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EXPERIMENTAL ARTICLES

The Effects of the O-(2-R-Oxime 4-Benzoyl) Pyridine Derivate GIZh-298 and Topiramate on the Contents of Monoamines and Their Metabolites in Rat Brain Structures: A Neurochemical Study

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Abstract—The effects of the putative antiepileptic drug GIZh-298 and the reference standard topiramate on the concentrations of monoamines and their metabolites in the frontal cortex, hypothalamus, nucleus accumbens, striatum, and hippocampus of Wistar rats was investigated using HPLC. It was shown that topiramate at a dose of 100 mg/kg induces an increase in dopamine concentration and a decrease in its metabolism rate in the frontal cortex, a decrease in the level of its metabolites in the dorsal striatum, and an increase in concentrations of dopamine and its metabolites in the hypothalamus 30 minutes after injection. GIZh-298 at a dose of 60 mg/kg caused an increase in the serotonin and dopamine concentration in the frontal cortex and a decrease in the dopamine metabolism rate in the dorsal striatum 30 minutes after injection, which may be considered as one of the components of the antiepileptic effect of this drug.

Keywords: GIZh-298, topiramate, monoamines, brain structures, HPLC

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INTRODUCTION

According to the World Health Organization, the rate of epilepsy varies in a wide range across different countries, from 1.5 to 50 cases per 1000 population. In Russia, the abundance of epilepsy is 2.98 per 1000 people [1].

Most antiepileptic drugs (AEDs) are manufactured outside Russia, therefore Russian patients sometimes do not have proper access to necessary AEDs, and additionally, imported AEDs are characterized by their high price, which is among the causes of insufficient healthcare [2]. In accordance to this, the development of novel Russian AEDs is important.

Preliminary studies have demonstrated that GIZh-298 (O-(2-R-oxime 4-benzoyl) pyridine derivate), as synthesized in the Chemistry Department of our Institute, possesses a clear antiepileptic effect in the maximum electroshock seizure model [3] (patent claim no. 201611336 from April 8, 2016). However, the effects of this drug on the activity of brain neurotransmitter systems whose dysfunction is known to play an essential role in seizures development have not been determined thus far.

Therefore, the aim of our study was to compare the effects of GIZh-298 and topiramate (fructose derivate), taken as a reference drug, on the contents of neurotransmitter monoamines and their metabolites in the structures of the rat brain.

MATERIALS AND METHODS

The study was performed with 24 male Wistar rats weighing 200–240 g (Stolbovaya breeding center) kept in a laboratory vivarium under a 12-hr light regime with free access to water and standard food. To avoid the influence of circadian rhythms on the rate of neurotransmitter biosynthesis and metabolism all experiments were performed between 10 and 12 a.m. Experiments were conducted in accordance with the ethics rules of animal care established by the ethics committee of the Institute.

Animals were divided into three experimental groups: Group 1 (control group, 0.9% NaCl), Group 2 (GIZh-298, 60 mg/kg, i.p.), Group 3 (topiramate, 100 mg/kg, i.p., Topamax, Janssen–Silag AG, Switzerland). Each experimental group consisted of eight animals.

At 30 minutes after the injection of 0.9% NaCl, topiramate, or GIZh-298 the animals were sacrificed by decapitation and the frontal cortex (FC), hypothal-

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Table 1. The concentrations of monoamines and their metabolites in the structures of the intact Wistar rat brain (nmol/g of tissue)

Brain structure	NE	DA	DOPAA	HVA	3-MT	5-HT	5-HIAA	DOPAA/DA	HVA /DA	5-HIAA/5-HT
Hypothalamus	15.2 ± 1.79	4.1 ± 0.94	1.04 ± 0.21	0.17 ± 0.06	0.33 ± 0.17	23.92 ± 1.92	27.97 ± 3.25	0.26 ± 0.04	0.04 ± 0.01	1.17 ± 0.09
Hippocampus	1.07 ± 0.33	0.04 ± 0.03	0.04 ± 0.01	–	–	2.54 ± 0.62	4.87 ± 1.09	1.59 ± 1.4	–	1.95 ± 0.25
Frontal cortex	1.01 ± 0.16	0.20 ± 0.05	0.07 ± 0.02	–	–	2.61 ± 0.23	2.33 ± 0.33	0.32 ± 0.06	–	0.90 ± 0.14
Nucleus accumbens	1.69 ± 0.27	27.25 ± 4.63	3.65 ± 0.48	1.38 ± 0.33	0.10 ± 0.07	4.42 ± 0.51	7.49 ± 0.89	0.14 ± 0.03	0.05 ± 0.01	1.71 ± 0.24
Striatum	0.24 ± 0.05	38.62 ± 3.58	3.72 ± 0.44	2.55 ± 0.43	0.18 ± 0.05	2.92 ± 0.31	7.98 ± 0.97	1.6 ± 0.28	1.1 ± 0.26	45.53 ± 9.52

The data are presented as mean ± standard deviation (M ± SD). *Statistically significant difference vs control group at $p < 0.05$. In the hippocampus and frontal cortex the concentrations of HVA, 3-MT, and the HVA/DA ratio are not given due to insufficient concentrations in these structures.

amus, nucleus accumbens (NA), striatum, and hippocampus were isolated on ice, frozen in liquid nitrogen and weighed. Prior to neurotransmitter analysis, the samples were homogenized using a hand homogenizer (Teflon-glass) in 20 volumes of 0.1 N $HClO_4$ with 3,4-dioxybenzylamine (0.5 nmol/mL) as an internal standard. The samples were centrifuged at 10000 *g* for 10 min. The concentration of monoamines was measured by HPLC using a LC-304T chromatograph (BAS, West Lafayette, United States) equipped with a LC-4B electrochemical detector (BAS, West Lafayette, United States) and a ReproSil-Pur ODS analytical column (C18, 100 × 4 mm, 3 μm) (Dr. Maisch, Germany) [4]. Registration of samples was performed using a Mul'tikhrom programmed apparatus complex v. 1.5 (Ampersend, Russia). Statistical analysis of the data was performed using Statistica 10.0 software (StatSoft Inc., United States). Normality was tested using the Shapiro–Wilk criterion, while the equality of the mean values was tested using the unpaired t-test.

RESULTS

The concentrations of monoamines and their metabolites (nmol/g of tissue) are shown in Table 1.

The most prominent changes in the contents of monoamines and their metabolites that were induced by GIZh-298 and topiramate at a dosage exerting a seizure-suppressive effect (60 mg/kg and 100 mg/kg, respectively) were observed in the FC, striatum, and hypothalamus (Table 2).

In the FC of rats injected with GIZh-298 (60 mg/kg), we observed a statistically significant increase in dopamine (DA) and serotonin (5-HT) by 38 and 18%, respectively. In the striatum, GIZh-298 induced statistically significant changes in the activity of the dopaminergic system. Thus, the concentrations of 3,4-dioxyphenylacetic acid (DOPAA), homovanilic acid (HVA), and the HVA/DA and DOPAA/DA ratios, which characterize the intensity of DA transformation into its metabolites, were reduced by 19, 26, 29, and 23%, respectively ($p < 0.05$). In the hypothalamus, NA, and hippocampus, GIZh-298 had no significant influence on neurotransmitter content (Table 2).

In the FC topiramate induced a statistically significant decrease in the DOPAA/DA ratio (an index of DA metabolism) by 31% and an increase in the DA level by 37%. In the striatum, topiramate had no influence on DA exchange but decreased the level of its metabolite, 3-mthoxytyramine (3-MT), by 32%. In the hypothalamus, the concentrations of DA, DOPAA, and HVA increased by 35, 51, and 68%, respectively (Table 2). In the NA and hippocampus, there were no significant changes in the levels of the examined neurochemical parameters.

DISCUSSION

Our experiments demonstrated that GIZh-298 leads to accumulation of dopamine and serotonin in the FC. It has been proven that changes in the functioning of the dopaminergic system are present in both human epilepsy and its animal models. However, the role of dopamine in epileptogenesis has not been elucidated completely as yet. It is known that drugs that increase the activity of the dopaminergic system (e.g., antiparkinsonic drugs) possess antiepileptic and seizure-suppressing effects [5]. Moreover, it was found that D2 receptor agonists and D1 receptor blockers demonstrate anti-epileptic features (inhibit neuronal excitability), whereas D2 receptor antagonists and D1 receptor agonists decrease the threshold for neuronal depolarization, reduce the latent period before a sei-

Table 2. The effects of GIZh-298 (60 mg/kg) and topiramate (100 mg/kg) on the concentrations of monoamines and their metabolites in the structures of the intact Wistar rat brain (nmol/g of tissue)

Drug	NE	DA	DOPAA	HVA	3-MT	5-HT	5-HIAA	DOPAA/DA	HVA/DA	5-HIAA/5-HT
Hypothalamus										
GIZh-298 60 mg/kg	15.27 ± 2.59	3.68 ± 0.71	0.96 ± 0.18	0.17 ± 0.08	0.35 ± 0.12	27.74 ± 12.0	27.48 ± 4.79	0.26 ± 0.03	0.05 ± 0.03	1.06 ± 0.21
Topiramate 100 mg/kg	14.59 ± 2.8	**5.53 ± 1.37***	**1.57 ± 0.42***	**0.26 ± 0.1***	0.32 ± 0.11	26.13 ± 2.59	30.74 ± 4.64	0.29 ± 0.07	0.05 ± 0.02	1.18 ± 0.12
Hippocampus										
GIZh-298 60 mg/kg	1.23 ± 0.19	0.07 ± 0.08	0.04 ± 0.02	–	–	2.5 ± 0.31	4.76 ± 0.52	0.98 ± 0.62	–	1.92 ± 0.23
Topiramate 100 mg/kg	1.31 ± 0.24	0.04 ± 0.03	0.04 ± 0.02	–	–	2.98 ± 0.35	5.76 ± 0.75	1.56 ± 1.22	–	1.94 ± 0.24
Frontal cortex										
GIZh-298 60 mg/kg	1.13 ± 0.16	**0.26 ± 0.06***	0.07 ± 0.03	–	–	**3.09 ± 0.4***	2.54 ± 0.26	0.27 ± 0.08	–	0.83 ± 0.09
Topiramate 100 mg/kg	1.14 ± 0.18	**0.26 ± 0.06***	0.06 ± 0.02	–	–	2.84 ± 0.62	2.51 ± 0.39	**0.22 ± 0.09***	–	0.98 ± 0.33
Nucleus accumbens										
GIZh-298 60 mg/kg	1.44 ± 0.44	27.89 ± 7.09	3.28 ± 1.02	1.33 ± 0.46	0.06 ± 0.04	4.50 ± 1.19	7.69 ± 1.83	0.12 ± 0.01	0.04 ± 0.01	1.76 ± 0.32
Topiramate 100 mg/kg	1.53 ± 0.98	28.58 ± 9.14	4.30 ± 0.8	1.8 ± 0.62	0.05 ± 0.03	4.46 ± 1.28	7.88 ± 1.31	0.17 ± 0.1	0.07 ± 0.02	1.87 ± 0.49
Striatum										
GIZh-298 60 mg/kg	0.31 ± 0.13	37.10 ± 6.12	**3.03 ± 0.49***	**1.90 ± 0.49***	0.16 ± 0.05	2.47 ± 0.68	6.32 ± 2.03	**1.24 ± 0.19***	**0.78 ± 0.22***	38.12 ± 6.69
Topiramate 100 mg/kg	0.32 ± 0.11	40.77 ± 6.47	3.95 ± 0.59	2.84 ± 0.72	**0.12 ± 0.04***	3.24 ± 0.4	8.78 ± 1.43	1.61 ± 0.24	1.14 ± 0.2	44.83 ± 7.1

The data are presented as the mean ± standard deviation (M ± SD). *The statistically significant difference vs the control group at $p < 0.05$. In the hippocampus and frontal cortex, the concentrations of HVA, 3-MT, and HVA/DA ratio are not given due to insufficient concentrations in these structures.

zure, and may provoke seizures in people with no epileptic anamnesis [6–8]. Since GIZh-298 activates the dopaminergic system in the frontal cortex, and dopamine stimulates both D1 and D2 receptors equally [9], we have no ability to definitely claim which type of dopamine receptor is involved in the seizure-suppressing effect of GIZh-298. According to the literature data, in addition to the dopaminergic system, the serotoninergic system also actively participates in epileptogenesis and antiepileptic drugs alleviate the activity of this system [8, 10].

In the striatum, we observed a decreased concentration of DA metabolites along with a constant level of this neurotransmitter itself, which indicate either retarded synthesis of the DA or its release into the synaptic cleft under the influence of GIZh-298, or inhibition of the catecholamine degrading enzyme MAO. Along with this, studies have indicated elevated levels of DOPAA and HVA in the striatum after increased neuronal firing, and prevention or attenuation of epileptiform discharges and COMT inhibition by drugs that inhibit DA metabolism (likewise after GIZh-298) [11].

Taking all these facts into consideration, we could assume that the antiepileptic effect of GIZh-298 is possibly related to its inhibitory action on DA synthesis and metabolism in the striatum, and increased levels of DA and 5-HT in the frontal cortex.

A decreased rate of DA metabolism in the FC together with an increased DA level, which was observed after topiramate administration, indicates accumulation of DA, which, possibly acting via D2 receptors, leads to decreased neuronal depolarization and subsequently, to cessation of the spreading of excitation waves from neuron to neuron [9, 5]. In the dorsal striatum, the 3-MT level was decreased, but no changes in DA and HVA concentration were observed, which was reflected by inhibition of the DA degrading enzyme, catechol-O-methyl transferase (COMT). In addition, topiramate activated DA synthesis in the hypothalamus, as judged by the increased concentration of DA and its metabolites, while the question of whether this phenomenon is related to the antiepileptic effect of the drug remains open.

Summarizing our data, we may assume that the antiepileptic effects of GIZh-298 and topiramate occur through involvement of the FC and dorsal striatum, whereas the increased DA level in the hypothalamus after topiramate administration is likely associated with other brain effects of this drug.

CONCLUSIONS

(1) It has been shown that GIZh-298 at a dose of 60 mg/kg induces a statistically significant increase in dopamine and serotonin concentration in the frontal cortex of rats and a decrease in the dopamine metabolism rate in the dorsal striatum 30 minutes after administration, which may be regarded as one of the components of its antiepileptic effect.

(2) Topiramate at a dose of 100 mg/kg induces a statistically significant increase in the dopamine level and a decrease in its metabolism index in the frontal cortex, a decrease in the level of 3-MT in the dorsal striatum, and an increased dopamine level in the hypothalamus 30 minutes after injection.

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