Annals of Nuclear Medicine Vol. 17, No. 3, 249–253, 2003

Age-related changes of the [^{11}C]CFT binding to the striatal dopamine transporters in the Fischer 344 rats: a PET study

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We investigated the age-related changes of the binding of [^{11}C]CFT to striatal dopamine transporters (DATs) *in vivo* in Fischer 344 rats by positron emission tomography (PET). The tissue dissection method represented an age-related decrease in the uptake ratio of the striatum to the cerebellum and in the specific binding-to-nonspecific binding ratio of [^{11}C]CFT. PET demonstrated an age-dependent decrease in the striatal uptake of [^{11}C]CFT, however, the kinetic analysis represented the age-related decrease in both the association rate constant (k_3) and dissociation rate constant (k_4), but not the binding potential (k_3/k_4) that was a parameter including both of density and affinity of the binding sites. The PET finding was not necessarily coincident with the result investigated *in vitro* previously. Therefore, careful interpretation is necessary for PET studies using [^{11}C]CFT and small animals such as rats.

Key words: aging, dopamine transporter, [^{11}C]CFT, positron emission tomography

INTRODUCTION

IN VIVO IMAGING of dopamine transporters (DATs) by positron emission tomography (PET) and single photon emission computed tomography is a useful technique for the diagnosis of degenerative brain disorders involving dopaminergic systems such as Parkinson's disease.

PET studies with rats are useful for noninvasive characterization of neuroreceptor functions and directly compared with *in vitro* studies. Several PET studies have been applied to characterize the radioligand binding to dopamine receptors or dopamine transporters in the striatum of rat models.[1,4,7,14] As for PET measurement of age-related changes of neuroreceptors in rats, Suzuki et al.[13] reported that the binding potentials of [^{11}C]SCH23390 to dopamine D_1 receptors and of [^{11}C]raclopride to dopamine D_2 receptors in the striatum were decreased with age. We found that the [^{11}C]raclopride binding in the rat striatum was enhanced by the dopamine D_2 receptor gene transfer mediated by adenoviral vectors.[12,16]

The aim of present study clarified the age-related changes of the binding of [^{11}C]2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane ([^{11}C]CFT or [^{11}C]WIN 35,428) to striatal DATs *in vivo* in Fischer 344 rats by PET, in order to demonstrate the usefulness of PET application to the rat model. CFT is a cocaine analog lacking the ester bond between the phenyl group and the tropane ring. It has a selective affinity for DATs (K_i = 14.7 nM; DATs/serotonin transporters selectivity, 12.3; DATs/norepinephrine transporters selectivity, 43.2).[11] Its DATs binding characteristics and pharmacokinetic properties have been very well characterized *in vitro* and *in vivo* in rodents,[2] primates,[8] and humans.[10] *In vivo* accumulation of [^{3}H]CFT in the striatum was reduced by more than 50% in aged monkeys compared with young adult monkeys,[8] and a significant age-related decrease in the overall density (B_{max}) of [^{3}H]CFT binding sites in the striatum was observed *in vitro* in the Fischer 344 rats.[3]

MATERIALS AND METHODS

Six-month-old (311–363 g, N = 6), 12-month-old (376–450 g, N = 9) and 24-month-old (388–492 g, N = 7) virgin

Received October 9, 2002, revision accepted December 25, 2002.

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male Fischer-344 rats were supplied from the Department of Laboratory Animal Science at the Tokyo Metropolitan Institute of Gerontology. The animal studies were approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology.

[^{11}C]CFT was synthesized by the reaction of [^{11}C]methyl triflate and the demethyl compound as previously described.[5,9]

PET study

PET examinations were carried out as previously described.[6,7] A rat was anesthetized with isoflurane, and was placed in a prone position on the holder. [^{11}C]CFT (9.0–24.0 MBq/0.32–1.35 nmol) was intravenously injected through tail vein, and the PET scanning was performed over a period of 60 min (20 frames by 30 sec and 50 frames by 1 min). PET camera used was a model SHR 2000 (Hamamatsu Photonics K.K., Hamamatsu, Japan) which consists of four detector rings and acquires seven slices at a center-to-center interval of 6.5 mm with a resolution of 4.0 mm full width at half maximum in the transaxial plane. The regions of interest were placed on the striatum and cerebellum. The radioactivity levels are expressed as the percentage of injected dose per m*l* tissue volume (%ID/m*l*). Time-activity curve of the cerebellum as an input function and that in striatum were fitted to a two-parameter compartment model to estimate the kinetic parameters (k_3 and k_4).[4,7,16] The k_3 and k_4 values were association and dissociation rate constants, respectively, for the ligand-receptor binding. The estimation of kinetic parameters was performed on the nonlinear least-square method using UNIX workstation O2 (SGI, California, USA) and medical software Dr.View (Asahi Kasei Joho Systems, Tokyo, Japan). The values of the binding potential ($BP = k_3/k_4$) were calculated by determining the ratio of the estimated k_3 value to the estimated k_4 value.

Tissue dissection study

Rats were killed by cervical dislocation just after the end of PET scanning for 60 min. The brain was removed and dissected into the striatum, the cerebral cortex and the cerebellum. The tissues were measured for the radioactivity with an auto-gamma counter and weighed. The uptake was expressed as the percentage of the injected dose per gram of the tissue (%ID/g). The *in vivo* specific binding and the *in vivo* nonspecific binding were determined as the residual uptake after the subtraction of the cerebellar uptake from the striatal uptake (striatal uptake – cerebellar uptake) and the cerebellar uptake, respectively.

Statistical analysis

Results are expressed as mean ± standard deviation (S.D.). Comparison among three groups was performed by an one-way analysis of variance (ANOVA) followed by Turkey-Kramer post hoc test for multiple group comparison using Statview 5.0 software (SAS Institute Inc., Cary, NC, USA). $p < 0.05$ was considered statistically significant.

RESULTS

The [^{11}C]CFT-PET images of the brain in three groups of rats were shown in Figure 1. The age-related decrease of the [^{11}C]CFT binding in the striatum was observed. Time activity curves in the striatum, the cerebellum and the specific binding in the striatum are shown in Figure 2. The radioactivity level gradually decreased after initial uptake in the striatum in all groups of rats, but rapidly deceased in the cerebellum. The peak levels of [^{11}C]CFT uptake in both the striatum and the cerebellum tended to decrease with age. The specific binding increased for the first 30 min and then remained constant.

The rate constants and binding potentials in the striatum are summarized in Table 1. Both k_3 and k_4 values decreased with age. The k_3 value in the 24-month-old rats significantly decreased to 78% and to 82% of those in the 6- and 12-month-old rats, respectively. The k_4 value in

Table 1 Age-related changes of the kinetic parameter of the striatal binding of [^{11}C]CFT measured by PET in the Fischer 344 rats

	Age (month)		
	6	12	24
k_3	0.041 ± 0.007	0.039 ± 0.007	0.032 ± 0.007*,#
k_4	0.034 ± 0.008	0.031 ± 0.006	0.027 ± 0.006*
BP (= k_3/k_4)	1.23 ± 0.25	1.26 ± 0.14	1.21 ± 0.19

Mean ± S.D. (N = 6–9)
*$p < 0.05$: 6 months-old rats vs. 24 months-old rats.
#$p < 0.05$: 12 months-old rats vs. 24 months-old rats.

Table 2 Age-related changes of the uptake of [^{11}C]CFT in the cortex, striatum and cerebellum measured by the tissue dissection method in of Fischer 344 rats 60 min after injection

	Age (month)		
	6	12	24
	Uptake (%ID/g)		
Cortex	0.16 ± 0.04	0.16 ± 0.03	0.17 ± 0.03
Striatum	1.13 ± 0.31	1.05 ± 0.19	0.88 ± 0.16
Cerebellum	0.09 ± 0.02	0.09 ± 0.01	0.10 ± 0.02
Specific binding**	1.04 ± 0.29	1.02 ± 0.22	0.79 ± 0.15
	Uptake ratio		
Striatum/cerebellum	12.4 ± 2.0	12.1 ± 2.2	8.7 ± 0.4*,#
Specific binding/nonspecific binding**	11.4 ± 2.0	12.0 ± 2.5	7.7 ± 0.2*,#

Mean ± S.D. (N = 6–9)
*$p < 0.05$: 6 months-old rats vs. 24 months-old rats.
#$p < 0.05$: 12 months-old rats vs. 24 months-old rats.
**Specific binding and nonspecific binding were determined as the deference between the striatal uptake and cerebellar uptake and the cerebellar uptake, respectively.

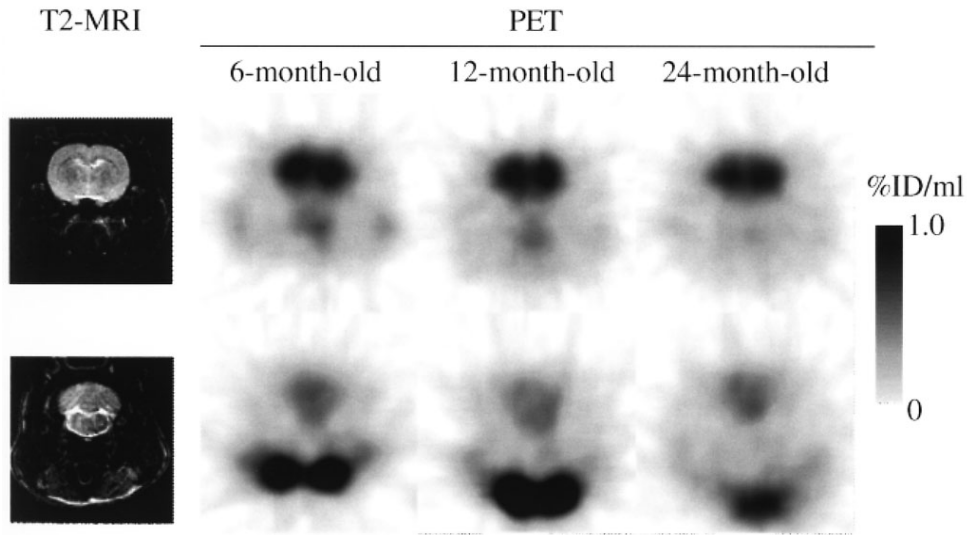

Fig. 1 Brain PET images with [^{11}C]CFT of three groups of Fischer 344 rats (6-, 12- and 24-month-old), and reference MRI images corresponding to these PET images. The [^{11}C]CFT-PET images were acquired for 10 min starting 50 min after injection.

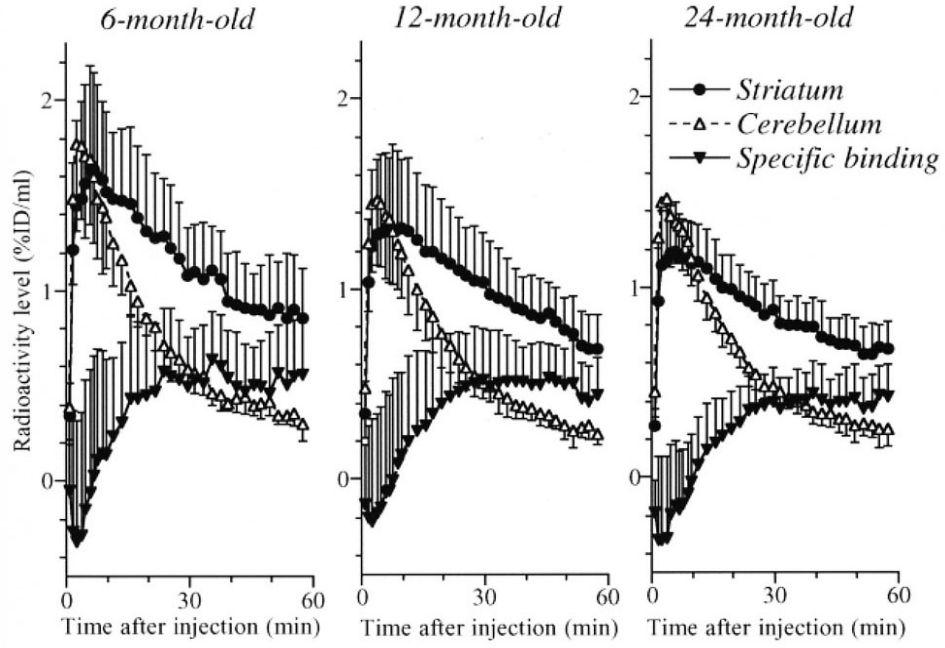

Fig. 2 Time-activity curves on the striatum, cerebellum and the specific binding in the striatum of three groups of Fischer 344 rats (6-, 12- and 24-month-old) after intravenous injection of [^{11}C]CFT. The specific binding in the striatum was determined as the difference between the striatal uptake and the cerebellar uptake. The radioactivity levels are expressed as the percentage of injected dose per m*l* tissue volume (%ID/m*l*).

the 24-month-old was 79% and 87% of those in the 6- and 12-month-old rats, respectively. However, there is no significant change in the binding potential between each of the three groups.

The uptake of [^{11}C]CFT was directly measured by tissue dissection. Table 2 showed a tendency that the uptake decreases with age in the striatum but not in the cerebellum and the cortex. The uptake ratio of the striatum to the cerebellum, and the ratio of the specific binding to the nonspecific binding significantly decreased in 24-month-old rats when compared with those in 6- and 12-month-old rats, although there is no significant change in the *in vivo* specific binding of [^{11}C]CFT in the striatum among three groups.

DISCUSSION

The age-related changes of the [^{11}C]CFT binding to DATs was investigated *in vivo* in Fischer 344 rats. The tissue dissection method represented an age-related decrease in the uptake ratio of the striatum to the cerebellum and in the specific binding-to-nonspecific binding ratio of [^{11}C]CFT. A tendency of age-related decrease in the *in vivo* specific binding of [^{11}C]CFT was also observed in the striatum. The evaluation of the specific binding of [^{11}C]CFT by the tissue dissection at a single time point after the PET measurement may be reasonable, because the specific binding of [^{11}C]CFT remained in an equilibrium state from 30 to 60 min (Fig. 2). The finding is coincidence with that obtained *in vitro*. Hebert et al.[3] reported an age-related decrease in the density (B_{max}) of [^{3}H]CFT binding sites in the rat striatum by *in vitro* autoradiography. PET also demonstrated a tendency of an age-dependent decrease in the striatal uptake of [^{11}C]CFT (Figs. 1 and 2), however, the kinetic analysis represented the age-related decrease in both the association rate constant (k_3) and the dissociation rate constant (k_4), but not the binding potential ($BP = k_3/k_4$) that was a parameter including both of density and affinity of the binding sites.

Previously, Ishiwata et al.[6] suggested that the tracer kinetic measured by PET for ^{11}C-labeled dopamine receptor ligands was not necessarily parallel to that measured by the tissue dissection method, because of low spatial resolution of PET camera used for small brain structures of rat. In the present study, the striatal uptake values (%ID/g) evaluated by tissue dissection at 60 min and the corresponding values evaluated by PET at 50–60 min for the different age of rats were obtained as 1.13 ± 0.31 vs. 0.84 ± 0.26 in the 6-month-old rats, 1.05 ± 0.19 vs. 0.68 ± 0.18 in the 12-month-old rats and 0.88 ± 0.16 vs. 0.63 ± 0.14 in the 24-month-old rats, respectively. The PET values were underestimated to be 65–74% of those of evaluated by tissue dissection. Notwithstanding the technical problem, Suzuki et al.[13] found the age-related decrease in the [^{11}C]SCH 23390 binding to dopamine D_1 receptors or in the [^{11}C]raclopride binding to dopamine D_2 receptors using the same PET camera.

An important factor inducing the discrepancy between PET and *in vitro* findings may be anesthesia. Isoflurane anesthesia scarcely changed the [^{11}C]raclopride binding to dopamine D_2 receptors when compared with consciousness,[6] however, it may affect the binding of [^{11}C]CFT to DATs. Tsukada et al.[15] found that isoflurane anesthesia markedly enhanced the BP of [^{11}C]CFT in the monkey brain, and that the degrees of reduction of BP by preadministration of DATs inhibitors were marked less than those observed in the conscious monkey brain. The isoflurane anesthesia may affect differently the binding of [^{11}C]CFT to DATs between the aged and young rats, which resulted in no age-related change in the BP values.

In conclusion, the present study demonstrated the age-related decrease in the DATs-specific binding of [^{11}C]CFT in the striatum of Fischer 344 rats by tissue dissection. By the kinetic analysis of PET measurement, the age-related decrease was also found in the association and dissociation rate constants in the [^{11}C]CFT DATs binding, however, the age-related change was not found for the BP. The finding by PET was not necessarily coincident with the result investigated *in vitro* previously. Therefore, careful interpretation is necessary for PET studies using [^{11}C]CFT and small animals such as rats. Further improvements in anesthesia techniques and precise correction for the partial volume effects for small animal brain structures may be required for appropriate use of PET technology in this setting.

ACKNOWLEDGMENTS

The authors thank Dr. W. F. Wang and Y. Koyanagi for their assistance in the PET study, and also thank the staff of Positron Medical Center, Tokyo Metropolitan Institute of Gerontology.

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