Decreased Dopamine D₂ Receptor Binding in the Anterior Cingulate Cortex in Schizophrenia

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Background: The clinical efficacy of dopamine D₂ receptor antagonism on the psychotic symptoms of schizophrenia has been widely demonstrated. However, most in vivo imaging studies have not been able to detect significant changes in striatal D₂ receptors in schizophrenia. On the other hand, a number of studies have reported abnormalities in the cerebral cortex of schizophrenia. The aim of this study was to examine the extrastriatal D₂ receptors of patients with schizophrenia.

Methods: Eleven drug-naive male patients with schizophrenia were examined with positron emission tomography using carbon 11–labeled FLB 457. Symptoms were assessed using the Brief Psychiatric Rating Scale. Eighteen healthy controls were used for comparison. Region-of-interest analysis was performed using the reference tissue method, and binding potential (BP) was used for the index of dopamine D₂ receptor binding.

Results: The BP value was significantly lower, by about 12.5%, in the anterior cingulate cortex in drug-naive patients with schizophrenia than in healthy controls. A significant negative correlation was observed between BP in the anterior cingulate cortex and the positive symptom score on Brief Psychiatric Rating Scale.

Conclusions: The lower BP values indicate fewer D₂ receptors in the anterior cingulate cortex in patients with schizophrenia. Alterations in D₂ receptor function in the extrastriatal region may underlie the positive symptoms of schizophrenia.

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Schizophrenia is believed to involve altered dopaminergic transmission. This is supported by the fact that long-term use of dopamine agonists such as amphetamine can cause psychotic symptoms and dopamine D₂ receptor antagonists are the most widely used drugs for the treatment of schizophrenia. The symptoms of schizophrenia are usually subdivided into positive and negative symptoms. Positive symptoms such as hallucinatory behavior and unusual thought content are more effectively treated with D₂ receptor blockers than are negative symptoms. The therapeutic potency of antipsychotic drugs correlates with the affinity for D₂ receptors. These observations imply that D₂ receptors are critically involved in the pathophysiology of positive symptoms.

Increased density of striatal D₂ receptors has been reported in several postmortem studies. However, most in vivo imaging studies with drug-free or drug-naive patients could not detect a significant increase in striatal D₂ receptors. On the other hand, neuropathological studies have demonstrated abnormalities in the extrastriatal regions of schizophrenia. Moreover, an increasing body of evidence favors the crucial role of extrastriatal regions in the pathophysiology of positive symptoms, and extrastriatal D₂ receptors are thought to be common sites of action of antipsychotic drugs. We have recently reported the abnormality of dopamine D₁ receptor binding in the prefrontal cortex of patients with schizophrenia and its relation to negative symptoms. In this study, we performed positron emission tomography (PET) scans using carbon 11–labeled FLB 457 to examine extrastriatal D₂ receptors and their relation to clinical symptoms in drug-naive patients with schizophrenia.

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RESULTS

The multivariate analyses of BP values in the extrastriatal regions indicated a significant group difference between patients with...
SUBJECTS AND METHODS

SUBJECTS

Eleven drug-naïve male schizophrenic patients with a mean±SD age of 28.1±7.9 years who met the DSM-IV criteria for schizophrenia or schizoaffective disorder were studied. Those with schizophreniform disorder at study entry met the criteria for schizophrenia at the 6-month follow-up. Exclusion criteria were current or past substance abuse and a history of alcohol-related problems, mood disorders, organic brain disease, and antipsychotic or antidepressant medication use. Initial diagnoses were made by the physicians in charge (Y.O., Y.N., K.N., or others), second diagnoses were made by secondary physicians (T.S. and F.Y. or M.I. and another physician) on the day of the PET scan with reference to the written history of the present illness by the physician in charge, and final diagnoses were made by 2 of the secondary physicians (T.S. and F.Y.) several months after the PET scan with reference to the follow-up information from the physicians in charge. All the physicians involved in these steps and procedures were psychiatrists. The patients were recruited from the outpatient units of 5 university-affiliated psychiatric hospitals and the psychiatric divisions of general hospitals in Tokyo and Chiba prefecture in Japan. The average±SD onset age was 25.8±8.4 years (range, 14.0-38.0 years), and the duration of illness ranged from 1 month to 8 years (average, 2.1 years). Psychopathology was assessed by the 18-item Oxford version of the Brief Psychiatric Rating Scale (BPRS) translated into Japanese (item score range, 0-6 points). The BPRS scores completed by 2 of the authors (T.S. and F.Y.) and 1 other psychiatrist. The ratings were reviewed by these 2 authors (T.S. and F.Y.) and 1 other psychiatrist after the patient interview, and disagreements were resolved by consensus; the consensus ratings were then used in this study. Positive and negative symptom scores were calculated as the sum of the following items. Positive symptom subscales were conceptual disorganization, mannerisms and posturing, hostility, grandiosity, suspiciousness, hallucinatory behavior, unusual thought content, and excitement. Negative symptom subscales were emotional withdrawal, motor retardation, and blunted affect.

Eighteen healthy male subjects (mean±SD age, 27.3±6.2 years) were recruited as controls from among university students and hospital employees. The controls were free of any criteria for neuropsychiatric disorders and had no relatives with neuropsychiatric disorders based on unstructured psychiatric screening interviews. Subjects were examined by magnetic resonance imaging (MRI) to rule out any organic brain diseases. After description of the study to the subjects, written informed consent was obtained from all patients and healthy subjects. This study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan.

IMAGE ACQUISITION AND ANALYSIS

A scanner system (ECAT EXACT HR+; CTI-Siemens, Knoxville, Tenn) was used to observe the radioactivity. The system provides 63 planes and a 15.5-cm field of view. To minimize head movement, a head fixation device (Fixster, Stockholm, Sweden) was used. A transmission scan for attenuation correction was performed using a germanium 68-gallium 68 source. Acquisitions were done in 3-dimensional mode with the interplane septa retracted. A bolus of 108.4 to 232.4 MBq (mean±SD, 169.7±36.6 MBq) of [11C]FLB 457 with high specific radioactivities (64.9-483.2 GBq/µmol) was injected intravenously from the antecubital vein with a 20-ml saline flush. Dynamic scans were performed for 80 minutes immediately after the injection.

The MRIs were acquired on Gyroscan NT (Philips Medical Systems, Best, the Netherlands) (1.5 T). T1-weighted images of the brain were obtained for all subjects except 3 patients who refused to participate in the MRI scan. The scan parameters were 1-mm-thick, 3-dimensional

schizophrenia and healthy controls (F1,70 = 2.60; P = .04) with no significant interaction between group and age (F1,70 = 0.52; P = .81). Follow-up univariate ANCOVA revealed that BP values in the anterior cingulate cortex were significantly lower in patients with schizophrenia compared with healthy controls (F1,26 = 6.40; P = .02) (Table 1 and Figure 2). A significant negative correlation was observed between BP values and clinical rating for positive symptom score on the BPRS in the anterior cingulate cortex (r = .81, P = .002) (Table 2 and Figure 3). There was no significant correlation between BP and clinical rating for negative symptom score in any of the brain regions. No significant correlations were observed between BP values and age at onset or duration of illness.

The R1 value in the anterior cingulate cortex, which is the ratio of the delivery in the anterior cingulate cortex to that in the cerebellum (ratio of influx), did not differ significantly between the patients (mean±SD, 0.90±0.09) and the controls (mean±SD, 0.94±0.06) by ANCOVA with age as the covariate (F1,26 = 2.20; P = .15).

The volume of the anterior cingulate cortex was measured from the slice where the genu of the corpus callosum appeared to the slice where the corpus callosum was no longer seen. The relative volume (mean±SD) of the anterior cingulate cortex was 0.25%±0.05% in patients with schizophrenia and 0.27%±0.04% in healthy controls. No significant difference was observed between patients and controls, using ANCOVA with age as covariate (F1,23 = 1.54; P = .23). The intraclass correlation coefficient was 0.96 for intracranial volume and 0.89 for the anterior cingulate cortex.

Comment

The present study indicated that the BP values for [11C]FLB 457 binding were significantly lower in the anterior cingulate cortex of the patients with schizophrenia than those in healthy controls.

Several confounding factors need to be considered, including the selectivity of FLB 457, reliability of the reference tissue, change in blood flow, partial volume effect, and the effect of endogenous dopamine. Although FLB 457 has affinity for both dopamine D2 and D3 receptors, the distinct anatomic distribution of D3 receptors supports the
T1 images with a transverse plane (repetition time/echo time, 19/10 milliseconds; flip angle, 30°; scan matrix, 256 × 256 pixels; field of view, 256 × 256 mm; and number of excitations, 1).

All emission scans were reconstructed with a Hann- ing filter cutoff frequency of 0.4 (full width at half maximum, 7.5 mm). The tissue concentration of radioactivity was obtained from 8 regions of interest (ROIs) defined on the PET images of summed activity for 80 minutes with reference to the available individual MRIs and brain atlas. Because of technical reasons and the lack of the 3 MRI data, the coregistration of the MRI on the PET image was not ready for use in this study. The regions were the anterior cingulate cortex, prefrontal cortex, temporal cortex, thalamus, parietal cortex, occipital cortex, hippocampus, and cerebellar cortex. Although [11C]FLB 457 accumulates to a high degree in the striatum, high-affinity ligands show very slow clearance from high-density receptor regions, where radioligand delivery can be rate limiting. 13 In this study, we did not evaluate the striatal data since [11C]FLB 457 is not a suitable ligand for quantitative analysis of the striatum. 10,11

Circular ROIs were set at 8 mm diameter to cover 7 slices for the cerebellum; 6 slices for the thalamus; 10 to 11 slices for the anterior cingulate cortex, prefrontal cortex, temporal cortex, and occipital cortex; 8 to 9 slices for the parietal cortex; and 4 to 5 slices for the hippocampus (Figure 1). Two investigators (F.Y. and Y.S.), masked to the identity of the scans, agreed on the ROI positions. The average values of right and left ROIs were used to increase the signal-to-noise ratio for the calculations.

Quantitative tracer kinetic modeling was performed using a 3-parameter (simplified) reference tissue compartmental model. 16,19 The cerebellum was used as the reference tissue because it is nearly devoid of D2 receptors. 13,19 The model allows the estimation of binding potential (BP), which is defined as follows: BP = f1Bmax/[Kd (1 + S F/Kd)], where f1 is the free fraction of unbound radioligand, Bmax is the density of receptor, Kd is the dissociation constant for the radioligand, and F and Kd are the free concentration and the dissociation constant of i competing ligands, respectively. The model also allows the estimation of R1, which is the ratio of the delivery in the ROI tissue to that in the reference region (ratio of influx). 16

Neuroradiometric measurement was carried out for consideration of the partial volume effect on the region where a significant difference was observed compared with the controls. Morphologic analysis was performed on MRI data of 8 patients with schizophrenia and 18 healthy controls using National Institutes of Health image software (National Institutes of Health, Research Service Branch, Bethesda, Md). Gray matter was manually traced on every other slice, separating it from the cerebrospinal fluid and white matter by using the thresholding technique available in the image software. The size of the area across the slices was summed and multiplied by the slice thickness (2 mm) to obtain the approximate volume. After correction of differences in head size by dividing the measured volume by intracranial volume, the relative volumes (measured volume/intracranial volume × 100) were used for the analysis. 19

STATISTICAL ANALYSIS

Group differences in BP values of the extrastriatal regions between patients and controls were compared using a multivariate analysis of covariance with all the brain regions as dependent variables. Age was a covariate since there was an age effect on BP in the regions. 21 Follow-up serial 1-way analysis of covariance (ANCOVA) with age as covariate was performed to specify the regional differences. P = .05 (2-tailed) was chosen as the significance threshold. The relationship between regional BP values adjusted for age and positive and negative symptom scores of the patients were evaluated using the Pearson correlation method. For correlation analysis, we used Bonferroni correction for the P level for 7 variables (P < .007 [.05/7]).

Several lines of evidence have indicated that the positive symptoms of schizophrenia could be related to the hyperdopaminergic state, and worsening of positive symptoms by amphetamine challenge was reported to be positively correlated with the magnitude of dopamine release. 22 As shown in the present study, the BP values in the anterior cingulate cortex correlated negatively with the positive symptom score, ie, a higher dopamine concentration might be expected in the anterior cingulate cortex when the binding of [11C]FLB 457 is low. This could be explained by the direct competition with increased endogenous dopamine. 23 But the detectability of dopamine release in the extrastriatal regions is controversial. 24 and the susceptibility of [11C]FLB 457 to endogenous dopamine has not yet been examined. Our monkey experiments indicated that the extrastriatal [11C]FLB 457 binding was not sensitive to endogenous dopamine. We observed that [11C]FLB 457 binding in the cortex and thalamus was not significantly affected by 1 mg of intravenous methamphetamine challenge, whereas the striatal binding of [11C]clozapine was decreased by more than 20%. 25 Therefore, providing that the affinity did not differ, our finding might

view that [11C]FLB 457 binding in the anterior cingulate cortex represents binding to D2 receptors. 13,19 The functional abnormality of the cerebellum in schizophrenia might hamper its use as a reference, but the cerebellar time activity curves did not differ significantly between patients and controls when the radioactivity was adjusted for injected dose; there was no main effect of group or group-by-time interaction with repeated-measures analysis of variance with Greenhouse-Geisser correction (group F1,26 = 0.02; P = .88; group-by-time interaction F2.13,55.47 = 0.34; P = .88). Patients with schizophrenia may have alterations in regional cerebral blood flow. 26,27 However, the reduction of BP is unlikely to be an effect of altered blood flow, since the R1 value (ratio of the delivery) did not differ significantly between the patients and controls. In addition, the BP values are minimally dependent on tracer delivery over the average of R1 values obtained in this study. 18 Although atrophy can affect the BP value, no significant difference was observed in the volume of the anterior cingulate cortex between 8 patients and controls. Therefore, the alteration in gross brain anatomy is less likely to be responsible for the reduction of BP.
be attributable to the lower D2 receptor density in the anterior cingulate cortex. A reduction in the number of available receptors might occur as a result of an increased concentration of endogenous dopamine.26 On the other hand, the reduced concentration of cortical D2 receptors in schizophrenia has also been discussed in relation to abnormal brain development.2 In any case, the present data do not allow us to determine the onset of the reduction.

Cellular localization of dopamine D2 receptors has been demonstrated on both pyramidal neurons and nonpyramidal interneurons, which use γ-aminobutyric acid (GABA) as an inhibitory transmitter.27,28 Based on the finding of the deficit of inhibitory interneurons in the anterior cingulate cortex,3 less GABAergic inhibitory transmission has been proposed in the cortical local circuit of schizophrenic brain.3,4 It has been demonstrated by animal studies that dopamine release in the prefrontal cortex can be regulated by GABA interneurons that have D2 receptors.29 Although the binding of [11C]FLB 457 cannot discriminate D2 receptors on different types of neurons, the reduction of D2 receptors might represent an altered regulatory function of interneurons.

Antipsychotic treatment is reported to change the activity in the anterior cingulate cortex in schizophrenia30,31 and increase the D2 receptor density in the rat medial prefrontal cortex.32 Antipsychotic drugs may act on pyramidal neurons in both a direct and an indirect manner. Direct effects may be mediated by blocking D2 receptors on pyramidal neurons, whereas indirect effects might be regulated through interneurons.

The anterior cingulate cortex has also been noted in the pathophysiology of positive symptoms. Significant activation is observed during auditory verbal hallucinations.9-10 The role of the anterior cingulate cortex in executive function, selective attention, and error detection13 may suggest a contribution to conceptual disorganization and hallucinatory behavior.34 The dysfunction of the anterior cingulate cortex in schizophrenia has been suggested to be linked to the dysfunction of dopaminergic transmission because neural response to a cognitive activation of the anterior cingulate cortex was significantly modulated by a manipulation of dopaminergic transmission in schizo-
Table 2. Pearson Correlation Coefficients Between Carbon 11–Labeled FLB 457 Binding Potential Values and Positive and Negative Symptom Scores on the Brief Psychiatric Rating Scale*  

<table>
<thead>
<tr>
<th>Regions</th>
<th>Positive Symptom Score</th>
<th>Negative Symptom Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>−0.81</td>
<td>0.002†</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>−0.59</td>
<td>0.06</td>
</tr>
<tr>
<td>Temporal cortex</td>
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<td>0.35</td>
</tr>
<tr>
<td>Parietal cortex</td>
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<td>0.19</td>
</tr>
<tr>
<td>Occipital cortex</td>
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<td>0.45</td>
</tr>
<tr>
<td>Hippocampus</td>
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<td>0.72</td>
</tr>
<tr>
<td>Thalamus</td>
<td>−0.42</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*[^11C]FLB 457 binding potential values were adjusted for age. †P<.007 (0.05/7).

The dysfunction of the anterior cingulate cortex may also affect other cortical regions, since the anterior cingulate cortex has direct anatomic links with the temporal cortex and prefrontal cortex, hippocampus, and thalamus, and in fact, disorders of corticocortical integration in schizophrenia have been suggested by PET studies.7,35,39

Our findings suggest that the abnormal functional connectivity with aberrant regulation of dopaminergic transmission in the anterior cingulate cortex5 might be relevant to the pathophysiology of schizophrenia. However, because of the limited number of patients and the moderate illness severity in this study, it could be possible that regions other than the anterior cingulate cortex, such as the thalamus, might also show significant difference in BP if there were a larger number of patients. Thus, further study is needed to determine whether the decrease in D2 receptor binding is specific for the anterior cingulate cortex and whether this decrease changes with the psychiatric course of the illness.

**REFERENCES**


