0026-895X/91/010049-06$03.00/0

MOLECULAR PHARMACOLOGY, 39:49-54

Characterization of the Binding of a Novel Nonxanthine Adenosine Antagonist Radioligand, [^{3}H]CGS 15943, to Multiple Affinity States of the Adenosine A_1 Receptor in the Rat Cortex

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Received May 23, 1990; Accepted October 18, 1990

SUMMARY

The triazoloquinazoline CGS 15943 is the first reported nonxanthine adenosine antagonist that has high affinity for brain adenosine receptors. In the present study, the binding of [^{3}H]CGS 15943 to recognition sites in rat cortical membranes was characterized. Saturation experiments revealed that [^{3}H]CGS 15943 labeled a single class of recognition sites with high affinity (K_d = 4 nM) and limited capacity (B_{max} = 1.5 pmol/mg of protein). Competition studies revealed that the binding of [^{3}H]CGS 15943 was consistent with the labeling of brain adenosine A_1 receptors. Adenosine agonists inhibited 1 nM [^{3}H]CGS 15943 binding with the following order of activity N^6-cyclopentyladenosine (IC_{50} = 15 nM) > 2-chloroadenosine > (*R*)-N^6-phenylisopropyladenosine > 5′-N^6-ethylcarboxamidoadenosine > (*S*)N^6-phenylisopropyladenosine > CGS 21680 > CV 1808 (IC_{50} > 10,000 nM). The potency order for adenosine antagonists was CGS 15943 (IC_{50} = 5 nM) > 8-phenyltheophylline > 1,3-dipropyl-8-(4-amino-2-chloro)phenylxanthine > 1,3-diethyl-8-phenylxanthine > theophylline = caffeine (IC_{50} > 10,000 nM). Antagonist inhibition curves were steep and best described by a one-site binding model. In contrast, adenosine A_1 agonist competition curves were shallow, as indicated by Hill coefficients less than unity. Computer analysis revealed that these inhibition curves were best described by a two-site binding model. Agonist competition curves generated in the presence of 1 mM GTP resulted in a rightward shift and steepening of the inhibition-concentration curves, whereas antagonist binding was not altered in the presence of GTP. The complex binding interactions found with adenosine agonists indicate that [^{3}H]CGS 15943 labels both high and low affinity components of the adenosine A_1 receptor in the rat cortex. Additionally, the present data also provide some evidence that [^{3}H]CGS 15943 may also recognize an additional low affinity binding component, which may represent a putative low affinity A_{2b} receptor in this tissue.

There is considerable evidence to indicate that adenosine specifically modulates neurotransmission via interactions with two cell surface receptor subtypes, termed A_1 and A_2 (1, 2). Specific interactions at these different receptors can be determined on the basis of their agonist pharmacological profiles with N^6-substituted adenosine analogs, such as CHA, showing selectivity and high affinity for the A_1 receptor. NECA, a 5′-substituted adenosine analog (3), and CGS 21680, a 5′,2-substituted adenine nucleoside (4–6), in contrast, have high affinity for the A_2 receptor. Based on the differential pharmacology of NECA and CGS 21680, there is growing evidence for a further subclassification of the A_2 receptor into high (A_{2a}) and low (A_{2b}) affinity subtypes (3–5). Alkylxanthines such as caffeine and theophylline are prototypic adenosine receptor antagonists; however, these compounds have relatively low potency and nonselective antagonist actions at both adenosine receptor subtypes, in addition to being phosphodiesterase inhibitors (7).

The use of more potent xanthine adenosine receptor antagonists, such as DPX, as radioligands has been of limited utility due to their high lipophilicity, as well as their low specific activity, selectivity, and affinity for either adenosine receptor subtype (8). More recently, however, several adenosine A_1-selective xanthine derivatives have been developed that offer significant technical advantages over DPX. [^{3}H]xanthine car-

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ABBREVIATIONS: CHA, N^6-cyclohexyladenosine; CPA, N^6-cyclopentyladenosine; 2-CADO, 2-chloroadenosine; CGS 8216, 2-phenylpyrazolo[4,3-*c*]quinolin-3-(5*H*)-one; CGS 9896, 2-(*p*-chlorophenyl)-pyrazolo[4,3-*c*]-quinolin-3-(5*H*)-one; CGS 15943, 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-amine; CGS 20625, 2-(4-methoxyphenyl)-2,3,6,7,8,9,10-octahydrocyclohepta[*b*]pyrazolo-[3,4-*d*]pyridin-3-one; CGS 21680, 2-[*p*-(2-carboxyethyl)-phenethylamino]-5′-*N*-ethylcarboxamido adenosine; CV 1808, 2-phenylaminoadenosine; DPX, 1,3-diethyl-8-phenylxanthine; NECA, 5′-N^6-ethylcarboxamidoadenosine; PACPX, 1,3-dipropyl-8-(4-amino-2-chloro)phenylxanthine; 8-PT, 8-phenyltheophylline; 8-PST, 8-(*p*-sulfophenyl) theophylline; PIA, N^6-phenylisopropyladenosine; Gpp(NH)p, guanyl-5′-yl-imidophosphate; G protein; guanine nucleotide-binding protein; CPX, 8-cyclopentyl-1,3-dipropylxanthine.

boxylic acid congener and [^{3}H]xanthine amine congener represent functionalized congeners of DPX that can selectively label A_1 receptors in brain with nanomolar affinity (9–11). [^{3}H]CPX (PD116,948) also has been shown to selectively label A_1 receptors with high affinity (12, 13). Interestingly, agonist competition curves obtained with some of these radiolabeled antagonists have revealed complex binding, indicative of high and low affinity states of the A_1 receptor (12, 13). There is now further evidence indicating that multiple affinity states of adenosine receptors exist, which are regulated by membrane-bound G proteins (3, 14–19).

CGS 15943 (Fig. 1) was one of the first potent nonxanthine adenosine receptor antagonists (20, 21), being a derivative of the pyrazoloquinazoline benzodiazepine receptor inverse agonist, CGS 8216 (22). Although CGS 15943 was originally described as being 7-fold selective for the A_2 receptor, this adenosine antagonist has low nanomolar affinity for both A_1 and A_2 adenosine receptor subtypes (20). In contrast to other xanthine adenosine antagonists, CGS 15943 is not a phosphodiesterase inhibitor and does not interact with adenosine transporter sites (20). This compound is a potent and specific adenosine receptor antagonist *in vivo* (23), with a solubility/affinity ratio greater than 1000 (24). Given the fact that GCS 15943 has been shown to be a competitive antagonist at the brain A_1 receptor and to have noncompetitive antagonist actions at the high affinity striatal A_2 receptor (20), the purpose of the present study was to investigate the binding of [^{3}H]CGS 15943 to rat cortical membranes, which contain a high concentration of adenosine A_1 receptors and essentially no high affinity A_2 receptors (5, 25, 26).

Materials and Methods

Radioligand binding. Binding of [^{3}H]CGS 15943 to rat brain cortical membranes was measured by a modification of the method previously described for [^{3}H]CHA (27). Rat cortical tissue was homogenized in 20 volumes (w/v) of 50 mM Tris·HCl buffer (pH 7.4 at 25°), using a Brinkman Polytron (setting 6, 30 sec). The homogenate was centrifuged at 48,000 × *g* at 4° for 10 min (Beckman J-2-2IM centrifuge, JA 20.1 rotor). The resultant pellets were resuspended to 20 mg/ml tissue in buffer to which adenosine deaminase (2 IU/ml) was added to remove endogenous adenosine. The homogenate was incubated at 37° for 30 min and then recentrifuged. The final pellets were frozen and stored at −70°.

All binding assays were run in triplicate in a final volume of 1 ml, using 12 × 75-mm polypropylene tubes. For the saturation studies, 8–10 concentrations (0.01–30 nM) of [^{3}H]CGS 15943 were included in the assay. Nonspecific binding was determined in the presence of 200 μM 2-CADO. For the competition studies, 10–15 concentrations (0.001 nM to 100 μM) of inhibitor, guanine nucleotide, or buffer were included in the assay with 1 nM [^{3}H]CGS 15943. Incubations were initiated by the addition of tissue at a final protein concentration of 100–200 μg/ml, and binding reactions were continued for 2 hr at 23°. Bound radioactivity was isolated by filtration under reduced pressure over Whatman GF/B filter strips, using a Brandel cell harvester (M-24R; Brandel, Gaithersburg, MD), and unbound radioactivity was removed with three 5-ml washes of ice-cold buffer. Bound radioactivity was subsequently determined by conventional liquid scintillation counting, at an efficiency of approximately 45%. Protein concentrations were determined by the method of Bradford (28), using bovine serum albumin as the reference standard.

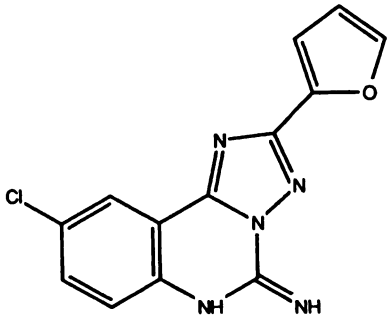

Fig. 1. Structure of CGS 15943.

All binding data were analyzed using the nonlinear curve-fitting program RS/1 (Bolt, Beranak, and Newman, Boston, MA), with equations that describe the competitive interactions of a drug with two noninterconvertible recognition sites (29). Accordingly, a partial *F* test ($p < 0.01$) was used to determine reliable estimates of whether a one-component or two-component binding model offered the best description of the binding data (5).

Materials. CGS 8216, CGS 9896, CGS 15943, CGS 20625, and CGS 21680 were synthesized in the Chemistry Department of CIBA-GEIGY Corp. (Summit, NJ), as described previously (6, 21, 30). [^{3}H]CGS 15943 (21 Ci/nmol) was obtained, by reduction with tritium gas (Dupont-NEN, Boston, MA), from the precursor 9-chloro-2-(5-bromo-2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine. Other adenosine reference standards were obtained from Research Biochemicals, Inc. (Natick, MA). All other assay reagents were obtained from Fisher Scientific, Inc. (Springfield, NJ).

Results

Preliminary experiments revealed that, after 2 hr at 23°, [^{3}H]CGS 15943 (1 nM) binding reached equilibrium, with specific binding amounting to approximately 70% of total binding (data not shown). Ligand saturation experiments (five experiments) revealed that [^{3}H]CGS 15943 bound to a single class of recognition sites in rat cortical membranes, with high affinity (K_d = 4 ± 2 nM) and limited capacity (apparent B_{max} = 1.5 ± 0.5 pmol/mg of protein). A representative saturation isotherm and Scatchard plot are shown in Fig. 2.

Given the close similarity in the chemical structures of CGS 15943 and the benzodiazepine inverse agonist CGS 8216 and the weak adenosine antagonist activity of the latter entity (31), several compounds in this chemical series known to interact with the central benzodiazepine receptor were evaluated, together with diazepam, for their ability to inhibit the binding of [^{3}H]CGS 15943 (Table 1). Of these compounds, only CGS 8216 was found to have marginal activity, with an IC_{50} value of 9.2 μM. The micromolar affinity of CGS 8216 in inhibiting the binding of [^{3}H]CGS 15943 is in reasonable agreement with its activity in competing for A_1 agonist binding (IC_{50} value of 3.6–5.5 μM) (31, 32).

In competition studies, CGS 15943 showed the greatest activity (IC_{50} = 5 nM) in inhibiting the binding of [^{3}H]CGS 15943 (Table 2). The rank order of activity for a series of adenosine receptor antagonists was CGS 15943 > PACPX > 8-PT > DPX > 8-PST. Caffeine and theophylline were found to be relatively inactive in inhibiting the binding of [^{3}H]CGS 15943 (approximately 25% inhibition at 10 μM). Computer analysis revealed that all antagonist inhibition curves were best described by a one-component binding model (Table 2 and Fig. 3).

Adenosine agonists were found to inhibit binding of [^{3}H]CGS 15943 in a manner consistent with the labeling of the adenosine A_1 receptor (Table 2). The rank order of these agonists was CPA > (*R*)-PIA > NECA = 2-CADO > (*S*)-PIA > CGS 21680 > CV 1808. [^{3}H]CGS 15943 binding was stereoselective, with (*R*)-PIA (IC_{50} = 125 nM) being approximately 8

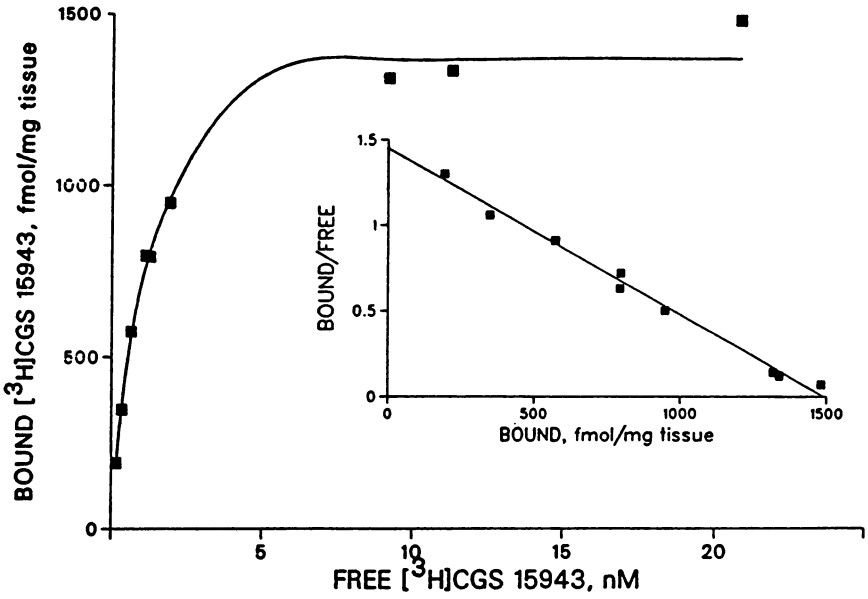

Fig. 2. Representative saturation isotherm and Scatchard plot (*inset*) of specific [³H]CGS 15943 binding to rat cortical membranes.

TABLE 1

Activity of several anxiomodulators in inhibiting the binding of CGS 15943

Values represent means determined in triplicate observations from three separate experiments.

Compound	IC_{50}
	nM
CGS 8216	9,250 ± 880
CGS 9896	>10,000
CGS 20625	>10,000
Diazepam	>10,000

TABLE 2

Inhibition of [³H]CGS 15943 binding to rat cortical membranes

Values represent mean ± 1 SE, obtained from at least three separate experiments.

Compound	IC_{50}	n_H
	nM	
Antagonists		
CGS 15943	5 ± 0.05	1.01 ± 0.02
PACPX	20 ± 3	1.00 ± 0.05
8-PT	128 ± 10	0.86 ± 0.05
DPX	393 ± 37	0.75 ± 0.04
8-PST	4,240 ± 210	0.76 ± 0.05
Caffeine	>10,000	
Theophylline	>10,000	
Agonists		
CPA	15 ± 2	0.36 ± 0.02*
(*R*)-PIA	125 ± 15	0.42 ± 0.02*
2-CADO	338 ± 30	0.56 ± 0.02*
NECA	224 ± 12	0.63 ± 0.01*
(*S*)-PIA	981 ± 50	0.51 ± 0.03*
CGS 21680	7,840 ± 650	0.81 ± 0.05
CV1808	>10,000	

* Hill coefficient was found to be significantly ($p < 0.01$) different from unity.

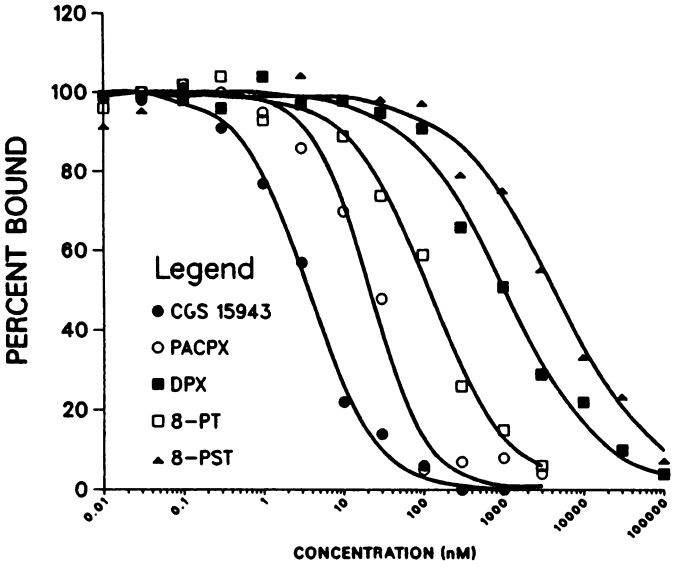

Fig. 3. Representative antagonist competition curves for the inhibition of 1 nM [³H]CGS 15943 binding. Computer analysis revealed that a one-component binding model best described all antagonist inhibition curves.

times more active than its stereoisomer, (*S*)-PIA (IC_{50} = 980 nM). Interestingly, agonist compounds with high affinity for the A_1 receptor produced shallow inhibition curves, as indicated by Hill coefficients less than unity (Table 2 and Fig. 4). Computer analysis of the binding data revealed that a two-component model described the data significantly better than a one-component model. The binding constants generated by this analysis are shown in Table 3. The A_2-selective agonists CV 1808 and CGS 21680 showed relatively weak activity in inhibiting the binding of [³H]CGS 15943, consistent with its selective labeling of the A_1 receptor, and these inhibition curves were best described by a one-component binding model.

To assess adenosine A_1 receptor-G protein interactions, additional inhibition curves were generated for the A_1 agonists in the presence of GTP. Inclusion of either GTP or the nonhydrolyzable analog Gpp(NH)p did not significantly affect [³H]CGS 15943 binding to rat cortical membranes at concentrations from 0.01 to 1 mM. Consistent with this observation, the ability of PACPX to inhibit the binding of [³H]CGS 15943 was not affected by the addition of 1 mM GTP (IC_{50} = 25 ± 2 nM). In contrast, adenosine A_1 agonists were 2–3-fold less active in the presence of 1 mM GTP, although the same order of potency was observed as that seen in the absence of GTP. GTP also increased the Hill coefficients from a mean of 0.49 ± 0.04 to 0.85 ± 0.03. Computer analysis of the binding curves revealed that, in the presence of GTP, a one-component binding model adequately described the agonist binding curves. Fig. 5 shows

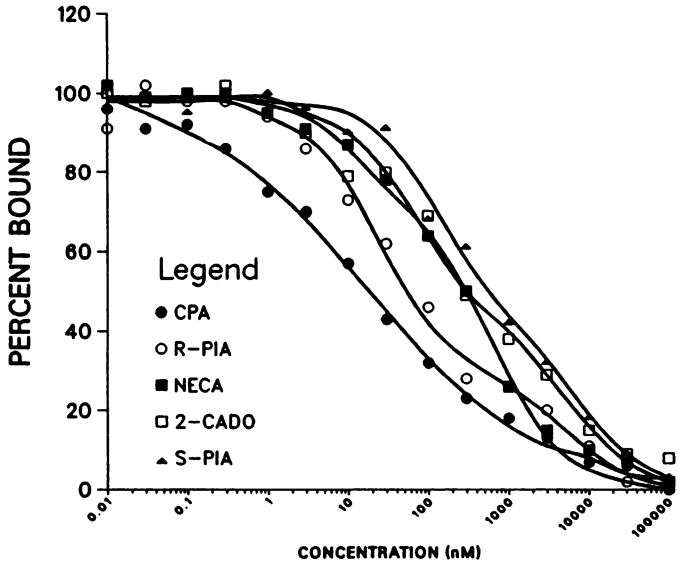

Fig. 4. Representative agonist competition curves for the inhibition of 1 nM [^{3}H]CGS 15943 binding. Inhibition of [^{3}H]CGS 15943 binding by A_1 agonists produced shallow competition curves (i.e., Hill coefficients less than unity), which were best described by a two-site binding model.

TABLE 3

Two-site analysis of inhibition curves for potent A_1 agonist in [^{3}H]CGS 15943 binding

Values represent mean binding parameters obtained from computer analysis of the high (B_H) and low (B_L) affinity components of each inhibition curve.

Compound	B_H	IC_{50_H}	B_L	IC_{50_L}
	%	nM	%	nM
CPA	61 ± 2	6 ± 1	36 ± 5	5000 ± 1000
(*R*)-PIA	58 ± 2	20 ± 2	39 ± 2	6735 ± 900
2-CADO	44 ± 4	41 ± 9	54 ± 4	2050 ± 300
NECA	48 ± 3	40 ± 5	51 ± 3	1200 ± 140
(*S*)-PIA	46 ± 3	91 ± 15	53 ± 3	8200 ± 1200

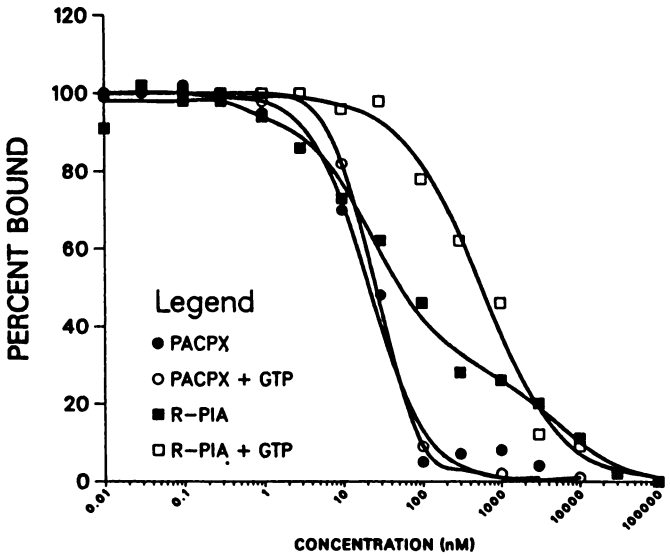

Fig. 5. Representative antagonist and agonist inhibition of [^{3}H]CGS 15943 binding in the absence and presence of 1 mM GTP. The ability of PACPX to compete for the binding of [^{3}H]CGS 15943 was unaffected by the inclusion of GTP in the assay. However, in the presence of GTP, the inhibition curve produced by (*R*)-PIA was shifted approximately 20-fold to the right and steepened such that a one-component binding model best described the competition curve.

representative inhibition curves for PACPX and (*R*)-PIA in the absence and presence of 1 mM GTP.

Discussion

The present results indicate that the triazoloquinazoline [^{3}H] CGS 15943 specifically binds to rat cortical tissue membranes with nanomolar affinity and limited capacity. The ability of adenosine agonists and antagonists to inhibit the binding of [^{3}H]CGS 15943 revealed a pharmacological profile indicative of labeling of adenosine A_1 receptors in this tissue. The rank order of potency of adenosine agonists to compete for the binding of [^{3}H]CGS 15943 was consistent with similar data obtained with A_1-selective agonist radioligands (i.e., inhibition of [^{3}H]CHA and [^{3}H]CPA binding in mammalian cortical tissue) (27, 32).

Although GCS 15943 is structurally similar to the benzodiazepine inverse agonist CGS 8216, CGS 15943 has been shown to be a pharmacologically specific adenosine receptor antagonist, with an approximately 500-fold greater activity at the adenosine receptor as compared with the benzodiazepine receptor (20). Additionally, several structural analogs of CGS 15943 that are known to have high affinity for the central benzodiazepine receptor were inactive in inhibiting the binding of [^{3}H] CGS 15943. CGS 8216 did show weak affinity in competing for the binding of [^{3}H]CGS 15943; however, this degree of activity is consistent with its ability to also inhibit the binding of highly A_1-selective agonists (20).

The apparent B_{max} for [^{3}H]CGS 15943 binding to rat cortical membranes was found to be approximately 2–3-fold greater than typically obtained with potent A_1-selective agonists (27, 32); however, it is in the range reported for some other xanthine adenosine antagonists (12, 33). This finding is consistent with the ability of an antagonist radioligand to label both high and low affinity states of a G protein-linked receptor and may be the result of a ternary complex formation, wherein the G protein is a determining factor in the nature of the receptor-ligand interaction (19, 34). Thus, the apparent increased B_{max} obtained with [^{3}H]CGS 15943 is probably due to the fact that an adenosine antagonist can recognize both high and low affinity states of the receptor, whereas adenosine agonists may preferentially recognize only the high affinity state of the A_1 receptor.

The finding that shallow inhibition curves were produced by potent A_1 agonists but steep inhibition curves were produced by antagonist compounds in competing for the binding of a radiolabeled antagonist is also suggestive of multiple affinity states of the A_1 receptor (34). The IC_{50} values obtained for the high affinity components of the agonist inhibition curves appear to reflect an interaction at a high affinity state of the A_1 receptor, because they are in good agreement with the activity of these agonists in inhibiting the binding of several high affinity and A_1-selective agonist radioligands (27, 32). In the presence of GTP, the potency of A_1 agonists to inhibit the binding of [^{3}H]CGS 15943 was decreased 2–3-fold and the Hill coefficients for these inhibition curves were increased, whereas the binding of the antagonist PACPX remained unaffected. These results are consistent with the ability of GTP to selectively decrease the binding affinity of agonists for adenosine A_1 receptors (8, 14, 35).

The complex nature of the inhibition by adenosine agonists of the binding of [^{3}H]CGS 15943 to rat cortical membranes agrees with other data derived for several novel xanthine aden-

osine antagonists. Potent A_1 agonists have been shown to produce complex inhibition curves in competing for the binding of [^{3}H]CPX (12, 13, 36) and ^{125}I-BW-A844U (37) to rat brain membranes and of [^{3}H]xanthine amine congener to rat adipocytes (33). Additionally, the activity of A_1 agonists to inhibit antagonist binding can be reduced in the presence of guanine nucleotides (33, 35). Together, these findings indicate that A_1-selective receptor antagonist radioligands can be used to label multiple affinity states of the A_1 receptor.

As an adenosine receptor antagonist, CGS 15943 has been shown to have an approximately 7-fold greater affinity for inhibiting the binding of [^{3}H]NECA to the adenosine A_2 receptor, as compared with its affinity for inhibiting the binding of [^{3}H]CHA to the A_1 receptor (20). Contrary to these observations, in the present study, using rat cortical membranes, it does not appear that [^{3}H]CGS 15943 had any significant activity at the high affinity adenosine A_2 receptor (A_{2a}) in this tissue, because the A_2-selective compounds CV 1808 and CGS 21680 were both inactive. These data are consistent with previous observations, from receptor autoradiography studies, that the rat cerebral cortex contains few, if any, high affinity A_2 receptors (25, 26).

Although the present data clearly demonstrate that [^{3}H]CGS 15943 can recognize multiple affinity states of the A_1 receptor, several additional questions remain concerning the nature of the low affinity component of [^{3}H]CGS 15943 binding to rat cortical membranes. GTP did not produce a complete shift in the agonist competition curves, a result similar to that obtained with other xanthine adenosine antagonists (8, 12, 35). If only high and low affinity states of the A_1 receptor were present, it might be expected that the IC_{50} values in the presence of GTP (Table 4) would correspond to the IC_{50_L} values for the low affinity components in the absence of GTP (Table 3). Clearly, this is not the case.

These observations raise the question of whether CGS 15943 is only labeling the high and low affinity states of the A_1 receptor. One possibility, as suggested previously by Leung *et al.* (38), is that [^{3}H]CGS 15943, or any adenosine receptor antagonist, may be able to differentiate between an A_1 receptor coupled to a G protein and an uncoupled state of the A_1 receptor, which might account for the inability of GTP to completely shift the inhibition curves. It should be noted that several recent studies have shown more pronounced GTP effects using a variety of xanthine radioligands with different assay conditions, solubilized receptor preparations, and/or different species (13, 39, 40). Additionally, the apparent incomplete conversion of high to low affinity binding may reflect species-dependent intrinsic differences in the degree of coupling of the receptor/G protein complex (41).

Another possibility is that, in addition to labeling multiple affinity states of the A_1 receptor, [^{3}H]CGS 15943 may also label a low affinity A_{2b} receptor in rat cortex. Evidence for this comes from the observation that there is a good correspondence between the IC_{50_L}/IC_{50_H} ratios for the adenosine agonists in Table 3 and their respective A_2/A_1 selectivity ratios derived from recent data from our laboratory (5) (see Table 5). One important difference in these ratios, however, is that the A_2 values were generated using [^{3}H]CGS 21680, an apparent A_{2a}-selective agonist radioligand (5, 38). This would suggest that the IC_{50_L} values contained in Table 3 do not represent activity at the A_{2a} receptor. Nevertheless, a rank ordering of the IC_{50_L} values reveals an A_2-like pharmacological profile, and CGS 15943 has previously been shown to completely block an A_2 receptor-mediated increase in adenylate cyclase activity in brain (20).

The possibility that [^{3}H]CGS 15943 may recognize a low affinity A_2 receptor in the rat cortex could account for the similar selectivity ratios in Table 5 and the A_2-like activity profile of the low affinity agonist binding components in Table 3. The relatively weak activity of CGS 21680 in inhibiting the binding of [^{3}H]CGS 15943 is also consistent with this hypothesis. In this situation, the IC_{50_H} and IC_{50_L} values shown in Table 3 would represent binding to the high affinity state of the A_1 receptor and the IC_{50} value for the A_{2b} receptor, respectively. The IC_{50} values generated in the presence of GTP (Table 4) would then represent binding to the low affinity state of the A_1 receptor.

In this and other studies (8, 12, 35), it remains unclear why a complete GTP-induced shift of the IC_{50_H} values to the IC_{50_L} values was not observed. In reality, GTP may actually be doing this, where the IC_{50} value for the low affinity state of the A_1 receptor is "a hidden third component" of the agonist inhibition curves generated in the absence of GTP. At present, however, only two binding components could be reliably discriminated by computer modeling techniques in the absence of GTP. This could be attributable to a number of factors, including the relatively small 10–20-fold difference in the IC_{50} values for the high affinity state (Table 3) and the low affinity state (Table 4) of the A_1 receptor in rat cortex. According to this model, however, two binding components might be expected to be present when the competition curves were generated in the presence of GTP. Whether GTP is eliminating binding to the A_{2b} receptor remains unknown.

In conclusion, the present data provide the first evidence that a potent non-xanthine adenosine receptor antagonist radioligand, [^{3}H]CGS 15943, can be used to characterize agonist interactions at multiple affinity states of the A_1 receptor in rat cortical membranes. Additionally, there is some evidence that [^{3}H]CGS 15943 may be labeling an additional low affinity

TABLE 4

Activity of potent A_1 agonists in inhibiting the binding of [^{3}H]CGS 15943 in the presence of 1 mM GTP

Values represent means ± 1 SE, determined from at least three separate experiments. Computer analysis of the binding data revealed that a two-component binding model no longer offered a significant improvement over a one-component binding model in describing the competition curves.

Compound	IC_{50}	N_H
	nM	
CPA	66 ± 2	0.84 ± 0.02
(*R*)-PIA	391 ± 52	0.79 ± 0.07
NECA	332 ± 22	0.86 ± 0.09
2-CADO	914 ± 72	0.91 ± 0.05
(*S*)-PIA	1915 ± 160	0.89 ± 0.06

TABLE 5

Comparison of the ratios of IC_{50_L}/IC_{50_H} and A_2/A_1 activity for several prototypic adenosine agonists

Values for the IC_{50} ratios were taken from Table 3, and the values for A_2/A_1 were derived from Jarvis *et al.* (5). A positive correlation ($r = 0.98$, $p < 0.01$) was observed between the ratios of the agonist high and low affinity components for the inhibition of [^{3}H]CGS 15943 binding to rat cortical membranes and their respective A_1 receptor selectivity ratios.

Compound	IC_{50_L}/IC_{50_H}	A_2/A_1
CPA	833	890
(*R*)-PIA	337	205
(*S*)-PIA	90	104
2-CADO	50	86
NECA	30	2

binding component, which may represent the low affinity A_{2b} receptor in this tissue. Further work is required to determine whether [^{3}H]CGS 15943 (or any other adenosine receptor antagonist) can be used to specifically label this low affinity A_{2b} receptor in brain. Interestingly, the chemically related [1,2,4] triazolo[4,3-a]quinoxalin-4-amines also have been recently identified as potent adenosine receptor antagonists (42). Based on these potent antagonist compounds, there appears to be some degree of similarity between the structure activity requirements for potent adenosine A_1 receptor antagonists and compounds that have therapeutic potential as novel anxiolytics and/or antidepressants (20, 42, 43). This observation is further supported by the many reports regarding the similarities in the behavioral pharmacology of purines, benzodiazepines, and antidepressants (20, 44). The development of a potent and selective A_2 receptor antagonist is needed for further elucidation of the physiological significance of the A_2 receptor subtypes.

Acknowledgment

We thank Dr. Saul Maayani for his helpful comments on an earlier version of this manuscript.

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