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Evaluation of Carbon-11-Labeled KF17837: A Potential CNS Adenosine A2a Receptor Ligand

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The ^{11}C-labeled KF17837 ([7-methyl-^{11}C](E)-8-(3,4-dimethoxy-styryl)-1,3-dipropyl-7-methylxanthine) was evaluated as a PET ligand for mapping adenosine A2a receptors in the central nervous system (CNS). **Methods:** The regional brain distribution of [^{11}C]KF17837 and the effect of adenosine antagonists on the distribution were measured in mice by the tissue sampling method. In rats, the regional brain uptake of [^{11}C]KF17837 and the effect of carrier KF17837 was visualized by autoradiography. Imaging of the monkey brain with [^{11}C]KF17837 was performed by PET. **Results:** In mice, a high uptake of [^{11}C]KF17837 was found in the striatum in which A2a receptors were highly enriched. The uptake was decreased by co-injection of carrier KF17837 or a xanthine-type A2a antagonist CSC but not by nonxanthine-type A2a antagonists ZM 241385 or SCH 58261, or an A1 antagonist KF15372. In the rat brain, [^{11}C]KF17837 was accumulated higher in the striatum than in other brain regions, and the uptake was blocked by co-injection of carrier KF17837. In a monkey PET study, a high striatal uptake of radioactivity was observed. **Conclusion:** Carbon-11-KF17837 binds to adenosine A2a receptors in the striatum. However, the presence of an unknown but specific binding site for xanthine-type compounds also was suggested in the other brain regions. The results also suggested that the in vivo receptor-binding sites of xanthine-type ligands are slightly different from those of nonxanthine-type A2a antagonists.

Key Words: carbon-11-KF17837; xanthine; adenosine A2a receptor; central nervous system; PET

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Adenosine is an endogenous modulator of synaptic functions in the central nervous system (CNS) as well as in the peripheral system. The effect is mediated by two major subtypes of receptors: adenosine A1 receptors, which exhibit higher affinity to adenosine and inhibit adenylyl cyclase, and A2 receptors, which exhibit lower affinity to adenosine and stimulate adenylyl cyclase. Recent advances in molecular biology and pharmacology have demonstrated the presence of at least five subtypes, i.e., A1, A2a, A2b, A3 and A4 receptors. They act with guanosine triphosphate-binding proteins and are coupled not only to adenylyl cyclase but also to ion channels and phospholipases.
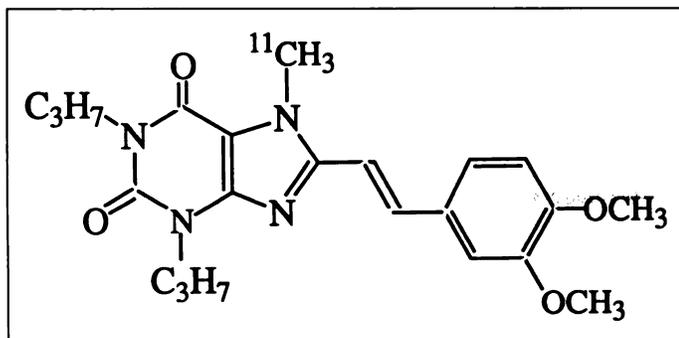
FIGURE 1. Structural formula of [^{11}C]KF17837.

The current status of the adenosine receptors has been reviewed (1–5).

In the CNS, A$_1$ receptors are present throughout the presynaptic region of excitatory neurons, being rich in the hippocampus, cerebral cortex, thalamic nuclei, basal ganglia and cerebellar cortex in animals (6–9) and humans (10). A$_{2a}$ receptors are highly enriched in the striatum, nucleus accumbens and olfactory tubercle in which dopamine D1 and D2 receptors also are localized with very high densities (11–13), whereas A$_{2b}$ receptors show a ubiquitous distribution. By the in situ hybridization technique, adenosine A$_{2a}$ receptor mRNA and dopamine D2-receptor mRNA were found to be mainly expressed in striatopallidal γ-aminobutyric acid (GABA)-enkephaline neurons (14–16). In the patients with Huntington's chorea with a selective degeneration of the striatopallidal neurons, the A$_{2a}$ receptor density is significantly reduced in the striatum, whereas the density is not affected significantly in the patients with Parkinson's disease characterized by a selective degeneration of nigrostriatal dopamine neurons (17). Recent pharmacological and biochemical data suggest the presence of a receptor-receptor interaction between adenosine A$_{2a}$ and dopamine D2-receptors in the striatum (18). Adenosine A$_{2a}$ receptors also mediate striatal GABA and acetylcholine release (19,20).

During the last decade, many neuroreceptors in humans and other animals have been visualized in vivo by PET with corresponding radioligands. Although adenosine itself is not a neurotransmitter, PET assessment of the adenosine receptor system offers the opportunity to understand the general neurotransmission system because of its physiological functions of modulating the neuroreceptor system nonspecifically. Recently, we have developed PET ligands for the two adenosine receptor subtypes: [^{11}C]KF15372 ([3-propyl-^{11}C]8-dicyclopropylmethyl-1,3-dipropylxanthine) (21,22) and its methyl and ethyl derivatives (23) for adenosine A$_1$ receptors, and [^{11}C]KF17837 {[7-methyl-^{11}C](E)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine} (Fig. 1) for the adenosine A$_{2a}$ receptors (24). The selectivity and usefulness of [^{11}C]KF15372 and its derivatives as PET A$_1$ receptor ligands have been demonstrated in the previous studies (22,23). In a preliminary tissue distribution study of [^{11}C]KF17837, a higher striatal uptake of the tracer was confirmed in rodents (24). In vitro autoradiography with [^{3}H]KF17837 also indicated that the compound is a selective adenosine A$_{2a}$ ligand (25). In this study, we characterize the properties of [^{11}C]KF17837 in vivo as a PET tracer in mice, rats and monkeys.

MATERIALS AND METHODS

(E)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (KF17837) and (E)-8-(3,4-dimethoxystyryl)-1,3-dipropylxanthine, 8-(3-chlorostyryl) caffeine (CSC), (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) 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261) and 8-dicyclopropylmethyl-1,3-dipropylxanthine (KF15372) were synthesized by Kyowa Hakko Kogyo Company. Alloxazine was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI) and 1,3-dipropyl-7-methylxanthine (DPMX) was obtained from Research Biochemical, Inc. (Natic, MA).

Radiosynthesis of [^{11}C]KF17837 (Fig. 1) was performed by ^{11}C-methylation of (E)-8-(3,4-dimethoxystyryl)-1,3-dipropylxanthine as described previously (24). The specific activity was 37–64 GBq/μmol.

Regional Brain Distribution Study in Mice

We used three groups of male ddY mice (29–38 g). In the first group, [^{11}C]KF17837 (450–550 kBq/9.2–20 pmol) was injected intravenously into mice; and the second group was given [^{11}C]KF17837 (450–550 kBq/9.2–20 pmol) together with carrier KF17837 (50 nmol). These mice were killed at 5, 15, 30 and 60 min after injection by cervical dislocation (n = 4). In the third group, [^{11}C]KF17837 (540–1100 kBq/22–91 pmol) was co-injected with one of the following adenosine antagonists into mice. KF17837 (50 nmol, Ki: A$_1$, 62 nM; A$_{2a}$, 1.0 nM) (26), CSC (100 nmol, Ki: A$_1$, 28 μM; A$_{2a}$, 54 nM) (27,28) and DPMX (100 nmol, Ki: A$_1$,7 μM; A$_{2a}$, 1.2 μM) (29) are xanthine type A$_2$ antagonists. ZM 241385 (375 nmol, Ki: A$_1$, 510 nM; A$_{2a}$, 0.91 nM) (30), SCH 58261 (50 nmol, Ki: A$_1$, 121 nM; A$_{2a}$, 2.3 nM) (31) and alloxazine (100 nmol, Ki: A$_1$, 5.25 μM; A$_{2a}$, 2.72 μM) (11) are nonxanthine A$_2$ antagonists. KF15372 (100 nmol, Ki: A$_1$, 3.0 nM; A$_{2a}$, 430 nM) (32) is a xanthine type A$_1$ antagonist. The mice were killed 15 min after injection (n = 22 for control and n = 4 for each of the other groups). Blood was collected by heart puncture. The brain was removed and divided into the striatum, cerebellum and cortex. The tissue uptake of radioactivity was expressed as the percent injected dose per gram of tissue (% ID/g).

Autoradiographic Study in Rats

Male Wistar rats (220–270 g) were killed 15 min after intravenous injection of [^{11}C]KF17837 (67–216 MBq/0.84–13.2 nmol, n = 4). The effect of co-injected carrier KF17837 (100 nmol/animal, n = 3; and 700 nmol/animal, n = 3) on the brain uptake also was measured at 15 min after injection. Coronal and sagittal brain sections were prepared as described previously (22). For the coronal section, the right brain hemisphere of the rat injected with [^{11}C]KF17837 and the left brain hemisphere of the rat given [^{11}C]KF17837 with carrier KF17837 were put side by side to make the hybrid brain for comparative demonstration. Regional brain uptake was analyzed using an imaging plate and a bioimaging analyzer of type BAS 3000 (FUJIX, Fuji Photo Film Co., Tokyo, Japan). The regional brain uptake of radioactivity was measured as the photo-stimulated luminescence (PSL) and expressed as the PSL/mm^2/MBq in which the injected dose was decay-corrected at the contact time.

PET Study in a Monkey

A female rhesus monkey (22 yr, 3.4 kg) was anesthetized with 0.01–0.05% isoflurane. Carbon-11-KF17837 (97 MBq/2.2 nmol) was injected intravenously into the monkey, and the time sequential tomographic scanning was performed in the coronal section of the brain for 60 min (10 frames for 1 min, 4 frames for 5 min and 3 frames for 10 min). The PET camera used was a model SHR 2000 (Hamamatsu Photonic, Hamamatsu, Japan), which consists of four ring detectors and accomodates seven slices with a resolution of 4.0 mm FWHM in the transaxial plane (33). Regions of interest (ROI) were placed on the striatum, cortex, cerebellum and thalamus, and the time-activity curve in the ROI was obtained for each
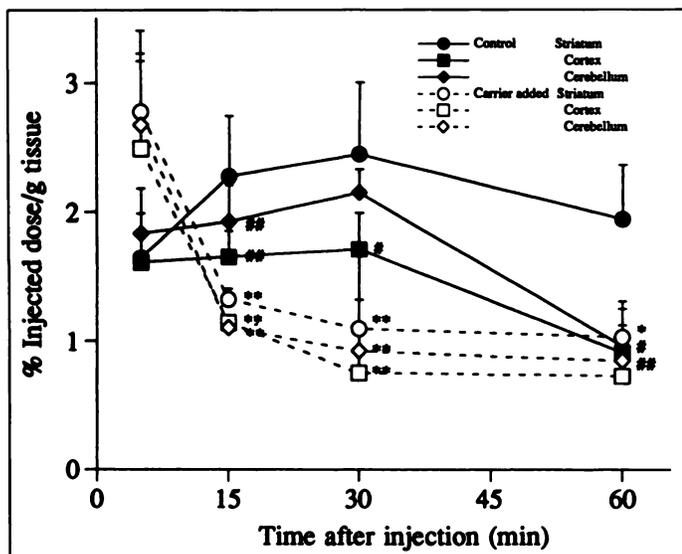
FIGURE 2. Regional brain distribution of radioactivity and effect of co-injected carrier KF17837 after an intravenous injection of [^{11}C]KF17837 into mice. Injection dose was 450–550 kBq/9.2–20 pmol. Student's t-test was performed between the striatum and cortex or cerebellum in the control, * represents p < 0.1 and ** represents p < 0.01. Between the control and carrier-loading group, * represents p < 0.1 and ** represents < 0.01. Solid and open symbols represent the control and carrier-loading group, respectively. Circle = striatum; square = cortex; and diamond = cerebellum.

scan of the brain as described (34). The radioactivity value was expressed as the standardized uptake value [SUV, (regional activity/milliliter volume)/injected activity/gram body weight].

RESULTS

Regional Brain Distribution Study in Mice

Figure 2 shows the regional brain uptake of [^{11}C]KF17837 in mice and the effect of co-injected carrier KF17837 on the uptake. In the control, the uptake of [^{11}C]KF17837 by the striatum, cortex and cerebellum gradually increased up to 30 min and then decreased. The uptake was much higher in the striatum than in the cortex and cerebellum after 15 min. By co-injection of carrier KF17837, the tracer uptake by the striatum, cortex and cerebellum was enhanced at 5 min after injection but was reduced markedly 15 min after injection in the three regions. At 30 min, the tracer uptake was decreased to the lowest level, one-third of the controls. At 60 min, no difference in the uptake by the cortex and cerebellum was found between the control and carrier-loading group, although the striatal uptake was significantly reduced by the carrier-loading.

Table 1 shows the effects of co-injection of several adenosine antagonists. The tracer uptake by the striatum, cortex and cerebellum was decreased by carrier KF17837 or a xanthine-type A_{2a} antagonist CSC but was not affected by a weak xanthine-type A_{2a} antagonist DPMX. The uptake was not affected by nonxanthine-type A_{2a} antagonists ZM 241385, SCH 58261 or alloxazine or an A_1 antagonist KF15372.

Autoradiographic Study in Rats

Figure 3 shows the autoradiographic images of coronal and sagittal brain sections of the rats. In the coronal section (Fig. 3A), [^{11}C]KF17837 was accumulated higher in the striatum than in the cortex. In the sagittal section (Fig. 3B), the regional brain distribution was visualized clearly. The mean uptake assessed as the PSL/mm^2/MBq was 0.89 (n = 4) in the striatum, 0.59 (n = 4) in the cortex and 0.74 (n = 3) in the cerebellum. When carrier KF17837 (700 nmol/animal) was co-injected (Figs. 3A and 3C), the brain structure disappeared, and the mean uptake values were decreased to 0.61 (n = 3) in the striatum, 0.45 (n = 3) in the cortex and 0.51 (n = 2) in the cerebellum. In the control brain, the mean striatum-to-cortex ratio was 1.51 and the mean striatum-to-cerebellum ratio was 1.20. By co-injection of carrier KF17837 (700 nmol), the striatum-to-cortex ratio was 1.36 and the striatum-to-cerebellum ratio was 1.20. When 100 nmol of KF17837 was co-injected, the image was similar to that of the control (data not shown) and the mean striatum-to-cortex ratio was 1.56 (n = 3) and the mean striatum-to-cerebellum ratio was 1.24 (n = 2).

PET Study in a Monkey

Figure 4 shows images of the rhesus monkey brain acquired by PET with [^{11}C]KF17837. A high uptake of radioactivity was observed in the striatum. The uptake also was visualized in the cortex, cerebellum and thalamus. The time-activity curves in the these regions during 60 min are shown in Figure 5. The radioactivity level (SUV) in the striatum was slightly higher than those in other brain regions. The striatal activity level was retained for the initial 20 min and then gradually decreased with time. The uptake ratio of striatum-to-cortex, striatum-to-cerebellum and striatum-to-thalamus was 1.38, 1.32 and 1.38, respectively, 30 min after injection.

TABLE 1
Effect of Adenosine Antagonists on the Regional Brain Distribution of Radioactivity at 15 Minutes After Intravenous Injection of Carbon-11-KF17837 into Mice

Antagonist (nmol)*	Percent injection dose/gram tissue			
	Blood	Striatum	Cortex	Cerebellum
Control	0.84 ± 0.13	2.28 ± 0.47	1.65 ± 0.20	1.93 ± 0.28
KF17837 (50)	1.19 ± 0.05	1.32 ± 0.09‡	1.14 ± 0.02†	1.10 ± 0.03‡
CSC (100)	1.16 ± 0.18	2.02 ± 0.51	1.27 ± 0.26†	1.38 ± 0.29†
DPMX (100)	0.97 ± 0.21	2.33 ± 0.41	1.93 ± 0.24	2.20 ± 0.26
ZM 241385 (375)	0.96 ± 0.10	2.60 ± 0.05	2.08 ± 0.10	2.37 ± 0.22
SCH 58261 (50)	1.07 ± 0.08	2.75 ± 0.24	2.45 ± 0.39	2.77 ± 0.55
Alloxazine (100)	0.80 ± 0.13	1.96 ± 0.39	1.74 ± 0.42	1.96 ± 0.50
KF15372 (100)	1.06 ± 0.09	2.31 ± 0.37	1.83 ± 0.17	2.07 ± 0.32

*Co-injected doses of antagonists are indicated in parentheses.
Student's t-test was performed between the control and the group given antagonists:
†p < 0.01 and ‡p < 0.001.
Mean ± s.d. (control, n = 22 and other groups, n = 4). Injection dose: 540–1100 MBq/22–91 pmol.

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DISCUSSION

In this study, we have characterized the in vivo properties of [^{11}C]KF17837 as a PET tracer. In mice, rats and a monkey, the tracer was taken up at a higher level by the striatum, which is rich in the adenosine A_{2a} receptors, than by the other brain regions. This is consistent with the distribution of adenosine A_{2a} receptors demonstrated by an in vitro binding assay with the brain membrane fraction (11) or by autoradiography (ARG) (12,13) and with that of A_{2a} receptor mRNA measured by the in situ hybridization technique (14–16).

In the competition study, the tracer uptake was enhanced in 5 min by carrier loading in the striatum, cortex and cerebellum. This phenomenon may be derived from the altered peripheral absorption or pharmacological effect of a large amount of KF17837. On the other hand, after 5 min the striatal uptake of the tracer was significantly decreased. While the uptake in the cortex and cerebellum was also clearly blocked at 15 and 30 min, the tracer has washed out from the cortex and cerebellum but not from the striatum at 60 min, leaving the binding of the tracer in the cortex and cerebellum at a nonspecific level. The binding of the tracer in the striatum and in the cortex and cerebellum may have differing kinetics. The slight reduction also was observed by co-injection of a xanthine type A_{2a} antagonist CSC, though the difference was not significant in case of the striatum, but not by co-injection of a weak A_{2a} antagonist DPMX or a strong A_1 antagonist KF15372. These findings suggested that [^{11}C]KF17837 has a selective affinity for adenosine A_{2a} receptors.

In the regional brain distribution study, the tracer uptake by the cortex and cerebellum, which lack A_{2a} receptors, also was decreased by co-injection of KF17837 or CSC. The phenomenon also was detected in the present ex vivo and a previous in vitro ARG studies (25). In the in vitro ARG study on [^{3}H]KF17837S, in which an active E-form (18%) of [^{3}H]KF17837 and an inactive Z-isomer (82%) were mixed, the specific binding in vitro was 26% of the total binding in the cerebral cortex and 12% in the cerebellum (25). The corresponding figures assessed by the blocking effect on the ex vivo ARG were 24% in the cortex and 31% in the cerebellum. These findings suggest the presence of unknown but specific binding sites for xanthine-type compounds in the cortex and cerebellum, considering both the differing kinetics of the tracer binding in the cortex and cerebellum and in the striatum as described

FIGURE 3. Autoradiograms of the brain section at 15 min after an intravenous injection of [^{11}C]KF17837 with and without carrier KF17837 into rats. (A) Coronal section of rat brain in which the right hemisphere of the control and the left hemisphere of the rat given carrier-loading [^{11}C]KF17837 were put side-by-side for comparison. The left side shows a control image and the right side shows a carrier-added image. (B) Control sagittal section. (C) Carrier-added sagittal section.

above. A possibility is that KF17837 may have an affinity for A_{2b} receptor in vivo as well. We tried alloxazine as a weak A_{2b} antagonist (35) because a selective adenosine A_{2b} antagonist was not available, but it did not reduce the uptake of [^{11}C]KF17837. However, we cannot determine whether the [^{11}C]KF17837 binds to the A_{2b} receptors or not.

The in vitro ARG has shown that the total [^{3}H]KF17837S-binding was concentrated 2.3–3.0 times higher in the striatum than in the other regions (25). The specific binding assessed by competition with a large amount of carrier KF17837S was 64% in the striatum. When the cortex and cerebellum were assumed to be the reference region free of A_{2a} receptors, the specific binding (striatum minus reference region) was estimated to be 56–67% from their data. On the other hand, the present ex vivo ARG study has shown that the total [^{11}C]KF17837 uptake was 1.2–1.5 times higher in the striatum than in the other regions at 15 min postinjection (Fig. 3B). The A_{2a} receptors-specific uptake by the striatum was 31% of the total uptake assessed by the self-competition or 17–34% assessed by the difference between the striatum and reference regions. The investigated time may not be an ideal point for comparing in vitro and in vivo data. We performed the blocking studies in the regional brain distribution in mice and in the ARG in rats at 15 min after tracer injection because of the short-half life of ^{11}C. In mice, the specific uptake assessed by the self-competition at this time was approximately two-thirds that of the highest specific uptake at 30 min postinjection. Because of the susceptibility of a xanthine compound to metabolical alterations (22,23), the labeled metabolites possibly affect the assessment of the specific binding of the tracer in vivo.

The co-injection of nonxanthine-type adenosine antagonists ZM 241385 or SCH 58261, which have a high affinity for the A_{2a} receptors, did not reduce the uptake of [^{11}C]KF17837, although the affinity of ZM 241385 (30) and SCH 58261 (31) for the A_{2a} receptors is 180 times and 20 times, respectively, higher than that of xanthine-type CSC (27,28) in the membrane binding assay. One possibility is that a sufficient amount of ZM 241385 or SCH 58261 to block the receptor binding of the tracer did not enter the brain. However, both compounds are lipophilic. Therefore, it is doubtful that both compounds would have had difficulty crossing the blood-brain barrier. On the other hand, if all the ZM 241385 and SCH 58261 penetrated the blood-brain barrier, the blocking effect of both compounds on the different regions of the brain would still be less than CSC based on our finding that [^{11}C]KF17837 also has an affinity for the myocardial adenosine A_{2a} receptors (24). The blocking effect of ZM 241385 and SCH 58261 on the myocardial uptake of [^{11}C]KF17837 was smaller than that of CSC (data will be described elsewhere). There is no barrier in the heart that corresponds to the blood-brain barrier in the CNS. Therefore, the most logical explanation would be that xanthine-type and nonxanthine-type antagonists may recognize different binding sites besides the common binding site within the A_{2a} receptor in vivo. Alternatively, the in vivo configuration of the receptor molecule itself or of the receptor-containing region of the plasma membrane may provide different binding sites for the xanthine-type and nonxanthine-type structures.

This study demonstrated the adenosine A_{2a} receptors in the primate brain in vivo by PET. By the PET scanning, the striatal uptake of [^{11}C]KF17837 was visualized clearly, and the SUV value was larger in the striatum than in the cortex, cerebellum and thalamus of the monkey. When the uptake in reference brain regions besides the striatum was assumed to be the nonspecific binding, the A_{2a} receptor-specific binding of the

FIGURE 4. PET images of the anesthetized monkey brain with [^{11}C]KF17837. The images were acquired for 50 min starting at 10 min after the injection. Injection dose was 97 MBq/2.2 nmol. The values in the color scale are SUV by 10^4 times.

[^{11}C]KF17837 was approximately 28% of the total striatal uptake.

CONCLUSION

The regional brain distribution and blocking studies in mice, ex vivo ARG with blocking studies in rats and PET imaging in primates have suggested that [^{11}C]KF17837 binds adenosine A_{2a} receptors in the striatum. However, the presence of an unknown but specific binding site for the xanthine-type compounds also was suggested in the other brain regions.
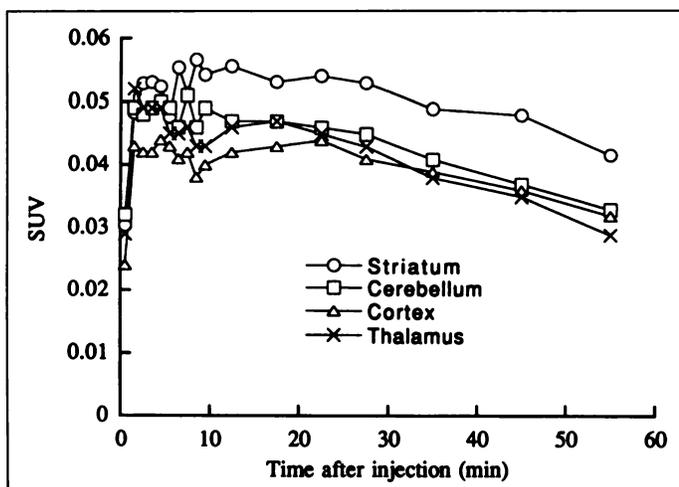
ADDENDUM

During discussion of the manuscript, Stone-Elander et al. reported the PET study of the same compound in the rhesus monkeys (*Nucl Med Biol* 1997;24;187–191). They stated the limited usefulness of the tracer because of low penetration across the blood-brain barrier and the high nonspecific binding.

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FIGURE 5. Time-activity curves in the rhesus monkey brain [^{11}C]KF17837.

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Fully Automated Establishment of Stereotaxic Image Orientation in Six Degrees of Freedom for Technetium-99m-ECD Brain SPECT

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Anatomical localization requires establishing an anatomical space within the image matrix. We developed a fast, fully automated method to establish the image orientation for ^{99m}Tc-ethylcysteinate dimer (ECD) brain SPECT images. **Methods:** The image orientation of ECD brain SPECT images was established in four stages. First, the brain surface was edge-detected as an isosurface at an adaptive threshold. Second, a "convex hull" was determined for the isosurface to minimize regional variability in brain shape. A principal axis transformation and a symmetry vector analysis were applied to the convex hull to resolve the craniocaudal direction and to estimate the midsagittal plane. Third, the brain orientation was refined from this estimate by location of the interhemispheric fissure, the tentorial groove and the frontotemporal groove on the isosurface. Last, the intercommissural (anterior commissure–posterior commissure, or AC–PC) line was detected on the midsagittal slice, and the Talairach grid was scaled to fit the maximal brain dimensions from the AC–PC line. **Results:** The average absolute errors were 2.3° ± 1.5° and 1.08 mm ± 1.11 mm for the midsagittal plane (n = 24) and 2.04° ± 0.80°, 2.0% ± 1.8% of the brain length and 2.3% ± 2.2% of the brain height for the AC–PC line (n = 8). In addition, this program successfully established the image orientation in 94 of 100 clinical ECD brain SPECT studies. Processing time was <40 sec for 128 × 128 × 50 matrices on a DEC Alpha workstation. **Conclusion:** We have developed a fast, robust and fully automated method that determines the orientation of ECD brain SPECT images. This objective method of standardizing the image orientation should be useful for anatomical localization and clinical interpretation of these images.

Key Words: technetium-99m-ECD; brain SPECT; image orientation; automation; image processing

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In recent years, there has been increasing recognition of the clinical application of functional brain imaging using SPECT and PET. In interpreting these images, it is often essential to accurately localize detected functional signals in the anatomical space of the brain. This anatomical localization requires establishing an anatomical space within the image matrix. The spatial relationship between the anatomical space and the image matrix is referred to as the image orientation, and it is defined by six degrees of freedom (three translational coordinates along and three rotational angles around the x-, y- and z-axes). A common approach to anatomical localization has been the concurrent use of morphological imaging such as MRI or x-ray CT, which provides detailed anatomical information that functional images may lack (1–4). Image coregistration between the two types of imaging modalities allows the image orientation of SPECT or PET to be established in the anatomical space of MRI or CT.

Alternatively, anatomical localization can be achieved by establishing a standard anatomical space, such as the Talairach stereotaxic coordinate system (5), in the functional image. An advantage of this approach is that MRI or CT is not required. Furthermore, this approach enables intersubject comparison of corresponding functional signals by matching signals associated with the same stereotaxic locations. In several reports (6–8), a standard anatomical space has been established by using fiducial markers for the canthomeatal or orbitomeatal lines to estimate the stereotaxic baseline that passes through the anterior and posterior commissures of the brain (the AC–PC line). However, the limitation of this and other fiducial marker-based methods is the significant variability between the external and the true brain orientations (9). An alternative method is to derive information about image orientation, such as the AC–PC line, retrospectively from internal landmarks within the functional image (10–12).