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Increased plasma agmatine levels in patients with schizophrenia

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Agmatine is an endogenous substance, synthesized from L-arginine, and it is proposed to be a new neurotransmitter. Preclinical studies indicated that agmatine may have an important role in the pathophysiology of schizophrenia. This study was organized to investigate plasma agmatine in patients with schizophrenia and in healthy controls. Eighteen patients with schizophrenia and 19 healthy individuals constituted the subjects. Agmatine levels in the plasma were measured using the HPLC method. The S100B protein level, which is a peripheral biomarker for brain damage, was also measured using the ELISA method. While plasma levels of agmatine in patients with schizophrenia were significantly increased ($p < 0.0001$) compared to those of healthy individuals (control), there were no significant changes in the levels of S100B protein ($p = 0.660$). An ROC (receiver operating characteristic) curve analysis revealed that measuring plasma agmatine levels as a clinical diagnostic test would significantly differentiate between patients with schizophrenia and those in the control group (predictive value: 0.969; $p < 0.0001$). The predictive value of S100B measurements was not statistically significant ($p > 0.05$). A multiple regression analysis revealed that the age of the patient and the severity of the illness, as indicated by the PANSS score, significantly contributed the plasma agmatine levels in patients with schizophrenia. These results support the hypothesis that an excess agmatine release is important in the development of schizophrenia. The findings also imply that the plasma agmatine level may be a potential biomarker of schizophrenia.

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1. Introduction

Agmatine is a cationic polyamine that is synthesized through the decarboxylation of the conditionally essential amino acid L-arginine. It is produced in bacteria, plants and invertebrates and is highly conserved in nature (Tabor and Tabor, 1984). The presence of agmatine in the brain has been shown, and agmatine was proposed to be a new neurotransmitter (Reis and Regunatan, 1998). Agmatine, much like other central neurotransmitters, is synthesized in the brain, stored in the synaptic vesicles of neurons, inactivated by reuptake, degraded by specific enzymes (namely, agmatinase and diamine oxidase), and released from axon terminals by Ca^{2+}-dependent depolarization (Reis and Regunatan, 1998). Recently, agmatinase was found to be localized in pre- and postsynaptic terminals, and proposed to regulate agmatine activity in neurons (Madai et al., 2012). Agmatine binds some specific receptors, such as glutamatergic N-methyl-D-aspartate (NMDA) and α2-adrenergic and imidazoline receptors (Reis and Regunatan, 1998; Halaris and Piletz, 2007). Thus, agmatine extensively meets the criteria for neurotransmitters in the central nervous system (CNS) (Reis and Regunatan, 2000; Uzbay, 2012). Agmatine has also been proposed to be an endogenous antidepressant substance, moreover in a postmortem study, agmatinase, the enzyme inactivates agmatine, was found to be strongly upregulated in hippocampal interneurons of subjects with mood disorders (Bernstein et al., 2012).

Recently, we reported that a high dose of agmatine disrupted the prepulse inhibition (PPI) of the acoustic startle reflex and dramatically potentiated the apomorphine-induced disruption of the PPI in rats (Uzbay et al., 2010a). Measurement of the PPI is an important tool to better understand the impairments in information processing of schizophrenia spectrum disorders (Geyer and Braff, 1987); disruptions of the PPI are related to the cognitive symptoms of schizophrenia, as well as its negative and positive

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symptoms (Braff et al., 1999). Thus, the results from our study (Uzbay et al., 2010a) implied that agmatine may play a key role in the pathogenesis of schizophrenia. Moreover, the end products of agmatine metabolism, such as spermidine and spermine, were found to be increased in postmortem brains from patients with schizophrenia (Richardson-Andrews, 1983; Andrews, 1985; Das et al., 1989; Ramchand et al., 1994; Gilad et al., 1995). Falayeh (1988) reported that serum spermidine oxidase activity was elevated in patients with schizophrenia. Moreover, asymmetric dimethylarginine (ADMA), which is an endogenously formed precursor of cell-signaling molecules, such as nitric oxide (NO) and agmatine, was also detected at higher levels in the plasma of patients with schizophrenia than in healthy controls (Das et al., 1996; Kopieczna-Grzebieniak and Goss, 2005). Overall, these findings support our hypothesis that an increased brain agmatine level may play an important role in the pathogenesis of schizophrenia.

The main objective of the present study was to test the agmatine hypothesis and to acquire additional clinical evidence to support the role of agmatine in the pathogenesis of schizophrenia. Thus, we measured the plasma agmatine levels in patients with schizophrenia and in healthy volunteers. Because agmatine is an endogenous substance that may be secreted in response to stressful conditions of the brain, we also assessed the levels of the S100B protein to evaluate whether the agmatine levels are changed as a result of astroglial cell activation. The S100B protein is produced by astrocytes in the brain, and an increase in the levels of this protein is related to immune activation of these cells. Immune activation in the brain and increase in S100B protein have been related to neuronal damage and several neurodegenerative disorders (Rothermundt et al., 2009). S100B has also been related to neurons (Steiner et al., 2007). Previous studies showed an increase in S100B protein levels in the cerebrospinal fluid (CSF) and serum of patients with schizophrenia, and proposed that there is an association between high S100B levels and poor therapeutic response (Rothermundt et al., 2001, 2009; Schmitt et al., 2005; Zhang et al., 2010).

2. Material and methods

2.1. Participants and ethical conditions

By means of a semi-structured interview, based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), patients and their relatives were investigated. Eighteen patients with schizophrenia (11 male and 7 female) were recruited from the admissions to the Department of Psychiatry in Uludag University, Faculty of Medicine, Bursa, Turkey, from March 2011 to January 2012. None of them had taken any psychotropic medicine during the previous 6 months. The selection criteria included the DSM-IV. The exclusion criteria included a history of other diseases of the central nervous system, such as dementia, Parkinson's, major depressive disorder, and bipolar disorder; other chronic serious disorders, such as cancer, hypertension, and diabetes; unstable medical conditions, such as metabolic syndrome; and some special conditions, such as pregnancy and breast feeding. None of the healthy volunteers (11 male and 8 female) had serious disorders including mental, endocrine or cardiovascular problems. The patients' ages ranged between 23 and 66 years. The 6 of the subjects were the first-episode patients, and the other patients were the drug-free for the last 6 months. The demographic features of the patients and healthy volunteers are shown in Table 1.

The symptoms of schizophrenia were assessed using the Positive and Negative Syndrome Scale (PANSS). Considering the circadian rhythm, blood samples were obtained between 08:30 and 10:00 a.m. from antecubital veins into heparinized tubes just before the breakfast. The blood was centrifuged at 2000× g for 15 min at 4 °C. Then, the plasma samples were stored at −80 °C until the day of the assay.

Table 1
Demographical features of the patients and healthy volunteers.

	Patients with schizophrenia	Healthy controls
Subject number (sex)	18 (11 male, 7 female)	19 (11 male, 8 female)
Age (years) (range)	37.39 ± 2.97 (23–65)	33.89 ± 1.42 (23–43)
Illness duration (years)	9.83 ± 2.37	
PANSS score	87.17 ± 3.13	
Agmatine levels (ng/mL)	34.96 ± 3.74*	7.98 ± 1.59
S100B levels (pg/ml)	7.81 ± 2.50	6.26 ± 1.80

Data were expressed as mean ± S.E.M., *$p < 0.05$, Mann–Whitney U test.

The ethical committee of the Uludag University approved this study (Date 09 September, 2009; No: 2009-11/104). Written informed consent was also obtained from all participants.

2.2. Chemicals and reagents

Agmatine sulfate salt, potassium dihydrogen sulfate, potassium hydroxide, boric acid, o-phthalaldehyde (OPA), 2-mercaptoethanol (ME), octyl sulfate sodium salt, perchloric acid, hydrochloric acid, high-pressure liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Sample preparation and derivatization

Each 2 ml of plasma obtained from the patients and healthy volunteers was divided into two separate tubes, each containing 1 ml. One hundred microliters of deionized water was added to the first tube, and an equal volume of agmatine standard was added to the second tube to serve as an internal standard (2 μM, final concentration). Then, each plasma sample was deproteinized with 700 μL of 1 M perchloric acid plus 0.1 M hydrochloric acid and left on ice for 1 h before centrifugation for 15 min at approximately 1000× g at 4 °C. Supernatant samples of 1.5 ml were neutralized with 5 M NaOH and mixed with 750 μL of derivatization reagent. The OPA and ME derivatizing reagent was prepared daily by dissolving 50 mg of OPA in 1 ml of methanol and then adding 53 μL of ME into 9 ml of 3% KOH and 3% H_2BO_4.

2.4. Extraction, concentration, and measurement of plasma agmatine

Because of the different substances in the plasma that react with OPA, derivatized samples were extracted using the C_{18} extraction cartridge (Chromoband®, Machery − Nagel, Duren, Germany) before injection onto the HPLC system, as indicated by Molderings et al. (2004) with some modifications. Earlier, this cartridge had been washed with water and methanol, and 2.25 ml of the derivatized samples was applied to the cartridge. Then, the cartridges were washed with a mixture of 3 ml of water, methanol, and acetonitrile [1/1/1 (vol/vol/vol), pH 13, adjusted with NaOH)] and a mixture of 100 μL of water, methanol, and acetonitrile [1/3/5 (vol/vol/vol) pH 3.5, adjusted with acetic acid]. Agmatine was eluted with a second 100 μL aliquot of water, methanol, and acetonitrile [1/3/5 (vol/vol/vol) pH 3.5, adjusted with acetic acid]. A volume of 20 μL of the eluate was injected into the HPLC immediately after the extraction and concentration procedures.

The plasma agmatine level was determined using an HPLC system, as previously described (Raasch et al., 1995; Feng et al., 1997). The HPLC system (HP 1100 series, Hewlett–Packard, Palo

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