PHARMACOLOGY AND TOXICOLOGY

Effect of Cyclic GABA Derivative TZ-146 on the Content of Neurotransmitters in Rat Brain Stem

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The effect of cyclic GABA derivative TZ-146 on the content of catecholamines, serotonin, their metabolites, and neurotransmitter amino acids in rat hypothalamus and brain stem was studied by high-performance liquid chromatography with electrochemical detection. Opposite changes in the content of homovanillic acid, aspartate, glutamate, and glycine in the examined structures were accompanied by inhibition of dopamine metabolism. Possible participation of cerebral glutamatergic system in the effect of TZ-146 is discussed.

Key Words: *dopamine; serotonin; neurotransmitter aminoacids; brain stem structures*

GABA-derivatives are in the focus of clinical and experimental studies due to a variety of their biological activities and, specifically, their cardioprotective potency [1,6]. The directed search for GABA-ergic agents with antiischemic activity yielded original cyclic GABA derivative TZ-146, which possesses antianginal [3] and antiarrhythmic [4] properties. Taking into consideration that the effect of GABA derivatives could be mediated via modulation of not only on GABA-ergic synapses, but also the metabolism of other neurotransmitters [10], our aim was to study the effect of TZ-146 on the neurotransmitter mechanisms in the brain.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 220-280 g. TZ-146 was injected intraperitoneally in a dose of 50 mg/kg 10 min before euthanasia. The rats were decapitated and the hypothalamus and brain stem were extracted and washed in ice-cold physiological saline. Tissue specimens were stored in liquid nitrogen.

The content of catecholamines, serotonin, their metabolites, and neurotransmitter amino acids was determined by high-performance liquid chromatography with electrochemical detection (HPLC/ED) [5]. Brain tissue specimens were homogenized in 0.1 N $HClO_4$ with dioxybenzylamine (0.5 nM/ml) as the internal standard. The samples were centrifuged at 10,000*g* for 10 min. To determine the content of catecholamines, 20 μl supernatant was directly injected into an Ultrasphera analytical column (C_{18}, 4.6×250 mm, 5 μM). Norepinephrine, 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine, homovanillic acid (HVA), 5-hydroxyindole acetic acid, and serotonin (5-HT) were separated on a thermostatically controlled (25°C) column with 0.1 M citrate-phosphate buffer as the mobile phase containing sodium octane sulfonate (0.3 mM), EDTA (0.1 mM), and 8% acetonitrile (pH 3.2). Monoamides and their metabolites were determined on carbon-glass electrode at +0.85 V with an Ag/AgCl reference electrode the elution rate of 1 ml/min [5]. The content of inhibitory (GABA, glycine, taurine) and excitatory (aspartate and glutamate) neu-

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TABLE 1. Effect of TZ-146 on Content of Catecholamines, Serotonin, and Their Metabolites (nM/g tissue) in Rat Brain Stem ($M\pm m$, n=8)

Substance	Hypothalamus		Brain stem	
	control	TZ-146	control	TZ-146
Norepinephrine	8.328±0.437	8.149±0.241	5.424±0.496	5.479±0.408
DOPAC	0.357±0.057	0.320±0.062	0.204±0.042	0.247±0.015
Dopamine	2.495±0.122	2.854±0.175	1.674±0.728	0.841±0.051
5-Hydroxyindole acetic acid	7.953±0.464	7.697±0.340	7.234±0.709	7.982±0.410
HVA	0.139±0.019	0.086±0.010*	0.163±0.019	0.261±0.031*
5-HT	10.126±0.525	10.592±0.263	7.038±0.651	8.198±0.350
DOPAC/dopamine	0.144±0.024	0.166±0.022	0.214±0.042	0.297±0.019
HVA/dopamine	0.056±0.007	0.032±0.005*	0.171±0.025	0.308±0.028*
5-Hydroxyindole acetic acid/5-HT	0.787±0.023	0.726±0.026	1.028±0.019	0.986±0.057

Note. Here and in Table 2: $p<0.05$ *compared to the control.

TABLE 1. Effect of TZ-146 on the Content of Catecholamines, Serotonin, and Their Metabolites (nM/g tissue) in Rat Brain Stem ($M\pm m$, n=8)

Amino acid	Hypothalamus		Brain stem	
	control	TZ-146	control	TZ-146
Aspartate	3.436±0.296	2.371±0.145*	1.272±0.061	1.497±0.183
Glutamate	10.297±0.466	8.260±0.524*	2.815±0.176	3.074±0.265
Glycine	2.189±0.394	1.091±0.116*	0.516±0.039	0.715±0.073*
Taurine	2.924±0.144	2.526±0.101	1.064±0.067	1.188±0.061
GABA	3.278±0.553	3.436±0.474	4.574±0.407	5.030±0.566
GABA/glutamate	0.312±0.052	0.406±0.042	1.606±0.093	1.627±0.084

rotransmitter amino acids was routinely determined by HPLC/ED [7]. The supernatant (50 µl) was incubated for 20 min at 37°C with L-homoserine (0.01 mg/µl, 50 µl in 0.2 N NaOH, internal standard) and o-phthalaldehyde sulfite reagent (25 µl) in borate buffer (0.1 M, pH=9.5). The analyzed mixture (5 µl) was transferred to a Zorbax chromatographic column (C_{18}, 4.6×250 mm, 5 µM) and the elution profile was recorded using an LC-4B (BAC) electrochemical detector at +0.85 mV with a carbon glass measuring and Ag/AgCl reference electrodes. The mobile phase contained phosphate buffer (0.05 M, pH= 5.6), EDTA (0.025 mM), and methanol (5%).

The results were statistically analyzed using Student's t test.

RESULTS

In the hypothalamus and brain stem, injection of TZ-146 significantly changed only HVA content ($p<0.05$, Table 1): this parameter decreased in hypothalamus and increased in the brain stem. An important parameter is the HVA/dopamine ratio characterizing the rate of extracellular dopamine turnover. Injection of TZ-146 inhibited dopamine turnover in the hypothalamus, but activated it in the brain stem.

The most pronounced changes in the content of neurotransmitter amino acids were observed in the hypothalamus (Table 2). In the brain stem, only glycine concentration significantly increased, while in the hypothalamus the content of all amino acids (except GABA) was changed. The greatest changes in both cerebral structures involved glycine (increased by 101% in the hypothalamus and decreased by 39% in the brain stem), while GABA underwent only minor changes. The directionality of changes in each structure was similar for excitatory (aspartate and glutamate) and inhibitory (glycine, GABA, and taurine) amino acids.

The changes in the content of neurotransmitter amino acids and catecholamines in the examined structures were opposite: the content of aspartate, glutamate, glycine, and taurine significantly or insignifi-

cantly increased in the brain stem and decreased in the hypothalamus.

Although both structures are considered as parts of the brain stem, the opposite changes in the content of neurotransmitter amino acids and catecholamines observed by us suggest different roles of these morphological subdivisions in mediating the pharmacological effect of TZ-146.

Therefore, despite TZ-146 is a cyclic GABA derivative, its effect seems to be mediated via glutamatergic, rather than GABA-ergic neurotransmitter system. This conclusion is supported by the increase in aspartate and glutamate contents observed in our experiments and reported by others. For example, piracetam, a well-known cyclic GABA derivatives, does not affect GABA-receptors, synaptosomal utilization, and GABA content in the brain and plasma [8,9]. Oxiracetam, another GABA derivative, decreases glutamate content in hippocampal sections from rat brain [10].

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