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Increased serum levels of glutamate in adult patients with autism

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Abstract

Background: Precise mechanisms underlying the pathophysiology of autism are currently unknown. Given the major role of glutamate in brain development, we have hypothesized that glutamatergic neurotransmission plays a role in the pathophysiology of autism. In this study, we studied whether amino acids (glutamate, glutamine, glycine, D-serine, and L-serine) related to glutamatergic neurotransmission are altered in serum of adult patients with autism.
Methods: We measured serum levels of amino acids in 18 male adult patients with autism and age-matched 19 male healthy subjects using high-performance liquid chromatography.
Results: Serum levels (mean = 89.2 μM, S.D. = 21.5) of glutamate in the patients with autism were significantly ($t = -4.48$, $df = 35$, $p < 0.001$) higher than those (mean = 61.1 μM, S.D. = 16.5) of normal controls. In contrast, serum levels of other amino acids (glutamine, glycine, D-serine, L-serine) in the patients with autism did not differ from those of normal controls. There was a positive correlation ($r = 0.523$, $p = 0.026$) between serum glutamate levels and Autism Diagnostic Interview–Revised (ADI-R) social scores in patients.
Conclusions: The present study suggests that an abnormality in glutamatergic neurotransmission may play a role in the pathophysiology of autism.

Keywords: Amino acids; Autism; D-Serine; Glutamate; HPLC; Human serum

1. Introduction

Autism is a neuropsychiatric disorder characterized by severe and sustained impairment in social interaction, deviance in communication, and patterns of behavior and interest that are restricted, stereotyped, or both (Volkmar and Pauls, 2003). Although genetic and environmental factors are implicated in the pathophysiology of autism, the precise mechanisms underlying the pathophysiology of this disorder remain to be determined (Volkmar and Pauls, 2003; Baron-Cohen and Belmonte, 2005; Polleux and Lauder, 2004; McDougle et al., 2005).

Glutamate, the major excitatory neurotransmitter in the brain, plays a major role in brain development, affecting neuronal migration, neuronal differentiation, axon genesis, and neuronal survival (Coyle et al., 2002). Accumulating evidence suggests that abnormalities in glutamatergic neurotransmission may play a role in the pathophysiology of autism (McDougle et al., 2005). First, cDNA microarray technology has demonstrated that the glutamate neurotransmitter system is abnormal in postmortem brain samples of autism (Purcell et al., 2001). The mRNA levels of genes, including excitatory amino acid transporter 1 (EAAT 1) and AMPA-type glutamate receptor, are significantly increased in the brain of autism, suggesting abnormalities of glutamatergic neurotransmission in the pathogenesis of this disorder (Purcell et al., 2001). Genetic studies have demonstrated the involvement

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of single nucleotide polymorphisms (SNPs) in the genes encoding both metabotropic and ionotropic glutamate receptors in autism (Jamain et al., 2002; Serajee et al., 2003). Furthermore, a strong association of autism with SNPs within SLC25A12, a gene encoding the mitochondrial aspartate/glutamate carrier (AGC1), has been demonstrated, suggesting the potential etiological role of AGC1 in autism (Ramoz et al., 2004; Segurado et al., 2005). However, recent two studies using large samples did not confirm the association of SLC25A12 gene and autism, suggesting that the SLC25A12 gene is not a major contributor to genetic susceptibility of autism (Blasi et al., 2006; Rabionet et al., 2006).

Second, it has been reported that blood levels of glutamate are altered in patients with autism (Rolf et al., 1993; Moreno-Fuenmayor et al., 1996; Aldred et al., 2003). Rolf et al. (1993) have reported that plasma levels of glutamate in children (8–14-year-olds) with autism are significantly decreased compared to age-matched healthy controls. In contrast, Aldred et al. (2003) have reported that plasma levels of glutamate in patients (4–29 year-olds) with autism or Asperger's syndrome are significantly increased compared with controls. One of the reasons for such contradictory findings could be a difference in sample composition; the study by Aldred et al. (2003) incorporated a wider age range. Nonetheless, previous studies indicate alterations in the glutamatergic system expressed at the periphery level. The studies reporting on blood levels of glutamate in autistic patients present inconsistent results. Therefore, it is of great interest to examine whether levels of amino acids such as glutamate are altered in autistic patients.

Several lines of evidence suggest that D-serine, an endogenous co-agonist at the NMDA receptors, plays a role in the pathophysiology of schizophrenia, which is a neurodevelopmental disorder (Snyder and Ferris, 2000; Coyle and Tsai, 2004; Hashimoto et al., 2005a). We have previously reported that serum levels of D-serine are significantly decreased in patients with schizophrenia (Hashimoto et al., 2003; Yamada et al., 2005). However, to our knowledge, serum D-serine levels have never been investigated in relation to autism.

The purpose of the present study was, therefore, to examine whether individuals with autism have aberrant serum levels of D-serine as well as other amino acids (glutamate, glutamine, glycine, and L-serine) associated with glutamatergic neurotransmission. Furthermore, we also examined any relationship between amino acid levels and clinical symptoms in autistic patients.

2. Methods

2.1. Participants

Eighteen male autistic subjects (mean age = 21.2 years, S.D. = 2.1, range = 18–26) and age-matched 19 male healthy control subjects (mean = 22.2 years, S.D. = 2.2, range = 18–26) were included in this study (Table 1). All participants for both groups were Japanese. The autistic subjects were recruited through advocacy groups in Nagoya and Hamamatsu cities, which are located in the middle of the mainland of Japan. For the diagnosis of autism, the recruited individuals were initially assessed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)(American Psychiatric Association, 1994), followed by assessment using the Autism Diagnostic Interview–Revised (ADI-R)(Lord et al., 1994) by trained child psychiatrists clinicians (KJT and AS). Participants were excluded from the study, if they had a diagnosis of fragile X syndrome, epileptic seizures, obsessive–compulsive disorder, affective disorders, or any additional psychiatric or neurological diagnoses. All the autistic subjects were drug-naive or had been free of psychoactive medications for at least 6 months. Healthy controls were recruited from Hamamatsu City by advertisement. All control-group participants underwent a comprehensive assessment of medical history to eliminate individuals with any neurological or other medical disorders. The Structured Clinical Interview for the DSM-IV (SCID) was also conducted to scrutinize any personal or familial history of past or present mental illness. None of the comparison subjects initially recruited was found to fulfill these exclusion procedures. After the participants were given a complete description of the study, written informed consent was obtained from all subjects before they entered the study. This study received approval from the ethics committee of the Hamamatsu University School of Medicine and Chiba University Graduate School of Medicine.

Table 1
Clinical characteristics of 18 adult patients with autism

Characteristics	Mean ± S.D. (range)
Age at onset (years)	3.72 ± 1.07 (1–5)
Duration of illness (years)	17.5 ± 2.23 (14–22)
ADI-R	
A. Social	22.11 ± 4.96 (14–29)
B. Communication	15.44 ± 4.84 (6–21)
C. Stereotype	5.22 ± 1.77 (3–10)
Y-BOCS	11.28 ± 5.39 (2–26)
Obsession	6.44 ± 3.13 (1–14)
Compulsion	4.94 ± 3.62 (0–14)
AQ-Aggression	50.56 ± 12.3 (34–69)
Theory of Mind—Faux Pas Test	23.44 ± 8.16 (6–34)
IQ	
Full-scale IQ	96.83 ± 20.33 (62–140)
Verbal IQ	95.11 ± 19.87 (53–131)
Performance IQ	100.4 ± 18.4 (75–137)

ADI-R: Autism Diagnostic Interview–Revised, Y-BOCS: Yale–Brown Obsessive–Compulsive Scale, AQ: Aggression Questionnaire, IQ: Intellectual Quotient.

2.2. Psychological measures

ADI-R is a semi-specially formulated structured psychiatric interview with a parent, especially a mother, which is administered to the parent. It is used to confirm diagnosis and also to evaluate the core symptoms of autism. ADI-R is based on three separate scores. Score A quantifies impairment in social interaction (the range of score: 0–32), score B quantifies impairment in communication (the range of score: 0–26), and score C quantifies restricted, repetitive, and stereotyped patterns of behavior and interests (the range of score: 0–16). Higher scores on each indicate worse performance.

Obsessional/repetitive behavior was rated using the Yale-Brown Obsessive–Compulsive Scale (Y-BOCS)(Goodman