Quantitative Analysis of Norepinephrine Transporter in the Human Brain Using PET with (S,S)-^{18}F-FMeNER-D_2

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(S,S)-^{18}F-FMeNER-D_2 was recently developed as a radioligand for the measurement of norepinephrine transporter imaging with PET. In this study, a norepinephrine transporter was visualized in the human brain using this radioligand with PET and quantified by several methods. **Methods:** PET scans were performed on 10 healthy men after intravenous injection of (S,S)-^{18}F-FMeNER-D_2. Binding potential relative to nondisplaceable binding (BP_{ND}) was quantified by the indirect kinetic, simplified reference-tissue model (SRTM), multilinear reference-tissue model (MRTM), and ratio methods. The indirect kinetic method was used as the gold standard and was compared with the SRTM method with scan times of 240 and 180 min, the MRTM method with a scan time of 240 min, and the ratio method with a time integration interval of 120–180 min. The caudate was used as reference brain region. **Results:** Regional radioactivity was highest in the thalamus and lowest in the caudate during PET scanning. BP_{ND} values by the indirect kinetic method were 0.54 ± 0.19 and 0.35 ± 0.25 in the thalamus and locus coeruleus, respectively. BP_{ND} values found by the SRTM, MRTM, and ratio methods agreed with the values demonstrated by the indirect kinetic method (r = 0.81–0.92). **Conclusion:** The regional distribution of (S,S)-^{18}F-FMeNER-D_2 in our study agreed with that demonstrated by previous PET and postmortem studies of norepinephrine transporter in the human brain. The ratio method with a time integration interval of 120–180 min will be useful for clinical research of psychiatric disorders for estimation of norepinephrine transporter occupancy by antidepressants without requiring arterial blood sampling and dynamic PET.

Key Words: norepinephrine transporter; (S,S)-^{18}F-FMeNER-D_2; positron emission tomography; human brain; thalamus

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Norepinephrine, one of the monoamine neurotransmitters in the central nervous system, has been reported to be related to several functions such as memory, cognition, consciousness, and emotion and to play important roles in psychiatric disorders (*1–4*). Norepinephrine transporter is responsible for the reuptake of norepinephrine into presynaptic nerves. Norepinephrine reuptake inhibitors are used for the treatment of depression and attention deficit hyperactivity disorder (ADHD) (*4–7*). Thus, changes in norepinephrine transporter functions in several psychiatric disorders can be expected, but in vivo estimation has not been performed because of a lack of suitable radioligands for norepinephrine transporters.

(S,S)-^{18}F-FMeNER-D_2 has recently been developed as a radioligand for the measurement of norepinephrine transporter for PET (*8*). (S,S)-^{18}F-FMeNER-D_2 is a reboxetine analog and has high affinity for norepinephrine transporter and high selectivity from other monoamine transporters. Tracer distribution and dosimetry of (S,S)-^{18}F-FMeNER-D_2 were reported in monkey (*8,9*) and human studies (*10,11*). Another monkey study showed that (S,S)-^{18}F-FMeNER-D_2 binding decreased by the administration of atomoxetine, a selective norepinephrine reuptake inhibitor (*12*). However, quantitative analyses of (S,S)-^{18}F-FMeNER-D_2 bindings using an arterial input function have not yet, to our knowledge, been performed.

In this study, we aimed to quantify the norepinephrine transporter bindings in the human brain using (S,S)-^{18}F-FMeNER-D_2 with arterial blood sampling and also to validate noninvasive methods for quantification without arterial blood sampling.

MATERIALS AND METHODS

Subjects

Ten healthy men (age range, 21–26 y; mean ± SD, 22.7 ± 1.6 y) participated in this study. All subjects were free of any somatic, neurologic, or psychiatric disorders, and they had no

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history of current or previous drug abuse. Written informed consent was obtained from all subjects following a complete description of this study. The study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiologic Sciences, Chiba, Japan.

PET Procedure

(S,S)-^{18}F-FMeNER-D_2 was synthesized by fluoromethylation of nor-ethyl-reboxetine with ^{18}F-bromofluoromethane-d_2 as previously described (*8*). A PET scanner system (ECAT EXACT HR+; CTI-Siemens) was used for all subjects, with a head holder used to minimize head movement. A transmission scan for attenuation correction was performed using a ^{68}Ge–^{68}Ga source. Dynamic PET scans were performed after a 1-min intravenous slow bolus injection of 353.4–382.7 MBq (mean ± SD, 368.1 ± 9.1 MBq) of (S,S)-^{18}F-FMeNER-D_2. The specific radioactivity of (S,S)-^{18}F-FMeNER-D_2 was 144.8–390.2 GBq/μmol (312.8 ± 76.2 GBq/μmol). Brain radioactivities were measured from 0 to 90 min (1 min × 10, 2 min × 15, and 5 min × 10), from 120 to 180 min (10 min × 6), and from 210 to 240 min (10 min × 3). MR images of the brain were acquired with a 1.5-T MRI scanner (Gyroscan NT; Philips). T1-weighted images were obtained at 1-mm slices.

Arterial Blood Sampling and Metabolite Analysis

To obtain the arterial input function, arterial blood samples were taken manually 42 times during the PET scan. Each blood sample was centrifuged to obtain plasma and blood cell fractions, and the concentrations of radioactivity in whole blood and in plasma were measured.

The percentage of unchanged (S,S)-^{18}F-FMeNER-D_2 in plasma was determined by high-performance liquid chromatography in 22 of the blood samples. Acetonitrile was added to each plasma sample, and samples were centrifuged. The supernatant was subjected to high-performance liquid chromatography radiodetection analysis (column: XBridge Prep C18, mobile phase, 90% acetonitrile/50 mM ammonium acetate = 48/52; Waters). Plasma input function was defined as radioactivity of plasma multiplied by the percentage of unchanged radioligand.

Regions of Interest

All MR images were coregistered to the PET images using a statistical parametric mapping system (SPM2; The Wellcome Trust Centre for Neuroimaging, University College London). Regions of interest were drawn manually on summed PET images, with reference to coregistered MR images, and were defined for the thalamus, locus coeruleus, hippocampus, anterior cingulate gyrus, and caudate head. Regional radioactivity was calculated for each frame, corrected for decay, and plotted versus time.

Kinetic Model of ^{18}F-FMeNER-D_2

To describe the kinetics of (S,S)-^{18}F-FMeNER-D_2 in the brain, the 3-compartment model with 4 first-order rate constants was used. The 3 compartments were defined as follows: C_P was the radioactivity concentration of unchanged radioligand in plasma (arterial input function), C_{ND} was the radioactivity concentration of nondisplaceable radioligand in the brain, including nonspecifically bound and free radioligand, and C_S was the radioactivity concentration of radioligand specifically bound to transporters. The rate constants K_1 and k_2 represent the influx and efflux rates, respectively, for radioligand diffusion through the blood–brain barrier, and the rate constants k_3 and k_4 are the radioligand transfers between the compartments for nondisplaceable and specifically bound radioligand, respectively. This model can be described by the following equations:

$$\frac{dC_{ND}(t)}{dt} = K_1C_P(t) - (k_2+k_3)C_{ND}(t) + k_4C_S(t),$$

$$\frac{dC_S(t)}{dt} = k_3C_{ND}(t) - k_4C_S(t), \text{ and}$$

$$C_T(t) = C_{ND}(t)+C_S(t).$$

$C_T(t)$ is the total radioactivity concentration in any brain region measured by PET.

Calculation of (S,S)-^{18}F-FMeNER-D_2 Binding Potential

(S,S)-^{18}F-FMeNER-D_2 binding was quantified by the indirect kinetic, simplified reference-tissue model (SRTM), multilinear reference-tissue model (MRTM), and ratio methods. In these methods, (S,S)-^{18}F-FMeNER-D_2 bindings were expressed as binding potentials relative to nondisplaceable binding (BP_{ND}) (*13*). We used the caudate as the reference brain region because of its

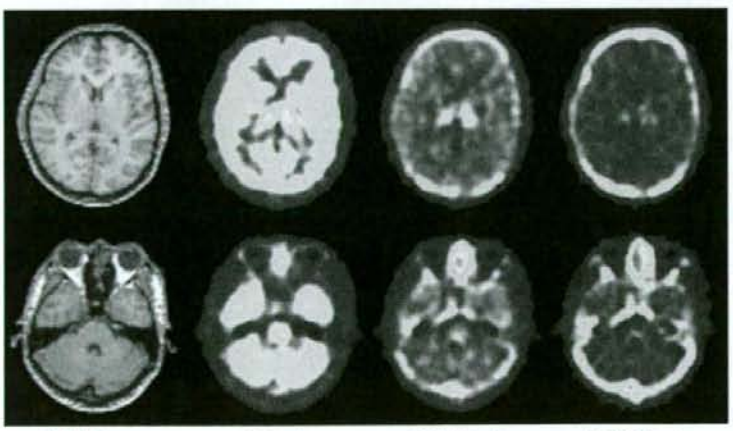

FIGURE 1. Typical summed PET images of (S,S)-^{18}F-FMeNER-D_2 and T1-weighted MR images. Upper panel shows slice of caudate and thalamus, and lower panel shows slice of locus coeruleus.

negligible norepinephrine transporter density (*14–16*). Software (PMOD; PMOD Technologies) was used for these analyses.

Indirect Kinetic Method

With the caudate as reference region, BP_{ND} can be expressed as:

$$BP_{ND} = \frac{V_{T(regions)}}{V_{T(caudate)}} - 1,$$

where $V_{T(regions)}$ is the total distribution volume ($= [K_1/k_2][k_3/k_4 + 1]$) of target regions and $V_{T(caudate)}$ is the total distribution volume of the caudate. The K_1, k_2, k_3, and k_4 values were determined by nonlinear least-squares curve fitting to the regional time–activity curves. In this analysis, blood volume (V_b), which depends on the first-pass extraction fraction of the tracer, was also estimated using the radioactivity of whole blood to diminish the influence of the tracer remaining in the blood. In this study, the indirect kinetic method was used as the standard method (*17*).

SRTM Method

Assuming that both target and reference regions have the same level of nondisplaceable binding, the SRTM method can be used to describe time–activity data in the target region as follows (*18*):

$$C_T(t) = R_1 C_R(t) + \left(k_2 - R_1 \frac{k_2}{1+BP_{ND}}\right) C_R(t) \otimes \exp\left(\frac{-k_2}{1+BP_{ND}} t\right),$$

where R_1 is the ratio of K_1/K_1' (K_1, influx rate constant for the brain region; K_1', influx rate constant for the reference region), $C_R(t)$ is the radioactivity concentration in the reference region (caudate), and $\otimes$ denotes the convolution integral. Using this model, 3 parameters (R_1, k_2, and BP_{ND}) were estimated by a nonlinear curve-fitting procedure. Scan data up to 180 or 240 min were used.

MRTM Method

The MRTM method is one of the variations of the graphical approaches (*19*). After a certain equilibrium time (t*), the following multilinear regression is obtained:

$$C_T(T) = -\frac{V_{T(regions)}}{V_{T(caudate)}} \int_0^T C_R(t)dt + \frac{1}{b} \int_0^T C_T(t)dt - \frac{V_{T(regions)}}{V_{T(caudate)} k_2' b} C_R(T),$$

where k_2' is the efflux rate constant for the reference region. In this analysis, t* was determined so that the maximum error from the regression within the linear segment would be 10% for each time–activity curve. BP_{ND} for the MRTM method was calculated using the same equation as described previously for the indirect kinetic method ($= V_{T(regions)}/V_{T(caudate)} - 1$). Scan data up to 240 min were used.

Ratio Method

In the ratio method, BP_{ND} can be expressed as:

$$BP_{ND} = \frac{AUC_{(regions)}}{AUC_{(caudate)}} - 1,$$

where $AUC_{(regions)}$ is the area under the time–activity curve of the target regions and $AUC_{(caudate)}$ is the area under the time–activity curve of the caudate. The integration interval of 120–180 min was used in this method.

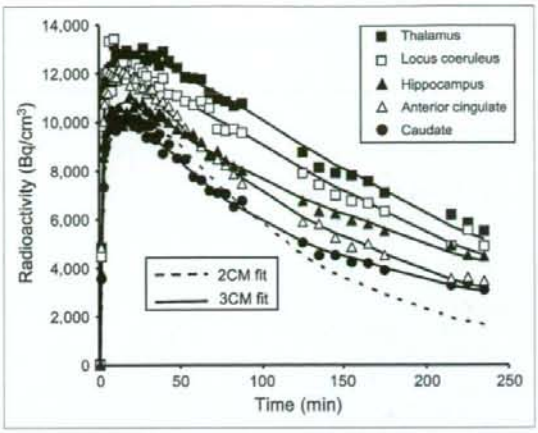

FIGURE 2. Typical time–activity curves of (S,S)-^{18}F-FMeNER-D_2 in brain. Time–activity curves of all regions could be described by 3-compartment model (3CM). Time–activity curve of caudate could also be described by 2-compartment model (2CM).

Simulation Study

A simulation study was performed to estimate errors in BP_{ND} calculated by the SRTM and ratio methods. Tissue time–activity curves for the thalamus were generated using the 3-compartment model. The rate constant values K_1, k_2, and k_4 of the thalamus were assumed to be 0.157, 0.037, and 0.016, respectively. The value of k_3 ranged from 0.019 to 0.039 in 6 steps. Tissue time–activity curves for the caudate were also generated using the 3-compartment model, assuming that the rate constant values K_1, k_2, k_3, and k_4 were 0.124, 0.032, 0.010, and 0.010, respectively. These assumed values were taken from the results obtained by the kinetic approach. The average of arterial input function for all

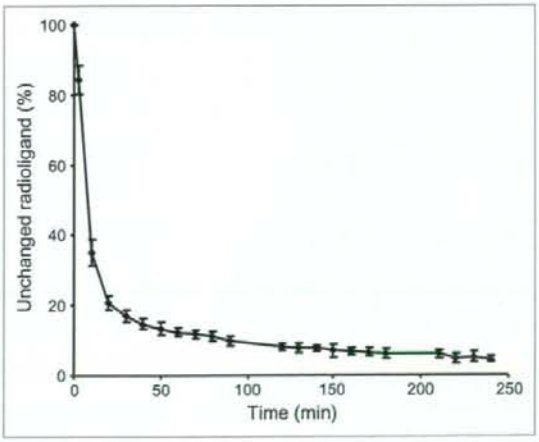

FIGURE 3. Average percentage of unchanged (S,S)-^{18}F-FMeNER-D_2 in plasma. Bars indicate 1 SD.

TABLE 1
Data for Each Brain Region Determined by Kinetic Approach Using 3-Compartment Model with Arterial Input Function

Region	V_b	Rate constant				V_{ND}	V_T
		K_1 (mL/mL/min)	k_2 (min^{-1})	k_3 (min^{-1})	k_4 (min^{-1})		
Thalamus	0.045 ± 0.016	0.157 ± 0.025	0.037 ± 0.005	0.027 ± 0.007	0.016 ± 0.002	4.36 ± 0.90	11.45 ± 2.29
Locus coeruleus	0.032 ± 0.008	0.154 ± 0.026	0.040 ± 0.009	0.020 ± 0.008	0.013 ± 0.004	4.06 ± 1.01	9.89 ± 1.78
Hippocampus	0.038 ± 0.010	0.119 ± 0.013	0.035 ± 0.011	0.022 ± 0.016	0.015 ± 0.005	3.73 ± 0.83	8.48 ± 1.14
Anterior cingulate	0.053 ± 0.011	0.144 ± 0.019	0.032 ± 0.005	0.010 ± 0.005	0.012 ± 0.004	4.61 ± 1.04	8.33 ± 1.70
Caudate (3-compartment model)	0.031 ± 0.008	0.124 ± 0.018	0.032 ± 0.005	0.010 ± 0.005	0.010 ± 0.004	3.92 ± 0.80	7.51 ± 1.51
Caudate (2-compartment model)	0.045 ± 0.010	0.109 ± 0.017	0.019 ± 0.001			5.77 ± 0.98	

Values are mean ± SD. V_{ND} is defined as K_1/k_2 and V_T as $(K_1/k_2)(k_3/k_4 + 1)$.

subjects was used to generate the time–activity curves. With these generated time–activity curves, BP_{ND} values were calculated by the SRTM (scan time of 240 min), MRTM, and ratio methods. The calculated BP_{ND} values for the simulation study were compared with those calculated by the indirect kinetic method.

RESULTS

Typical summed PET images of 3 time periods and T1-weighted MR images are shown in Figure 1. Typical time–activity curves in the brain showed that regional radioactivity was highest in the thalamus and lowest in the caudate (Fig. 2). Time–activity curves for all regions could be described by the 3-compartment model. The time–activity curve for the caudate could also be described by the 2-compartment model. The average percentage of unchanged (S,S)-^{18}F-FMeNER-D_2 in plasma was 84.4% ± 3.9% at 3 min, 35.1% ± 3.7% at 10 min, 10.0% ± 1.4% at 90 min, 6.1% ± 1.3% at 180 min, and 4.5% ± 0.9% at 240 min (Fig. 3).

The blood volume, rate constants, nondisplaceable distribution volume (V_{ND}), and total distribution volume (V_T) for each brain region determined by the kinetic approach using the 3-compartment model with arterial input function are shown in Table 1. For the caudate, the 2-compartment model without a specific binding compartment was also applied. Akaike information criteria of the 3-compartment model were lower than those of the 2-compartment model (143 ± 16 vs. 227 ± 6, $P < 0.0001$; paired t statistics).

The BP_{ND} values of the thalamus calculated by all methods are shown in Table 2. BP_{ND} values in the thalamus by the MRTM method showed the best correlation with those by the indirect kinetic method ($r = 0.92$) (Fig. 4C). The SRTM method with scan times of 180 and 240 min and the ratio method also agreed with the BP_{ND} values by the indirect kinetic method ($r = 0.81$–0.91) (Figs. 4A, 4B, and 4D). However, BP_{ND} values in brain regions other than the thalamus could not be estimated by the SRTM and MRTM methods because of failed curve fitting, showing no convergence. The BP_{ND} values of each brain region by the indirect kinetic and ratio methods are shown in Table 3. The correlation of BP_{ND} values in all target regions between the indirect kinetic and the ratio methods is shown in Figure 5A. The Bland–Altman plot of BP_{ND} values by these 2 methods is shown in Figure 5B.

In the simulation study, estimated BP_{ND} values, compared with assumed BP_{ND} values, by the SRTM (scan time of 240 min), MRTM, and ratio methods were slightly overestimated (Fig. 6).

DISCUSSION

After intravenous injection of (S,S)-^{18}F-FMeNER-D_2, radioactivity was highest in the thalamus and lowest in the caudate. BP_{ND} in the thalamus using the ratio method was 0.67 ± 0.15, almost the same value as found in a previous human PET study (*10*). The locus coeruleus showed relatively high uptake, and the hippocampus and anterior cingulate cortex showed relatively low uptake. This result was in agreement with previous reports that the thalamus and locus coeruleus showed high densities of norepinephrine transporters (*14–16,20*). Previous autoradiographic studies with human postmortem brains reported that norepinephrine transporter density in the locus coeruleus was higher by about 10 times than that in the thalamus (*14,15*). However,

TABLE 2
BP_{ND} Values in Thalamus by All Methods

Method	BP_{ND}
Indirect kinetic	0.54 ± 0.19
SRTM (240 min)	0.61 ± 0.14
SRTM (180 min)	0.64 ± 0.14
MRTM	0.61 ± 0.14
Ratio	0.67 ± 0.15

Values are mean ± SD.

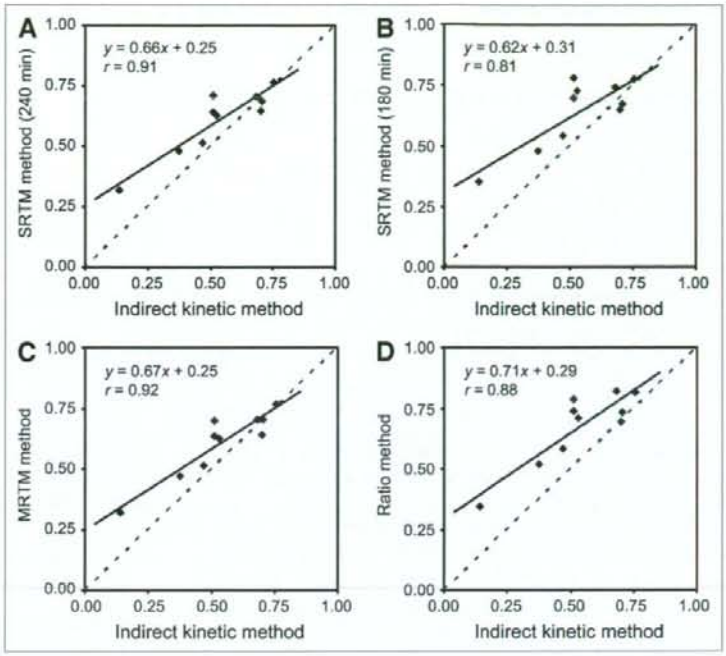

FIGURE 4. Correlation between BP_{ND} values in thalamus estimated by indirect kinetic method and SRTM method with scan time of 240 min (A) or 180 min (B), MRTM method (C), or ratio method (D).

previous and present PET studies reported almost the same values between the locus coeruleus and thalamus (*10,16*). One possible reason for the discrepancy was the partial-volume effect due to the limited spatial resolution of the PET scanner, the locus coeruleus being a very small structure.

In the current study, the indirect kinetic method with arterial blood sampling was used as the standard method (*17*). The BP_{ND} values in the thalamus by the other 3 methods—the SRTM with scan times of 240 and 180 min, MRTM, and ratio methods—were in agreement with those found by the indirect kinetic method. Although the indirect kinetic method was considered the standard method, it required a long PET time as well as arterial blood sampling, an invasive procedure particularly unsuitable for patients with psychiatric disorders. Because the ratio method does not require long PET and arterial blood sampling, this method would be preferable for clinical investigation. The SRTM and MRTM methods can estimate only the thalamus, as curve fitting failed in other brain regions. The MRTM2 method (*19*) may be able to estimate BP_{ND} in regions other than thalamus; however, weighted k_2' value among brain regions could not be calculated in this tracer. The possible reasons of failed curve fitting might be the small differences of time–activity curves between target and reference regions and the noise in time–activity curves. The ratio method could reveal BP_{ND} values in brain regions other than the thalamus. The BP_{ND} values by the ratio method were in agreement with those by the indirect kinetic method for all brain regions (Fig. 5A). Although bias was observed by the ratio method, this bias did not change according to the BP_{ND} values (Fig. 5B). The ratio method could estimate norepinephrine transporter binding in the thalamus and also other brain regions.

The time–activity curves in the caudate were better described by the 3-compartment model than the 2-compartment model. Similar results were reported for several PET radioligands; the kinetics in the reference region were also evaluated using the 3-compartment model (*17,21,22*). The results could be explained if the caudate contained specific binding for norepinephrine transporters. However, previous autoradiographic studies showed that the density of norepinephrine transporters in the caudate was very low (*14–16*). Another possible explanation is that the compartments of free and nonspecific binding could be separated by the kinetic analysis. Moreover, spillover from

TABLE 3
BP_{ND} Values for Each Brain Region by Indirect Kinetic and Ratio Methods

Region	Indirect kinetic method	Ratio method
Thalamus	0.54 ± 0.19	0.67 ± 0.15
Locus coeruleus	0.35 ± 0.25	0.42 ± 0.13
Hippocampus	0.13 ± 0.14	0.23 ± 0.09
Anterior cingulate	0.13 ± 0.16	0.15 ± 0.09

Values are mean ± SD.

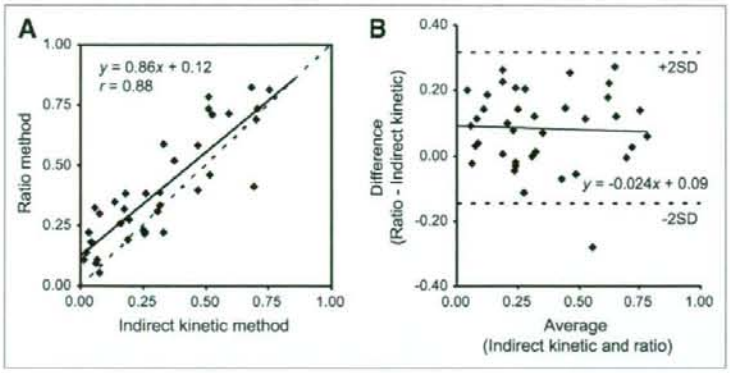

FIGURE 5. Correlation between BP_{ND} values in all target regions estimated by indirect kinetic method and ratio method (A) and Bland–Altman plot (B).

other brain regions to the caudate may affect the results because the caudate is a small structure and is surrounded by regions with specific binding.

In this study, we investigated norepinephrine transporter binding in only limited regions. Other brain regions such as the cerebral cortex and cerebellum are also considered to possess norepinephrine neurons and norepinephrine transporters (*14–16*). However, (S,S)-^{18}F-FMeNER-D_2 showed that defluorination and uptake of ^{18}F in the skull influenced cerebral radioactivity (*8*). Although (S,S)-^{18}F-FMeNER-D_2 had reduced defluorination by the dideuteration, compared with (S,S)-^{18}F-FMeNER, estimation in the cerebral cortex or cerebellum adjacent to the skull was considered difficult.

In this study, occupancy of norepinephrine transporter by antidepressants was not evaluated. Previous animal studies showed dose-dependent norepinephrine transporter occupancy by atomoxetine (*12*). However, a human study using [^{11}C](S,S)-MRB reported no differences in occupancy between different doses of atomoxetine (*16*). Further, occupancy studies in humans to estimate the clinical effects of antidepressants, similar to occupancy studies for dopamine D_2 receptor and serotonin transporters (*23,24*), will be needed.

In the simulation study, BP_{ND} values by the SRTM with a scan time of 240 min, MRTM, and the ratio methods were overestimated, compared with assumed BP_{ND} (Fig. 6). Such overestimation was also observed in measured PET data, especially in regions with low specific binding (Figs. 4A, 4C, and 4D). The degree of overestimation of BP_{ND} was larger in measured data than that in the simulation, especially in regions with low specific binding. Noise in measured data might cause such discrepancy, and therefore further studies using simulated data with added noise may be required. Although linear correlation was observed in BP_{ND} values between the ratio and indirect kinetic methods, this overestimation may cause errors in the calculation of occupancy by antidepressants. When baseline BP_{ND} is 0.6, estimated occupancy by the ratio method is 22%, 43%, and 65%, corresponding to the assumed occupancy of 25%, 50%, and 75%, respectively (Fig. 6). The SRTM and MRTM methods also showed the underestimation of occupancy, 21%, 43%, and 64% by the former and 21%, 42%, and 63% by the latter.

CONCLUSION

(S,S)-^{18}F-FMeNER-D_2 is a suitable radioligand for PET measurement of norepinephrine transporters in the human brain. The 3-compartment model could well describe the brain kinetics of (S,S)-^{18}F-FMeNER-D_2. Because the ratio method does not require long PET imaging times and arterial blood sampling, this method would be useful for clinical research of psychiatric disorders.

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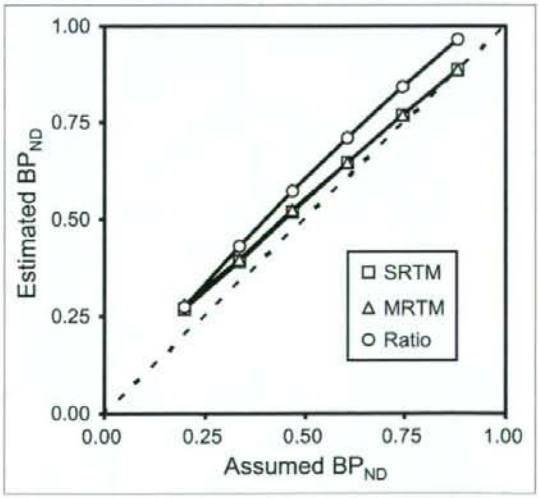

FIGURE 6. Correlations between assumed BP_{ND} values by indirect kinetic method and those by SRTM with scan time of 240 min, MRTM method, or ratio method in simulation studies.

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REFERENCES

1. Harmer CJ, Shelley NC, Cowen PJ, Goodwin GM. Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. *Am J Psychiatry.* 2004;161:1256–1263.
2. Strange BA, Hurlemann R, Dolan RJ. An emotion-induced retrograde amnesia in humans is amygdala- and beta-adrenergic-dependent. *Proc Natl Acad Sci USA.* 2003;100:13626–13631.
3. Southwick SM, Davis M, Horner B, et al. Relationship of enhanced norepinephrine activity during memory consolidation to enhanced long-term memory in humans. *Am J Psychiatry.* 2002;159:1420–1422.
4. Nutt DJ. The role of dopamine and norepinephrine in depression and antidepressant treatment. *J Clin Psychiatry.* 2006;67(suppl 6):3–8.
5. Cheng JY, Chen RY, Ko JS, Ng EM. Efficacy and safety of atomoxetine for attention-deficit/hyperactivity disorder in children and adolescents: meta-analysis and meta-regression analysis. *Psychopharmacology (Berl).* 2007;194:197–209.
6. Chamberlain SR, Del Campo N, Dowson J, et al. Atomoxetine improved response inhibition in adults with attention deficit/hyperactivity disorder. *Biol Psychiatry.* 2007;62:977–984.
7. Shelton RC. The dual-action hypothesis: does pharmacology matter? *J Clin Psychiatry.* 2004;65(suppl 17):5–10.
8. Schou M, Halldin C, Sovago J, et al. PET evaluation of novel radiofluorinated reboxetine analogs as norepinephrine transporter probes in the monkey brain. *Synapse.* 2004;53:57–67.
9. Seneca N, Andree B, Sjoholm N, et al. Whole-body biodistribution, radiation dosimetry estimates for the PET norepinephrine transporter probe (*S,S*)-[^{18}F]FMeNER-D2 in non-human primates. *Nucl Med Commun.* 2005;26:695–700.
10. Takano A, Gulyas B, Varrone A, et al. Imaging the norepinephrine transporter with positron emission tomography: initial human studies with (*S,S*)-[^{18}F]FMeNER-D_2. *Eur J Nucl Med Mol Imaging.* 2008;35:153–157.
11. Takano A, Halldin C, Varrone A, et al. Biodistribution and radiation dosimetry of the norepinephrine transporter radioligand (*S,S*)-[^{18}F]FMeNER-D_2: a human whole-body PET study. *Eur J Nucl Med Mol Imaging.* 2008;35:630–636.
12. Seneca N, Gulyas B, Varrone A, et al. Atomoxetine occupies the norepinephrine transporter in a dose-dependent fashion: a PET study in nonhuman primate brain using (*S,S*)-[^{18}F]FMeNER-D2. *Psychopharmacology (Berl).* 2006;188:119–127.
13. Innis RB, Cunningham VJ, Delforge J, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab.* 2007;27:1533–1539.
14. Schou M, Halldin C, Pike VW, et al. Post-mortem human brain autoradiography of the norepinephrine transporter using (*S,S*)-[^{18}F]FMeNER-D2. *Eur Neuropsychopharmacol.* 2005;15:517–520.
15. Donnan GA, Kaczmarczyk SJ, Paxinos G, et al. Distribution of catecholamine uptake sites in human brain as determined by quantitative [^{3}H] mazindol autoradiography. *J Comp Neurol.* 1991;304:419–434.
16. Logan J, Wang GJ, Telang F, et al. Imaging the norepinephrine transporter in humans with (*S,S*)-[^{11}C]O-methyl reboxetine and PET: problems and progress. *Nucl Med Biol.* 2007;34:667–679.
17. Ito H, Sudo Y, Suhara T, Okubo Y, Halldin C, Farde L. Error analysis for quantification of [^{11}C]FLB 457 binding to extrastriatal D_2 dopamine receptors in the human brain. *Neuroimage.* 2001;13:531–539.
18. Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. *Neuroimage.* 1996;4:153–158.
19. Ichise M, Liow JS, Lu JQ, et al. Linearized reference tissue parametric imaging methods: application to [^{11}C]DASB positron emission tomography studies of the serotonin transporter in human brain. *J Cereb Blood Flow Metab.* 2003;23:1096–1112.
20. Ordway GA, Stockmeier CA, Cason GW, Klimek V. Pharmacology and distribution of norepinephrine transporters in the human locus coeruleus and raphe nuclei. *J Neurosci.* 1997;17:1710–1719.
21. Lundberg J, Odano I, Olsson H, Halldin C, Farde L. Quantification of ^{11}C-MADAM binding to the serotonin transporter in the human brain. *J Nucl Med.* 2005;46:1505–1515.
22. Farde L, Ito H, Swahn CG, Pike VW, Halldin C. Quantitative analyses of carbonyl-carbon-11-WAY-100635 binding to central 5-hydroxytryptamine-1A receptors in man. *J Nucl Med.* 1998;39:1965–1971.
23. Takano A, Suzuki K, Kosaka J, et al. A dose-finding study of duloxetine based on serotonin transporter occupancy. *Psychopharmacology (Berl).* 2006;185:395–399.
24. Arakawa R, Ito H, Takano A, et al. Dose-finding study of paliperidone ER based on striatal and extrastriatal dopamine D_2 receptor occupancy in patients with schizophrenia. *Psychopharmacology (Berl).* 2008;197:229–235.

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Error analysis for PET measurement of dopamine D_2 receptor occupancy by antipsychotics with [^{11}C]raclopride and [^{11}C]FLB 457

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ABSTRACT

Dopamine D_2 receptor occupancy by antipsychotic drugs has been measured with positron emission tomography (PET) by comparing the binding potential (BP) values before and after drug administration. This occupancy has been found to be related to clinical effects and side effects. In this study, we evaluated the uncertainty of the quantitative analysis for estimating the dopamine D_2 receptor occupancy by antipsychotics in simulation and human studies of [^{11}C]raclopride and for the high affinity ligand [^{11}C]FLB 457. Time–activity curves of [^{11}C]raclopride and [^{11}C]FLB 457 were simulated, and the reliability of BP estimated by a simplified reference tissue model and the calculated occupancy were investigated for various noise levels, BP values, and scan durations. Then, in the human PET study with and without antipsychotics, the uncertainty of BP and occupancy estimates and the scan duration required for a reliable estimation were investigated by a bootstrap approach. Reliable and unbiased estimates of [^{11}C]raclopride BP_{ND} could be obtained with recording as short as 32 min, with the relative standard deviation (SD) of the striatal occupancy remaining less than 10%. Conversely, in [^{11}C]FLB 457 studies, the mean value increased and SD of the temporal cortex and thalamus exceeded 10% when the scan duration was shorter than 60 min. These results demonstrated that dopamine D_2 receptor occupancy by antipsychotics can be estimated precisely with an optimal scan duration with [^{11}C]raclopride and [^{11}C]FLB 457.

Introduction

Neuroreceptor imaging with positron emission tomography (PET) has made it possible to measure dopamine D_2 receptor occupancy by antipsychotic drugs in the living human brain (Farde et al., 1988, 1990). Raclopride has been widely used as a radioligand for PET measurement of striatal dopamine D_2 receptor binding (Farde et al., 1985). Using this competition paradigm, it has been shown that dopamine D_2 receptor occupancy in the striatum measured with [^{11}C] raclopride in antipsychotic drug-treated patients was related to clinical effects including antipsychotic effects and extrapyramidal symptoms (Farde et al., 1992a,b; Farde and Nordstrom, 1993; Nordstrom et al., 1993; Nyberg et al., 1995; Kapur et al., 2000). However, due to its low affinity for dopamine D_2 receptors, [^{11}C]raclopride is not suitable for measuring dopamine D_2 receptor binding in extrastriatal regions with low receptor densities. Extrastriatal regions, in particular cerebral cortices with low density of dopamine D_2 receptors, have been suggested to be the important sites for antipsychotic action, especially for the so-called atypical antipsychotics (Lidow et al., 1998; Pilowsky et al., 1997). [^{11}C] FLB 457, which has a high affinity for dopamine D_2 receptors, was shown to be suitable for the quantification of low-density extrastriatal dopamine receptors using PET (Halldin et al., 1995; Farde et al., 1997; Delforge et al., 1999; Olsson et al., 1999; Suhara et al., 1999), and has been applied for the measurement of receptor occupancy in extrastriatal regions (Farde et al., 1997; Talvik et al., 2001; Agid et al., 2007; Mizrahi et al., 2007). However, because of its slow kinetics, the time point of reaching equilibrium in receptor-rich regions is beyond the end of the data acquisition time, and thus the binding of [^{11}C]FLB 457 cannot be measured in the striatum for the practical data with noise (Olsson and Farde, 2001).

Receptor occupancy can be estimated from reductions in the observed binding potential evoked by competition from antipsychotic medication. Several quantitative methods have been proposed for estimating binding potential, and recently a

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Table 1
Subjects and procedure of [^{11}C]raclopride study

Antipsychotic drug	Number of subjects	Age mean±SD	Dose of antipsychotic [mg]	Scan start time after antipsychotic administration [h]
Risperidon	5	28.6±6.2	2	2
Quetiapin	3	25.0±6.9	400	8
Perospirone	3	28.3±4.0	16	8

simplified reference tissue model (Lammertsma and Hume, 1996) has often been used in [^{11}C]raclopride and [^{11}C]FLB 457 studies, a method employing a reference region with negligible specific binding to avoid arterial blood sampling. Because binding potential varies widely in occupancy studies, accuracy of the measurement of wide range binding potential should be confirmed.

In this study, we evaluated errors in quantitative analysis for estimating dopamine D_2 receptor occupancy by antipsychotics with [^{11}C]raclopride and [^{11}C]FLB 457 in simulation and human studies. The effect of scan duration on the error of estimates was also evaluated.

Materials and methods

Estimation of receptor occupancy

Simplified reference tissue model (SRTM)

This method yields the binding potential value by eliminating the arterial plasma time–activity curve (TAC) arithmetically from model equations by using a TAC from a reference region where specific bindings are negligible, under the assumptions that the distribution volume of the nondisplacable compartment was equal in the target and reference regions, and that a target region can be described with the one-tissue compartment model shown in Eq. (1) (Lammertsma and Hume, 1996).

$$C_t(t) = R_1 C_r(t) + \left(k_2 - \frac{R_1 k_2}{1 + BP_{ND}}\right) e^{-\frac{k_2}{1+BP_{ND}}} \otimes C_r(t)$$
$$R_1 = K_1/K_1^r,\ BP_{ND} = k_3/k_4, \quad (1)$$

where C_t and C_r are the radioactivity concentrations in the target and reference regions, respectively, K_1 and K_1^r describe the rate constants for the transfer of radioligand from plasma to target and reference regions, respectively, k_2 describes the rate constant for the transfer of free radioligand from target region to plasma, and k_3 and k_4 represent the association and dissociation rate constants of the specific binding. This SRTM estimates three parameters, the delivery ratio of the target region to the reference region (R_1), the clearance rate constant of the target region (k_2), and binding potential, referred to as BP_{ND} by nonlinear least squares.

Table 2
Subjects and procedure of [^{11}C]FLB 457 study

Antipsychotic drug	Number of subjects	Age mean±SD	Dose of antipsychotic [mg]	Scan start time after antipsychotic administration [h]
Risperidon	5	28.6±6.2	2	4
Sultopride	3	25.3±2.3	25	2
Sulpiride	3	27.7±6.7	200	3

Receptor occupancy

Receptor occupancy is calculated from BP_{ND} of two scans, with and without antipsychotics, as follows:

$$\text{Occupancy}\,(\%) = 100^*(BP_{control} - BP_{drug})/BP_{control}, \quad (2)$$

where $BP_{control}$ represents the BP_{ND} value derived from a scan without antipsychotics and BP_{drug} represents that from a scan with antipsychotics (Farde et al., 1988).

Simulation study

Simulated TACs calculated for [^{11}C]raclopride and [^{11}C]FLB 457 with several BP_{ND} values and noise levels were generated to investigate the bias and variation of parameter estimates caused by statistical noise. Dynamic tracer concentrations for 90-min scans in the putamen of [^{11}C]raclopride and the temporal cortex of [^{11}C]FLB 457 were derived from Eq. (1) using a measured TAC of the cerebellum as a reference input function and assuming population mean input parameter values obtained previously in a study of untreated humans in which BP_{ND} values were varied to 100%, 50%, and 20% of the

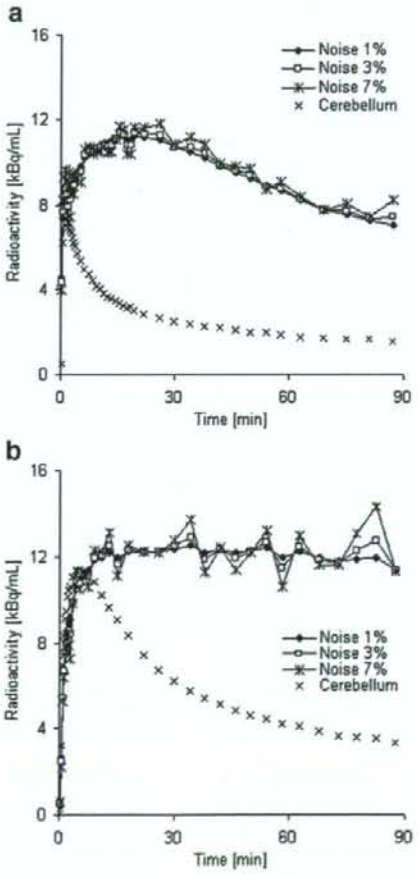

Fig. 1. Examples of simulated time–activity curves for the putamen with [^{11}C]raclopride (R_1=0.94, k_2=0.22, BP_{ND}=3.0) (a) and the temporal cortex with [^{11}C]FLB 457 (R_1=0.81, k_2=0.048, BP_{ND}=2.1) (b) at noise levels of 1%, 3%, and 7%. The time–activity curves for the cerebellum used as an input function were also shown.

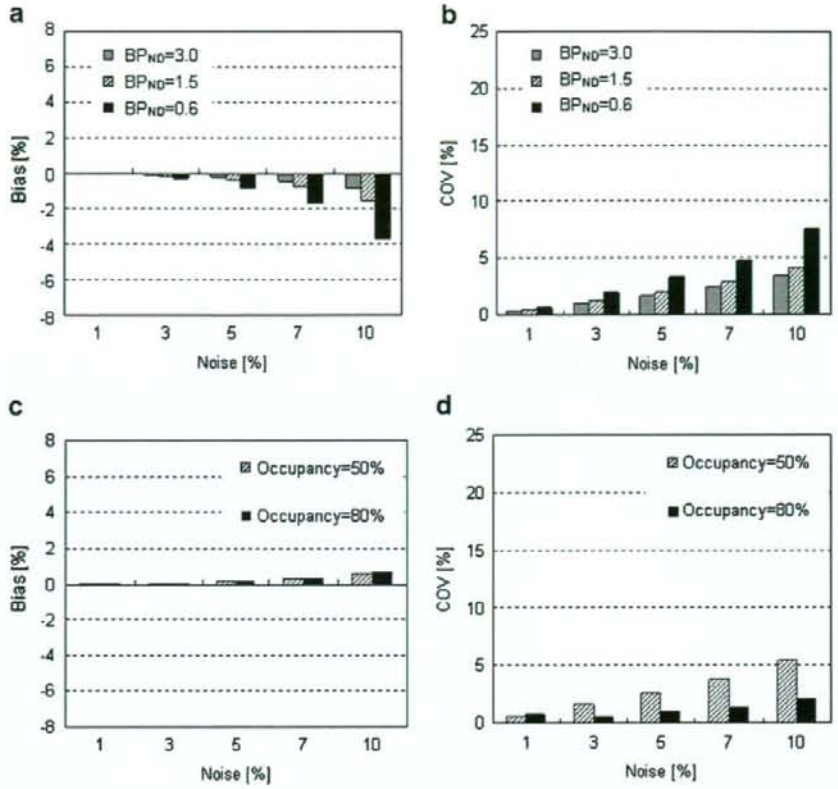

Fig. 2. Relation between noise level and reliability of BP_{ND} estimates (a, b) and between noise level and reliability of occupancy estimates (c, d) for simulated data of the putamen in [^{11}C]raclopride with R_1 = 0.94, k_2 = 0.22, BP_{ND} = 3.0, 1.5 and 0.6 at noise levels of 1%, 3%, 5%, 7%, and 10%. Occupancy was calculated by BP_{ND} estimated from the time–activity curves with BP_{ND} = 3.0 and 1.5 (occupancy = 50%), and those with BP_{ND} = 3.0 and 0.6 (occupancy = 80%). Deducing from the residual error of TAC fitting by SRTM, the noise level of human ROI analysis was 1–5%.

original value (R_1 = 0.94, k_2 = 0.22, BP_{ND} = 3.0, 1.5, and 0.6 for [^{11}C]raclopride, and R_1 = 0.81, k_2 = 0.048, BP_{ND} = 2.1, 1.05, and 0.42 for [^{11}C]FLB 457).

The Gaussian-distributed mean-zero noise with variance proportional to the true TAC was added to the non-decaying tissue activity for each frame by Eq. (3) adopted from Logan et al. (2001).

$$SD\,(\%) = 100 \cdot \sqrt{N_i}/N_i, \tag{3}$$

$$N_i = \int_{t_i-\frac{\Delta t_i}{2}}^{t_i+\frac{\Delta t_i}{2}} C_t(t) \cdot e^{-\lambda t} \cdot dt \cdot F, \tag{4}$$

where i is the frame number, C_t is the non-decaying tissue radioactivity concentration derived from the k-values and the input function, t_i is the midpoint time of the ith frame, Δt_i is the data collection time, λ is the radioisotope decay constant, and F is a scaling factor representing the sensitivity of the measurement system, introduced here to adjust the noise level. It should be noted that this equation assumes that noise, which is added to the TAC, is determined by the count of the curve itself. In fact, noise is determined by the total counts in the slice, and is affected by random counts, dead time, etc. The level of noise for the dynamic data was expressed as the mean of percent SD described in Eq. (3) from 1 to 90 min. In this simulation study, F was chosen so that the level of noise would be 1, 3, 5, 7, and 10% for [^{11}C]raclopride and [^{11}C]FLB 457 TAC without BP_{ND} reduction, and one thousand noisy data sets were generated for each.

In these simulated TACs, kinetic parameters including BP_{ND} were calculated using the SRTM, and the relationship between the reliability of BP_{ND} estimates and noise level was investigated for TACs with various true BP_{ND}s. In these estimations, starting parameter values of the iteration for the fitting varied by ±25% from the true value (Ichise et al., 2003), and parameter estimates were considered invalid if R_1, k_2, BP_{ND} were negative or more than three times the true value. Occupancy was also calculated from Eq. (2) by using BP_{ND}s estimated from TACs with BP_{ND} = 3.0 ([^{11}C]racloporide) or BP_{ND} = 2.1 ([^{11}C]FLB 457) as $BP_{control}$ conditions. Reliability of the estimated parameters was evaluated by the bias of the mean value from the true value and coefficient of variation (COV; SD / mean[%]) of the estimates excluding outliers. In this evaluation, it was assumed that the TACs of [^{11}C]raclopride and [^{11}C]FLB 457 could be described with SRTM and that BP_{ND} estimated by SRTM represented binding potential estimated by a two-tissue compartment model with arterial input function.

Next, the effect of scan duration on BP_{ND} and occupancy estimates was investigated. In 90-min simulated TACs of 3%

noise level that corresponds to the noise level of human region-of-interest (ROI) analysis, the duration of the scan used for the fitting of parameter estimation was progressively reduced from 90 to 20 min for [^{11}C]raclopride and from 90 to 32 min for [^{11}C]FLB 457, and parameters were estimated by SRTM for each. The relationship between the reliability of parameter estimates and scan duration was evaluated by the COV of estimates and bias of the mean value from the true value for each fitting interval.

Human study

Subjects

Each of the [^{11}C]raclopride and [^{11}C]FLB 457 studies was based on eleven healthy male volunteers, some of whom were not the same in the two studies (Tables 1, 2). None of the subjects had a history of psychiatric, neurological, or somatic disorders, and none had alcohol- or drug-related problems. The study was approved by the ethics and radiation safety committees of the National Institute of Radiological Sciences, Chiba, Japan. Written informed consent was obtained from each subject.

Positron emission tomography procedure

For each subject, two PET scans were performed, before and after antipsychotic drug administration (Tables 1, 2), on separate days using the ECAT EXACT HR+ (CTI-Siemens, Knoxville, TN, USA), which provides 63 planes and a 15.5-cm axial field of view. A 10-min transmission scan with 3-rod source of ^{68}Ga–^{68}Ga was performed to correct for attenuation. Dynamic PET scans of [^{11}C]raclopride were carried out for 60 min (20 s × 12, 60 s × 16, 240 s × 10) in 3-dimensional mode with a bolus injection of 217.3 ± 14.1 MBq of [^{11}C]raclopride. The specific radioactivity was 198.9 ± 54.4 GBq/μmol at the time of injection. PET scans of [^{11}C]FLB 457 were carried out for 90 min (20 s × 9, 60 s × 5, 120 s × 4, 240 s × 11, 300 s × 6) in 3-dimensional mode with a bolus injection of 220.0 ± 19.5 MBq of [^{11}C]FLB 457. The specific radioactivity was 174.7 ± 48.1 GBq/μmol at the time of injection. There were no striking differences between the amount of cold raclopride or FLB 457 administered at baseline and after treatment of antipsychotic medication. Arterial blood sampling was not carried out in either study. All emission data were reconstructed by filtered-back projection using a Hanning filter with a cut-off frequency of 0.4 (full width at half maximum = 7.5 mm).

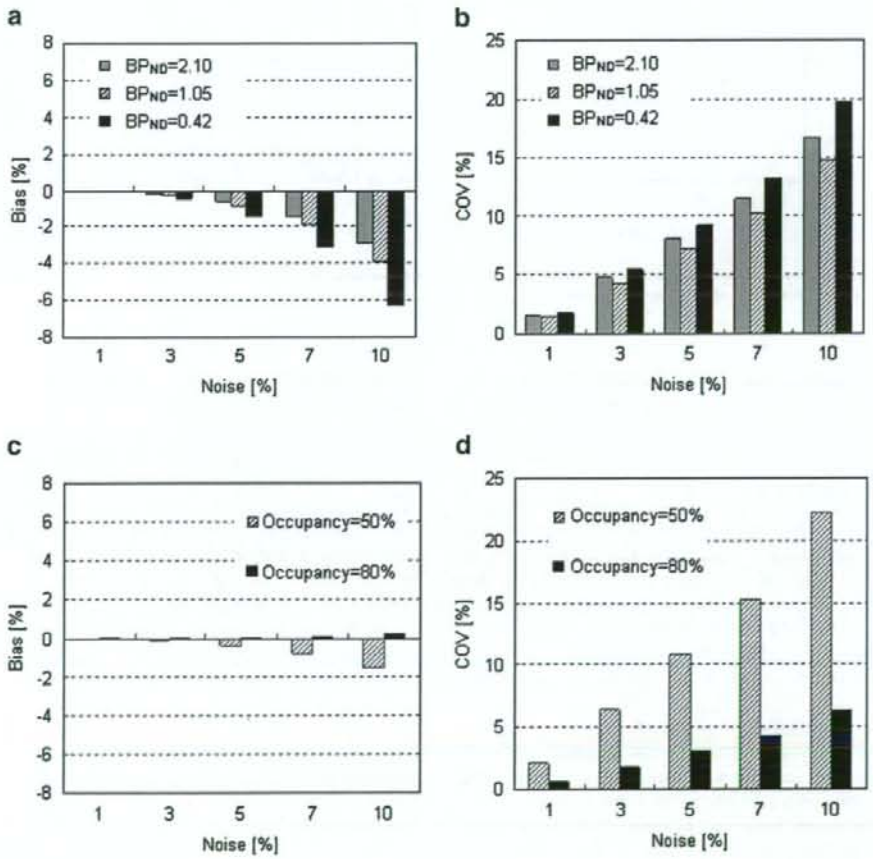

Fig. 3. Relation between noise level and reliability of BP_{ND} estimates (a, b) and between noise level and reliability of occupancy estimates (c, d) for simulated data of the temporal cortex with [^{11}C]FLB 457 with $R_1 = 0.81$, $k_2 = 0.048$, $BP_{ND} = 2.1$, 1.05 and 0.42 at noise levels of 1%, 3%, 5%, 7%, and 10%. Occupancy was calculated by BP_{ND} estimated from the time-activity curves with $BP_{ND} = 2.1$ and 1.05 (occupancy = 50%), and those with $BP_{ND} = 2.1$ and 0.42 (occupancy = 80%). Deducing from the residual error of TAC fitting by SRTM, the noise level of human ROI analysis was 1–5%.

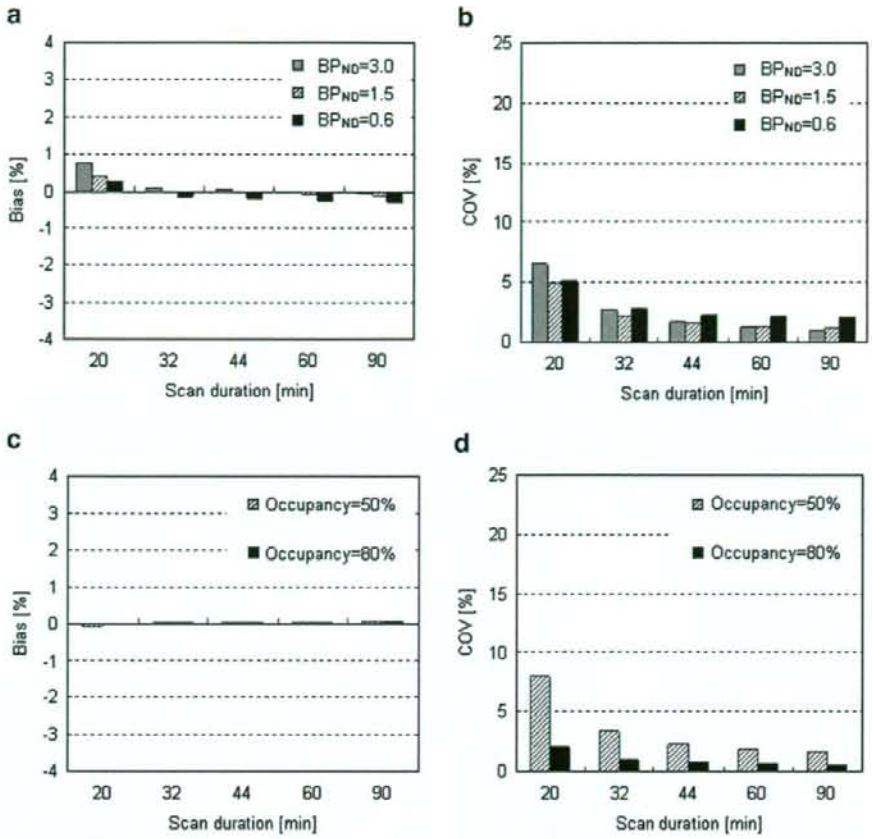

Fig. 4. Relation between scan duration and reliability of BP_{ND} estimates (a, b) and between scan duration and reliability of occupancy estimates (c, d) for simulated data of the putamen in [^{11}C]raclopride with R_1=0.94, k_2=0.22, BP_{ND}=3.0, 1.5, and 0.6 at a 3% noise level.

T1-weighted magnetic resonance imaging (MRI) of the brain was performed with Gyroscan NT, 1.5 T (Phillips Medical Systems, Best, The Netherlands).

Positron emission tomography data analysis

The summed PET images for all frames were coregistered to individual MR images, and ROIs were defined manually over the caudate, putamen, and cerebellum for [^{11}C]raclopride studies, and temporal cortex, thalamus, and cerebellum for [^{11}C]FLB 457 studies. In the TACs of these ROIs, R_1, k_2, and BP_{ND} were estimated by SRTM using the cerebellum as reference region, selected on the basis of its low specific binding (Olsson et al., 1999; Suhara et al., 1999).

In each scan, the reliability of parameter estimates was evaluated by a bootstrap approach with residual errors of fitting (Turkheimer et al., 1998; Kukreja and Gunn, 2004; Ogden and Tarpey, 2006). Briefly, weighted residual errors between measured and model-predicted TACs were calculated in each frame. Next, on the assumption that these errors of each frame $\varepsilon=[\varepsilon_1, \varepsilon_2, \varepsilon_3,\ldots, \varepsilon_F]$ are independent, identically distributed, and of zero mean, resampled versions of the errors $\varepsilon^*=[\varepsilon_1^*, \varepsilon_2^*, \varepsilon_3^*,\ldots,\varepsilon_F^*]$ were generated by random resampling of the original error ε. The resampling procedure for ε_i^* involved random selecting from ε with an equal probability associated with each of the F elements. Bootstrap replication TACs were generated by adding these resampling errors ε^* with appropriate weighting to the model-predicted TACs. In this study, the residual error of each frame was weighted with the SD derived from the TAC expressed as Eq. (3). It should be noted that the SD of each frame is not in a strict sense expressed by the counts of the TAC, as mentioned in the section above on Simulation study. Five hundred replication TACs were generated, parameters were estimated by SRTM for each, and mean and SD of these 500 estimates were calculated.

The relationship between the scan duration and COV of estimates and between the scan duration and bias was also investigated by shortening the interval of fitting of the bootstrap replication TACs from 60 to 20 min in [^{11}C] raclopride studies, and from 90 to 32 min in [^{11}C]FLB 457 studies. We define bias as the difference between the mean magnitude of BP_{ND} calculated for each truncated fitting interval and that of the 60-min fitting interval in [^{11}C] raclopride studies, and that of the 90-min fitting interval in [^{11}C]FLB 457 studies.

Analysis of simulation and human data was performed using Dr. View (AJS, Tokyo, Japan).

Results

Simulation study

Examples of simulated TACs for [^{11}C]raclopride and [^{11}C]FLB 457 with several noise levels and the cerebellum TAC used as reference input function are shown in Fig. 1. Deducing from the residual error of TAC fitting by SRTM, the noise level of human ROI analysis was about 1–5% in both [^{11}C]raclopride and [^{11}C]FLB 457 studies. BP_{ND} was estimated by SRTM using these simulated TACs, and occupancy was also calculated. In both [^{11}C]raclopride and [^{11}C]FLB 457 studies, the extent of the downward bias in BP_{ND} estimates became larger as noise increased or BP_{ND} decreased (Figs. 2a and 3a). However, in occupancy estimates, there was little bias even with a high noise level and with small occupancy (Figs. 2c and 3c). COV of BP_{ND} and occupancy became larger as noise increased (Figs. 2b, 2d, 3b, 3d). However, in both [^{11}C]raclopride and [^{11}C]FLB 457 studies, COV was under 6% at a 3% noise level. COV of occupancy estimates became larger when occupancy was smaller.

Next, the relationship between the reliability of BP_{ND} estimates, occupancy estimates, and scan duration was investigated. In [^{11}C]raclopride studies, there was little bias and COV was under 3% at a 3% noise level with both BP_{ND} and occupancy estimates with scan duration longer than 32 min (Fig. 4). Meanwhile, in [^{11}C]FLB 457 studies, COV was over 13% and bias from the 90-min scan became over 2% with BP_{ND} estimates when scan duration was shorter than 44 min (Figs. 5a, b). Although bias of occupancy estimates was small even if the scan duration became short, COV of occupancy estimates was also over 10% when occupancy was 50% and scan duration was shorter than 60 min (Figs. 5c, d). We had no outliers even when the scan duration was 20 min in [^{11}C]raclopride studies and when the scan duration was longer than 44 min in [^{11}C]FLB 457 studies.

Human study

An example of measured tissue-TACs at baseline and after administration of antipsychotic medication is shown in Fig. 6. The shape of the TACs was similar between before and after antipsychotic administration in the cerebellum, while the accumulation of radioactivity in the post-antipsychotic scan decreased at late times in other regions. Occupancies by antipsychotics were 27–79% with [^{11}C]raclopride and 18–86% with [^{11}C]FLB 457.

Bias and COV were estimated by a bootstrap approach. In the [^{11}C]raclopride studies there was no bias for either BP_{ND}

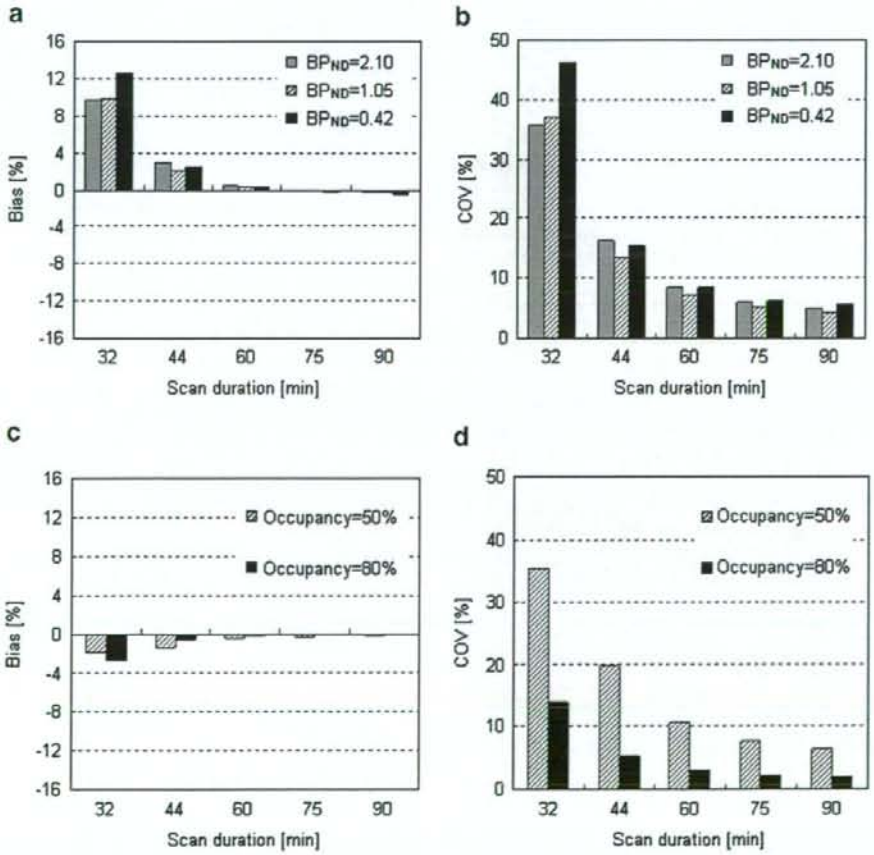

Fig. 5. Relation between scan duration and reliability of BP_{ND} estimates (a, b) and between scan duration and reliability of occupancy estimates (c, d) for simulated data of the temporal cortex in [^{11}C]FLB 457 with R_1=0.81, k_2=0.048, BP_{ND}=2.1, 1.05, and 0.42 at a 3% noise level.

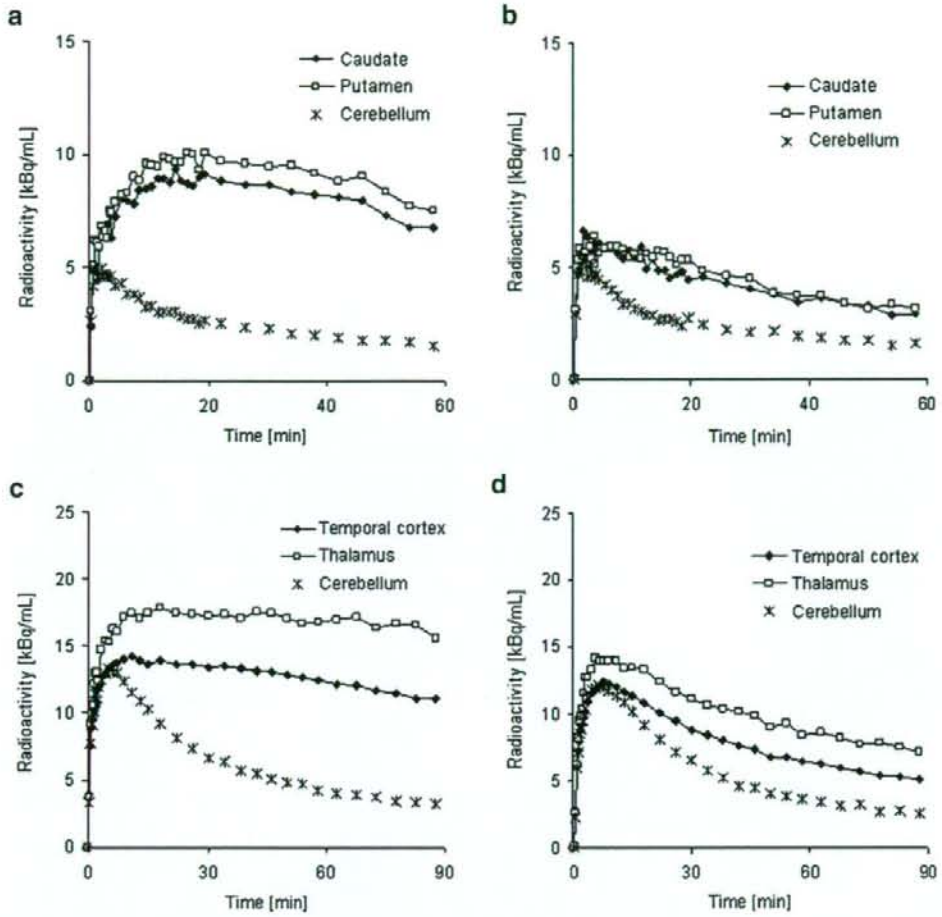

Fig. 6. Time–activity curves of pre-antipsychotic (left) and post-antipsychotic of risperidon (right) with [^{11}C]raclopride (a, b) and [^{11}C]FLB 457 (c, d). Occupancy of [^{11}C]raclopride was 71% in both the caudate and putamen, and that of [^{11}C]FLB 457 was 64% in the temporal cortex and 58% in the thalamus.

and occupancy estimates when scan duration was longer than 32 min. The COV in studies after antipsychotic administration were larger than that at baseline. When the scan duration was 32 min, COV of BP_{ND} was under 4% in the caudate and under 3% in the putamen at baseline, under 6% in the caudate and under 4% in the putamen after antipsychotic administration, and COV of occupancy was also under 6% in both regions (Figs. 7a, b). On the other hand, in [^{11}C]FLB 457 studies, the bias became larger when scan duration was shorter than 60 min. COV was about 5% for the 90-min scan, and when scan duration was shorter than 60 min, COV of BP_{ND} was greater than 9% in the thalamus, and greater than 5% in the temporal cortex at baseline, and greater than 13% in the thalamus, 9% in the temporal cortex after antipsychotic administration, whereas the COV of occupancy was also over 9% (Figs. 7c, d).

Discussion

In the quantification of receptor occupancy, it is necessary for binding potential to be quantified precisely before and after the administration of antipsychotics, since occupancy is derived from the binding potential values between these two scans. The simplified reference tissue model (SRTM) is often used in [^{11}C]raclopride and [^{11}C]FLB 457 studies. However, BP_{ND} varies widely in occupancy studies, and it can be difficult to obtain a solution for SRTM when specific binding is low, even given favorable noise levels (Gunn et al., 1997). Moreover, with a high noise level, the estimated parameters are affected by the noise in tissue TAC (Ichise et al., 2003). Therefore, in this study, we evaluated the effects of noise on the BP_{ND} estimates with SRTM for a range of BP_{ND} values likely to be encountered in occupancy studies with antipsychotic medication. As noise included in a reference TAC used as an input function may bring about an error in parameter estimates, in this study, the reference region was determined over the whole cerebellum, such that the resultant TAC is essentially noise-free.

On the other hand, Olsson and Farde simulated TACs with a plasma input curve and a two-tissue compartment model for various B_{max} values without noise and estimated occupancy by the peak equilibrium method, end time method, and SRTM (Olsson and Farde, 2001). They demonstrated that the drug occupancy estimated from SRTM was close to the assumed

value for simulated TACs of both [^{11}C]raclopride and [^{11}C]FLB 457 when the occupancy was 25, 50, and 70%, even when the assumed B_{max} extends over a considerable range. Therefore, SRTM is thought to be useful for the quantification of receptor occupancy with antipsychotics in which binding potential varies over a wide range both in striatal regions with a high density of dopamine D_2 receptors and extrastriatal regions with a low density of receptors.

Most investigations concerning the assessment of errors in parameter estimates have assumed that the reference regions has no specific binding, and can thus be fitted with a one-tissue compartment model. However, it was recently reported that a non-negligible density of dopamine D_2/D_3 receptors in the cerebellum leads to some underestimation of BP_{ND} as well as erroneous estimation of differential occupancies in an [^{11}C] FLB 457 study (Asselin et al., 2007). Therefore, interpretation of the estimated occupancy must be made with some caution.

Reliability of estimated parameters

In the simulation study, bias and COV became larger as the noise increased, the magnitude of BP_{ND} decreased, or occupancy decreased (Figs. 2 and 3). In the human study with [^{11}C] raclopride, COV of estimated BP_{ND} and occupancy was evaluated for the caudate and putamen, and COVs were larger for the caudate than for the putamen. This was because the noise level of TAC for the caudate was higher than that of the putamen which had a larger ROI volume than did the caudate, a result consistent with the tendency observed in the simulation. In addition, COV of BP_{ND} estimates for caudate and putamen after antipsychotic administration, in which the magnitude of the BP_{ND} was low, were larger than that in the baseline study, which was also consistent with the result of the simulation study. Similarly, in the [^{11}C]FLB 457 study, COV of estimated BP_{ND} and occupancy for the temporal cortex was smaller than that of the thalamus because the ROI size of the temporal cortex was larger, and COV of BP_{ND} estimates in the study with the administration of antipsychotics was larger than that without it. However, in all cases, COV of BP_{ND} estimates was less than 7%, which seems sufficient for the sensitive evaluation of receptor occupancy. In this study, we evaluated the reliability of parameter estimates for ROI-based estimation. However, in voxel-based estimation, the noise level is usually higher, so COV of estimates can be expected to become larger.

In human studies, reliability of BP_{ND} and occupancy estimates was evaluated by non-parametric bootstrap approach in

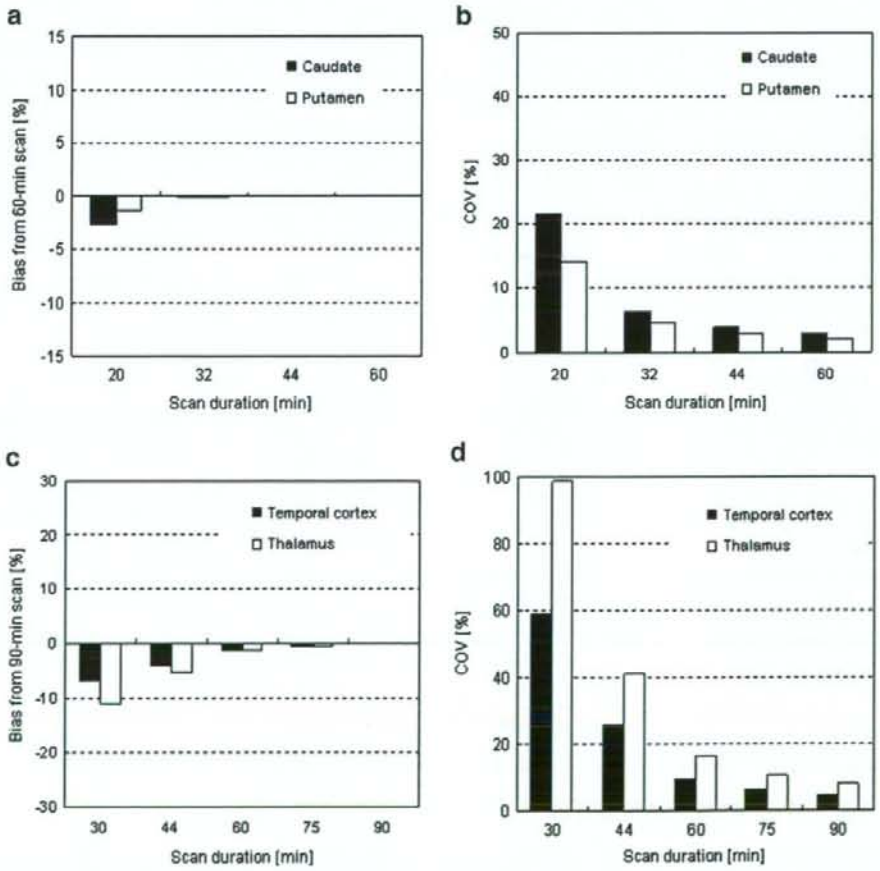

Fig. 7. Relation between scan duration and reliability of occupancy estimates derived from a bootstrap approach for human data of the putamen and caudate with [^{11}C]raclopride (a, b) and of the temporal cortex and thalamus in [^{11}C]FLB 457 (c, d).

which the uncertainty of BP_{ND} estimated from a ROI was evaluated from many replicated TACs generated by resampling weighted residual errors between measured and model-predicted TACs (Turkheimer et al., 1998; Kukreja and Gunn, 2004). Correct use of this method requires determination of the appropriate weights, and the model-predicted TAC should agree with the true TAC. This is often difficult, especially for a TAC with high noise. However, in this study, parameters were estimated for ROI-based TACs by SRTM, so the effect of noise was likely negligible. The bootstrap approach is useful for assessment of the analysis method or the required scan duration in PET dynamic studies (Ogden et al., 2007), as it can be done from the measured data without the need to specify the noise distribution in PET measurements.

Effect of scan duration

In the human studies, a 32-min scan duration gave unbiased and reliable BP_{ND} estimates in [^{11}C]raclopride studies both at baseline, and after treatment with antipsychotic medication (Fig. 7). Conversely, results of the [^{11}C]FLB 457 studies show that a 60 min scan duration would be required for the temporal cortex and a 75-min scan for the thalamus to estimate BP_{ND} and occupancy within 10% COV (Fig. 7). This difference in required scan durations between [^{11}C]raclopride and [^{11}C]FLB 457 may be related to the kinetics of each ligand. Especially, the value of k_2 is remarkably different between the tracers (k_2 = 0.38 in putamen for [^{11}C]raclopride (Ito et al., 1998), k_2 = 0.09 in temporal cortex for [^{11}C]FLB 457 (Olsson et al., 1999)), meaning that the washout from the brain with [^{11}C]raclopride is faster than that with [^{11}C]FLB 457.

The required scan duration for a reliable estimation depends on the properties of ligand kinetics, estimation method, receptor density, noise level according to injection dose, ROI size, sensitivity of the measurement system, and so on. Therefore, evaluation of the effect of the scan duration on the reliability of parameter estimates is very important.

In summary, the uncertainty and required scan duration in PET quantitative analysis of dopamine D_2 receptor occupancy by antipsychotic drugs were evaluated in simulation and human studies with [^{11}C]raclopride and [^{11}C]FLB 457. In [^{11}C]raclopride human studies, a 32-min scan duration provided unbiased and reliable BP_{ND} and occupancy estimates, as did a 60-min scan duration, and COVs of the caudate and putamen were under 10% in case of ROI-based estimation. Conversely, in [^{11}C]FLB 457 studies, the mean value increased and COVs of the temporal cortex and thalamus were over 10% when the scan duration was shorter than 60 min, since the kinetics of this radioligand are slower. Dopamine D_2 receptor occupancy by antipsychotics can be estimated precisely with [^{11}C]raclopride and [^{11}C]FLB 457 if applying an optimal scan duration.

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References

Agid, O., Mamo, D., Ginovart, N., Vitcu, I., Wilson, A.A., Zipursky, R.B., et al., 2007. Striatal vs extrastriatal dopamine D2 receptors in antipsychotic response--a double-blind PET study in schizophrenia. Neuropsychopharmacology 32, 1209–1215.

Asselin, M.C., Montgomery, A.J., Grasby, P.M., Hume, S.P., 2007. Quantification of PET studies with the very high-affinity dopamine D2/D3 receptor ligand [11C]FLB 457: re-evaluation of the validity of using a cerebellar reference region. J. Cereb. Blood Flow Metab. 27, 378–392.

Delforge, J., Bottlaender, M., Loc'h, C., Guenther, I., Fuseau, C., Bendriem, B., et al., 1999. Quantitation of extrastriatal D2 receptors using a very high-affinity ligand (FLB 457) and the multi-injection approach. J. Cereb. Blood Flow Metab. 19, 533–546.

Farde, L., Nordstrom, A.L., 1993. PET examination of central D2 dopamine receptor occupancy in relation to extrapyramidal syndromes in patients being treated with neuroleptic drugs. Psychopharmacol. Ser. 10, 94–100.

Farde, L., Ehrin, E., Eriksson, L., Greitz, T., Hall, H., Hedstrom, C.G., et al., 1985. Substituted benzamides as ligands for visualization of dopamine receptor binding in the human brain by positron emission tomography. Proc. Natl. Acad. Sci. USA 82, 3863–3867.

Farde, L., Wiesel, F.A., Halldin, C., Sedvall, G., 1988. Central D_2 dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. Arch Gen Psychiatry 45, 71–76.

Farde, L., Wissel, F.A., Stone-Elande, S., Halldin, C., Nordstorm, A.L., Hall, H., et al., 1990. D_2 dopamine receptors in neuroleptic-naïve schizophrenic patients. Arch. Gen. Psychiatry 47, 213–219.

Farde, L., Nordstrom, A.L., Halldin, C., Wiesel, F.A., Sedvall, G., 1992a. PET studies of dopamine receptors in relation to antipsychotic drug treatment. Clin. Neuropharmacol. 15, 468A–469A.

Farde, L., Nordstrom, A.L., Wiesel, F.A., Pauli, S., Halldin, C., Sedvall, G., 1992b. Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Arch. Gen Psychiatry 49, 538–544.

Farde, L., Suhara, T., Nyberg, S., Karlsson, P., Nakashima, Y., Hietala, J., et al., 1997. A PET-study of [^{11}C]FLB 457 binding to extrastriatal D_2-dopamine receptors in healthy subjects and antipsychotic drug-treated patients. Psychopharmacology (Berl) 133, 396–404.

Gunn, R.N., Lammertsma, A.A., Hume, S.P., Cunningham, V.J., 1997. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. Neuroimage 6, 279–287.

Halldin, C., Farde, L., Hogberg, T., Mohell, N., Hall, H., Suhara, T., et al., 1995. Carbon-11-FLB 457: a radioligand for extrastriatal D2 dopamine receptors. J. Nucl. Med. 36, 1275–1281.

Ichise, M., Liow, J.S., Lu, J.Q., Takano, A., Model, K., Toyama, H., et al., 2003. Linearized reference tissue parametric imaging methods: application to [^{11}C]DASB positron emission tomography studies of the serotonin transporter in human brain. J. Cereb. Blood Flow Metab. 23, 1096–1112.

Ito, H., Hietala, J., Blomqvist, G., Halldin, C., Farde, L., 1998. Comparison of the transient equilibrium and continuous infusion method for quantitative PET analysis of [^{11}C] raclopride binding. J. Cereb. Blood Flow Metab. 18, 941–950.

Kapur, S., Zipursky, R., Jones, C., Remington, G., Houle, S., 2000. Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am. J. Psychiatry 157, 514–520.

Kukreja, S.L., Gunn, R.N., 2004. Bootstrapped DEPICT for error estimation in PET functional imaging. Neuroimage 21, 1096–1104.

Lammertsma, A.A., Hume, S.P., 1996. Simplified reference tissue model for PET receptor studies. Neuroimage 4, 153–158.

Lidow, M.S., Williams, G.V., Goldman-Rakic, P.S., 1998. The cerebral cortex: a case for a common site of action of antipsychotics. Trends Pharmacol. Sci. 19, 136–140.

Logan, J., Fowler, J.S., Volkow, N.D., Ding, Y.S., Wang, G.J., Alexoff, D.L., 2001. A strategy for removing the bias in the graphical analysis method. J. Cereb. Blood Flow Metab. 21, 307–320.

Mizrahi, R., Rusjan, P., Agid, O., Graff, A., Mamo, D.C., Zipursky, R.B., et al., 2007. Adverse subjective experience with antipsychotics and its relationship to striatal and extrastriatal D2 receptors: a PET study in schizophrenia. Am. J. Psychiatry 164, 630–637.

Nordstrom, A.L., Farde, L., Wiesel, F.A., Forslund, K., Pauli, S., Halldin, C., et al., 1993. Central D2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. Biol. Psychiatry 33, 227–235.

Nyberg, S., Farde, L., Halldin, C., Dahl, M.L., Bertilsson, L., 1995. D2 dopamine receptor occupancy during low-dose treatment with haloperidol decanoate. Am. J. Psychiatry 152, 173–178.

Ogden, R.T., Tarpey, T., 2006. Estimation in regression models with externally estimated parameters. Biostatistics 7, 115–129.

Ogden, R.T., Ojha, A., Erlandsson, K., Oquendo, M.A., Mann, J.J., Parsey, R.V., 2007. In vivo quantification of serotonin transporters using [(11)C]DASB and positron emission tomography in humans: modeling considerations. J. Cereb. Blood Flow Metab. 27, 205–217.

Olsson, H., Farde, L., 2001. Potentials and pitfalls using high affinity radioligands in PET and SPECT determinations on regional drug induced D2 receptor occupancy — a simulation study based on experimental data. Neuroimage 14, 936–945.

Olsson, H., Halldin, C., Swahn, C.G., Farde, L., 1999. Quantification of [^{11}C]FLB 457 binding to extrastriatal dopamine receptors in the human brain. J. Cereb. Blood Flow Metab. 19, 1164–1173.

Pilowsky, L.S., Mulligan, R.S., Acton, P.D., Ell, P.J., Costa, D.C., Kerwin, R.W., 1997. Limbic selectivity of clozapine. Lancet 350, 490–491.

Suhara, T., Sudo, Y., Okauchi, T., Maeda, J., Kawabe, K., Suzuki, K., et al., 1999. Extrastriatal dopamine D2 receptor density and affinity in the human brain measured by 3D PET. Int. J. Neuropsychopharmacol. 2, 73–82.

Talvik, M., Nordstrom, A.L., Nyberg, S., Olsson, H., Halldin, C., Farde, L., 2001. No support for regional selectivity in clozapine-treated patients: a PET study with [(11)C] raclopride and [(11)C]FLB 457. Am. J. Psychiatry 158, 926–930.

Turkheimer, F., Sokoloff, L., Bertoldo, A., Lucignani, G., Reivich, M., Jaggi, J.L., et al., 1998. Estimation of compartment and parameter distribution in spectral analysis. J. Cereb. Blood Flow Metab. 18, 1211–1222.

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ORIGINAL INVESTIGATION

Dose-finding study of paliperidone ER based on striatal and extrastriatal dopamine D_2 receptor occupancy in patients with schizophrenia

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Abstract
Rationale Paliperidone ER is a novel antipsychotic drug in an extended-release (ER) formulation. As with all antipsychotics, careful dose setting is necessary to avoid side effects.
Objectives In this study, we measured striatal and extrastriatal dopamine D_2 receptor occupancy during paliperidone ER treatment in patients with schizophrenia using positron emission tomography (PET) to compare regional occupancy and to estimate the optimal dose.
Materials and methods Thirteen male patients with schizophrenia participated in this 6-week multiple-dose study. Six of them took 3 mg of paliperidone ER per day, four took 9 mg, and three took 15 mg. Two to 6 weeks after first drug intake, two PET scans, one with [^{11}C]raclopride and one with [^{11}C]FLB 457, were performed in each patient on the same day. The relationship between the dose or plasma concentration of paliperidone and dopamine D_2 receptor occupancy was calculated.
Results The dopamine D_2 receptor occupancies in the striatum measured with [^{11}C]raclopride and the temporal cortex measured with [^{11}C]FLB 457 were 54.2–85.5% and 34.5–87.3%, respectively. ED_{50} values of the striatum and temporal cortex were 2.38 and 2.84 mg/day, respectively. There was no significant difference in dopamine D_2 receptor occupancy between the striatum and the temporal cortex.
Conclusions The data from this study suggest that paliperidone ER at 6–9 mg provides an estimated level of dopamine D_2 receptor occupancy between 70–80% and that the magnitude of dopamine D_2 receptor occupancy is similar between the striatum and temporal cortex.

Keywords Paliperidone ER · Dopamine D_2 receptor occupancy · Striatum · Extrastriatum · Positron emission tomography · Schizophrenia

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Introduction

Paliperidone is a novel antipsychotic drug for the treatment of schizophrenia. It is an active metabolite of risperidone (9-OH risperidone) and shows almost the same pharmacological profile, with high affinity for dopamine D_2 receptor and serotonin 5-HT_2 receptor (Leysen et al. 1988; Leysen et al. 1994). Paliperidone ER is the extended-release (ER) formulation of paliperidone, which offers low peak-to-trough

fluctuations, and a significant clinical effect over placebo has been reported (Davidson et al. 2007; Kane et al. 2007; Kramer et al. 2007).

Although the term 'limbic selectivity' has been attributed to second-generation antipsychotics based upon regional differences of dopamine D_2 receptor occupancy between the striatum and extrastriatal regions (Bigliani et al. 2000; Bressan et al. 2003a,b; Grunder et al. 2006; Kessler et al. 2006; Pilowsky et al. 1997; Stephenson et al. 2000; Xiberas et al. 2001), inconsistent results have been reported (Agid et al. 2007; Kessler et al. 2005; Talvik et al. 2001; Yasuno et al. 2001). There are no data in the literature concerning dopamine D_2 receptor occupancy in the striatum and extrastriatal regions by paliperidone.

In this study, we investigated the degree of dopamine D_2 receptor occupancy over a wide dose range of paliperidone ER (3–15 mg) and also compared the striatal and extrastriatal dopamine D_2 receptor occupancy in patients with schizophrenia using positron emission tomography (PET).

Materials and methods

Subjects and study protocol

Thirteen male patients (age range, 22–40 years; mean ± SD, 29.4±5.4 year) diagnosed with schizophrenia, according to the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition criteria, participated in the study (Table 1). This study was conducted as part of an open-label phase II trial of paliperidone ER in Japan (JNS007ER-JPN-S21; Janssen Pharmaceutical K.K.). After complete explanation of the study, written informed consent was obtained from all patients. Exclusion criteria were current or past substance abuse, organic brain disease, epilepsy, or diabetes mellitus. Subjects with severe liver or renal dysfunction, prolonged QTc interval, and treatment with electroconvulsive therapy within 90 days before screening were also excluded. The inclusion criteria were less than 120 of the positive and negative symptom scale (PANSS) score at screening and patients well controlled by only one oral antipsychotic drug during the 4 weeks before the study. Administration of paliperidone ER started on the day after the last administration of the previous drug. The paliperidone ER dose was 3 mg/day in six patients, 9 mg/day in four patients, and 15 mg/day in three patients, given once a day after breakfast for 6 weeks at the same dosage. Clinical symptoms were assessed with PANSS before and 6 weeks after the start of treatment with paliperidone ER. Occurrence of extrapyramidal symptoms (EPS) was assessed by clinical observations without using the standard rating scale. After 2 to 6 weeks, two PET scans per patient were done on the same day, one with [^{11}C]raclopride for striatal dopamine D_2 receptor occupancy and one with [^{11}C]FLB 457 for extrastriatal dopamine D_2 receptor occupancy. The reason for the use of different radioligands was that [^{11}C]raclopride is suitable only for a high-density region such as the striatum, and [^{11}C]FLB 457 is suitable for a low-density extrastriatal region, but its affinity is too high for a high-density region (Ito et al. 1999; Okubo et al. 1999). This

Table 1 Characteristics of the patients, positive and negative symptom scale (PANSS), dopamine D_2 receptor occupancy, plasma concentration of paliperidone ER, and EPS

Patient number	Age (year)	Duration of illness (year)	PANSS		Dose (mg/day)	[^{11}C]raclopride		[^{11}C]FLB 457		EPS
			Before	After		Plasma concentration (ng/ml)	Receptor occupancy (%)	Plasma concentration (ng/ml)	Receptor occupancy (%)	
1	28	7.9	59	55	3	7.04	54.2	7.44	58.9	−
2	21	2.2	36	34	3	7.78	58.4	7.5	34.5	−
3	28	5.5	49	46	3	6.32	55.1	6.62	53.3	−
4	35	13	68	67	3	8.33	66.7	8.84	63.0	−
5	22	0.2	77	73	3	12.8	56.2	12.3	37.5	−
6	28	8.1	70	61	3	9.9	56.8	10.2	71.1	−
7	22	7.9	99	96	9	21.4	71.4	20.6	78.7	−
8	33	7.9	60	56	9	57	81.8	51.9	64.6	−
9	25	7.8	43	42	9	27.1	72.1	23.2	74.1	−
10	39	5.4	79	71	9	59.9	84.3	65.2	87.3	+
11	28	0.2	55	38	15	48.2	85.5	43.6	79.6	+
12	33	12.3	65	65	15	14.5	73.7	13.4	74.3	+
13	31	6.9	58	56	15	54.2	82.1	51.7	79.1	−
mean	29	6.6	62.9	58.5						
SD	5.4	3.9	16.5	16.8						

study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan.

PET procedure

A PET scanner system, ECAT EXACT HR + (CTI-Siemens, Knoxville, TN, USA), was used to measure regional brain radioactivity. To minimize head movement, a head fixation device (Fixter, Stockholm, Sweden) was used. A transmission scan for attenuation correction was performed using a ^{68}Ge-^{68}Ga source before each scan. Dynamic PET scanning was performed for 60 min after intravenous bolus injection of 214.3–260.0 MBq of [^{11}C]raclopride. The specific radioactivity of [^{11}C]raclopride was 118.7–294.2 GBq/μmol (mean ± SD, 201.9±45.2 GBq/μmol). One hour after the end of the [^{11}C]raclopride PET measurement, dynamic PET scanning was performed for 80 min after intravenous bolus injection of 218.0–237.4 MBq of [^{11}C]FLB 457. The specific radioactivity of [^{11}C]FLB 457 was 104.7–418.6 GBq/μmol (mean ± SD, 299.3±112.2 GBq/μmol). Magnetic resonance (MR) images of the brain were acquired with 1.5 T MR imaging, Gyroscan NT (Philips Medical Systems, Best, The Netherlands). T_1-weighted MR images at 1-mm slices were obtained. Venous blood samples were obtained immediately before tracer injection for each PET scan to measure the plasma concentration of paliperidone.

Data analysis

All emission scans were reconstructed with a Hanning filter cut-off frequency of 0.4. Regions of interest (ROIs) were defined for the striatum ([^{11}C]raclopride), temporal cortex ([^{11}C]FLB 457), and cerebellum ([^{11}C]raclopride and [^{11}C]FLB 457). The ROIs were drawn manually on the summated PET images with reference to the individual MR images. The average values of right and left ROIs were used for the analysis. Dopamine D_2 receptor binding was quantified using a three-parameter simplified reference tissue model (Ito et al. 2001; Lammertsma and Hume 1996). The cerebellum was used as the reference tissue given its negligible density of dopamine D_2 receptors (Suhara et al. 1999). This model allows the estimation of binding potential (BP_{ND}), which was defined as $f_{ND} \times B_{max}/K_d$, where f_{ND} is the free fraction of ligand in the nondisplaceable tissue compartment, B_{max} is the receptor density, and K_d is the dissociation constant (Innis et al. 2007).

The dopamine D_2 receptor occupancy by paliperidone was estimated using the following equation: occupancy(%) = $(BP_{base} - BP_{drug})/BP_{base} \times 100$, where BP_{base} is the BP_{ND} in the drug-free state, and BP_{drug} is the BP_{ND} after administration of paliperidone (Takano et al. 2004; Takano et al. 2006a, b; Yasuno et al. 2001). In this study, the mean BP_{ND} in age-matched normal male subjects (n=13; age range 22–40 years; mean ± SD, 29.2±5.5 years) was used as BP_{base}, as BP_{ND} in the striatum measured with [^{11}C]raclopride or in the temporal cortex measured with [^{11}C]FLB 457 in patients with schizophrenia is not significantly different from that in the normal control (Farde et al. 1990; Suhara et al. 2002; Talvik et al. 2003). The PET procedure and data analysis for the BP_{ND} estimation of normal subjects were the same as those for the patients. The relationship between the dose or plasma concentration of paliperidone and dopamine D_2 receptor occupancy is described by the following equation: occupancy(%) = $C/(C + ED_{50}) \times 100$, where C is the dose or plasma concentration of paliperidone, and ED_{50} is the dose or plasma concentration required to induce 50% occupancy (Nyberg et al. 1999; Takano et al. 2004; Takano et al. 2006a, b; Yasuno et al. 2001). In this study, maximum occupancy was fixed at 100%, the same as previous occupancy studies of risperidone (Nyberg et al. 1999; Yasuno et al. 2001).

Measurement of plasma concentration of paliperidone

Blood samples were collected in heparinized tubes and centrifuged for 10 min at 3,000 rpm. Separated plasma samples were stored at −20°C. Plasma concentrations of paliperidone were determined using a validated liquid chromatography coupled to mass spectrometry/mass spectrometry (LC–MS/MS) method with a target lower limit of quantification of 0.10 ng/ml (Johnson & Johnson Pharmaceutical Research and Development L. L. C., Beerse, Belgium).

Statistical analysis

Correlations between dose or plasma concentration of paliperidone and dopamine D_2 receptor occupancy in the striatum and temporal cortex were assessed. Correlations between striatal occupancy and age or duration of illness were also assessed. Paired t tests were performed to compare (1) dopamine D_2 receptor occupancies between the striatum and temporal cortex and (2) plasma concentrations of paliperidone between the two PET scans, with [^{11}C]raclopride and [^{11}C]FLB 457, in each individual subject. In all tests, a p value<0.05 was considered statistically significant.

Results

The dopamine D_2 receptor occupancy in the striatum measured with [^{11}C]raclopride was 54.2 to 85.5% (Table 1). Mean dopamine D_2 receptor occupancies in the striatum were 57.9±4.5% at 3 mg/day, 77.4±6.6% at 9 mg/day, and 80.4±6.1% at 15 mg/day. ED_{50} in the striatum was 2.38 mg/day (r=0.86) and 6.65 ng/ml (r=0.82; Fig. 1).