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EFFECT OF PHENIBUT ON MONOAMINE CONTENT AND THEIR METABOLITES, AS WELL AS NEUROTRANSMITTER AMINO ACIDS IN RAT BRAIN

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The effect of the nootropic drug Phenibut, a structural analogue of gamma ma-aminobutyric acid (GABA) for the content of monoamines and their metabolites, and also neurotransmitter amino acids in the structures of the brain of Wistar rats. It was found that phenibut at a dose of 25 mg / kg with a single intraperitoneal injection nii causes a statistically significant increase in the content of the dopamine metabolite 3,4-dioxyphenylacetic acid (DOPAC), as well as the inhibitory amino acid tau rina in the striatum. The absence of a statistically significant effect of Phenib the level of GABA, serotonin, and dopamine in various brain structures and moderate a significant decrease under the action of the studied drug in the content of norepinephrine in hypo pokampa.

Key words : monoamines, neurotransmitter amino acids, brain, no offspring, memory, GABA derivatives, phenibut, rats

INTRODUCTION

Currently, great importance is attached to study of mechanisms of action and implementation in clinics practical practice for diseases of the nervous system nootropic drugs that facilitate learning memory and memory affecting metabolism neurons with vasoactive and antihypoxic physical properties [1, 3]. Among the most important effects of nootropics should be attributed to their neuroprotective strong action and the ability to facilitate reparative new recovery of brain tissue in case of damage of various genesis [4, 5, 8].

Among the drugs of the nootropic series, in particular, cyclic derivatives of gamma-aminobutyric acid lots (GABA), of considerable interest a GABA derivative phenibut (g-amino hydrochloride β-phenylbutyric acid). The latter is the original ginal domestic nootropic drug with a wide range of concomitant pharmacological sky (including anxiolytic) activity [1, 5]. Fer nibut increases mental and physical performance ability, reduces emotional stress, anxiety, improves sleep; reduces the manifestations of asthenia and vaso-vegetative symptoms (headache, sensory heaviness in the head), irritability, emotional lability [1, 5], exhibits neuroprotective properties under conditions of ischemic damage to the brain [8], stress of various origins [4], etc.

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Despite the fact that this drug is widely some application in the clinic of asthenic and anxiety no-neurotic states, molecular mechanisms we have poorly studied its actions. In particular, studying the effect of phenibut on the content of monoamines - diators of excitation in the central nervous system [norepinephrine (NA), dopamine (DA) and serotonin (5-hydroxytryptamine, 5-OT), as well as their metabolites], allowing the dissolution of cover the mechanisms of neuropsychotropic action of Chemistry [6, 7] has not been carried out to date.

The question of the effects of these connection to the parameters of neurotransmission between the amount of amino acids in various structures of the brain, a fractional study of which would reveal functional formation of the brain, which is property target of pharmacological actions of phenibut [2]. Currently the key the role of neurotransmitter amino acids in the brain in the pathogen without a variety of disorders of the central nervous systems - epileptic seizures, ischemia, hyr poxia, depressive and psychotic disorders, Alzheimer's disease, Parkinson's disease and several others proven by numerous experimental and clinical studies [2, 3].

In this study, using the method of calculating juice-performance liquid chromatography with electro chemical detection (HPLC / ED) was used to study the effect of phenibut on the content of monoamines and their metabolites, as well as neurotransmitter amino acids in the brain structures of Wistar rats.

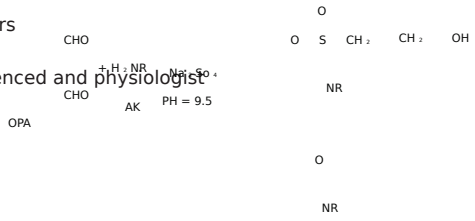
RESEARCH METHODS

In the experiment, 28 male rats were used Wistar lines weighing 200-220g (nursery of the Russian Academy of Medical Sciences Stolbovaya). The animals were kept in standard vivarium conditions under natural light conditions.

Experiments were carried out in the interval from 10 to 14 hours day. The animals were kept in individual boxes.
sugar 30 min before the introduction of phenibut by an experienced and physiologist gic solution to control animals.

Phenibut was used at a dose of 25 mg / kg. Phenibut and saline was injected 45 min before decar animal nutrition. Brain structures [frontal cortex (FC), hippocampus, striatum], removed on ice and frozen in liquid nitrogen. Then highlighted structures were crushed in a glass-tef bosom ”(0.2 mm) at 10 ° C at the rotation speed of the pestle ka 3000 rpm. As a selection medium, we used called 0.1 N. $HClO_4$ with addition of 500 picomole / ml internal standard dioxybenzylamine (DOBA). Brain structures were homogenized in 20 medium volumes. dy allocation. Samples were centrifuged at 10,000 g within 10 minutes. The supernatant was used further neish for the determination of monoamines, their metabolites comov, as well as neurotransmitter amino acids.

The content of monoamines and their metabolites was determined was measured by the method of high-performance liquid chromium tographies with electrochemical detection (HPLC / ED) on an LC-304T chromatograph (BAS, “West Lafayette ”, USA) with Rheodyne 7125 injector with petr Leu for 20 µL for applying samples. The studied items The substances were separated on a reversed-phase column (Ker proSilrPur, ODSr3, 4 ´ 100 mm, grain diameter 3 µm, Dr. Majsch GMBH, Germany, Elsiko, Moscow). Uterine standards were prepared monthly in 0.1 n $HClO_4$ in centering 500 nmol / ml with addition of 0.2 mM



Amino acid derivatization scheme.
OPA - orthophthalaldehyde (orphthalaldehyd), AK - amino acid.

sodium metabisulfite as a preservative. Worker whose standards were prepared from stock solutions daily dilution in 0.1 n $HClO_4$, 1: 1000. Measurement carried out on a glass-carbon electrode (+0.85 V) against the Ag / AgCl reference electrode. All used the reagents used for the analysis were of a high degree purity: high purity KH_2PO_4 anhydrous ("Fluka") - 0.069 M, citric acid monohydrate ("Fluka") - 0.27M, sodium salt of ethylenediaminetetraacetic acids - (EDTA Na_2, “Sigma”) - 0.27 mM, octylsur lfat Na_2 (ion-pair reagent “Diapharm”) - 1.9mM, acetonitrile (Merck) - 1.871M, pH 3.0. To calibrate the chromatograph, mixtures were used. working standards of analytes in the ratio nii 500 picomole / ml. The concentration values of the catheter cholamines (CA) in the test samples were calculated,

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Substance	ON	YES	DOFUK	GVK	5rOT	5-OIUK	DOFUK / YES	GVK / YES	5rOIUK / 5rOT
					Frontal cortex				
Phys. pprr (0.9% NaCl)	100.0 ± 10.3	100.0 ± 11.0	100.0 ± 13.4	100.0 ± 16.0	100.0 ± 9.4	100.0 ± 8.4	100.0 ± 9.4	100 .0 ± 7.1	100.0 ± 5.8
Phenibut (25 mg / kg a / b)	74.7 ± 3.4s	86.7 ± 3.2	82.1 ± 5.9	51.4 ± 6.2s	85.8 ± 2.5	93.8 ± 3.5	94.4 ± 7.4	60.3 ± 6.6 s	108.3 ± 5.4
					Striatum				
Phys. pprr (0.9% NaCl)	100.0 ± 12.9	100.0 ± 6.6	100.0 ± 5.4	100.0 ± 5.5	100.0 ± 6.7	100.0 ± 5.5	100.0 ± 5.3	100 .0 ± 2.0	100.0 ± 3.4
Phenibut (25 mg / kg a / b)	82.9 ± 9.2	108.2 ± 2.1	116.8 ± 5.7s	95.4 ± 5.6	101.5 ± 4.7	107.5 ± 4.9	106.1 ± 3.2	87.5 ± 4.2s	105.4 ± 4.6
					Hippocampus				
Phys. pprr (0.9% NaCl)	100.0 ± 9.6	100.0 ± 54.4	100.0 ± 26.5	100.0 ± 13.0	100.0 ± 4.2	100.0 ± 4.1	100.0 ± 23.0	100 .0 ± 23.4	100.0 ± 3.6
Phenibut (25 mg / kg a / b)	65.4 ± 3.9s	43.1 ± 5.4	37.5 ± 8.7	61.3 ± 7.4 s	93.1 ± 4.4	101.9 ± 3.6	42.4 ± 11.0	67.6 ± 11.9	110.1 ± 5.2

Note . s - Differences are significant in comparison with the content of monoamines in the brain of Wistar rats treated with nat. prp (0.9% NaCl) at $p < 0.05$ (Student's *t*- criterion). i / b - intraperitoneally.

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based on the ratios of the heights of the peaks in the standard si and in the sample, according to the following formula:

$$C_{KAion} = \frac{H_{KAion}}{H_{dGBAop}} \times F \text{ pg / ml},$$

where $f = \frac{C_{KAist}}{H_{Cast} \quad H_{dGBast}}$,

Φ_i is the conversion factor for the concentration Kai, N_{CAist} - peak height KAi standard, H_{KAiop} - peak height

and 5% acetonitrile. The speed of the mobile phase is set 1.5 ml / min.

The mobile phase was filtered using a vacuum pump through a cellulose filter (pore diameter - 0.2 µm) and before each chromatographic the determination was thoroughly degassed under vacuum.

As a standard for quantification amino acids in rat brain structures used solution containing aspartate, glutamate, glycine, tau rin, GABA at a concentration of 0.5 mmol / l (see diagram).

KAi determined in the sample, H dGBAst - peak height dGBA in standard solution, H dGBAop - peak height dGBA determined in the sample, C KAist - concentration CAi in the standard, C CAiop is the concentration of CAi, determined taken in the sample.

Determination of the content of excitatory (aspartate, glutamate) and inhibitory (GABA, glycine, taurine) neuro mediator amino acids carried out method HPLC / ED according to standard method (Pearson and et al., 1991). Since amino acids in native form are very weak chromophores (not absorbed by the UV spectrum) and do not exhibit electrochemical their detection, it is necessary to preliminary chemical modification rationing - derivatization. for this we used the orr tophthalic aldehyde (OFA) capable of fluorescence to bind when binding with amino acid.

GABA, aspartate, glutamate, taurine, glycine in concentration a concentration of 0.1 μM / ml in 0.1 N. HClO 4 was used in the as a standard mixture for calibration. Across 15 min after incubation at room temperature 20 μL of the solution was loaded onto an Agilent Hypersil column ODS 5 mkM, 4.6 ´ 250. Product registration section measurements were carried out on an Agir fluorescence detector lent 1100 (USA) at an excitation wavelength of 230 nm and emission waves of 392 nm. The mobile phase consisted of 0.05 M phosphate buffer (pH 5.6) with 0.025 mM EDTA

Statistical processing of research results were performed using standard methods. parametric statistical analysis of trcr rere Student; one-way dispersion analysis, Student's test with Bonferro's correction nor for multiple comparisons.

RESULTS AND ITS DISCUSSION

When studying the effects of Phenibut on maintenance monoamines and their metabolites were found to indicate the substance involved statistically significantly reduces the vein HA in the hippocampus by 35%. Phenibut did not affect the content of dA, however, caused a statistically significant correct change in the content of its metabolites, increased high concentration of DOPAA in the striatum and, on the contrary, reducing the content of HVA in the hippocampus. Effects of phenyr buta to the level of 5-OT and its metabolite 5-OIAA, It was not ruined. It was not noted also statistically significant effect of this compound on the complex indicators characterizing the speed of the circuit rota dA and 5-OT, except for the parameter GVK / dA, which decreased slightly in the striatum (Table 1).

When studying the effect of phenibut on the content excitatory (aspartate, glutamate) and inhibitory (GABA, glycine, taurine) neurotransmitter amino acids of the most significant effects were noted in striatum. At the same time, phenibut caused a small, but

Table 2. **Effect of the effect of the effect on the content of non-transmitter amino acids in the low-voltage supply (M ± SEM)% Ratio to Control (0.9% NaCl)**

A drug	Asp	Glu	Gly	Tau	GABA
			Frontal cortex		
Phys. prr (0.9% NaCl)	100.0 ± 4.258	100.0 ± 4.085	100.0 ± 3.155	100.0 ± 5.695	100.0 ± 3.999
Phenibut (25 mg / kg a / b)	102.383 ± 4.066	102.517 ± 2.511	106.834 ± 5.422	103.552 ± 3.805	102.673 ± 2.102
			Striatum		
Phys. prr (0.9% NaCl)	100.0 ± 5.241	100.0 ± 4.891	100.0 ± 2.922	100.0 ± 4.455	100.0 ± 6.706
Phenibut (25 mg / kg a / b)	113.134 ± 4.493	113.413 ± 4.921	110.738 ± 4.734	114.102 ± 4.547s	103.092 ± 5.893
			Hippocampus		
Phys. prr (0.9% NaCl)	100.0 ± 2.397	100.0 ± 1.864	100.0 ± 2.401	100.0 ± 3.616	100.0 ± 1.688
Phenibut (25 mg / kg a / b)	98.475 ± 1.249	94.831 ± 1.477s	95.477 ± 1.968	102.871 ± 3.763	120.178 ± 23.187

Note . s - Differences are significant compared to control at p <0.05 (Student's t-criterion). i / b - intraperitoneally.

statistically significant increase in concentration taurine (Table 2).

Effects in other structures of the rat brain phenibut practically did not appear, only insignificant the concentration of glutamate in the hippopotamus decreased significantly. campe.

The results obtained indicate that phenibut causes a significant increase in the content of dopamine metabolite 3,4-dioxyphenylacetic acid, as well as the inhibitory amino acid taurine. We did not observe the effect of phenibut on GABA levels. was obtained in spite of the fact that the studied substance is is a structural analogue of the latter. Not noted and the significant effect of this nootropic drug on the serotonergic system.

CONCLUSIONS

1. Phenibut causes a marked increase in retention of dopamine metabolite 3,4-dioxyphenyl acetic acid and inhibitory amino acid taurine in striatum.

2. The drug has no significant effect for GABA, serotonin, and dopamine in various structures rax of the brain and moderately reduces the content norepinephrine in the hippocampus.

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EFFECT OF PHENIBUT ON THE CONTENT OF MONOAMINES, THEIR METABOLITES, AND NEUROTRANSMITTER AMINO ACIDS IN RAT BRAIN STRUCTURES

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Effects of the nootropic drug phenibut, which is a structural analog of gamma-aminobutyric acid (GABA), on the content of monoamines, their metabolites, and neurotransmitter amino acids in brain structures have been studied on Wistar rats. It is established that a single administration of phenibut in a dose of 25 mg / kg (ip) produces a statistically significant increase in the content of dopamine metabolite (3,4-dioxyphenylacetic acid) and the retarding amino acid taurine in striatum. At the same time, phenibut did not significantly influence the levels of GABA, serotonin, and dopamine in various brain structures and produce a moderate decrease in the level of norepinephrine in the hippocampus.

Keu words : Phenibut, monoamines, neurotransmitter amino acids, brain, nootropes, GABA derivatives, memory