (diazepam) and by various purine derivatives.* In the presence of 10 μM GABA, the potency of diazepam, a benzodiazepine agonist, in inhibiting the binding of [^{3}H]β-CCE was markedly increased (Table 2). Under these conditions, GABA did not markedly alter [^{3}H]β-CCE binding. In contrast to diazepam, the inhibition by 2-amino-6-methylthiopurine, 2-amino-6-butyldithiopurine and 2-amino-6-pentyldithiopurine of the binding of [^{3}H]β-CCE was not markedly altered by 10 μM GABA.

DISCUSSION

Previously we have found that, among various purine derivatives, 2-amino-6-methylthiopurine was the most potent in displacing the specific binding of [^{3}H]diazepam [5]. In the present study, we have shown that some of the more hydrophobic derivatives of 2-amino-6-methylpurine had higher potencies than 2-amino-6-methylthiopurine. The derivative with five carbons in the alkyl chain seemed to have the highest potency, with a K_i value of 0.92 μM (Table 1). In addition to hydrophobicity, there seemed to be a steric effect on the activity of alkyl derivatives as inhibitors of [^{3}H]diazepam binding, since alkyl groups with ten and more carbons had much lower activities. As reported with 6-methylthioguanine [5], these 6-alkyldithiopurines inhibited competitively the specific binding of [^{3}H]diazepam.

The inhibition of [^{3}H]diazepam binding to rat brain membranes by these 2-amino-6-alkyldithiopurine derivatives was not due to the thio- or dithio-moiety in the molecule, since 2-amino-6-thiocyanatopurine, 6-benzyldithiopurine and 6-*p*-chlorobenzyldithiopurine, at concentrations of 50 μM, caused only 28.9, 28.9 and 31.5% inhibition, respectively, under the same assay conditions. Furthermore, the 2-amino group in the purine moiety seemed essential for the inhibition by these 2-amino-6-alkyldithiopurines, since compounds without the 2-amino group were much less potent than the parent compounds.

Qualitative differences in the modulation of benzodiazepine receptors have been reported when these sites are occupied by benzodiazepine agonists or antagonists [6–8]. It appears that affinities of

Table 2. Effect of GABA on the potencies of inhibitors of [^{3}H]β-CCE binding by rat cerebral cortical membranes*

	% Inhibition of binding activity	
	− GABA	+ 10 μM GABA
30 nM Diazepam	51.0 ± 3.4	67.7 ± 2.7
50 μM 2-Amino-6-methylthiopurine	50.0 ± 2.5	48.8 ± 3.0
5 μM 2-Amino-6-*n*-butyldithiopurine	50.2 ± 3.0	49.4 ± 3.2
5 μM 2-Amino-6-*n*-pentyldithiopurine	61.8 ± 3.0	62.5 ± 2.4

* Values are means ± S.D. with four separate analyses.

agonists are increased in the presence of GABA, while the affinities of antagonists are not altered significantly. In contrast to the ability of diazepam to displace [^{3}H]β-CCE binding being increased by GABA, none of the potencies of 2-amino-6-methylthiopurine, 2-amino-6-butyldithiopurine and 2-amino-6-pentyldithiopurine were increased significantly by GABA (Table 2). Therefore, these compounds would be "antagonists" by this type of GABA test.

Much higher concentrations of purine derivatives were required for the inhibition of [^{3}H]β-CCE binding. Inosine, for example, at a concentration of 2 mM caused 42.3% inhibition of [^{3}H]β-CCE binding. The inhibition by inosine was somewhat potentiated in the presence of GABA, to 51.7%.

It is difficult to extrapolate the effects of these 6-alkyldithiopurines *in vitro* on benzodiazepine receptor binding to the *in vivo* situation. Therefore, it would be useful to have information concerning some of the behavioral properties of these compounds. Future behavioral studies should show whether these alkyldithioguanine derivatives are antagonists or act like benzodiazepine. In addition, further modification of the purine molecule may lead to potentially useful compounds as drugs.

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