ceptors (27). We then investigated whether there were correlations between subregional binding and psychopathology score as assessed with the Brief Psychiatric Rating Scale (BPRS) (28).

Method

Subjects

This study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan. After complete description of the study, written informed consent was obtained from all subjects. The patients were recruited from the outpatient units of five university-affiliated psychiatric hospitals and the psychiatric divisions of general hospitals in the prefectures of Tokyo and Chiba. Ten drug-naive right-handed male patients with schizophrenia (mean age=29.5 years, SD=7.8) who met DSM-IV criteria for schizophrenia or schizophreniform disorder were included. One patient satisfying the criteria for schizophreniform disorder (duration of illness was 1 month at the time of study entry) met the criteria for schizophrenia at 6-month follow-up. His behavioral ratings were evaluated at the time of study entry. Eight of the 11 patients in a previous study (1) had been examined with magnetic resonance imaging (MRI) and could thereby be included in the present analysis that also included two newly recruited patients. The other three patients had refused to participate in the MRI scan (1), and we could not include them in the present analysis. The duration of illness ranged from 1 month to 7 years, with a median of 2 years. Psychopathology was assessed by the 18-item Oxford version of the BPRS translated into Japanese (item score range=0–6 points) (28). Sum scores for positive and negative symptoms were calculated (1, 29), with the positive symptom subscale including the following eight items: conceptual disorganization, mannerisms and posturing, hostility, grandiosity, suspiciousness, hallucinatory behavior, unusual thought content, and excitement. The negative symptom subscale included these three items: emotional withdrawal, motor retardation, and blunted affect. BPRS total scores ranged from 14 to 42 (mean=29.3, SD=8.9), the mean positive symptom score was 14.6 (SD=4.6), and the mean negative symptom score was 5.5 (SD=4.6). The healthy subjects were recruited through notices on bulletin boards at universities and their affiliated hospitals where the patients were diagnosed. The 19 healthy right-handed male comparison subjects were age-matched (mean=29.6 years, SD=7.5). Parental socioeconomic status was determined on the basis of the Hollingshead-Redlich scale, and no significant differences between patients (mean=2.6, SD=0.7) and comparison subjects (mean=2.3, SD=0.5) were found ($t=1.30$, $df=27$, $p>0.20$). The comparison subjects did not meet criteria for any psychiatric or neurological disorder and had no first-degree relatives with neuropsychiatric disorders.

PET and MRI Procedures

The PET system ECAT EXACT HR+ (CTI-Siemens, Knoxville, Tenn.) was used to measure radioactivity in the brain. The field of view of this system is 15.5 cm. To minimize head movement, a head fixation device (Fixster, Stockholm) was used. A transmission scan for attenuation correction was performed using a ^{68}Ge-^{68}Ga source. Acquisitions were performed in three-dimensional mode with the interplane septa retracted. A bolus of 89.5–249.0 MBq (mean=172.5, SD=40.0) of [^{11}C]FLB457 with high specific radioactivity (64.9–534.9 GBq/μmol) was injected intravenously into a cannula inserted in an antecubital vein. The cannula was then flushed by the rapid injection of 20 ml of saline. Radioactivity in brain was measured in a series of scans for 80 minutes starting immediately after the injection. The emission scans were reconstructed with a Hanning filter cutoff frequency of 0.4 (full width at half maximum=7.5 mm). Images from the reconstructed volume were displayed as 67 horizontal sections. MR images were acquired on a Phillips Gyroscan NT, 1.5 tesla. T_1-weighted images of the brain were obtained to allow for differentiation between white and gray matter. The scan parameters for 1-mm-thick, three-dimensional T_1 images in the transversal plane were as follows: TR/TE=19/10 msec, flip angle=30°, matrix=256×256, field of view=256×256 mm, number of excitations=1.

Quantitative Analysis of [^{11}C]FLB457 Binding

Quantitative analysis was performed using the three-parameter simplified reference tissue model (30, 31). The cerebellum was used as a reference region because it has been shown to be almost devoid of D_2 receptors (23, 31). The model provides an estimation of the binding potential, which is defined by the following equation:

$$BP=k_3/k_4=f_2\,B_{max}/\{K_d\,[1+\Sigma_i\,F_i/K_{di}]\},$$

where k_3 and k_4 describe the bidirectional exchange of tracer between the free compartment and the compartment representing specific binding, f_2 is the "free fraction" of nonspecifically bound radioligand in brain, B_{max} is the receptor density, K_d is the equilibrium dissociation constant for the radioligand (32), and F_i and K_{di} are the free concentration and the dissociation constant of competing endogenous dopamine, respectively. The model also provides the parameter R_1, which represents the ratio of radioligand delivery in the region of interest to that in the reference region (influx ratio).

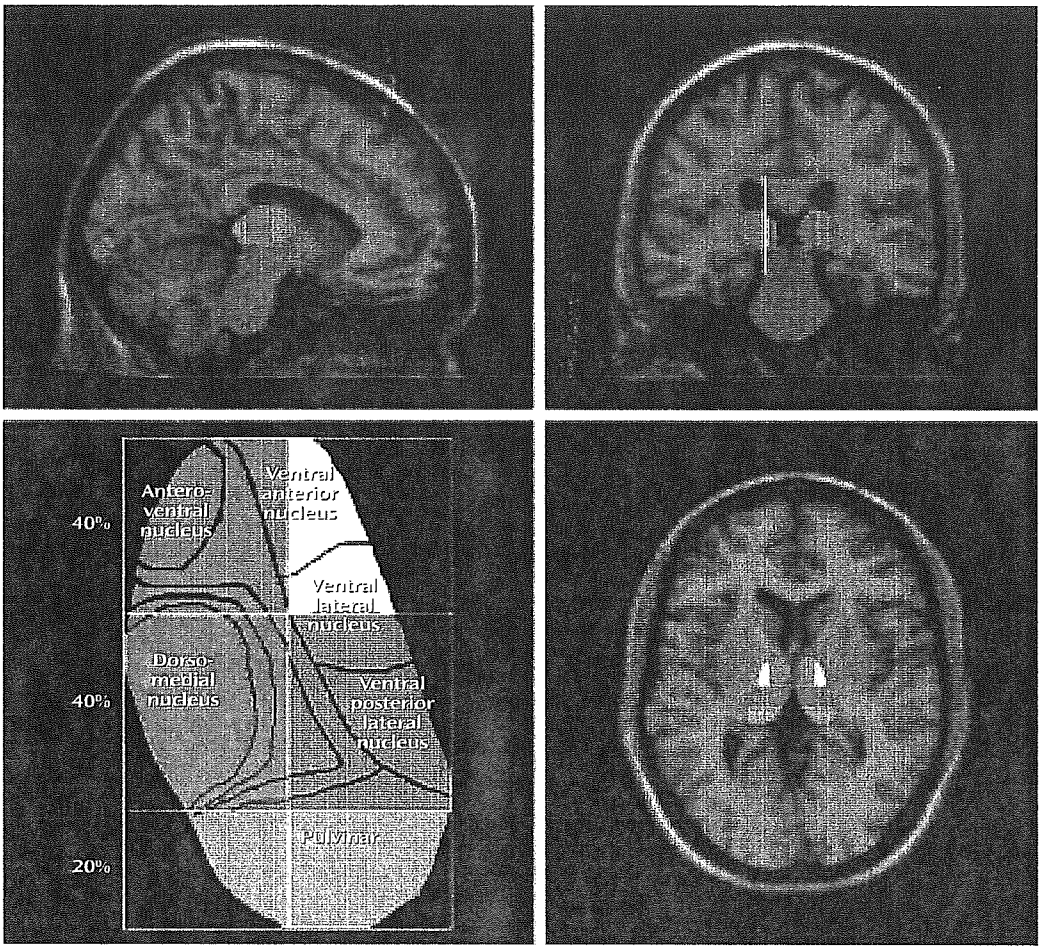
Thalamic Subdivisions

Regions of interest for five operationally defined subregions of the thalamus were defined on MR images according to a manual tracing technique that has been described in the literature and applied previously for the study of thalamic volumes in schizophrenia (5, 7). The regions of interest were delineated three-dimensionally on MR images and displayed by a distinct color as described previously (33) (Figure 1).

In the first step, the boundaries of the whole thalamus were identified. The mamillary body was used as the anterior boundary. The internal capsule was the lateral boundary, the third ventricle the medial boundary, and the inferior border of the third ventricle the inferior boundary. The posterior boundary was defined as the location where the hemispheres of the thalamus merged under the crux fornix. The superior boundary was the main body of the lateral ventricle (7). In the second step, the thalamus was subdivided into five distinct regions. The thalamus was first divided into medial and lateral parts. A line drawn parallel to the lateral border of the midbrain, the interhemispheric fissure, and the cerebral aqueduct represented the vertical bisection (coronal view in Figure 1). This line was continued through all thalamic slices to create a plane of bisection parallel to the interhemispheric fissure. The individual number of contiguous coronal slices in which the thalamus appeared was then calculated.

The thalamus was divided into anterior, central, and posterior divisions that were defined as fixed percentages of the total number of coronal slices. The anterior and central divisions each contained 40% of the total number of slices, and the posterior division contained 20%. Using this approach, the thalamus was divided into six subregions. The medial and lateral portions of the posterior thalamus were then combined, since they both corresponded to the pulvinar (axial view in Figure 1). In the final step, the regions of interest were linearly transformed using the parameters obtained from the coregistration of the individual MRI and PET images. This was done using SPM 99 (34), with the default parameter option of mutual information (35). After transformation of the regions of interest from MRI to PET, the regional radioactivity of each region of interest was calculated for each frame, corrected for decay, and plotted against time. The average values for

FIGURE 1. Thalamic Subdivisional Regions of Interest[a]



[a] Regions of interest were defined on T_1-weighted MR images by a manual tracing technique as described in a previous article examining the thalamic volumes in schizophrenia (7). Approximate regions of specific thalamic nuclei are depicted in the representation of the axial view of the thalamus (lower left) (5, 7). The line drawn in the coronal view of the MRI—which is parallel to the lateral border of the midbrain, interhemispheric fissure, and cerebral aqueduct—represents the line of vertical bisection of each thalamus.

regions of interest in the right and left hemisphere were used to increase the signal-to-noise ratio for the calculations. Subdivision of the thalamus and measurement of binding potential values was performed in duplicate by a single investigator (F.Y.) in 10 of the healthy subjects, and intrarater reliability was assessed. High intraclass correlations (ICCs) for subregions were seen for the two sets of measurements (anterior medial: ICC=0.96, anterior lateral: ICC=0.94, central medial: ICC=0.92, central lateral: ICC=0.90, posterior: ICC=0.82).

Morphological analysis was performed on the volume of the region of interest defined on MRI images. The size of the area was calculated, summed across slices, and multiplied by the slice thickness (1 mm), yielding approximate volumes. Intracranial volume was used as a covariate when comparing volumetric measures between the groups.

Statistical Comparisons

The binding potential and influx ratio (R_1) values for the whole thalamus were compared between patients and healthy subjects by Student's t test. The volumes for the whole thalamus were compared between patients and comparison subjects using one-way analysis of covariance (ANCOVA) with the intracranial volume as covariate. Group differences in the binding potential values of the thalamic subregions were compared by using multivariate analysis of variance (MANOVA). Follow-up serial one-way analyses of variance (ANOVAs) were performed to specify regional differences. To examine the influence of regional differences of blood flow and volumes on those of the binding potential values, serial one-way ANOVAs and ANCOVAs with intracranial volume as covariate were performed to specify regional differences of the influx ratios and volumes, respectively. A p value of 0.05 (two-tailed) was chosen as the significance threshold. The relationship between regional binding potential values and BPRS scores (total score as well as positive and negative symptom subscale scores) was evaluated in the correlation analysis. In consideration of the effect of the duration of illness, we examined the relationship of its variables to regional binding potential values. We also evaluated the relationships of age and parental socioeconomic status to the binding potential values in healthy subjects and patients. In the correlation analysis, we used the Pearson correlation method, and $p<0.01$ [0.05/5] was considered as significant to avoid type I errors due to the multiplicity of statistical analyses.

TABLE 1. [^{11}C]FLB457 Binding Potential[a] in Thalamic Subregions of Patients With Schizophrenia and Healthy Comparison Subjects

Thalamic Subregion	Binding Potential[b]					
	Healthy Subjects (N=19)		Schizophrenia Patients (N=10)		Analysis of Variance	
	Mean	SD	Mean	SD	F (df=1, 27)	p
Anterior medial	3.72	0.70	3.67	0.23	0.05	0.83
Anterior lateral	2.69	0.51	2.55	0.25	0.69	0.41
Central medial	3.95	0.48	3.53	0.43	5.21	0.03
Central lateral	2.56	0.38	2.43	0.28	0.84	0.37
Posterior	2.64	0.26	2.33	0.23	10.15	0.004

[a] Index of dopamine D_2 receptor binding.
[b] Multivariate analysis of variance was performed to test for group differences between comparison subjects and patients (Wilks's lambda=0.56; F=3.64, df=5, 23, p=0.01).

Results

With regard to the binding potential values for [^{11}C]FLB457 binding in the whole thalamus, there was no significant difference between the two groups (schizophrenia patients: mean=3.31, SD=0.33; comparison subjects: mean=3.54, SD=0.45) (t=1.45, df=27, p>0.15). However, multivariate analyses of the binding potential values in the thalamic subregions showed significant differences between patients with schizophrenia and comparison subjects. Follow-up ANOVAs revealed that the binding potential values in the central medial region and the posterior region were significantly lower in the patients (Table 1, Figure 2).

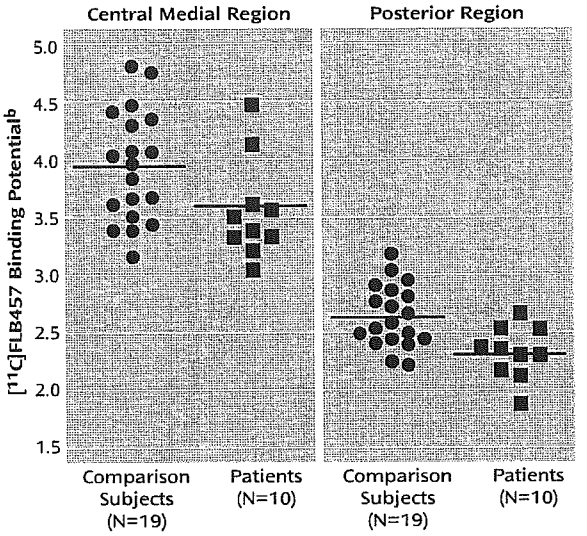
The influx ratio (R_1) for the whole thalamus (schizophrenia patients: mean=0.84, SD=0.50; comparison subjects: mean=0.87, SD=0.57) did not differ significantly between patients and comparison subjects (t=1.62, df=27, p>0.12) nor did it differ in any subregion (Table 2). The volume of the whole thalamus was not significantly different between patients (mean=8.65, SD=1.16) and healthy subjects (mean=8.65, SD=1.19) (F=0.005, df=1, 26, p>0.94), and no significant difference was found for any of the thalamic subregions (Table 2).

For the central medial region and the posterior region there was a statistically significant negative correlation between binding potential and positive symptom subscores on the BPRS (Table 3, Figure 3). There was no significant correlation between binding potential and the BPRS total scores or negative symptom subscore for any region. Further, no significant relationship was observed between regional binding potential values and the duration of illness, age, or parental socioeconomic status in comparison subjects and patients.

Discussion

In a previous PET study, we reported low dopamine D_2 receptor binding in the anterior cingulate in patients with schizophrenia and a statistical trend for low binding in the thalamus (1). In the present study with a partly overlapping patient group, a more detailed analysis of the thalamus indicated low [^{11}C]FLB457 binding potential in the central medial and posterior subregions of the thalamus in patients with schizophrenia. Highly elevated levels of dopamine have been found postmortem in the thalamus of schizophrenia (36), and the reduction in binding could be attributed to an increase in endogenous dopamine. However, our previous study indicated that extrastriatal [^{11}C]FLB457 was not sensitive to endogenous dopamine, as [^{11}C]FLB457 binding in the cortex and thalamus was not significantly affected by a 1-mg/methamphetamine challenge (37). Therefore, the present findings may be attributed to the receptor density. A longitudinal study will be required to settle the issue of whether low density is acquired during the course of disease or whether it represents abnormal brain development (38).

FIGURE 2. [^{11}C]FLB457 Binding Potential in the Central Medial and Posterior Subregions of the Thalamus in Patients With Schizophrenia (N=10) and Healthy Comparison Subjects (N=19)[a]



[a] The horizontal line represents group mean.
[b] Index of dopamine D_2 receptor binding.

The subregions with low D_2 receptor binding comprise primarily the dorsomedial nucleus and pulvinar, two important components in circuitries previously suggested in the pathophysiology of schizophrenia (5, 7). The thalamus has long been suggested to have a gating function that filters the sensory input to the cortex, thereby providing protection against sensory overload and hyperarousal (39). Startle prepulse inhibition, a sensitive measure of sensory gating, has indeed been suggested as being related to dopamine neurotransmission in the thalamus (40). Both animal and human studies have provided evidence that the mediodorsal thalamus has a particular role in the regulation of startle prepulse inhibition (41–43). In experimental studies, it has been shown that prepulse inhibition of startle can be disrupted after microinjection of the dopamine

TABLE 2. Influx Ratios and Volumes of Thalamic Subregions in Patients With Schizophrenia and Healthy Comparison Subjects

	Influx Ratio[a]						Volume (cm^3)					
	Healthy Subjects (N=19)		Schizophrenia Patients (N=10)		Analysis of Variance		Healthy Subjects (N=19)		Schizophrenia Patients (N=10)		Analysis of Covariance[b]	
Thalamic Subregion	Mean	SD	Mean	SD	F (df=1, 27)	p	Mean	SD	Mean	SD	F (df=1, 26)	p
Anterior medial	0.87	0.06	0.83	0.07	2.20	0.15	2.25	0.38	2.29	0.32	0.05	0.83
Anterior lateral	0.80	0.08	0.77	0.08	1.32	0.26	0.80	0.25	0.77	0.28	0.11	0.75
Central medial	0.92	0.08	0.88	0.04	1.38	0.25	2.39	0.51	2.27	0.50	0.29	0.59
Central lateral	0.90	0.06	0.87	0.06	2.07	0.16	1.89	0.41	1.93	0.33	0.02	0.89
Posterior	0.80	0.06	0.78	0.05	0.60	0.45	1.33	0.23	1.39	0.26	0.37	0.55

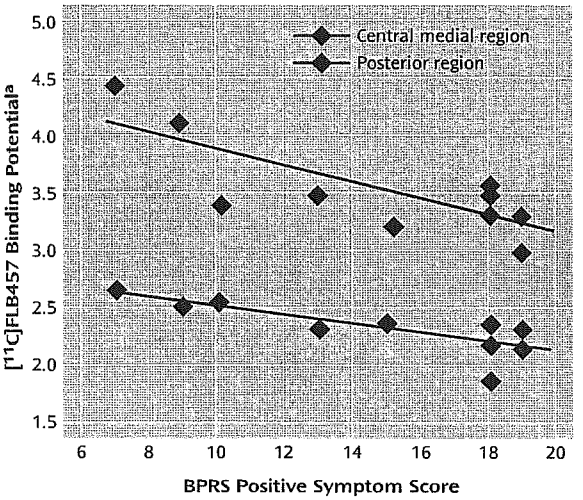
[a] The ratio of radioligand delivery in the region of interest to that in the reference region (cerebellum).
[b] Intracranial volume entered as covariate.

TABLE 3. Correlations Between [^{11}C]FLB457 Binding Potential[a] and BPRS Total and Positive and Negative Symptom Scores in Patients With Schizophrenia (N=10)

	Correlation Between Binding Potential and BPRS Score					
	Total Scale		Positive Symptoms		Negative Symptoms	
Thalamic Subregion	r	p	r	p	r	p
Anterior medial	–0.06	0.86	–0.18	0.62	0.23	0.52
Anterior lateral	0.06	0.88	0.39	0.27	–0.41	0.24
Central medial	–0.64	0.05	–0.78	0.008	0.01	0.97
Central lateral	–0.03	0.93	–0.11	0.77	0.31	0.38
Posterior	–0.58	0.08	–0.80	0.006	0.18	0.61

[a] Index of dopamine D_2 receptor binding.

FIGURE 3. Relationship Between [^{11}C]FLB457 Binding Potential in the Central Medial and Posterior Subregions of the Thalamus and BPRS Positive Symptom Score in Patients With Schizophrenia (N=10)



[a] Index of dopamine D_2 receptor binding.

agonist apomorphine into the medial thalamus (43). The dorsomedial nucleus has connections to the prefrontal cortex and anterior cingulate (44). Both these regions have also been included in discussions on the gating function (39). An abnormality of the dorsomedial nucleus in schizophrenia may be attributed to functional disturbances in any of the brain regions discussed in relation to sensory gating.

The other subnuclei with low binding potential are found in the pulvinar. This is one of several structures implicated in attentional processing and salience (45–47). The pulvinar has connections with the visual and auditory cortex as well as the prefrontal cortex and temporal association areas (48). A primary abnormality in the pulvinar may thus induce unusual associations and sensory disturbances in schizophrenia (26). Taken together, these two regions may have a functional role consistent with several of the disturbed functions in schizophrenia and underlie the correlation with BPRS positive symptom score.

The observation of a significant negative correlation between subregional binding potential and positive symptom score is similar to that previously reported for [^{11}C]FLB457 binding in the anterior cingulate. The anterior cingulate has direct connections with the dorsomedial nucleus and the pulvinar (48–50). When we examined the relationship between dopamine D_2 receptor binding in the central medial and posterior regions of the thalamus and that of the anterior cingulate using the Pearson correlation method, we found a significant interrelationship between those regions in patients with schizophrenia ($p<0.05$). The abnormality shown in the thalamic subregions might have a similar background to that in the anterior cingulate. However, the cellular localization of D_2 receptors in the thalamic subregions that might allow speculation about the altered regulatory function of interneurons with D_2 receptors has not yet been determined, as discussed in the previous study (1).

Reduced regional blood flow has been reported in the thalamus of schizophrenia (51). However, the reduction of binding potential is unlikely to be a result of altered blood flow, since the R_1 value did not differ significantly between the healthy subjects and patients. While morphological changes have been reported in the thalamic mediodorsal nucleus and pulvinar in schizophrenia (7, 8), and this can affect binding potential, no significant difference was observed in the volumes of thalamic subdivisions, including the central medial and posterior regions, between our examined patient and comparison groups. The relatively moderate severity and short duration of illness in our pa-

tient group may explain the lack of volume change (52, 53). In any case, low dopamine D_2 receptor binding can therefore not be attributed to reductions in gross brain anatomy.

There are several confounding factors in this study. First, the number of patients was small, raising the question of adequate statistical power, and thus it cannot be ruled out that a larger study population might reveal that the binding potential values of other thalamic subregions and volumetric measurements will also show significant differences. In addition, [^{11}C]FLB457 has high affinity not only for dopamine D_2 receptors but also for dopamine D_3 receptors (27). Dopamine D_3 receptors are distributed mainly to the ventral striatum and the islands of Calleja in the postmortem human brain, but they have as yet not been distinctly identified in the thalamus (54–56). Thus, there is a possibility that our findings could be partly explained by the reduction of dopamine D_3 receptors, but this will have to await the outcome of future studies on the amount of dopamine D_3 receptors in the thalamus. Another factor is that psychopathology was assessed by the 18-item BPRS, but this scale mainly measures the affective component of the negative symptoms and does not cover well the additional components that identify cognitive, anergic, and social dimensions (57).

Finally, our measurement of thalamic subdivisions has several limitations. We were unable to delineate and employ an intrathalamic marker as a consistent landmark for our regional subdivisions. Rather, we relied upon approximate percentage-based divisions of the total thalamic area as a means of dividing the thalamus. This automated method reduced some of the subjectivity and systematic bias involved in defining subthalamic areas with limited resolution imaging. However, without manual editing, the assumptions that all thalamic nuclei are consistently represented by these rigid subdivisions cannot be assured, and the volume of each subdivision would not be comparable with data from carefully edited volumetric studies.

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PRIORITY COMMUNICATION

Decreased 5-HT_{1A} Receptor Binding in Amygdala of Schizophrenia

Fumihiko Yasuno, Tetsuya Suhara, Tetsuya Ichimiya, Akihiro Takano, Tomomichi Ando, and Yoshiro Okubo

Background: *On the basis of postmortem data and the pharmacological action of atypical antipsychotics, serotonin-1A receptors are of interest in the study of the pathophysiology of schizophrenia. To investigate serotonin-1A receptors in schizophrenia and their relation to symptoms, we measured the availability of serotonin-1A receptors in patients with schizophrenia using positron emission tomography with [carbonyl-^{11}C]WAY-100635.*

Methods: *Serotonin-1A receptor binding of 11 patients with schizophrenia (8 drug-naive and 3 drug-free) was compared with that of 22 age-matched and gender-matched healthy control subjects. Symptoms were assessed using the Positive and Negative Syndrome Scale. Serotonin-1A receptor binding in selected regions of interest was quantified by binding potential obtained by the reference tissue method.*

Results: *The regional binding potential value was lower in the amygdala by about 19% in patients with schizophrenia than in normal controls. A significant negative correlation was observed between binding potential in the amygdala and the negative and depression/anxiety symptom scores on the five-symptom subscale of the Positive and Negative Syndrome Scale.*

Conclusions: *Decreased serotonin-1A receptor binding in the amygdala may underlie the affective components included in the symptoms of negative and depression/anxiety in schizophrenia.*

Key Words: 5-HT_{1A}, schizophrenia, amygdala, anxiety, depression, negative symptoms

Several lines of evidence have suggested that serotonin-1A (5-HT_{1A}) receptor function may be involved in the pathophysiology of schizophrenia. Results of most postmortem studies have reported a change in frontal 5-HT_{1A} receptor density in patients with schizophrenia using [^{3}H]8-hydroxydipropylaminotetralin ([^{3}H]8-OH-DPAT) or [^{3}H]WAY-100635 as ligands (Hashimoto et al 1993; Burnet et al 1996; Sumiyoshi et al 1996; Simpson et al 1996), and the pharmacological administration of 5-HT_{1A} agonist response showed dysfunction of 5-HT_{1A} receptors in patients with schizophrenia (Lee and Meltzer 2001). A recent human positron emission tomography (PET) study showed increased binding potential of 5-HT_{1A} receptors in the medial temporal cortex (Tauscher et al 2002).

The function of 5-HT_{1A} receptors has been suggested to modulate human cognition (Yasuno et al 2003) and affect (Tauscher et al 2001). In relation to the mechanism of therapeutic drugs, atypical antipsychotic drugs such as clozapine, quetiapine, and ziprasidone were reported to exhibit a partial agonistic effect on 5-HT_{1A} receptors (Elliott and Reynolds 1999; Newman-Tancredi 1998), and this may be one of the mechanisms that improves diverse symptoms such as negative symptoms of schizophrenia (Meltzer 1999).

In this study, we performed PET scans using [carbonyl-^{11}C]WAY-100635 ([^{11}C]WAY-100635) to examine the 5-HT_{1A} receptor and its relation to clinical symptoms in patients with schizophrenia.

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Methods and Materials

Subjects

This study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiologic Sciences, Chiba, Japan. After complete explanation of the study, written informed consent was obtained from all subjects. Demographic and clinical data on subjects are presented in Table 1. The patients were recruited from the outpatient units of five university-affiliated psychiatric hospitals and the psychiatric divisions of general hospitals in the urban environments of Tokyo and Chiba prefecture. Eleven patients with schizophrenia (two women and nine men; mean age = 31.1 years, range = 18–50) meeting the DSM-IV criteria for schizophrenia or schizophreniform disorder were studied. Eight of the patients (mean age = 29.6 years, range = 18–38) were neuroleptic-naive and three (mean age = 35.0 years, range = 26–50) had been drug-free for at least 1 month before the PET measurement. Six patients satisfying criteria for schizophreniform disorder (duration of illness was 1 to 4 months at the time of study entry) met the criteria for schizophrenia at 6-month follow-up. The duration of illness ranged from 1 month to 5 years. Exclusion criteria were current or past substance abuse, mood disorders, organic brain disease, and antipsychotic, antidepressant medication within 1 month before PET measurement. No patients except one received any benzodiazepine medication within 1 month before PET measurement. The patients were screened for the exclusion criteria before study entry at the hospitals where they were recruited.

Psychopathology was assessed by the Positive and Negative Syndrome Scale (PANSS) (Kay et al 1987). Positive and Negative Syndrome Scales were completed by three experienced psychiatrists (FY, TI, AT, or TA). The ratings were reviewed by them after the interview and disagreements were resolved by consensus; the consensus ratings were used in this study. The symptom scores were calculated as the sum, positive, negative, and general subscores of PANSS. The PANSS sum score ranged from 71 to 124 (mean ± SD; 92.5 ± 21.8); the mean positive score was 24.6 ± 4.5; the negative score was 21.3 ± 7.2; and the general score was 46.5 ± 11.4.

To assess and measure more accurately the discrete domains

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Table 1. Demographic and Clinical Characteristics at Study Entry

	n	Age (Years)	Male/ Female	Duration of Illness (Months)	Schizophrenia/ Schizophreniform	PANSS Positive/Negative/General
Normal Controls	22	31.0 ± 8.5	18/4	–	–	–
Patients						
Drug-naive	8	29.6 ± 7.0	6/2	1–48	2/6	23.8 ± 4.8/20.8 ± 8.5/44.8 ± 12.9
Drug-free[a]	3	35.0 ± 13.1	3/0	24–60	3/0	27.0 ± 2.6/22.7 ± 2.1/51.3 ± 4.5
Total	11	31.1 ± 8.7	9/2	1–60	5/6	24.6 ± 4.5/21.3 ± 7.2/46.5 ± 11.4

PANSS, Positive and Negative Syndrome Scale; PET, positron emission tomography.
[a]Patient 1: Taking sulpiride (600 mg/day) 5 years before PET examination; Patient 2: Taking haloperidol (2.25 mg/day) and biperiden (3 mg/day) 2 years before PET examination; Patient 3: Taking risperidon (4 mg/day) 2 years before PET examination.

of the psychopathology, symptom scores were also calculated as the sums of the following items from PANSS according to the five-syndrome model (Lindenmayer et al 1994, 1995): positive: delusion and grandiosity; negative: emotional withdrawal, poor rapport, and social withdrawal; excitement: excitement and hostility; cognition: conceptual disorganization and abstract thinking; depression and anxiety: anxiety, guilt feeling, and depression. The mean positive score was 5.9 ± 1.6; the negative score was 9.4 ± 2.9; the excitement score was 5.5 ± 1.9; the cognition score was 7.1 ± 2.8; and the depression and anxiety score was 10.3 ± 3.9.

Normal control subjects were recruited through notices on bulletin boards at the universities and from the staffs of the affiliated hospitals where the patients were ascertained. Control subjects were 22 age-matched and gender-matched healthy subjects (4 women and 18 men; mean age = 31.0 years, range = 22–51) who did not meet criteria for any neuropsychiatric disorders and did not have relatives with neuropsychiatric disorders based on unstructured laboratory psychiatric screening interviews, which covered all axis I diagnostic categories of DSM-IV. They were examined by magnetic resonance imaging (MRI) to rule out any organic brain diseases.

PET Study Procedure

After transmission scan with a ^{68}Ge-^{68}Ga source, a bolus of 176.5 MBq to 237.9 MBq of [^{11}C]WAY-100635 was injected with specific radioactivity of 38.9 GBq/μmol to 400.0 GBq/μmol at the time of injection. Radioactivity was measured for 90 minutes using CTI-Siemens ECAT EXACT HR+ (CTI-Siemens, Knoxville, Tennessee) in three-dimensional (3D) mode, which provides 63 planes and a 15.5-cm field of view. All emission scans were reconstructed with a Hanning filter cutoff frequency of .4 (full-width half-maximum [FWHM] = 7.5 mm). T1-weighted MRI was acquired by Phillips Intera, 1.5 tesla (Philips Medical Systems, Best, The Netherlands). T1-weighted images of the brain were obtained from all subjects. The scan parameters were 1 mm thick, 3D, T1 images with a transverse plane (repetition time [TR]/echo time [TE] 21/9.2 milliseconds, flip angle 30, matrix 256 × 256, field of view [FOV] 256 mm × 256 mm).

Quantification of 5-HT$_{1A}$ Receptors

Regional radioactivity of 9 brain regions (cerebellum, anterior cingulate, prefrontal cortex, temporal cortex, parietal cortex, occipital cortex, amygdala, hippocampus, and midbrain raphe) were obtained with a template-based method for defining volumes of interest (VOIs), as described in our recent article (Yasuno et al 2002) with the exception of the midbrain raphe. In short, the template-based method consisted of two major steps. The first step comprised the spatial transformation of the template of a VOI from a model MRI to an individual MRI. The second step was to refine the transformed VOI to the individual segmented gray matter of the MRI using the intensity characteristics of these images (Figure 1A). The final refined VOIs were linearly transformed with the parameters obtained from the coregistration of the individual MRIs to PET images. The VOI on the midbrain raphe nuclei region was defined as follows: a circular region of interest (ROI) (5-mm radius) was centered over the midbrain identified in the coregistered MRI where raphe nuclei were evident in the PET summation images (Figure 1B), based on the method described in a previous study (Drevets et al 1999). Comparison of the radioactivities of the right and left sides of VOIs in patient and control groups revealed no significant

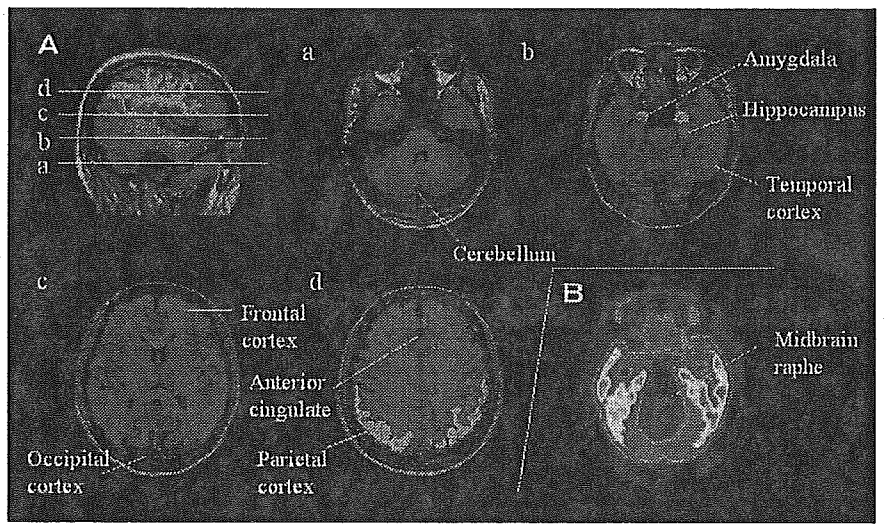

Figure 1. (A) The transformed VOI template placed on the axial slices of MRI of one subject after refining to segmented gray matter image. The horizontal lines through the sagittal section indicate the point of intersection for each of the corresponding axial sections. **(B)** The manually delineated circular ROI overlying the midbrain area containing raphe nuclei in the axial plane of summated PET images of one subject. VOI, volume of interest; MRI, magnetic resonance imaging; ROI, region of interest; PET, positron emission tomography.

differences in any of the regions in both groups. The average values of right and left VOIs were used to increase the signal-to-noise ratio for the calculations.

The [^{11}C]WAY-100635 binding was quantified with a reference tissue compartmental model using the cerebellum as reference tissue (simplified reference tissue method [SRTM]) (Gunn et al 1998). This model allows the estimation of binding potential (BP), which is defined as follows: BP (k3/k4) = f_2 Bmax/[Kd [1 + Σ_i F_i/Kd_i]], where k3 and k4 describe the exchange of tracer between the free compartment and a specifically bound ligand compartment, f_2 is the "free fraction" of unbound radioligand, Bmax is the density of receptor, Kd is the dissociation constant for the radioligand, and F_i and Kd_i are the free concentration and the dissociation constant of the competing endogenous ligand, respectively. The model also allows the estimation of R_1, the ratio of the delivery in the tissue ROI compared with that in the reference region (ratio of influx).

The quantification of [^{11}C]WAY-100635 binding to 5-HT_{1A} receptors has been demonstrated using both SRTM and kinetic analysis with metabolite-corrected plasma input (Farde et al 1998). There is general agreement on the utility of both SRTM and kinetic analysis in the quantification of [^{11}C]WAY-100635 binding, but there has been some debate on the preferred method, as discussed in previous studies (Gunn et al 1998; Parsey et al 2000; Slifstein et al 2000).

Some previous studies have indicated that SRTM has yielded lower results than kinetic analysis, with the relative underestimation appearing to be an increasing function of binding potential (Parsey et al 2000; Slifstein et al 2000). Yet, these problems should not preclude the use of SRTM in clinical studies for several reasons. First, the errors associated with this method might be low compared with the between-subject or within-subject differences associated with the experimental conditions. Second, the bias described here might be compensated for by higher test/retest reproducibility resulting from the fact that this method does not require measurement of arterial input function and the propagation of error associated with it. Third, the placement of an arterial catheter is the most invasive part of the procedure and might be associated with subject anxiety or discomfort (Slifstein et al 2000). Under these considerations, we used the SRTM approach in this study while keeping in mind its limitations.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows 11.0 (SPSS Japan Inc., Tokyo, Japan). Group differences in regional BP and R1 values between two groups were compared using repeated measures analysis of variance (ANOVA) with Greenhouse-Geisser correction. Follow-up unpaired t tests were performed for BP and R1 values of individual ROIs. All statistical tests were 2-tailed and reported at $p < .05$.

Multiplying the area of each ROI by the number of slices and their respective thickness provided an approximation for the actual VOI. Additional ANOVA was performed to compare the VOI values between groups. Follow-up unpaired t tests were performed for individual VOI values. To compare the uptake in the cerebellum between patients and controls, analysis of covariance (ANCOVA) was performed using the area under the cerebellar time activity curves as the dependent variable and injected dose as covariate.

The relationship between regional BP values and symptom scores was evaluated by the Pearson correlation method. In the correlation analysis, $p < .006$ (.05/8) was considered as significant to avoid type I errors in the multiplicity of statistical analysis.

Table 2. Binding Potential Values for Volumes of Interest

Region	BP Values[a] Controls (n = 22)	Patients (n = 11)	t Test (df = 31)[b] t Score	p
Prefrontal Cortex	4.36 ± .93	3.90 ± .71	1.47	.15
Temporal Cortex	4.27 ± .91	4.00 ± .56	.88	.38
Parietal Cortex	4.13 ± .94	3.68 ± .57	1.43	.16
Occipital Cortex	3.13 ± .80	2.96 ± .49	.66	.52
Anterior Cingulate	5.32 ± 1.18	4.84 ± .77	1.20	.24
Hippocampus	7.33 ± 1.53	6.36 ± 1.48	1.74	.09
Amygdala	6.09 ± 1.30	4.95 ± 1.27	2.39	.02[c]
Midbrain Raphe	2.74 ± .86	2.68 ± .99	.18	.86

Values are mean ± SD.
BP, binding potential; ANOVA, analysis of variance.
[a]Repeated measures ANOVA revealed a significant group-by-region interaction ($F_{2.7, 85.0}$ = 2.96, p = .04).
[b]Minimum level of statistical significance, $p < .05$.
[c]Significant difference between groups.

Results

Analysis of variance of the BP values for controls versus patients with schizophrenia revealed a significant group-by-region interaction [group-by-region interaction, F(2.7,85.0) = 2.96, p = .04; main effect of group, F(1,31) = 2.41, p = .13]. Follow-up unpaired t tests revealed that BP values in the amygdala were significantly lower, by 19%, in patients with

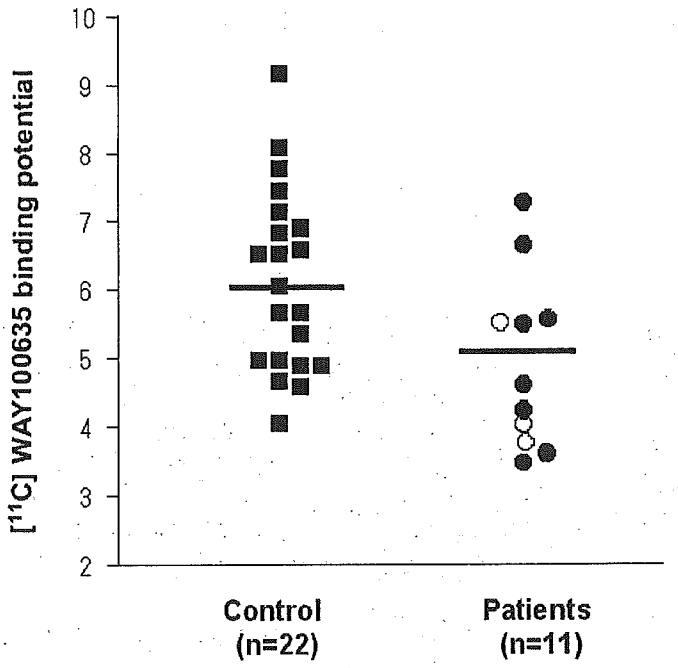

Figure 2. The [^{11}C]WAY-100635 binding potentials in the amygdala in 11 patients with schizophrenia and in 22 healthy control subjects. Closed circles represent drug-naive patients (n = 8), and open circles represent drug-free patients (n = 3). Binding potential values in the amygdala were significantly lower (by 19%) in patients with schizophrenia when compared with normal control subjects.

Table 3. Pearson's Correlation Coefficients Between Binding Potential Values and Symptom Scores According to the Five Syndrome Model

	Positive		Negative		Excitement		Cognition		Depression/Anxiety	
Region	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*
Prefrontal Cortex	.34	ns	−.23	ns	−.18	ns	−.10	ns	−.11	ns
Temporal Cortex	.37	ns	−.49	ns	−.26	ns	−.09	ns	−.18	ns
Parietal Cortex	.27	ns	−.48	ns	−.29	ns	−.03	ns	−.25	ns
Occipital Cortex	.27	ns	−.22	ns	−.09	ns	−.09	ns	−.19	ns
Anterior Cingulate	.32	ns	−.24	ns	−.18	ns	.10	ns	−.20	ns
Hippocampus	−.21	ns	−.66	ns	−.31	ns	−.15	ns	−.63	ns
Amygdala	−.32	ns	−.79	.004[a]	−.61	ns	−.34	ns	−.78	.005[a]
Midbrain Raphe	.22	ns	−.27	ns	−.36	ns	−.10	ns	−.36	ns

[a] $p < .006$ (0.05/8).

schizophrenia when compared with normal controls ($t_{31} = 2.39$, $p = .023$) (Table 2, Figure 2).

For R1 values, there was no main effect of group or group-by-region interaction with 2-way ANOVA [group, $F(1,31) = .50$, $p = .48$; group-by-region interaction, $F(4.3,132.9) = 1.56$, $p = .19$], and the value in the amygdala did not differ significantly between the patients and controls by unpaired t test (controls, .74 ± .08; patients, .74 ± .10; $t_{31} = .01$, $p = .99$).

For VOI values, there was no main effect of group or group-by-region interaction with 2-way ANOVA [group, $F(1,31) = .88$, $p = .36$; group-by-region interaction, $F(3.0,93.0) = 1.25$, $p = .30$], and the VOI value in the amygdala did not differ significantly between the patients and controls by unpaired t test (controls, 1.9 ± .8 cm^3; patients, 1.9 ± .3 cm^3; $t_{31} = .34$; $p = .74$). There was no significant difference in the area under the cerebellar time activity curves between groups [$F(1,30) = .32$, $p = .58$].

There were significant negative correlations between BP values and clinical rating for negative and depression/anxiety symptom scores in the amygdala, calculated according to the five-syndrome model (Lindenmayer et al 1994, 1995) (Table 3, Figure 3); however, there was no significant relationship between BP values and PANSS sum, positive, negative, and general scores. No significant correlations were observed between BP values and clinical rating for other scores in any brain region.

Discussion

The present study showed that 5-HT_{1A} receptor binding in the amygdala was significantly lower in patients with schizophrenia than in healthy controls as measured by PET with [^{11}C]WAY-100635. A significant negative correlation was observed between 5-HT_{1A} receptor binding and the clinical rating for negative and depression/anxiety symptom scores on the five-symptom scale of PANSS in the amygdala.

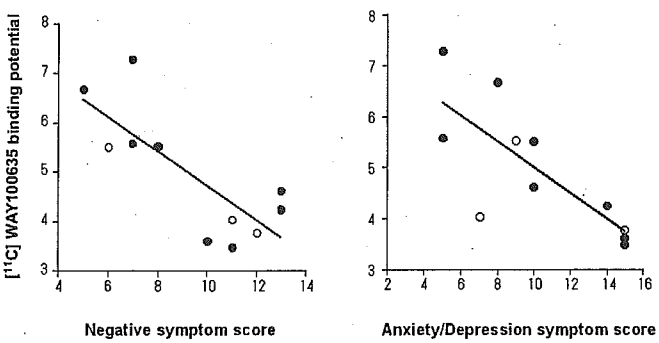

Figure 3. Relationship between [^{11}C]WAY-100635 BP in the amygdala and negative and anxiety/depressive symptom scores on PANSS in 11 patients with schizophrenia. Closed circles represent drug-naive patients, and open circles represent drug-free patients. Significant negative correlations between binding potential values and clinical ratings for negative and depression/anxiety symptom scores were observed in the amygdala. PANSS, Positive and Negative Syndrome Scale.

Several confounding factors need to be considered, including change in blood flow, partial volume effect, and the effect of endogenous serotonin. Patients with schizophrenia may have alterations in regional cerebral blood flow (Gur et al 2002; Taylor et al 2002); however, the reduction of BP of [^{11}C]WAY-100635 is unlikely to be an effect of altered blood flow, since the R1 value did not differ significantly between the controls and patients and BP from the reference tissue method is minimally dependent on tracer delivery over the R1 values obtained in this study (Lammertsma and Hume 1996). Morphologic changes have been reported in schizophrenia (Pegues et al 2003; Lawrie et al 2003) and they can affect BP, but no significant difference was observed in VOI values between our patient and control groups. Decreased 5-HT_{1A} bindings are therefore unlikely to be attributable to alterations in gross brain anatomy. The reduction in binding in this study cannot be ascribed to a change in endogenous serotonin, since our previous study showed that [^{11}C]WAY-100635 binding was not sensitive to endogenous serotonin concentration (Maeda et al 2001).

In this study, the cerebellum was chosen as the reference tissue because Hall et al (1997) reported that there was no evidence of specific binding of WAY-100635 in the cerebellum from autoradiography studies of postmortem adult humans and Burnet et al (1996) found no 5-HT_{1A} receptor messenger RNAs (mRNAs) in postmortem human cerebellum. There was no significant difference in the areas under the cerebellar time activity curves between controls and patients, indicating that there was no significant difference in the distribution of the radiotracer in the subject's reference tissue in our study. Despite the report of aberrant specific 5-HT_{1A} binding in the cerebellum of patients with schizophrenia (Slater et al 1998), it was reported only in the vermis of the cerebellum and not in the cerebellar hemisphere. The VOI of the cerebellum in our study contained only the gray matter of the cerebellar hemisphere and did not include the cerebellar vermis.

In this PET study of 5-HT_{1A} receptors in patients with schizophrenia, we could not detect an elevation of 5-HT_{1A} receptor binding in the prefrontal cortex, as had been reported in postmortem studies (Hashimoto et al 1993; Burnet et al 1996; Simpson et al 1996; Sumiyoshi et al 1996). The mean duration of untreated psychosis in our sample was 17 ± 22 months (range 1 month to 5 years) at the time of examination, whereas the patients in the postmortem studies had an average illness duration of approximately 20 years and all had a history of drug

treatment. Differences in sample populations, including medication and duration of illness, may account for the differences between our results and those of the previous postmortem studies.

One recent PET study reported the elevation of BP of [^{11}C]WAY-100635 in the medial temporal cortex of schizophrenia (Tauscher et al 2002). In contrast to that study, the mean duration of untreated psychosis in our sample was relatively short, including six schizophreniform patients with disease duration of only 1 to 4 months at the time of study entry. There was a possibility that the shorter duration of untreated psychosis of our sample affected the difference in receptor binding result. Further, schizophrenia is considered to be a complex and heterogeneous disorder that is characterized by a diversity of symptoms. Given that there was a relationship between 5-HT$_{1A}$ receptor binding and negative and depression/anxiety symptoms, differences in severity of symptoms, including negative and affective symptoms, may explain the difference in the receptor binding result. The relatively small number of patients in PET studies must also be considered as an important confounding factor.

Several lines of evidence have indicated that anxiety and depression could be related to functional alterations in the amygdala. The amygdala is believed to be involved in the acquisition and expression of emotional memories and the evaluation of the emotional content of stimuli (LeDoux and Muller 1997; Phelps and Anderson 1997). Abnormal activation in the amygdala has been shown during symptom provocation in anxiety disorders, and this activation was normalized after treatment with selective serotonin reuptake inhibitors (SSRIs) (Fredrikson and Furmark 2003). Previous studies suggest that emotional/stress-response systems that include the amygdala are abnormally activated in depressive subgroups (Drevets 2003).

The 5-HT$_{1A}$ receptors have been shown to have a relation with anxiety and depression. Our findings are in line with previous PET studies, showing that people with lower 5-HT$_{1A}$ receptor density are more likely to display higher levels of anxiety (Tauscher et al 2001) and also that patients with major depressive disorder are associated with a widespread reduction in 5-HT$_{1A}$ receptor binding (Drevets et al 1999; Sargent et al 2000). In the present study, we also found this relation to negative symptoms on five symptom scales, which might be multidimensional (Welham et al 1999) and include the affective component related to the emotional/stress-response systems that include the amygdala (Anand and Shekhar 2003). We did not find the relation between BP of the amygdala and the negative scale of PANSS. The PANSS negative scale includes not only affective but also cognitive and thinking components, such as abstract thinking and stereotyped thinking, which were not directly related to the emotional/stress-response systems. The inclusion of nonaffective components could result in the absence of correlation between BP of the amygdala and the negative scale of PANSS.

In animal models, it has been demonstrated that the stimulation of postsynaptic 5-HT$_{1A}$ receptors resulted in neuronal hyperpolarization and inhibition of neuronal activity (Pugliese et al 1998). It can be assumed that the decrease in function or density of postsynaptic 5-HT$_{1A}$ receptors in the amygdala may result in an excess of postsynaptic cell activity by reduced inhibitory effect of 5-HT$_{1A}$ receptors in the amygdala. This absence of inhibition in the amygdala may be related to depression/anxiety and the affective component of the negative symptoms. The present study supports the view that the 5-HT$_{1A}$ receptor can be a target for atypical drug action, and its ability to improve negative and affective symptoms may, in part, be mediated by agonistic activity on the 5-HT$_{1A}$ receptors in the amygdala.

Some of the methodological limitations of our study need to be recognized. The number of patients was small, raising the question of statistical power adequacy, and thus it cannot be ruled out that a larger study population might reveal a change in BP values in other regions, such as the hippocampal region and parietal/prefrontal cortical regions. Further, the majority of patients showed only a mild to moderate level of severity. Future work with larger patient populations will be necessary to discuss the issue of the degree of localized change of 5-HT$_{1A}$ receptors.

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ARTICLE

Estimation of the time-course of dopamine D_2 receptor occupancy in living human brain from plasma pharmacokinetics of antipsychotics

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Abstract

Although the kinetic profile of antipsychotics at dopamine D_2 receptor sites has been suggested to be important for antipsychotic action and dosing schedule, the kinetic profiles of the respective antipsychotic drugs in the brain have not yet been clearly defined. We aimed to estimate the time-course of dopamine D_2 receptor occupancy from plasma pharmacokinetics and the apparent in-vivo affinity parameter (ED_{50}; concentration required to induce 50% occupancy). Dopamine D_2 receptor occupancies and plasma concentrations of risperidone were measured in five patients with schizophrenia using positron emission tomography with [^{11}C]FLB 457. Measured dopamine D_2 occupancies were compared with those estimated from plasma kinetics and in-vivo ED_{50}. The time-course of dopamine D_2 receptor occupancy was simulated with altered plasma kinetics or apparent in-vivo affinity parameters of the drug. Mean half-life of dopamine D_2 receptor occupancy of risperidone was 80.2 h while that of the plasma concentration was 17.8 h. Dopamine D_2 receptor occupancy estimated from plasma pharmacokinetics and in-vivo ED_{50} was within 1 S.D. of the mean measured occupancy. When the ED_{50} value was changed to one-tenth and 10-fold, the simulated half-life of receptor occupancy changed to 117.6 h and 27.3 h respectively. Using plasma pharmacokinetics and in-vivo ED_{50}, the time-course of receptor occupancy could be calculated. Simulation of drug kinetics at receptors would provide useful information for the evaluation of antipsychotics.

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Key words: Antipsychotics, dopamine D_2 receptor, occupancy, pharmacokinetics, positron emission tomography.

Introduction

The application of positron emission tomography (PET) and single photon emission computed tomography (SPECT) to the receptor-imaging field has made it possible to measure the dopamine D_2 receptor occupancy with antipsychotic drugs (Bench et al., 1993; Bigliani et al., 1999; Farde et al., 1988, 1990). The clinical effect of antipsychotic drugs has been reported to be associated with a striatal dopamine D_2 receptor occupancy level higher than 70% (Kapur et al., 2000; Nordström et al., 1993).

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Relatively rapid kinetics of dopamine D_2 receptor occupancy with transient high occupancy were shown in some antipsychotics such as quetiapine (Kapur et al., 2000), and the kinetic profile at receptors has been suggested to represent an important profile of antipsychotic action (Kapur et al., 2000; Seeman and Tallerico, 1999; Suhara et al., 2002a). Plasma concentrations of antipsychotics have been relied upon as objective indicators of drugs in vivo, and plasma pharmacokinetics have been used for determining rational dosage regimens. On the other hand, significant dissociation of antipsychotic kinetics between plasma and the brain has been reported, and the conventional approach of relying on plasma elimination half-lives for dosing schedules of antipsychotics has been questioned (Tauscher et al., 2002). Although the kinetic profile at the dopamine D_2 receptor site has

been increasingly focused upon, those of the individual antipsychotic drugs still need to be clarified, and they can be expected to be of great value for dosing schedules in clinical situations as well as for drug developments. In addition, cortical regions have been suggested to be the important sites for antipsychotic action, especially for the so-called atypical antipsychotics (Lidow et al., 1998; Pilowsky et al., 1997).

We aimed to estimate the time-course of dopamine D_2 receptor occupancy by risperidone by a combination of the values of the present plasma pharmacokinetics and the in-vivo ED_{50} value (concentration required to induce 50% occupancy) calculated from our previous data; and to simulate the time-course of dopamine D_2 receptor occupancy induced by antipsychotics with different pharmacological profiles and changing pharmacokinetic and apparent in-vivo affinity parameters (ED_{50}).

Methods

Patients

Five male patients (age range 24–45 yr; mean ± S.D., 35.2 ± 9.6 yr) meeting the DSM-IV criteria for schizophrenia participated in this study. The patients were recruited from the outpatient units of Tokyo Medical and Dental University affiliated psychiatric hospitals in the Tokyo and Chiba prefectures in Japan. They had received risperidone for more than 7 months without other medication. Four were maintained on 4 mg and one on 6 mg risperidone once every night.

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

Radioligand

The precursors for the synthesis of [^{11}C]FLB 457 were kindly supplied by Astra Arcus (Sodertalje, Sweden). [^{11}C]FLB 457 was synthesized by *O*-methylation of the corresponding precursors with [^{11}C]methyliodide with high specific radioactivity, which was obtained by a reduction of [^{11}C]CO_2 with $LiAlH_4$ in an inert atmosphere with specially designed equipment (Halldin et al., 1995; Suzuki et al., 1999). The radiochemical purities were more than 95%.

PET procedure

Dynamic scans were performed for 80 min using ECAT EXACT HR+ (CTI-Siemens, Knoxville, TN, USA) immediately after the bolus injection of 155.0–238.7 (mean ± S.D., 207.7 ± 28.5) MBq of [^{11}C]FLB 457 with high specific radioactivities (139.3–394.8 GBq/μmol; mean ± S.D., 237.6 ± 69.5 GBq/μmol).

MRIs were acquired on Gyroscan NT (Phillips Medical Systems, Best, The Netherlands) (1.5 T) to obtain T1-weighted images of the brain.

On the day before the first PET scan (day 0), the patients stopped taking risperidone that night. On the day of the first PET scan (day 1), the patients took breakfast around 07:00 hours and took their usual daily dose of risperidone orally at 10:00 hours. The first PET scan was performed at 15:00 hours (5 h post-risperidone). The second PET scan was performed at 10:00 hours on the next day (day 2) (24 h post-risperidone). The third PET scan was performed at 15:00 hours on the following day (day 3) (53 h post-risperidone). Blood samples were taken to measure the concentrations of risperidone and 9-OH-risperidone just before and after each PET scan.

Data analysis

All emission scans were reconstructed with a Hanning filter cut-off frequency of 0.4. The temporal cortex, the area for calculating ED_{50} in the previous study (Yasuno et al., 2001), was chosen for the region of interest (ROI). Following the previous study (Yasuno et al., 2001), circular ROIs were set at 8 mm diameter to cover 7 slices for the cerebellum and 10–11 slices for the temporal cortex on the PET images of summated activity for 80 min with reference to the individual MR images and Brain Atlas. The average values of right and left ROIs were used to increase the signal–noise ratio for the calculations. Quantification was performed using a three-parameter simplified reference tissue model (Lammertsma, 1996). The cerebellum was used as the reference tissue because of its negligible density of dopamine D_2 receptors (Suhara et al., 1999). This model allows the estimation of binding potential (BP), which was defined as the ratio of receptor density (B_{max}) to dissociation constant (K_d).

The occupancy of risperidone at the dopamine D_2 receptor was estimated using the following equation:

$$Occ = (BP_{baseline} - BP_{drug}) \times 100/BP_{baseline},$$

where Occ is the receptor occupancy, $BP_{baseline}$ is the BP in the drug-free state, and BP_{drug} is the BP of the patient on the drug. In this study, because we could not perform PET scans in a drug-free state for four of the patients, the age-corrected mean BP in the temporal cortex ($BP = -0.0245 \times age + 2.474$) of 11 drug-naive patients with schizophrenia (age range 19–40 yr;

Table 1. Characteristics of the patients, the time-course of dopamine D_2 receptor occupancy, and the half-life of plasma concentration and dopamine D_2 receptor occupancy of risperidone (Ris.)

Patient no.	Age (yr)	Ris. (mg/d)	D_2 receptor occupancy (%)			Half-life (h)	
			5 h	24 h	53 h	Plasma concentration	D_2 receptor occupancy
1	24	4	81.1	55.6	36.5	11.5	45.5
2	39	4	69.1	62.6	41.1	16.9	62.0
3	42	4	86.2	71.7	57.2	17.7	73.8
4	45	4	87.7	82.6	70.9	23.7	127.3
5	26	6	85.6	77.3	62.8	19.3	92.2
Mean (±S.D.)	35.2 (±9.6)	4.4 (±0.9)	82.0 (±7.6)	70.0 (±10.9)	53.7 (±14.5)	17.8 (±4.4)	80.2 (±31.4)

mean ± S.D., 28.1 ± 7.9 yr) reported in our previous study (Suhara et al., 2002b), was used as $BP_{baseline}$. Individual $BP_{baseline}$ was used for one patient with 4 mg risperidone (patient 2 in Table 1), who had been drug-free for 15 months at the time of the baseline PET scan. Dopamine D_2 receptor occupancies at three time-points were fitted to a linear regression, that can be described by

$$y = o + a \times t,$$

where o is the estimated maximal receptor occupancy at 0 h (Tauscher et al., 2002). Time to reach half of the estimated maximal receptor occupancy was defined as $T_{\frac{1}{2}}$ of receptor occupancy. The R^2 values of linear regression analysis ranged from 0.95 to 0.99.

Plasma concentration of risperidone

The plasma concentrations of risperidone and its active metabolite, 9-OH-risperidone, were determined by HPLC, and their sum was used as the plasma concentration of risperidone because they both have a similar pharmacological profile (Dollery, 1999). The range of the observation time (5–53 h) was considered to equal the elimination phase of the drug, since the plasma concentration of risperidone was reported to reach a peak within 2 h of its oral administration (Dollery, 1999), and oral absorption of risperidone was reportedly not significantly affected by food (Dollery, 1999). The time-course of the plasma concentration was fitted to one-exponential function (Gefvert et al., 1998; Tauscher et al., 2002). The time required to reach half of the plasma concentration of risperidone was defined as $T_{\frac{1}{2}}$ of plasma concentration. The R^2 values of the exponential fitting ranged from 0.95 to 0.99.

Simulation study

A simulation study was performed to estimate the time-course of dopamine D_2 receptor occupancy from the plasma pharmacokinetics and in-vivo ED_{50} value.

The relationship between dopamine D_2 receptor occupancy and plasma concentration of antipsychotics was expressed by the following equation (Fitzgerald et al., 2000; Kapur and Remington, 1996):

$$D_{2,occ} = 100 \times D/(ED_{50} + D), \quad (1)$$

where $D_{2,occ}$ is dopamine D_2 receptor occupancy, D is the concentration of the drug in proximity to the dopamine D_2 receptor, and ED_{50} is the concentration required to induce 50% occupancy.

The decrease in plasma concentration was expressed by the following equation (Gefvert et al., 1998; Tauscher et al., 2002):

$$C = me^{-bt}, \quad (2)$$

where C is the plasma concentration, m is the estimated maximal plasma concentration at 0 h, b is a constant, and t is the time after the drug administration.

Plasma concentration was used as a functional surrogate of D (Fitzgerald et al., 2000; Kapur and Remington, 1996). Therefore $C = D$.

Combining equations (1) and (2),

$$D_{2,occu} = 100 \times me^{-bt}/(ED_{50} + me^{-bt}), \quad (3)$$

The ED_{50} value in the temporal cortex calculated from our previous data was 6.4 ng/ml (Yasuno et al., 2001).

The plasma concentrations of the five patients were averaged at each time-point and the mean plasma

concentration was fitted to one-exponential function and used in this simulation:

$$\text{plasma concentration (ng/ml)} = 45.0 \times e^{-0.036t} \quad (R^2 = 0.969)$$

($T_{\frac{1}{2}}$ of the mean plasma concentration = 19.3 h).

Dopamine D_2 receptor occupancy derived from this equation with mean plasma concentration was compared to the mean of occupancy calculated from the present consecutive PET data. In addition, dopamine D_2 receptor occupancy estimated from individual plasma data was also compared to measured occupancy from individual PET data by repeated-measures ANOVA.

The time-course of dopamine D_2 receptor occupancy was simulated by varying the ED_{50} value of risperidone from one-tenth to 10-fold (0.1, 0.2, 0.5, 2, 5, 10) of 6.4 ng/ml with fixed plasma kinetics (plasma concentration $= 45.0 \times e^{-0.036t}$). The time-course of dopamine D_2 receptor occupancy was also simulated by varying $T_{\frac{1}{2}}$ of the plasma concentration from one-tenth to 5-fold (0.1, 0.2, 0.5, 2, 5) of $T_{\frac{1}{2}}$ of the mean plasma concentration of five patients with fixed maximal plasma concentration (45.0 ng/ml) and ED_{50} value (6.4 ng/ml). The time required to reach half of the receptor occupancy from simulated time 0 was defined as simulated $T_{\frac{1}{2}}$ of receptor occupancy.

Results

The half-life ($T_{\frac{1}{2}}$) of dopamine D_2 receptor occupancy of the five patients varied from 45.5 to 127.3 h, with a mean value of 80.2 ± 31.4 h (Table 1). The $T_{\frac{1}{2}}$ of the plasma concentration of risperidone was 11.5–23.7 h, with a mean value of 17.8 ± 4.4 h (Table 1). The patient with the shortest $T_{\frac{1}{2}}$ of dopamine D_2 receptor occupancy had the shortest $T_{\frac{1}{2}}$ of plasma concentration, and the patient with the longest $T_{\frac{1}{2}}$ of dopamine D_2 receptor occupancy had the longest $T_{\frac{1}{2}}$ of plasma concentration (Table 1). Figure 1 shows the time-courses of dopamine D_2 receptor occupancy and plasma concentration of patient 3 with the nearest $T_{\frac{1}{2}}$ to the mean value.

The estimated dopamine D_2 receptor occupancies from the mean plasma pharmacokinetics of the five patients (plasma concentration (ng/ml) = $45.0 \times e^{-0.036t}$) and the in-vivo ED_{50} value (6.4 ng/ml) were 85.4% at 5 h, 74.6% at 24 h and 50.5% at 53 h, which were within 1 S.D., respectively, of the mean dopamine D_2 receptor occupancies of the five patients (82.0 ± 7.6% at 5 h, 70.0 ± 10.9% at 24 h and 53.7 ± 14.5% at 53 h).

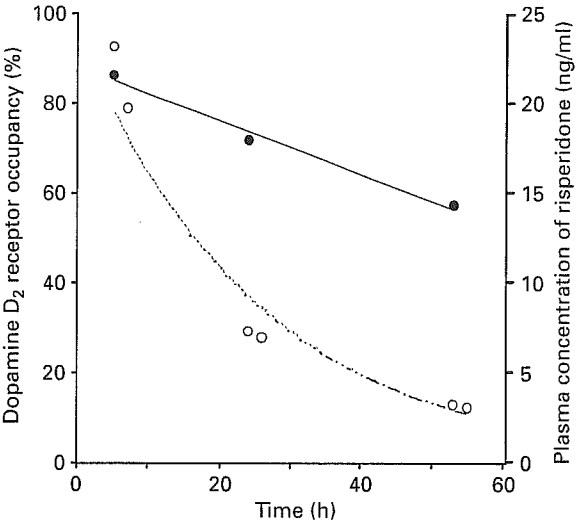

Figure 1. Time-course of dopamine D_2 receptor occupancy in the temporal cortex (●) and the plasma concentrations (○) after taking 4 mg risperidone (patient 3). The sum of the plasma concentrations of risperidone and 9-OH-risperidone was used as the plasma concentration of risperidone. The $T_{\frac{1}{2}}$ of plasma concentration (17.7 h) was shorter than that of dopamine D_2 receptor occupancy (73.8 h).

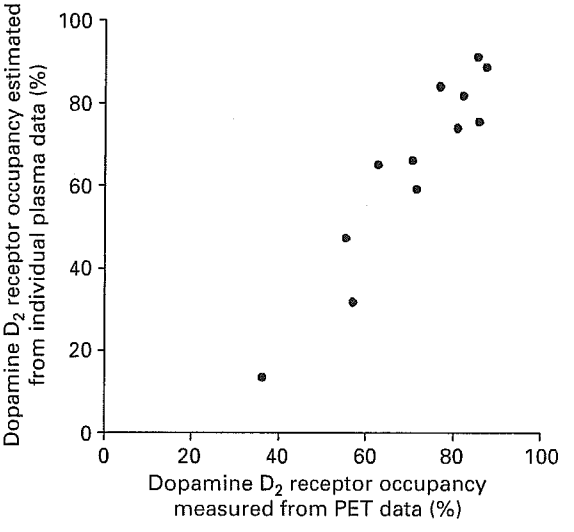

Figure 2. The relationship between the dopamine D_2 receptor occupancy measured from PET data and the dopamine D_2 receptor occupancy estimated from individual plasma data.

Figure 2 shows the relationship between the estimated occupancies from individual plasma data and the individual measured dopamine D_2 receptor occupancies. The estimated occupancies were not significantly different from the measured occupancies ($p > 0.05$).

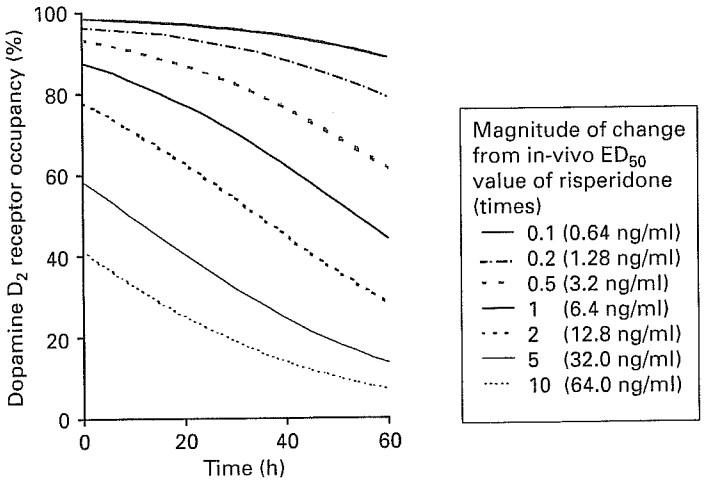

Figure 3. Effect of ED_{50} value change on the time-course of simulated dopamine D_2 receptor occupancy. Values are the magnitudes of change from the ED_{50} value of risperidone (6.4 ng/ml). Estimated dopamine D_2 receptor occupancy increased and the slope of the curves became gentler as the ED_{50} value became smaller.

In the simulation study, when the ED_{50} value was changed to one-tenth (0.64 ng/ml) with fixed plasma pharmacokinetics [plasma concentration (ng/ml) = $45.0 \times e^{-0.036t}$], the simulated dopamine D_2 receptor occupancy was 98.6% at 0 h and 91.0% at 53 h, and the simulated $T_{\frac{1}{2}}$ of dopamine D_2 receptor lengthened to 117.6 h (Figure 3). When ED_{50} was increased to 10-fold (64 ng/ml) with fixed plasma pharmacokinetics, the simulated dopamine D_2 receptor occupancy became 41.3% at 0 h, and 9.3% at 53 h, and the simulated $T_{\frac{1}{2}}$ of dopamine D_2 receptor occupancy shortened to 27.3 h (Figure 3). The simulated $T_{\frac{1}{2}}$ of the time-course of dopamine D_2 receptor occupancy became longer as ED_{50} became smaller, and vice versa.

When the $T_{\frac{1}{2}}$ of the plasma concentration was changed to one-tenth (1.9 h) with fixed ED_{50} (6.4 ng/ml) and the estimated maximal plasma concentration, the simulated dopamine D_2 receptor occupancy was 87.6% at 0 h and 0% at 53 h, and the simulated $T_{\frac{1}{2}}$ of dopamine D_2 receptor occupancy shortened to 6.0 h. When the $T_{\frac{1}{2}}$ of the plasma concentration was changed to 5-fold (96.3 h), the simulated dopamine D_2 receptor occupancy became 87.6% at 0 h and 82.7% at 53 h, and the $T_{\frac{1}{2}}$ of dopamine D_2 receptor occupancy lengthened to 302.2 h.

Discussion

In this study, we demonstrated that the time-course of dopamine D_2 receptor occupancy by various antipsychotics could be estimated from the combination of the plasma pharmacokinetics data and the apparent in-vivo affinity parameter (ED_{50}). The estimated time-course of dopamine D_2 receptor occupancy from the mean pharmacokinetics data and the in-vivo ED_{50} value fitted well with the data from the consecutive PET scans of our patients. Since consecutive PET scans for each antipsychotic drug are not readily performed in routine clinical situations, this estimation of the time-course of dopamine D_2 receptor occupancy from plasma pharmacokinetics, with separately measured apparent in-vivo affinity parameter, would be of great value in the clinical setting in terms of both dosing schedule, and drug development and evaluation. For example, the in-vivo ED_{50} value of striatal D_2 receptor occupancy by haloperidol was reported to be approx. 0.51 ng/ml using [^{11}C]raclopride (Kapur et al., 1997). Although data concerning the time-course of dopamine D_2 receptor occupancy by haloperidol is limited, striatal D_2 receptor occupancy in one volunteer was reported to be 92% at 3 h and 76% at 27 h after single oral administration of 7.5 mg (Nordström et al., 1992). From the reported data (Nordström et al., 1992), the $T_{\frac{1}{2}}$ of the plasma concentration of haloperidol was estimated to be approx. 13 h. Using our equation, the simulated dopamine D_2 receptor occupancy by the oral administration of 7.5 mg haloperidol would be 95% at 3 h and 84% at 27 h. Quetiapine, an antipsychotic drug with low affinity for dopamine D_2 receptor, was reported to show 64% occupancy at 2 h after 450 mg of oral administration, and almost no occupancy on striatal D_2 receptor at 24 h (Gefvert et al., 2001; Kapur et al., 2000). The in-vivo ED_{50} value of quetiapine has not been investigated thoroughly, but it was estimated

Table 2. Reported (Rep.) time-course of dopamine D_2 receptor occupancy and estimated (Est.) occupancy values

Drug	Dose (mg)	in-vivo ED_{50} of drug (ng/ml)	Plasma $T_{\frac{1}{2}}$ of drug (h)	Dopamine D_2 receptor occupancy (%)			
					2–3 h	1 day	2 days
Haloperidol	7.5[a]	0.51[b]	13[a]	Rep.	92[a]	76[a]	–
				Est.	95	84	–
Quetiapine	450[c]	330–770[d]	3[c]	Rep.	64[c]	0[c]	–
				Est.	69–83	1.3–1.5	–
Risperidone	3[e]	6.87[f]	19.5[e]	Rep.	–	72±9[e]	47±16[e]
				Est.	–	65	45
Olanzapine	15[e]	10[g]	20.9[e]	Rep.	83[e]*	78[e]	61[e]
				Est.	86*	77	60

– Indicates that the data was not available.

[a] Data from Nordström et al. (1992); [b] data from Kapur et al. (1997); [c] data from Gefvert et al. (1998); [d] data from Kapur et al. (2000); [e] data from Tauscher et al. (2002); [f] data from Nyberg et al. (1999); [g] data from Kapur et al. (1999).

* Data at 6 h after drug administration.

to be in the range of 330–770 ng/ml (Kapur et al., 2000), and the $T_{\frac{1}{2}}$ of plasma concentration was estimated to be approx. 3 h (Gefvert et al., 2001). The simulated dopamine D_2 receptor occupancy was calculated to be 69–83% at 2 h and 1.3–1.5% at 24 h. Thus, the transient high occupancy reported for the clinical dose of quetiapine can be simulated with its affinity parameter and pharmacokinetics data. Using the reported plasma concentration data from a discontinuation experiment with 3 mg risperidone (Tauscher et al., 2002), the $T_{\frac{1}{2}}$ of mean plasma concentration of risperidone was estimated to be 19.5 h. The striatal D_2 receptor occupancy can be simulated with the reported in-vivo ED_{50} value (6.87 ng/ml) (Nyberg et al., 1999). The simulated value was 65% at 24 h and 45% at 48 h, which was within 1 S.D. of the reported values from the PET measurements (72±9% at 24 h and 47±16 at 48 h) (Tauscher et al., 2002). Although variations in plasma data and in-vivo ED_{50} values can result in deviations in the results of the estimation, the estimated values seemed to be consistent with the reported clinical results (Table 2).

The present results indicated that dopamine D_2 receptor occupancy by risperidone remained high even after the plasma concentration had decreased. This was consistent with a recent report that the kinetics of dopamine D_2 receptor occupancy in the brain and the plasma pharmacokinetics of antipsychotics are dissociated (Tauscher et al., 2002). The dissociation of plasma pharmacokinetics and receptor occupancy was also shown in a dopamine D_1 receptor occupancy study with the dopamine D_1 receptor antagonist NNC 756 (Karlsson et al., 1995). Our simulation method would be useful for investigating the pharmacodynamics of various drugs with specific binding. In this study we used the ED_{50} value measured in vivo because a disparity between in-vivo and in-vitro dissociation rates under different conditions has been reported; environmental factors such as temperature and incubation time can affect the in-vitro data of receptor binding (Kapur et al., 2001; Kessler et al., 1993). Thus, although direct comparative affinity parameters for FLB 457 between in vitro and in vivo were not available, the in-vivo ED_{50} value would be more reliable for estimating the time-course of receptor occupancy in the living human brain.

Several confounding factors must be noted in the present study. First, dopamine D_2 receptor occupancy was calculated using age-corrected mean BP values of other drug-naive patients with schizophrenia as baseline. The absence of the patients' own baseline values introduced a potential error. The coefficient of variance of dopamine D_2 binding potential in the temporal cortex was reported as approx. 15% in schizophrenia using [^{11}C]FLB 457 (Suhara et al., 2002b). If the BP value at baseline were changed by 15%, the occupancy in this study would change from −11% to +8.3%. Secondly, we measured only three time-points to evaluate the time-course of receptor occupancy and six time-points for the plasma pharmacokinetics, and linear regression was used to estimate the half-life of dopamine D_2 receptor occupancy. Obviously, the use of more time-points would be additionally favourable for a more precise fitting. But as shown in Figure 3, as the time-course of dopamine D_2 receptor occupancy was not an exponential or a linear function, the

meaning of the $T_{\frac{1}{2}}$ of receptor occupancy was not equal to that of plasma. Thirdly, in our study we started to measure occupancy and plasma concentration 5 h after oral administration. Although this could simplify the estimation to the elimination phase, estimation of the total kinetics including the absorption phase is needed for more detailed results. Fourthly, in the simulation study we assumed that the same mathematical model could be applied regardless of the degree of in-vivo affinity. However, discussions concerning the effect of the in-vivo environment such as endogenous transmitters on the binding of drugs with different characteristics have been reported (Farde et al., 1990; Seeman and Tallerico, 1999). Fifthly, [^{11}C]FLB 457 has high affinity for both dopamine D_2 and dopamine D_3 receptors in vitro (Halldin et al., 1995). Although distinct anatomical localization has been reported for dopamine D_3 receptors (Hall et al., 1996), further study will be necessary to determine the contribution of dopamine D_3 receptor binding in the human temporal cortex.

In conclusion, the time-course of dopamine D_2 receptor occupancy by risperidone can be estimated from plasma pharmacokinetics and the in-vivo ED_{50} value, and this estimated relationship would be applicable to other antipsychotics. Moreover, the estimation of drug kinetics at the receptor site would undoubtedly provide information useful for the evaluation of new antipsychotics.

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Statement of Interest

None.

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