Table 2. Results of Case–Control and Family-Based Association Studies Between *SRR* and Schizophrenia

Sample Set A	Schizophrenia (n = 50)	Control (n = 52)	Case–Control p Value	
Minor Allele Frequency			Allele	Genotype
IVS1a+465G>C	.220	.241	.743	.038
IVS1b+15G>A	.190	.087	.041	.026
Sample Set B	**Schizophrenia (n = 570)**	**Control (n = 570)**	**Case–Control p Value**	
Minor Allele Frequency			Allele	Genotype
IVS1a+465G>C (SNP1)	.273	.264	.635	.321
IVS1b+15G>A (SNP2)	.105	.096	.529	.802
LD between the two SNPs				
D'	1.000	1.000		
r^2	.044	.038		
Haplotype Frequency (SNP1–SNP2)			Global p value	
G-A	.105	.097		
G-G	.622	.640	.643	
C-G	.272	.263		

Sample Set C (Family Panel)	p for PDT-SUM	p for PDT-AVE	p for ETDT	Global p for TRANSMIT
IVS1a+465G>C	.592	.439	.786	.890
IVS1b+15G>A	.250	.310	.837	

PDT, pedigree disequilibrium test; PDT-SUM places greater weight on larger families; PDT-AVE places equal weight on all families.

To evaluate the abundance of each transcript in human brain, we performed an RNase protection assay (RPA). We designed four probes specific to each isoform, which shared 143 nt downstream and harbored upstream a 60–75 nt region specific to each isoform (Figure 2). When probe 1a, 1c, or 1d was used, the 143 nt protection fragment was clearly detected, whereas the expected > 200 nt fragment could not be detected (Figure 2), suggesting that isoforms a, c, and d are the minority in brain tissue, and that isoform b, containing the common 143 nt region, forms the majority of *SRR* in the brain. This idea is supported by two lines of evidence: 1) we detected a weak but apparent signal around 218 (= 143 + 75) nt and no signal around 143 nt (Figure 2), and 2) when the 5' region of the *SRR* transcript was amplified by 5'-RACE, clones with fragments corresponding to isoform 1b were predominant but those corresponding to the other isoforms were very scarce (data not shown). The lower signal intensity of the 218 nt band detected with probe b, relative to the signal of the 143 nt fragment detected with probes 1a, 1c, or 1d, could be due to the high G/C content in the cDNA sequence of probe b (60/75 = 80% G/C). Increased G/C content can produce complex secondary structures in both probe and target sequences, leading to decreased signal-to-noise ratios. The results were not improved by altering the probe sequence.

The *SRR* spans a genomic region of more than 50 kb. Mutation screening of all exons including the newly identified ones in this study, splice boundaries and 5' flanking regions of exons 1a–1d identified four polymorphisms, two SNPs (IVS1a+465G>C, rs408067, IMS-JST095603; IVS1b+15G>A, rs3744270, IMS-JST095602) surrounding exon 1b, and two novel SNPs, one in intron 5 (IVS5-34A>G) and the other in the 3'-untranslated region (Stop+302G>A; Figure 1). The IVS1a+465G>C is located next to the 5' end nucleotide of exon 1b that was defined by the present 5'-RACE analysis. The SNP databases display an exonic SNP (Stop+821T>C, rs2273983, IMS-JST069708), but in our sample it was not polymorphic (only the T allele was detected). Because only IVS1a+465G>C and IVS1b+15G>A were genetically informative (minor allele frequencies ≥ .10), we examined these two SNPs in the subsequent genetic study.

Serum D-Serine Levels in Schizophrenia and Control Subjects

In a prior study, we examined 42 schizophrenia patients and 42 healthy control subjects for their serum levels of serine isomers (Hashimoto et al 2003). Among them, DNA was available from only 19 patients and 8 control subjects. To determine both serum levels of serine and genotypes in the same individuals, we newly recruited 31schizophrenia patients and 46 normal control subjects and established sample set A (50 schizophrenia and 52 control subjects). Using sample set A, we were able to replicate the previous findings: significant decreases in the concentration of serum D-serine (p = .0211) and the percentage of D-serine in total serine (p < .0001), and significant increases in total serine (p = .0029) and L-serine (p = .0026) concentrations in the schizophrenia group compared with the control group (Figure 4). When we analyzed these measures using only the newly recruited samples, we observed similar trends in the comparison of schizophrenia and control groups, with the following p values: p = .7221 for serum D-serine concentration, p = .0145 for percentage of D-serine in total serine, p = .0072 for total serine level, and p = .0075 for L-serine concentration.

As in the prior study (Hashimoto et al 2003), we detected no correlation between serum total serine (Pearson correlation coefficient r = –.183, p = .202), L-serine (r = –.184, p = .020), and D-serine (r = –.077, p = .597) levels, and age of onset of any of the patients (the detailed characteristics of individual subjects are partly described elsewhere [Hashimoto et al 2003], and the remainder are available on request]. Likewise, we confirmed no significant correlation between the duration of illness and any of the serum levels of total serine (r = .093, P = 521), L-serine (r = .091, P = .531), and D-serine (r =.205, P = .154). Gender, age, medication use, and smoking had no effect on any of the three serine-related measures, in the healthy control subjects or patients from the present sample panel A, which is consistent with our previous report (Hashimoto et al 2003). Current thinking suggests that medication by typical and atypical antipsychotic drugs is unlikely to influence serum levels of D-serine (Tsai et al 1999).

Table 3. Results of Case–Control (Using Sample Set B) and Family-Based (Using Sample Set C) Association Studies Between *DAO* and Schizophrenia

Marker No.	Polymorphism[a]	Case-Control *p* Value		PDT *p* Value		ETDT *p* Value	2 SNP-Based Haplotype *p*-value[b] Case-Control/Family-Based						3 SNP-Based Haplotype *p* Value[b] Case–Control/Family-based				
		Allele	Genotype	AVE	SUM												
DAO-01	ATG−6732T>C	.250	.198	.138	.216	0.402	.403/ .483										
DAO-02	IVS1−27T>G	.498	.768	.683	.938	0.550		.350/ .591					.334/ .639				
DAO-03	IVS3−544C>T	.310	.547	.708	1.000	0.618			.198/ .549					.412/ .710			
DAO-04	IVS4+282T>G	.734	.938	.941	1.000	0.720				.192/ .490					.198/ .489		
DAO-05	IVS6−55A>G	.309	.565	.547	.870	.454					.046/ .685					.011/ .689	
DAO-06	Stop+38851G>A	.020	.023	.157	.089	.350											

ETDT, extended transmission disequillibrium test; PDT, pedigree disequilibrium test; SNP, single nucleotide polymorphisms.
[a]Second allele is a minor allele.
[b]*p* value represents a global *p* value for both case–control and family-based association tests.

Genetic Association Studies

The two nucleotide variants of *SRR* were first typed using sample set A (50 schizophrenics, 52 control subjects), whose serum data was available (Table 2). Genotypic frequencies for IVS1a+465G>C and genotypic and allelic frequencies for IVS1b+15G>A displayed nominally significant differences between schizophrenia and control subjects.

To follow up these initial findings, a second independent sample set B (570 schizophrenia patients, 570 control subjects) was typed for the two SNPs (Table 2). We failed to replicate positive association results in the second, larger sample groups. The IVS1a+465G>C and IVS1b+15G>A polymorphisms were in substantial LD (Table 2). The reason $D' = 1.00$ in both schizophrenia and control groups is due to the complete linkage between the C allele of IVS1a+465G>C and the G allele of IVS1b+15G>A, and the relatively small values of r^2 could be a result of the major G allele of IVS1a+465G>C being present against a background of both alleles of the IVS1b+15G>A (Table 2). Distributions of haplotypes constructed by the two SNPs did not differ significantly between the schizophrenia and control groups (global $p = .643$) (Table 2).

Table 2 also shows the results of family-based association tests. The PDT program computes two statistical measures, PDT-SUM and PDT-AVE. Briefly, PDT-SUM gives more weight to larger families, whereas PDT-AVE places equal weight on all families. The suitability of both statistical methods depends on family structure and genetic models (Martin et al 2001). The two SNPs in *SRR* showed no significant association with schizophrenia by either of the two PDT statistics. We performed another family-based association test, ETDT analysis, on the 80 complete triad families. Again, this did not detect a significant association between either SNP of *SRR* and schizophrenia (Table 2). Moreover, haplotype transmission analysis found no haplotypes that were preferentially transmitted to schizophrenia subjects (specific haplotype *p*s are not shown; global $p = .890$; Table 2).

Genetic analysis of *DAO* demonstrated that the SNPs DAO-02 to DAO-05 form a single haplotype block, with the DAO-01 marker being in moderate LD with the block, with an obvious LD gap between the DAO-06 and the haplotype block (Figure 3). In the sample panel A, significant association between *DAO* and schizophrenia was not detected (Supplement 1). The allelic, genotypic, and haplotypic analyses using the sample sets B and C also failed to reveal genetic association between *DAO* and schizophrenia, except for the DAO-06 marker in the case–control sample set B [allelic $p = .020$, genotypic $p = .023$, global *p* for the (DAO-04)-(DAO-05)-(DAO-06) haplotype was .011] (Table 3).

The analyzed polymorphisms from the *SRR* and *DAO* genes

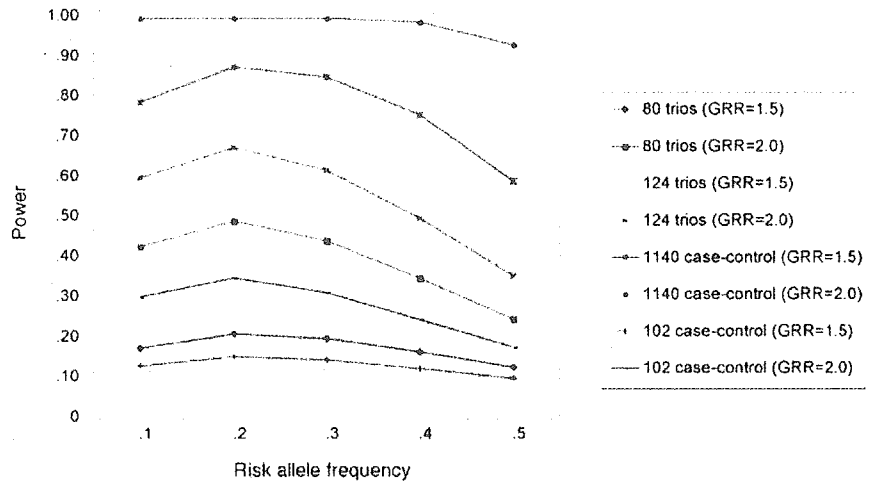

Figure 5. Results of power analysis under varying degrees of risk allele frequency and GRR (genetic relative risk). The family samples used for ETDT (extended transmission disequilibrium test) analysis are designated "80 trios," and those used for PDT (pedigree disequilibrium test) "124 trios"; "1140 case–control" is sample set B and "102 case–control" is sample set A.

Table 4. Relationship Between Ratio of D-Serine to Total Serine (%) and *SRR* Genotypes

Sample	D-Serine % According to IVS1a+465G>C Genotype (Mean ± SE)			*p* Value	D-Serine % According to IVS1b+15G>A Genotype (Mean ± SE)		*p* Value	D-Serine % According to Combined IVS1a+465G>C and IVS1b+15G>A Genotypes (Mean ± SE)				*p* Value
	G/G	G/C	C/C		G/G	G/A		G/G - G/G	G/G - G/A	G/C - G/G	G/C - G/A	C/C - G/G	
Control Group (*n* = 52)	1.39 ± .07 (*n* = 32)	1.07 ± .07 (*n* = 15)	1.41 ± .19 (*n* = 5)	.033	1.27 ± .06 (*n* = 43)	1.43 ± .12 (*n* = 9)	.179	1.38 ± .08 (*n* = 26)	1.49 ± .15 (*n* = 6)	1.02 ± .05 (*n* = 12)	1.30 ± .22 (*n* = 3)	1.41 ± .19 (*n* = 5)	.052
Schizophrenia Group (*n* = 50)	.97 ± .05 (*n* = 28)	1.02 ± .06 (*n* = 22)	None	.383	1.02 ± .04 (*n* = 31)	.94 ± .08 (*n* = 19)	.177	.94 ± .04 (*n* = 17)	1.02 ± .11 (*n* = 11)	1.12 ± .06 (*n* = 14)	.83 ± .09 (*n* = 8)	None	.082
Combined Group (*n* = 112)	1.19 ± .05 (*n* = 60)	1.04 ± .04 (*n* = 37)	1.41 ± .19 (*n* = 5)	.063	1.17 ± .04 (*n* = 74)	1.10 ± .08 (*n* = 28)	.258	1.20 ± .06 (*n* = 43)	1.19 ± .10 (*n* = 17)	1.07 ± .04 (*n* = 26)	.96 ± .11 (*n* = 11)	1.41 ± .19 (*n* = 5)	.145

p values were calculated using the two-tailed Mann–Whitney *U* test in the comparison of two groups or the Kruskal–Walls test for comparison of three or more groups.

were in Hardy–Weinberg equilibrium in all the sample sets. The results of power analysis under various assumptions of parameters are shown in Figure 5. The case–control sample set B had a power of approximately .6–.9 under GRR (genetic relative risk) = 1.5 and > .9 under GRR = .2, when α = .05 and a dominant mode were assumed. The family sample sets and case–control sample set A showed lower power compared with the sample panel B.

Phenotype–Genotype Correlation Analysis

We examined whether the serum levels of serine enantiomers could be affected by the genotypes of *SRR* or *DAO*. Among the serine-related measures, the ratio of D-serine to total serine (%) was most significantly different between schizophrenia and the control subjects. Table 4 shows the relationship between the ratio of D-serine and the genotypes of two *SRR* polymorphisms. The mean values of this measure were not significantly different among the genotypes, except for the IVS1a+465G>C genotypes in the control group. Other measures including the D-serine, L-serine, and total serine concentrations were not significantly affected by *SRR* genotype (data not shown). Table 5 shows the correlation between the ratio of D-serine levels and genotypes of the six *DAO* polymorphisms. None of these *DAO* genetic markers significantly affected the D-serine parameter.

Discussion

Our previous findings of disturbed serine metabolism, including D-serine reduction and decreased D-serine/total serine ratio, in schizophrenia serum (Hashimoto et al 2003) have here been verified in larger samples. Recently, we also found that the ratio of D-serine to total serine was reduced in cerebrospinal fluids of drug-naïve schizophrenia compared with normal subjects (Hashimoto et al, in press). Therefore, the dysregulation of synthesis, metabolism, or both of D-serine in schizophrenia is likely to underlie the pathophysiology of at least some part of schizophrenia (Hashimoto et al 2004). D-serine is generated from L-serine by serine racemase, a pyridoxal-5'-phosphate-dependent enzyme enriched in brain astrocytes (De Miranda et al 2000; Dunlop and Neidle 1997; Wolosker et al 1999a, 1999b). The recent report of Coyle et al (unpublished data) that disruption of the serine racemase gene in mice resulted in not detectable D-serine in brain confirms the role of this gene. In the study we report here, we first focused on the human *SRR* gene as a potential major determinant for regulating D-serine levels not only in serum but also in brain for the following reasons: 1) serine racemase protein levels are highest in the brain, with a lower level in liver and faint or no detectable expression in other peripheral tissues (Wolosker et al 1999a); 2) liver expresses a large amount of D-amino acid oxidase, which almost completely metabolizes D-serine in liver (Hashimoto and Oka 1997; Wolosker et al 1999a); and 3) administration of D-serine or L-serine leads to the elevation of both stereoisomers of serine in the brain, suggesting a possible shuttling of the compounds through the blood brain barrier (Takahashi et al 1997). These lines of evidence imply that D-serine in the blood may originate from the brain and that reduced serum levels of D-serine in schizophrenia may reflect a decrease in the enzymatic activity of SRR in the brain of patients.

We revealed the existence of four 5' end untranslated exons of the human *SRR* gene in the brain, generating four mRNA isoforms coding for an identical protein sequence. The major transcript uses exon 1b, and our mutation search identified two SNPs in the vicinity of this exon. A database search demonstrated that exon 1b and its flanking intronic sequences that span

Table 5. Relationship Between Ratio of D-Serine to Total Serine (%) and *DAO* Genotypes

Polymorphism and Genotype	% D-Serine According to Genotype (Mean ± SE)		
	Control Group (n = 52)	Schizophrenia Group (n = 50)	Combined Group (n = 112)
DAO-01 (ATG−6732T>C)			
T/T	1.27 ± .38 (n=32)	.99 ± .28 (n=30)	1.13 ± .36 (n=62)
T/C	1.34 ± .40 (n=19)	.99 ± .26 (n=18)	1.17 ± .38 (n=37)
C/C	1.39 ± .00 (n= 1)	1.07 ± .00 (n= 1)	1.23 ± .23 (n= 2)
p	.675	.817	.621
DAO-02 (IVS1−27T>G)			
T/T	1.32 ± .37 (n=13)	.96 ± .27 (n=14)	1.13 ± .37 (n=27)
T/G	1.30 ± .35 (n=31)	1.05 ± .27 (n=24)	1.19 ± .34 (n=55)
G/G	1.25 ± .53 (n= 8)	.90 ± .22 (n=11)	1.05 ± .41 (n=19)
p	.724	.360	.138
DAO-03 (IVS3−544C>T)			
C/C	1.32 ± .37 (n=13)	.96 ± .26 (n=15)	1.13 ± .37 (n=28)
C/T	1.30 ± .35 (n=31)	1.06 ± .27 (n=24)	1.19 ± .34 (n=55)
T/T	1.25 ± .53 (n= 8)	.88 ± .22 (n=10)	1.04 ± .42 (n=18)
p	.724	.179	.103
DAO-04 (IVS4+282T>G)			
T/T	1.18 ± .46 (n=11)	.92 ± .22 n =13)	1.04 ± .37 (n=24)
T/G	1.30 ± .35 (n=30)	1.05 ± .28 (n=23)	1.19 ± .34 (n=53)
G/G	1.4 ± .36 (n=11)	.96 ± .28 (n=13)	1.16 ± .38 (n=24)
p	.225	.462	.125
DAO-05 (IVS6−55A>G)			
A/A	1.32 ± .37 (n=13)	.99 ± .23 (n=14)	1.15 ± .35 (n=27)
A/G	1.30 ± .35 (n=31)	1.04 ± .29 (n=25)	1.18 ± .35 (n=56)
G/G	1.25 ± .53 (n= 8)	.88 ± .22 (n=10)	1.04 ± .42 (n=18)
p	.724	.306	.140
DAO-06 (Stop+36851G>A)			
G/G	1.39 ± .42 (n=12)	.95 ± .33 (n=15)	1.15 ± .43 (n=27)
G/A	1.26 ± .39 (n=23)	1.04 ± .23 (n=25)	1.14 ± .33 (n=48)
A/A	1.29 ± .34 (n=17)	.94 ± .26 (n= 8)	1.18 ± .35 (n=25)
p	.592	.509	.632

p values were calculated using the Kruskal–Wallis test.

IVS1a+465G>C and IVS1b+15G>A are not conserved among species, suggesting human-specific gene regulation. The GC-richness in the genomic sequences of exon 1b and its 5'-flanking region and the lack of a TATA box suggest that mRNA isoform 1b can be transcribed from multiple transcription start sites by contribution of Sp1 motifs. The G-to-C transversion at the IVS1a+465G>C site and G-to-A transition at the IVS1b+15G>A position are predicted to abolish Sp1 binding sites (TFSEARCH: http://mbs.cbrc.jp/research/db/TFSEARCHJ.html). Therefore, these polymorphisms could bear functional consequences. The genetic association analysis using the small sample size of panel A detected nominally significant genotypic or allelic associations (or both) between the two variants and schizophrenia. These initial weak associations could not be replicated in the 10-fold larger case–control panel B or family sample set C, however. These results indicate that the polymorphisms in the 5' portion of *SRR* do not play a major role in the susceptibility to schizophrenia in the Japanese population, although we cannot exclude the possibility that a subset of people with schizophrenia are biologically affected by the *SRR* gene.

Analysis of *DAO*, a gene involved in the metabolic pathway of D-serine, also showed no evidence of association with schizophrenia, with the exception of the DAO-06 SNP. The weak association between this marker and schizophrenia in the large case–control panel B is difficult to interpret, however, because 1) DAO-06 is located more than 36 kb downstream from the last exon of the *DAO* gene, 2) there are no annotated genes within a 20-kb range of the DAO-06 marker, 3) in the small-scale sample set A, DAO-06 showed a trend of allelic association with schizophrenia (p = .094), but the disease-associated alleles were different between the sample sets A and B.

The current study again confirmed the robustness of serum levels of D-serine against the confounding clinical parameters of gender, age, and treatment and duration of illness, suggesting a cogent contribution of genetic variance to D-serine regulation. We failed to detect firm correlation between the genotypes of two 5' SNPs in *SRR* and the D-serine-related measurements, however. The ratio of D-serine to total serine concentrations were slightly different among the genotypes of IVS1a+465G>C in the control group. The statistical difference was due to the reduced mean value in heterozygotes (IVS1a+465G/C) compared with both types of homozygotes (IVS1a+465G/G, IVS1a+465C/C), thus making the results difficult to interpret. It would be more reasonable to interpret the difference as being attributable to statistical fluctuation caused by small sample size (N = 52). Additionally, the *DAO* genetic variants showed no significant correlation with serum D-serine measure.

As stated previously, some enzymes responsible for serine metabolism (e.g. SRR and serine hydroxymethyltransferase) require pyridoxal-5'-phosphate as a cofactor (Schell 2004), it would be intriguing to examine the serum levels of this compound, although Muntjewerff et al (2003) reported no significant differences in levels of pyridoxal-5'-phosphate between schizophrenia and control groups.

In summary, we observed no major genetic effects of *SRR* and *DAO* polymorphisms in the predisposition to schizophrenia and prediction of serum levels of serine isomers in Japanese cohorts. The present results do not, however, exclude the possibility that SRR (DAO) protein or its enzymatic activity is decreased (increased) in schizophrenia by other mechanisms including disturbed functional integrity of D-serine-producing cells (e.g., astrocyes; Hashimoto et al, in press; Steffek et al, unpublished data), but even the genetic contributions of *SRR* and *DAO* in other ethnic populations. In future studies, it would be worth pursuing these possibilities and analyzing other genes involved in the relevant metabolic pathways, for example, genes for serine hydroxymethyltransferase and 3-phosphoglycerate dehydrogenase (committed in L-serine biosynthesis in the phosphorylated pathway; Mitoma et al 2004) and genes for serine racemase-interacting proteins including the glutamate receptor interacting protein (Kim et al 2005) and PICK1 (protein interacting with C kinase 1; Hikida et al, unpublished data).

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Supplementary material cited in this article is available online.

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Short communication

Reduced D-serine to total serine ratio in the cerebrospinal fluid of drug naive schizophrenic patients

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Abstract

Several lines of evidence suggest that D-serine, an endogenous agonist of the glycine site on the NMDA receptors, might play a role in the pathophysiology of schizophrenia. The purpose of this study was to determine whether levels of D- and L-serine or D-serine ratio (D-serine/total serine) in cerebrospinal fluid (CSF) were altered in first episode and drug-naive schizophrenic patients. The CSF levels of D- and L-serine in 25 male first episode and drug-naive schizophrenic patients and 17 age-matched male healthy subjects were measured using a column-switching high performance liquid chromatography system. The percentage of D-serine in the total serine of patients was significantly ($z=-2.01$, $p=0.044$) lower than that of controls. This study suggests that synthetic or metabolic pathways of D-serine may be abnormal in the brain of drug-naive schizophrenic patients, supporting the NMDA receptor dysfunction hypothesis of schizophrenia.

Keywords: Cerebrospinal fluid (CSF); D-Serine; L-Serine; NMDA receptor; Schizophrenia

1. Introduction

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 (Javitt and Zukin, 1991; Olney and Farber, 1995; Tamminga, 1998; Goff and Coyle, 2001; Hashimoto et al., 2004, 2005). Several animal studies have demonstrated that levels of free D-serine in the brain are about one third of levels of L-serine, and that the extracellular concentration of D-serine is higher than many common L-amino acids (Hashimoto and Oka, 1997). It has also been shown that D-serine is synthesized by a glial serine racemase, a novel enzyme converting L-serine to D-serine in the mammalian brain (Snyder and Ferris, 2000). Biochemical and electrophysiological studies suggest that endogenous D-serine is a physiological modulator at the co-agonist site of NMDA-type of glutamate receptors (Snyder and Ferris, 2000). Furthermore, administration with D-serine could improve cognition and decrease negative symptoms in schizophrenic patients receiving typical antipsychotics (Goff and Coyle, 2001).

We reported that serum levels of D-serine and the ratio of D-serine to total serine were lower in patients with schizophrenia, suggesting that the activity of serine racemase may be reduced in schizophrenic patients (Hashimoto et al., 2003). Although L- and D-serine are known to pass through the blood–brain barrier, it is still necessary to verify changes of L- and D-serine levels in the cerebrospinal fluid

Abbreviations: CSF, cerebrospinal fluid; HPLC, high performance liquid chromatography; NBD-F, 4-fluoro-7-nitro-2,1,3-benzoxadiazole; NMDA, *N*-methyl-D-aspartate; TFA, trifluoroacetic acid.

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(CSF). In this study, we investigated whether the levels of D- and L-serine in the CSF of first episode and drug-naive schizophrenic patients are different from those of age-matched healthy controls.

2. Methods

2.1. Subjects

Twenty-five male patients with schizophrenia (mean age, 26.1 years; range, 18–39) and 17 age-matched male healthy subjects (mean age, 27.3 years; range, 22–44) with no psychiatric disease were enrolled in Linkoping University Hospital and Uppsala University, Sweden. All patients diagnosed according to the DSM-III-R (American Psychiatric Association, 1987) were first episode and drug-free, i.e. they had never been treated with antipsychotic drugs. All patients did not have any neurological symptoms. The ethics committee of each institute approved the present study, and we received the informed consent from the participants of the study.

2.2. Measurement of total, L-, and D-serine concentration

Measurement of total, L-, and D-serine levels were carried out according to established methods using a column-switching high performance liquid chromatography (HPLC) system (Shimadzu Corporation, Kyoto, Japan). 10 μL of the human CSF was added with 10 μL of 0.1 M borate buffer (pH 8.0) and 30 μL of 50 mM 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F; Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) in CH_3CN. The reaction mixture was then heated at 60 °C for 1 min, and immediately supplemented with 100 μL of H_2O/CH_3CN (90/10) containing 0.1% trifluoroacetic acid (TFA) to stop the reaction. 10 μL of the resultant solution was injected into the HPLC system. A reverse-phase ODS column (TSKgel ODS-80Ts (Tosoh Corporation, Tokyo, Japan) as Column 1) was used for the separation and quantification of total (D+L) serine, and the gradient elution of the mobile phase was kept at a constant flow rate of 0.8 mL/min. Mobile phase 1a consisted of H_2O/CH_3CN (90/10) containing 0.1% TFA, and phases 1b and 1c, of H_2O/CH_3CN (10/90) containing 0.1% TFA and CH_3CN, respectively. The time program for gradient elution was programmed as follows: 0–25.0 min 1a/1b/1c=92:8:0, 25–35 min 1a/1b/1c=0:100:0, and 35–45 min, 1a/1b/1c=0:0:100. The chiral column (Column 2) used for the separation and quantification of D- and L-serine with NBD-F comprised two Sumichiral OA-2500 columns (*S*) (Sumika Chemical Analysis Service Ltd., Osaka, Japan), which were connected in tandem. The mobile phase was 15 mM citric acid in MeOH. The flow rate was isocratically pumped at 0.8 mL/min. The column temperature of all columns was maintained at 35 °C. Fluorescence detection was made at 530 nm with an excitation wavelength at 470 nm.

2.3. Data analysis

Differences between groups were analyzed using the non-parametric Mann–Whitney *U*-test. A $p<0.05$ level was considered significant.

3. Results

The CSF levels (median, 21.5 μM; mean±S.D., 22.8±8.01 μM) of L-serine in the schizophrenic patients were higher ($z=-1.82$, $p=0.069$) than those (median, 18.6 μM; mean±S.D., 18.2±4.78 μM) of normal controls, although statistical analysis was no significant (Table 1). The CSF levels (median, 1.26 μM; mean±S.D., 1.39±0.54 μM) of D-serine in the patients were slightly lower ($z=-0.282$, $p=0.778$) than those (median, 1.43 μM; mean±S.D., 1.39±0.29 μM) of normal controls (Table 1). However, the percentage (median, 5.55%; mean±S.D., 6.01±2.15%) of D-serine in the total serine in the patients was significantly ($z=-2.01$, $p=0.044$) lower than that (median, 7.64%; mean±S.D., 7.41±1.83%) of controls (Table 1).

4. Discussion

The major findings of the present study are that ratio of D-serine to total serine in CSF of first episode and drug naive schizophrenic patients was significantly decreased as compared with those of healthy controls, consistent with our serum results (Hashimoto et al., 2003). This is a first report measuring CSF levels of D- and L-serine in first episode and drug naive patients with schizophrenia. D-Serine is formed from L-serine by serine racemase, a pyridoxal-5′-phosphate dependent enzyme enriched in brain astrocytes (Snyder and Ferris, 2000). Therefore, it is conceivable that the pronounced reduction of the ratio (D-serine/total serine) in the patients may reflect the reduction of enzymatic activity of serine racemase in the brains of drug-naive schizophrenic patients, although further studies of on the synthetic and metabolic pathways (e.g. serine racemase, D-amino acid oxidase, serine hydroxymethyltransferase) and the release

Table 1
CSF levels of L- and D-serine and ratio (D-serine/total serine) in normal controls and drug naive schizophrenic patients

	Controls ($n=17$)	Schizophrenia ($n=25$)	p value
L-Serine (μM)	18.6 (18.2±4.78)	21.5 (22.8±8.01)	0.069
D-Serine (μM)	1.43 (1.39±0.29)	1.26 (1.39±0.54)	0.778
Ratio [%: D/(D+L)]	7.64 (7.41±1.83)	5.55 (6.01±2.15)	0.044*

The values of L-serine, D-serine, and ratio were shown in the median (mean±S.D.).
The comparison among two groups was performed using Mann–Whitney *U*-test.
* $p<0.05$.

and uptake of D-serine are required for investigation of its pathological roles in schizophrenia.

We also found a slightly increase of L-serine levels in the CSF of drug-naive schizophrenic patients, although statistical analysis was no significant. It has already been reported that levels of total serine and glycine in the brain from schizophrenic patients are higher when compared to controls, suggesting a possible abnormality in the pyridoxal-5′-phosphate dependent enzyme, serine hydroxymethyltransferase, which catalyzes the interconversion of serine and glycine (Waziri et al., 1992). Thus, it is suggested that elevated levels of L-serine may, in part, be a result of elevated glycine levels due to the synthesis of serine from glycine. Therefore, it is likely that the synthetic or metabolic pathway of L-serine may be abnormal in schizophrenia, although the exact mechanisms of increased L-serine levels in schizophrenic patients are currently unknown.

In the current study, we could not find significant changes of absolute CSF levels of D-serine in the drug-naive patients although CSF levels of D-serine in patients were slightly lower than those of controls. It is suggested that CSF levels of amino acids (e.g., glutamate) may be sensitive enough to reflect the changes in the defined brain regions (Tsai et al., 1998; Hashimoto et al., 2005). It is, therefore, unclear whether CSF levels of D-serine reflect the actual synaptic activity in the brain regions. However, it is likely that abnormality for synthetic and metabolic pathways of D-serine may be shown in brain of schizophrenia. Furthermore, it should be noted that the sample size (17 controls and 25 patients) for this study was small and that it should be considered exploratory in nature. One should take caution in approaching negative findings in such small samples. Therefore, further studies using a larger sampling of CSF or postmortem brain samples from schizophrenia will be necessary to determine the role of D-serine in the pathophysiology of schizophrenia.

5. Conclusion

The present study demonstrates that ratio of D-serine to total serine may be decreased in the brain of drug-naive schizophrenic patients, suggesting that abnormality for synthetic and metabolic pathways of D-serine in the glia–neuron interaction may be implicated in pathophysiology of schizophrenia.

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The X-box binding protein 1 (XBP1) gene is not associated with methamphetamine dependence

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Abstract

Bipolar disorder has known as a high risk factor for substance abuse and dependence such as alcohol and illegal drugs. Recently, Kakiuchi et al. reported that the −116C/G polymorphism in the promoter region of the X-box binding protein 1 (XBP-1) gene, which translates a transcription factor specific for endoplasmic reticulum stress caused by misfolded proteins, was associated with bipolar disorders and schizophrenia in a Japanese population. Abuse of methamphetamine often produces affective disorders such as manic state, depressive state, and psychosis resembling paranoid-type schizophrenia. To clarify a possible involvement of XBP-1 in the etiology of methamphetamine dependence, we examined the genetic association of the −116C/G polymorphism of the XBP-1 gene by a case–control study. We found no significant association in allele and genotype frequencies of the polymorphism either with methamphetamine dependence or any clinical phenotype of dependence. Because the polymorphism is located in the promoter region of the XBP-1 gene and affects transcription activity of the gene, it is unlikely that dysfunction of XBP-1 may induces susceptibility to methamphetamine dependence.

Keywords: Methamphetamine dependence; Genetics; Association study; X-box binding protein 1

Substance abuse has increased dramatically worldwide. The incidence and prevalence of methamphetamine dependence especially has become increasingly widespread both in the Western world and in Asia. In these epidemic phenomena, genetic contributions to the etiology of substance abuse have been demonstrated by family, twin and adoption studies [2,13,27]. Kendler et al. [13] showed that twin resemblance increases from occasional use to abuse or dependence in a population-based study of twins. The estimated heritability of cocaine use was 0.39, whereas the heritabilities of its abuse and dependence were 0.79 and 0.65, respectively [14]. Analysis of these data may contribute to the understanding of the significance of genetic involvement in substance abuse.

In recent years, bipolar disorders have been focused on as a risk factor for substance abuse. The National Institute of

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Mental Health Epidemiologic Catchment Area study found that over 60% of patients with type-1 bipolar disorder developed a substance abuse during their lifetime [21]. Similar high rates of substance abuse in bipolar disorders have been reported from many different countries [25,30]. In addition, previous studies have suggested that substance abuse might contribute to either the development of bipolar disorders or to a worse prognosis in bipolar disorder subjects [24,26]. Furthermore, acute and chronic amphetamine use, including methamphetamine exposure, can precipitate a psychotic state, with hallucinations and delusions resembling schizophrenia [23,28]. Amphetamine consumption also worsens positive symptoms of schizophrenia and liability to relapse [15,29].

X-box binding protein 1 (XBP-1) was basically extracted for the basic regional leucine zipper transcription factor, which is essential for terminal B-cell differentiation, forming a stable heterodimer with the protein encoded by the proto-oncogene c-fos but not c-jun [7,18]. XBP-1 also functions as a pivotal molecule in the endoplasmic reticulum (ER) stress response [7,18]. Recently, Kakiuchi et al. [10,11] reported that a polymorphism (−116C → G) in the promoter region of the XBP-1 gene, affecting its putative binding site, which impairs the XBP-1 loop in the ER stress response, was associated with bipolar disorders and schizophrenia in a Japanese population. They also indicated that the −116G allele in the promoter region of the XBP-1 gene has lower XBP-1-dependent transcription activity than the −116C allele, and that the dysfunction of XBP-1 may contribute to decreased ER stress response [11]. In addition, Chen et al. [4] reported that a functional polymorphism −197C/G in the XBP-1 gene is also genetic risk factor for schizophrenia in a Chinese population. On the other hand, several studies indicated that there was no significant association between bipolar disorders and the −116G allele of the XBP-1 promoter polymorphism [5,6].

The ER is a subcellular compartment that plays a pivotal role in cellular calcium storage and signaling [1]. When unfolded proteins are accumulated in the ER, ER chaperons such as heat shock protein (Hsp)40, Hsp70, and Hsp90, assist in re-folding them. Accumulation of unfolded proteins in the ER lumen is a warning signal that activates a stress response [12]. Kakiuchi et al. [11] indicated that elevated ER stress due to dysfunction of the XBP-1 loop may enhance susceptibility to bipolar disorders and schizophrenia. Finally, impairment of ER functioning contributes to the pathological processes culminating in neuronal cell death induced by methamphetamine [9,20]. Based on these rationales, we examined a possible genetic association of the XBP-1 −116C/G polymorphism in patients with methamphetamine dependence in a Japanese population.

The subjects were 153 patients with methamphetamine dependence (124 males and 29 females; mean age, 37.8 years; S.D. 12.1 years) meeting the ICD-10-DCR criteria (F15.2), who were outpatients or inpatients in psychiatric hospitals of the Japanese Genetics Initiative for Drug Abuse (JGIDA), and 200 age-, gender-, and geographical origin-matched normal controls (162 males and 38 females; mean age, 37.3 years; S.D. 12.1 years), who were mostly medical staff members without a past individual or family history of drug dependence or psychotic disorders. One hundred and forty-one of the patients also suffered from methamphetamine psychosis. The patients with methamphetamine dependence and/or psychosis were divided into several subgroups by clinical phenotype according to the age at first consumption, multisubstance abuse status, latency of psychosis, prognosis, and spontaneous relapse of psychotic symptoms. In Japan, people becoming 20 years old or more are admitted adult categories. Adult people are legally allowed to consume alcohol and cigarette. So, we divided the patients with methamphetamine dependence into two categories, before and after the age of 20 years. Seventy-six patients (49.7%) had consumed methamphetamine before the age of 20 years, and 77 patients (50.3%) after 20 years old. Forty-eight patients (31.4%) had abused only methamphetamine during their lifetime, and 105 patients (68.6%) had abused drugs other than methamphetamine in the past or present. Besides methamphetamine, organic solvents were the most frequently abused drugs, followed by marijuana. Cocaine and heroin were rarely abused. Sixty patients (42.6%) had developed psychosis less than 3 years after the first methamphetamine consumption and 81 patients (57.4%) after 3 or more years. The course of methamphetamine psychosis varied among patients, and some patients showed sustained psychotic symptoms even after methamphetamine discontinuance, as previously reported [23,28]. Therefore, patients were divided into two categories of psychosis prognosis, the transient type and the prolonged type, which showed remission of psychotic symptoms within 1 month and after more than 1 month, respectively, after the discontinuance of methamphetamine consumption and beginning of treatment with neuroleptics; 85 patients (60.3%) were the transient type, and 56 patients (39.7%) were the prolonged type. It has been well documented that once methamphetamine psychosis has developed, patients in the remission state become liable to spontaneous relapse without reconsumption of methamphetamine [28]. Sixty-five patients had a history of spontaneous relapse and 76 patients did not. This study was performed after obtaining approval from the ethics committees of each institute of JGIDA, and all subjects provided written informed consent for the use of their DNA samples in this research.

The genomic DNA was extracted from peripheral leukocytes using the standard phenol/$CHCl_3$ method. A −116C → G polymorphism of the XBP-1 gene was amplified by polymerase chain reaction (PCR), with 3% dimethyl sulfoxide and 0.75 units of Taq DNA polymerase in a total volume of 15 μl reaction mixture using the following primer sets: 5′-GGT CAT TTT CCG CGG GGG GTT AC-3′and 5′-TTC GTC AGT CTG GAA AGC TCT CG-3′. Initial denaturation was performed for 5 min at 95 °C. Then, 35 cycles were performed (30 s of denaturing at 95 °C, 30 s of annealing at the appropriate temperature, and 30 s of extension at

72 °C), followed by a final extension at 72 °C for 5 min. The PCR products were then digested with Acc65I and analyzed on 3.0% agarose gels. All genotyping was performed in a blinded fashion, with the control and case samples mixed randomly.

Deviation of the genotype counts from Hardy–Weinberg equilibrium was tested using a χ^2 goodness-of-fit test. The statistical significance of differences in the genotype distribution and allele frequency between patients and controls was assessed by a χ^2 test or Fisher's exact test at a significance level of 0.05.

The genotype distribution and allele frequencies of the XBP-1 −116C/G polymorphism for patients with methamphetamine dependence and controls are shown in Table 1. The genotype distribution of all patients and controls subjects did not deviate significantly from Hardy–Weinberg equilibrium at the polymorphic locus. No significant differences were found in the frequency of the genotype or allele of the XBP-1 −116C/G polymorphism between patients with methamphetamine dependence and controls (genotype, $\chi^2 = 2.59$, d.f. = 2, $P = 0.28$; allele, $\chi^2 = 2.71$, d.f. = 1, $P = 0.11$). Neither were found significant differences in the frequency of the genotype or allele between subcategories by clinical phenotype of methamphetamine-dependent patients: age at first methamphetamine consumption less 20 years and more than 20 years (genotype, $\chi^2 = 1.31$, d.f. = 2, $P = 0.53$; allele, $\chi^2 = 0.01$, d.f. = 1, $P = 1$), with and without multiple substance abuse (genotype, $\chi^2 = 1.86$, d.f. = 2, $P = 0.39$; allele, $\chi^2 = 1.93$, d.f. = 1, $P = 0.19$), latency of methamphetamine-induced psychosis less and more than 3 years (genotype, $\chi^2 = 1.74$, d.f. = 2, $P = 0.46$; allele, $\chi^2 = 0.80$, d.f. = 1, $P = 0.44$), psychosis prognosis of transient and prolonged type (genotype, $\chi^2 = 0.37$, d.f. = 2, $P = 0.85$; allele, $\chi^2 = 0.29$, d.f. = 1, $P = 0.6$), or with and without of spontaneous relapse of psychotic symptoms (genotype, $\chi^2 = 1.12$, d.f. = 2, $P = 0.61$; allele, $\chi^2 = 0.44$, d.f. = 1, $P = 0.52$).

XBP-1 is expressed ubiquitously at high levels in the lung, liver, spleen, testis and especially in the prefrontal cortex of the brain [11]. XBP-1 is essential in ER stress response signaling. ER is a subcellular compartment that plays a pivotal role in cellular calcium storage and signaling, whose functioning contributes to the pathological processes culminating in neuronal cell death [1]. Dysregulation of intracellular calcium homeostasis can cause ER stress and ER-induced apoptosis [19]. Calcium-mediated cell death is associated with the activation of various proteases [17] that can cleave substrates, including actin and fodrin, some of which are essential for maintenance of cellular homeostasis [8,16]. Kakiuchi et al. [11] reported that the −116G allele in the promoter region of the XBP-1 gene has about 50% lower XBP-1-dependent transcription activity than the −116C allele. Since they found a significant excess of −116G allele in patients with bipolar disorders and schizophrenia, they hypothesized that disturbance of the ER stress-signaling induced by the impaired XBP-1 feedback system may contribute to susceptibility to these diseases [10,11].

Several reports indicated that methamphetamine-induced nerve terminal degeneration and cell death occurs consequent to interactions of ER stress and mitochondrial death pathways [3]. Neurotoxicity due to methamphetamine administration

Table 1
Distributions and allele frequency of the X-box binding protein 1 (XBP-1) −116C/G polymorphism genotype in patients with methamphetamine (METH) dependence

Group	*N*	Genotype				Allele		
		C/C (%)	C/G (%)	G/G (%)	*P* value	C (%)	G (%)	*P* value
Control	200	32 (16.0)	88 (44.0)	80 (40.0)		152 (38.0)	248 (62.0)	
METH dependence/psychosis	153	17 (11.1)	64 (41.8)	72 (47.1)	0.28	98 (32.0)	208 (68.0)	0.11
Subcategories of METH dependence/psychosis								
Age at first use								
20 years<	76	7 (9.2)	35 (46.1)	34 (44.7)		49 (32.2)	103 (67.8)	
20 years≥	77	10 (13.0)	29 (37.7)	38 (49.4)	0.53	49 (31.8)	105 (68.2)	1
Multisubstance abuse								
No	48	7 (14.6)	22 (45.8)	19 (39.6)		36 (37.5)	60 (62.5)	
Yes	105	10 (9.5)	42 (40.0)	53 (50.5)	0.39	62 (29.5)	148 (70.5)	0.19
Latency of psychosis								
3 years<	60	4 (6.7)	26 (43.3)	30 (50.0)		34 (28.3)	86 (71.7)	
3 years≥	81	11 (13.6)	32 (39.5)	38 (46.9)	0.46	54 (33.3)	108 (66.7)	0.44
Prognosis of psychosis								
Transient	85	8 (9.4)	35 (41.2)	42 (49.4)		51 (30.0)	119 (70.0)	
Prolonged	56	7 (12.5)	23 (41.1)	26 (46.4)	0.85	37 (33.0)	75 (67.0)	0.6
Spontaneous relapse of psychotic symptoms								
No	76	10 (13.2)	30 (39.5)	36 (47.4)		50 (32.9)	102 (67.1)	
Yes	65	5 (7.7)	28 (43.1)	32 (49.2)	0.61	38 (29.2)	92 (70.8)	0.52

Numbers in parentheses indicate percentages. Statistical analysis was performed by a χ^2 test or Fisher's exact test.

is considered to be one of the neural mechanisms underlying methamphetamine dependence, withdrawal, and psychosis. However, the present study did not find any significant association between the XBP-1 −116C/G polymorphism and methamphetamine dependence. We also examined a possible association between clinical phenotypes of methamphetamine dependence and psychosis, such as age at first methamphetamine consumption, latency to onset of psychosis, prognosis, spontaneous relapse, and multisubstance abuse status; however, there was no significant association with any clinical phenotype either. The present results indicate that XBP-1 dysfunction is not involved in the pathophysiology of methamphetamine dependence and psychosis.

ER has many sophisticated stress-signaling pathways besides XBP-1 systems that enable it to adapt to a whole host of stress factors. Recent studies demonstrated that other ER-stress-related molecules are involved in methamphetamine neurotoxicity. Thus, methamphetamine activates calpain, a Ca^{2+}-responsive protease, caspase-12, GRP78/Bip, and CHOP/GADD153 (C/EBP homology protein/growth arrest and DNA damage 153) and results in ER-dependent cell death [3]. CHOP can form functionally negative heterodimers with members of the C/EBP family [22], and it has also been implicated in apoptosis [31]. Methamphetamine induced high expression of CHOP protein, and CHOP down-regulated Bcl-2 expression and up-regulated the proapoptotic Bcl-2 family members BAX and BAK [9]. Increased BAX and BAK accumulate in ER and mitochondria, followed by caspase-independent Bcl-2 sensitive release of Ca^{2+} from ER and subsequent Ca^{2+} accumulation in mitochondria, which disrupts cellular metabolism and induces cell death via release of cytochrome c and associated activation of the caspase-dependent cell death pathway. Our genetic results also indicate that molecules other than XBP-1 may be involved in a methamphetamine-induced ER-dependent stress pathway and the susceptibility to methamphetamine neurotoxicity of patients with methamphetamine dependence and/or psychosis.

The power analysis showed that the present sample size had a power of 0.93 and 0.92 to detect a small effect size ($w = 0.184$ and 0.128) at an alpha value of 0.05 to detect significant genotypic and allelic associations between controls and all methamphetamine patients. The present total sample size can be, therefore, considered large enough statistically. However, the statistical power deteriorated in the analysis of the subgroups of patients divided by clinical phenotypes, and it is possible that negative results about clinical phenotypes of methamphetamine dependence and psychosis result from a type I error.

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Tropisetron improves deficits in auditory P50 suppression in schizophrenia

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Abstract

Physiological deficits in inhibition of the P50 auditory evoked potential in schizophrenia have been related to diminished expression of α7 nicotinic acetylcholine receptors. Diminished P50 inhibition is correlated with neuropsychological deficits in attention, one of the principal neurocognitive disturbances in schizophrenia. Nicotine administration improves P50 inhibition, presumably by achieving additional activation of these diminished receptors, but its toxicity and marked tachyphylaxis make it an ineffective therapeutic. Nicotine also has weak positive effects on several neurocognitive deficits in schizophrenia, which raises the possibility that the α7 nicotinic receptor is a clinically relevant therapeutic target that should be addressed by less toxic agents. Tropisetron, a drug already approved for clinical use outside the United States as an anti-emetic, is a partial agonist at α7 nicotinic receptors and an antagonist at 5-HT_3 receptors. As an initial proof-of-principle study, we determined that a single administration of tropisetron significantly improves P50 inhibition in schizophrenia. These data are consistent with biological activity at a pathophysiological mechanism in schizophrenia and support further trials of this drug as a possible therapeutic for neurocognitive deficits in schizophrenia.

Keywords: Nicotinic receptors; Serotonin receptors; Evoked potentials auditory; Schizophrenia; Tropisetron; Drug therapy

1. Introduction

Inadequate modulation of response to sensory input is a hallmark of schizophrenia (Venables, 1967; Braff and Freedman, 2002). Deficits in sensory gating in schizophrenia can be measured by recording

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the P50 auditory evoked potential in response to repeated stimuli. At a 500 ms interval between the first, or conditioning stimulus, and the second, or test stimulus, most normal subjects significantly decrease the amplitude of the test P50 response compared to the conditioning response, whereas patients with schizophrenia have diminished suppression (Adler et al., 1982; Braff and Geyer, 1990). Abnormal P50 suppression has been linked to genetic markers at the locus of the α7 nicotinic acetylcholine receptor subunit gene on chromosome 15q13–14 (Freedman et al., 1997; Raux et al., 2002).

Additional evidence indicates involvement of the α7 nicotinic receptor in schizophrenia. Postmortem brain tissue from schizophrenic subjects has diminished numbers of α7 nicotinic receptors labeled by [^{125}I]α-bungarotoxin in the hippocampus (Freedman et al., 1995). A similar decrease in α-bungarotoxin binding is reported in the nucleus reticularis thalami (Court et al., 1999). Decreased α7 nicotinic receptor peptide immunoreactivity has been found in the frontal cortex of schizophrenic patients (Guan et al., 1999). Diminished expression of α7 nicotinic receptors may lead to failure of cholinergic activation of inhibitory interneurons that is manifest physiologically as deficits of P50 suppression in schizophrenia (Freedman et al., 2000).

Abnormal P50 suppression in patients and their relatives normalizes following administration of the archetypal nicotinic receptor agonist, nicotine (Adler et al., 1992, 1993). Nicotine also improves some aspects of neuropsychological performance in schizophrenia (Smith et al., 2002; Myers et al., 2004; Jacobsen et al., 2004; Harris et al., 2004). However, because the effects of nicotine are short lived and accompanied by significant receptor desensitization and other toxic effects, nicotine itself has no practical therapeutic effect in schizophrenia. Nonetheless, less toxic agents that activate α7 nicotinic receptors be a new class of therapeutic agents for potential use in the treatment of schizophrenia (Simosky et al., 2002).

Both α7 nicotinic receptors and serotonin 5-HT$_3$ receptors are members of the superfamily of ligand-gated ion channels. These two receptors have the greatest similarity within the family, with approximately 30% sequence homology (Maricq et al., 1991). Tropisetron is a potent 5-HT$_3$ receptor antagonist that is widely used in the treatment of patients with chemotherapy-induced or postoperative nausea and vomiting (Simpson et al., 2000). Recently, it has been demonstrated that tropisetron is also a partial agonist at α7 nicotinic receptors with a high affinity (K_i=6.9 nM for α7 nicotinic receptors, K_i=5.3 nM for 5-HT$_3$ receptors) (Macor et al., 2001; Papke et al., 2004). Because tropisetron is already approved for human use outside the United States, we were able to examine the effects of tropisetron on P50 suppression in schizophrenic patients in the present study.

2. Methods

2.1. Subjects

Twenty-two schizophrenic patients (14 men, 8 women) were recruited from Chiba University Hospital and Kimura Hospital, Chiba, Japan. All patients fulfilled American Psychiatric Association DSM-IV criteria for schizophrenia. Age of the patients was 39.8±14.0 years (mean±standard deviation). They did not have current or recent histories of alcohol or drug abuse. Ten patients had never smoked and two patients had abstained from tobacco for many years. One patient was medication-free. Antipsychotic drugs administered for treatment were olanzapine (5–20 mg/day, n=4), risperidone (4–11 mg/day, n=12), perospirone (16–48 mg/day, n=6), quetiapine (500–750 mg/day, n=2), levomepromazine (25 mg/day, n=1), propericyazine (80 mg/day, n=1), fluphenazine decanoate (25 mg/4 weeks, n=1), pimozide (2 mg/day, n=1) and zotepine (50 mg/day, n=1). The mean dosage in chlorpromazine equivalents was 640±432 mg/day. Eleven male and four female healthy controls (age 21 to 44 years) were also studied. The ethics committee of Chiba University Graduate School of Medicine approved the study. All subjects provided written informed consent for their participation in the study after the procedure had been fully explained to them.

2.2. Experimental protocol

P50 auditory evoked potentials were recorded before administration of tropisetron. Two capsules (10 mg total dose) of tropisetron (Navoban®, Novartis Pharmaceuticals Ltd., Tokyo, Japan) were adminis-

trated, and 1 h later P50 auditory evoked potentials were again recorded. Oral administration of tropisetron (5 mg) in healthy subjects has a half-life ($T_{1/2}$) of 5.7 h and time of maximum plasma concentration (T_{max}) of 2.6 h (Kees et al., 2001).

2.3. Measurement of P50 suppression

Subjects were seated in a comfortable recliner and instructed to relax with their eyes open and to focus on a fixation point. The testing took place in a quiet, lighted room. The subject was monitored visually and by electroencephalogram (EEG) for signs of sleep or slow wave activity, which prompted the experimenter to speak briefly with the subject. The 120 pairs of auditory clicks were presented every 10 s, with a 500-ms interclick interval. The stimuli were 1 ms square waves amplified to 70 dB SPL. Recordings were performed with gold disc electrodes at Fz, Cz and Pz with a forehead ground and linked earlobe reference. Data are presented from Cz site; recordings at Cz best discriminate schizophrenia patients from normal subjects (Clementz et al., 1998). Eye movements and blinks were monitored by electrooculographic (EOG) recording. The resistance of all electrodes was less than 10 kΩ. EEG activity was recorded by SYNAFIT2500 amplifiers (NEC) with filters at 0.5 and 100 Hz. Data were acquired at a 500 Hz digitization rate and individual trials were stored to disk for analysis. Individual trials were rejected if EEG or EOG voltage was greater than ±70 μV, generally indicative of excessive muscle activity, eye movements or other artifacts.

The conditioning P50 wave was identified by a rater blind to subject identity and treatment condition as the most positive peak between 40 and 90 ms after the conditioning stimulus. The test P50 wave was identified as the positive peak after the test stimulus that was closest in latency to the conditioning P50. Amplitude was defined as the difference between the positive peak and the preceding negative trough for both waves. The P50 T/C ratio was calculated by dividing the test P50 amplitude by the conditioning P50 amplitude.

2.4. Statistical analysis

The data are presented as means±standard deviation. Data analysis was performed using SPSS version 12.0 (SPSS Inc., Chicago, IL). The effect of tropisetron was assessed with Student's paired sample *t*-test. Relationships between two variables were examined using Pearson's correlation coefficients. *P* values less than 0.05 (two-tailed) were considered statistically significant.

3. Results

The 22 patients with schizophrenia had a mean P50 T/C ratio at baseline of 0.84±0.55, which was

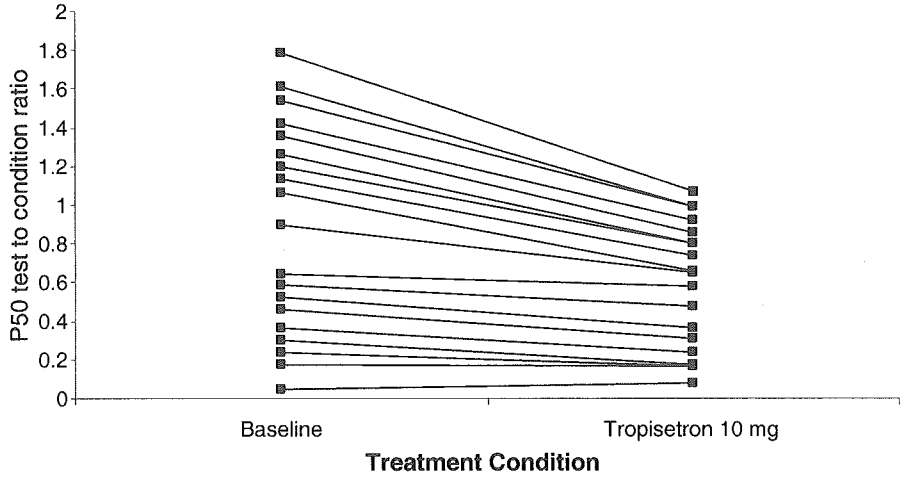

Fig. 1. The ratio of test P50 amplitude to conditioning amplitude before and after tropisetron administration in schizophrenia patients.

significantly greater than the mean P50 T/C ratio recorded in 11 control subjects (0.36±0.30, t=2.68, df=31, P=0.012). The P50 T/C ratios of three patients were lower than 0.50 (0.07, 0.25 and 0.28). Because their values were already within the normal range, the probability of these patients responding to tropisetron was considered too low to justify their exposure to the drug. For the remaining 19 patients with schizophrenia, the P50 T/C ratios were significantly decreased after administration of tropisetron. The mean baseline ratio was 0.94±0.53, compared to 0.61±0.33 after tropisetron administration (paired t-test=2.32, df=18, P=0.033). The change in P50 T/C ratio from baseline to post drug administration was significantly correlated with the baseline value (r=0.85, t=6.60, df=17, P<0.001; Fig. 1). Although there was a trend towards increased conditioning P50 amplitude and decreased test P50 amplitude after tropisetron administration, the amplitudes and latencies of the conditioning and test P50 were not significantly different between the two time points (Table 1).

Baseline P50 T/C ratios were higher, but not significantly, in the 11 schizophrenic patients who did not smoke (1.09±0.63), compared to the 8 patients who did smoke (0.74±0.26, t=1.64, df=18, P=0.117). The effect of tropisetron was significant only for the non-smokers, who decreased their P50 ratios by 0.54±0.69 (paired t-test=2.61, df=10, P=0.026). The smokers decreased their P50 ratios by 0.04±0.38, a non-significant change.

Eight control subjects were recorded twice at the same interval experienced by the patients with schizophrenia during drug administration; their ratios increased slightly, but non-significantly, over the same interval (mean change 0.085±0.11, paired t-test=2.13, df=7, P=0.070).

Table 1
Effects of tropisetron on auditory sensory gating in schizophrenia

P50 parameters	Baseline		Topisestron 10 mg	
	Mean	S.D.	Mean	S.D.
Amplitude (μV)				
Conditioning	2.40	1.31	2.65	1.47
Test	1.93	0.96	1.45	1.24
Latency (ms)				
Conditioning	58	13	60	13
Test	56	16	59	14
P50 T/C ratio[a]	0.94	0.53	0.61	0.33

[a] Significant treatment difference, paired t=2.32, df=18, P=0.033.

4. Discussion

Tropisetron, a partial agonist at α7 nicotinic receptors, improved deficits in P50 suppression in schizophrenic patients. Suppression in patients with initially high P50 T/C ratios was improved by administration of tropisetron; ratios in some patients with lower P50 T/C ratios were not improved. Only four patients recovered to normal levels (P50 T/C ratio <0.5). Further studies using higher doses (e.g., 20–30 mg) of tropisetron are needed to establish the full effect of tropisetron, since only a low dose (10 mg) of tropisetron was used in this study.

Tropisetron is also a potent antagonist at 5-HT_3 receptors. Therefore, it is unclear whether improvement of P50 suppression by tropisetron is mediated via direct agonist effects on α7 nicotinic receptors or via effects on 5-HT_3 receptors. We have found recently that tropisetron normalizes deficient auditory gating in DBA/2 mice and that this effect of tropisetron is blocked by co-administration of the selective α7 nicotinic receptor antagonist methyllycaconitine, suggesting that activation of α7 nicotinic receptors is necessary for normalization of auditory gating deficits by tropisetron (Hashimoto et al., submitted). A nicotinic cholinergic mechanism is also supported by the finding in the present study that tropisetron's effects are diminished in patients who smoke. Chronic exposure to nicotine desensitizes nicotinic receptors and thereby effectively blocks the effects of additional nicotinic receptor stimulation (Harris et al., 2004). However, tropisetron, like other 5-HT_3 antagonists, increases release of acetylcholine (Ach) in the brain (Consolo et al., 1994; Giovannini et al., 1998; Maura et al., 1992). Therefore, tropisetron's stimulation of nicotinic receptors could be an indirect effect of 5-HT_3 antagonism. Recently, Adler et al. (in press) have demonstrated that ondansetron, a highly selective 5-HT_3 receptor antagonist, improves deficits of P50 suppression in schizophrenic patients. Thus, the effects of the α7-nicotinic receptor stimulation and 5-HT_3 antagonism may be synergistic.

Indirect activation of nicotinic receptors has also been proposed as a mechanism of action of some atypical neuroleptics. Several atypical antipsychotic drugs (e.g., clozapine, olanzapine, risperidone) improve P50 suppression in schizophrenic patients, whereas typical antipsychotic drugs do not have this effect (Light et al., 2000; Adler et al., 2004). P50 suppression for patients receiving clozapine, olanzapine, and risperidone was 82.6%, 75.0% and 39.2%, respectively (Light et al., 2000), indicating that the effect of risperidone on deficits of P50 suppression is weaker than that of clozapine and olanzapine. In the present study, 3 patients treated with olanzapine had normal P50 suppression (T/C ratio <0.5), whereas 12 patients treated with risperidone had deficits in P50 suppression (T/C ratio >0.5), consistent with this previous report (Light et al., 2000). The mechanisms underlying the difference between clozapine (or olanzapine) and risperidone are currently unknown. One possibility is their difference in affinity for 5-HT_3 receptors. Clozapine and olanzapine have moderate to high affinity for 5-HT_3 receptors (clozapine: K_i=108 nM, olanzapine: K_i=84 nM), whereas risperidone has no affinity for 5-HT_3 receptors (K_i>10,000 nM) (Leysen et al., 1996). Clozapine, like ondansetron, increases Ach levels in rat brain (Ichikawa et al., 2002), and it is possible that this increased Ach activates α7 nicotinic receptors. This hypothesis is in agreement with Simosky et al. (2003), who observed that normalization of sensory inhibition in DBA/2 mice by clozapine was reversed by the α7 nicotinic receptor antagonist α-bungarotoxin.

The neurobiological effect of tropisetron demonstrated in the present study suggests future studies to assess the effects of tropisetron on cognitive dysfunction in schizophrenia, because P50 deficits have been previously associated with attentional deficits in schizophrenia (Cullum et al., 1993). These attentional deficits are in turn associated with psychosocial deficits that comprise the long-term poor outcome of schizophrenia (Green, 1996).

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