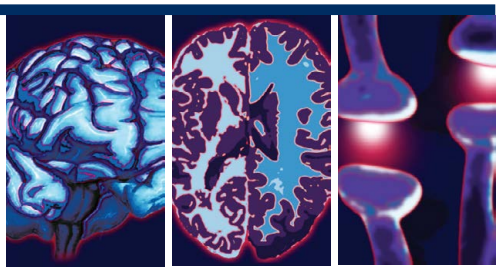

Agmatine mediated hypertonic stress development in Schizophrenia: a Novel study

Veysel Kenan Çelik[1,†], Ethem Erdal Erşan[2], Hasan Kiliçgün[3], Serkan Kapancik[1], Serpil Erşan[4]

SUMMARY

The aim of this study was to investigate precursors such as agmatine, ornithine involved in the synthesis of polyamines, enzymes level of these pathways and their correlation with diseases. In this study, 37 patients with schizophrenia and 37 healthy individuals no systemic disease (diabetes, hypertension, schizophrenia and mental illness) were taken as the control group. Determination of Ornithine decarboxylase, Arginine Decarboxylase, and Agmatinase levels were determined according to the each standard enzyme curve graph by using ELISA kits, Arginase activity, and ornithine levels were measured spectrophotometrically. When arginase activity was compared to the control group, it was determined that arginase activity was dropped significantly in the patient group ($p=0.001$ $p<0.05$). On the other hand, statistically significant increase in ornithine and arginine decarboxylase levels was found ($p=0.001$ $p<0.05$) ($p=0.044$ $p<0.05$) respectively. Even though the increase in ornithine decarboxylase and agmatinase levels was found, it was not statistically significant ($p>0.05$). The increase in ornithine levels in schizophrenia is mediated by increasing levels of agmatine just as hypo glutamatergic hypothesis put forward in schizophrenia. As a result, it can be suggested that agmatine may play a fundamental role in the development of osmolarity and hypertonic stress in schizophrenia and may cause a negative impact on the prognosis of the disease

Keywords

Schizophrenia, Polyamine, Agmatine, Ornithine, Hypertonic stress

Introduction

According to the neurodevelopmental hypothesis, schizophrenia is defined as a severe mental illness which leads to the complex table in the brain by creating a problem in almost all functions in the brain such as thinking, perception, cognitive functions, and mood. Because schizophrenia starts at an early age (15-25 for men, while in women 25-35 years of age) by showing a chronic course. For many years, it causes the quality of life of patients; it is one of the most important disability diseases in the world that limits a person's movements, senses, or activities [1]. The reports of the World Health Organization noted that more than 21 million people are affected all over the world. The basic hypothesis of schizophrenia is associated with over-stimulation of dopamine (D2) receptors in the associative striatum and deficiencies of (D1) dopamine receptors in the prefrontal cortex. Therefore, for therapeutic purposes, drugs blocking D2 receptors (antipsychotics) were developed. However, due to the inability of current treatment to improve the disease [2,3], new hypotheses to the understanding of the pathogenesis and treatment of schizophrenia has

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been proposed. For this purpose, the nerve cell membrane abnormality [4] which previously the theory argue that methylated compounds affect the mental health [5]. Later it was converted to a single carbon hypothesis [6] and the nicotinic and / or such as the presence of muscarinic acetylcholine receptors concentrations [7], several new approaches have been developed. Most recently among these is N-methyl-D-aspartate (NMDA) receptors hypofunction hypothesis [8-11] in which a polyamine (PA) such as agmatine interacted with these receptors causes the hypofunction of these receptors [2,12]. The principal members of polyamines which are aliphatic and polyvalent cationic peptides are the putrescine, spermidine, and spermine which carry 2, 3, and 4 amine groups in their structure, respectively. Polyamines are essential for normal cell growth and development in Eukaryotes, and they are tightly regulated by very little characterized transport systems, a dynamic network via the biosynthetic and catabolic enzymes and the intracellular concentration of them under normal physiological conditions. Polyamines affect the whole process of various cellular events such gene transcription, synthesis and cell growth and differentiation [13,14]. Two reactions with arginine precursors in the pathway for the biosynthesis of polyamines come to the forefront. One of them is ornithine decarboxylase (ODC) of the enzyme-catalyzed steps limiting the speed of arginase in the polyamine synthesis reaction, another is the arginine decarboxylase (ADC) step catalysed by agmatinase enzymes **(Figure 1)**. Polyamines have an important role in the etiology of schizophrenia, the role played by the spermidine as first and interacts with various structures of the brain, have a regulatory role in synaptic transmission even influence uptake of neurotransmitters. Those were known since70-ies [15].

Agmatine was firstly discovered as part of the spermine and the endogenous ligand as imidazoline receptors (IR) and interest due to the binding of α2-adrenergic receptors and originally referred agmatine as a neurotransmitter [16-18] further year with the detection of an interaction with NMDA receptors, another result of the polyamine hypo glutamate which led to born of the hypothesis in schizophrenia. Thus, agmatine has been receiving more attention polyamine than spermidine in schizophrenia. In fact, although agmatine is found in the brain as a very little amount, present endogenous agmatine is sufficient to interact with the receptors [19,20]. Agmatine is synthesised with arginine as endogenous, it is also absorbed in the small intestine with food. If we are to sort the main sources of agmatine: a) Agmatine coming from endogenous synthesis b) Agmatine produced and the released by the intestinal microflora including pathogens such as Helicobacter pylori, c) Agmatine- absorbed from digested food. d) Agmatine spilt from gastro endothelial cells, e) Agmatine from accumulated cells and tissues and absorbed from the gut, it is shaped re-absorption as part of enterohepatic circulation [21,22]. We aimed to investigate the role of agmatine caused the hypofunction of NMDA receptors appeared for the understanding of the pathogenesis and treatment of schizophrenia in this study. For this purpose, it was investigated critical enzyme involved in the synthesis of polyamines and the metabolism of agmatine (Arginine decarboxylase, Agmatinase, Arginase, and ornithine decarboxylase the rate-limiting enzyme) and the plasma levels of ornithine.

Also, this study was indicated by the first pilot study. Thus, it will help to bridge the gap in the literature. Another object was to shed light on new review or approaches in schizophrenia

Materials and Methods

This study was conducted with the cooperation of Sivas Numune Hospital Psychiatric Department and Sivas Cumhuriyet University Faculty of Medicine Department of Medical Biochemistry. Chemicals used in this study are analytical purity and the quality of Sigma.

■ Patients

Patients who meet the following criteria at the start of treatment are eligible for the study:

- Patients diagnosed and followed up by with schizophrenia in Sivas Numune Hospital Psychiatry Clinic
- At least 18 years of age signed written informed consent
- Patients who, at the start of study, meet any of the following criteria are not eligible for the study
- Body weight under 50 kg
- Inability to fully comprehend and/or perform study procedures in the investigator's opinion.
- Immunosuppressive therapy (for organ transplantation) or renal dialysis (current or planned within the next 12 months)

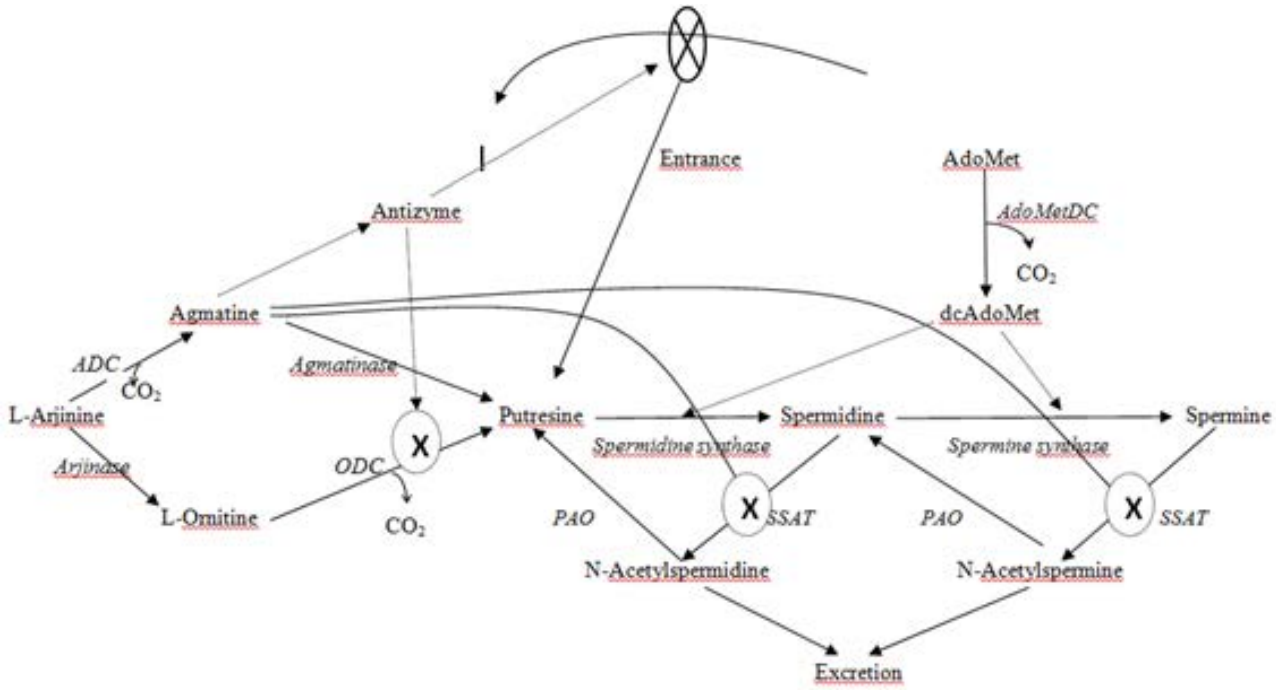

Figure 1: Polyamine Biosynthesis in Mammalian.

Arginine decarboxylase: (ADC), Ornithine decarboxylase: (ODC), s-adenosylmethionine decarboxylase: (AdometDC), decarboxylated s-adenosylmethionine (dcAdoMet), polyamineoxidase (PAO) and spermidine-spermine acetyltransferase (SSAT), inhibisyon (X)

In this study, 37 outpatients diagnosed and followed up by with schizophrenia in Sivas Numune Hospital Psychiatry Clinic as in the experimental group and 37 healthy individuals no systemic disease (diabetes, hypertension, schizophrenia and mental illness) as in the control group were taken. When the patient and individuals in the control were compared in terms of gender and age distribution, there was no significant statistical difference between the two groups (t=0.23, p=0.812; p>0.05) **(Table 1)**. Questionnaire form to all patients and controls included in the study was completed by questions and answers with mutual negotiations.

■ Collection and preparation of samples

10 ml blood samples from patients diagnosed with schizophrenia and controls were taken. After centrifugation at 4000 rpm for 15 minutes obtained serums placed in the Eppendorf tubes and they were stored at -80°C to be worked with related parameters.

■ Enzyme levels and activity studies

Ornithine decarboxylase, arginine decarboxylase, and agmatinase levels were determined according to the standard curve graph of each enzyme by using ELISA kits (Cusa¬bio Biotech, Wuhan, China).

Arginase activity was determined spectrophotometrically according to the method as described by Geyer et al and it was given as µmol urea / ml / hour [23]. Serum ornithine levels were measured spectrophotometrically as µmol / L according to the method of Chinard [24].

Statistical Analysis

Data of current study were loaded in SPSS software program (14.0 data analysis program), Mann-Whitney U Test was used to the evaluation of data and error level was taken as 0.05.

Results

There was no significant difference between social and demographic data of patients group (e.g. Age, sex, and smoking habits) and control group. There was a homogeneity between groups as seen in **Table 1** (p>0.05). Arginase enzyme activity showed a very effective reduction according to the control group levels as seen in **Table 2**. This reduction was statistically

significant (p=0.001 p<0.05). Although this reduction of arginase levels ornithine levels increased in schizophrenic patients and that increasing was statistically highly significant also. (p=0.001 p<0.05). Another finding was an increase in levels of ADC, which involves the synthesis of agmatine. The increase in levels of ADC was statistically significant (p=0.044 p<0.05). ODC and agmatinase levels also have shown an increase compared to the control group. But those increases were not statistically significant (p>0.05).

Discussion

In this study, the levels of ADC and agmatinase, which are responsible for the production and destruction of agmatine, were examined. Also, the levels ODC enzyme and ornithine, which are responsible for rate-limiting enzyme in polyamine synthesis, were examined. In this sense, this study is the first study on this topic. Although, changes in agmatinase and ODC enzyme levels were not statistically significant, when the control group of patients was compared with schizophrenia increases of ADC (p=0.044 p<0.05) and ornithine (p=0.001 p<0.05) levels and decrease in the arginase activity (p=0.001 p<0.05) were statistically highly significant (Table 2). There is strong evidence on changes in various neurotransmitters systems involved in the pathophysiological process (dopaminergic, glutamatergic, GABAergic, opioid, such as cholinergic or serotonergic systems) that leads to the formation of schizophrenia. On the other hand, the etiology of schizophrenia still remains a mystery in today [25-27]. As a matter of fact, 11.355 cases and 16,416 controls were used in the most recent and comprehensive study. The relationship between chromosomal copy number (CNV) and the disorder of chromosome with the load and between the rich parts of the GABAergic genes were examined. Therefore, it was concluded that the etiology and pathophysiology of schizophrenia were associated with glutamatergic signal changing [27]. Nevertheless, factors causing deterioration of the signal is still not revealed. In the current study, it was observed that the increased ornithine levels naturally are to increase the synthesis of glutamate and GABA. Thus, this situation points more irregular and complex signalling. It is apparent that as an osmolyte, ornithine leads to chronic osmotic pressure and also GABA receptor family may cause tonic inhibition and change plasticity in the hippocampal area [27-29]. Because increased ornithine urea cycle reaction increases the amount of citrulline, this increasing will contribute to the urea synthesis and increase of osmolarity as indirectly in many parts of the brain such as the cerebellum, cerebral cortex and brain stem [30]. In this way, agmatine- mediated increased ornithine levels lead to the development of hypertonic stress and it will have a negative effect on patients with schizophrenia. Also, it may lead to worsening the prognosis of the disease [31]. Our results showed that although, ODC rate-determining enzyme levels in the ornithine metabolism and polyamine synthesis were increased in serum, the reason of high levels of ornithine was agmatine. Our experimental results indicated that statistically a significant increase in ADC enzyme levels, which is involved in the synthesis of agmatine, will consistently lead to high serum agmatine level. Agmatine is an important regulator of the homeostatic balance of polyamines, this balance is made by agmatine through antizyme protein which is a natural inhibitor of the ODC (Figure 1) [32-34]. This inhibition naturally causes an increase in serum levels of ornithine. Moreover, it will lead to increased synthesis of urea in the brain [30]. Thus, this increase will result in the sustained increase of brain ornithine and as a result of activation of urea synthesis. Also, increased osmotic pressure disrupts the isotonic structure and will induce the hypertonic effect.

Based on these results, the current study was made with the purpose of contribution to the pathogenesis of schizophrenia. Findings obtained

Table 1: Characteristics of the patient and control groups.

	Schizophrenia (N = 37)	Control (N= 37)	p
Age ($\overline{X}$ ± sd)	38.45 ± 11.78	39.10 ± 11.53	> .05
Sex (male/female)	25/12	25/12	> .05
Smoking (%)	16 (43.2%)	15 (40.5%)	> .05

Table 2: Evaluation of measured parameters in patients and the control group.

	Schizophrenia (N=37)		Control (N=37)		U	p
	$\overline{X}$	sd	$\overline{X}$	sd		
ADC (pg/ml)	21.18	16.74	15.30	8.66	498	.044*
ODC (pg/ml)	13.53	13.24	9.47	8.64	554	.158
Agmatinase (pg/ml)	11.19	13.40	6.80	4.71	654	.742
Arjinaz (µmolurea/mL/h)	9.90	4.58	16.14	4.10	223	.000*
Ornitine (µmol/mL)	0.14	0.02	0.09	0.03	138	.000*
* p< .05						

in this pilot study demonstrated that agmatine leads to the hypo glutamate effect by causing increased levels of N-methyl-D-aspartate receptor hypofunction. Also, agmatine mediates the development of hypertonic stress in the brain by increasing the levels of ornithine. This study may help develop insight into the life-world of those suffering from schizophrenia. But it needs to uncover and clear understanding of the impact of the role of agmatine and other polyamines for the etiology and pathogenesis of schizophrenia. Similar studies with larger patient groups will contribute to better understanding the role agmatine in the pathogenesis of schizophrenia and contribute to development precautions against to schizophrenia.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

List of abbreviations

N-methyl-D-aspartate receptors: (NMDA)
Ornithine decarboxylase: (ODC)
Arginine decarboxylase: (ADC)
Imidazoline receptors: (IR)
Chromosomal copy number: (CNV)

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