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Further characterization of a CNS adenosine A_{2a} receptor ligand [^{11}C]KF18446 with *in vitro* autoradiography and *in vivo* tissue uptake

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PET assessment of the adenosine A_{2a} receptors localized in the striatum offers us a potential new diagnostic tool for neurological disorders. In the present study, we carried out *in vitro* receptor autoradiography of a newly developed PET ligand [^{11}C]KF18446 ([7-methyl-^{11}C]-(*E*)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine) with rat brain sections. [^{11}C]KF18446 showed a high striatum/cortex binding ratio (5.0) and low nonspecific binding (<10%), suggesting that [^{11}C]KF18446 has characteristics comparable or slightly superior to [^{3}H]CGS 21680 or [^{3}H]SCH 58261, which are currently available representative A_{2a} receptor ligands. Scatchard analysis indicated a K_d of 9.8 nM and a B_{max} of 170 fmol/mm^3 tissue in the striatum and a K_d of 16.4 nM and a B_{max} of 33 fmol/mm^3 tissue in the cortex. Seven xanthine-type and four nonxanthine-type adenosine receptor ligands with an affinity for the adenosine A_{2a} receptors significantly reduced the *in vitro* binding of [^{11}C]KF18446 to the brain section. The blocking effects were much stronger in the striatum than in the cortex, but did not necessarily parallel their affinity. On the other hand, four xanthine-type ligands and one nonxanthine-type ligand (SCH 58261) of the 11 ligands studied reduced the *in vivo* uptake of [^{11}C]KF18446 in mice, but other ligands, including A_1-selective and nonselective ligands and three nonxanthine-type A_{2a}-selective antagonists did not. We conclude that [^{11}C]KF18446 is a promising adenosine A_{2a} receptor ligand for PET study.

Key words: [^{11}C]KF18446, adenosine A_{2a} receptor, striatum, PET

INTRODUCTION

ADENOSINE is an endogenous modulator of a number of physiological functions in the central nervous system (CNS) as well as in peripheral organs. Recent advances in molecular biology and pharmacology have demonstrated the presence of at least four subtypes i.e., A_1, A_{2a}, A_{2b}, and A_3 receptors.[1–3]

In the CNS, adenosine A_1 receptors which exhibit higher affinity for adenosine and inhibit adenylyl cyclase are present both pre- and postsynaptically in many regions, being rich in the hippocampus, cerebral cortex, thalamic nuclei, the basal ganglia and the cerebellar cortex in animals[4–7] and humans.[8,9] Adenosine A_{2a} receptors which exhibit lower affinity for adenosine and stimulate adenylyl cyclase are highly enriched in the striatum, nucleus accumbens and olfactory tubercle, in which dopamine D_1 and D_2 receptors are localized at very high densities.[10–12] Recent studies also demonstrated the presence of A_{2a} receptors in the hippocampus and cortex.[13–17] Adenosine A_{2b} receptors show a ubiquitous distribution.[10–12] By the *in situ* hybridization technique, adenosine A_{2a} receptor mRNA and dopamine D_2 receptor mRNA are found to be mainly expressed in striatopallidal γ-aminobutyric acid (GABA)-ergic-enkephaline neurons.[18,19] The adenosine A_{2a} receptor density is significantly reduced in the striatum of patients with Huntington's chorea with selective degeneration of the

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striatopallidal neurons, but not in patients with Parkinson's disease with selective degeneration of nigrostriatal dopamine neurons.[20]

Recently, adenosine receptors in the CNS have been considered as targets for new drugs for many neurological and psychiatric disorders such as Parkinson's disease and schizophrenia.[21–23] In patients with Parkinson's disease, L-dopa treatment is partially effective, and its chronic use over several years may lead to lose efficacy. Therefore, adenosine therapy is expected to be used as an alternative or adjunct therapy.[21] In treating schizophrenia, chronic administration of neuroleptics frequently results in the development of severe movement disorders.[24] Animal experiments suggest that adenosine A_{2a} receptors are associated with antipsychotic activity of the neuroleptics through interaction with dopamine receptors.[25]

In general, development of selective receptor agonists and antagonists is essential for investigating neuroreceptor systems. As for adenosine A_{2a} receptors, a selective A_{2a} receptor agonist [^{3}H]-2-[*p*-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine ([^{3}H]CGS 21680) is currently used as a standard radiolabeled ligand for *in vitro* studies of adenosine A_{2a} receptors. As selective A_{2a} antagonists, xanthine-type compounds have been used as selective probes, since the first proposal of KF17837 ((*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine) by Shimada et al.[26] Nonxanthine-type antagonists have also been proposed,[27–29] including 5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyridine (SCH 58261)[30] and 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-yl amino]ethyl)phenol (ZM 241385)[31] which have a high affinity and selectivity for the A_{2a} receptors. Their ^{3}H- or ^{125}I-labeled analogs are used as radiolabeled ligands.[32–34]

For the purpose of studying the adenosine A_{2a} receptors in humans by positron emission tomography (PET), we have recently prepared [^{11}C]KF17837.[35,36] This was preferentially taken up by the striatum in mice, rats and monkeys, but to a smaller extent by the cerebral cortex and cerebellum, suggesting that the selectivity of [^{11}C]KF17837 for the adenosine A_{2a} receptors may not be sufficient as a PET tracer. In the continuing search for a more selective ligand, we found that [7-methyl-^{11}C]-(*E*)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine ([^{11}C]KF18446) showed much more selective affinity and lower nonspecific uptake *in vivo* than [^{11}C]KF17837.[37,38] In a blocking study to characterize the selectivity of [^{11}C]KF18446 in mice, the striatal uptake was blocked by co-injection of any of four high affinity xanthine-type A_{2a} receptor antagonists, but the blocking effect of a nonxanthine-type antagonist SCH 58261 was weaker than that of xanthine-type A_{2a} antagonists.[38] Another nonxanthine-type A_{2a} antagonist ZM 241385 did not block the striatal uptake of [^{11}C]KF18446 (unpublished data). Also the uptake of [^{11}C]KF17837 was not significantly decreased *in vivo* by SCH 58261 or ZM 241385.[36]

In the present study, we carried out *in vitro* receptor autoradiography (ARG) of [^{11}C]KF18446 to characterize the new PET ligand. The blocking effects of various xanthine-type and nonxanthine-type adenosine receptor ligands on the *in vitro* binding of [^{11}C]KF18446 to the rat brain section are described. We also examined the *in vivo* blocking effects of the ligands on the brain uptake of [^{11}C]KF18446 in mice by the tissue dissection method. A part of this study was presented at the 6th International Symposium on Purines and Pyrimidines held in May 19–24 1998, in Ferrara, Italy.[39]

MATERIALS AND METHODS

Materials

Four xanthine-type adenosine A_{2a} receptor antagonists, KF17837,[26] KF18446,[38] (*E*)-1,3-diallyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthine (KF19631)[38] and 8-chlorostyryl-1,3,7-trimethylxanthine (CSC),[40] four nonxanthine-type adenosine A_{2a} receptor antagonists, SCH 58261, ZM 241385, 8-chloro-1-phenyl[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (CP-66713)[41] and 2-(2-furyl)-5-[2-(morpholino)ethylamino][1,2,4]triazolo[1,5-*a*][1,3,5]triazine-7-amine (ZD9255) (Jones, WO 94/14812 1994), and an adenosine A_1 antagonist 8-dicyclopropylmethyl-1,3-dipropylxanthine (KF15372)[42] were prepared by Kyowa Hakko Kogyo Company. 3,7-Dimethyl-1-propargylxanthine (DMPX),[43] 8-[4-[[[[(2-aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine (XAC),[42] and CGS 21680 were purchased from Research Biochemical, Inc. (Natick, MA, USA).

[^{11}C]KF18446 was prepared by ^{11}C-methylation of the demethyl compound with [^{11}C]methyl iodide by the known method.[37,38] The specific radioactivity was 10–72 TBq/mmol.

Male Wistar rats (8–9 weeks old) and male ddY mice (7–9 weeks old) were obtained from Tokyo Laboratory Animals Company (Tokyo, Japan). The animal studies were approved by the Animal Care and Use Committee of Tokyo Metropolitan Institute of Gerontology.

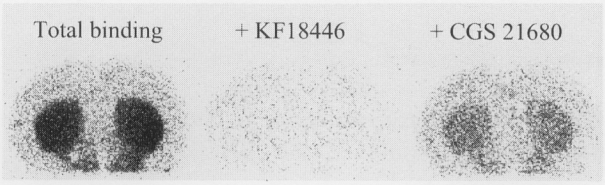

Fig. 1 *In vitro* autoradiograms of the binding of [^{11}C]KF18446 to the rat brain section. Left side, total binding; middle, blockade with cold KF18446; and right, blockade with CGS 21680. Tracers, 2.0–3.5 nM; and nonradioactive KF18446 and CGS 21680, 20 μM.

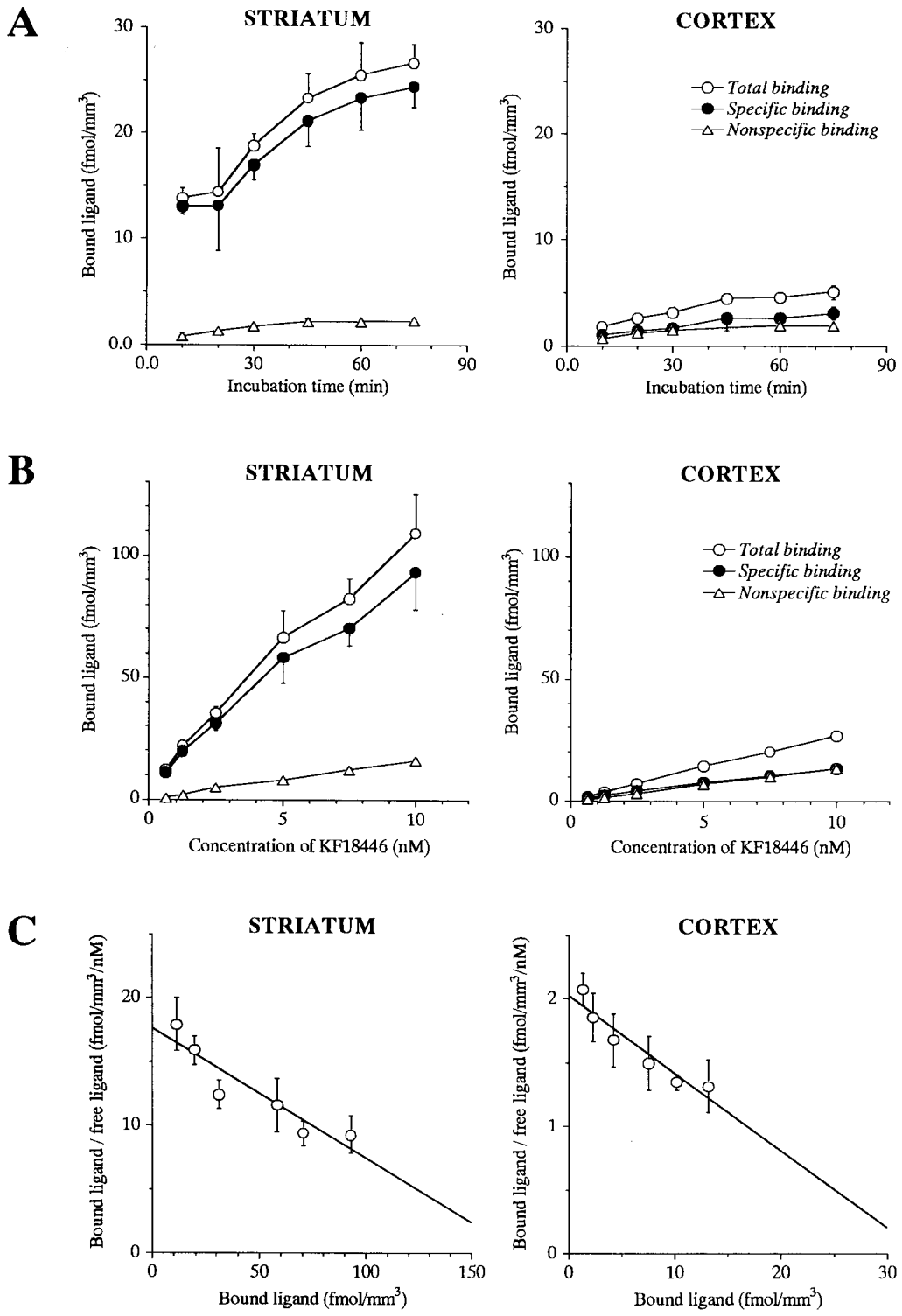

Fig. 2 *In vitro* binding of [^{11}C]KF18446 to the rat brain section. A, time-course; B, dose-dependence; and C, Scatchard analysis.

In vitro *binding of [^{11}C]KF18446 to rat brain section*
In vitro ARG of [^{11}C]KF18446 was performed by radioluminography as described previously.[44] Three sets of 20 μm thick adjacent coronal rat brain sections near the bregma were prepared. According to the method of Przedborski et al.,[45] two sets of the brain sections were pre-incubated in 50 mM Tris-HCl, pH 7.4, containing 10 mM $MgCl_2$ and 0.2 IU/mL adenosine deaminase for 30 min at room temperature to remove endogenous adenosine, and then the sections were incubated in the same

Table 1 Blockade of [^{11}C]KF18446 binding to the rat brain by various adenosine antagonists (20 μM) measured with *in vitro* autoradiography, summarized with their lipophilicity and affinity

	Blockade* (% decrease in binding)		Lipophilicity$ cLog P	Affinity, K_i (nM)#			
	Striatum	Cortex		A_1	A_{2a}	A_1/A_{2a}	Ref.
Xanthine-type A_{2a} adenosine receptor antagonists							
KF17837	80.5 ± 1.9	27.0 ± 2.7	3.81	62	1.0	62	(38)
KF19631	84.7 ± 0.2	41.7 ± 2.8	2.84	860	3.5	250	(30)
KF18446	91.0 ± 0.7	60.4 ± 1.3	1.13	1600	5.9	270	(30)
CSC	57.5 ± 3.2	31.4 ± 1.0	2.95	28000	54	520	(31)
Xanthine-type nonselective adenosine receptor antagonists							
XAC	71.6 ± 2.5	28.7 ± 3.1	2.67	11	21	0.52	(33)
DPMX	64.3 ± 1.3	25.7 ± 1.9	–0.36	12000	8600	1.4	(34)
Xanthine-type A_1 adenosine receptor antagonists							
KF15372	76.7 ± 2.5	24.3 ± 6.5	4.14	3.0	430	0.0070	(33)
Nonxanthine-type A_{2a} adenosine receptor antagonists and agonist							
ZM 241385	74.1 ± 2.4	14.2 ± 7.3	2.29	510	0.91	560	(unpublished)
SCH 58261	78.3 ± 4.7	23.3 ± 3.7	2.94	121	2.3	53	(22)
CP-66713	77.8 (n = 2)	21.8 (n = 2)	3.57	6900	5.1	1400	(33)
ZD9255	67.0 (n = 2)	9.4 (n = 2)	1.19	1300	13	100	(4)
Agonist							
CGS 21680	70.0 (n = 2)	16.0 (n = 2)		420	4.5	93	(38)

*Mean ± s.d. (n = 3 or 2).

$Calculated from the structure.[40]

#The affinity of all compounds for the adenosine A_1 and A_{2a} receptors was determined using the rat forebrain membrane and [^{3}H]N^6-cyclohexyladenosine as a radioligand and the rat striatal membrane and [^{3}H]CGS 21680, respectively (see each Ref.).

buffer containing 1.54 nM (100 kBq/mL) [^{11}C]KF18446 for 75 min at room temperature. Nonspecific binding was determined by incubating the other set of the sections in the medium, to which cold KF18446 had been added to a final concentration of 20 μM. The assay was stopped by washing the sections with ice-cold 50 mM Tris-HCl (pH 7.4) containing 120 mM NaCl. These procedures were carried out under dim light to prevent isomerization of radioactive and cold KF18446.[35,46] Then the brain sections were dried on a hot plate at 60°C, and apposed on an imaging plate until the activity decayed out. Regional radioactivity was measured as PSL/mm^2 over the regions of interest placed on the striatum and on the cerebral cortex. The specific binding was determined by subtracting the activity under cold KF18446 loading. The radioactivity (cpm) of the other set of sections was measured with a gamma counter for calibration of PSL. The ^{11}C activity was then converted to the concentration of receptor-bound ligand (fmol/mm^3 tissue) by using the specific activity of the ligand and efficiency of the gamma counter (cpm/Bq) as previously described.[44]

To measure the dissociation constant (K_d) and maximal receptor binding (B_{max}), the buffer containing [^{11}C]KF18446 at various concentrations (0.625, 1.25, 2.50, 5.0, 7.5 and 10 nM) of cold KF18446 was prepared, and the 20 μm thick brain sections were incubated for 30 min at 37°C in each buffer as described above. Nonspecific binding was determined as described above. The concentration of receptor-bound ligand (fmol/mm^3 tissue) in the striatum and cortex was measured as described above. The bound ligand (fmol/mm^3 tissue) was plotted against the ratio of bound ligand to free ligand (fmol/mm^3 tissue/nM), as a Scatchard plot.

Blocking effects of adenosine receptor ligands on the in vitro *binding of [^{11}C]KF18446 to rat brain section*

As described above, the *in vitro* binding assay was carried out in the same buffer containing 2.0–3.5 nM (100 kBq/mL) [^{11}C]KF18446 with or without one of the following 11 antagonists or an agonist CGS 21680 at 20 μM for 30 min at room temperature. The antagonists used were four xanthine-type adenosine A_{2a} antagonists, KF17837, KF18446, KF19631, and CSC, four nonxanthine-type adenosine A_{2a} antagonists, ZM 241385, SCH 58261, CP-66713 and ZD9255; two nonselective xanthine-type antagonists XAC and DMPX; and a xanthine-type A_1 antagonist KF15372. The binding was measured in PSL/mm^2, and the ratio of the binding measured with each blocker to that without blockers was calculated.

In vitro *affinity of KF18446 for other neuroreceptors*

The *in vitro* affinity of KF18446 for other neuroreceptors: adrenergic α_1, α_2 and β_1; dopamine D_1 and D_2; agonist site of $GABA_A$ and benzodiazepine site of $GABA_A$; histamine H_1 and H_2; non-selective muscarine; nicotinic acetylcholine; and serotonin 5-HT_{1A} and 5-HT_2 was determined using rat striatal membranes and 10 μM KF18446, as described.[47]

Blocking effects of adenosine receptor ligands on the in vivo *mouse brain uptake of [^{11}C]KF18446*

[^{11}C]KF18446 (0.54–1.3 MBq/14–72 pmol) with or without one of the 11 antagonists was intravenously injected into mice (33–41 g). The amounts of the co-injected antagonist were 50 nmol/animal for KF17837 and 100 nmol/animal for the others. The mice were killed by cervical dislocation at 15 min after injection. The brain was removed and dissected into the striatum, cerebellum and cerebral cortex. The ^{11}C-radioactivity in the samples was counted in an auto-gamma counter and decay-corrected. After weighing the tissues, the ^{11}C-radioactivity level in tissues was expressed as the percent injected dose per gram tissue (%ID/g).

Lipophilicity of adenosine receptor ligands

Lipophilicity (cLog P) of adenosine receptor ligands was mathematically calculated to evaluate the penetration of the ligands across the blood-brain barrier *in vivo*.[48]

RESULTS

In vitro *binding of [^{11}C]KF18446 to rat brain section*

Figure 1 shows representative *in vitro* autoradiograms of [^{11}C]KF18446. In the presence of 20 μM KF18446, the binding of [^{11}C]KF18446 to the striatum disappeared. The time course of the [^{11}C]KF18446 binding to the striatum and cerebral cortex increased over 75 min (Fig. 2A). The specific binding was approximately 90% and 60% of the total binding in the striatum and cortex, respectively. The total binding and specific binding in the striatum were 5.0 and 8.2 times, respectively, as high as in the cortex at 45–75 min.

A saturation binding experiment showed a specific binding of [^{11}C]KF18446 to the striatum and cortex (Fig. 2B). Scatchard analysis (Fig. 2C) indicated a K_d of 9.8 nM and an apparent B_{max} of 170 fmol/mm^3 tissue in the striatum and a K_d of 16.4 nM and a B_{max} of 33 fmol/mm^3 tissue in the cortex.

Blocking effects of adenosine receptor ligands on the in vitro *binding of [^{11}C]KF18446 to rat brain section*

The blocking effects of various adenosine receptor ligands on the *in vitro* binding of [^{11}C]KF18446 are summarized in Table 1. Table 1 also represents the affinity of the ligands for the adenosine A_1 and A_{2a} receptors obtained from the literature. All ligands investigated significantly reduced the binding of [^{11}C]KF18446 to the striatum and to the cortex. The blocking effects in general were much larger in the striatum than in the cortex, but the effects did not necessarily parallel their *in vitro* affinity. KF18446 had the largest blocking effect in both the striatum and cortex. An A_1 antagonist KF15372 with a weak affinity for A_{2a} receptors (K_i = 430 nM)[41] and a non selective antagonist DPMX (K_i = 8600 nM)[43] also had blocking effects.

Table 2 Affinity of KF18446 to various neuroreceptors measured with *in vitro* radioligand membrane binding assay

Receptor	% inhibition by 10 μM KF18446
Adrenergic α_1'	−5
Adrenergic α_2'	−13
Adrenergic β_1	8
Dopamine D_1	−3
Dopamine D_2	−13
$GABA_A$, agonist site	12
$GABA_A$, benzodiazepine site	5
Histamine H_1	2
Histamine H_2	18
Muscarine, non-selective	−13
Nicitinic acetylcholine	9
Serotonin 5-HT_{1A}	9
Serotonin 5-HT_2	16

Assays were run according to the standard protocol documented in the references. Rat or guinea pig brain membranes were used as receptor sources. All assays were validated using appropriate reference standards.

Figure 1 shows the *in vitro* ARG images of [^{11}C]KF18446 blocked by 20 μM CGS 21680. Although KF18446 and CGS 21680 have a similar affinity for adenosine A_{2a} receptors using [^{3}H]CGS 21680 as a radioligand (Table 1), the blockade of striatal binding of [^{11}C]KF18446 by CGS 21680 was incomplete.

Lipophilicity of adenosine receptor ligands

Lipophilicity (cLog P) of the 11 adenosine receptor ligands is summarized in Table 1. Among the xanthine-type compounds the highest lipophilicity was found for KF15372, followed by KF17837 and CSC. The cLog P for KF18446 was relatively low. All four nonxanthine-type ligands had lipophilicity ranging from that for KF18446 to that for KF17837.

In vitro *affinity of KF18446 for other neuroreceptors*

We performed an *in vitro* membrane binding assay of KF18446 for several other neuroreceptors: adrenergic α_1, α_2, and β_1; dopamine D_1 and D_2; agonist site of $GABA_A$ and benzodiazepine site of $GABA_A$; histamine H_1 and H_2; non-selective muscarine; nicotinic acetylcholine; and serotonin 5-HT_{1A} and 5-HT_2. As shown in Table 2 affinity of KF18446 for any of these receptors was not detected (< 10 μM of K_d values).

Blocking effects of adenosine receptor ligands on the in vivo *mouse brain uptake of [^{11}C]KF18446*

At 15 min after injection of [^{11}C]KF18446 into mice, the uptake of the radioactivity (%ID/g) was 4.39 ± 0.97 in the striatum, 1.55 ± 0.25 in the cortex and 1.62 ± 0.24 in the cerebellum (Fig. 3).

The blocking effects of the various adenosine receptor antagonists on the regional brain uptake of [^{11}C]KF18446

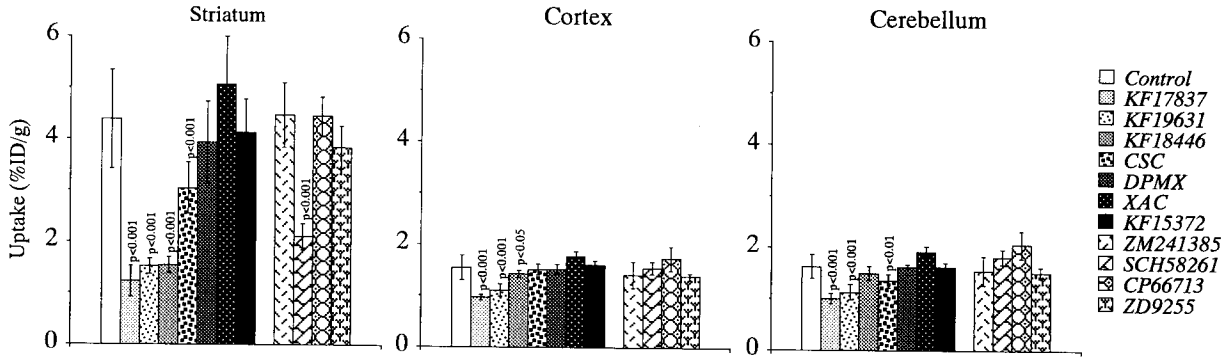

Fig. 3 Effect of various adenosine receptor ligands on the regional brain uptake of [^{11}C]KF18446 at 15 min after injection of the tracer into mice. Mean ± s.d. (n = 4, except for control, n = 23; CSC, n = 8; and SCH 58261, n = 9). Student's t-test was carried out against control.

are summarized in Figure 3. The striatal uptake was reduced by each of the four xanthine-type A_{2a} antagonists, and the order of the blocking effect (KF17837 > KF19631 > KF18446 > CSC) paralleled their *in vitro* affinity. The uptake by the cortex and cerebellum was also slightly reduced by each of these four antagonists in the same way as was observed in the striatal uptake. The blocking effects were much smaller in the cortex and cerebellum than in the striatum. The nonselective antagonists DPMX and XAC and an A_1 antagonist KF15372 did not block the uptake of the tracer in any region. Among the four nonxanthine-type adenosine A_{2a} antagonists, SCH 58261 significantly reduce the uptake of [^{11}C]KF18446 in the striatum, but not in the cortex or cerebellum. The other three antagonists did not reduce the uptake in any region.

DISCUSSION

From the first proposal of KF17837 as a selective A_{2a} antagonist by Shimada et al.,[26] several xanthine-type compounds have been used as selective probes for pharmacological studies of the adenosine A_{2a} receptors.[27–29] Recently we prepared ^{11}C-labeled KF17837 and three other xanthine analogs and found that [^{11}C]KF18446 was a promising candidate as an *in vivo* probe for studying adenosine A_{2a} receptors by PET.[35–39] In the present study, we further characterized the new radioligand [^{11}C]KF18446 by *in vitro* receptor ARG in rats and *in vivo* tissue uptake in mice.

A selective A_{2a} receptor agonist, [^{3}H]CGS 21680, is now widely used as a standard ligand for *in vitro* studies of adenosine A_{2a} receptors, but CGS 21680 does not cross the blood-brain barrier and cannot be used as a PET tracer. The characteristics of [^{11}C]KF18446 were comparable or slightly superior to those of [^{3}H]CGS 21680. In the membrane binding studies, the A_{2a}/A_1 selectivity was 270 for KF18446 and 93 for [^{3}H]CGS 21680 (Table 1). By *in vitro* ARG, nonspecific binding of [^{11}C]KF18446 in the striatum was 9% of the total binding, whereas the corresponding value for [^{3}H]CGS 21680 was estimated to be 19%.[47] The total binding ratio of striatum to cortex was 5.0 for [^{11}C]KF18446 and 4.6 for [^{3}H]CGS 21680.[13] It was also found that KF18446 scarcely had any affinity for at least 13 other neuroreceptors (Table 2). Recently nonxanthine-type [^{3}H]SCH 58261 is proposed as a selective A_{2a} receptor radioligand. The A_{2a}/A_1 selectivity was approximately 800 but 53 with [^{3}H]CGS 21680, and the nonspecific binding was < 15% below a concentration of 3 nM,[34] which are not as good as for KF18446. Taken together with a previous *in vivo* study[38] and the present results, [^{11}C]KF18446 is a promissing PET ligand for mapping adenosine A_{2a} receptors.

The *in vitro* binding of [^{11}C]KF18446 to the rat striatum was inhibited by all the adenosine receptor ligands used in the present study at 20 μM (Table 1). This is reasonable because each ligand had an affinity for A_{2a} receptors, which was assessed by the K_i value for the binding of [^{3}H]CGS 21680 to A_{2a} receptors on the rat striatal membrane (Table 1), but the blocking effects did not necessarily parallel the affinity. The largest blocking effect was found for KF18446 and SCH 58261 among xanthine and nonxanthine antagonists, respectively. The blocking effect of CGS 21680 was relatively weak among the ligands with affinity of the same magnitude. These findings suggest that those ligands have multiple binding sites: a common binding site regarded as the typical adenosine A_{2a} receptor and their own unknown binding site suggested to be in the cortex and hippocampus.[13–17]

On the other hand, the *in vivo* result showed a striking difference from the *in vitro* result. The blocking effects of xanthine-type ligands on the *in vivo* mouse brain uptake of [^{11}C]KF18446 were apparently different from those of nonxanthine-type ligands with the only exception of SCH 58261 (Fig. 3). Although the *in vitro* and *in vivo* experiments were carried out in rats and mice, respectively, the species difference is not likely to exist for the adenosine A_{2a} receptors.[36,38] The blocking effect of xanthine ligands on the striatal uptake of the tracer paralleled their *in vitro*

affinity: KF17837 > KF19631 > KF18446 > CSC, except for DPMX having a weak affinity and XAC and KF15372 having a high affinity for A_1 receptors.

The discrepancy between *in vitro* and *in vivo* findings cannot be explained by the lipophilicity of the ligands (Table 1). A possible explanation is multiple binding sites of the ligands. When the ligands have different binding sites besides the typical adenosine A_{2a} receptors as suggested by *in vitro* ARG, it is possible that the binding to the undefined sites might hinder the blocking effect on the uptake of [^{11}C]KF18446. Another explanation is that the association rates of the A_{2a} receptor antagonists binding are different for xanthine-type and nonxanthine-type antagonists. In living animals the receptor ligands are delivered into the brain and washed out by the blood flow, so that the receptor-ligand binding is not necessarily kept in a state of equilibrium, which is quite different from the *in vitro* binding assay. The antagonists with a low association rate may not effectively block the receptor binding of [^{11}C]KF18446.

The present study suggests the presence of saturable binding sites for [^{11}C]KF18446 in the cortex. Recent *in vitro* studies also showed that A_{2a} receptors are found in the hippocampus and cortex.[13–17] The binding of a standard A_{2a} receptor ligand [^{3}H]CGS 21680 in these tissues may be different from the classical adenosine A_{2a} receptors present in the striatum and from other defined receptor subtypes as well. The B_{max} and K_d values reported are 353 pmol/mg protein and 58 nM in the hippocampus, 264 pmol/mg protein and 58 nM in the cortex and 419 pmol/mg protein and 17 nM in the striatum.[15] The cortical binding site of [^{3}H]CGS 21680 was clearly discriminated from the striatal binding site by using another selective adenosine A_{2a} receptor antagonist SCH 58261.[49] For [^{11}C]KF18446 we found the specific binding sites with a K_d of 16.4 nM and a B_{max} of 33 fmol/mm^3 tissue in the cortex, whereas K_d and B_{max} values in the striatum were 9.8 nM and 170 fmol/mm^3 tissue, respectively.

As a radioligand for biochemical and pharmacological studies radiolabeled KF18446 has a disadvantage because photoisomerization from a high affinity *E*-isomer to a low affinity *R*-isomer easily occurs in the xanthine derivatives with 8-styryl groups.[46] Nevertheless, the disadvantage is easily overcome in PET studies, where the radiolabeled ligand is used immediately after preparation.[35] We conclude that [^{11}C]KF18446 is a promissing adenosine A_{2a} receptor ligand for PET study.

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