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

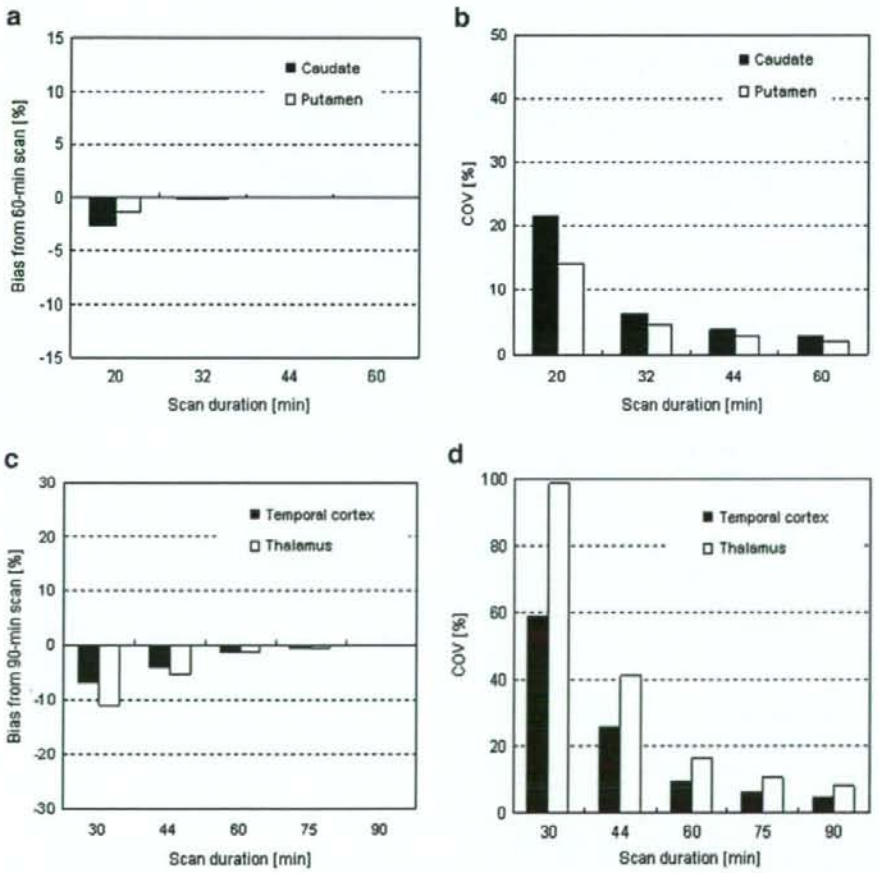

Fig. 7. Relation between scan duration and reliability of occupancy estimates derived from a bootstrap approach for human data of the putamen and caudate with [^{11}C]raclopride (a, b) and of the temporal cortex and thalamus in [^{11}C]FLB 457 (c, d).

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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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).

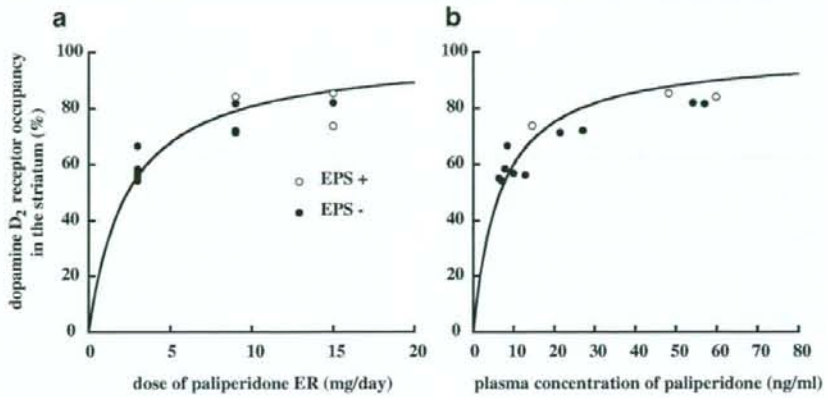

Fig. 1 Relationship between dopamine D_2 receptor occupancy in the striatum and dose (**a**) or plasma concentration (**b**) of paliperidone ER in the [^{11}C] raclopride study. ED_{50} in the striatum was 2.38 mg/day (r=0.86) and 6.65 ng/ml (r=0.82)

The dopamine D_2 receptor occupancy in the temporal cortex measured with [^{11}C]FLB 457 was 34.5 to 87.3%. Mean dopamine D_2 receptor occupancies were 53.1±14.5% at 3 mg/day, 76.2±9.5% at 9 mg/day, and 77.7±3.0% at 15 mg/day in the temporal cortex. ED_{50} in the temporal cortex was 2.84 mg/day (r=0.73) and 7.73 ng/ml (r=0.61; Fig. 2). There were no significant differences in plasma concentrations of paliperidone between the two scans (p= 0.24) and in dopamine D_2 receptor occupancy between the striatum and temporal cortex at any dose (p=0.30).

There were no correlations between striatal occupancy and age (p=0.07) or duration of illness (p=0.90).

Average PANSS scores of all patients were 62.9±16.5 before taking paliperidone ER and 58.5±16.8 after 6 weeks. Three patients, two taking 15 mg and one 9 mg (no. 10, 11, 12), showed EPS (Table 1).

Discussion

The present study demonstrated that the ED_{50} of striatal dopamine D_2 receptor occupancy of paliperidone ER was 2.38 mg/day and that of the temporal cortex was 2.84 mg/day. Previous studies reported that the striatal ED_{50} of risperidone was 1.2 mg/day (Nyberg et al. 1999) and that the limbic-cortical ED_{50} was 1.46 mg/day (Yasuno et al. 2001). These studies indicate that the equivalent ratio for a daily dose between risperidone and paliperidone ER seems to be about 1:2. The striatal and temporal ED_{50} values of plasma concentration of paliperidone were 6.65 and 7.73 ng/ml, respectively, almost matching the values previously reported for risperidone active moiety (6.87 ng/ml, Nyberg et al. 1999; 7.43 ng/ml, Yasuno et al. 2001) for striatal and limbic–cortical regions, respectively. The therapeutic dose ranges of paliperidone ER calculated from ED_{50} were 5.6–9.5 mg/day and 15.5–26.6 ng/ml. In two previous studies (Nyberg et al. 1999; Yasuno et al. 2001), the sum of risperidone and paliperidone was regarded as risperidone active moiety. Because paliperidone shows almost the same affinity for dopamine D_2 receptor as risperidone, the effect for dopamine D_2 receptor was about the same between risperidone active moiety and paliperidone. This suggests that similar dopamine D_2 receptor occupancy is achieved with comparable plasma concen-

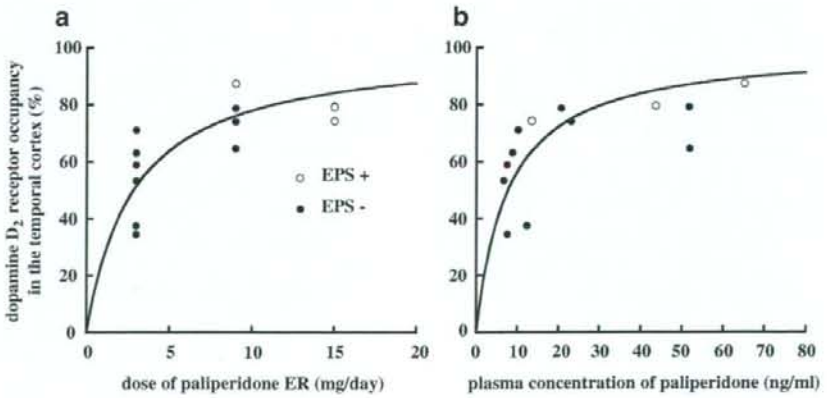

Fig. 2 Relationship between dopamine D_2 receptor occupancy in the temporal cortex and dose (**a**) or plasma concentration (**b**) of paliperidone ER in the [^{11}C]FLB 457 study. ED_{50} in the temporal cortex was 2.84 mg/day (r=0.73) and 7.73 ng/ml (r=0.61)

trations of paliperidone or risperidone active moiety. This finding confirms that paliperidone is as effective in crossing the blood–brain barrier as the active moiety of risperidone.

In the previous PET study that administered a single dose of paliperidone ER at 6 mg to four healthy Caucasian subjects, the striatal dopamine D_2 receptor occupancy fluctuation derived was 75–78%, and ED_{50} was 4.4 ng/ml (Karlsson et al., presented at WWS 2006). The differences between the two studies may be explained by the small number of observations and/or ethnicity. In the present study, occupancy was measured at steady-state drug levels (after multiple doses), whereas the previous study was carried out after a single dose.

There were no significant differences between striatal and extrastriatal dopamine D_2 receptor occupancy by paliperidone. Although the interval between the two scans was 2 h, the difference in plasma concentrations of paliperidone between them was about 7%, statistically not different as paliperidone ER tablets were made for flat plasma concentrations at a steady state. There have been discussions about the concept of 'limbic selectivity,' i.e., low dopamine D_2 receptor occupancy in the striatum and high occupancy in the extrastriatum (Pilowsky et al. 1997). It was reported in some second-generation antipsychotics such as clozapine (Grunder et al. 2006; Kessler et al. 2006; Pilowsky et al. 1997; Xiberas et al. 2001), olanzapine (Bigliani et al. 2000; Xiberas et al. 2001), amisulpiride (Bressan et al. 2003a; Xiberas et al. 2001), and quetiapine (Kessler et al. 2006; Stephenson et al. 2000) using [^{123}I] epidepride, [^{76}Br]FLB 457 or [^{18}F]fallypride. However, no significant difference between the striatum and extrastriatal regions have been reported using two different ligands, [^{11}C]raclopride and [^{11}C]FLB 457 (Agid et al. 2007; Talvik et al. 2001), or one ligand, [^{18}F]fallypride (Kessler et al. 2005). Human dopamine D_2 receptor occupancy by risperidone also showed inconsistent results. Two studies showed higher occupancy in the temporal cortex than in the striatum using [^{123}I]epidepride (75% in the temporal cortex and 50% in the striatum; Bressan et al. 2003b) and [^{76}Br] FLB 457 (91.6% in the temporal cortex and 63.3% in the striatum; Xiberas et al. 2001). On the other hand, similar occupancy values by risperidone were reported in the striatum (53–85%) using [^{11}C]raclopride (Nyberg et al. 1999) and extrastriatal regions (38–80%) using [^{11}C]FLB 457 (Yasuno et al. 2001). Because several factors such as scanning time, ligand selection, kinetic modeling, etc. need to be considered (Erlandsson et al. 2003; Olsson and Farde 2001), we used two different ligands to measure the different receptor density regions with appropriate scanning time and kinetic modeling for each ligand (Olsson and Farde 2001). Our results indicated no significant difference in regional occupancy (Agid et al. 2007; Kessler et al. 2005; Talvik et al. 2001; Yasuno et al. 2001). Although extrastriatal regions are suggested to be sites for antipsychotic action (Lidow et al. 1998), a recent study reported that extrastriatal dopamine D_2 receptor occupancy did not correlate with the antipsychotic effect (Agid et al. 2007).

In the present study, three patients complained of EPS. Average striatal occupancy of these three patients was 80.8%, a level in line with that known to increase the likelihood for EPS (Farde et al. 1992; Kapur et al. 2000; Nordstrom et al. 1993).

Previous studies indicated that over 70% of dopamine D_2 receptor occupancy is required for antipsychotic effects in patients with schizophrenia in the acute phase (Kapur et al. 2000; Nordstrom et al. 1993). In chronic treatment, haloperidol decanoate showed 73% occupancy at 1 week after injection and 52% occupancy at 4 weeks (Nyberg et al. 1995). Long-acting injectable risperidone showed 25–83 or 53–79% occupancy at a steady state (Gefvert et al. 2005; Remington et al. 2006). It is difficult to link the degree of dopamine D_2 receptor occupancy to a clinical effect, as almost all our patients (except nos. 5 and 11) had been undergoing long-term treatment when they entered the study. However, in all patients, these scores decreased with treatment or remained stable (Table 1) irrespective of dose. Furthermore, in all patients, striatal dopamine D_2 receptor occupancies above 50% were noted. This indicates that, for maintenance therapy of patients with schizophrenia, over 70% dopamine D_2 receptor occupancy might not necessarily be required. However, as this was an open-label study, further studies (such as randomized controlled trials) would be needed for an exact estimation of the threshold of dopamine D_2 receptor occupancy in the treatment of chronic patients with schizophrenia.

The half-life of paliperidone is about 28 h (data on file). High receptor occupancy is sustained when the plasma half-life of the treatment is long (Takano et al. 2004). Sustained high dopamine D_2 receptor occupancy can be expected at dosages of 9 or 15 mg/day of paliperidone ER. As EPS are a frequent reason for interruption of drug treatment (Lieberman et al. 2005), although the therapeutic dose range of paliperidone ER calculated from ED_{50} was 5.6–9.5 mg/day, for chronic treatment, lower doses might be useful, avoiding dopamine D_2 receptor occupancy rates above 80%. The estimated dopamine D_2 receptor occupancy at 6 mg/day of paliperidone ER was about 72%, in a range associated with efficacy (dopamine D_2 receptor occupancy above 70%) but not above a level associated with increased risks of extrapyramidal side effects (dopamine D_2 receptor occupancy above 80%).

To calculate the dopamine D_2 receptor occupancy in this study, we used BP_{ND} of normal control subjects as a surrogate for BP_{ND} in the drug-free state. Although previous studies showed no difference in dopamine D_2 receptor density in the striatum (Farde et al. 1990) or in the

temporal cortex (Suhara et al. 2002; Talvik et al. 2003) between the normal subjects and the patients with schizophrenia, individual differences in dopamine D_2 receptor density might potentially lead to an error in the estimation of dopamine D_2 receptor occupancy (Farde et al. 1992). For example, if BP_{base} changes from −13% to +15%, the range of the present study, the calculated 50% occupancy could be changed from 43 to 57%. The effect of a small portion of displaceable binding in the cerebellum (Delforge et al. 2001; Hall et al. 1996) may lead to an underestimation from 50% of [^{11}C]FLB 457 occupancy to 46% (Olsson et al. 2004). These factors may explain the differences in dopamine D_2 receptor occupancy between the striatum and temporal cortex in some patients.

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%. The magnitude of dopamine D_2 receptor occupancy is similar between the striatum and temporal cortex.

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$GABA_A$/Benzodiazepine receptor binding in patients with schizophrenia using [^{11}C]Ro15-4513, a radioligand with relatively high affinity for α5 subunit

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Abstract

Dysfunction of the GABA system is considered to play a role in the pathology of schizophrenia. Individual subunits of $GABA_A$/Benzodiazepine (BZ) receptor complex have been revealed to have different functional properties. α5 subunit was reported to be related to learning and memory. Changes of α5 subunit in schizophrenia were reported in postmortem studies, but the results were inconsistent. In this study, we examined $GABA_A$/BZ receptor using [^{11}C]Ro15-4513, which has relatively high affinity for α5 subunit, and its relation to clinical symptoms in patients with schizophrenia.

[^{11}C]Ro15-4513 bindings of 11 patients with schizophrenia (6 drug-naïve and 5 drug-free) were compared with those of 12 age-matched healthy control subjects using positron emission tomography. Symptoms were assessed using the Positive and Negative Syndrome Scale. [^{11}C]Ro15-4513 binding was quantified by binding potential (BP) obtained by the reference tissue model. [^{11}C]Ro15-4513 binding in the prefrontal cortex and hippocampus was negatively correlated with negative symptom scores in patients with schizophrenia, although there was no significant difference in BP between patients and controls. $GABA_A$/BZ receptor including α5 subunit in the prefrontal cortex and hippocampus might be involved in the pathophysiology of negative symptoms of schizophrenia.

Keywords: γ-Amino-butyric acid; Schizophrenia; Negative symptoms; Prefrontal cortex; Hippocampus; PET

1. Introduction

γ-Amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system.

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$GABA_A$/Benzodiazepine (BZ) receptors are heteropentamiric GABA-gated chloride channels, and mediate fast synaptic inhibition (Moss and Smart, 2001). Benzodiazepines enhance the action of the neurotransmitter GABA at $GABA_A$/BZ receptors by interaction with their modulatory benzodiazepine sites.

Dysfunction of GABA neurotransmission in the brain is thought to play a role in the pathology of schizophrenia (Simpson et al., 1989; Reynolds et al., 1990). Postmortem studies using [^{3}H]muscimol showed that binding was increased in the hippocampal formation (Benes et al., 1996a), anterior cingulate cortex (Benes et al., 1992) and prefrontal cortex (Benes et al., 1996b; Dean et al., 1999) in patients with schizophrenia. The axon terminals of chandelier GABA neurons are reported to be reduced substantially in the middle layers of the prefrontal cortex in schizophrenia (Lewis et al., 1999).

$GABA_A$/BZ receptor chloride channel complex consists of two α subunits, two β subunits and one γ subunit (Barnard et al., 1998; Lüddens et al., 1995; Mehta and Ticku, 1999). It has been reported that the diversity of α subunits is responsible for various functional properties and ligand selectivity to the $GABA_A$/BZ receptor (Barnard et al., 1998; Low et al., 2000; Mehta and Ticku, 1999; Tobler et al., 2001). α1 subunit has been suggested to be related to hypnotic and sedative amnesic actions, whereas α2, α3 and α5 subunits to anxiolytic, anticonvulsant, and antipsychotic actions, and to the function of learning and memory (Crestani et al., 2001; Mohler et al., 2001; Serwanski et al., 2006).

Alterations in individual subunits of $GABA_A$/BZ receptor in schizophrenia have been the focus of recent postmortem studies. Expression of α1 subunit was reported to increase in the prefrontal cortex of patients with schizophrenia (Ohnuma et al., 1999; Ishikawa et al., 2004), α2 subunit was reported to increase in the prefrontal cortex (Volk et al., 2002), and α5 subunit expression was reported to show no significant change (Akbarian et al., 1995) or increase (Impagnatiello et al., 1998).

Several ligands such as [^{11}C]flumazenil and [^{11}C]Ro15-4513 were developed to visualize $GABA_A$/BZ receptors by positron emission tomography (PET) (Inoue et al., 1992; Halldin et al., 1992; Pappata et al., 1988). Both [^{11}C]flumazenil and [^{11}C]Ro15-4513 have the imidazobenzodiazepine core structure. However, flumazenil is a $GABA_A$/BZ receptor antagonist while Ro15-4513 is known as a $GABA_A$/BZ receptor partial inverse agonist. A different distribution pattern has been reported for the binding of [^{11}C]Ro15-4513 compared to that of [^{11}C]flumazenil (Inoue et al., 1992; Halldin et al., 1992). Ro15-4513 was reported to have relatively higher affinity for the α5 subunit-containing $GABA_A$/BZ receptor *in vitro* (Lüddens et al., 1994; Wieland and Lüddens, 1994). [^{11}C]Ro15-4513 bindings in the cingulate and temporal cortical regions showed relatively higher binding to α5 subunit of $GABA_A$ receptor (Lingford-Hughes et al., 2002; Maeda et al., 2003).

A simplified method without arterial blood sampling for [^{11}C]Ro15-4513 in the living human brain has been evaluated recently, and it can be used in clinical studies (Asai et al., in press).

In this study, we measured [^{11}C]Ro15-4513 binding to examine $GABA_A$/BZ receptors with α5 subunit and their relation to clinical symptoms in patients with schizophrenia.

2. Methods and materials

2.1. Subjects

Eleven patients with schizophrenia (5 women, 6 men; 32.8±10.2 years old, mean±SD) meeting DSM-IV criteria for schizophrenia or schizophreniform disorder were enrolled in this study. Demographic and clinical data on subjects are shown in Table 1. Six of the patients (3 women, 3 men; 29.2±7.3 years old) were neuroleptic-naïve and five (2 women, 3 men; 37.2±12.2 years old) had been neuroleptic-free for at least one year before the PET measurement except one subject who took

Table 1
Demographic and clinical characteristics asstudy entry

	N	Age (years)	Male/female	Duration of illness (months)	Schizophrenia/schizophreniform	PANSS			
						Positive	Negative	General	Total
Patient	11	32.8±10.2	6/5	1–444	9/3	24.4±5.1	21.4±6.0	44.6±10.2	90.4±19.6
Drug-naïve	6	29.2±7.3	3/3	1–36	3/3	24.8±3.9	20.3±8.0	45.3±12.0	90.5±23.0
Drug-free	5	37.2±12.2	3/2	24–444	6/0	23.8±6.8	22.6±2.5	43.8±8.8	90.2±17.4
Normal controls	12	29.0±10.2	12/0	–	–	–	–	–	–

neuroleptics two weeks before the PET measurement. Three neuroleptic-naïve patients satisfying criteria for schizophreniform disorder (duration of illness 1 to 4 months at the time of PET measurement) met criteria for schizophrenia at 6-month follow-up. The patients were recruited from the outpatient units of university-affiliated psychiatric hospitals, psychiatric divisions of general hospitals, and a mental clinic in the urban environments of Tokyo and Chiba prefectures in Japan. Exclusion criteria were current or past substance or cannabis or alcohol abuse, mood disorders, organic brain disease, and medication of antipsychotics, antidepressants, or benzodiazepines or mood stabilizers within two weeks before PET measurement. Five out of 11 subjects were smokers.

Psychopathology was assessed by the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). PANSS was completed by three experienced psychiatrists on the same day as PET measurements was performed. They reviewed the ratings after the interviews, and disagreements were resolved by consensus; the consensus ratings were used in this study. The symptom scores were calculated as the total scores, positive symptom, negative symptom, and general symptom subscores of PANSS. The total PANSS score ranged from 60 to 124 (90.4±19.6, mean±SD), mean positive symptom scores were 24.4±5.1, negative symptom scores were 21.4±6.0, and general symptom scores were 44.6±10.2.

Normal control subjects (12 men, 29.0±10.2 years old) were recruited through notices on bulletin boards at the universities and among the staffs of the affiliated hospitals where the patients had been diagnosed. None of the controls had a history of psychiatric or neurological illness, brain injury, chronic somatic illness, or substance abuse. None had taken any drug including benzodiazepines within two weeks before PET measurements. Seven out of 12 subjects were smokers. All the subjects were examined by T1-weighted magnetic resonance image (MRI) using 1.5 T Philips Gyroscan NT to rule out organic brain diseases. This study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan. Written informed consent was obtained from all subjects.

2.2. PET measurement

[^{11}C]Ro15-4513 was synthesized by *N*-methylation of a corresponding *N*-desmethyl precursor with [^{11}C] methyl iodide. The reaction mixtures were purified by liquid chromatography, eluted with CH_3CN/6mM-phosphoric acid=175/325. The radiochemical purities were more than 95%.

The PET system used was ECAT EXACT HR+(CTI-Siemens, Knoxville, TN, USA), which provides 63 planes and a 15.5-cm field of view and was used in 3-dimensional mode. After a 10-minute transmission scan, a bolus of 352.3±66.9 MBq (mean±SD) of [^{11}C] Ro15-4513 with high specific radioactivities (103.4±38.9 GBq/μmol; mean±SD) was injected into the antecubital vein with a 20-ml saline flush. Radioactivity in the brain was measured in a series of sequential frames up to 60 min (total 28 frames).

2.3. PET data analysis

All emission scans were reconstructed with a Hanning filter cut-off frequency of 0.4 (FWHM 7.5 mm). Regions-of-interest (ROIs) were delineated on PET/MRI coregistered images for ten target regions (anterior cingulate, hippocampus, amygdala, thalamus, temporal cortex, prefrontal cortex, insula, caudate, putamen, cerebellum) and the pons as a reference region. Regional binding potentials were calculated using a simplified reference tissue model (SRTM)(Lammertsma and Hume, 1996). In brief, based on the three-compartment model, regional radioactivities in a target region (C_T) can be described by the following equation:

$$C_T(t) = R_1 C_R(t) + (k_2 - R_1\theta_3) C_R(t)^* e^{-\theta_3 t},$$

where C_R represents the radioactivity in the reference region, R_1 is the ratio of K_1 in a target region to the reference region, $\theta_3 = k_2/(1+BP)$, K_1 and k_2 are rate constants corresponding to the influx and efflux rates from plasma to the tissue compartments, and * is the

Table 2
Binding potentials for regions of interest

	BP values		*T* test (df=21)	
	Controls (*N*=12)	Patients (*N*=11)	*T* score	*p*
Anterior cingulated	6.08±0.72	6.14±0.63	−0.213	0.833
Hippocampus	5.43±0.77	4.95±0.80	1.432	0.167
Amygdala	5.49±0.56	5.25±0.48	1.118	0.276
Thalamus	2.00±0.28	1.83±0.24	1.534	0.14
Temporal cortex	4.20±0.52	4.12±0.38	0.438	0.666
Prefrontal cortex	3.60±0.35	3.59±0.34	0.09	0.929
Insula	5.79±0.63	5.56±0.46	1.011	0.324
Caudate	2.99±0.43	3.32±0.81	−1.199	0.249
Putamen	2.86±0.36	3.10±0.45	−1.445	0.165
Cerebellum	1.32±0.25	1.34±0.23	0.148	0.883

Values are mean±SD.

convolution operator. In this study, the pons was chosen as the reference tissue because this region is almost devoid of $GABA_A$/BZ receptor complex (Abadie et al., 1992).

2.4. Statistical analysis

Statistical analysis of the difference of regional BP or R_1 for each ROI between patients and controls was performed by repeated measures analysis of variance (ANOVA). When any interaction was found, post hoc Bonferroni correction was used for multiple comparisons. $p<0.05$ was considered significant.

Correlations between regional BP and PANSS scores were analyzed with Pearson's correlation method. $p<0.05$ was considered significant.

3. Results

Regarding regional BP values of [^{11}C]Ro15-4513, two-way repeated ANOVA revealed significant group-region interaction [$F_{4.3,90.6}=2.6$, $p=0.037$]. However, post hoc Bonferroni correction showed no significant differences of BPs for 10 ROIs between patients and controls (Table 2). As for R_1 values, two-way repeated ANOVA revealed no significant main effect of the groups [$F_{4.9,103.9}=1.613$, $p=0.164$] nor group-region interaction [$F_{1,21}=1.532$, $p=0.229$].

For the reference tissue, time activity curves of the pons between patients with schizophrenia and controls were compared with repeated-measures ANOVA with Green–Geisser correction. There was no significant main effect of groups [$F_{1,21}=1.027$, $p=0.323$] or no significant group by time interaction [$F_{2.09,43.9}=0.203$, $p=0.826$].

Regarding the relation to clinical symptoms, there were significant negative correlations between [^{11}C]Ro15-4513 binding in the prefrontal cortex and negative symptom scores ($R=-0.733, p=0.010$)(Fig. 1A), general symptom scores ($R=-0.655$, $p=0.029$) (Fig. 1C), and total PANSS scores ($R=-0.690$, $p=0.019$) (Fig. 1D). There was also a negative correlation between [^{11}C]Ro15-4513 binding in the hippocampus and negative symptom scores ($R=-0.605, p=0.048$) (Fig. 1B). No other regions

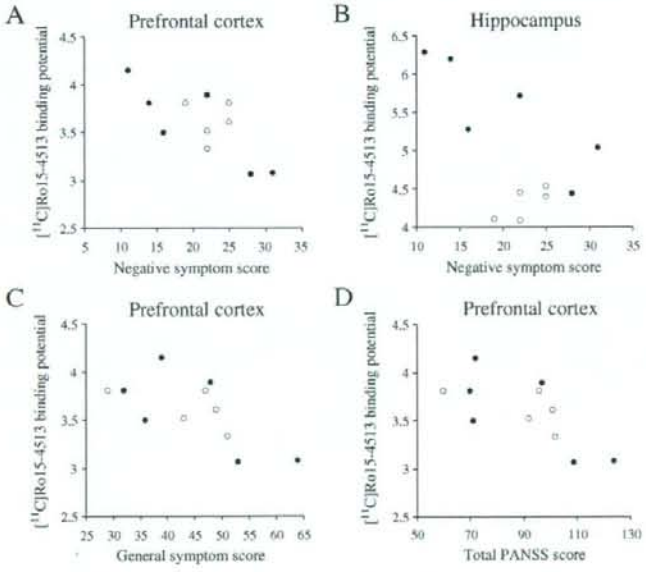

Fig. 1. Relationship between regional [^{11}C]Ro15-4513 binding potentials and PANSS scores in 11 patients with schizophrenia. Filled circles indicate neuroleptic-naïve patients ($N=6$). Open circles indicate drug-free patients ($N=5$). Total PANSS scores consist of positive symptom scores, negative symptom scores, and total symptom scores. There were significant negative correlations between [^{11}C]Ro15-4513 binding in the prefrontal cortex and negative symptom scores ($R=-0.733$, $p=0.010$)(A), general symptom scores ($R=-0.655$, $p=0.029$) (C), and total PANSS scores ($R=-0.690$, $p=0.019$) (D). There was also a negative correlation between [^{11}C]Ro15-4513 binding in the hippocampus and negative symptom scores ($R=-0.605$, $p=0.048$) (B).

Table 3
Correlation between regional [11C]Ro15-4513 binding potentials and PANSS scores

	Positive symptoms		Negative symptoms		General symptoms		Total scores	
Region	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*
Anterior cingulate	−0.123	0.718	−0.312	0.350	−0.079	0.817	−0.169	0.620
Hippocampus	−0.008	0.982	−0.605	0.048*	−0.221	0.513	−0.302	0.367
Amygdale	−0.394	0.231	−0.307	0.359	−0.282	0.401	−0.343	0.302
Thalamus	−0.298	0.373	−0.163	0.633	0.005	0.987	−0.125	0.714
Temporal cortex	−0.415	0.205	−0.594	0.054	−0.564	0.070	−0.583	0.060
Prefrontal cortex	−0.485	0.131	−0.733	0.010*	−0.655	0.029*	−0.690	0.019*
Insula	−0.146	0.668	−0.541	0.085	−0.281	0.403	−0.349	0.292
Caudate	−0.164	0.630	−0.118	0.729	0.031	0.929	−0.063	0.854
Putamen	−0.383	0.245	−0.287	0.393	−0.184	0.587	−0.283	0.398
Cerebellum	0.057	0.868	−0.120	0.725	−0.010	0.976	−0.027	−0.937

showed significant correlation with clinical symptom scores (Table 3).

4. Discussion

In this study, significant negative correlation between clinical symptoms (especially negative symptoms) and $GABA_A$/BZ receptor binding in the prefrontal cortex (Fig. 1A, C, D) and the hippocampus (Fig. 1B) of the patients with schizophrenia was found. The significant relation between $GABA_A$/BZ receptor binding and clinical symptoms would suggest dysfunctions of the GABA system in schizophrenia.

Our results showed no significant difference of $GABA_A$/BZ receptor binding between patients with schizophrenia and controls (Table 2). This is consistent with some of the previous postmortem studies (Akbarian et al., 1995; Impagnatiello et al., 1998). However, inconsistent results have also been reported (Benes et al., 1996a, 1996b; Dean et al., 1999). Inconsistency can be attributed to methodological differences between PET study and postmortem study, as well as to the effects of prolonged antipsychotic and benzodiazepine administration. None of the patients in this study had taken any antipsychotics or benzodiazepines for at least two weeks before PET measurement. On the other hand, most of the subjects investigated in the postmortem studies had taken antipsychotics and/or benzodiazepines on a long-term basis. Recently, it was suggested from an animal experiment that antipsychotic drug administration would result in a "reshuffling" of $GABA_A$ receptor subtypes (Skilbeck et al., 2007).

Although there was no significant difference in [^{11}C] Ro15-4513 binding between patients and controls, [^{11}C] Ro15-4513 binding was found to be negatively correlated with clinical symptom scores. Although some previous SPECT studies using [^{123}I]iomazenil showed no significant difference of benzodiazepine binding between patients and controls (Abi-Dargham et al., 1999, Verhoeff et al., 1999), some reported that there were significant negative correlations between benzodiazepine binding and the severity of negative symptoms (Busatto et al., 1997), or cognitive impairment (Ball et al., 1998) in patients with schizophrenia. Our results were consistent with those studies, despite [^{11}C]Ro15-4513 having relatively high affinity for α5 subunit of $GABA_A$/BZ receptor while [^{123}I]iomazenil binds to $GABA_A$/BZ receptor non-selectively.

α5 subunit-containing $GABA_A$ receptors are reported to be concentrated in the apical dendrites of pyramidal neurons (Akbarian et al., 1995). In a postmortem study, α2 subunit of GABA in the axonal initial segment of pyramidal neurons was reported to be increased in patients with schizophrenia (Volk et al., 2002). The expression of subunits of $GABA_A$/BZ receptor was reported to be changed following chronic administration of phencyclidine, which induces schizophrenia-like symptoms in rats (Abe et al., 2000). Combining our results with these reports, the imbalance among α subunits in pyramidal neurons could be expected in patients with schizophrenia.

Dopamine receptors in the prefrontal cortex have been suggested to be involved in the pathophysiology of schizophrenia. Dopamine D1 receptor plays a key role in negative symptoms and cognitive dysfunctions of schizophrenia (Abi-Dargham et al., 2002; Okubo et al., 1997). Reduced prefrontal pyramidal neuron output could change the activity of dopamine neurons in the prefrontal cortex in schizophrenia (Lewis and Gonzalez-Burgos, 2006). The possible change of α5 subunit in the prefrontal cortex might cause the change of pyramidal neuron output, which might interact with dopamine D1 receptor.

Not only the prefrontal cortex but also the hippocampus was found to be correlated negatively with negative symptoms of patients with schizophrenia in this study (Fig. 1B). Hippocampal-dependent spatial learning was improved in α5 subunit of $GABA_A$ receptor-knockout mice (Collinson et al., 2002), or by systemic treatment of an inverse agonist selective for α5 $GABA_A$ receptors (Chambers et al., 2003). The change of α5 subunit of $GABA_A$ receptors in the prefrontal cortex in patients with schizophrenia might affect hippocampal function because of the plastic neuronal connections between the hippocampus and prefrontal cortex (Goldman-Rakic et al., 1984; Laroche et al., 2000; Maccotta et al., 2007; Tierney et al., 2004; Takahashi et al., 2007).

There has been some interest in treating negative symptoms and cognitive dysfunctions in schizophrenia with GABA-modulating drugs (Guidotti et al., 2005; Lewis et al., 2004; Menzies et al., 2007). Imidazenil, which selectively allosterically modulates cortical $GABA_A$ receptors containing α5 subunit, was reported to contribute to amelioration of the behavioral deficits without producing sedation or tolerance liability in mice (Guidotti et al., 2005), and it increased locomotor activity in a social isolation mouse model (Pinna et al., 2006).

There were several limitations to this preliminary study. The number of subjects was small, and five of the eleven patients were previously treated. Further study would be needed with a larger population of drug-naïve patients. Although age correction was not performed, we previously reported no significant age effect of [^{11}C] Ro15-4513 binding (Suhara et al., 1993). We also compared with age-matched subgroup of drug naïve paitents (N=6) with controls (N=12) and two-way repeated ANOVA revealed no significant group-region interaction of [^{11}C]Ro15-4513 binding.

Sex was not matched between patients and controls, but sex differences of [^{11}C]Ro15-4513 binding have not been reported.

In conclusion, the present study showed that [^{11}C] Ro15-4513 binding was negatively correlated with negative symptom scores in schizophrenia. $GABA_A$/BZ receptor including α5 subunit might be involved in the pathophysiology of schizophrenia with negative symptoms.

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Contributors

Y Asai, T Takano and T Suhara designed the study and wrote the protocol. Y Okubo, M Matsuura, A Otsuka, H Takahashi, T Ando, and S Ito recruited the subjects and made psychiatric evaluations. Y Asai, T Takano, and R Arakawa performed the data analysis. Y Asai wrote the first draft of the manuscript. H Ito gave fruitful comments to finalize the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All the authors have no conflict of interest.

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Normal database of dopaminergic neurotransmission system in human brain measured by positron emission tomography

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The central dopaminergic system is of interest in the pathophysiology of schizophrenia and other neuropsychiatric disorders. Both pre- and postsynaptic dopaminergic functions can be estimated by positron emission tomography (PET) with different radiotracers. However, an integrated database of both pre- and postsynaptic dopaminergic neurotransmission components including receptors, transporter, and endogenous neurotransmitter synthesis has not yet been reported. In the present study, we constructed a normal database for the pre- and postsynaptic dopaminergic functions in the living human brain using PET. To measure striatal and extrastriatal dopamine D_1 and D_2 receptor bindings, dopamine transporter binding, and endogenous dopamine synthesis rate, PET scans were performed on healthy men after intravenous injection of [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, [^{11}C]PE2I, or L-[β-^{11}C]DOPA. All PET images were anatomically standardized using SPM2, and a database was built for each radiotracer. Gray matter images were segmented and extracted from all anatomically standardized magnetic resonance images using SPM2, and they were used for partial volume correction. These databases allow the comparison of regional distributions of striatal and extrastriatal dopamine D_1 and D_2 receptors, dopamine transporter, and endogenous dopamine synthesis capability. These distributions were in good agreement with those from human postmortem studies. This database can be used in various researches to understand the physiology of dopaminergic functions in the living human brain. This database could also be used to investigate regional abnormalities of dopaminergic neurotransmission in neuropsychiatric disorders.

Introduction

The central dopaminergic system is of major interest in the pathophysiology of schizophrenia and other neuropsychiatric disorders. Both pre- and postsynaptic dopaminergic functions can be estimated by positron emission tomography (PET) and single-photon emission computed tomography (SPECT) with the use of several radiotracers. The binding of dopamine receptors representing postsynaptic functions can be measured for each of D_1 and D_2 subtypes. For measurement of dopamine D_1 receptor binding, [^{11}C]SCH23390 (Farde et al., 1987a; Halldin et al., 1986) and [^{11}C]NNC112 (Halldin et al., 1998) are widely used. To measure the binding of striatal and extrastriatal dopamine D_2 receptors, which are quite different in densities, [^{11}C]raclopride (Farde et al., 1985; Ito et al., 1998; Kohler et al., 1985) and [^{11}C]FLB457 (Halldin et al., 1995; Ito et al., 2001; Suhara et al., 1999), respectively, are widely used. The bindings of striatal (Farde et al., 1987b, 1990; Nordstrom et al., 1995) and extrastriatal (Suhara et al., 2002; Yasuno et al., 2004) dopamine D_2 receptors in schizophrenia have been investigated. For estimation of the presynaptic dopaminergic function, dopamine transporter binding is measured by [^{11}C]β-CIT (Farde et al., 1994; Muller et al., 1993), [^{11}C]PE2I (Emond et al., 1997; Hall et al., 1999), and other radioligands. The endogenous dopamine synthesis rate measured by 6-[^{18}F]fluoro-L-DOPA (Gjedde, 1988; Gjedde et al., 1991; Huang et al., 1991) and L-[β-^{11}C]DOPA (Hartvig et al., 1991; Tedroff et al., 1992) can also indicate the presynaptic dopaminergic function. Dopamine transporter binding (Laakso et al., 2000; Laruelle et al., 2000) and the endogenous dopamine synthesis rate (Hietala et al., 1995; Laruelle, 1998; Lindstrom et al., 1999; Reith et al., 1994) in schizophrenia have been investigated.

An anatomic standardization technique, consisting of the transformation of brain images of individual subjects into a standard brain shape and size in three dimensions, allows inter-subject averaging of PET images (Fox et al., 1988; Friston et al., 1990). Using this technique with calculation of PET parametric images, a database of the regional distribution of neurotransmission functions can be constructed, and from this, group comparisons between normal control subjects and patients on a voxel-by-voxel basis can be performed. Previously, we built a normal database for the striatal and extrastriatal dopamine D_2 receptor bindings representing the postsynaptic dopaminergic neurotransmission components in the living human brain (Ito et al., 1999; Okubo et al., 1999). The in vivo regional distribution of dopamine D_2 receptor binding was in good agreement with that known from in vitro studies. For presynaptic dopaminergic function, a normal database for the endogenous dopamine synthesis rate in the living human brain has also been constructed with the use of 6-[^{18}F]fluoro-L-DOPA (Nagano et al.,

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2000). However, an integrated database for both pre- and postsynaptic dopaminergic neurotransmission components including receptors, transporter, and endogenous neurotransmitter synthesis, which allows to compare regional distributions between neurotransmission components on a same coordinate, has not been reported. In the present study, we constructed a normal database for pre- and postsynaptic dopaminergic neurotransmission components in the living human brain using PET and the anatomic standardization technique. Striatal and extrastriatal dopamine D_1 and D_2 receptor bindings, dopamine transporter binding, and endogenous dopamine synthesis rate were measured in healthy volunteers using the radiotracers [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, [^{11}C]PE2I, and L-[β-^{11}C]DOPA.

Materials and methods

Subjects

The study was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Sciences, Chiba, Japan. A total of 37 healthy men were recruited, and they gave their written informed consent for participation in the study (Table 1). The subjects were free of somatic, neurological or psychiatric disorders on the basis of their medical history and magnetic resonance (MR) imaging of the brain. They had no history of current or previous drug abuse and had not taken dopaminergic drugs in the past two weeks. Both PET studies with [^{11}C]raclopride and [^{11}C]FLB457 were performed in the same subjects on the same day. For [^{11}C]SCH23390, [^{11}C]PE2I, and L-[β-^{11}C]DOPA studies, each subject underwent only one PET study except three subjects: two underwent L-[β-^{11}C]DOPA, [^{11}C]raclopride, and [^{11}C]FLB457 studies; one underwent L-[β-^{11}C]DOPA and [^{11}C]SCH23390 studies. L-[β-^{11}C]DOPA studies were conducted without L-DOPA decarboxylase inhibitor premedication.

PET procedures

All PET studies were performed with a Siemens ECAT Exact HR+ system, which provides 63 sections with an axial field of view of 15.5 cm (Brix et al., 1997). The intrinsic spatial resolution was 4.3 mm in-plane and 4.2 mm full-width at half maximum (FWHM) axially. With a Hanning filter (cutoff frequency: 0.4 cycle/pixel), the reconstructed in-plane resolution was 7.5 mm FWHM. Data were acquired in three-dimensional mode. Scatter was corrected (Watson et al., 1996). A head fixation device with thermoplastic attachments for individual fit minimized head movement during PET measurements. A 10-min transmission scan using a ^{68}Ge-^{68}Ga line source was performed for correction of attenuation. After intravenous rapid bolus injection of [^{11}C]raclopride, [^{11}C]FLB457, or [^{11}C]PE2I, data were acquired for 90 min in a consecutive series of time frames. For [^{11}C]SCH23390 and L-[β-^{11}C]DOPA studies, data were acquired for 60 and 89 min after intravenous rapid bolus injection, respectively. The frame sequence consisted of twelve 20-s frames, sixteen 1-min frames, ten 4-min frames, and five 6-min frames for [^{11}C]raclopride, and nine 20-s frames, five 1-min frames, four 2-min frames, eleven 4-min frames, and six 5-min frames for [^{11}C]FLB457 and [^{11}C]PE2I. For [^{11}C]SCH23390, the frame sequence consisted of thirty 2-min frames. The frame sequence for L-[β-^{11}C]DOPA studies consisted of seven 1-min frames, five 2-min frames, four 3-min frames, and twelve 5-min. Injected radioactivity was 197–235 MBq, 213–239 MBq, 212–242 MBq, 197–230 MBq, and 320–402 MBq for [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, [^{11}C]PE2I, and L-[β-^{11}C]DOPA, respectively. Specific radioactivity was 23–81 GBq/μmol, 133–285 GBq/μmol, 72–371 GBq/μmol, 55–1103 GBq/μmol, and 29–82 GBq/μmol at the time of injection for [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, [^{11}C]PE2I, and L-[β-^{11}C]DOPA, respectively.

Table 1
Number of subjects per study and average age

Study	Number of subjects	Age (years, mean±SD)
[^{11}C]SCH23390	10	27.3±4.6
[^{11}C]raclopride	10	26.9±3.9
[^{11}C]FLB457	10	26.9±3.9
[^{11}C]PE2I	10	24.2±3.1
L-[β-^{11}C]DOPA	10	23.4±3.3

All subjects are male.
[^{11}C]raclopride and [^{11}C]FLB457 PET were conducted on same subjects.
Two subjects underwent L-[β-^{11}C]DOPA, [^{11}C]raclopride, and [^{11}C]FLB457 studies; one underwent L-[β-^{11}C]DOPA and [^{11}C]SCH23390 studies.

MR imaging procedures

All MR imaging studies were performed with a 1.5-T MR scanner (Philips Medical Systems, Best, The Netherlands). Three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of thin transverse sections (TE: 9.2 ms; TR: 21 ms; flip angle: 30°; field of view: 256 mm; acquisition matrix: 256×256; slice thickness: 1 mm).

Calculation of parametric images

For PET studies with [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, and [^{11}C]PE2I, binding potential (BP) was calculated by the reference tissue model method on a voxel-by-voxel basis (Lammertsma et al., 1996; Lammertsma and Hume, 1996). By this method, the time–activity curve in the brain region is described by that in the reference region with no specific binding, assuming that both regions have the same level of nondisplaceable radioligand binding:

$$C_i(t) = R_1 \cdot C_r(t) + \{k_2 - R_1 \cdot k_2/(1+\mathrm{BP})\} \cdot C_r(t) \otimes \exp\{-k_2 \cdot t/(1+\mathrm{BP})\}.$$

where C_i is the radioactivity concentration in a brain region; $C_r(t)$ is the radioactivity concentration in the reference region. 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). k_2 is the efflux rate constant for the brain region, and ⊗ denotes the convolution integral. In this analysis, three parameters (BP, R_1, and k_2) were estimated by the basis function method (Cselenyi et al., 2006; Gunn et al., 1997). The cerebellum was used as a reference region. We used in-house written software to calculate parametric images.

For the L-[β-^{11}C]DOPA study, the dopamine synthesis index (I) was calculated on a voxel-by-voxel basis as follows (Dhawan et al., 2002; Hoshi et al., 1993; Ito et al., 2007):

$$I = \frac{\int_{t_1}^{t_2} C_i(t)\mathrm{d}t}{\int_{t_1}^{t_2} C_i'(t)\mathrm{d}t} - 1$$

where C_i is the radioactivity concentration in a brain region, and C_i' is the radioactivity concentration in a brain region with no