Decreased Dopamine D\textsubscript{2} Receptor Binding in the Anterior Cingulate Cortex in Schizophrenia

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**Background:** The clinical efficacy of dopamine D\textsubscript{2} 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 D\textsubscript{2} 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 D\textsubscript{2} 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 D\textsubscript{2} 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 D\textsubscript{2} receptors in the anterior cingulate cortex in patients with schizophrenia. Alterations in D\textsubscript{2} 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 D\textsubscript{2} 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 D\textsubscript{2} receptor blockers than are negative symptoms. The therapeutic potency of antipsychotic drugs correlates with the affinity for D\textsubscript{2} receptors. These observations imply that D\textsubscript{2} receptors are critically involved in the pathophysiology of positive symptoms.

Increased density of striatal D\textsubscript{2} 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 D\textsubscript{2} receptors.\textsuperscript{1} On the other hand, neuropathological\textsuperscript{2,3} and neuroimaging\textsuperscript{4,5} 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,\textsuperscript{6,7} and extrastriatal D\textsubscript{2} receptors are thought to be common sites of action of antipsychotic drugs.\textsuperscript{12,13} We have recently reported the abnormality of dopamine D\textsubscript{1} receptor binding in the prefrontal cortex of patients with schizophrenia and its relation to negative symptoms.\textsuperscript{14} In this study, we performed positron emission tomography (PET) scans using carbon 11–labeled FLB 457\textsuperscript{15} to examine extrastriatal D\textsubscript{2} receptors and their relation to clinical symptoms in drug-naive patients with schizophrenia.

**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-naive male schizophrenic patients with a mean±SD age of 28.1±7.9 years who met the DSM-IV criteria for schizophrenia or schizophreniform 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 outpatients 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 were 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, mania, delusions, halluciniations, 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...
view that \([^{11}C]\text{FLB 457}\) binding in the anterior cingulate cortex represents binding to D2 receptors.\textsuperscript{15,19} The functional abnormality of the cingulum in schizophrenia might hamper its use as a reference, but the cingulum 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 \(F_{1,27}=0.02; P=.88\); group-by-time interaction \(F_{2,13,55}=0.34; P=.73\)). Patients with schizophrenia may have alterations in regional cerebral blood flow.\textsuperscript{6,8} However, the reduction of BP is unlikely to be an effect of altered blood flow, since the \(R_1\) 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 \(R_1\) values obtained in this study.\textsuperscript{18} Al-though 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.

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.\textsuperscript{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 \([^{11}C]\text{FLB 457}\) is low. This could be explained by the direct competition with increased endogenous dopamine,\textsuperscript{23} but the detectability of dopamine release in the extrastriatal regions is controversial.\textsuperscript{24} and the susceptibility of \([^{11}C]\text{FLB 457}\) to endogenous dopamine has not yet been examined. Our monkey experiments indicated that the extrastriatal \([^{11}C]\text{FLB 457}\) binding was not sensitive to endogenous dopamine. We observed that \([^{11}C]\text{FLB 457}\) binding in the cortex and thalamus was not significantly affected by 1 mg of intravenous methamphetamine challenge, whereas the striatal binding of \([^{11}C]\text{raclopride}\) was decreased by more than 20%.\textsuperscript{25} Therefore, providing that the affinity did not differ, our finding might

\[
\text{BP} = f_2 \frac{B_{\text{max}}}{K_d(1+\Sigma_i F/K_i)},
\]

which is defined as follows: \(BP = f_2 \frac{B_{\text{max}}}{K_d(1+\Sigma_i F/K_i)}\), where \(f_2\) is the free fraction of unbound radioligand, \(B_{\text{max}}\) is the density of receptor, \(K_d\) is the dissociation constant for the radioligand, and \(F_i\) and \(K_{di}\) are the free concentration and the dissociation constant of \(i\) competing ligands, respectively. The model also allows the estimation of differences in head size by dividing the measured volume by intracranial volume, the relative volumes (\(\text{measured volume/intracranial volume} \times 100\)) were used for the analysis.\textsuperscript{26}

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where \(f_2\) is the free fraction of unbound radioligand, \(B_{\text{max}}\) is the density of receptor, \(K_d\) is the dissociation constant for the radioligand, and \(F_i\) and \(K_{di}\) are the free concentration and the dissociation constant of \(i\) competing ligands, respectively. The model also allows the estimation of differences in head size by dividing the measured volume by intracranial volume, the relative volumes (\(\text{measured volume/intracranial volume} \times 100\)) were used for the analysis.\textsuperscript{26}

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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. On the other hand, the reduced concentration of cortical D2 receptors in schizophrenia has also been discussed in relation to abnormal brain development. 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. Based on the finding of the deficit of inhibitory interneurons in the anterior cingulate cortex, less GABAergic inhibitory transmission has been proposed in the cortical local circuit of schizophrenic brain. It has been demonstrated by animal studies that dopamine release in the prefrontal cortex can be regulated by GABA interneurons that have D2 receptors. 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 schizophrenia and increase the D2 receptor density in the rat medial prefrontal cortex. 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. The role of the anterior cingulate cortex in executive function, selective attention, and error detection may suggest a contribution to conceptual disorganization and hallucinatory behavior. 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-
The anterior cingulate cortex (ACC) has direct anatomic links with the temporal cortex and prefrontal cortex, and it is hypothesized to play a role in the regulation of cognitive and emotional functions. PET studies have shown decreased hypoperfusion or hyperperfusion in the ACC in schizophrenia, which may be related to the pathophysiology of the illness. For example, decreased perfusion in the ACC has been associated with the severity of negative symptoms in schizophrenia.

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>.002†</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>-0.59</td>
<td>.06</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>-0.31</td>
<td>.35</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>-0.43</td>
<td>.19</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>-0.26</td>
<td>.45</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>-0.12</td>
<td>.72</td>
</tr>
<tr>
<td>Thalamus</td>
<td>-0.42</td>
<td>.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.

Our findings suggest that the abnormal functional connectivity with aberrant regulation of dopaminergic transmission in the anterior cingulate cortex 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.


