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Evaluation of iodinated and brominated [^{11}C]styrylxanthine derivatives as *in vivo* radioligands mapping adenosine A_{2A} receptor in the central nervous system

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In vivo assessment of the adenosine A_{2A} receptors localized in the striatum by PET or SPECT offers us a new diagnostic tool for neurological disorders. In the present study, we evaluated the potential of iodinated and brominated styrylxanthine derivatives labeled with ^{11}C as an *in vivo* probe. [7-Methyl-^{11}C]-(*E*)-3,7-dimethyl-8-(3-iodostyryl)-1-propargylxanthine ([^{11}C]IS-DMPX) and [7-methyl-^{11}C]-(*E*)-8-(3-bromostyryl)-3,7-dimethyl-1-propargylxanthine ([^{11}C]BS-DMPX) were prepared by the ^{11}C-methylation of corresponding 7-demethyl derivatives. An *in vitro* membrane binding study showed a high affinity (Ki values) of the two ligands for A_{2A} receptor: 8.9 nM for IS-DMPX and 7.7 nM for BS-DMPX, and a high A_{2A}/A_1 selectivity: >1100 for IS-DMPX and 300 for BS-DMPX. In mice, [^{11}C]IS-DMPX and [^{11}C]BS-DMPX were taken up slightly more in the striatum than in the reference regions such as the cortex and cerebellum. The uptake ratios of striatum to cortex and striatum to cerebellum gradually increased but were very small: 1.6–1.7 for the striatum-to-cortex ratio and 1.2 for the striatum-to-cerebellum ratio at 60 min postinjection. The uptake by these three regions was reduced by co-injection of an excess amount of carrier or an A_{2A} antagonist KF17837, but not by an A_1 antagonist KF15372. The blocking effects in the three regions were greater for [^{11}C]BS-DMPX (32–57%) than for [^{11}C]IS-DMPX (6–29%). *Ex vivo* autoradiography confirmed that the two ligands were slightly concentrated in the striatum. [^{11}C]BS-DMPX showed more selective affinity for adenosine A_{2A} receptors than [^{11}C]IS-DMPX, but these results have shown that the two tracers were not suitable as *in vivo* ligands because of low selectivity for the striatal A_{2A} receptors and a high nonspecific binding.

Key words: halogenated xanthine, adenosine A_{2A} receptor, PET, SPECT

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 enriched 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, whereas A_{2B} receptors have a ubiquitous distribution.[10–12] Several reports said that these receptors are altered in patients with neurological diseases. Decreased density of the A_1 receptors was

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found in the hippocampus of patients with Alzheimer's disease[13,14] and in temporal lobe epilepsy.[15] In patients with Huntington's chorea with a selective degeneration of the striatopallidal neurons, adenosine A_{2A} receptor density is significantly reduced in the striatum, but it is not significantly affected in patients with Parkinson's disease characterized by a selective degeneration of nigrostriatal dopamine neurons.[16]

Positron emission tomography (PET) assessment of the adenosine receptor system probably offers us a new diagnostic tool for several neurological disorders. For this purpose, we have proposed several PET ligands for the two adenosine receptor subtypes.[17-24] All ligands were developed based on the findings by Suzuki, Shimada and co-workers showing that xanthine derivatives have a high and selective affinity for adenosine A_1 or A_{2A} receptors.[25,26] [7-Methyl-^{11}C]-(*E*)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine ([^{11}C]KF18446) had promising properties as a PET ligand, although [7-methyl-^{11}C]-(*E*)-1,3-dipropyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthine ([^{11}C]KF17837) had the highest affinity. As for the xanthine-type A_{2A} receptor ligands, the xanthine derivatives including a styryl group at C8 is the most potent and selective.[26] Recently Müller et al. reported that brominated and chlorinated styrylxanthine derivatives, (*E*)-8-(3-bromostyryl)-3,7-dimethyl-1-propargylxanthine (BS-DMPX) and (*E*)-8-(3-chlorostyryl)-3,7-dimethyl-1-propargylxanthine, had similar affinity for the adenosine A_{2A} receptors as KF17837.[27,28] We expected that the iodinated derivative, (*E*)-3,7-dimethyl-8-(3-iodostyryl)-1-propargylxanthine (IS-DMPX), may also have a high affinity for the A_{2A} receptors.

In the present study, we prepared ^{11}C-labeled IS-DMPX and BS-DMPX and evaluated their potential as *in vivo* radioligands for mapping the adenosine A_{2A} receptors in the CNS. BS-DMPX is potentially labeled with another positron-emitter such as ^{76}Br, and the ^{123}I-labeled IS-DMPX can be applied to studies with single-photon emission computed tomography (SPECT). We measured the regional brain distribution of the tracers in mice by tissue dissection and *ex vivo* autoradiography, and determined the receptor-specific binding *in vivo* by a blocking study.

MATERIALS AND METHODS

Materials

An adenosine A_{2A} receptor antagonist KF17837 and an adenosine A_1 antagonist 8-dicyclopropylmethyl-1,3-dipropylxanthine (KF15372) were prepared by Kyowa Hakko Kogyo Company.

Synthesis of IS-DMPX, BS-DMPX and their 7-demethyl derivatives

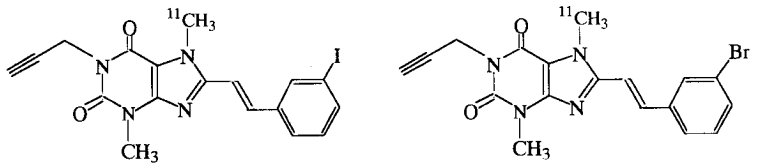
To a stirred suspension of 3-iodocinnamic acid (8.8 g) in methylene chloride (210 mL) were added thionyl chloride (2.8 mL) and pyridine (3 mL) at 0°C, and the reaction mixture was stirred for a further 3 hours at room temperature and concentrated. The residue was dissolved in *N,N*-dimethylformamide (160 mL), and 5,6-diamino-1-methyluracil (5 g) and pyridine (3.7 mL) was added to the mixture. The reaction mixture was stirred overnight and poured into ice-water (800 mL). Neutralization (pH 6) gave (*E*)-6-amino-1-methyl-5-(3-iodocinnmamoylamino)uracil (9.4 g) as a colorless powder. To the solution of the uracil obtained (9.0 g) in *N,N*-dimethylformamide (500 mL) were added Cs_2CO_3 (8.5 g) and propargyl bromide (5.4 mL, 80 w/v% toluene solution), and the reaction mixture was stirred for 3 days at room temperature. The mixture was poured into ice-water (1 L), and the crude material obtained was collected. Recrystallization from ethanol gave (*E*)-6-amino-1-methyl-5-(3-iodocinnamoylamino)-3-propargyluracil (2.6 g) as a colorless powder. The mixture of the obtained uracil (2.6 g), aqueous 4 N NaOH (160 mL), and ethanol (180 mL) was refluxed for 2 hours. The mixture was poured into ice-water (500 mL) and neutralized with conc. HCl (pH 6.2). Recrystallization of the crude material obtained from dioxane-water gave (*E*)-3-methyl-8-(3-iodostyryl)-1-propargylxanthine (0.6 g, 7-demethyl IS-DMPX) as a colorless powder: mp >270°C; ^{1}H NMR (270 MHz, CF_3CO_2D) δ ppm: 11.35 (1H, s), 7.92 (1H, s), 7.82 (1H, d, J = 7.9 Hz), 7.71 (1H, d, J = 16.5 Hz), 7.56 (1H, d, J = 7.9 Hz), 7.26–7.19 (1H, m), 7.07 (1H, d, J = 16.5 Hz), 4.87 (2H, d, J = 2.6 Hz), 3.75 (3H, s), 2.26 (1H, t, J = 2.6 Hz); Elementary Analysis ($C_{17}H_{13}N_4O_2I$); Calcd for (%): C, 47.24; H, 3.03; N, 12.96, found (%): C, 47.57; H, 3.06; N, 12.99.

To a suspension of 7-demethyl IS-DMPX (455 mg) in *N,N*-dimethylformamide (20 mL) was added K_2CO_3 (400 mg) and methyl iodide (0.1 mL) and the mixture was stirred for a hour at 50°C. The mixture was poured into ice-water (100 mL), and the crude material obtained was collected. Recrystallization from dioxane-water gave IS-DMPX (400 mg) as a pale yellow powder: mp 215–218 °C (decomp); ^{1}H NMR (270 MHz, CF_3CO_2D) δ ppm: 7.97 (1H, s), 7.84 (1H, d, J = 9.6 Hz), 7.79 (1H, d, J = 16.5 Hz), 7.50 (1H, d, J = 7.6 Hz), 7.26–7.19 (1H, m), 6.93 (1H, d, J = 16.5 Hz), 4.84 (2H, d, J = 2.6 Hz), 4.26 (3H, s), 3.72 (3H, s), 2.24 (1H, t, J = 2.6 Hz); Elementary Analysis ($C_{18}H_{15}N_4O_2I$); Calcd for (%): C 48.45, H 3.39, N 12.56; Found (%): C 48.24, H 3.43, N 12.24.

BS-DMPX and 7-demethyl BS-DMPS were prepared as previously described.[27]

Radiosynthesis of [^{11}C]IS-DMPX and [^{11}C]BS-DMPX

[^{11}C]IS-DMPX and [^{11}C]BS-DMPX were prepared by ^{11}C-methylation of the corresponding 7-demethyl compounds with [^{11}C]methyl iodide in the presence of Cs_2CO_3 by the known method used for the synthesis of [^{11}C]KF17837 and [^{11}C]KF18446,[19,23] but the radiochemical yields were very low (0.7–4.2% based on [^{11}C]methyl



[^{11}C]IS-DMPX (Ki = 8.9 nM) [^{11}C]BS-DMPX (Ki =7.7 nM)

Fig. 1 Chemical structures of [^{11}C]IS-DMPX and [^{11}C]BS-DMPX. The affinity for adenosine A_{2A} receptors was measured in the present study.

Table 1 Tissue distribution of radioactivity after intravenous injection of [^{11}C]IS-DMPX into mice

	Uptake (%ID/g)			
	5 min	15 min	30 min	60 min
Blood	0.40 ± 0.06	0.29 ± 0.02	0.20± 0.03	0.22 ± 0.02
Brain	1.88 ± 0.26	1.77 ± 0.18	1.63 ± 0.28	1.22 ± 0.04
Heart	15.19 ± 1.42	10.98 ± 1.56	7.89 ± 0.58	5.19 ± 0.56
Lung	2.73 ± 0.32	1.88 ± 0.20	1.61 ± 0.24	1.29 ± 0.21
Liver	5.65 ± 1.31	4.32 ± 0.47	2.96 ± 0.37	3.14 ± 0.11
Pancreas	3.33 ± 0.45	2.18 ± 0.38	1.48 ± 0.30	0.94 ± 0.19
Spleen	2.02 ± 0.20	1.13 ± 0.29	0.94 ± 0.19	0.74 ± 0.16
Kidney	11.48 ± 1.82	10.55 ± 0.56	8.47 ± 0.79	5.34 ± 0.25
Small intestine	7.91 ± 0.74	11.91 ± 0.84	8.11 ± 1.80	7.37 ± 2.04
Muscle	3.09 ± 0.54	2.59 ± 0.46	2.14 ± 0.07	2.10 ± 0.15

Mean ± s.d. (n = 4)

Table 2 Tissue distribution of radioactivity after intravenous injection of [^{11}C]BS-DMPX into mice

	Uptake (%ID/g)			
	5 min	15 min	30 min	60 min
Blood	0.42 ± 0.08	0.35 ± 0.06	0.32 ± 0.03	0.23 ± 0.03
Brain	2.46 ± 0.24	2.35 ± 0.27	2.25 ± 0.21	1.79 ± 0.25
Heart	15.45 ± 3.23	11.20 ± 1.43	9.00 ± 1.30	5.63 ± 2.08
Lung	2.70 ± 0.45	2.07 ± 0.39	1.92 ± 0.51	1.46 ± 0.37
Liver	5.44 ± 0.35	5.91 ± 1.04	4.72 ± 0.60	3.24 ± 0.70
Pancreas	3.13 ± 0.22	2.68 ± 0.71	1.64 ± 0.40	1.00 ± 0.39
Spleen	1.24 ± 0.24	1.38 ± 0.24	1.07 ± 0.11	0.60 ± 0.03
Kidney	10.33 ± 0.90	11.31 ± 2.36	8.20 ± 0.97	5.34 ± 1.56
Small intestine	5.39 ± 1.16	5.81 ± 1.18	5.50 ± 1.68	7.51 ± 1.65
Muscle	3.40 ± 0.49	2.97 ± 0.68	2.81 ± 0.55	2.05 ± 0.60

Mean ± s.d. (n = 4)

iodide). The specific radioactivity was 24–56 GBq/μmol.

Male ddY mice (8 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.

In vitro *affinity for the adenosine A_{2A} and A_1 receptors*

The *in vitro* affinity of IS-DMPX and BS-DMPX for the adenosine A_{2A} and A_1 receptors was determined by using rat striatal membrane and [carboxyethyl-^{3}H(N)]-2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamido adenosine hydrochloride as a radioligand and the rat forebrain membrane and N^6-[^{3}H]cyclohexyladenosine, respectively, as described.[29] All assays were performed in the dark to prevent photoisomerization.

Biodistribution in mice

Each ^{11}C-labeled tracer (1.0 MBq/25–49 pmol) was intravenously injected into mice. They were killed by cervical dislocation at 5, 15, 30 and 60 min post injection. The blood was collected by heart puncture, and the tissues were harvested and the brain was divided into the striatum, cerebellum, cortex and the rest. The ^{11}C in the samples was counted with an auto-gamma counter and the tissues were weighed. The tissue uptake of ^{11}C was expressed as

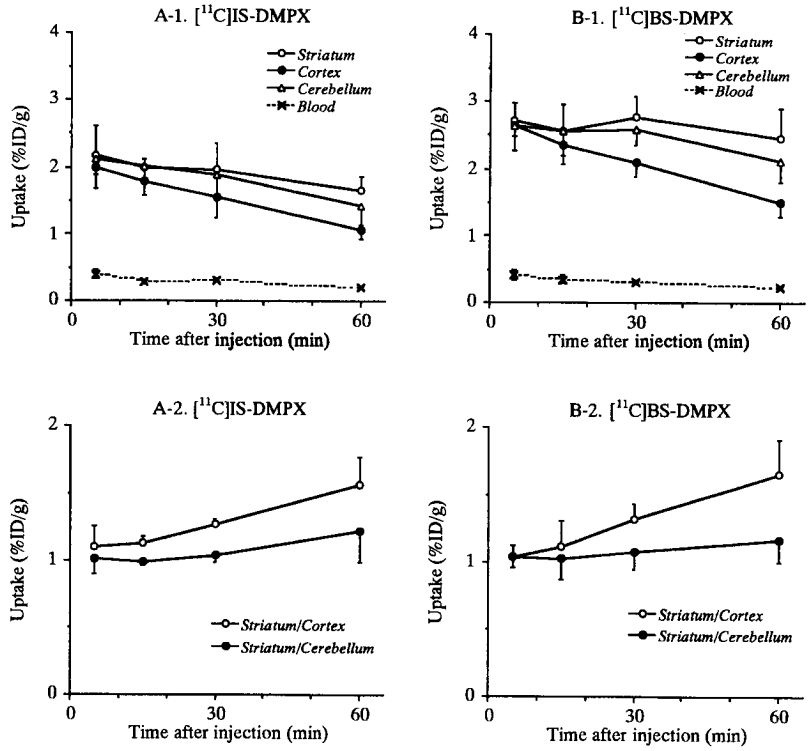


Fig. 2 Time course of regional brain radioactivity uptake and its ratios of striatum to cortex and striatum to cerebellum after an intravenous injection of [^{11}C]IS-DMPX (A) or [^{11}C]BS-DMPX (B) into mice.

the percent injected dose per gram tissue (%ID/g).

In another group of mice, the tracer was co-injected together with one of three adenosine antagonists: carrier IS-DPMX or BS-DMPX, an adenosine A_{2A} antagonist KF17837[26] and an A_1 antagonist KF15372,[23] and the radioactivity levels in the regional brain and blood were measured at 15 min after injection. The amount of the co-injected compounds was 50 nmol/animal.

Ex vivo *autoradiographic study*

Mice were killed 30 min after intravenous injection of [^{11}C]IS-DMPX (46 MBq/1.4 pmol) or [^{11}C]BS-DMPX (57 MBq/1.3 pmol). The brain was rapidly dissected, frozen and sagittally cut into 20 μm-thick sections with a cryotome (Bright Instrument Co., Ltd., Huntingdon, UK). The brain sections were dried on a hot plate at 60°C and were apposed to a storage phosphor screen (PhosphorImager SI system, Molecular Dynamics, Sunnyvale CA, USA) until complete decay. The regional brain distribution was visualized.

RESULTS

In vitro *affinity for the adenosine A_{2A} receptors*

The Ki values of IS-DMPX were 8.9 ± 0.87 nM for the adenosine A_{2A} receptors and >10000 nM for the adenosine A_1 receptors. The Ki values of BS-DMPX were 7.7 ± 0.37 nM for the adenosine A_{2A} receptors and 2300 ± 120 nM for the adenosine A_1 receptors. The two compounds had a similar affinity for A_{2A} receptors, but IS-DMPX was more selective for the A_{2A} receptors than BS-DMPX: A_{2A}/A_1 ratios were >1100 and 300 for IS-DMPX and BS-DMPX, respectively.

Tissue distribution of [^{11}C]IS-DMPX and [^{11}C]BS-DMPX in mice

[^{11}C]IS-DMPX and [^{11}C]BS-DMPX showed a similar tissue distribution (Tables 1 and 2). Blood clearance of the radioactivity was very rapid. At 5 min after the injection, the highest uptake of the two tracers was found in the heart, followed by the kidney, liver or small intestine. In all tissues the radioactivity levels were decreased after 15 min except for the uptake of [^{11}C]BS-DMPX by the small intestine. The brain uptake was low but relatively retained for 60 min among the tissues investigated.

Figure 2 shows the regional brain uptake and the uptake ratio of the striatum to reference tissues. The uptake of the two tracers was slightly higher in the striatum than in the cortex and cerebellum. The uptake ratios of the striatum to reference regions were slightly increased but low. The striatum-to-cortex and striatum-to-cerebellum ratios were 1.6 ± 0.2 and 1.2 ± 0.2, respectively, for [^{11}C]IS-DMPX and 1.7 ± 0.3 and 1.2 ± 0.2, respectively, for [^{11}C]BS-DMPX at 60 min postinjection.

As shown in Figure 3, the uptake of [^{11}C]BS-DMPX by these three regions was significantly reduced by co-

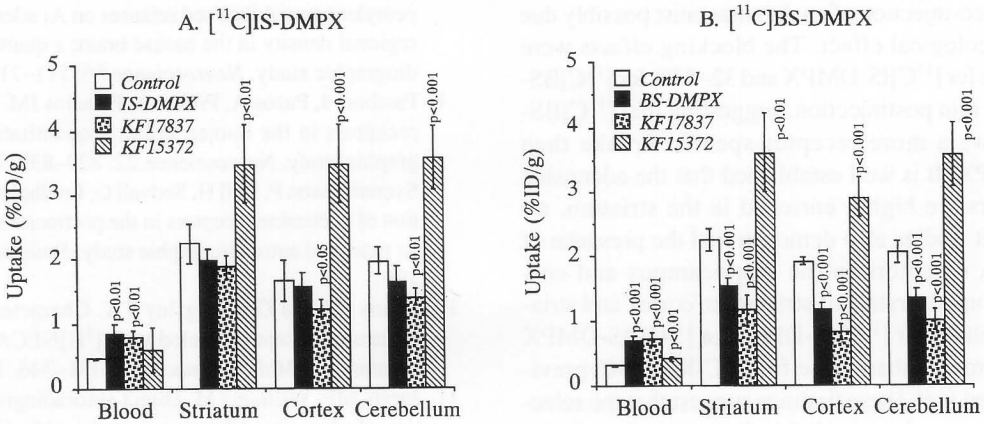


Fig. 3 Effects of adenosine receptor ligands on the regional brain uptake of [^{11}C]IS-DMPX (A) and [^{11}C]BS-DMPX (B) at 15 min after an intravenous injection of the tracer into mice. A Student's t-test was carried out against control.

injection of 50 nmol of carrier or an adenosine A_{2A} antagonist KF17837 (43–68% of the control). It is noted that the uptake by the three regions was significantly increased by an A_1 antagonist KF15372. The blocking effect of the uptake of [^{11}C]IS-DMPX was smaller (71–94% of the control), and only the cortex and cerebellum with KF17837 showed a statistically significant reduction.

Ex vivo *autoradiographic study*

Figure 4 shows the *ex vivo* autoradiographic image of a sagittal brain section of the mouse at 30 min after the tracer injection. A slightly high uptake of [^{11}C]IS-DMPX and [^{11}C]BS-DMPX was found in the striatum, but a high uptake was also observed in other brain regions as shown on tissue dissection.

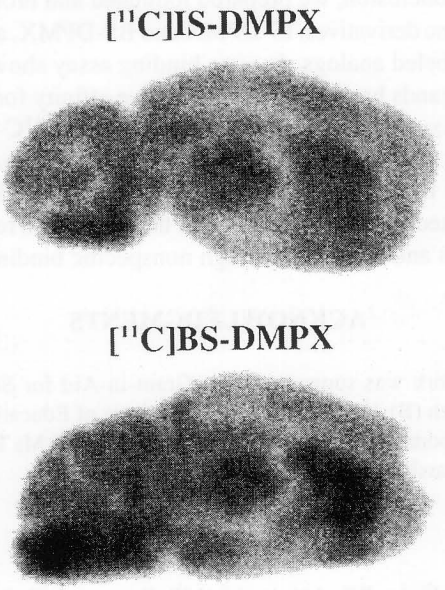


Fig. 4 *Ex vivo* autoradiograms of the rat brain section of [^{11}C]IS-DMPX and [^{11}C]BS-DMPX. The *ex vivo* autoradiography was performed at 30 min after an intravenous injection of ^{11}C-labeled tracers.

DISCUSSION

From the first proposal of KF17837 by Shimada et al., several xanthine derivatives have been widely used as selective antagonists for adenosine A_{2A} receptors.[26–28] Recently we found that [^{11}C]KF18446 is a promising PET ligand for mapping adenosine A_{2A} receptors.[23,24] In the present study, we prepared ^{11}C-labeled new xanthine derivatives containing iodine or bromine and evaluated their *in vivo* characteristics for the purpose of developing radioligands for studying adenosine A_{2A} receptors with PET and SPECT.

In the present membrane binding studies, the two ligands have a similar affinity for A_{2A} receptor: 8.9 ± 0.87 nM for IS-DMPX and 7.7 ± 0.37 nM for BS-DMPX. The affinity of BS-DMPX was comparable to the previously reported value (Ki, 8.2 nM for A_{2a} receptors and 1200 nM for the A_1 receptors).[28] We found that IS-DMPX showed better A_{2A}/A_1 selectivity than BS-DMPX and other A_{2A} ligands[26–28]: >1100 for IS-DMPX versus 300 for BS-DMPX. As compared with [^{11}C]KF18446, which is a previously proposed PET ligand,[23,24] the two ligands had a slightly lower affinity for A_{2A} receptors than KF18446, and IS-DMPX showed more selectivity than KF18446: the Ki value of KF18446 for the A_{2A} receptors was 5.9 nM, and A_{2A}/A_1 selectivity was 270.

In mice both tracers were taken up slightly higher by the striatum than by the cortex and cerebellum, but the uptake by all three regions was reduced by co-injection of carrier or an adenosine A_{2A} receptor antagonist KF17837, suggesting specific uptake by the cortex and cerebellum as well as by the striatum. The uptake was unexpectedly

increased by co-injection of an A_1 antagonist possibly due to a pharmacological effect. The blocking effects were small: 6–29% for [^{11}C]IS-DMPX and 32–57% for [^{11}C]BS-DMPX at 15 min postinjection, suggesting that [^{11}C]BS-DMPX shows a more receptor-specific uptake than [^{11}C]IS-DMPX. It is well established that the adenosine A_{2A} receptors are highly enriched in the striatum, although recent studies also demonstrated the presence of atypical A_{2A} receptors in the hippocampus and cortex.[30–34] The uptake ratios of striatum to cortex and striatum to cerebellum for [^{11}C]IS-DMPX or [^{11}C]BS-DMPX were much smaller than those for [^{11}C]KF18446 previously proposed.[23,24] These findings suggest that the selectivity of the tracers is not suitable for *in vivo* ligands for studying adenosine A_{2A} receptors in the CNS. On the other hand, peripherally both tracers were highly taken up by the heart (Tables 1 and 2), suggesting that they may be used in myocardial imaging as [^{11}C]KF17837.[21]

In conclusion, we prepared iodinated and brominated xanthine derivatives, IS-DPMX and BS-DPMX, and their ^{11}C-labeled analogs. *In vitro* binding assay showed that two ligands have a high and selective affinity for adenosine A_{2A} receptors. Nevertheless, the two ^{11}C-labeled tracers were not suitable ligands for studying A_{2A} receptors of the CNS *in vivo*, because of the low selectivity evaluated by the uptake ratios of the striatum to reference regions and because of a high nonspecific binding.

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REFERENCES

1. Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden TK, Jacobson KA, et al. Nomenclature and classification of purinoceptors. *Pharmacol Rev* 46: 143–156, 1994.
2. Palmer TM, Stiles GL. Adenosine receptors. *Neuropharmacology* 34: 683–694, 1995.
3. Ongini E, Fredholm BB. Pharmacology of adenosine A_{2A} receptors. *Trends Pharmacol Sci* 17: 364–372, 1996.
4. Lewis ME, Patel J, Moon Edley S, Marangos PJ. Autoradiographic visualization of rat brain adenosine receptors using N^6-cyclohexyl[^{3}H]adenosine. *Eur J Pharmacol* 73: 109–110, 1981.
5. Goodman RR, Snyder SH. Autoradiographic localization of adenosine receptors in rat brain using [^{3}H]cyclohexyladenosine. *J Neurosci* 2: 1230–1241, 1982.
6. Fastbom J, Pazos A, Palacios JM. The distribution of adenosine A1 receptors and 5′-nucleotidase in the brain of some commonly used experimental animals. *Neuroscience* 22: 813–826, 1987.
7. Pagonopoulou O, Angelatou F, Kostopoulos G. Effect of pentylentetrazol-induced seizures on A_1 adenosine receptor regional density in the mouse brain: a quantitative autoradiographic study. *Neuroscience* 56: 711–716, 1993.
8. Fastbom J, Pazos A, Probst A, Palacios JM. Adenosine A1 receptors in the human brain: a quantitative autoradiographic study. Neuroscience 22: 827–839, 1987.
9. Svenningsson P, Hall H, Sedvall G, Fredholm BB. Distribution of adenosine receptors in the postmortem human brain: an extended autoradiographic study. *Synapse* 27: 322–335, 1997.
10. Bruns RF, Lu GH, Pugsley TA. Characterization of A_2 adenosine receptor labeled with [^{3}H]NECA in rat striatal membranes. *Mol Pharmacol* 29: 331–346, 1986.
11. Jarvis MF, Williams M. Direct autoradiographic localization of adenosine A2 receptors in the rat brain using the A_2-selective agonist, [^{3}H]-CGS 21680. *Eur J Pharmacol* 168: 243–246, 1989.
12. Parkinson FE, Fredholm, BB. Autoradiographic evidence for G-protein coupled A_2-receptors in rat neostriatum using [^{3}H]-CGS21680 as a ligand. *Naunyn-Schmied Arch Pharmacol* 342: 85–89, 1990.
13. Jansen K, Faull RLM, Dragunow M, Synek BJL. Alzheimer's disease: changes in hippocampal *N*-methyl-D-aspartate, quisqualate, neurotensin, adenosine, benzodiazepine, serotonin and opioid receptors—an autoradiographic study. *Neuroscience* 39: 613–627, 1990.
14. Ulas J, Brunner LC, Nguyen L, Cotman CW. Reduced density of adenosine A1 receptors and preserved coupling of adenosine A1 receptors to G proteins in Alzheimer hippocampus: a quantitative autoradiographic study. *Neuroscience* 52: 843–854, 1993.
15. Glass M, Faull RLM, Bullock JY, Jansen K, Mee EW, Walker EB, et al. Loss of A1 adenosine receptors in human temporal lobe epilepsy. *Brain Res* 710: 56–58, 1996.
16. Martinez-Mir MI, Probst A, Palacios JM. Adenosine A2 receptors: selective localization in the human basal ganglia and alterations with disease. *Neuroscience* 42: 697–706, 1991.
17. Ishiwata K, Furuta R, Shimada J, Ishii S, Endo K, Suzuki F, et al. Synthesis and preliminary evaluation of [^{11}C]KF15372, a selective adenosine A_1 antagonist. *Appl Radiat Isot* 46: 1009–1013, 1995.
18. Furuta R, Ishiwata K, Kiyosawa K, Ishii S, Saito N, Shimada J, et al. Carbon-11 labeled KF15372; a potential CNA adenosine A_1 recepter ligand. *J Nucl Med* 37: 1203–1207, 1996.
19. Ishiwata K, Noguchi N, Toyama H, Shimada J, Suzuki F, Senda M. Synthesis and preliminary evaluation of [^{11}C]KF17837, a selective adenosine A_{2A} antagonist. *Appl Radiat Isot* 47: 507–511, 1996.
20. Noguchi J, Ishiwata K, Furuta R, Simada J, Kiyosawa M, Ishii S, et al. Evaluation of carbon-11 labeled KF15372 and its ethyl and methyl derivatives as a potential CNS adenosine A_1 receptor ligand. *Nucl Med Biol* 24: 53–59, 1997.
21. Ishiwata K, Sakiyama Y, Sakiyama T, Shimada J, Toyama H, Oda K, et al. Myocardial adenosine A_{2a} receptor imaging of rabbit by PET with [^{11}C]KF17837. *Ann Nucl Med* 11: 219–225, 1997.
22. Noguchi J, Ishiwata K, Wakabayashi S, Nariai T, Shumiya S, Ishii S, et al. Evaluation of carbon-11 labeled KF17837:

a potential CNS adenosine A_{2a} receptor ligand. *J Nucl Med* 39: 498–503, 1998.
23. Ishiwata K, Noguchi N, Wakabayashi S, Shimada J, Ogi N, Nariai T, et al. ^{11}C-Labeled KF18446: a potential CNS adenosine A_{2a} receptor ligand. *J Nucl Med* 41: 345–354, 2000 .
24. Ishiwata K, Ogi N, Shimada J, Nonaka H, Tanaka A, Suzuki F, et al. Further characterization of a CNS adenosine A_{2a} receptor ligand [^{11}C]KF18446 with *in vitro* autoradiography and *in vivo* tissue uptake. *Ann Nucl Med* 14: 81–89, 2000.
25. Suzuki F, Shimada J, Mizumoto H, Karasawa A, Kubo K, Nonaka H, et al. Adenosine A_1 antagonists. 2. Structure-activity relationships on diuretic activities and protective effects against acute renal failure. *J Med Chem* 35: 3066–3075, 1992.
26. Shimada J, Suzuki F, Nonaka H, Ishii A, Ichikawa S. (*E*)-1,3-Dialkyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthine: potent and selective adenosine A_2 antagonists. *J Med Chem* 35: 2342–2345, 1992.
27. Müller CE, Geis U, Hipp J, Schobert U, Frobenius W, Pawlowski M, et al. Synthesis and structure-activity relationship of 3,7-dimethyl-1-propargylxanthine derivatives, A_{2A}-selective adenosine receptor antagonists. *J Med Chem* 40: 4396–4405, 1997.
28. Müller CE, Sandoval-Ramírez J, Schobert U, Geis U, Frobenius W, Klotz K-N. 8-(Sulfosytryl)xanthines; water-soluble A_{2A}-selective adenosine receptor antagonists. *Bioorg Med Chem* 6: 707–719, 1998.
29. Nonaka H, Ichimura M, Takeda M, Nonaka Y, Shimada J, Suzuki F, et al. KF17837 ((*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine) a potent and selective adenosine A_2 receptor antagonist. *Eur J Pharmacol* 267: 335–341, 1994.
30. Johansson B, Georgiev V, Parkinson FE, Fredholm BB. The binding of the adenosine A_2 selective agonist [^{3}H]CGS 21680 to rat cortex differs from its binding to rat striatum. *Eur J Pharmacol* 247: 103–110, 1993.
31. Cunha RA, Johansson B, van der Ploeg I, Sebastião AM, Ribeiro JA, Fredholm BB. Evidence for functionally important adenosine A_{2A} receptors in the rat hippocampus. *Brain Res* 649: 208–216, 1994.
32. Cunha RA, Johansson B, Constantino MD, Sebastião AM, Fredholm BB. Evidence for high-affinity binding sites for the adenosine A_{2A} receptor agonist [^{3}H]CGS 21680 in the rat hippocampus and cerebral cortex that are different from striatal A_{2A} receptors. *Naunyn-Schmied Arch Pharmacol* 353: 261–271, 1996.
33. Kirk IP, Richardson PJ. Further characterization of Adenosine A_{2a} receptor-mediated modulation of [^{3}H]CGS 21680 binding sites in the rat striatum and cortex. *Br J Pharmacol* 114: 537–543, 1995.
34. Parkinson FE, Fredholm BB. Further characterization of the binding of the adenosine receptor agonist [^{3}H]CGS 21680 to rat brain using autoradiography. *Neuropharmacology* 34: 393–403, 1995.