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ORIGINAL INVESTIGATION

Dopamine D_2 receptor occupancy by perospirone: a positron emission tomography study in patients with schizophrenia and healthy subjects

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Abstract
Rationale Perospirone is a novel second-generation antipsychotic drug with high affinity to dopamine D_2 receptor and short half-life of plasma concentration. There has been no investigation of dopamine D_2 receptor occupancy in patients with schizophrenia and the time course of occupancy by antipsychotics with perospirone-like properties.
Objective We investigated dopamine D_2 receptor occupancy by perospirone in patients with schizophrenia and the time course of occupancy in healthy subjects.
Materials and methods Six patients with schizophrenia taking 16–48 mg/day of perospirone participated. Positron emission tomography (PET) scans using [^{11}C]FLB457 were performed on each subject, and dopamine D_2 receptor occupancies were calculated. Moreover, baseline and three serial PET using [^{11}C]raclopride were performed at 1.5, 8, and 25.5 h after administration of a single dose of 16 mg of perospirone on four healthy male subjects, and occupancy was calculated for each scan.
Results Dopamine D_2 receptor occupancy in the temporal cortex of patients ranged from 39.6% to 83.8%. Especially, occupancy in two patients who took 16 mg of perospirone 2.5 h before PET was over 70%. Mean occupancy in the striatum of healthy subjects was 74.8% at 1.5 h, 60.1% at 8 h, and 31.9% at 25.5 h after administration.
Conclusion Sixteen milligrams of perospirone caused over 70% dopamine D_2 receptor occupancy near its peak level, and then occupancy dropped to about half after 22 h. The time courses of receptor occupancy and plasma concentration were quite different. This single dosage may be sufficient for the treatment of schizophrenia and might be useful as a new dosing schedule choice.

Keywords Dopamine D_2 receptor occupancy · Perospirone · Positron emission tomography · Schizophrenia · Time course

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Introduction

Perospirone is a novel second-generation antipsychotic drug used in Japan (Onrust and McClellan 2001). This drug shows high affinity to dopamine D_2 receptor (K_i= 1.77 nM) and serotonin 5-HT_2 receptor (K_i=0.06 nM; Takahashi et al. 1998), and its plasma concentration has a short half-life ($T_{1/2}$=1.9 h; Yasui-Furukori et al. 2004). A previous positron emission tomography (PET) study using [^{11}C]raclopride and [^{11}C]NMSP in healthy subjects with single 8 mg of perospirone showed blockage of both dopamine D_2 receptor and serotonin 5-HT_2 receptor (Sekine et al. 2006), but the optimal dose of perospirone in patients with schizophrenia has not been investigated.

Kapur et al. (2000b) reported that transient high dopamine D_2 receptor occupancy by quetiapine showed clinical effects for patients with schizophrenia. They suggested that this transient occupancy was related to "atypical" features of second-generation antipsychotics

with low affinity for dopamine D_2 receptor (Kapur and Seeman 2001). Plasma pharmacokinetics and affinity for receptor were considered to affect the time course of receptor occupancy (Kapur and Seeman 2001; Takano et al. 2004). However, the time course of receptor occupancy by antipsychotics with high affinity for dopamine D_2 receptor and a short half-life of plasma concentration has not been investigated.

In this study, we investigated dopamine D_2 receptor occupancy by several doses of perospirone in patients with schizophrenia. Moreover, we investigated the time course of dopamine D_2 receptor occupancy by perospirone with serial PET scanning in healthy subjects.

Materials and methods

This study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan. After complete explanation of this study, written informed consent was obtained from all subjects.

Patient study

Subjects and study protocol

Six patients aged 26–44 years (34.9±7.1, mean ± SD), diagnosed with schizophrenia according to Diagnostic and Statistical Manual of Mental Disorders-IV criteria, participated in this study (Table 1). Exclusion criteria were current or past substance abuse, brain tumor or vascular disease, and history of severe head injury or epilepsy. Subjects with severe liver or renal dysfunction, or having undergone electroconvulsive therapy within 90 days prior to this study were also excluded. The patients had been taking fixed dosages of perospirone for more than 2 weeks before this study. Doses of perospirone were 16 mg/day in one patient, 24 mg/day in two patients, and 48 mg/day in three patients. The interval between the last administration of perospirone and PET scan was from 2.5 to 17.5 h. Clinical symptoms were assessed by positive and negative symptom scale (PANSS). Venous blood samples were taken before and after PET scanning to measure the plasma concentration of perospirone and ID-15036, an active metabolite of perospirone (hydroxyperospirone). The average values of pre- and post-PET scanning were used.

PET procedure

A PET scanner system, ECAT EXACT HR+ (CTI-Siemens, Knoxville, TN, USA), was used for all subjects. A head fixation device was used to minimize head movement. Before the dynamic scan, a transmission scan for attenuation correction was performed using a ^{68}Ge-^{68}Ga source. The dynamic PET scan was performed for 90 min after intravenous bolus injection of 204.0–225.0 MBq (218.5± 7.7 MBq, mean ± SD) of [^{11}C]FLB 457. The specific radioactivity of [^{11}C]FLB 457 was 129.6–219.4 MBq/nmol (175.4±34.3 MBq/nmol, mean ± SD). Magnetic resonance images of the brain were acquired with 1.5 Tesla magnetic resonance imaging (MRI), Gyroscan NT (Philips Medical Systems, Best, Netherlands). T1-weighted images at 1-mm slices were obtained.

Data analysis

All emission scan data were reconstructed with a Hanning filter. Regions-of-interest (ROIs) were defined for the temporal cortex and cerebellar cortex. ROIs were drawn manually on PET images with reference to the individual MR images. The values of ROIs for right and left sides were averaged. Binding potential (BP_{ND}), defined as the specific binding compared to nondisplaceable uptake, of dopamine D_2 receptor in the temporal cortex was calculated using a three-parameter simplified reference tissue model (SRTM; Innis et al. 2007; Lammertsma and Hume 1996). The cerebellum was used as reference tissue because of its negligible density of dopamine D_2 receptors (Suhara et al. 1999).

Table 1 Patient characteristics, plasma concentration, and dopamine D_2 receptor occupancy

Number	Age (year)	Sex	PANSS	Dose (mg/day)	Interval: last dose–PET (h)	Last dose (mg)	Plasma concentration		Receptor occupancy (%)
							Perospirone (ng/ml)	ID-15036 (ng/ml)	
1	38	M	59	16	2.5	16	4.5	23.3	83.8
2	30	F	69	24	7.5	8	0.6	3.05	61.8
3	44	F	62	24	9.0	8	0	0.75	39.6
4	26	M	81	48	2.5	8	1.25	8.45	60.8
5	30	F	46	48	2.5	16	0.25	8.35	70.1
6	42	F	80	48	17.5	32	0.85	2.1	65.0

Receptor occupancy of perospirone is expressed as follows: Occupancy(%) = $(BP_{baseline} - BP_{drug})/BP_{baseline} \times 100$, where $BP_{baseline}$ is BP_{ND} in the drug-free state, and BP_{drug} is BP_{ND} after administration of the drug. Mean BP_{ND} of age-matched ten normal male subjects (age range 25–43 years; 34.8±6.7 years, mean ± SD) measured by the same procedure as for the patients was used as BP_{base} because of the lack of individual baseline BP_{ND}.

The relationship between receptor occupancy and plasma concentration of antipsychotic drug can be expressed as follows: Occupancy(%) = $C/(C + EC_{50}) \times 100$, where C is the plasma concentration of perospirone or ID-15036, and EC_{50} is the concentration required to induce 50% occupancy.

Measurement of plasma concentration of perospirone

Plasma concentrations of perospirone and ID-15036 were determined using a validated high performance liquid chromatography method (Yasui-Furukori et al. 2003; MP-Technopharma Corporation, Fukuoka, Japan). The lower limit of quantification was 0.1 ng/ml for both perospirone and ID-15036.

Healthy subject study

Subjects and study protocol

Four healthy male subjects aged 22–32 years (26.8±4.1, mean ± SD) participated in the other part of this study. None had a history of psychiatric, neurological, or somatic disorders. None had taken any medication for at least 2 weeks prior to this study. The baseline PET scan was performed within 2 weeks before taking perospirone. All subjects took a single dose of 16 mg of perospirone, and then three serial PET scans were performed at 1.5, 8, and 25.5 h after its administration. Venous blood samples were taken 11 times, at 0.5, 1.0, 1.5, 2.5, 3.5, 5.0, 6.5, 8.0, 9.0, 25.5, and 26.5 h after perospirone administration, to measure the plasma concentrations of perospirone and ID-15036.

PET procedure

A PET scanner system, ECAT EXACT HR+, was used for all subjects. A head fixation device was used to minimize head movement. Before the dynamic scan, a transmission scan for attenuation correction was performed using a ^{68}Ge-^{68}Ga source. The dynamic PET scan was performed for 60 min after intravenous bolus injection of 179.6–246.8 MBq (217.0±16.5 MBq, mean ± SD) of [^{11}C] raclopride. The specific radioactivity of [^{11}C]raclopride was 138.0–320.9 MBq/nmol (235.4±65.8 MBq/nmol, mean ± SD). T1-weighted images at 1-mm slices of the brain were acquired with 1.5 Tesla MRI, Gyroscan NT.

Data analysis

All emission scan data were reconstructed with a Hanning filter. ROIs were defined for the striatum and cerebellar cortex and were drawn manually on the PET images with reference to individual MR images. The values of ROIs for right and left sides were averaged. BP_{ND} of dopamine D_2 receptor in the striatum was calculated using SRTM. The cerebellum was used as reference tissue. Receptor occupancy was calculated using the individual BP_{ND} values of baseline and drug administration.

Results

Patient study

Dopamine D_2 receptor occupancy of patients with schizophrenia in the temporal cortex ranged from 39.6% to 83.8% (Table 1). Plasma concentrations of perospirone and ID-15036 ranged from 0 to 4.5 and 0.75 to 23.3 ng/ml, respectively. The plasma concentrations of perospirone and ID-15036 were fitted curvilinearly to the dopamine D_2 receptor occupancy (Fig. 1a, b). Estimated EC_{50} values of perospirone and ID-15036 were 0.31 and 1.90 ng/ml, respectively. The total PANSS score ranged from 46 to 81, and the average score of all patients was 66.2±13.4.

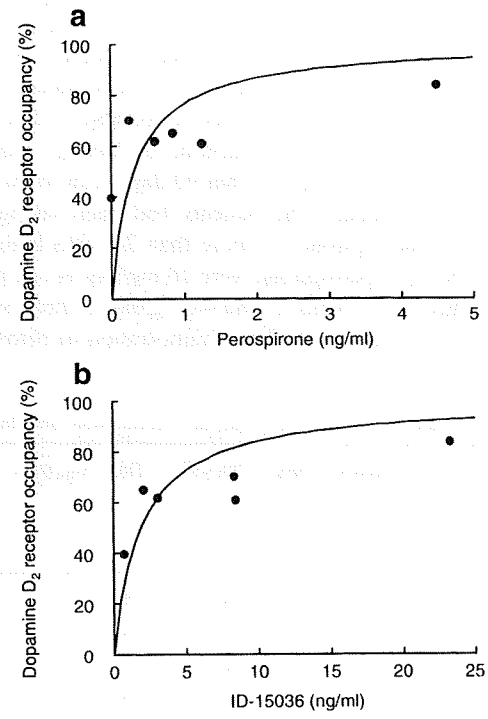

Fig. 1 Relationship between dopamine D_2 receptor occupancy and perospirone (**a**) and ID-15036 (**b**) in the patients study

Healthy subject study

Mean dopamine D_2 receptor occupancies in the striatum were 74.8±8.0% at 1.5 h, 60.1±5.6% at 8 h, and 31.9±6.4% at 25.5 h after administration of 16 mg of perospirone in healthy subjects (Fig. 2). The mean plasma concentrations of both perospirone and ID-15036 reached a peak at 1 h after administration, then rapidly decreased, and were not detectable at 25.5 h after (Fig. 3a, b). Estimated half-lives of plasma concentrations of perospirone and ID-15036 were 2.2 and 1.9 h, respectively. No subject complained of severe side effects such as extrapyramidal symptoms or sleepiness.

Discussion

Clinical dose of perospirone

A previous study reported that dopamine D_2 receptor occupancy using [^{11}C]raclopride was 44.4% with 8 mg of perospirone at 1 h post-administration (Sekine et al. 2006). PET studies have suggested that more than 70% dopamine D_2 receptor occupancy is necessary for antipsychotic effect and that 80% occupancy causes extrapyramidal symptoms (Farde et al. 1992; Kapur et al. 2000a; Nordstrom et al. 1993). Two patients (numbers 1 and 5) administered perospirone at 16 mg 2.5 h before PET scanning showed over 70% occupancy. On the other hand, one patient (number 4) taking 8 mg did not reach 70% occupancy in spite of a short interval between the last administration and PET scan. In healthy subjects, a peak of about 75% occupancy was also obtained with 16 mg of perospirone. Although some patients could be maintained at less than 70% occupancy, 16 mg of perospirone seems to be the necessary dose for achieving antipsychotic effect. The plasma concentrations of perospirone and ID-15036 inducing 70% occupancy (EC_{70}) were 0.72 and 4.43 ng/ml, respectively. Side effects could not be evaluated in this study because some patients were taking

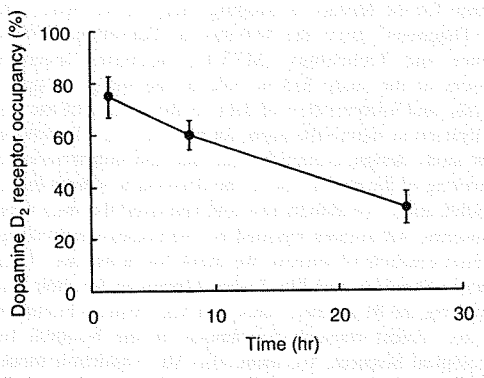

Fig. 2 Time course of mean dopamine D_2 receptor occupancy in healthy subject study. *Bars* represent standard deviation of mean

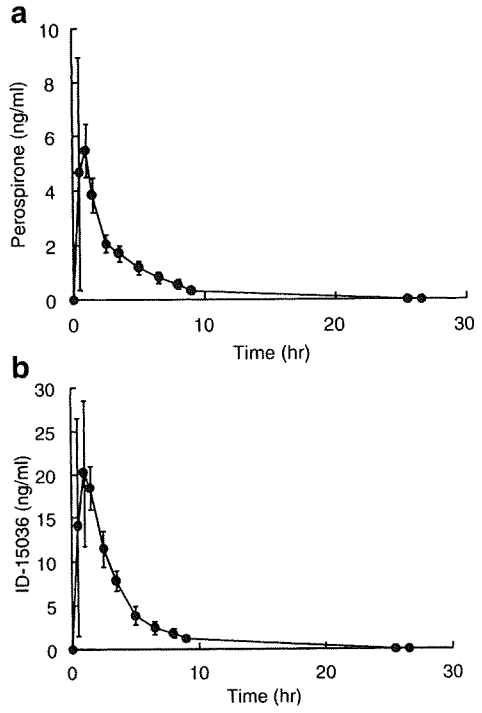

Fig. 3 Time course of mean plasma concentrations of perospirone (**a**) and ID-15036 (**b**) in healthy subjects study. *Bars* represent standard deviation of mean

benzodiazepines or anti-Parkinson drugs, and plasma prolactin levels were not measured.

Pharmacokinetics and contributions to receptor occupancy of perospirone and ID-15036

In healthy subjects, plasma concentrations of perospirone and ID-15036 peaked at 1 h after administration, with the half-lives of plasma concentrations being 2.2 and 1.9 h, respectively. The plasma concentration of ID-15036 was fourfold that of perospirone. These results were in good agreement with the previous study showing that the T_{max} values were 0.8 (perospirone) and 1.1 h (ID-15036), and $T_{1/2}$ was 1.9 h (perospirone; Yasui-Furukori et al. 2004). As ID-15036 has affinity for the dopamine D_2 receptor (K_i= 5.84 nM) and blocks the dopamine D_2 receptor of the in vivo rat brain (Takahashi et al. 1998), both perospirone and ID-15036 contributed to dopamine D_2 receptor occupancy, and the plasma concentrations of both were fitted to the occupancy curve.

Effects of affinity and pharmacokinetics of antipsychotics on time course of receptor occupancy

Dopamine D_2 receptor occupancy was about 75% at 1.5 h after perospirone administration and then showed a rela-

tively rapid decline. After 25.5 h, about 30% occupancy remained, although plasma concentrations of perospirone and ID-15036 were not detectable. The time to reach half of the peak occupancy of 75% was 22 h. The time courses of receptor occupancy and plasma concentration were quite different. In comparison, risperidone and olanzapine showed sustained occupancy; about 80% occupancy 5 or 6 h after administration decreased to only 70% after 24 h (Takano et al. 2004; Tauscher et al. 2002). On the other hand, quetiapine showed transient occupancy; 64% occupancy after 2 h decreased to 0% after 24 h (Kapur et al. 2000b). Some factors such as the time course of plasma concentration of antipsychotics or affinity for dopamine D_2 receptor were considered to affect the time course of receptor occupancy (Kapur and Seeman 2001; Takano et al. 2004). For example, high affinity and long half-life of plasma concentration (e.g., risperidone (K_i=1.1 nM, $T_{1/2}$= 17.8 h) and olanzapine (K_i=5.1 nM, $T_{1/2}$=19.5 h)) expressed sustained occupancy, and low affinity and short half-life of plasma concentration (e.g., quetiapine (K_i= 122 nM, $T_{1/2}$=3.2 h)) expressed transient occupancy (Gefvert et al. 1998; Seeman 2002; Takano et al. 2004; Tauscher et al. 2002). Perospirone has high affinity for dopamine D_2 receptor and a short half-life of plasma concentration (Takahashi et al. 1998; Yasui-Furukori et al. 2004). These features may cause relatively rapid decrease in occupancy, from 75% at 1.5 h of perospirone administration to 32% after 25.5 h, but the occupancy did not completely disappear within a day. In patients taking 32 mg perospirone (number 6), dopamine D_2 receptor occupancy was 65% at 17.5 h after, supporting an intermediate time course between sustained and transient occupancy.

Possibility of new dosing schedule with perospirone

There are several opinions concerning the dosing schedule of antipsychotics. A recent clinical study reported that extended antipsychotic dosing (every second or third day) was effective and decreased side effects for chronic patients with schizophrenia (Remington et al. 2005). An animal study reported that transient antipsychotic medication was more effective for amphetamine-induced behavioral abnormality than continuous one (Samaha et al. 2008). These findings indicate that sustained occupancy might not necessarily be required for antipsychotic therapy of schizophrenia. In prodromal episode-based intervention, antipsychotic drugs were used occasionally, and long antipsychotic-free periods were sometimes inserted. However, some studies reported that intermittent medication increased the relapse rate in schizophrenia (Gaebel et al. 2002; Herz et al. 1991; Schooler et al. 1997). Because perospirone shows an intermediate time course between sustained and transient occupancy, its single administration may become a new dosing schedule choice for an antipsychotic drug. Indeed, the administration of perospirone once a day indicated antipsychotic effects and preventions from relapse for chronic patients with schizophrenia (Kusumi et al. 2008). Four patients in the present study (numbers 1, 4, 5, and 6) taking 16 mg or more at least once a day were maintained for more than 6 months. Further study of relationships between clinical response and receptor occupancy of various dosing schedules in patients with schizophrenia will be needed.

Regional difference of dopamine D_2 receptor occupancy

Regional differences of dopamine D_2 receptor occupancy between the striatum and extrastriatum in some second-generation antipsychotic drugs have been discussed (Arakawa et al. 2008; Ito et al. 2009; Pilowsky et al. 1997; Talvik et al. 2001). In the present study, the mean occupancy of four healthy subject and two patients (number 1 and 5) in a short interval between the administration of 16 mg of perospirone and PET scanning seemed to differ very little (75.1% in the striatum with [^{11}C]raclopride and 77.0% in the temporal cortex with [^{11}C]FLB 457). It is suggested that there were no regional differences of dopamine D_2 receptor occupancy between the striatum and extrastriatum with perospirone despite the subjects, study protocols, and radioligands being different.

Conclusion

Sixteen milligrams of perospirone caused over 70% dopamine D_2 receptor occupancy near its peak level, then becoming about half after 22 h. The time courses of receptor occupancy and plasma concentration were quite different. This single dosage may be sufficient for the treatment of schizophrenia and might be useful as a new dosing schedule choice.

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Positron Emission Tomography Measurement of Dopamine D_2 Receptor Occupancy in the Pituitary and Cerebral Cortex: Relation to Antipsychotic-Induced Hyperprolactinemia

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Objective: Hyperprolactinemia is a common side effect of antipsychotic drugs used in the treatment of schizophrenia. However, the magnitude of hyperprolactinemia differs among antipsychotics, and there is no reliable mechanism-related marker for the risk of hyperprolactinemia that would allow us to characterize antipsychotics.

Method: In this study, 11 healthy male subjects taking different doses of sulpiride and 24 male patients with *DSM-IV*–diagnosed schizophrenia taking different antipsychotic drugs (risperidone, olanzapine, haloperidol, and sulpiride) participated. Positron emission tomography scanning using [^{11}C]FLB 457 was performed on all subjects. The dopamine D_2 receptor occupancy of antipsychotics in the pituitary and temporal cortex was calculated. Correlations between plasma concentration of prolactin and dopamine D_2 receptor occupancies were evaluated. The ratio of drug concentration of cerebral receptor site to that of pituitary receptor site (brain/plasma concentration ratio; B/P ratio) was calculated from the receptor occupancies in the 2 regions. Data were collected between November 2001 and September 2007.

Results: Significant positive correlation was observed between the plasma concentration of prolactin and dopamine D_2 receptor occupancy in the pituitary by all 4 antipsychotics ($P = .001$). Dopamine D_2 receptor occupancies of sulpiride were markedly different between the pituitary and temporal cortex, and the B/P ratio for sulpiride (0.34) was significantly lower than for olanzapine ($P = .007$) and risperidone ($P = .015$). Olanzapine had a relatively high B/P ratio (2.70), followed by haloperidol (2.40) and risperidone (1.61).

Conclusions: Dopamine D_2 receptor occupancy in the pituitary is a good indicator of hyperprolactinemia. B/P ratio, indicating the penetrating capability across the blood-brain barrier, seems to be a good characteristic biomarker of each antipsychotic drug for the risk of hyperprolactinemia at therapeutic dose.

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Hyperprolactinemia is a commonly encountered side effect of antipsychotic drugs in the treatment of schizophrenia,[1,2] and several deficits, such as galactorrhea and sexual dysfunction, can result. For women, amenorrhea and infertility are severe adverse effects, and long-term hyperprolactinemia causes osteoporosis in relation to hypogonadism.[1,3] Hyperprolactinemia is one of the major reasons for discontinuing antipsychotic drugs,[4] but the risk for this condition varies among them.[5–8] It has been reported that antipsychotics such as risperidone and amisulpride showed a high risk, and several factors have been discussed in relation to this risk,[5–9] such as affinity for dopamine D_2 receptors[6,8] and the pharmacodynamics in plasma and brain.[9]

Prolactin secretion is controlled by tonic inhibition of dopamine on tuberoinfundibular neurons.[10,11] Hyperprolactinemia is reported to be induced by the blocking of dopamine D_2 receptors in the pituitary. As the pituitary is located outside of the blood-brain barrier, drug effects on dopamine D_2 receptors would differ between it and the brain parenchyma.[9] However, there has been no report about this relation in the living human brain.

Previous positron emission tomography (PET) studies focused on extrapyramidal side effects of antipsychotics induced by over 80% of striatal dopamine D_2 receptor occupancy.[12–14] Since dopamine D_2 receptor density in the pituitary is low ($B_{max} = 1.3$ pmol/g tissue) compared to the striatum ($B_{max} = 16.6$ pmol/g tissue),[15] measurement of dopamine D_2 receptor binding in the pituitary is difficult using a radioligand with relatively low affinity such as [^{11}C]raclopride. [^{11}C]FLB 457 has very high affinity for dopamine D_2 receptors,[16] and since it is used to measure dopamine D_2 receptors in extrastriatal regions where their density is very low,[17–21] it can also be used to measure dopamine D_2 receptor binding in the pituitary. Although some studies have

reported the visualization or occupancy of human pituitary dopamine D_2 receptors,[22-24] the quantification of dopamine D_2 receptor occupancy in the pituitary by several antipsychotics using PET has not been reported.

In this study, we aimed to investigate biomarkers for the potential risk of antipsychotic drug–induced hyperprolactinemia in the living human brain. Dopamine D_2 receptor occupancies in the pituitary and temporal cortex were measured using [^{11}C]FLB 457 by different doses of sulpiride in healthy subjects to examine the dose-occupancy relationship in the 2 regions and by various antipsychotic drugs in patients with schizophrenia to examine the relation with hyperprolactinemia.

METHOD

Subjects and Study Protocol

Study of healthy subjects receiving different doses of sulpiride. Eleven healthy male subjects (age range, 21–40 years; mean ± SD = 27.1 ± 5.8 years) participated in this study. Two PET scans using [^{11}C]FLB 457 were performed before and 3 hours after a single dose of sulpiride at 200 mg (n = 3), 400 mg (n = 3), 600 mg (n = 3), and 800 mg (n = 2). Just before the second PET scan, venous blood samples were taken to measure the plasma concentration of prolactin.

Study of patients with schizophrenia receiving different antipsychotics. Twenty-four male patients (age range, 21–49 years; mean ± SD = 37.1 ± 8.9 years) diagnosed with schizophrenia according to *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (*DSM-IV*)[25] criteria participated in this study. Exclusion criteria were current or past substance abuse, organic brain disease, or epilepsy. Subjects with severe liver or renal dysfunction or who had undergone electroconvulsive therapy within 90 days prior to this study were also excluded. The patients had been taking 1 oral antipsychotic drug at fixed dosage for at least 2 weeks before the start of this study (range, 2 weeks to 11 years; mean, 25 months). Seven patients took risperidone at 2 mg (n = 2), 4 mg (n = 4), and 6 mg (n = 1); 7 patients took olanzapine at 5 mg (n = 2), 10 mg (n = 3), 15 mg (n = 1), and 20 mg (n = 1); 4 patients took haloperidol at 6 mg (n = 2), 9 mg (n = 1), and 12 mg (n = 1); and 6 patients took sulpiride at 200 mg (n = 2), 400 mg (n = 2), 600 mg (n = 1), and 900 mg (n = 1). Antipsychotic treatment was continued during the performance of the PET scans using [^{11}C]FLB 457. The duration between PET scan and the last administration of antipsychotic drug was between 2 hours and 20 hours. Just before PET scan, venous blood samples were taken to measure the plasma concentration of prolactin.

After complete description of this study, written informed consent was obtained from all subjects. The study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiologic Sciences, Chiba, Japan. Data were collected between November 2001 and September 2007.

Positron Emission Tomography Procedure

A PET scanner system, ECAT EXACT HR+ (CTI-Siemens, Knoxville, Tennessee), was used for all subjects. A head fixation device was used to minimize head movement. A transmission scan for attenuation correction was performed using a ^{68}Ge-^{68}Ga source before each scan. Dynamic PET scan was performed for 90 minutes after intravenous bolus injection of 155.0–240.1 MBq (mean ± SD = 228.5 ± 72.5 MBq) of [^{11}C]FLB 457. The specific radioactivity of [^{11}C]FLB 457 was 81.6–339.9 GBq/μmol (174.8 ± 63.4 GBq/μmol). Magnetic resonance images (MRIs) of the brain were acquired with 1.5 Tesla MRI, Gyroscan NT (Philips Medical Systems, Best, The Netherlands). T1-weighted images were obtained at 1-mm slices. All subjects were free of organic brain or pituitary lesions.

Data Analysis

All emission scan data were reconstructed with a Hanning filter. Regions of interest (ROIs) were defined for the pituitary, temporal cortex, and cerebellar cortex. The ROIs were drawn manually on PET images with reference to the individual magnetic resonance images. The values of ROIs for the right and left sides were averaged. The temporal cortex was used as the representative brain region because there was little difference in dopamine D_2 receptor occupancy among extrastriatal brain regions.[26] Binding potential (BP_{ND}) of dopamine D_2 receptors was calculated from the ratio of the area under the time-activity curve (AUC):

$$BP_{ND} = (AUC_{region} / AUC_{cerebellum}) - 1 \quad \text{(Equation 1).}$$

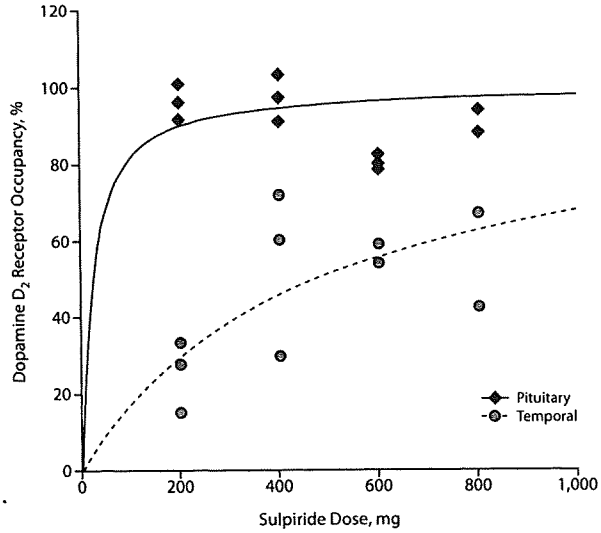
The subscript "region" denotes the pituitary and temporal cortex. The cerebellum was used as reference tissue given its negligible density of dopamine D_2 receptors.[27] In this study, an integration interval of 60 to 90 minutes was used for the calculation of AUC.[20]

The receptor occupancy of antipsychotic drug is expressed as follows[14]:

$$\text{Occupancy (\%)} = (BP_{baseline} - BP_{drug}) / BP_{baseline} \times 100 \quad \text{(Equation 2),}$$

in which $BP_{baseline}$ is the baseline BP_{ND} in the drug-free state and BP_{drug} is BP_{ND} after the administration of antipsychotic drug. In the healthy subjects study, both BP_{ND} values obtained from each individual were used. In the patients study, the mean BP_{ND} of 15 age-matched healthy male subjects (age range, 21–49 years, mean ± SD = 34.2 ± 8.4 years) was used as $BP_{baseline}$ because of the lack of individual baseline BP_{ND} values. There was no difference in age between healthy subjects and patients (2-tailed *t* test; $P = .32$). There was no age effect of BP_{ND} in the temporal cortex ($P = .37$) and pituitary ($P = .61$) within this age range (21–49 years) of healthy subjects.

Figure 1. Relationship Between Dose of Sulpiride and Dopamine D_2 Receptor Occupancy in the Pituitary or Temporal Cortex in Healthy Subjects[a]



[a]Dopamine D_2 receptors in the pituitary were almost fully occupied even at the lowest dose of sulpiride (200 mg), at which occupancy in the temporal cortex was around 25%. Curves were fitted according to this equation: Occupancy (%) = $D/(D+ED_{50})\times 100$. ED_{50} for the pituitary was 22.2 mg and 475.6 mg for the temporal cortex.

The relationship between receptor occupancy and antipsychotic drug dose can be expressed as follows[18,21]:

$$\text{Occupancy}\ (\%) = D/(D+ED_{50})\times 100 \quad \text{(Equation 3)},$$

in which D is the dose of drug and ED_{50} is the dose required to induce 50% occupancy.

We calculated the ratio of drug concentration in the cerebral cortex to that in plasma (brain/plasma ratio; B/P ratio), which indicates the penetrating capability of antipsychotic drugs across the blood-brain barrier. Equation 3 can be rewritten as follows:

$$C = IC_{50}/([100/\text{Occupancy}]-1) \quad \text{(Equation 4)},$$

in which C is the drug concentration in the brain or plasma. IC_{50} is the drug concentration required to induce 50% occupancy, reflecting the affinity of each antipsychotic drug to dopamine D_2 receptor, and, therefore, IC_{50} can be assumed to be the same value between the pituitary and the temporal cortex. Because the pituitary exists outside the blood-brain barrier, the B/P ratio can be expressed as follows:

$$\text{B/P ratio} = C_{brain}/C_{pituitary} = ([100/\text{Occupancy}_{pituitary}]-1)/([100/\text{Occupancy}_{temporal}]-1) \quad \text{(Equation 5)},$$

in which $C_{pituitary}$ is the drug concentration in the vicinity of receptors in the pituitary, and C_{brain} is the drug concentration in the vicinity of receptors in the temporal cortex. $\text{Occupancy}_{pituitary}$ is the dopamine D_2 receptor occupancy in the pituitary, and $\text{Occupancy}_{temporal}$ is that in the temporal cortex. The B/P ratio of each antipsychotic drug was calculated.

Prolactin Measurement

The plasma concentration of prolactin was measured by chemiluminescent immunoassay at a commercial laboratory (SRL Inc, Tokyo, Japan). The normal range for males is 3.6–12.8 ng/mL. Values exceeding 12.8 ng/mL were defined as hyperprolactinemia.

Simulation Study

A simulation study was performed in order to estimate the relationship between the B/P ratio and prolactin. First, pituitary occupancy was calculated by Equation 5 according to changes in the B/P ratio when occupancy in the temporal cortex was set at 60%, 70%, and 80%, which was the range of clinical dosage.[12–14] Next, assumed prolactin values were estimated using linear regression obtained from the patients study. The measured prolactin values in patients (mean temporal cortex occupancy, 66.5 ± 13.9%) were plotted in this simulation graph against the mean B/P ratio of each antipsychotic drug.

Statistics

Correlations between plasma concentration of prolactin and dopamine D_2 receptor occupancy in the pituitary or temporal cortex by the 4 antipsychotic drugs were evaluated using Pearson correlation coefficient. The relationship between occupancy in the pituitary and hyperprolactinemia was evaluated using Fisher exact test. Group differences of B/P ratio among the 4 antipsychotics were evaluated by Kruskal-Wallis test. Multiple comparisons of B/P ratio between the respective antipsychotics were evaluated using the Mann-Whitney *U* test with Ryan method.

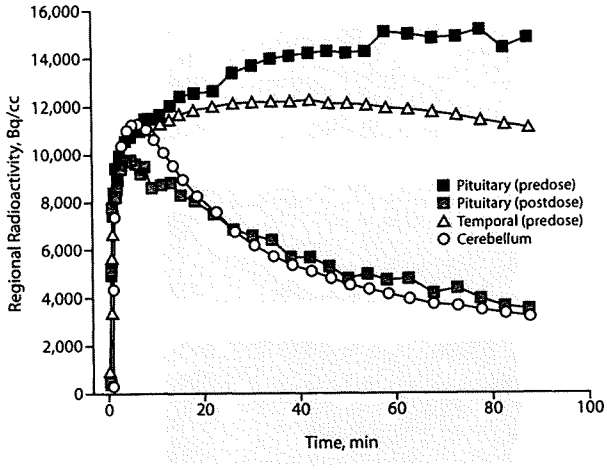
RESULTS

Study of Healthy Subjects Receiving Different Doses of Sulpiride

Dopamine D_2 receptor occupancy in the pituitary by sulpiride ranged from 78.4% to 103.2% (mean ± SD = 96.1 ± 4.6% for 200 mg, 97.1 ± 6.1% for 400 mg, 80.3 ± 1.9% for 600 mg, and 91.1 ± 4.2% for 800 mg), and the occupancy in the temporal cortex ranged from 15.2% to 71.9% (25.4 ± 9.3% for 200 mg, 54.0 ± 21.8% for 400 mg, 55.9 ± 2.9% for 600 mg, and 54.8 ± 17.5% for 800 mg) (Figure 1). ED_{50} for the pituitary was 22.2 mg and for the temporal cortex was 475.6 mg. The plasma concentration of prolactin ranged from 19.1 to 41.7 ng/mL. The plasma concentration of prolactin for all subjects reached the level of hyperprolactinemia.

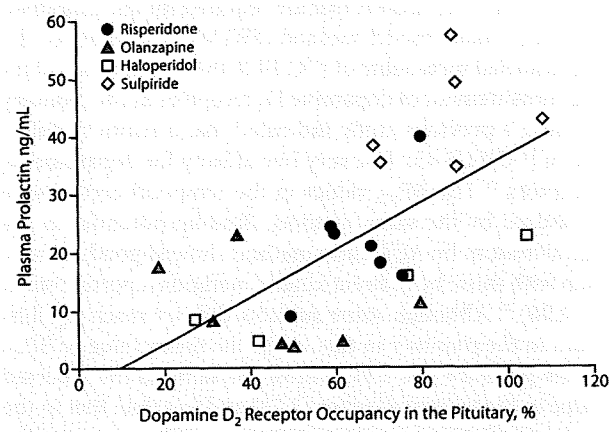
Figure 2 shows the mean values of the time-activity curves of the pituitary (predose and postdose of sulpiride),

Figure 2. Time-Activity Curves of the Pituitary (Predose and Postdose), Temporal Cortex (Predose), and Cerebellum in Mean Values of Eleven Healthy Subjects[a]



[a]In the pituitary of postdose, the mean value of all dosages was used. The postdose time-activity curve of the pituitary was decreased to a level similar to that of the cerebellum.

Figure 3. Relationship Between Dopamine D_2 Receptor Occupancy in the Pituitary and Plasma Concentration of Prolactin in Patients With Schizophrenia[a]



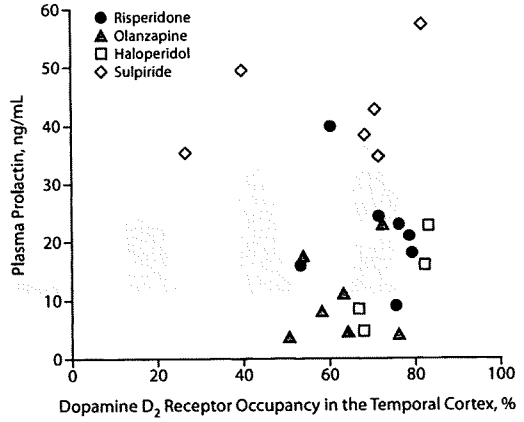
[a]Significant positive correlation was observed between the plasma concentration of prolactin and dopamine D_2 receptor occupancy in the pituitary by different doses of risperidone, olanzapine, haloperidol, and sulpiride ($Y = 0.41X - 4.0$; $P = .001$).

temporal cortex (predose), and cerebellum of the 11 healthy subjects. The curve of the pituitary at postdose was decreased to a level similar to that of the cerebellum.

Study of Patients Receiving Different Antipsychotics

Dopamine D_2 receptor occupancies in the pituitary by risperidone, olanzapine, haloperidol, and sulpiride were 49.2%–80.1%, 18.6%–79.5%, 27.2%–104.4%, and

Figure 4. Relationship Between Dopamine D_2 Receptor Occupancy in the Temporal Cortex and Plasma Concentration of Prolactin in Patients With Schizophrenia[a]



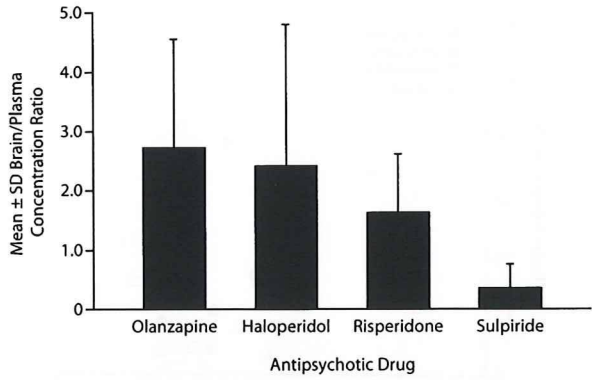
[a]No correlation was observed between the plasma concentration of prolactin and dopamine D_2 receptor occupancy in the temporal cortex ($P = .65$).

68.9%–108.4%, respectively, and those in the temporal cortex were 53.4%–79.5%, 50.7%–76.2%, 66.9%–83.2%, and 26.9%–81.8%, respectively. Plasma concentrations of prolactin of patients with risperidone, olanzapine, haloperidol, and sulpiride were 8.9 to 39.9 ng/mL, 3.5 to 23.0 ng/mL, 4.7 to 22.7 ng/mL, and 34.7 to 57.4 ng/mL, respectively. The percentages of hyperprolactinemia of olanzapine, haloperidol, risperidone, and sulpiride were 29% (2/7), 50% (2/4), 86% (6/7), and 100% (6/6), respectively.

Significant positive correlation was observed between the plasma concentration of prolactin and dopamine D_2 receptor occupancy in the pituitary by the 4 antipsychotic drugs ($Y = 0.41X - 4.0$; $r = 0.62$; $P = .001$) (Figure 3). However, no correlation was found between the plasma concentration of prolactin and dopamine D_2 receptor occupancy in the temporal cortex by the 4 antipsychotic drugs ($r = -0.097$; $P = .65$) (Figure 4). When the threshold was set with every 10% of occupancy in the pituitary, patients with hyperprolactinemia could be estimated using Fisher exact test at 50% with the lowest P value ($P = .005$).

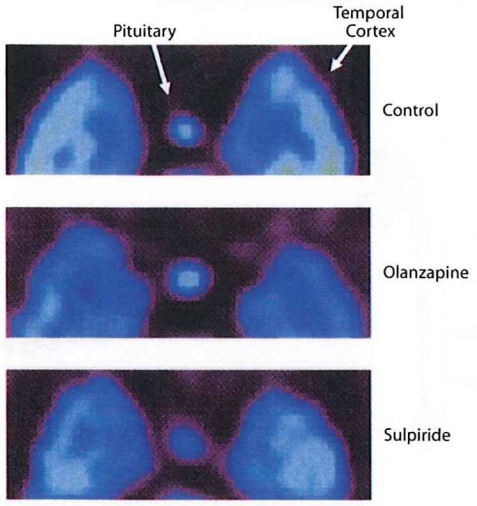
The B/P ratio of the 4 antipsychotics differed significantly ($\chi^2_3 = 8.54$; $P = .036$). The mean ± SD B/P ratios of olanzapine, haloperidol, risperidone, and sulpiride were 2.70 ± 1.84, 2.40 ± 2.40, 1.61 ± 1.00, and 0.34 ± 0.42, respectively (Figure 5), the same order as the percentages of hyperprolactinemia. The B/P ratios were significantly different in olanzapine versus sulpiride ($U = 2$; $P = .007 < 0.05/6$) and risperidone versus sulpiride ($U = 4$; $P = .015 < 0.05/2$), but haloperidol versus sulpiride did not reach significance ($U = 6$; $P = .20$). Figure 6 shows the PET images of 1 healthy subject and 2 patients, 1 taking olanzapine and

Figure 5. Antipsychotic Drug Concentration Ratio in the Brain to Plasma (B/P ratio) of 4 Drugs in Patients With Schizophrenia[a]



[a]B/P ratios of olanzapine, haloperidol, risperidone, and sulpiride were mean ± SD = 2.70 ± 1.84, 2.40 ± 2.40, 1.61 ± 1.00, and 0.34 ± 0.42, respectively. B/P ratios were significantly different in olanzapine versus sulpiride (P = .007) and risperidone versus sulpiride (P = .015), but haloperidol versus sulpiride did not reach significance (P = .20).

Figure 6. Positron Emission Tomography Images of 1 Healthy Subject and 2 Patients Taking Olanzapine or Sulpiride[a]



[a]The 3 positron emission tomography images were axial slices at the pituitary and temporal cortex. High brightness indicates high binding of [^{11}C]FLB 457, meaning less occupied dopamine D_2 receptors. Sulpiride blocked dopamine D_2 receptors in the pituitary more preferentially than in the temporal cortex, whereas olanzapine showed relatively less occupied dopamine D_2 receptors in the pituitary.

the other sulpiride. Sulpiride blocked dopamine D_2 receptors to a greater extent in the pituitary than in the temporal cortex, and olanzapine showed relatively less effect in the pituitary.

In the simulation study, drugs with a low B/P ratio induced a high prolactin level at clinical doses with 60%–80% of dopamine D_2 receptor occupancy in the temporal cortex. The measured prolactin values of patients also showed the same tendency (Figure 7). The simulated plasma prolactin level increased when the set value of occupancy in the temporal cortex was increased.

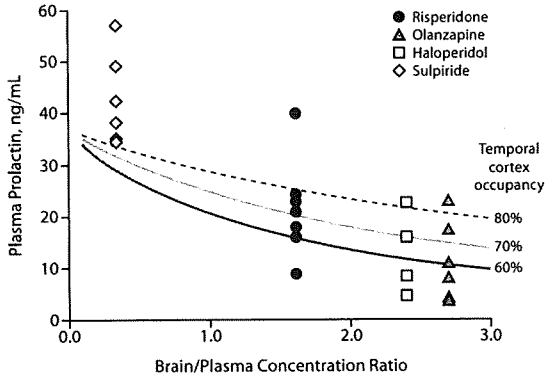
DISCUSSION

In the present study healthy subjects treated with different doses of sulpiride, a marked difference in dopamine D_2 receptor occupancy was confirmed between the pituitary and temporal cortex using the baseline of each. Dopamine D_2 receptors in the pituitary were almost fully occupied even at the lowest dose (200 mg), at which occupancy in the temporal cortex was around 25% (Figure 1). Lipophilicity is a major determinant of the penetrating ability into the brain.[28] Since the log P value of sulpiride is reported to be 0.42–1.31, the low brain uptake was considered to be due to lower lipophilicity.[29,30]

Although nonspecific binding in the pituitary may not be the same as that of the brain parenchyma, the fully occupied time-activity curve of the pituitary was at almost the same level as that of the cerebellum (Figure 2), suggesting that the cerebellum could be used as a measure of nonspecific binding in the pituitary. In the calculation of BP_{ND} in the pituitary, we used the AUC ratio method, which does not require the assumptions that are required for the simplified reference tissue model method (SRTM). The effect of the radiolabeled metabolite of [^{11}C]FLB 457 would be small for the measurement of dopamine D_2 receptors in the pituitary because a previous study indicated that a major metabolite of [^{11}C]FLB 457 had very low affinity for dopamine D_2 receptors.[16] The BP_{ND} values in the temporal cortex were measured by the same method. Binding potential in the extrastriatum by AUC ratio method showed good correlation with those by indirect kinetic method (reported value; r = 0.96).[20] Although some subjects did not reach equilibrium in the pituitary in this study, the mean value of BP_{ND} in the pituitary was 2.11, almost the same as the reported value in the temporal cortex (2.23) and less than that in the thalamus (3.67), suggesting that the equilibrium would be around the measured time range. Furthermore, the occupancies in the pituitary of patients (n = 24) calculated by the SRTM method showed good correlation with those by the AUC ratio method (r = 0.94, data not shown). These data suggested that the AUC ratio method can also be used for quantification of the pituitary.

The present study demonstrated that dopamine D_2 receptor occupancy in the pituitary by 4 antipsychotic drugs was significantly correlated with the plasma concentration of prolactin (Y = 0.41X – 4.0; P = .001) (Figure 3), but no such correlation was found in the temporal cortex (Figure 4). It has been reported that hyperprolactinemia was induced when dopamine D_2 receptor occupancy in the

Figure 7. Simulated Curve Between Antipsychotic Drug Concentration Ratio in the Brain to Plasma (B/P ratio) and Estimated Prolactin Level at Clinical Dosage[a]



[a]Drug with a low B/P ratio induces a high prolactin level at clinical dosage. Occupancy in the temporal cortex was set at 60%–80%. Prolactin values measured in patients were also plotted against mean B/P ratio, which can be a characteristic index of each antipsychotic drug.

striatum exceeded 50% for raclopride[31] and 72% for haloperidol.[12] However, no correlation was reported between hyperprolactinemia and striatal or temporal cortex occupancy by amisulpride.[32] In this study using Fisher exact test, the patients with hyperprolactinemia could be estimated at 50% with the lowest P value ($P=.005$). This indicated that 50% of dopamine D_2 receptor occupancy in the pituitary might represent a threshold level of hyperprolactinemia.

The magnitude of hyperprolactinemia differs among antipsychotics. It has been reported that atypical antipsychotics showed a low risk of hyperprolactinemia as compared to typical antipsychotics.[1,5,7] Although olanzapine, clozapine, and quetiapine reportedly showed a relatively low risk,[5–8] risperidone and amisulpride, regarded as atypical antipsychotics, presented a high risk.[5–8,32] This difference was discussed in relation to the affinity to dopamine D_2 receptors.[6,8] Risperidone has relatively high affinity, whereas those of olanzapine, clozapine, and quetiapine are medium or low.[33] This order is apparently in accordance with the risk of hyperprolactinemia, although amisulpride has a high risk despite its medium affinity.[33] Thus, after all, the affinity for dopamine D_2 receptors could not conclusively explain the risk of hyperprolactinemia.

A previous animal study suggested that the dissociation between central and peripheral dopamine D_2 receptor occupancy could be a marker of prolactin elevation.[9] The reported ED_{50} ratios of the pituitary to the striatum were 654 for amisulpride, 11 for risperidone, and 0.7 for olanzapine, indicating that their respective permeabilities were low, medium, and high. In this study, we calculated the B/P ratio defined as the ratio of antipsychotic drug concentration at the temporal cortex receptor site to that at the pituitary receptor site to explain the ED_{50} difference between that in the pituitary and the temporal cortex. It was based on the assumption of fixed IC_{50}, although several factors should be considered concerning the IC_{50} determination. First, endogenous dopamine may affect the BP_{ND} values of [^{11}C] FLB 457. Some studies reported that dopamine manipulation such as amphetamine challenge did not change BP_{ND},[34–36] but other studies reported a different conclusion.[37–42] In this study, we assumed that, compared with amphetamine challenge, antipsychotic drug did not substantially change endogenous dopamine. Second, antipsychotic concentration change during the PET scan may affect the occupancy values according to a previous study.[24] Our previous studies reported that there was no regional difference of dopamine D_2 receptor occupancy between the striatum and extrastriatum.[43] Moreover, dopamine D_2 receptor densities showed similar values between the temporal cortex and pituitary ($B_{max}=0.4$ and 1.3 pmol/g tissue, respectively).[15] In this study, the drug washout rate could be ignored because patient treatment was in the steady-state and receptor densities were close between the 2 sites. Taken together, we assumed that IC_{50} values were the same between the pituitary and the temporal cortex, and that the antipsychotic drug concentration difference could be estimated.

The order of the B/P ratio in our results was consistent with that of the ED_{50} ratio in the above-mentioned animal study. The B/P ratio can be the characteristic index of antipsychotic drugs. The concentration of sulpiride in the temporal cortex was one-third of that in the pituitary, while those of olanzapine and haloperidol were about double or triple. Sulpiride had significantly low permeability compared to risperidone ($P=.015$) and olanzapine ($P=.007$), indicating the high risk of hyperprolactinemia for sulpiride.[5] Although no significant difference of B/P ratio between haloperidol and sulpiride was observed, possible reasons were small sample size and large SD. The order of the B/P ratio was the same as the percentage of hyperprolactinemia in this study. In the simulation study, a drug with low B/P ratio induced a high prolactin level at clinical dosage with 60%–80% of dopamine D_2 receptor occupancy in the temporal cortex (Figure 7). The measured prolactin values of patients showed a similar tendency to the simulated ones. Thus, the B/P ratio seems to be a useful index for predicting the risk of each antipsychotic drug for hyperprolactinemia.

Brain permeability differences are the result of several factors. The Log P values of risperidone, olanzapine, and haloperidol are 3.04, 2.89, and 3.36–3.52, respectively,[30,44,45] and the higher permeability of risperidone, olanzapine, and haloperidol compared to sulpiride (or amisulpride; Log $P=1.10–1.70$[29,45]) can be ascribed to high lipophilicity. However, risperidone, in fact, has a slightly higher Log P value than olanzapine despite its slightly lower permeability, a seeming contradiction explainable by the fact that risperidone is reportedly a substrate of P-glycoprotein, one of the efflux transporters at the blood-brain barrier.[46,47]

There were several confounding factors in this study. In the patients study, we used the mean BP_{ND} of healthy control subjects as baseline because previous studies showed no differences in dopamine D_2 receptors in the temporal cortex between patients and healthy subjects[17,19] or lower binding in patients.[48,49] A variety of baseline BP_{ND} values can lead to large SD of occupancy or B/P ratio. For example, if BP_{base} changes by ± 15%,[17] the calculated 50% occupancy could be changed from 41% to 57%. The variety would affect the statistics significantly, especially with the small number of subjects like the haloperidol cases. Furthermore, possible change in the pituitary of patients with schizophrenia[50] could lead to potential errors in the estimation of occupancy values.

In conclusion, dopamine D_2 receptor occupancies in the pituitary by 4 different antipsychotics were well correlated with the plasma concentration of prolactin. The B/P ratios of the 4 antipsychotics were significantly different. The magnitude of hyperprolactinemia of various antipsychotics can be predicted by the B/P ratio, which indicates the permeability of antipsychotics into the brain. Thus, especially in the area of new drug development, the B/P ratio of each antipsychotic drug might prove to be useful for the early evaluation of the risk of hyperprolactinemia.

Drug names: clozapine (FazaClo, Clozaril, and others), haloperidol (Haldol and others), olanzapine (Zyprexa), quetiapine (Seroquel), risperidone (Risperdal and others).

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ORIGINAL INVESTIGATION

Norepinephrine transporter occupancy by antidepressant in human brain using positron emission tomography with (S,S)-[^{18}F]FMeNER-D$_2$

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Abstract
Rationale Central norepinephrine transporter (NET) is one of the main targets of antidepressants. Although the measurement of NET occupancy has been attempted in humans, the outcomes have been inconclusive.
Objective In this study, the occupancy of NET by different doses of an antidepressant, nortriptyline, was measured using positron emission tomography (PET) with (S,S)-[^{18}F]FMeNER-D$_2$.
Materials and methods PET scans using (S,S)-[^{18}F]FMeNER-D$_2$ were performed on six healthy men before and after oral administration of a single oral dose of nortriptyline (10–75 mg). After a bolus i.v. injection of (S,S)-[^{18}F]FMeNER-D$_2$, dynamic scanning was performed for 0–90 min, followed by scanning for 120–180 min. The ratio of the thalamus-to-caudate areas under the curve (120–180 min) minus 1 was used as the binding potential (BP$_{ND}$) for NET. NET occupancy was calculated as the percentage reduction of BP$_{ND}$. Venous blood samples were taken to measure the concentrations of nortriptyline just before injection of the tracer and at 180 min after the injection.
Results Mean NET occupancies by nortriptyline were 16.4% at 10 mg, 33.2% at 25 mg, and 41.1% at 75 mg. The mean plasma concentration of nortriptyline was less than the lower limit of detection at 10 mg, 23.7 ng/mL at 25 mg, and 50.5 ng/mL at 75 mg. Estimated ED$_{50}$ was 76.8 mg of administration dose and 59.8 ng/mL of plasma concentration.
Conclusions NET occupancy by nortriptyline corresponding to the administration dose of 10–75 mg or plasma concentration was observed from 16% to 41%.

Keywords Norepinephrine transporter · (S,S)-[^{18}F]FMeNER-D$_2$ · Positron emission tomography · Occupancy · Nortriptyline

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Introduction

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 (Harmer et al. 2004; Southwick et al. 2002; Strange et al. 2003). It plays important roles in the pathophysiology of depression (Nutt 2006). Norepinephrine transporter (NET) is responsible for the reuptake of norepinephrine into presynaptic nerves and is one of the main targets of antidepressants. Serotonin transporter (5-HTT) is another main target of antidepressants. Positron emission tomography (PET) studies, using radioligands such as [^{11}C]McN(+)5652 and [^{11}C]DASB, have made it possible to measure the occupancy of 5-HTT by antide-

pressants in living human brain (Meyer et al. 2001, 2004; Suhara et al. 2003). 5-HTT occupancy was reported to be over 80% at clinical doses of selective serotonin reuptake inhibitors (SSRIs) during the treatment of depression (Meyer et al. 2001, 2004; Suhara et al. 2003; Takano et al. 2006). On the other hand, although the measurement of NET occupancy has been attempted in humans, the outcomes have been inconclusive (Logan et al. 2007). (S,S)-[^{18}F]FMeNER-D_2 was recently developed as a radioligand for the measurement of NET binding with PET (Schou et al. 2004), allowing estimation of NET bindings quantitatively in the human brain (Arakawa et al. 2008; Takano et al. 2008a, b).

Nortriptyline is one of the tricyclic antidepressants and is widely used for treatment of depression. The binding affinity of nortriptyline for NET is higher (Ki=1.49 nmol/L) than other monoamine transporters, e.g., 5-HTT (Ki=18.0 nmol/L) and dopamine transporter (Ki=1,200 nmol/L) (Vaishnavi et al. 2004). Thus, nortriptyline can be considered a relatively NET-selective antidepressant. In this study, we investigated the degree of NET occupancy by different doses of the antidepressant, nortriptyline, using PET with (S,S)-[^{18}F]FMeNER-D_2.

Materials and methods

Subjects

Six healthy male subjects (age range, 22–39 years; mean ± SD, 30.5±6.3 years) participated in this study. All subjects were free of any somatic, neurological, or psychiatric disorders, and they had no history of current or previous drug abuse. After complete description of this study, written informed consent was obtained from all subjects. The study was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Sciences, Chiba, Japan.

PET procedures

The first PET scan with (S,S)-[^{18}F]FMeNER-D_2 was performed before nortriptyline administration. The second PET scan was performed 5 h after single-dose oral administration. These two scans were carried out at the same time on separate days. The average interval between these two scans was 6.5 days (range, 2–14 days). The nortriptyline doses were 10, 25, and 75 mg in two subjects each. An ECAT EXACT HR+ (CTI-Siemens, Knoxville, TN, USA) PET scanner system was used for all measurements. A head fixation device was 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 bolus injection of 183.0–196.5 MBq (mean ± SD, 188.9±4.2 MBq) of (S,S)-[^{18}F]FMeNER-D_2. Specific radioactivity of (S,S)-[^{18}F]FMeNER-D_2 was 65.4–344.1 GBq/μmol (192.6±86.4 GBq/μmol). Brain radioactivities were measured from 0 to 90 min (1 min×10, 2 min×15, and 5 min×10), followed by scanning for 120–180 min (10 min×6).

Magnetic resonance (MR) images of the brain were acquired with a 1.5 Tesla MR scanner, Gyroscan NT (Philips Medical Systems, Best, Netherlands). T1-weighted images were obtained at 1-mm slices.

Measurement of plasma concentration of nortriptyline

Venous blood samples were taken to measure the plasma concentrations of nortriptyline just before injection of the radioligand and at 180 min after the injection. The average values of these two samples were used in this study. The plasma concentrations of nortriptyline were determined by gas chromatography–mass spectrometry with a lower limit of quantification of 20.0 ng/mL (Mitsubishi Chemical Medience Corporation, Tokyo, Japan).

Data analysis

All emission scans were reconstructed with a Hanning filter (cutoff frequency: 0.4 cycle/pixel). All MR images were co-registered to the PET images using a statistical parametric mapping (SPM2) system. Regions of interest were drawn manually on the thalamus, hippocampus, anterior cingulate cortex (ACC), locus coeruleus (LC), and the caudate in each of the summated PET images with reference to the co-registered MR image. Regional radioactivity was calculated for each frame, corrected for decay, and plotted versus time.

(S,S)-[^{18}F]FMeNER-D_2 bindings were expressed as binding potentials relative to non-displaceable binding (BP_{ND}) (Innis et al. 2007). BP_{ND} of (S,S)-[^{18}F]FMeNER-D_2 in the thalamus, hippocampus, ACC, and LC were calculated using the simplified reference tissue model method (SRTM; Lammertsma and Hume 1996), multilinear reference tissue model method (MRTM; Ichise et al. 2003), and the area under the curve (AUC) ratio method. We used the caudate as reference brain region in all methods because of its negligible NET density (Donnan et al. 1991; Logan et al. 2007; Schou et al. 2005). In the AUC 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 that of the caudate. An integration interval of 120–180 min was used in this

method because the specific binding (target region minus caudate) has a peak at this time during PET measurement (Ito et al. 1998). AUC of the caudate adjusted by the injected dose of (S,S)-[^{18}F]FMeNER-D_2 did not significantly differ between pre- and post-administration of nortriptyline (paired t test; p=0.27). For these analyses, the software package PMOD (PMOD Technologies, Zurich, Switzerland) was used.

NET occupancy was calculated by the following equation:

$$\text{Occupancy}(\%) = \frac{\text{BP}_{\text{baseline}} - \text{BP}_{\text{drug}}}{\text{BP}_{\text{baseline}}} \times 100,$$

where $\text{BP}_{\text{baseline}}$ is BP_{ND} in the drug-free state, and BP_{drug} is BP_{ND} after administration of nortriptyline. The relationship between the dose or plasma concentration of nortriptyline and NET occupancy is described by the following equation:

$$\text{Occupancy}(\%) = \frac{C}{C + \text{ED}_{50}} \times 100,$$

where C is the dose or plasma concentration of nortriptyline, and ED_{50} is the dose or plasma concentration required to induce 50% occupancy (Suhara et al. 2003; Takano et al. 2006).

Results

Typical summated PET images (120–180 min) before and after administration of 75 mg of nortriptyline are shown in Fig. 1. Typical time-activity curves of the thalamus and caudate before and after administration of 75 mg of nortriptyline are shown in Fig. 2. In the AUC ratio method, the mean NET occupancies in the thalamus by nortriptyline doses were 16.4% at 10 mg, 33.2% at 25 mg, and 41.1% at 75 mg. NET occupancies in the LC ranged from 41.6% to 90.3%, but they were not dose-dependent. Those in the hippocampus and ACC by the AUC ratio method and in all regions by SRTM and MRTM could not be estimated because occupancies or BP_{ND} revealed very high or negative values in many cases. The mean plasma concentration of nortriptyline was less than the lower limit (<20.0 ng/mL) at 10 mg, 23.7 ng/mL at 25 mg, and 50.5 ng/mL at 75 mg. NET occupancy in the thalamus by the AUC ratio method and plasma concentration of nortriptyline before and after the

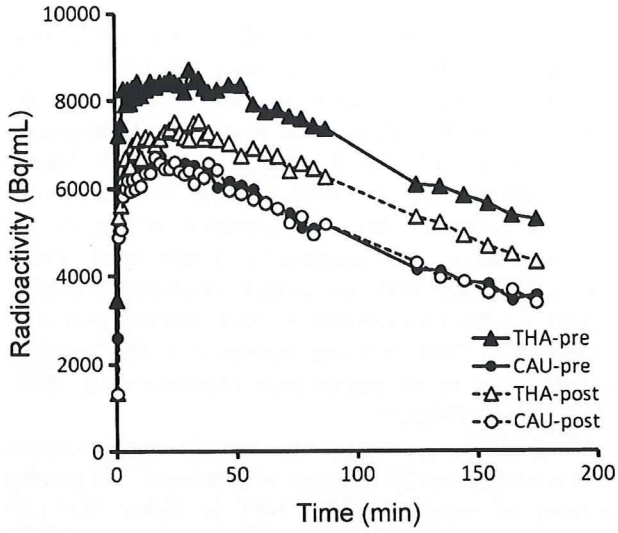

Fig. 2 Typical time-activity curves of thalamus and caudate before and after administration of 75 mg of nortriptyline. *THA-pre* thalamus before administration, *CAU-pre* caudate before administration, *THA-post* thalamus after administration, *CAU-post* caudate after administration

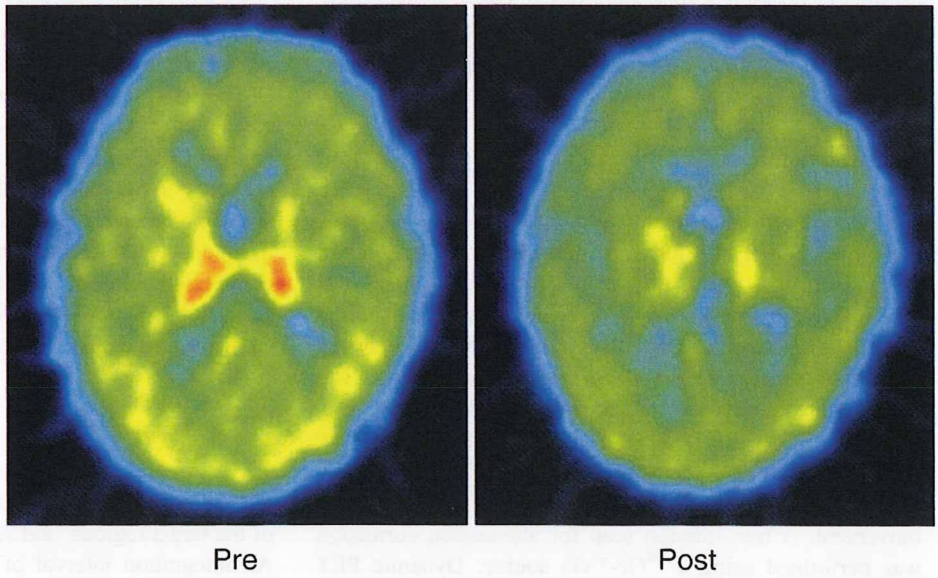

Fig. 1 Typical summated positron emission tomography images (120–180 min) before and after administration of 75 mg of nortriptyline

Table 1 Norepinephrine transporter occupancy in the thalamus by area under the curve ratio method and plasma concentration of nortriptyline

Plasma concentration *pre* is plasma concentration of nortriptyline just before injection of the tracer. Plasma concentration *post* is plasma concentration of nortriptyline at 180 min after injection

Nortriptyline dose (mg)	NET occupancy (%)	Plasma concentration (ng/mL)	
		Pre	Post
10	22.8	–	–
10	10.0	–	–
25	36.9	25.5	21.6
25	29.4	26.3	21.2
75	33.8	30.4	45.6
75	48.3	69.9	56

scans are shown in Table 1. Estimated ED_{50} was 76.8 mg of administration dose and 59.8 ng/mL of plasma concentration (Figs. 3 and 4).

Discussion

NET is an important site of action of antidepressants such as tricyclic antidepressants and serotonin-norepinephrine reuptake inhibitors. Several studies have been reported regarding 5-HTT occupancy by antidepressants (Meyer et al. 2001, 2004; Suhara et al. 2003; Takano et al. 2006). However, NET occupancy by antidepressants in human brain has not been reported because of a lack of suitable radioligands for NET. One human study using [^{11}C](S,S)-MRB reported no significant dose dependency of NET occupancy at different doses of atomoxetine (Logan et al. 2007). (S,S)-[^{18}F]FMeNER-D_2 has recently been developed as a radioligand for the measurement of NET binding with PET (Schou et al. 2004). Quantification methods of NET bindings in the human brain using this ligand have been established (Arakawa et al. 2008; Takano et al. 2008a, b). Previous animal studies using (S,S)-[^{18}F]FMeNER-D_2 showed dose-dependent change in NET occupancy up to almost 100% after steady-state infusion of different doses of atomoxetine (0.003–0.12 mg/kg per hour) (Seneca et al. 2006; Takano et al. 2009). Consistent with animal experiments, the present human studies showed that NET occupancy in the thalamus by nortriptyline occurred in a dose-dependent manner. On the other hand, dose-dependent NET occupancy was not observed in the LC. This might in part reflect the difference in NET inhibitory effect by antidepressants between the LC and thalamus.

SSRIs are widely considered as the first choice of treatment for depression. However, it is known that about one third of the patients with major depression do not respond to SSRIs (De Wilde et al. 1983; Dick and Ferrero 1983; Guelfi et al. 1987; Martin et al. 1987; Stokes 1993). Recent studies suggest that the treatment of depression with newer antidepressants that simultaneously enhance both serotonergic and norepinephrinergic neurotransmissions can be expected to result in higher response and remission rates compared to SSRIs (Kampf-Sherf et al. 2004; Papakostas et al. 2007; Thase et al. 2007).

5-HTT occupancy was reported to be over 80% at clinical doses of SSRIs during the treatment of depression (Meyer et al. 2001, 2004; Suhara et al. 2003). However, the percentage of NET occupancy during clinical optimal dosing has not yet been reported. A previous study reported that a plasma concentration range between 50 and 139 ng/mL of nortrip-

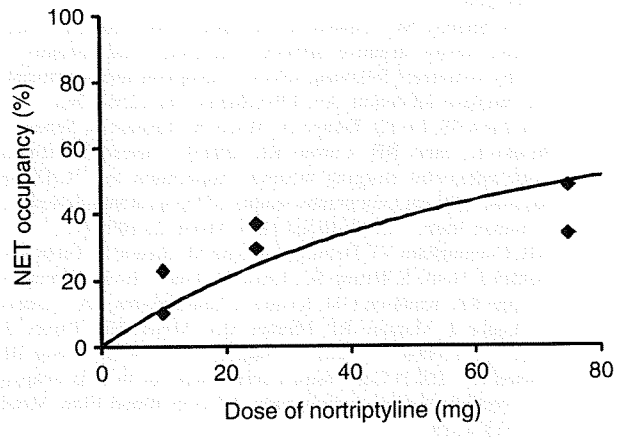

Fig. 3 Relationship between norepinephrine transporter occupancy and administration dose of nortriptyline. ED_{50} was 76.8 mg

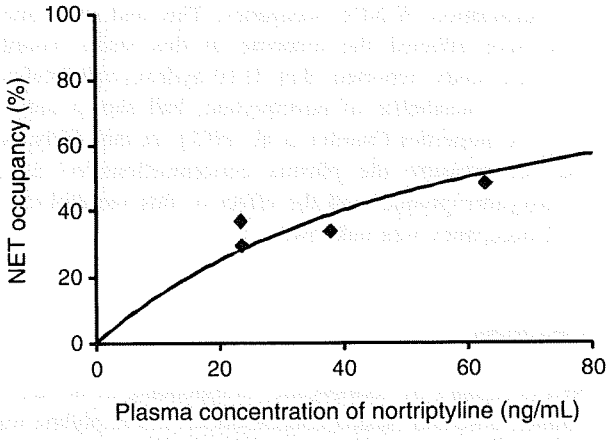

Fig. 4 Relationship between norepinephrine transporter occupancy and plasma concentration of nortriptyline. ED_{50} was 59.8 ng/mL

tyline was effective after 2 weeks of treatment for patients with depression (Asberg et al. 1971). Another double-blind, randomized study suggested that less than 150 ng/mL of nortriptyline was effective compared with greater than 180 ng/mL after 6 weeks of treatment (Kragh-Sorensen et al. 1976). Thus, it was generally appreciated that the effective plasma concentration of nortriptyline was between 50 and 150 ng/mL (Sadock and Sadock 2007). Extrapolating from these plasma concentrations and ED_{50} value of the present study, the expected NET occupancy of nortriptyline can be assumed to be in a range of 50–70%. Further, NET occupancy studies in humans will be needed to evaluate the relation with the clinical effects of antidepressants.

There are several limitations in this study. First, we measured healthy subjects with a single oral dose of nortriptyline, and the dosing schedule was not randomized. Moreover, the test–retest reproducibility of (S,S)-[^{18}F]FMeNER-D_2 has not been reported. The present study was a pilot and preliminary study for further studies on patients with chronic treatment to allow discussion of the clinical conditions. Second, we could only estimate NET occupancy in the thalamus and LC by the AUC ratio method. The reason for not estimating other occupancies would be the PET procedures in this study. The present PET scan time was short, and the injected dose of (S,S)-[^{18}F]FMeNER-D_2 was small compared with previous studies. Another reason would be the characteristics of (S,S)-[^{18}F]FMeNER-D_2, which showed relatively low specific bindings. Additionally, arterial blood samplings, which would allow conducting the occupancy plot (Cunningham et al. 2010), could not be performed. Further study with arterial input function will be needed to estimate the accuracy of NET occupancy obtained by the AUC ratio method. Third, although we have reported that a linear correlation was observed in BP_{ND} values between the ratio method and kinetic analysis using arterial blood data (Arakawa et al. 2008), the ratio method can induce systemic underestimation of NET occupancy. This underestimation may have affected the outcome of this study. Fourth, previous study reported that E-10-hydroxynortriptyline, an active metabolite of nortriptyline, had shown antidepressant properties (Nordin et al. 1991). In this study, we did not measure the plasma concentrations of E-10-hydroxynortriptyline, and the effect of this metabolite on NET occupancy was unknown.

Conclusion

NET occupancy by nortriptyline corresponding to the administration dose and plasma concentration of nortriptyline was observed from 16.4% at 10 mg, 33.2% at 25 mg, and 41.1% at 75 mg in human brain using PET with (S,S)-[^{18}F]FMeNER-D_2. Estimated ED_{50} was 76.8 mg of administration dose and 59.8 ng/mL of plasma concentration.

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