Effect of Combined Administration of Afobazole and 5-HT$_{2b/2c}$ Receptor Antagonist SB-200646A on Neurochemical Profile of Brain Structures in C57Bl/6 and BALB/c Mice

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The effects of combined administration of afobazole and 5-HT$_{2b/2c}$ receptor antagonist SB-200646A on the content of monoamines and their metabolites in brain structures of C57Bl/6 and BALB/c mice were studied by the methods of HPLC with electrochemical detection. Combined administration of afobazole and SB-200646A increased the content of epinephrine in the striatum of BALB/c mice (to 230% of the control) and in the hippocampus of both mouse strains. The content of dihydroxyphenylacetic and homovanillic acids and parameters of dopamine metabolism in these structures were reduced. The content of dopamine in the hypothalamus and amygdala was elevated in C57Bl/6, but not in BALB/c mice. These findings attest to the involvement of monoamine systems of the brain in the mechanism of afobazole action and suggest that the enhanced anxiolytic effect of afobazole combination with SB-200646A can be interpreted as a positive modulation of the effect of anxiolytic determined by blockade of 5-HT$_2$ serotonin receptors.

Key Words: *afobazole; mouse strains; selective serotonin 5-HT$_{2b/2c}$ receptor antagonist SB-200646A; liquid chromatography*

High prevalence of anxiety disorders in modern society necessitates elucidation of the nature of these pathological states and pharmacological aspects of their therapy, including the mechanisms of action of anxiolytic drugs. Serotoninergic systems of the brain, in particular, functionally different serotonin (5-HT) receptors, attract special interest in this respect. More than 15 subtypes of 5-HT receptors with different functional characteristics have been identified in the brain structures [8]. Their involvement in the regulation of emotional behavior is beyond doubt [11,13,14].

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Recent data suggest that various subtypes of serotonin receptors considerably contribute to the pathogenesis of anxious states [15]. For instance, 5-HT$_{1a}$ agonist buspirone exhibits pronounced anxiolytic effects and therefore after preclinical trials it was introduced into clinical practice [4]. The 5-HT$_2$ receptor family, in particular, 5-HT$_{2a}$ and 5-HT$_{2c}$ receptors, is also involved into anxiety development [7]. Antagonists of 5-HT$_{2a}$ receptors, *e.g.* ritanserin, eliminate anxiety symptoms in some behavioral models [6,12]. The blockade of 5-HT$_{2c}$ receptors produces similar anxiolytic effects and abolishes the anxiogenic effects of 5-HT$_{2c}$ receptor agonist mCPP [10]. This group includes also selective antagonist of 5-HT$_{2b/2c}$ receptors SB-200646A, the preparation that possesses potent anxiolytic activity [5,7,10] and produces no antidepressant and neuroleptic effects [9].

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Among new selective anxiolytic preparations, an imidazole derivative afobazole (5-ethoxy-2-[2-(morpholino)-ethylthio]-benzimidazole dihydrochloride) deserves special attention; this preparation created at V. V. Zakusov Institute of Pharmacology, Russian Academy of Sciences, differs from classical anxiolytic drugs (including benzodiazepine derivatives) by the absence of undesired side effects. It is hypothesized that the anxiolytic effects of afobazole is mediated via σ_1-receptor complex that modulates dopaminergic, serotoninergic, and cholinergic systems of the brain under conditions of emotional stress reaction [2].

Published data suggest that C57Bl/6 and BALB/c mice considerably differ by the phenotype of the emotional reaction to stress at both the behavioral and neurochemical levels. For instance, we have previously reported selective effects of afobazole on different neurotransmitter systems of the brain in these mouse strains. This preparation in a dose of 1 mg/kg more markedly reduced dopamine (DA) content in BALB/c mice than in animals with active reaction to stress [1].

At the same time, the nature of afobazole interaction with the serotoninergic systems of the brain remains poorly studied. There are no data on the contribution of various 5-HT receptor subtypes into the anxiolytic effect of this compound.

In the present neurochemical study, the effects of combined administration of afobazole and 5-$HT_{2b/2c}$ receptor antagonist SB-200646A on the content of monoamines and their metabolites in brain structures of C57Bl/6 and BALB/c mice with different emotional reaction to stress were studied by the methods of HPLC with electrochemical detection.

MATERIALS AND METHODS

The experiments were performed on BALB/c and C57Bl/6 mice weighing 20-22 g (Stolbovaya nursery, Russian Academy of Sciences) maintained under standard vivarium conditions with 12-h day-night regimen and free access to water and food. 5-$HT_{2b/2c}$ antagonist SB-200646A (N-(1-methyl-1H-indol-5-yl)-N'-3-pyridinyl urea hydrochloride; Sigma) was administered in a dose of 10 mg/kg and afobazole (Department of Chemistry, Institute of Pharmacology), Russian Academy of Medical Sciences) in a dose of 5 mg/kg. Both substances were dissolved in 0.9% NaCl and injected intraperitoneally. Controls received 0.9% NaCl. The substances were injected 60 min before decapitation.

Brain structures (frontal cortex, hypothalamus, amygdala, striatum, and hippocampus) were isolated on ice, frozen in liquid nitrogen, and weighed. Before neurotransmitter assay, the samples were homogenized in a Teflon-glass homogenizer in 20 volumes of 0.1 n $HClO_4$ in the presence of dihydroxybenzylamine (0.5 nmol/ml) as an internal standard. The samples were centrifuged at 10,000 rpm for 10 min. The content of monoamines and their metabolites was measured by HPLC with electrochemical detection on a LC-304T chromatograph (BAS, West Lafayette) with a Repro-Sil-Pur ODS analytic column (C_{18}, 100×4 mm, 3.3 μ; Dr.Maisch) [1]. The data were processed statistically using Student *t* test.

RESULTS

The content of monoamines and their metabolites (Table 1) in brain structures of control mice was taken as 100% in further experiments. In C57Bl/6 mice, afobazole considerably increased DA content in the hypothalamus and amygdala and reduced the complex parameters of utilization, the ratios of dihydroxyphenylacetic and homovanillic acids to DA (DOPAC/DA and HVA/DA)reflecting the rate of DA degradation to metabolites DOPAC and HVA in the same structures and in the striatum (Table 2). In BALB/c mice, DA content increased in the amygdala. We also detected a decrease in the content of serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) and 5-HIAA/5-HT ratio in the frontal cortex and amygdala of C57Bl/6 mice. A similar decrease in the latter parameter was observed also in the striatum of BALB/c mice. The content of DOPAC and DOPAC/DA and HVA/DA ratios also decreased in the striatum and amygdala.

SB-200646A had practically no effect on monoamine content and metabolism in all studied brain structures in both mouse strains. The exclusions were an increase in HVA content in the amygdala and DOPAC in the striatum of C57Bl/6 mice and an increase in DOPAC/DA ratio in the hippocampus of BALB/c mice.

Combined administration of afobazole and SB-200646A increased the content of epinephrine in the striatum and hippocampus of both mouse strains; in the striatum of BALB/c mice, this parameter increased to 230% of the control. The content of DA, DOPAC, and HVA and the values of DA metabolism parameters in these structures decreased. Interestingly, DA content in the hypothalamus and amygdala of C57Bl/6 mice increased (the latter to 200% of the control), while in BALB/c mice these changes were absent.

These results are difficult to interpret due to the absence of published data on neurochemical mechanisms of SB-200646A action, in particular, on its effect on various neurotransmitter systems of the brain. Only the results of *in vivo* electrophysiological studies of have been published [3]. The SB-200646A-induced increase in the content of DOPAC in the striatum of C57Bl/6 mice, *i.e.* in the structure containing axons

TABLE 1. Content of Monoamines and Their Metabolites in Brain Structures of C57Bl/6 and BALB/c Mice (*M*±*SEM*)

Brain structure	NE	DA	DOPAC	HVA	5-HT	5-HIAA	DOPAC/DA	HVA/DA	5-HIAA/5-HT
Frontal cortex									
C57Bl/6	2.921±0.354*	0.393±0.030	1.077±0.067*	0.543±0.03*	4.085±0.335	1.212±0.135	2.959±0.477	1.448±0.146*	0.321±0.064
BALB/c	1.532±0.101	0.387±0.082	0.876±0.060	0.712±0.061	3.469±0.559	1.031±0.069	2.794±0.419	2.097±0.233	0.261±0.026
Hypothalamus									
C57Bl/6	9.849±0.389	1.484±0.084*	0.550±0.036*	1.175±0.046*	24.885±1.646	4.259±0.320	0.375±0.026	0.814±0.064	0.172±0.009
BALB/c	10.564±0.409	2.261±0.095	0.961±0.102	1.496±0.133	24.282±1.560	4.600±0.394	0.427±0.043	0.663±0.056	0.194±0.018
Amygdala									
C57Bl/6	5.821±0.658	12.720±2.516	3.725±0.394*	3.110±0.356*	24.044±2.403	3.901±0.317	0.391±0.077	0.328±0.074	0.170±0.019
BALB/c	4.200±0.821	24.472±4.619	5.225±0.462	5.615±0.485	20.346±1.883	3.885±0.362	0.282±0.063	0.303±0.067	0.195±0.014
Striatum									
C57Bl/6	0.939±0.163*	54,308±2.921*	4.477±0.24*	6.050±0.375	10.043±0.580	2.134±0.137	0.084±0.006*	0.111±0.002**	0.213±0.008
BALB/c	0.299±0.034	42.176±1.598	5.591±0.376	8.535±0.622	8.556±0.345	1.937±0.111	0.134±0.012	0.202±0.012	0.226±0.008
Hippocampus									
C57Bl/6	2.937±0.258**	0,450±0.135	0.957±0.151	0.160±0.030	4.357±0.195**	2.209±0.113**	2.874±0.610	0.461±0.124	0.512±0.029
BALB/c	1.170±0.105	0.506±0.101	0.933±0.027	0.179±0.033	2.721±0.141	1.597±0.071	2.335±0.386	0.464±0.100	0.597±0.037

Note. NE: norepinephrine. *$p<0.05$, **$p<0.01$ in comparison with C57Bl/6 mice.

TABLE 2. Effect of Afobazole (AB) and SB-200646A (SB) on the Content of Monoamines and Their Metabolites in Brain Structures of C57Bl/6 and BALB/c Mice (*M*±*SEM*)

Substance		NE	DA	DOPAC	HVA	5-HT	5-HIAA	DOPAC/DA	HVA/DA	5-HIAA/5-HT
Frontal cortex										
C57Bl/6	AB	90.60±2.47	79.23±5.29	113.54±10.83	75.87±4.472*	80.88±3.48*	86.09±5.30	140.31±13.14*	96.95±6.22	105.41±8.15
	SB	77.05±7.06*	83.16±9.15	125.09±13.05	94.02±8.33	42.87±6.81*	119.51±18.01	148.96±13.69*	119.84±13.25	177.32±40.33
	AB+SB	116.72±5.25	127.36±11.44	79.19±9.34	115.19±12.03^{+}	117.87±6.41^{+}	83.65±5.26	60.23±6.78^{+}	86.21±5.27	67.04±5.99
BALB/c	AB	125.42±17.57	95.82±6.57	82.38±9.78	161.1±59.33	117.77±6.69	101.28±13.01	74.52±11.31	133.19±34.91	105.55±22.30
	SB	106.78±7.86	119.14±14.42	119.68±14.64	106.76±7.0	123.7±3.41	101.11±6.0	89.76±12.98	85.36±9.55	92.72±3.9
	AB+SB	117.68±4.92	132.05±18.18	108.16±6.42^{+}	119.47±9.52	129.54±6.43	95.35±5.737	75.17±10.17	85.63±9.78	84.33±4.88
Hypothalamus										
C57Bl/6	AB	111.39±12.42	113.78±16.52	10.85±1.07	96.92±12.21	107.16±19.72	103.27±19.06	6.36±0.65	93.76±10.06	98.45±6.35
	SB	90.42±4.82	73.59±4.07*	13.19±0.58	92.21±3.67	87.69±5.53	112.39±12.38	11.09±0.30	129.57±7.25*	127.63±8.86*
	AB+SB	113.55±5.17	141.85±16.31	75.93±12.60	110.39±18.37	119.57±7.41	96.55±8.25	66.9±4.27	74.41±4.30	79.62±2.43
BALB/c	AB	99.56±3.21	103.91±7.49	55.25±3.55*	76.56±7.27	106.83±5.39	82.20±5.65	53.69±2.46**	77.97±11.98*	75.20±3.06
	SB	98.32±3.77	96.93±6.12	95.41±5.23	103.50±6.78	102.25±5.66	103.33±6.16	99.54±5.94	109.46±10.07	99.32±4.63
	AB+SB	98.84±6.51	101.5±7.27	64.71±6.82	95.34±2.70^{+}	109.99±4.88	70.58±9.12	70.58±15.73	98.19±9.53	90.25±9.59
Amygdala										
C57Bl/6	AB	81.80±14.56	199.30±33.46*	93.49±5.94	91.73±5.71	99.71±8.03	71.82±5.43*	42.40±6.67*	41.37±6.68*	69.87±3.43*
	SB	136.32±14.13	115.87±21.26	115.90±9.28	135.23±9.50*	117.49±8.21	119.65±15.83	98.67±22.98	116.51±27.41	95.29±5.76
	AB+SB	145.78±17.42^{+}	197.23±23.91	99.07±10.87	110.63±10.49	116.69±9.01	87.74±7.89	40.7±5.26	45.93±6.91	71.83±2.94
BALB/c	AB	157.76±16.72*	69.41±9.54	69.69±8.27*	63.04±4.95*	123.83±7.29	79.67±7.70	83.38±11.56	79.23±13.22	62.33±3.18
	SB	158.99±22.65	107.17±17.69	108.79±12.72	106.86±11.96	128.08±7.34*	108.58±7.74	100.68±24.77	102.09±27.87	83.20±5.25
	AB+SB	142.66±37.63	143.04±19.16^{+}	104.51±10.26	92.11±6.87	129.50±7.60	86.68±5.24	60.90±7.21	53.86±6.18	70.57±4.04
Striatum										
C57Bl/6	AB	62.87±9.27*	101.94±3.5	114.42±14.96	92.45±4.13	86.15±2.36*	82.8±3.67	110.34±10.61	91.60±3.29	95.89±2.74
	SB	53.27±5.85**	109.59±3.67	185.90±9.74**	146.10±7.75*	85.38±2.40*	103.40±7.16	171.19±12.43*	134.72±6.77*	120.72±6.88
	AB+SB	159.88±14.64^{+}	111.99±4.83	67.08±4.88	85.58±7.15	133.04±6.89^{+}	119.54±9.23	59.21±3.69	75.93±4.47	89.68±5.81
BALB/c	AB	172.62±18.62	103.62±4.23	55.15±3.21**	74.22±4.73*	100.80±7.98	87.18±6.60	52.74±3.30*	71.33±2.18*	87.76±4.38*
	SB	127.31±13.08	112.20±4.87	94.66±7.13	102.43±3.92	100.67±8.06	101.63±6.58	84.71±8.46	91.65±2.33	104.50±8.47
	AB+SB	231.92±27.34^{+}	116.25±1.58^{+}	54.84±3.86	82.47±3.69	129.99±7.59	103.45±5.80	46.41±3.03	70.97±3.01	80.26±3.23
Hippocampus										
C57Bl/6	AB	107.69±6.50	163.36±44.12	100.93±15.59	119.39±15.26	96.70±5.44	91.66±5.38	56.33±10.70	82.94±23.28	95.12±5.81
	SB	105.48±2.06	58.57±7.83	107.12±16.09	108.75±14.20	97.10±2.80	115.10±12.29	144.36±30.34	153.79±22.35	117.22±11.05
	AB+SB	128.05±6.04^{+}	92.74±17.13	99.93±15.28	197.61±54.88	121.54±4.84^{+}	127.41±13.58^{+}	97.42±29.24	226.57±69.00	103.60±8.80
BALB/c	AB	73.62±5.98	100.78±10.29	108.51±5.32	97.88±19.10	105.89±5.91	100.71±4.49	139.47±18.33	132.51±34.92	95.30±6.24
	SB	10 038±1234	61.29±18.30	122.12±13.16	126.29±23.30	104.39±8.09	119.42±10.30	221.10±32.29*	193.26±22.57	114.15±9.34
	AB+SB	149.08±10.97^{+}	66.49±16.25	93.66±4.66	133.86±16.72	133.96±8.20^{+}	122.09±5.80^{+}	173.89±48.62	193.33±34.63*	90.68±3.60

Note. The data are presented in percents of the correspondting control. *p<0.05, **p<0.001 in comparison with the corresponding control; ^{+}p<0.05 in comparison with SB-200646A.

of dopaminergic neurons lying in the substantia nigra, agrees with enhanced impulse activity of striatal and ventral tegmental neurons [3]. The observed effect can also be a result of enhanced DA release into synaptic cleft upon stimulation of its synthesis. No effects of SB-200646A on DOPAC concentration was observed in BALB/c mice. Combined administration of afobazole and SB-200646A was followed by a decrease in DOPAC content in the striatum of both mouse strains. This is most likely related to the inhibitory effect of afobazole on monoamine oxidases A and B [2]. Moreover, increased level of norepinephrine can be a result of activating effects of SB-200646A on its synthesis, which leads to accumulation of this neurotransmitter in the striatum of both mouse strains. Interestingly, the observed effect was more pronounced in BALB/c mice characterized by more pronounced behavioral reaction to the stress stimulus. The above data suggest the existence of a relationship between the degree of the stress reaction and norepinephrine content in the striatum. No significant effects on activity of the serotoninergic system were observed except the increase in 5-HT and 5-HIAA in the hippocampus of BALB/c mice, which can be interpreted as an activating influence of SB-200646A.

The results of our neurochemical experiments agree with the results of our previous behavioral elevated plus-maze experiments: combined administration of SB-200646A and afobazole considerably potentiated the anxiolytic effect of the latter in both mouse strains (data not shown). Our findings attest to the involvement of the monoamine systems of the brain into the mechanism of afobazole action and suggest that the enhanced anxiolytic effect of afobazole combination with SB-200646A previously observed in behavioral experiments can be interpreted as a positive modulation of the anxiolytic effect determined by blockade of 5-HT_2 serotonin receptors.

REFERENCES

1. P. M. Klodt, V. S. Kudrin, V. B. Narkevich, *et al., Psikhofarmakol. Biol. Narkol*., **5**, No. 3, 1012-1015 (2005).
2. S. B. Seredenin and M. V. Voronin, *Eksp. Klin. Farmakol*., **72**, No. 1, 3-10 (2009).
3. T. P. Blackburn, K. Suzuki, and C. R. Ashby Jr., *Synapse*, **59**, No. 8, 502-512 (2006).
4. F. Clenet, M. Hascoet, G. Fillion, *et al., Behav. Brain Res.,* **158**, No. 2, 339-348 (2005).
5. M. S. Duxon, G. A. Kennett, S. Lightowler, *et al., Neuropharmacolgy,* **36**, No. 4-5, 601-608 (1997).
6. S. Gleeson, S. T. Ahlers, R. S. Mansbach, *et al., J. Pharmacol. Exp. Ther*., **250**, No. 3, 809-817 (1989).
7. F. G. Graeff, C. F. Netto, and H. Zangrossi Jr., *Neurosci. Behav. Rev*., **23**, No. 2, 237-246 (1998).
8. D. Hoyer and G. R. Martin, *Neuropharmacology.* **36**, No. 6, 419-428 (1997).
9. F. Jenck, M. Bos, J. Wichmann, *et al., Expert Opin. Investig. Drugs*., **7**, No. 10, 1587-1599 (1998).
10. G. A. Kennett, M. D. Wood, A. Glen, *et al., Br. J. Pharmacol*., **111**, No. 3, 797-802 (1994).
11. J. Kent, S. J. Mathew, and J. M. Gorman, *Biol. Psychiatry,* **52**, No. 10, 1008-1030 (2002).
12. V. Nunes-de-Souza, R. L. Nunes-de-Souza, R. J. Rodgers, and A. Canto-de-Souza, *Behav. Brain Res.,* **187**, No. 1, 72-79 (2008).
13. D. J. Nutt, *CNS Spectr.,* **10**, No. 1, 49-56 (2005).
14. N. S. Pillay and D. J. Stein, *Expert Opin. Emerg. Drugs*., **12**, No. 4, 541-554 (2007).
15. J. F. Tallman, J. Casella, and J. Kehne, *Neuropsychopharmacology*: *The Fifth Generation of Progress,* Eds. K.L.Davis, D.Charney, J.T.Coyle, C.Nemeroff. Lippincott Williams & Wilkins. (2002), pp. 993-1005.