

The analysis of selected neurotransmitter concentrations in serum of patients with Tourette syndrome

Analiza stężenia wybranych neuroprzekazników w surowicy chorych z zespołem Tourette'a

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Abstract

Background and purpose: Metabolic disturbances of excitatory and inhibitory neurotransmitters are implicated in pathogenesis of Tourette syndrome (TS). The aim of the study was to measure serum concentrations of glutamic acid, γ -aminobutyric acid (GABA) and glycine in TS patients and evaluate any correlation between neurotransmitter levels and age at onset, actual age, gender, tic severity, duration of the disease and concomitant psychiatric disorders.

Material and methods: Sixty-seven TS patients, aged 16–59, and 57 healthy controls, aged 19–37, were enrolled in the study. Information regarding medical history and physical investigation was collected using a short questionnaire. Sixty-seven percent of patients were medication-free at the time of examination and the rest had withheld treatment for 24 hours before. Blood samples were taken after a 12-hour fasting period. HPLC technique was used.

Results: The TS group had higher glutamic acid and lower GABA levels. Glycine concentrations were comparable. No differences regarding neurotransmitter concentrations between treated and non-treated patients were found. Patients with concomitant obsessive-compulsive disorder and severe tics had higher glutamate levels. Glutamate concentrations correlated positively with the number of comorbid psychiatric disorders and GABA concentrations correlated negatively with the number of behavioural problems in patients with comorbidities. There was no correlation between

Streszczenie

Wstęp i cel pracy: Zaburzenia metabolizmu neuroprzekazników pobudzających i hamujących odgrywają rolę w patogenezie zespołu Tourette'a. Celem pracy była analiza stężenia kwasu glutaminowego, γ -aminomasłowego (GABA) i glicyny w surowicy chorych z zespołem Tourette'a oraz ocena zależności pomiędzy stężeniami neurotransmiterów a wiekiem lub płcią, wiekiem w chwili zachorowania, nasileniem tików, czasem trwania choroby i współistniejącymi zaburzeniami psychiatrycznymi.

Materiał i metody: Zbadano 67 chorych na zespół Tourette'a w wieku 16–59 lat oraz 57 zdrowych osób w wieku 19–37 lat. Wykorzystano ankietę obejmującą informacje z badania podmiotowego i przedmiotowego. Sześćdziesiąt siedem procent chorych nie przyjmowało żadnych leków w momencie badania, u pozostałych wstrzymano podawanie 24 godziny przed pobraniem krwi. Próbkę zebrano po 12-godzinym okresie na czczo. Oznaczeń dokonano metodą HPLC.

Wyniki: W grupie chorych stężenie kwasu glutaminowego było większe, zaś GABA – mniejsze. Stężenie glicyny było podobne. Nie stwierdzono istotnej różnicy stężeń neuroprzekazników pomiędzy grupą osób leczonych i nieleczonych. W grupie osób ze współistniejącym zaburzeniem obsesyjno-kompulsywnym oraz ciężkim nasileniem tików stężenie kwasu glutaminowego było większe. Stwierdzono dodatnią korelację pomiędzy stężeniem kwasu glutaminowego a liczbą dodatkowych zaburzeń psychiatrycznych oraz odwrotną kore-

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analysed neurochemicals and age, gender, age at onset or disease duration.

Conclusions: Imbalance between excitatory and inhibitory systems in the brains of TS patients may be reflected by glutamate and GABA serum level changes. Glutamate and GABA may be biomarkers of the disease and high concentration of glutamate may indicate more severe course of TS.

Key words: Tourette syndrome, amino acids, neurotransmitters, glutamate, γ -aminobutyric acid, glycine.

Introduction

Tourette syndrome (TS) consists of multiple motor tics and at least one vocal tic that are present for more than 12 months. Diagnostic criteria are given in the DSM-IV-TR classification [1]. TS affects both children and young adults and is frequently associated with other psychiatric disorders. The most common are attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), mood and conduct disorder, anger control problems and self-injurious behaviour [2].

The aetiology of TS remains unknown. It is proposed though that genetic and autoimmune factors play a significant role in the pathogenesis of the condition [3]. Excessive dopaminergic transmission seems to be of great importance in the pathophysiology of tics, but other neurotransmitter system disturbances (noradrenergic, cholinergic, GABA-ergic, glutaminergic, serotonergic, opioid) are also present [4]. This disorder results from improper function of neurotransmitter transmission in cortico-subcortical loops composed of cortex, striatum, globus pallidus and thalamus. GABA and glutamic acid transmit the information and dopamine modulates their function [5]. Thus, the symptoms in TS are provoked by an imbalance in the excitatory and inhibitory system in the cortico-striato-thalamo-cortical loop [4].

The aim of our study was to assess whether changes in neurotransmitter concentrations in TS patients' brains may be reflected by their levels in serum and whether serum levels of selected amino acids can be a marker of the severity or clinical phenotype of the disease.

lację pomiędzy stężeniem GABA a liczbą zaburzeń behawioralnych w grupie ze współistniejącymi zaburzeniami psychiatrycznymi. Nie stwierdzono korelacji pomiędzy stężeniami analizowanych neuroprzekaźników a wiekiem lub płcią pacjentów, wiekiem zachorowania ani czasem trwania choroby.

Wnioski: Zaburzenie równowagi pomiędzy układem pobudzającym i hamującym w mózgu chorych z zespołem Tourette'a może się manifestować zmianami stężeń kwasu glutaminowego i GABA w surowicy. Kwas glutaminowy i GABA mogą być markerami choroby, a duże stężenie glutaminianu we krwi chorych może oznaczać cięższy przebieg zespołu Tourette'a.

Słowa kluczowe: zespół Tourette'a, aminokwasy, neuroprzekaźniki, kwas glutaminowy, kwas γ -aminomasłowy, glicyna.

We chose glutamic acid and GABA as they are the main transmitting chemicals in the basal ganglia, and glycine, which plays a role similar to GABA, but mainly in the spinal cord.

Material and methods

Sixty-seven TS patients (55 males and 12 females) of mean age 24.8 ± 7.9 years (range: 16-59) were included in the study, as well as 57 healthy controls (29 males and 28 females) of mean age 25.7 ± 3.7 years (range: 19-37). No person under the age of 16 was included in the study. In 34.3% (23/67) of patients with TS, allergy to different agents was found in their medical history, but no acute allergic reaction was present and no anti-allergics were used in any TS patient at the time of examination. Each of the following: bronchial asthma, Hashimoto disease and essential tremor were found in one patient only. The remaining 61.2% (41/67) of patients with TS had negative medical history. Physical examination was performed in every patient and revealed no findings except tics and other TS symptoms. The average age at tic onset in the TS group was 8.2 ± 3.6 years (range: 3-17).

A short questionnaire was used to obtain basic demographic data (age, gender), details about TS (age of tic onset, its severity, family history and treatment), concomitant psychiatric disorders and behavioural problems.

The diagnosis of TS and other comorbid psychiatric disorders was made using DSM-IV-TR criteria [1]. The TS patient group was further divided in two subgroups: TS-plus (49/67, 73.1%), with comorbidities

parallel to tics, and TS-only (18/67, 26.9%), without psychiatric disorders. The TS-plus group included patients with attention deficit hyperactivity disorder (ADHD, 24/67, 35.8%), obsessive-compulsive disorder (OCD, 23/67, 34.3%), learning disorder (23/67, 34.3%), anxiety disorder (15/67, 22.4%), conduct disorder (6/67, 9.0%), mood disorder (11/67, 16.4%), enuresis not related to a general medical condition (5/67, 7.5%), stuttering (7/67, 10.4%) and trichotillomania (3/67, 4.5%).

Behavioural problems that have no strict definition in DSM-IV-TR were diagnosed on the basis of clinical experience of the investigator and included: sleep and anger control problems, sexually inappropriate behaviours, self-injurious behaviour and problems with social skills.

Tic intensity was described as peak tic severity any time in the course of the disease. Mild tics did not significantly impair normal daily living and never required treatment with neuroleptics. Severe tics significantly interfered with normal daily activities and prevented patients from leading a normal life despite treatment. The majority of individuals (81%; 54/67) had mild tics, and the other 19% of patients (13/67) had severe tics.

At the time of blood sample collection a minority of the patients were taking medicines (33%, 22/67), mainly for tics. The most common kind of medicines used were neuroleptics such as haloperidol (7 cases, range of daily dose: 2-5 mg per day), risperidone (3 cases, range: 1-4 mg), tiapride (3 cases; range: 200-600 mg) and olanzapine (2 cases, range: 5-10 mg). Popular non-neuroleptics were: clonidine (4 cases, range: 150-225 µg) and antidepressive agents: sertraline (2 cases, 50 mg), clomipramine (1 case, 75 mg), fluoxetine (1 case, 40 mg) and citalopram (1 case, 20 mg). At the time of blood sample collection, all of the participants were drug free for at least 24 hours.

Blood samples were collected after a 12-hour period of fasting. Only still water was allowed. Serum amino acids were analysed according to a modified isocratic Pico Tag method by high performance liquid chromatography (HPLC) on the Shimadzu HPLC system equipped with the Waters reverse phase column (Spherisorb ODS2 3 µm and 4.6 × 250 mm) [6]. UV detection was performed at 254 nm and standards of amino acids used for quantitation were purchased from Sigma.

The study was approved by the institutional ethical review board and all patients and controls signed informed consent. In the case of a patient below the age

of 18, the informed consent was obtained both from one of the parents and the child.

Statistical analysis

All statistical analyses were performed by means of the program Statistica v. 8.0. Quantitative data are shown as median (minimum, maximum) and the Mann-Whitney U-test was used to compare the results. Qualitative data were analysed using the χ^2 test. Spearman's rank correlation coefficient was used to evaluate the correlation. A *p*-value of less than 0.05 was considered as significant.

Results

The TS group had statistically higher concentration of glutamate than controls ($p < 0.001$) and lower concentration of GABA than controls ($p < 0.05$). No significant difference between the groups was found regarding glycine concentration ($p = 0.37$) (Fig. 1). No relationship between amino acid serum concentrations and patients' age, age at tic onset or disease duration was found. No differences were found between males and females regarding concentrations of examined amino acids in the TS group and controls (Table 1). Positive family history (52.2%, 35/67) did not influence amino acid concentrations compared to TS patients with negative family history (data not shown).

The amino acid concentrations did not show any statistical differences in the TS-only compared to the TS-plus group (Table 2). We compared groups of TS patients with regard to the presence or absence of each comorbid psychiatric disorder. Only individuals with OCD had higher concentrations of glutamate (median 1.21 µg/L [0.12; 4.79] vs. 0.48 µg/L [0.05; 5.87], respectively; $p < 0.05$) and those with learning disorders had lower concentrations of GABA (median 0.11 µg/L [0.00; 0.40] vs. 0.15 µg/L [0.00; 0.38], respectively; $p < 0.05$). Patients with ADHD, anxiety disorder, conduct disorder, mood disorder, enuresis, stuttering and trichotillomania had similar concentrations of amino acids compared to the group of patients not having the particular disorder.

Spearman correlation analysis showed that in the comorbid TS patients (TS-plus group) the concentration of glutamate increased along with the number of other psychiatric disorders (Fig. 2A), while the concentration of GABA decreased along with the number of behavioural problems per patient (Fig. 2B). The number of behavioural problems correlated positively

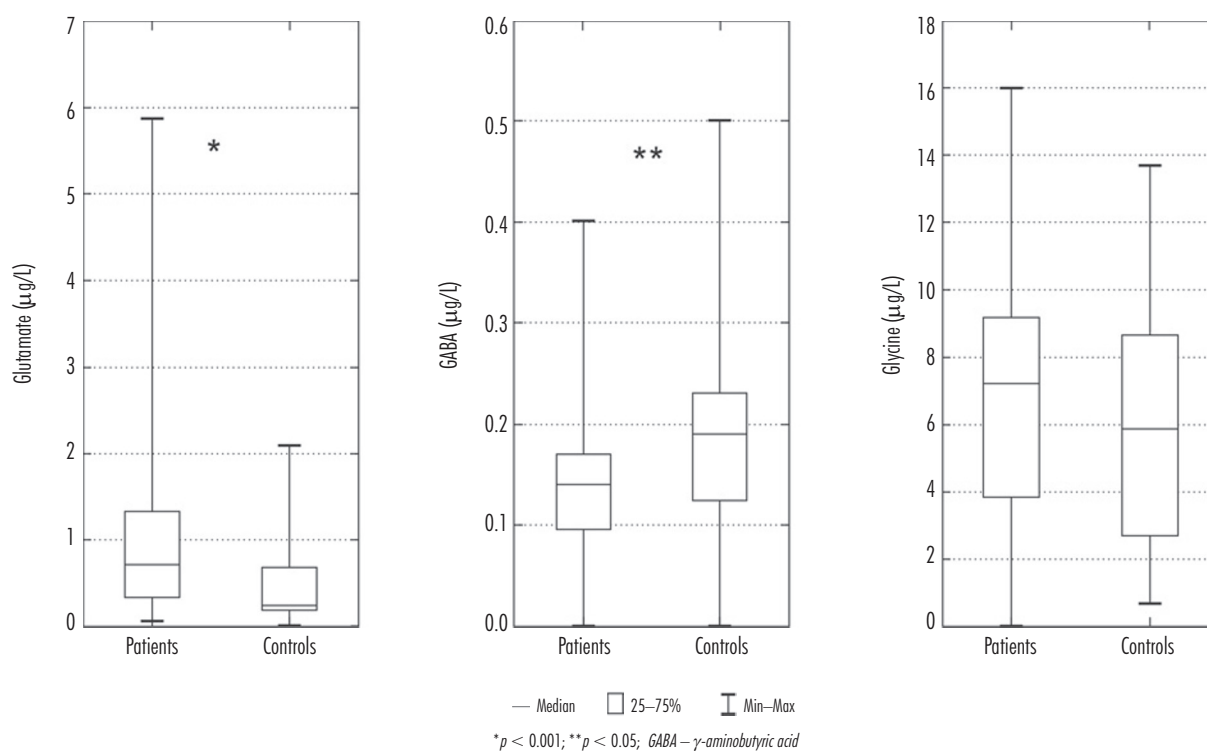


Fig. 1. Neurotransmitter serum concentrations in Tourette syndrome patients and control group. Values are shown as medians (minimum; maximum)

with the number of psychiatric disorders in the TS-plus group (Spearman $r = 0.60$; $p < 0.05$).

Glutamate appeared to be the only amino acid that had an association with severe tics compared to mild tics in the course of the disease (Fig. 3).

The comparison of patients pharmacologically treated and not treated at the time of examination revealed no statistically significant differences in relation to amino acid concentrations (Table 3).

Discussion

The main findings of our study are that the concentration of glutamate is higher and GABA is lower in TS patients' serum compared to controls. It remains unclear whether serum glutamate and GABA levels truly reflect their brain activity as we did not measure brain or cerebrospinal fluid levels of these amino acids. However, we speculate that the changes in serum glutamate and

Table 1. Neurotransmitter concentrations in males and females in TS and control groups. Values are shown as medians (minimum; maximum)

	Patients with Tourette syndrome			Controls		
	Males <i>n</i> = 55	Females <i>n</i> = 12	<i>p</i> -value	Males <i>n</i> = 29	Females <i>n</i> = 28	<i>p</i> -value
Glutamate (µg/L)	0.65 (0.06; 5.87)	0.85 (0.05; 2.9)	0.701	0.35 (0.0; 2.1)	0.24 (0.10; 1.84)	0.768
GABA (µg/L)	0.14 (0.0; 0.4)	0.13 (0.08; 0.27)	0.948	0.21 (0.0; 0.5)	0.16 (0.02; 0.30)	0.122
Glycine (µg/L)	7.29 (0.0; 15.98)	6.28 (1.85; 12.9)	0.452	7.84 (0.71; 13.7)	5.00 (1.03; 10.50)	0.091

GABA – γ -aminobutyric acid

GABA may reflect the changes in the brain. There are many papers in the literature on amino acid and neurotransmitter concentrations in serum of individuals with psychiatric disorders. High concentrations of serum glutamate have been associated with schizophrenia, autism, and alcohol withdrawal [7-9]. Low concentrations of serum GABA have been found in depression, post-traumatic stress disorder, and alcohol addiction [10]. The authors of these papers concluded that findings in serum amino acid concentrations were important in understanding the pathogenesis of these disorders because they may be related to brain levels.

The organization of the blood-brain barrier promotes the net removal of most amino acids including glutamate against a concentration gradient from brain to blood. Glutamate transporters (EAAT1, 2 and 3) are able to pump it from the brain into endothelial cells that form the blood-brain barrier and facilitative carrier xG for glutamate at the luminal (blood-facing) membrane provides a mechanism for removing it from brain to blood [11]. The lack of facilitative glutamate transport carriers on the abluminal (brain-facing) membrane prevents movement of glutamate into the brain [12]. When intracellular glutamate becomes greater than the plasma concentration, net transport of glutamate across the luminal membrane into the blood will occur. A similar mechanism exists for glutamine, which is converted to glutamate by glutaminase in endothelial cells [11]. Additionally, our results regarding glutamate in serum seem consistent with our previous findings in magnetic resonance spectroscopy of TS patients' brains, showing higher glutamate concentrations in the thalamus and

Table 2. Neurotransmitter concentrations in TS-only and TS-plus groups. Values are shown as medians (minimum; maximum)

	TS-only n = 18	TS-plus n = 49	p-value
Glutamate (µg/L)	0.54 (0.06; 3.64)	0.90 (0.05; 5.87)	0.611
GABA (µg/L)	0.15 (0.05; 0.31)	0.13 (0.0; 0.40)	0.362
Glycine (µg/L)	6.91 (1.60; 12.8)	7.21 (0.0; 15.98)	0.893

TS – Tourette syndrome, GABA – γ-aminobutyric acid

lenticular nucleus [13]. Considering sources of glutamate other than the brain, every patient underwent a thorough physical examination and in every case it was normal except for tics. No liver, kidney or alimentary tract signs were found. With regard to allergy, no patient with acute signs of allergic reaction was enrolled in the study.

The origin of plasma GABA in humans is difficult to demonstrate directly, but indirect evidence suggests that plasma GABA is largely derived from the brain. GABA is a very CNS-specific compound, with 99% of total body GABA and 95% of its synthesizing enzyme glutamate decarboxylase being located in the brain and the spinal cord [14]. GABA is transferred from the brain interstitial fluid to the circulating blood across the blood-brain barrier, which acts as an efflux pump for GABA to reduce the brain concentration [15]. Transport of GABA from the circulating blood to the brain is very small [16,17]. A high correlation between plas-

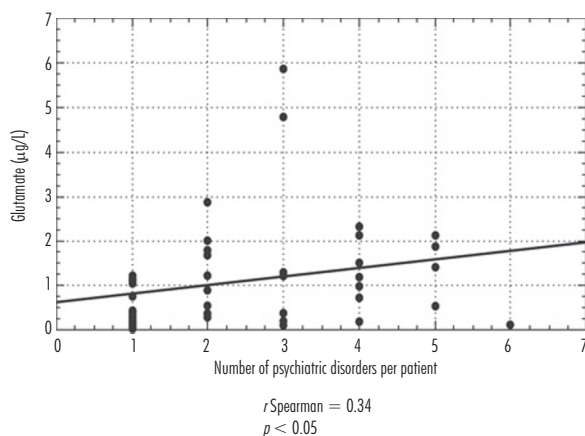


Fig. 2A. Correlation between glutamate concentration and number of psychiatric disorders per patient in Tourette syndrome-plus group

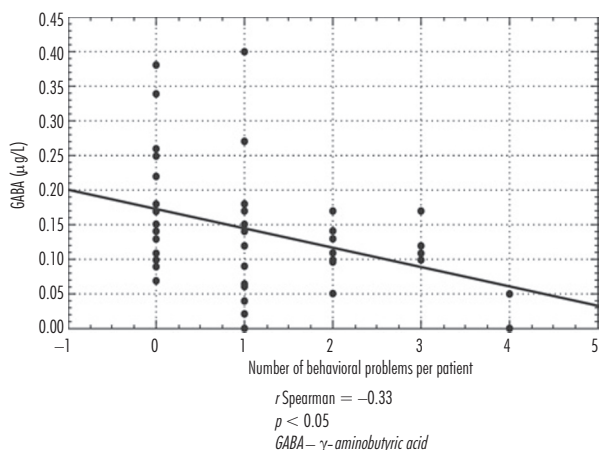


Fig. 2B. Correlation between GABA serum concentration and number of behavioural problems per patient in Tourette syndrome-plus group

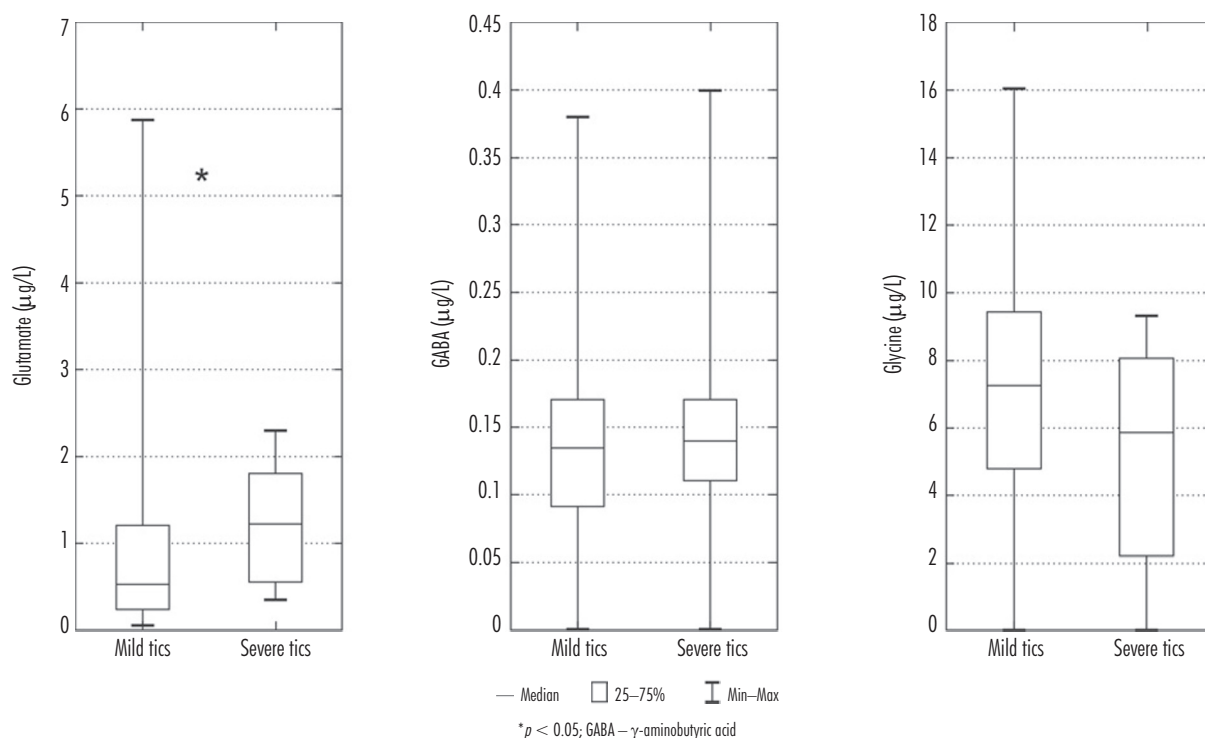


Fig. 3. Medians (minimum; maximum) of neurotransmitter concentrations according to tic severity

ma and CSF levels of GABA has been reported in rats [18], dogs [19] and normal humans [20]. Changes in plasma GABA appear to reflect changes in brain GABA [21] and cerebrospinal fluid GABA [22,23]. Thus, these data suggest that plasma GABA concentration is likely to reflect brain GABA-ergic activity.

To minimize the influence of food on amino acid concentrations, the individuals enrolled in the study were asked not to eat for 12 hours. We were aware that ceasing treatment 24 hours before collecting blood was not enough to eliminate its influence on amino acid levels in serum. Hence, to exclude the influence of pharmacological treatment on the findings we compared the treated and untreated group of TS patients and did not find any statistical difference (Table 3). Taking everything into account, we believe that serum glutamate and GABA derived from brain may reflect their brain levels and should be considered in pathophysiology of TS.

The dopaminergic transmission in TS is increased in basal ganglia [24,25]. Glutamic acid and GABA play a significant role in excitation and inhibition of the basal ganglia, yet dopamine is the main modulating chemical of this system. Dopamine-containing neurons of the substantia nigra pars compacta (SNc) modulate the activity of GABA-ergic striatal neurons. They exert their

action by way of D1 receptors (direct pathway, an increase of activity) and D2 receptors (indirect pathway, a decrease of activity) [5]. Blocking the neuronal transmission of the indirect pathway and/or increasing it in the direct pathway underlie the pathophysiology of involuntary movements such as tics or chorea. Mutual influence exists: the modulating function of dopamine on the GABA-ergic striatal neuronal projections' activity is exerted indirectly through GABA-ergic and cholinergic interneurons of the striatum [26]. In response, GABA released from neurons of the striatal striosome neurons inhibits dopaminergic neurons of the SNc. Low concentration of GABA could explain the excessive activity of dopamine-releasing neurons in SNc and thus would indicate the increased activity of the dopaminergic system in TS. However, there are only three papers in the literature dealing with GABA content in the CNS of TS patients. Two different studies conducted on the same four brains of TS patients showed normal glutamate decarboxylase activity [27] and GABA levels [28] when compared to the brains of control subjects. In the third one, cerebrospinal fluid GABA concentrations as well as serum GABA levels of 15 patients with TS were not significantly different from those of controls [29]. We do not confirm these findings as our study showed lower content of serum GABA in TS patients.

The dopaminergic system is closely connected with the glutaminergic system. Acting through D1 receptors dopamine enhances NMDA receptor-mediated responses and through D2 receptors inhibits non-NMDA receptor activity [30,31]. D1 receptor-mediated activity depends on the neuronal polarization state. In the case of a depolarized neuron, it enhances this state and at the same time causes insensitivity to any stimuli of the neuron in the non-active (hyperpolarized) state [32,33]. The excessive concentration of glutamate in TS may cause depolarization and this state could intensify the action of dopamine. Due to the existence of D1-NMDA heterodimer, the effect of up-regulation of the D1 receptors caused by glutamate acting through NMDA is greater [34]. This mechanism could explain the greater dopamine activity in the case of higher glutamate concentrations in TS. There are some data on glutamate levels in the brains of patients with TS. Autopsy of four brains of patients with TS revealed lower glutamate concentration in the internal globus pallidus (GPi) by 30% and to a smaller degree in the external globus pallidus (GPe) and substantia nigra pars reticulata (SNr) [28,35]. Because the GP and SNr receive their glutamatergic input from the subthalamic nucleus (STN), it suggests that STN output may be reduced in TS. Decreased excitatory input to the GPi would be expected to decrease activity in the inhibitory projection from the GPi to the thalamus, leading to increased activity of the thalamus and thalamo-cortical pathway. This hypothesis is consistent with our previous findings of higher concentration of glutamic acid in the thalami of 15 TS patients compared to normal humans in magnetic resonance spectroscopy of the brain [13]. It is possible that glutamate levels are different in various regions of the brains of TS patients and the net content of this amino acid is elevated. This may be reflected by high glutamate level in serum, as our present study has shown. However, to date no studies have been published on the concentration of glutamate in the cerebrospinal fluid and serum of TS patients to confirm this hypothesis.

There is no reliable marker of the disease that could indicate the prognosis or the course of TS. Our results may help to elaborate it. Glutamate level turned out to be higher in patients with severe tics and a larger number of concomitant psychiatric disorders, especially with obsessive-compulsive disorder. High concentration of glutamic acid may be a useful marker of the disease severity (its clinical phenotype and tic intensity), especially because it does not depend on the disease duration.

Table 3. Neurotransmitter concentrations in treated and untreated TS patients. Values are shown as medians (minimum; maximum)

	Treated n = 55	Untreated n = 12	p-value
Glutamate ($\mu\text{g/L}$)	0.54 (0.05; 5.87)	0.99 (0.10; 3.06)	0.42
GABA ($\mu\text{g/L}$)	0.14 (0.0; 0.40)	0.13 (0.0; 0.27)	0.67
Glycine ($\mu\text{g/L}$)	7.20 (0.0; 15.98)	7.01 (0.0; 12.90)	0.83

TS – Tourette syndrome, GABA – γ -aminobutyric acid

Children younger than 16 years of age were not studied, which might have influenced our results. It is known though that TS symptoms in children are more pronounced. Severity of tics and some of the concomitant psychiatric disorders (e.g. ADHD) usually decreases gradually after puberty [36]. In our study we did not find any correlation between glutamate or GABA and disease duration. This suggests that our findings should not be significantly different from those obtained in children. For ethical reasons we could not perform the lumbar puncture in patients with the diagnosis already stated and without any medical reasons. Due to the lack of information on the correlation between cerebrospinal fluid and serum concentrations of the neurotransmitters we cannot prove beyond doubt cerebral origin of the examined amino acids in serum. However, data from the literature mentioned above make it highly probable.

Our present results and previous findings of higher glutamate levels in the basal ganglia in TS patients [13] may suggest that in this condition the important provoking factor is the imbalance between the glutaminergic (excitatory) and GABA-ergic (inhibitory) systems. Normal values of glycine concentrations in TS patients, that is an inhibitory neurotransmitter in the spinal cord, support the notion that the problem does not lie in spinal cord function.

We conclude that the findings of this study may be important for understanding the pathogenesis of TS and predicting the course of the disease, though it should be considered with great caution.

Conclusions

1. Imbalance between excitatory and inhibitory systems in the brain of TS patients is reflected by concentrations of glutamate and GABA in serum.

- Concentrations of glutamate and GABA may represent markers of the disease, and high concentration of glutamate may predict more severe course of Tourette syndrome.

Disclosure

Authors report no conflict of interest.

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