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EXPERIMENTAL ARTICLES

Neurochemical Effects of Afobazol on the Level of Monoamines and Their Metabolites in Mice with Various Emotional Phenotypes with Serotonin Deficit

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Abstract—We studied the neurochemical effects of afobazol in the brain structures of BALB/C and C57BL/6 mice with serotonin deficit induced by *para*-chlorophenylalanine (PCPA), which inhibits tryptophan 5-hydroxylase, the main enzyme of serotonin synthesis. Interstrain differences were found in the level of norepinephrine (NE), serotonin (5-HT), and dopamine (DA) metabolism parameters in the frontal cortex (FC), amygdala, striatum, hypothalamus, and hippocampus. It was demonstrated that PCPA (350 mg/kg/3 days) caused a considerable decrease in the level of 5-HT and its metabolite 5-HIAA in the structures of the brain studied in both strains of mice, but in BALB/C mice the decrease in these indices was more intense (2–2.5 times). PCPA decreased the level of NE in the hypothalamus, amygdala, and striatum of C57BL/6 mice. In the 5-HT deficiency model, afobazol (5 mg/kg) influenced the parameters of dopaminergic neurotransmission by decreasing the level of DOPAC and the DOPAC/DA ratio in the hypothalamus and striatum of both strains. An increase in the content of 5-HT and NE was observed after a decrease caused by the administration of PCPA in the hypothalamus and amygdala of BALB/C mice and the hippocampus and amygdala of C57BL/6 mice. The indices of 5-HIAA/5-HT metabolism rate were decreased. The results of the current study confirm the previous data on the role of the serotonergic brain systems in the mechanism of action of afobazol. In PCPA-induced serotonin deficit, the drug influenced both stress-resistant (C57BL/6) and more emotionally labile animals (BALB/C) which is reflected by the restoration of serotonin and norepinephrine levels in the hypothalamus of BALB/C mice, as well as in the amygdala and hippocampus of C57BL/6 strain.

Keywords: afobazol, *para*-chlorophenylalanine (PCPA), mouse strains, brain structures, serotonin, norepinephrine, dopamine, HELC

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INTRODUCTION

Modeling neurotransmitter deficiency in animals with phenotypic variation in stress sensitivity is one of the most commonly used methods for studying the role of monoaminergic systems of the brain in anxiety disorders and the mechanism of action of anxiolytics. The main approach for modeling serotonin deficiency (5-hydroxitriptamine, 5-HT) in the brain is inhibiting tryptophan 5-hydroxylase (TPH), the key enzyme of the serotonin synthesis, using the irreversible inhibitor *para*-chlorophenylalanine (PCPA) or fenclonine, as well as alimentary consumption of amino acids lacking tryptophan which leads to a quick depletion of 5-HT in the body resulting in a considerable decrease in the content of this neurotransmitter [1]. BALB/C mice, which differ from C57BL/6 animals by their passive response to a modeled stress situation in an open field [2], bear a mutation in TPH2 gene, which determines a lower baseline level and synthesis rate of serotonin in the brain of these animals [3–5].

Afobazol (5-ethoxy-2-[2-(morpholino)-ethylthio]-benzimidazole dihydrochloride), synthesized in the Zakusov Institute of Pharmacology, has a high anxiolytic potential. The mechanism of anxiolytic activity of afobazol is attributed to its ability to bind to σ1 receptors, MT_1 and MT_3 melatonin receptors, as well as an MAO-A regulatory site exerting modulatory influence on the main neurotransmitter systems of the brain [6, 7]. We demonstrated the selectivity of afobazol action regarding different neurotransmitter systems in the brain of the indicated strains [8]. When co-administered with the aromatic amino acid decarboxylase inhibitor, NSD-1015, afobazol caused a decrease in the level of 3,4-dihydrophenylacetic acid (DOPAC) in the hypothalamus, and homovanillic acid (HVA) in the striatum of rats, which suggests an inhibitory effect of this substance on the main enzyme of DA biodegradation, monoamine oxidase B (MAO-B) [9]. Despite the fact that significant data have recently been col-

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lected on the pharmacological effects of afobazol, there is still very little information about its influence on the serotonergic system, which plays the leading role in the neurochemical mechanism of anxiety and depressive disorders.

For this reason, the present study was aimed at investigating the neurochemical aspects of the effect of afobazol on the content of monoamines and their metabolites in the brain structures of mouse strains with different emotional phenotypes, BALB/C and C57BL/6 under normal conditions and in PCPA-induced 5-HT deficiency.

MATERIALS AND METHODS

Animals. The experiments were performed with BALB/C and C57BL/6 male mice weighing 20–24 g ("Stolbovaya" vivarium) held under laboratory vivarium conditions with a 12-hour of light cycle with water and standard food ad libitum. To exclude the influence of circadian biorhythms on the rate of the neuromediator biosynthesis and metabolism, the tests were performed between 10 and 12 in the morning. Experiments were performed in compliance with "Rules of good laboratory practice of the Russian Federation" approved by the Order of the Ministry of Health of the Russian Federation № 199н of April 1, 2016. The animals were sustained in accordance with SP 2.2.1.3218-14 "Sanitary and epidemiological requirements to arrangement, equipment and maintenance of biological clinics (vivariums)" of August 29, 2014 no. 51. The experimental procedures were approved by Bioethics Commission of the Zakusov Institute of Pharmacology (protocol no. 6 of 16 April 2018).

Investigated substances. Afobazol (Zakusov Institute of Pharmacology) was dissolved in 0.9% NaCl and administered intraperitoneally at a dose of 5 mg/kg 1 hour prior to decapitating the animals. PCPA (Sigma) was administered intraperitoneally for three days at a dose of 150 mg/kg on the first day and 100 mg/kg on the following days. According to the literature, the decreased level of serotonin caused by PCPA develops gradually, reaches its peak after 3 days, and remains unchanged for 5–6 days [10].

Design of the experiment. The animals were divided into the following experimental groups (number of animals is given in brackets):

Group 1 BALB/C, control (0.9% NaCl) ($n = 9$);

Group 2 BALB/C, 0.9% NaCl + afobazol (5 mg/kg) ($n = 10$);

Group 3 BALB/C, PCPA at a total dose of 350 mg/kg ($n = 10$);

Group 4 BALB/C, 0.9% NaCl + afobazol + PCPA ($n = 10$);

Group 5 C57BL/6, control (0.9% NaCl) ($n = 10$);

Group 6 C57BL/6, 0.9% NaCl + afobazol (5 mg/kg) ($n = 9$);

Group 7 C57BL/6, PCPA at a total dose of 350 mg/kg ($n = 10$);

Group 8 C57BL/6, 0.9% NaCl + afobazol + PCPA ($n = 9$).

Neurochemical studies. The animals were decapitated 60 min after substance administration. The brain structures (frontal cortex (FC), hippocampus, hypothalamus, and striatum) were extracted on ice, frozen, weighed, and stored in liquid nitrogen. Prior to the experiments on the measurement of neurotransmitter levels, the samples were homogenized using a Potter homogenizer (teflon/glass) in 1 mL of 0.1 M $HClO_4$ with 3,4-dioxybenzylamine (0.5 nmol/mL) as the internal standard. The samples were centrifuged at 10000 *g* for 10 min. The content of monoamines and their metabolites (norepinephrine (NE), dopamine (DA), 3,4-dihydrophenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-hydroxytryptamine, 5-HT), and 5-hydroxyindoleacetic acid (5-HIAA)) was measured by high pressure liquid chromatography with electrochemical detection (HPLC/ED) using an LC-304T chromatograph (BSA, West Lafayette, United States) with a ReproSil-Pur ODS analytical column (C18, 100 × 4 mm, 3 μm) (Dr.Maisch, Germany) [11].

Statistical analysis of the data on the content of monoamines in the brain structures was conducted as follows: the data were analyzed using the Shapiro-Wilk test. Since the distribution of the results was close to normal, statistical significance of differences was analyzed using three-way ANOVA test followed by Duncan's multiple-range test with the critical level of significance at $\alpha = 0.05$. Mean values and standard errors of the mean are presented ($M \pm S.E.M.$).

RESULTS AND DISCUSSION

The content of monoamines and their metabolites in the brain structures of control intact BALB/C and C57BL/6 (0.9% NaCl) mice that were not exposed to external influence is presented in Table 1.

Comparison of the neurochemical indices in the two strains of animals demonstrated a statistically significant decrease in the levels of NE in the FC and hippocampus of BALB/C mice compared to C57BL/6 mice. In the hypothalamus NE content, on the contrary, was significantly higher. The level of DA metabolites DOPAC and HVA in the hypothalamus and amygdala of stress-sensitive mice, as well as the content of DA, were considerably higher than the corresponding values in C57BL/6 mice. The parameters of the serotonergic system of BALB/c mice significantly differed from C57BL/6 mice by higher 5-HT content in the amygdala and lower 5-HT content in in the hippocampus (Table 1).

A single dose of 5 mg/kg afobazol caused changes in the neurotransmitter levels in the brain structures of both mouse strains; the strongest effects were

Table 1. Content of monoamines and their metabolites in the brain structures of intact C57/BL and BA

Animal strains	NE	DA	DOPAC	HVA	DOPAC/DA	HVA/DA	5-HT	5-HIAA	5-HIAA/5-HT
				Frontal cortex					
C57/BL	2.97 ± 0.11**	0.88 ± 0.08	0.52 ± 0.04	0.50 ± 0.04	0.61 ± 0.03	0.61 ± 0.09	9.50 ± 0.30	1.18 ± 0.06*	0.07 ± 0.01
BALB/C	2.04 ± 0.15	0.86 ± 0.03	0.55 ± 0.03	0.58 ± 0.01	0.64 ± 0.03	0.68 ± 0.02	8.93 ± 0.80	0.98 ± 0.10	0.11 ± 0.00
				Hypothalamus					
C57/BL	5.12 ± 0.18*	1.15 ± 0.09*	0.51 ± 0.03*	0.53 ± 0.05*	0.46 ± 0.03	0.50 ± 0.07	21.63 ± 1.07	3.12 ± 0.18	0.14 ± 0.01
BALB/C	7.28 ± 0.42	1.87 ± 0.13	0.77 ± 0.04	0.83 ± 0.11	0.42 ± 0.02	0.43 ± 0.05	24.28 ± 1.75	3.41 ± 0.31	0.14 ± 0.00
				Amygdala					
C57/BL	4.15 ± 0.20	4.17 ± 0.87	1.20 ± 0.11*	1.16 ± 0.23*	0.33 ± 0.06	0.26 ± 0.03	32.50 ± 1.89*	3.07 ± 0.30	0.09 ± 0.01
BALB/C	4.49 ± 0.55	7.97 ± 1.64	2.21 ± 0.37	2.64 ± 0.55	0.28 ± 0.08	0.302 ± 0.06	44.49 ± 3.94	3.88 ± 0.36	0.08 ± 0.01
				Striatum					
C57/BL	0.59 ± 0.05	48.89 ± 2.82	2.33 ± 0.20	3.73 ± 0.26*	0.05 ± 0.00**	0.08 ± 0.00**	2.62 ± 0.12	1.21 ± 0.07	0.47 ± 0.03
BALB/C	0.87 ± 0.15	37.44 ± 5.43	2.98 ± 0.55	6.12 ± 1.12	0.06 ± 0.00	0.13 ± 0.01	3.30 ± 0.55	1.48 ± 0.32	0.44 ± 0.03
				Hippocampus					
C57/BL	2.18 ± 0.11**	0.44 ± 0.08	0.29 ± 0.04	0.15 ± 0.03	0.77 ± 0.118	0.37 ± 0.10	4.87 ± 0.22	2.09 ± 0.17	0.43 ± 0.03
BALB/C	1.424 ± 0.17	0.38 ± 0.04	0.27 ± 0.03	0.23 ± 0.03	0.65 ± 0.064	0.50 ± 0.12	3.30 ± 0.46*	1.80 ± 0.30	0.50 ± 0.03

The mean and standard error of the mean are presented ($M \pm S.E.M.$).
*Significant differences at $p < 0.05$ (three-way analysis with Duncan's multiple-range test).

observed for the serotonergic and norepinephrinergic systems. In the hypothalamus, amygdala, and hippocampus of C57BL/6 mice, we observed an increase in NE, whereas in BALB/C mice, its concentration showed a statistically significant decrease in the striatum and hippocampus (Table 2). Afobazol caused an increase in the content of 5-HT in almost all the studied brain structures (except the striatum) in C57BL/6 mice, whereas the content of 5-HIAA and the index of the 5-HT metabolism rate (5-HIAA/5-HT) in all the structures showed a statistically significant decrease. A similar decrease in the 5-HT metabolism rate was observed in the brain structures (except the amygdala) of BALB/C mice, however, it was not accompanied by the accumulation of the neurotransmitter; on the contrary, it showed a decrease in the striatum.

The effects of afobazol on the parameters of the dopaminergic system were characterized by a considerable (up to 264%) increase in the content of DA in the amygdala of mice with passive stress response. In the same structure of C57BL/6 animals, a significantly lesser increase (up to 145%) in the level of DA was observed. A decrease in the content of DOPAC and HVA was observed in the striatum of mice with active (47 and 45%, respectively) and passive (40 and 30%, respectively) stress response (Table 2).

PCPA at a total dose of 350 mg/kg for 3 days predictably caused a considerable decrease in the content of 5-HT and 5-HIAA in the studied brain structures in both mouse strains. Notably, mice with passive stress response (BALB/C) demonstrated a more profound decline in these indices—down to 70–80% of controls, which was 2–2.5 times lower than in C57BL/6 mice. PCPA considerably decreased the level of NE in the hypothalamus, amygdala, and striatum in stress-sensitive mice without affecting it in C57BL/6 mice. The values of the dopaminergic transmission parameters remained almost unchanged in the brain structures of both mouse strains (Table 2).

Afobazol in the model of serotonin deficit induced by subchronic administration of PCPA influenced the parameters of dopaminergic neurotransmission. In the hypothalamus and striatum of BALB/C and C57BL/6 mice, we observed a decrease in the content of DOPAC and a decrease in the DA metabolism rate (DOPAC/DA). The influence of afobazol on the content of 5-HT and 5-HIAA are of great interest. Afobazol increased the content of 5-HT, which was previously decreased as a result of PCPA administration, in the hypothalamus of mice with passive stress response (BALB/C) and in the FC, amygdala, and hippocampus of stress resistant mice (C57BL/6). The metabolism rate index (5-HIAA/5-HT) was decreased in the FC, hypothalamus, and amygdala of the both mouse strains similarly. However, the extent of serotonin deficiency compensation by afobazol was higher in C57BL/6 mice. A considerable increase in the NE content, which was previously decreased by PCPA, was registered in the amygdala and hippocampus of C57BL/6 mice and hypothalamus of stress-sensitive mice.

The decline in the level of 5-HT that we observed in the brain structures of BALB/C and C57BL/6 mice (Table 2) is consistent with the literature evidence that subchronical administration of PCPA for 3 days leads to an 80% decrease in the content of 5-HT compared to the initial values. This effect results from inhibition of the main enzyme of 5-HT biosynthesis tryptophan 5-hydroxylase by PCPA [10, 12]. In our study, PCPA caused a deeper (80%) decline in the level of serotonin and its metabolite in the brain structures of BALB/C mice, which points to a decreased rate of serotonin synthesis in these animals [13].

The data on the influence of afobazol on the parameters of the serotonergic system are consistent with the results of our previous studies. Thus, afobazol in the model of serotonin synthesis disturbance induced by aromatic amino acid decarboxylase inhibitor NSD-1015 caused an increase in the content of the serotonin synthesis precursor 5-hydroxytryptophan (5-HTP), 5-HT, and its metabolite 5-HIAA in the hypothalamus of rats by 50, 60, and 50%, respectively, which suggests that this anxiolytic influences tryptophan hydroxylase, the enzyme of 5-HTP synthesis [9]. In another of our studies, co-administering afobazol and the 5-HT2b/2c receptor antagonist SB-200646A caused an increase in the content of 5-HT and 5-HIAA in the hippocampus of BALB/C mice, which may be considered as positive modulation of the anxiolytic effect resulting from the blockade of 5-HT2 serotonin receptors, which points to the involvement of these receptors in the realization of the anxiolytic effect of afobazol [14]. In the present study, in PCPA-induced 5-HT deficiency the afobazol-induced increase in the level of neurotransmitter occurred due to its decreased metabolism, probably resulting from inhibition of monoamine oxidase A (MAO-A), an enzyme responsible for its biodegradation [6]. Note that afobazol to a larger extent mitigated PCPA-induced 5-HT deficiency in the brain structures of mice resistant to stress (C57BL/6) restoring its level to almost normal in the FC, hypothalamus, and amygdala. The weaker effect in stress-sensitive mice (BALB/C) can be attributed to genetically determined low levels of the enzymes MAO-B, MAO-A, and, probably, tryptophan 5-hydrozylase [4–6].

Interestingly, afobazol increases the NE concentration in the same structures where the increase in 5-HT was observed. Under conditions of PCPA-induced deficit, it occurs in the amygdala and hippocampus of C57BL/6 mice and in the hypothalamus of BALB/C mice, and under normal conditions, in the amygdala, hippocampus, and hypothalamus of stress-resistant mice. This increase in the concentration of NE and 5-HT in the synaptic cleft are caused by practically all antidepressants of differing structures. The

Table 2. The influence of PCPA (350 mg/kg, 3 days) and afobazol (5 mg/kg, 60 min) on the content of monoamines in the brain structures of C57/BL and BALB/C mouse strains

Substance		NE	DA	DOPAC	HVA	DOPAC/DA	HVA/ DA	5-HT	5-HIAA	5-HIAA/ 5-HT
Frontal cortex										
C57/BL	Afobazol	3.35 ± 0.13	0.84 ± 0.05	0.59 ± 0.07	0.29 ± 0.07	0.71 ± 0.08	0.37 ± 0.09	11.73 ± 0.62*	0.82 ± 0.05**	0.07 ± 0.00**
	PCPA	2.77 ± 0.08	0.75 ± 0.06	0.48 ± 0.04	0.46 ± 0.03	0.64 ± 0.02	0.65 ± 0.07	5.89 ± 0.37**	0.48 ± 0.04**	0.08 ± 0.00**
	Af + PCPA	2.81 ± 0.20	0.74 ± 0.11	0.45 ± 0.04	0.52 ± 0.14	0.55 ± 0.03	0.52 ± 0.14	**8.08 ± 0.58**$^{\#}$	0.40 ± 0.07**	**0.05 ± 0.01****$^{\#}$
BALB/C	Afobazol	1.83 ± 0.11	0.84 ± 0.03	0.59 ± 0.03	0.43 ± 0.04	0.70 ± 0.03	0.51 ± 0.04	6.69 ± 0.55*	0.58 ± 0.04**	0.09 ± 0.01
	PCPA	1.74 ± 0.14	0.69 ± 0.10	0.56 ± 0.07	0.44 ± 0.06	0.84 ± 0.06*	0.70 ± 0.08	2.58 ± 0.28**	0.22 ± 0.03**	0.09 ± 0.01*
	Af + PCPA	1.83 ± 0.08	0.78 ± 0.05	0.40 ± 0.04	0.38 ± 0.05	**0.52 ± 0.05**$^{\#\#}$	0.49 ± 0.06	2.65 ± 0.24**	0.13 ± 0.02**	**0.05 ± 0.01****$^{\#}$
HypothalamusC										
C57/BL	Afobazol	6.32 ± 0.40*	1.36 ± 0.04	0.37 ± 0.04*	0.40 ± 0.08	0.28 ± 0.03*	0.30 ± 0.06	26.38 ± 1.08*	2.32 ± 0.11*	0.09 ± 0.00**
	PCPA	4.69 ± 0.17	1.20 ± 0.07	0.49 ± 0.03	0.66 ± 0.04	0.41 ± 0.02	0.57 ± 0.04	12.10 ± 1.24**	1.07 ± 0.14**	0.09 ± 0.00**
	Af + PCPA	5.48 ± 0.17	1.19 ± 0.09	**0.27±0.04****$^{\#\#}$	0.55 ± 0.14	**0.24±0.03****$^{\#\#}$	0.47 ± 0.12	16.70 ± 1.93**	0.94 ± 0.11**	**0.06 ± 0.01**$^{\#}$
BALB/C	Afobazol	6.93 ± 0.51	1.84 ± 0.17	0.63 ± 0.06	0.57 ± 0.07	0.35 ± 0.03	0.31 ± 0.04	21.91 ± 1.65	2.26 ± 0.26*	0.10 ± 0.00**
	PCPA	5.56 ± 0.17*	1.48 ± 0.07**	0.55 ± 0.02**	0.62 ± 0.04	0.37 ± 0.02	0.43 ± 0.04	5.70 ± 0.23**	0.44 ± 0.05**	0.08 ± 0.01**
	Af + PCPA	**6.65 ± 0.38**$^{\#}$	1.74 ± 0.09	**0.27 ± 0.03***$^{\#}$	0.56 ± 0.06*	**0.20 ± 0.04****$^{\#}$	0.33 ± 0.04	**7.20 ± 0.43****$^{\#}$	0.36 ± 0.04*	**0.05 ± 0.01****$^{\#}$
Amygdala										
C57/BL	Afobazol	5.67 ± 0.27*	6.08 ± 0.80	1.21 ± 0.06	1.07 ± 0.15	0.23 ± 0.04	0.19 ± 0.04	46.18 ± 1.56*	2.33 ± 0.15	0.05 ± 0.00**
	PCPA	3.76 ± 0.31	5.85 ± 2.14	1.20 ± 0.21	1.30 ± 0.35	0.29 ± 0.04	0.26 ± 0.04	19.66 ± 1.30*	0.94 ± 0.13**	0.05 ± 0.01**
	Af + PCPA	**5.32 ± 0.28**$^{\#}$	4.12 ± 0.52	1.03 ± 0.08	1.15 ± 0.13	0.24 ± 0.03	0.29 ± 0.06	**31.19 ± 2.38**$^{\#}$	0.78 ± 0.11**	**0.02 ± 0.00****$^{\#}$
BALB/C	Afobazol	5.05 ± 0.75	21.05 ± 3.22**	2.10 ± 0.26	2.77 ± 0.33	0.11 ± 0.01*	0.16 ± 0.03*	38.28 ± 2.14	2.74 ± 0.19*	0.07 ± 0.00
	PCPA	2.21 ± 0.16**	5.81 ± 1.11	1.05 ± 0.06**	1.49 ± 0.21*	0.22 ± 0.03	0.29 ± 0.05	11.51 ± 0.70**	0.29 ± 0.05**	0.02 ± 0.00**
	Af + PCPA	**4.04 ± 0.27**$^{\#}$	9.36 ± 1.85	0.96 ± 0.08**	0.88 ± 0.19**	0.13 ± 0.03*	**0.10 ± 0.01***$^{\#}$	16.70 ± 1.58**	0.20 ± 0.08**	**0.01 ± 0.00****$^{\#}$
Striatum										
C57/BL	Afobazol	0.80 ± 0.08	54.21 ± 2.63	1.23 ± 0.07**	2.07 ± 0.18**	0.02 ± 0.00**	0.04 ± 0.00**	2.83 ± 0.32	0.70 ± 0.09*	0.25 ± 0.01**
	PCPA	0.89 ± 0.13	49.50 ± 4.18	2.58 ± 0.24	3.94 ± 0.29	0.05 ± 0.00	0.08 ± 0.00	1.66 ± 0.15**	0.36 ± 0.07**	0.21 ± 0.03**
	Af + PCPA	0.71 ± 0.07	58.20 ± 2.49	**0.97±0.08****$^{\#\#}$	3.46 ± 0.47	**0.02 ± 0.00****$^{\#}$	**0.06 ± 0.01***$^{\#}$	1.94 ± 0.11*	0.34 ± 0.08**	0.17 ± 0.04**
BALB/C	Afobazol	0.43 ± 0.05*	43.64 ± 1.79	1.77 ± 0.05*	4.22 ± 0.20**	0.04 ± 0.00**	0.10 ± 0.00**	2.04 ± 0.14*	0.68 ± 0.05*	0.34 ± 0.03*
	PCPA	0.51 ± 0.05*	38.12 ± 1.46	2.19 ± 0.10*	5.12 ± 0.17*	0.06 ± 0.00*	0.14 ± 0.00	0.82 ± 0.06**	0.10 ± 0.02**	0.13 ± 0.03**
	Af + PCPA	0.52 ± 0.09	35.50 ± 3.40	**0.67 ± 0.09****$^{\#}$	**2.06±0.22****$^{\#\#}$	**0.02±0.00****$^{\#\#}$	**0.06±0.00****$^{\#\#}$	0.49 ± 0.12**	0.17 ± 0.13**	0.08 ± 0.02**
Hippocampus										
C57/BL	Afobazol	2.82 ± 0.08*	0.40 ± 0.05	0.23 ± 0.01	0.12 ± 0.03	0.64 ± 0.08	0.35 ± 0.10	6.66 ± 0.20**	1.68 ± 0.08	0.25 ± 0.02*
	PCPA	1.96 ± 0.10	0.54 ± 0.10	0.27 ± 0.02	0.13 ± 0.02	0.63 ± 0.12	0.29 ± 0.06	2.50 ± 0.37**	0.41 ± 0.08**	0.15 ± 0.02**
	Af+PCPA	**2.46 ± 0.09**$^{\#}$	0.49 ± 0.07	0.42 ± 0.03	0.16 ± 0.04	0.96 ± 0.14	0.33 ± 0.10	**4.43 ± 0.37**$^{\#}$	0.51 ± 0.10**	0.11 ± 0.01**
BALB/C	Afobazol	1.03 ± 0.05*	0.65 ± 0.10	0.30 ± 0.07	0.35 ± 0.15	0.37 ± 0.11	0.24 ± 0.05	2.90 ± 0.34	1.01 ± 0.10**	0.36 ± 0.02*
	PCPA	1.30 ± 0.18	0.45 ± 0.06	0.29 ± 0.04	0.11 ± 0.02	0.76 ± 0.10	0.32 ± 0.08	0.74 ± 0.13**	0.22 ± 0.06**	0.29 ± 0.07*
	Af + PCPA	1.34 ± 0.09	0.47 ± 0.07	0.39 ± 0.08	0.11 ± 0.02	1.02 ± 0.22	0.26 ± 0.04	0.84 ± 0.18**	0.07 ± 0.02*	0.17 ± 0.09**

The mean and standard error of the mean are presented ($M \pm S.E.M.$).
*Significant differences compared to controls for the same strain at $p < 0.05$ (three-way analysis with Duncan's multiple-range test);
**$p < 0.001$ (three-way analysis with Duncan's multiple-range test);
#Significant differences compared to the group that received PCPA at $p < 0.05$;
##$p < 0.001$ (three-way analysis with Duncan's multiple-range test).

antidepressant mirtazapine blocks alpha-2 NE receptors and through a negative feedback increases the level of NE and 5-HT in the synaptic cleft. In our experiment, the effect of afobazol is likely related to the inhibition of neurotransmitter degradation due to a decrease in the activity of MAO-A. This assumption is substantiated by the data on the afobazol binding profile [6].

The influence of afobazol on the indices of dopaminergic neurotransmission is consistent with our previous data. Thus, it was demonstrated that, when co-administered with the aromatic amino acid decarboxylase inhibitor, NSD-1015, afobazol causes a decrease in the level of DOPAC in the hypothalamus, and HVA in the striatum of rats, which suggests that the former inhibits the main enzyme of DA biodegradation monoamine oxidase B (MAO-B) [9]. In the present work, the effects of afobazol on the DA metabolites DOPAC and HVA in the hypothalamus and striatum of the both mouse strains may be similarly explained by a decrease in the rate of DA utilization.

As for the interstrain differences in the behavioral effects of afobazol on animals with phenotypic variations in emotional reactions, it is known from the literature that the drug only influences one phenotype—animals with genetically determined fear response to emotional stress (BALB/C strain) and humans with individual typological traits of mental susceptibility to stressors [15].

The results of the present study substantiate our previous data on the involvement of the serotonergic brain systems in realizing the effects of afobazol and shed light on the role of the adrenergic system. In PCPA-induced serotonin deficiency, the drug influences both stress-resistant and emotionally labile animals, which is manifested in the compensatory restoration of the serotonin level in the frontal cortex, amygdala, and hippocampus of C57BL/6 mice and the hypothalamus of BALB/C mice. At the same time, afobazol increases the level of norepinephrine in the amygdala and hippocampus of C57BL/6 mice and in the hypothalamus and amygdala of BALB/C mice.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare no conflicts of interest.

Ethical approval. Experiments were performed in compliance with "Rules of good laboratory practice of the Russian Federation" approved by the Order of the Ministry of Health of the Russian Federation № 199 of April 1, 2016. The animals were maintained in accordance with SP 2.2.1.3218-14 "Sanitary and epidemiological requirements to arrangement, equipment and maintenance of biological clinics (vivariums)" of August 29, 2014. The experimental procedures were approved by the Bioethics Commission of the Zakusov Institute of Pharmacology , (protocol no. 6 of April 16, 2018).

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