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Binding of [^{3}H]KF17837S, a Selective Adenosine A_2 Receptor Antagonist, to Rat Brain Membranes

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SUMMARY

The potential of 8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-[^{3}H]methylxanthine ([^{3}H]KF17837S) as a highly selective antagonist radioligand for the adenosine A_{2A} receptor was examined and compared with the properties of the adenosine A_{2A} receptor agonist radioligand 2-[*p*-(2-[^{3}H]carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine ([^{3}H]CGS21680). [^{3}H]KF17837S specific binding to rat striatal membranes was saturable and reversible. Saturation studies showed that the binding of [^{3}H]KF17837S occurred at a single site, with high affinity (K_d, 7.1 ± 0.91 nM) and limited capacity (B_{max}, 1.3 ± 0.23 pmol/mg of protein). Adenosine receptor antagonist ligands competed with the binding of 1 nM [^{3}H]KF17837S with the following order of activity: CGS15943 > KF17837S > *N*-[2-(dimethylamino)ethyl]-*N*-methyl-4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)benzenesulfonamide ≥ xanthine amine congener > 8-cyclopentyl-1,3-dipropylxanthine > 8-(noradamantan-3-yl)-1,3-dipropylxanthine > caffeine. Adenosine receptor agonists inhibited [^{3}H]KF17837S binding in the following order: 5′-*N*-ethylcarboxamidoadenosine ≥ CGS21680 > 2-phenylaminoadenosine ≥ (*R*)-*N*^{6}-phenylisopropyladenosine > *N*^{6}-cyclopentyladenosine > (*S*)-*N*^{6}-phenylisopropyladenosine. The K_i values of the antagonists for [^{3}H]KF17837S binding and the rank order of potency were similar to those for [^{3}H]CGS21680 binding. The affinities of the agonists were lower with [^{3}H]KF17837S binding than with [^{3}H]CGS21680 binding. However, a strong positive correlation (r = 0.98) was observed between the pharmacological profiles for these two radioligand assays. The inhibition curve for CGS21680 was best fitted to a two-component binding model and addition of GTP shifted the inhibition curve to the right, suggesting that [^{3}H]KF17837S labeled two agonist coupling states. Other pharmacological agents had negligible affinities for the [^{3}H]KF17837S binding site. Autoradiographic study of [^{3}H]KF17837S binding using rat brain sections revealed that the binding site was highly enriched in the striatal region. These data indicate that [^{3}H]KF17837S labels the adenosine A_{2A} receptor in rat brain.

Adenosine, acting via specific cell surface receptors, modulates a number of physiological functions. Adenosine receptors have been classified into A_1, A_{2A}, A_{2B}, and A_3 subtypes based on molecular biology and pharmacology (1–3). All of these receptor subtypes have the structural features of receptors linked to G proteins and positively (A_{2A} and A_{2B}) or negatively (A_1 and A_3) coupled to adenylate cyclase (1–3). In the brain, the A_1 and A_2 receptor subtypes have unique tissue distributions, whereas the A_3 receptor has relatively low expression (3). A_{2A} receptors are mainly localized to the striatum, whereas A_1 and A_{2B} receptors show more ubiquitous distributions (4–6). The region-specific distribution of A_1 and A_{2A} receptors may explain the different behavioral actions elicited by selective adenosine receptor ligands. For example, whereas A_1 receptors are implicated in the modulation of hippocampal excitability and synaptic processes involved in some types of behavioral learning (7, 8), A_{2A} receptors are involved in the control of locomotor activity (9).

The study of adenosine A_1 receptors has been greatly facilitated by widely available, high affinity, radioligand agonists and antagonists that are selective for this receptor subtype, for example the agonist ligands [^{3}H]cyclohexyladenosine (10) and (*R*)-[^{3}H]PIA (11) and the antagonist ligand [^{3}H]DPCPX (12).

Brain A_{2A} receptors have been characterized using the nonselective agonist [^{3}H]NECA (13) and the nonselective antagonist [^{3}H]PD115,199 (14), in combination with an appropriate concentration of either the A_1-selective agonist CPA or the A_1-selective antagonist DPCPX, to block A_1 sites. These binding assays showed similar pharmacological profiles and regional distributions and were consistent with the specific labeling of the high affinity brain A_{2A} receptor (13–15). However, [^{3}H]NECA bound to different states and/or subtypes of the adenosine receptors (13, 16), as well as to other sites in addition to

ABBREVIATIONS: PIA, *N*^{6}-phenylisopropyladenosine; CGS15943, 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-amine; PD115,199, *N*-[2-(dimethylamino)ethyl]-*N*-methyl-4-(2,3,6,7- tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)benzenesulfonamide; XAC, xanthine amine congener; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; KW-3902, 8-(noradamantan-3-yl)-1,3-dipropylxanthine; NECA, 5′-*N*-ethylcarboxamidoadenosine; CV1808, 2-phenylaminoadenosine; CPA, *N*^{6}-cyclopentyladenosine; CGS21680, 2-[*p*-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine.

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the currently recognized adenosine cell surface receptor subtypes (13, 17, 18). Recently, the availability of the A_{2A}-selective agonist [^{3}H]CGS21680 has enabled the measurement of high affinity A_{2A} sites in rat striatum without the need for selective blockade of A_1 sites (19). However, [^{3}H]CGS21680 labeled high and low affinity sites in rat striatum (20). The high affinity site most likely represented the A_{2A} site, whereas the low affinity site appeared to be composed of a combination of both A_1-like and A_{2B}-like adenosine receptors.

KF17837, (*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (Fig. 1), has been recently described as the first adenosine A_2 receptor-selective antagonist (21, 22). KF17837 in dilute solution was rapidly isomerized by exposure to visible light to form the stable equilibrium mixture KF17837S (*E*-isomer, 18%; *Z*-isomer, 82%). Both KF17837 and KF17837S were found to have high affinity and selectivity for rat striatal adenosine A_{2A} receptors labeled by [^{3}H]CGS21680, with K_i values of 1.0 nM and 7.9 nM, respectively, whereas the *Z*-isomer was virtually inactive (22). In rat pheochromocytoma PC-12 cells, which contain only A_{2A} receptors (23), KF17837S antagonized cAMP accumulation induced by 1 μM CGS21680, with an IC_{50} value of 53 nM (22). *In vivo*, orally administered KF17837 ameliorated the cataleptic responses induced by CGS21680 in rats (24). Recently Jacobson *et al.* (25) reported another selective A_{2A} antagonist, 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine, but as yet 1,3,7-trimethyl-8-(3-chlorostyryl)-xanthine has not been used as a radioligand. Here we describe the use of [^{3}H]KF17837S as a selective antagonist radioligand for A_{2A} receptors. The binding of [^{3}H]KF17837S to rat striatal membranes is characterized and the pharmacological specificity of this antagonist radioligand for the A_{2A} receptor is described.

Materials and Methods

Membrane preparation. Binding of [^{3}H]KF17837S and [^{3}H]CGS21680 to rat brain striatal membranes was measured by a modification of the method described previously by Jarvis *et al.* (19). Striatal tissue from male Wistar rats (body weight, 150–200 g) was homogenized in ice-cold 50 mM Tris·HCl buffer, pH 7.7, using a Polytron homogenizer (setting 5, 30 sec). The homogenate was centrifuged at 50,000 × *g* for 10 min at 4°, and the pellet was washed in fresh buffer. The final pellet was stored at −80° until the binding assay experiments.

[^{3}H]KF17837S binding. The assay mixture contained 50 mM Tris·HCl buffer, pH 7.7, striatal membranes (0.1 mg of protein), 1 nM [^{3}H]KF17837S, 10 mM $MgCl_2$, and 0.1 unit/ml adenosine deaminase. Incubation was for 20 min at 25°. Nonspecific binding was determined in the presence of 1 μM KF17837S. Binding reactions were terminated by filtration through Whatman GF/B filters (which had been presoaked in 0.3% polyethylenimine) under reduced pressure using a MT-24 cell harvester (Brandel). Filters were washed three times with ice-cold buffer (5 ml) and placed in scintillation vials, and bound radioactivity was determined using a liquid scintillation counter (Packard Tri-Carb 4530).

[^{3}H]CGS21680 binding. The assay mixture contained 50 mM Tris·HCl buffer, pH 7.7, striatal membranes, 4 nM [^{3}H]CGS21680, 10 mM $MgCl_2$, and 0.1 unit/ml adenosine deaminase. Incubation was for 120 min at 25°. Nonspecific binding was determined in the presence of 100 μM CPA. Binding reactions were terminated by filtration through Whatman GF/C filters. The bound radioactivity was determined by the same procedure as for [^{3}H]KF17837S binding.

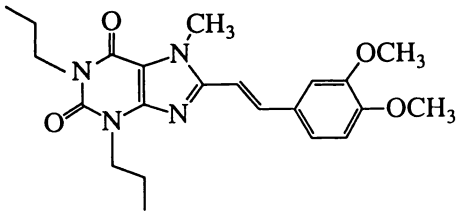

Fig. 1. Structure of KF17837.

The binding assays were performed in duplicate with seven to 11 concentrations of each drug. Protein concentrations were determined by the method of Lowry *et al.* (26), using bovine serum albumin as the reference standard.

Data analysis. Computer analyses with EBDA and LIGAND (27) were used to evaluate the kinetics constants (k_{obs} and k_{-1} values), dissociation constant (K_d value), and receptor density (B_{max} value). A partial *F* test was used to determine whether the binding was better fit by a one- or two-site model (27). IC_{50} values and Hill coefficients were determined from computerization of the logit-log curves. The Cheng-Prusoff equation (28) was used to calculate K_i values from IC_{50} values. Data represent means ± standard errors.

Autoradiography of [^{3}H]KF17837S binding. Autoradiographic studies were conducted as previously described by Jarvis and Williams (5). In brief, 20-μm coronal rat brain sections were thaw-mounted onto gelatin-coated microscope slides. Tissue sections were preincubated for 60 min at 25° in 50 mM Tris·HCl buffer, pH 7.7, containing 10 mM $MgCl_2$ and 1 unit/ml adenosine deaminase, to remove endogenous adenosine. Tissue sections were incubated for 30 min at 25° in buffer containing 10 nM [^{3}H]KF17837S. Nonspecific binding was determined from adjacent brain sections incubated in the added presence of 1 μM KF17837S. Binding reactions were terminated by washing brain sections for 1 min in ice-cold buffer twice, followed by a rinse with ice-cold distilled water. Brain sections were then rapidly dried and apposed to tritium-sensitive imaging plates (BAS-TR; Fuji Photo Film Co., Tokyo, Japan) for 4 days. After development, autoradiograms were analyzed with a bioimaging analyzer (BAS3000; Fuji).

Materials. [^{3}H]KF17837 was tritiated by Amersham International plc (Buckinghamshire, England). The tritiated compound (specific activity, 3150 GBq/mmol; radiochemical purity, 97%) was obtained from the precursor (*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropylxanthine by methylation with [^{3}H]methyl iodide. Exposure of [^{3}H]KF17837 to visible light swiftly yielded the ultimate equilibrium product, [^{3}H]KF17837S. [^{3}H]CGS21680 (specific activity, 1465.2 GBq/mmol) was purchased from New England Nuclear (Boston, MA). CGS21680 and CV1808 were from Research Biochemicals, Inc. (Natick, MA). NECA, (*R*)-PIA, CPA, (*S*)-PIA, and adenosine deaminase were from Sigma Chemical Co. (St. Louis, MO). KF17837, CGS15943, PD115199, XAC, DPCPX, and KW-3902 were synthesized at the Medicinal Chemistry Division of Kyowa Hakko Laboratories (21). Other reagents were from standard commercial sources. KF17837S was prepared as described in the previous report (22).

Results

Specific [^{3}H]KF17837S binding was found to increase linearly with increases in tissue concentration up to 0.5 mg of protein (data not shown). [^{3}H]KF17837S binding reached equilibrium after approximately 10 min at a radioligand concentration of 1.0 nM (Fig. 2A). The specific binding at equilibrium was 60–70% of total binding. With the addition of 1 μM KF17837S, [^{3}H]KF17837S binding was rapidly reversed (Fig. 2B). Both association and dissociation appeared monophasic. Association and dissociation kinetic rate constants were as follows: k_{obs} = 0.45 ± 0.073 min^{-1}, k_{-1} = 0.39 ± 0.091 min^{-1} from $t_{1/2}$ = 2.5 ± 1.0 min, and k_{+1} = 0.041 min^{-1} nM^{-1}. These values gave a kinetic dissociation constant, K_d, of 9.6 nM. Saturation experiments revealed that [^{3}H]KF17837S bound to a single class of receptors in rat striatal membranes, with a K_d

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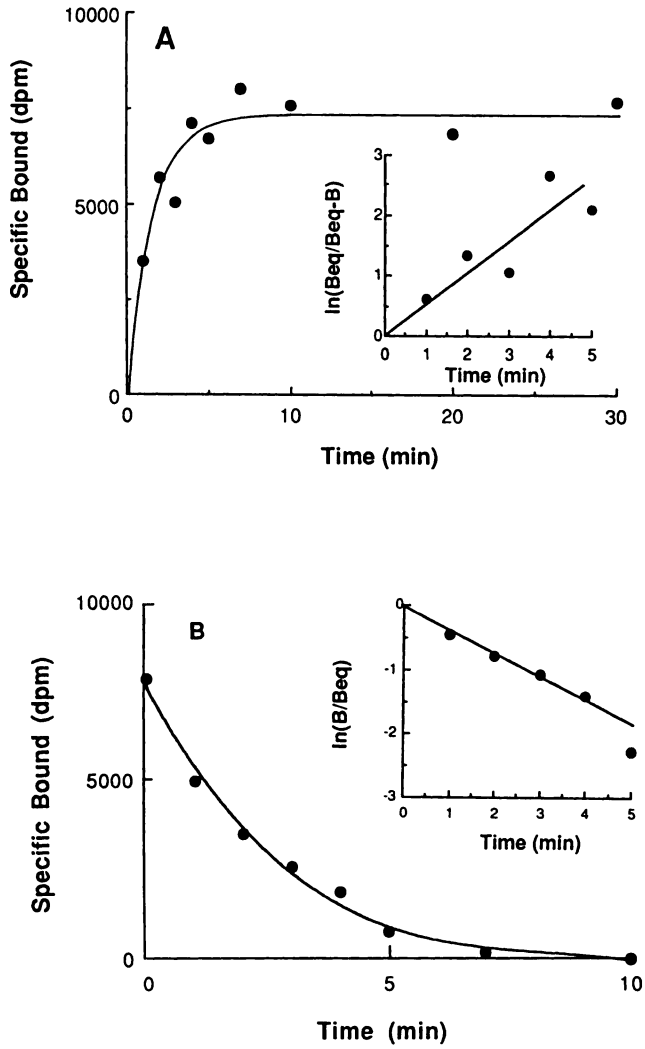

Fig. 2. Kinetics of [³H]KF17837S binding to rat striatal membranes, with representative curves for association (A) and dissociation (B). Dissociation was initiated by the addition of 1 μM unlabeled KF17837S after a 20-min incubation of ligand with the tissue at 25°. *Insets*, first-order plots of the [³H]KF17837S binding. *Beq*, amount of [³H]KF17837S bound at equilibrium; *B*, amount bound at each time. Association and dissociation kinetic rate constants were as follows: k_{obs} = 0.45 ± 0.073 min^{-1}, k_{-1} = 0.39 ± 0.091 min^{-1} from $t_{½}$ = 2.5 ± 1.0 min, and k_{+1} = 0.041 min^{-1} nM^{-1}. Values indicate means ± standard errors obtained from at least four separate experiments.

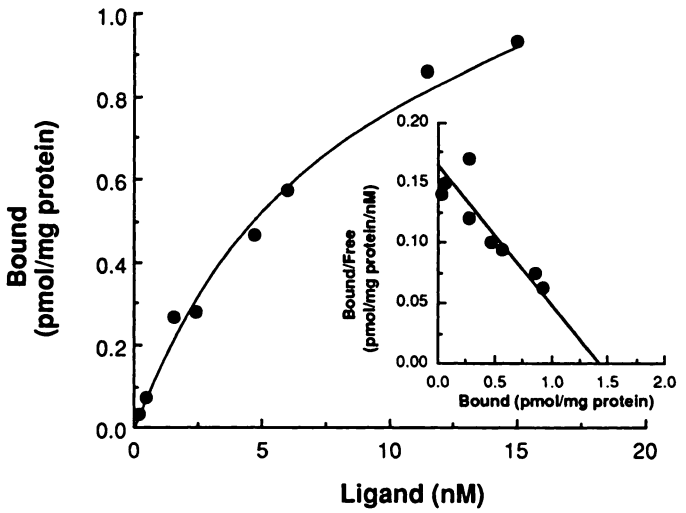

Fig. 3. Representative saturation curve for [³H]KF17837S binding to rat striatal membranes. *Inset*, corresponding Scatchard plot of the data. A K_d value of 7.1 ± 0.91 nM and a B_{max} value of 1.3 ± 0.23 pmol/mg of protein were determined. Values indicate means ± standard errors from four experiments.

TABLE 1

Comparison of the inhibition by various adenosine receptor agonists and antagonists of the binding of [³H]KF17837S and [³H] CGS21680 to rat striatal membranes

Rat striatal membranes were incubated with 1 nM [³H]KF17837S or 4 nM [³H] CGS21680 in the presence of varying concentrations of the agonists or antagonists. The Cheng-Prusoff equation (28) was used to calculate K_i values from IC_{50} values. Values are means ± standard errors of three to five separate experiments performed in duplicate

	[³H]KF17837S		[³H]CGS21680, K_i
	K_i	n_H[a]	
	nM		nM
Agonists			
NECA	59 ± 9.4	0.69 ± 0.15	3.1 ± 0.40[b]
CGS21680	130 ± 38	0.43 ± 0.011	4.5 ± 1.1[b]
CV1808	340 ± 71	0.79 ± 0.058	44 ± 2.4
(*R*)-PIA	510 ± 75	0.71 ± 0.10	54 ± 2.9
CPA	1,500 ± 240	0.67 ± 0.057	230 ± 31[b]
(*S*)-PIA	6,400 ± 1,100	0.84 ± 0.058	660 ± 54
Antagonists			
CGS15943	0.39 ± 0.013	1.00 ± 0.19	0.39 ± 0.13[b]
KF17837S	9.7 ± 1.7	0.97 ± 0.17	7.9 ± 0.055[b]
PD115,199	9.8 ± 2.9	1.02 ± 0.087	5.7 ± 0.88[b]
XAC	21 ± 4.0	0.95 ± 0.13	14 ± 4.0
DPCPX	160 ± 35	1.03 ± 0.18	120 ± 9.1
KW-3902	230 ± 12	0.96 ± 0.080	170 ± 16[b]
Caffeine	11,000 ± 2,100	1.28 ± 0.26	19,000 ± 910

[a] n_H, Hill coefficient.
[b] The K_i values for the [³H]CGS21680 binding assays were obtained from the report of Nonaka *et al.* (22).

value of 7.1 ± 0.91 nM and a B_{max} value of 1.3 ± 0.23 pmol/mg of protein (Fig. 3).

K_i values of various adenosine receptor agonists and antagonists for [³H]KF17837S binding and [³H]CGS21680 binding are listed in Table 1. The K_i values of antagonists in the two binding assays were in good agreement. The order of potency in both assays was the nonxanthine antagonist CGS15943 > KF17837S > PD115,199 ≥ XAC > DPCPX > KW-3902 > caffeine. The affinities of agonists for [³H]KF17837S binding were 1 order of magnitude lower than those for [³H]CGS21680 binding. However, for the order of potency there was a good correlation between the two assays, NECA ≥ CGS21680 > CV1808 > (*R*)-PIA > CPA > (*S*)-PIA. The correlation coefficient for the agonist K_i values between the two assays was 0.98 (Fig. 4).

The antagonists inhibited [³H]KF17837S binding with Hill coefficients close to unity, whereas the agonists showed shallow displacement curves with Hill coefficients between 0.43 and 0.84 (Table 1). Fig. 5 shows concentration-inhibition curves for KF17837S and CGS21680 for [³H]KF17837S binding. KF17837S competed with a slope factor of 0.97 ± 0.17. GTP (100 μM) did not affect the inhibition curve for KF17837S; the K_i values of KF17837S in the presence and absence of GTP were 13 ± 1.3 nM and 9.7 ± 1.7 nM, respectively. The displacement curve for CGS21680 was shallow, with a slope factor of 0.43 ± 0.011. It fitted significantly better to a two-site (rather than a single-site) model, with a high affinity K_i value (K_{iH}) of 47 nM and a low affinity K_i value (K_{iL}) of 35,000 nM (Table 2).

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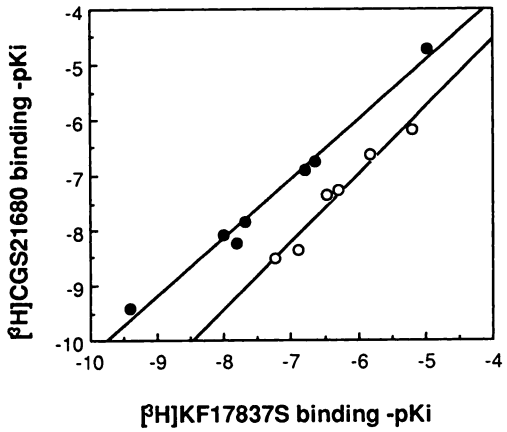

Fig. 4. Comparison of the K_i values of adenosine receptor antagonists (●) and agonists (○) measured using [³H]CGS21680 and [³H]KF17837S. Values were taken from Table 1. The correlation coefficients were 0.99 for the antagonists and 0.98 for the agonists.

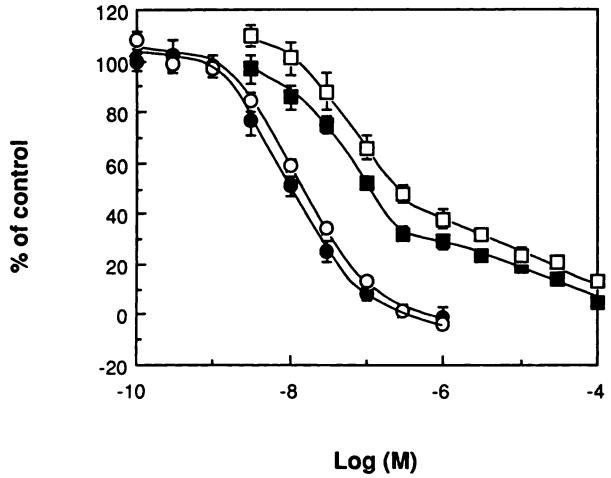

Fig. 5. Competition by CGS21680 (*squares*) and KF17837S (*circles*) with [³H]KF17837S binding to rat striatal membranes. Specific binding of [³H]KF17837S in the presence (*open symbols*) and in the absence (*closed symbols*) of 100 μM GTP was determined at various concentrations of CGS21680 or KF17837S. Data points represent means ± standard errors from at least four separate experiments conducted in duplicate.

TABLE 2

Inhibition by CGS21680 and KF17837S of [³H]KF17837S binding to rat striatal membranes in the presence and absence of 100 μM GTP

Data were analyzed by the LIGAND program applied to a one-site inhibition model, giving an apparent inhibition constant (apparent K_i), or to a model for two classes of noninteracting sites, giving a high affinity site (K_H) and a low affinity site (K_L). The percentages of receptors in the high affinity and low affinity states are shown in parentheses. Values represent means ± standard errors from at least four separate experiments conducted in duplicate

Compound	Apparent K_i	n_H[a]	K_H	K_L
	nM		nM	nM
CGS21680	130 ± 38	0.43 ± 0.011	47 (75%)	35,000 (25%)
CGS21680 + GTP	340 ± 96	0.42 ± 0.039	86 (74%)	32,000 (26%)
			($p < 0.001$)[b]	($p < 0.001$)[b]
KF17837S	9.7 ± 1.7	0.97 ± 0.17		
KF17837S + GTP	13 ± 1.3	0.97 ± 0.064		

[a] n_H, Hill coefficient.
[b] The inhibition curves for CGS21680 and CGS21680 in the presence of 100 μM GTP were found to fit significantly ($p < 0.001$) better to a two-site model.

GTP slightly shifted the inhibition curve to the right; the apparent K_i values were 340 ± 96 nM and 130 ± 38 nM in the presence and absence of GTP, respectively. However, the slope was hardly altered by the addition of GTP.

The adenosine transport inhibitors dipyridamole and nitrobenzylthioinosine, the purinergic P-site agonist 2′,5′-dideoxyadenosine, and the purinergic P_2 receptor agonists ATP, adenosine-5′-*O*-(3-thio)triphosphate, and α, β-methylene-ATP, at 10 μM, had no significant effects on [³H]KF17837S binding to the rat striatal membranes. Other pharmacological agents, including dopamine, the dopamine D_1 receptor antagonist SCH23390 [(*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol], the dopamine D_1 receptor agonist SKF38393 [2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine HCl], the dopamine D_1/D_2 receptor antagonist haloperidol, the muscarinic acetylcholine receptor antagonist atropine, the α_1-adrenergic receptor antagonist prazosin, the α_2-adrenergic receptor agonist clonidine, and the Ca^{2+} channel antagonists nifedipine and nitrendipine, all caused <50% inhibition even at 10 μM.

[³H]KF17837S binding to membranes derived from four different brain areas was compared (Table 3). It was apparent that this binding was concentrated in striatum, with lower levels of specific binding in the other three regions. In rat brain coronal sections, [³H]KF17837S binding was highly localized in the striatum (Fig. 6). Lower levels of binding were observed in the globus pallidus, and the superficial layers of the cortex were also weakly labeled.

Discussion

In the present study, we have demonstrated that [³H]KF17837S specifically binds to adenosine A_{2A} receptors corre-

TABLE 3

Comparison of the binding of [³H]KF17837S to rat striatal, cortical, hippocampal, and cerebellar membranes

Values indicate means ± standard errors obtained from triplicate observations from one representative experiment, in which 0.5 mg of membrane protein from each area was incubated with radioligand in the presence and absence of 1 μM KF17837S to define nonspecific binding

Tissue	Binding		Specific binding
	Total	Nonspecific	
	dpm		% of striatum value
Striatum	22,616 ± 1,423	7,597 ± 387	100
Cerebral cortex	8,300 ± 186	6,165 ± 154	18
Hippocampus	7,548 ± 196	6,113 ± 282	15
Cerebellum	9,957 ± 376	8,711 ± 291	10

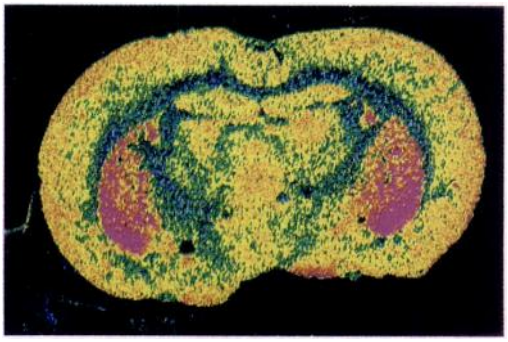

Fig. 6. Linearized autoradiographic image of 10 nM [³H]KF17837S binding to a rat brain section.

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sponding to the high affinity binding sites for [^{3}H]CGS21680 in rat striatal membranes. This conclusion is based on the following evidence. First, the ligand saturation studies revealed that [^{3}H]KF17837S bound to a single class of recognition sites in striatal membranes, with a K_d value of 7.1 nM. This value is consistent with the K_i value of KF17837S (7.9 nM) obtained from displacement studies using [^{3}H]CGS21680 as the radioligand (23). Second, the [^{3}H]KF17837S binding sites exhibit a pharmacological profile consistent with that of an A_{2A} receptor. The rank order of potency of adenosine receptor agonists and antagonists observed in the present study is identical to those observed using [^{3}H]CGS21680 (Table 1) and [^{3}H]NECA (13) as the radioligands. The correlation coefficients for the affinities measured using [^{3}H]KF17837S binding and [^{3}H]CGS21680 binding were 0.99 for antagonists and 0.98 for agonists (Fig. 4). Third, adenosine A_1 receptor-selective compounds exhibited 320–2500-fold lower affinity for the receptors labeled with [^{3}H]KF17837S than for adenosine A_1 receptors (Table 1) (13, 22, 29). Fourth, 15 other pharmacological agents, including adenosine transport inhibitors and purinergic P_2 receptor ligands, had very little effect on [^{3}H]KF17837S binding at concentrations up to 10 μM. Fifth, the regional distribution of [^{3}H]KF17837S binding was in good agreement with that reported for the A_{2A} receptor (5, 6, 19). Autoradiograms of [^{3}H]KF17837S binding to rat brain slices showed that the binding sites were highly enriched in the striatum, with lower binding in the globus pallidus and a lesser amount in the cerebral cortex (Fig. 6). [^{3}H]KF17837S binding to membranes derived from four different brain areas showed that this binding was concentrated in the striatum, with lower levels of specific binding in the other brain regions investigated (Table 3). This localization is similar to that of A_{2A} receptors determined in autoradiograms using [^{3}H]CGS21680 (5, 6) and by *in situ* hybridization using a probe for the mRNA coding for a cloned A_{2A} receptor (30), although nucleus accumbens and olfactory tubercle, where A_{2A} receptors are also highly localized (5, 6, 30), remain to be investigated. Recently, James *et al.* (31) and Johansson *et al.* (32) characterized the low level of specific binding of [^{3}H]CGS21680 in rat and human cerebral cortex, the binding characteristics of which appeared to be A_{2A}-like but different from those of striatal A_{2A} receptors.

Whereas the K_i values of adenosine receptor antagonists derived from competition studies using [^{3}H]KF17837S binding were in good agreement with those observed using [^{3}H]CGS21680 binding, adenosine receptor agonists were about 10-fold less potent in displacing [^{3}H]KF17837S than [^{3}H]CGS21680. These results are similar to those observed when the displacement of the nonselective antagonist [^{3}H]PD115,199 is compared with that of [^{3}H]NECA (13, 14). The antagonists produced steep inhibition curves, i.e., Hill coefficients not significantly different from unity. On the other hand, the Hill coefficients of the agonists were between 0.43 and 0.83 for [^{3}H]KF17837S binding (Table 1). Data from the competition studies using [^{3}H]KF17837S and unlabeled CGS21680 conformed significantly better to a two-component binding model (Table 2). With G protein-coupled receptors, agonists generally label both low and high affinity states of the receptors, depending on receptor-G protein coupling, whereas antagonists equally label both states of the receptors by eliminating receptor-G protein coupling. Addition of 100 μM GTP, which would be expected to cause dissociation of the receptor-G protein complex, shifted the inhibition curve for CGS21680 to the right, resulting in an apparent K_i value for CGS21680 approximately 3 times larger than that seen in the absence of GTP. These results suggest that [^{3}H]KF17837S labels two agonist coupling states without distinction. The differing susceptibility of [^{3}H]CGS21680 and [^{3}H]KF17837S to displacement by agonists supports the concept that A_{2A} receptors exist in two agonist coupling states, with [^{3}H]KF17837S labeling both states and [^{3}H]CGS21680 mainly labeling the high affinity state for agonists. As described in the introduction, Wan *et al.* (20) reported that [^{3}H]CGS21680 labeled high and low affinity sites in rat striatum. The low affinity sites labeled by [^{3}H]CGS21680, which were more abundant in the striatum than were the high affinity sites, were observed only with very high (100 μM) ligand concentrations, and they were composed of a combination of A_1-like and A_{2B}-like adenosine receptors. We have not yet determined the accurate affinities of KF17837S for A_{2B} and A_3 receptors in rat brain. The agonist order of potency for the A_{2B} receptor is NECA > (*R*)-PIA > CGS21680 and that for the A_3 receptor is (*R*)-PIA = NECA > CGS21680. These potency orders are different from that observed for [^{3}H]KF17837S binding, indicating that this compound has rather weaker activity for the A_{2B} and A_3 receptors than for the A_{2A} receptor. Under our assay conditions using 1 nM [^{3}H]KF17837S, A_1 and A_{2B} receptors would not be detected due to their low affinity for this ligand, i.e., for rat brain A_1 receptors the K_i is 390 nM, whereas the IC_{50} for inhibition of A_{2B} receptor-mediated, NECA-stimulated, adenylate cyclase in Jurkat cells (a human T cell line) is 1500 nM (22). It is therefore unlikely that significant radioligand would remain bound to these receptors using conventional binding techniques (33). Despite the rightward shift of the inhibition curve, the addition of GTP hardly affected the slope factor. Although the exact reason remains unknown, this may be related to an anomalous interaction of receptor and G protein, which has previously been reported for the A_{2A} receptor (33). Further investigations (perhaps using a solubilized membrane preparation), including studies of the effects of cations and guanine nucleotides, may reveal the reasons for the inability of GTP to affect the Hill coefficient (33).

In conclusion, the A_{2A}-selective antagonist [^{3}H]KF17837S directly labeled the high affinity A_{2A} receptor in rat brain. To our knowledge, [^{3}H]KF17837S is the first A_{2A} receptor-selective antagonist radioligand. Additional studies with [^{3}H]KF17837S will provide for biochemical and functional characterization of adenosine A_{2A} receptors both *in vitro* and *in vivo*.

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