Decreased Serum Levels of D-Serine in Patients With Schizophrenia

Evidence in Support of the N-Methyl-d-Aspartate Receptor Hypofunction Hypothesis of Schizophrenia

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Background: The hypofunction of the N-methyl-d-aspartate (NMDA) subtype of glutamate receptors has been implicated in the pathophysiology of schizophrenia. Several lines of evidence suggest that D-serine may function as an endogenous agonist of the glycine site of the NMDA receptor. The aim of this study was to examine whether serum levels of D- and L-serine in patients with schizophrenia are different from those of healthy controls.

Methods: Forty-two patients with schizophrenia and 42 age- and sex-matched healthy controls were enrolled in this study. Symptoms were assessed using the Brief Psychiatric Rating Scale. Serum levels of total serine and D- and L-serine were measured by high-performance liquid chromatography.

Results: Serum levels of D-serine in the patients with schizophrenia were significantly ($z = -3.30$, $P = .001$) lower than those of healthy controls. In contrast, serum levels of total (D and L) serine ($z = -2.40$, $P = .02$) and L-serine ($z = -2.49$, $P = .01$) in the schizophrenic patients were significantly higher than those of controls. In addition, the percentage of D-serine in the total serine in the schizophrenic patients was significantly ($z = -4.78$, $P < .001$) lower than that of controls, suggesting that the activity of serine racemase, an enzyme catalyzing the formation of D-serine from L-serine, may have been reduced in the schizophrenic patients.

Conclusions: Reduced levels of D-serine may play a role in the pathophysiology of schizophrenia, and serum D- and L-serine levels might provide a measurable biological marker for schizophrenia.

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Several lines of evidence suggest that a dysfunction in glutamatergic neurotransmission via the N-methyl-d-aspartate (NMDA) subtype of glutamate receptors might be involved in the pathophysiology of schizophrenia. This hypothesis has evolved from clinical findings that phencyclidine and its congener ketamine, which block the NMDA receptor ion channel, induce a schizophrenia-like psychosis, representing negative and positive symptoms and cognitive dysfunction in healthy humans, and that phencyclidine exacerbates such symptoms in patients with chronic schizophrenia. Although only L-amino acids generally play a physiologic role in species other than bacteria, the existence of some D-amino acids, such as D-serine in higher species, including humans, has been demonstrated. It has previously been shown that D-serine is selectively enriched in the forebrain, with immunocytochemical localization in type II astrocytes in gray matter areas also rich in the NMDA receptor, suggesting that D-serine may modulate the strychnine-insensitive glycine sites of the NMDA receptor. Interestingly, therapeutic trials with D-serine have been shown to significantly improve symptoms (positive, negative, and cognitive) in patients with schizophrenia, suggesting that D-serine may play an important role in the pathophysiology of schizophrenia. Therefore, it would be of great interest to clarify the potential contribution of endogenous D-serine to the pathophysiology of schizophrenia. Along these lines, the present study was undertaken to examine whether the serum levels of D- and L-serine of patients with schizophrenia are different from those of age- and sex-matched healthy controls.

METHODS

PATIENTS AND CLINICAL DATA
Forty-two patients with schizophrenia (mean ± SD age, 35.5 ± 15.0 years; range, 16-65 years; 22 men and 20 women) were recruited from the Chiba University Hospital and Kimura Hospital, Chiba, Japan. Forty-two age- and sex-
DETERMINATION OF TOTAL SERINE, \( L \)-SERINE, AND D-SERINE

\( D \)-Amino acids, including \( D \)-serine, are known to be present in processed foods.\(^{13}\) To avoid the effects of \( D \)-serine contained in food, serum samples of the patients and healthy controls were collected between 11 AM and noon, and the samples were stored at −80°C until used for the assay. Sample preparation and measurement of total, \( L \)-, and \( D \)-serine levels were performed according to previously described methods,\(^{14,15}\) using a column-switching high-performance liquid chromatography (HPLC) system, by researchers (T.F. and H.H.) blinded to the respective groups (healthy controls and schizophrenic patients). It has been reported previously that serum levels of \( D \)-serine correlate positively with serum levels of creatinine and blood urea nitrogen, suggesting that serum \( D \)-serine may be an indicator of renal dysfunction.\(^{13}\) Therefore, patients with renal dysfunction were excluded from this study.

Table 1. Characteristics and Test Scores of Study Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Controls</th>
<th>Patients With Schizophrenia</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>21/21</td>
<td>22/20</td>
<td>.83*</td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>35.5 ± 14.4 (20-70)</td>
<td>36.0 ± 14.7 (16-65)</td>
<td>.88†</td>
</tr>
<tr>
<td>Onset (range), y</td>
<td>NA</td>
<td>25.3 ± 11.6 (13-57)</td>
<td>NA</td>
</tr>
<tr>
<td>Illness duration (range), y</td>
<td>NA</td>
<td>10.2 ± 11.0 (0.41)</td>
<td>NA</td>
</tr>
<tr>
<td>BPRS scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>23.1 ± 14.5 (2-58)</td>
<td>NA</td>
</tr>
<tr>
<td>Positive</td>
<td>NA</td>
<td>13.3 ± 9.85 (2-37)</td>
<td>NA</td>
</tr>
<tr>
<td>Negative</td>
<td>NA</td>
<td>5.12 ± 4.02 (0-16)</td>
<td>NA</td>
</tr>
<tr>
<td>Total serine, mg/dL (µmol/L)</td>
<td>1.86 ± 0.32 (177.3 ± 30.7)</td>
<td>2.1 ± 0.49 (199.7 ± 46.6)</td>
<td>.02‡</td>
</tr>
<tr>
<td>( d )-Serine, mg/dL (µmol/L)</td>
<td>0.024 ± 0.006 (2.28 ± 0.59)</td>
<td>0.020 ± 0.006 (1.86 ± 0.53)</td>
<td>.001†</td>
</tr>
<tr>
<td>( l )-Serine, mg/dL (µmol/L)</td>
<td>1.84 ± 0.32 (175.0 ± 30.6)</td>
<td>2.08 ± 0.49 (197.9 ± 46.4)</td>
<td>.01‡</td>
</tr>
<tr>
<td>Ratio of ( d )-serine to total serine, %</td>
<td>1.31 ± 0.34</td>
<td>0.95 ± 0.26</td>
<td>&lt;.001‡</td>
</tr>
</tbody>
</table>

Abbreviations: BPRS, Brief Psychiatric Rating Scale; NA, not applicable.
*The comparison between 2 groups was performed using the \( \chi^2 \) test.
†The comparison between 2 groups was performed using the \( t \) test.
‡The comparison between 2 groups was performed using the Mann-Whitney \( U \) test.

The data are presented as mean ± SD. The \( \chi^2 \) test was used for categorical variables, and the \( t \) test (unpaired) was used for continuous variables. Because the values of serum serine levels did not have normal distribution, the differences between the 2 groups and among multiple groups were examined using the nonparametric Mann-Whitney \( U \) test and the Kruskal-Wallis test. The relationships between the 2 variables were examined using Pearson correlation coefficients for the following reasons: the parameters (clinical variations) other than the serum serine levels all revealed an approximately normal distribution and the correlations were examined by a parametric method, which can be applied to situations in which at least one variable has a normal distribution.\(^{16}\) \( P < .05 \) was considered statistically significant.

STATISTICAL ANALYSIS

Age and sex matching was successful, because there was neither a marked nor a significant difference between healthy controls (\( n = 42 \)) and schizophrenic patients (\( n = 42 \)) (Table 1). The characteristics of all study participants are given in Tables 1 and 2. The concentration of total (\( D \) and \( L \)) serine and \( L \)- and \( D \)-serine in the serum of healthy controls and schizophrenic patients was determined by using a column-switching HPLC system.\(^{14}\)

Serum levels of total serine of the patients (median, 2.1 mg/dL [203.5 µmol/L]; 2.1 ± 0.5 mg/dL [199.7 ± 46.6 µmol/L]) were significantly (\( z = −2.40, P = .02 \)) higher than those of the healthy controls (median, 1.9 mg/dL [176.2 µmol/L]; 1.9 ± 0.3 mg/dL [177.3 ± 30.7 µmol/L]) (Table 1). Serum levels of \( L \)-serine of the patients (median, 2.1 mg/dL [201.3 µmol/L]; 2.1 ± 0.5 mg/dL [197.9 ± 46.4 µmol/L]) were also significantly (\( z = −2.49, P = .01 \)) higher than those of the healthy controls (median, 1.8 mg/dL [174.1 µmol/L]; 1.8 ± 0.3 mg/dL [175.0 ± 30.6 µmol/L]) (Table 1). The percentage of \( D \)-serine in the human serum was approximately 0.5% to 2% of the total serine concentration, which was consistent with a previous report.\(^{15}\) In contrast, serum levels of \( D \)-serine of the schizophrenic patients (median, 0.019 mg/dL [1.84 µmol/L]; 0.020 ± 0.006 mg/dL [1.86 ± 0.53 µmol/L]) were significantly (\( z = −3.30, P = .001 \)) lower than those of the healthy controls (median, 0.024 mg/dL [2.27

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However, there was no correlation between serum total serine (median, 1.29%; 1.31%±0.34%; n=42; Table 1 and Figure, A). Moreover, in the schizophrenic patient serum, the percentage of d-serine in the total serine (median, 0.95%; 0.95%±0.26%; n=42) was significantly (z=−0.78, P<.001) lower than that in the serum of healthy controls (median, 1.29%; 1.31%±0.34%; n=42; Table 1 and Figure, B). However, there was no correlation between serum total serine (r=0.015, P=.92), L-serine (r=−0.016, P=.50), and d-serine (r=0.012, P=.94) levels and age of onset in any of the patients. In addition, the serum levels of total serine (r=0.045, P=.78), L-serine (r=−0.047, P=.77), and d-serine (r=0.144, P=.37) were not correlated with the duration of illness in any of the patients. No significant sex (or age) differences were detected in the serum levels of total serine, L-serine, and D-serine and the percentage of D-serine in the total serine.

The detailed characteristics of each schizophrenic patient are shown in Table 2. There was no age difference (t=−0.144, P=.89) between drug-naive patients and medicated patients. There was also no sex difference (χ²=0.933, P=.33) between drug-naive patients and medicated patients. As expected, the duration of illness (2.41±5.05 years) in the drug-naive patients (n=15) was significantly (t=−4.04, P<.001) lower than that of the medicated patients (14.6±11.0 years) (n=27). The total BPRS scores (29.5±16.7) among drug-naive patients were significantly (t=2.20, P=.03) higher than those of the medicated patients (19.7±12.0). Furthermore, the Positive Symptom subscale BPRS scores (19.6±11.2) of drug-
naive patients were significantly ($t=3.46$, $P=.001$) higher than those of the medicated patients ($9.78±7.10$), whereas the Negative Symptom subscale BPRS scores ($4.80±5.2$) of drug-naive patients were not significantly different ($t=0.379$, $P=.71$) from those of the medicated patients ($5.30±3.28$). The serum levels of total serine ($z=-1.01$, $P=.31$), L-serine ($z=-0.984$, $P=.32$), and D-serine ($z=-1.22$, $P=.22$) of the medicated patients were not significantly different than those of the drug-naive patients.

We then examined the correlation between serum total serine, L-serine, D-serine levels and BPRS scores of patients with schizophrenia. Interestingly, there was a significant positive correlation between serum D-serine levels and total scores ($r=0.542$, $P=.003$), positive symptom scores ($r=0.589$, $P<.001$), and negative symptom scores ($r=0.427$, $P=.02$) on the BPRS among the medicated patients, whereas no such correlation between serum total serine or L-serine levels and total or each subscale score on the BPRS was detected. Because these correlations might have been confounded by medication, we controlled for the dose of antipsychotics using partial correlation coefficients. Even when the administered antipsychotics (chlorpromazine equivalents) were adjusted for using partial correlation coefficients, the relationship among serum D-serine levels and total scores (partial correlation coefficient $=0.488$, $P=.01$), positive symptom scores (partial correlation coefficient $=0.545$, $P=.004$), and negative symptom scores (partial correlation coefficient $=0.401$, $P=.04$) on the BPRS remained significant. Moreover, no correlation between antipsychotic dosages (chlorpromazine equivalents) and serum total serine ($r=-0.109$, $P=.59$), L-serine ($r=-0.111$, $P=.58$), and D-serine ($r=0.270$, $P=.17$) levels was observed in the medicated patients. In contrast, no significant correlation between total serine (total scores: $r=0.278$, $P=.32$; positive symptoms scores: $r=0.465$, $P=.08$; negative symptoms scores: $r=0.019$, $P=.95$), L-serine (total scores: $r=0.281$, $P=.32$; positive symptoms scores: $r=0.467$, $P=.08$; negative symptoms scores: $r=0.022$, $P=.94$), or D-serine (total scores: $r=-0.051$, $P=.86$; positive symptoms scores: $r=0.127$, $P=.66$; negative symptoms scores: $r=-0.189$, $P=.52$) levels and the BPRS scores (total, positive, negative) was observed in the drug-naive patients. In addition, there was no significant difference regarding total serine (healthy controls: $z=-0.536$, $P=.59$; schizophrenic patients: $z=-1.43$, $P=.15$), L-serine (healthy controls: $z=-0.506$, $P=.61$; schizophrenic patients: $z=-1.49$, $P=.14$), or D-serine (healthy controls: $z=-0.904$, $P=.37$; schizophrenic patients: $z=-0.521$, $P=.60$) between smokers and nonsmokers among healthy controls and schizophrenic patients. Furthermore, no significant difference was observed among patients regarding total serine ($H=2.78$, $P=.60$), L-serine ($H=2.77$, $P=.60$), D-serine ($H=4.55$, $P=.34$), or the ratio of D-serine to total serine ($H=4.47$, $P=.35$) in terms of the disease subtypes (eg, catatonic, paranoid, residual, disorganized, and undifferentiated) determined by the DSM-IV criteria.

The major findings of this study are that: (1) serum levels of L-serine in the patients with schizophrenia are slightly higher than those of age- and sex-matched healthy controls, and (2) serum levels of D-serine and the ratio of D-serine to total serine in patients with schizophrenia are markedly decreased compared with those of controls. It is already known that glycine is converted to L-serine by the pyridoxal-5'-phosphate–dependent enzyme serine hydroxymethyltransferase. In addition, it has been reported previously that plasma levels of total serine and glycine in patients with schizophrenia are higher than those of controls and that levels of serine and glycine in the brain of schizophrenic patients are higher than those of controls, suggesting a possible abnormality in serine hydroxymethyltransferase. Thus, it seems that the synthetic or metabolic pathway of L-serine may be abnormal in schizophrenic patients, although the precise mechanism of increased L-serine levels in this population remains unknown.

D-Serine is formed from L-serine by serine racemase, a pyridoxal-5'-phosphate–dependent enzyme enriched in brain astrocytes. The brain distribution of serine...
racemase closely resembles that of D-serine, and pharmacologic inhibition of the enzyme diminishes D-serine levels in astrocytes, suggesting that serine racemase physiologically synthesizes D-serine, playing a part in the regulation of the NMDA receptor. It has been demonstrated previously by Northern blot analysis that the highest levels of serine racemase messenger RNA are in the liver and that the second-highest values are in brain tissue; low levels have been reported in the kidney, and slight amounts have been found in other tissues. It has also been shown that the liver expresses large amounts of D-amino acid oxidase, which completely metabolizes D-serine in most peripheral tissues, where D-serine is almost undetectable. It also has been shown that administration of D-serine or L-serine leads to the elevation of both D- and L-serine levels in the brain, suggesting that D- and L-serine can enter into the brain; elevation associated with the administration of D-serine or L-serine also suggests the possibility of direct racemization between D- and L-serine. It has been reported that levels of D-serine in the prefrontal cortex of schizophrenic patients are lower than those of healthy controls; however, in that study, no statistical analysis was performed due to the limited number of samples of brain tissue from patients with schizophrenia. Therefore, it is likely that D-serine in the blood may originate from the brain and that reduced levels of serum D-serine from patients may reflect decreased levels of D-serine in the brain, resulting in the hypofunction of the NMDA receptor in patients with schizophrenia. Furthermore, it is conceivable that the comparatively low ratio of D-serine to total serine in the serum from the patients observed in this study may reflect a reduction in the enzymatic activity of serine racemase in the patients. It is clear that further studies of the relevant metabolic pathways (eg, those involving D-amino acid oxidase, serine hydroxymethyltransferase, and 3-phosphoglycerate dehydrogenase) will be necessary, as will studies of the release and uptake of D-serine, to determine the potentially pathological role of decreased D-serine levels in schizophrenia.

As mentioned herein, treatment with D-serine revealed significant improvements in positive, negative, and cognitive symptoms in patients treated with antipsychotic drugs. In contrast, treatment with D-serine did not alter the symptoms of patients treated with clozapine; however, serum levels of D-serine had significantly increased 6 weeks after D-serine treatment, which suggests that any potential D-serine effects, or lack thereof, might have been due to the administration of antipsychotic drugs. In this study, a positive correlation between the total and subscale scores on the BPRS and serum D-serine levels in the medicated patients, but not in the drug-naive patients, was detected. It is possible that the serum levels of D-serine in patients who respond to antipsychotic drugs (eg, dopamine and serotonin receptor antagonists) may be lower than those of patients who do not respond to antipsychotic drugs; the mechanisms underlying such relationships between clinical symptoms and D-serine levels in medicated patients remain unknown.

In conclusion, there is a significant reduction in endogenous serum D-serine levels in schizophrenic patients. Furthermore, our findings suggest that serum D- and L-serine levels may serve as a convenient peripheral marker for schizophrenia. As an endogenous ligand for the glycine site of NMDA receptors, D-serine may play an important role in the pathophysiology of schizophrenia.

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