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NEUROPHARMACOLOGICAL ANALYSIS OF THE ANTIDEPRESSANT ACTION OF 2-[3-METHYL-7-(THIETAN-3-YL)-1-ETHYLXANTH-8-YLTHIO]ACETIC ACID HYDRAZIDE

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A compound with low toxicity and pronounced antidepressant activity upon single and long-term administration was discovered by us earlier among a series of new thietanylxanthine derivatives. The present article focused on the central mechanisms of action of 2-[3-methyl-7-(thietan-3-yl)-1-ethylxanth-7-ylthio]acetic acid hydrazide (laboratory code M-20). Neuropharmacological analysis showed that M-20 at doses of 0.97 and 12 mg/kg exhibited effects indicative of possible stimulatory action on adrenergic and inhibitory action on GABA-ergic neurotransmission in brains of outbred white mice. M-20 at a dose of 12 mg/kg produced an activating effect on the serotoninergic system in addition to action on the adrenergic and GABA-ergic systems and altered the activity of the cholinergic system. M-20 at doses of 0.97 and 12 mg/kg did not alter the effects of haloperidol and L-DOPA, which indicated that it did not influence dopaminergic neurotransmission and MAO-inhibiting activity. The results indicated that M-20 was promising (at the low dose) for use with depression associated with decreased activity of the serotoninergic system without side effects on the dopaminergic and cholinergic systems.

Keywords: thietanylxanthines, mice, mechanism of action.

The WHO reported that the worldwide total number of depression patients increased from 2005 to 2015 by 18.4% or 350 million [1, 2]. This disorder affects eight million Russians or 5.5% of the population. The first theory of the genesis of depression invoked a serotonin deficit, a deficiency of which was observed in the brains of depression patients [3, 4]. However, the discovery and development of new antidepressants showed that the adrenergic and dopaminergic systems were also involved in the genesis of depression [5]. The disadvantages of modern antidepressants and the increased incidence of more complicated forms of depression necessitated the search for and development of new drugs combining several types of psychotropic action. Multi-year research on new thietanylxanthine derivatives identified 2-[3-methyl-7-(thietan-3-yl)-1-ethylxanth-8-ylthio]acetic acid hydrazide (laboratory code M-20) with low toxicity and potent antidepressant activity after a single and long-term administration [6, 7]. Compounds containing a thietane ring were also shown to possess antidepressant activity by others [8, 9].

The present article reports studies of the neuropharmacological aspects of the mechanism of the antidepressant action of M-20.

EXPERIMENTAL PART

The following drugs and substances were used: clopheline (solution for i.v. injection, 0.1 mg/mL, JSC Organica, Russia); haloperidol (solution for i.v. and i.m. injection, 5 mg/mL, OOO Velfarm, Russia); L-DOPA (Sigma-Aldrich, Germany); 5-hydroxytryptophan (5-HTP, Sigma-Aldrich, Germany), picrotoxin (Sigma-Aldrich, Germany), and arecoline (Sigma-Aldrich, Germany). M-20 was synthesized as previously described [7]. Its constants and spectral characteristics agreed with those in the literature: mp = 181 – 183°C (*i*-PrOH). $C_{13}H_{18}N_6O_3S_2$. IR spectrum,

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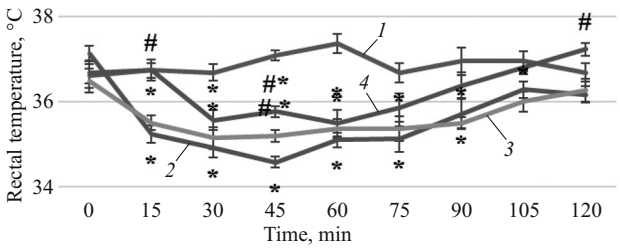

Fig. 1. Influence of M-20 on rectal temperature of mice administered clopheline at a dose of 0.1 mg/kg. Data are given as $M \pm m$; * $p < 0.05$, differences statistically significant vs. the group receiving normal saline; # $p < 0.05$, differences statistically significant vs. the group receiving clopheline (two-factor dispersion analysis with Bonferroni correction). Normal saline (*1*), clopheline + normal saline (*2*), M-20 (0.97 mg/kg) + clopheline (*3*), M-20 (12 mg/kg) + clopheline (*4*).

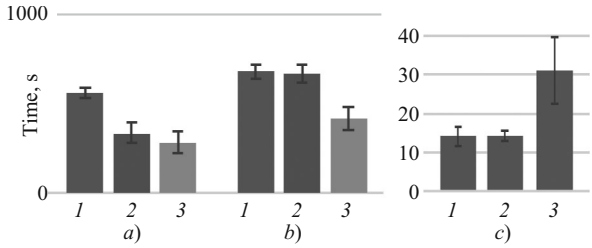

Fig. 2. Influence of M-20 on effects of picrotoxin. Data are given as $M \pm m$; * $p < 0.05$, differences statistically significant vs. the control group (two-factor dispersion analysis with Bonferroni correction). LP = latent period. LP of tremor (*a*), LP of convulsion (*b*), number of convulsions (*c*). Normal saline + picrotoxin (*1*), M-20 (0.97 mg/kg) + picrotoxin (*2*), M-20 (12 mg/kg) + picrotoxin (*3*).

ν_{max}, cm^{-1}: 1645, 1657, 1665, 1696, 1699 (C=C, C=N, C=O, N-H); 3100 – 3380 (N-H). PMR spectrum ($CDCl_3$), δ, ppm: 1.24 (t, 3H, J 6.8 Hz, CH_3); 3.24 – 3.35 (m, 2H, $S(CH)_2$); 3.53 (s, 3H, 3-CH_3); 3.82 – 3.98 (br.s, 4H, SCH_2 and NH_2); 4.12 (q, 2H, J 6.8 Hz, 1-CH_2); 4.28 – 4.39 (m, 2H, $S(CH)_2$); 5.74 – 5.88 (m, 1H, 7-CH); 8.24 – 8.35 (br.s, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 13.16 (CH_3); 29.72 (3-CH_3); 33.88 (SCH_2); 34.82 [$S(CH)_2$]; 36.97 (1-CH_2); 51.94 (7-CH); 109.10 (C^5); 148.88 (C^4); 149.01 (C^2); 150.69 (C^6); 153.79 (C^8); 168.43 (8-CO) [10].

The tests used 160 outbred male mice (22 – 24 g) raised at Immunopreparat Co. (Ufa). Animals were kept under standard vivarium conditions with natural lighting and free access to water and feed according to GOST R 50258–92.

Experiments were conducted in strict compliance with all requirements of the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (Strasbourg, 1986) and Ministry of Health of the RF Order No. 199n "On approval of good laboratory practice rules" dated Apr. 1, 2016.

TABLE 1. Influence of M-20 on L-DOPA Effects

Observation time, min	Stereotype potency, balls			
	control 1 (normal saline + L-DOPA 100 mg/kg)	control 2 (normal saline + L-DOPA 500 mg/kg)	M-20 (0.97 mg/kg) + L-DOPA (100 mg/kg)	M-20 (12 mg/kg) + L-DOPA (100 mg/kg)
30	0.0 ± 0.0*	3.0 ± 0.0	0.0 ± 0.0*	0.0 ± 0.0*
60	0.0 ± 0.0*	3.0 ± 0.0	0.0 ± 0.0*	0.0 ± 0.0*
90	0.0 ± 0.0*	3.0 ± 0.0	0.0 ± 0.0*	0.0 ± 0.0*

Data given as $M \pm m$; * $p < 0.05$, differences statistically significant vs. control 2 (two-factor dispersion analysis with Bonferroni correction).

M-20 was studied at doses of 0.97 and 12 mg/kg.

Neuropharmacological studies used methods given in handbooks [11, 12]. Animals were divided into control and test groups with eight animals each. M-20 was injected once i.p. at doses of 0.97 and 12 mg/kg, which were previously determined by us as the doses having the most potent antidepressant action [6]. Control animals were injected with normal saline. The necessary compounds except haloperidol, which was injected once with the test compound, were injected once 30 min after injection of M-20 or normal saline.

Test results were processed using the software suites Micro Office Excel 2007 (Microsoft, USA), Statistica 6.1. (StatSoft Inc., USA), and Biostat 2006 (USA). Variational series were described using medians and interquartile scatter of Me [25%, 75%] and arithmetic means and standard errors of means $M \pm m$. Statistical analysis used parametric and nonparametric criteria, i.e., the Dunn criterion for multiple comparisons and two-factor dispersion analysis with the Bonferroni correction. Differences were considered statistically significant for $p < 0.05$ for all types of analysis [13].

RESULTS AND DISCUSSION

Influence of M-20 on hypothermal effect of clopheline. Rectal temperatures decreased statistically significantly from 15 to 90 min of observation in control animals receiving i.p. clopheline at a dose of 0.1 mg/kg as compared to animals that received normal saline ($p < 0.05$) (Fig. 1). Preliminary injection of M-20 (at a dose of 0.97 mg/kg, at 45 min; at a dose of 12 mg/kg, at 15, 45, and 120 min of observation) prevented hypothermia cause by injection of the central α_2-adrenoreceptor agonist clopheline ($p < 0.05$).

Influence of M-20 on picrotoxin convulsions. Tremors developed after 9 min; the first convulsions, after 11 min in control animals receiving s.c. picrotoxin at a dose of 2.5 mg/kg. Fourteen episodes of convulsions were observed in the mice during the whole observation period (60 min) (Fig. 2).

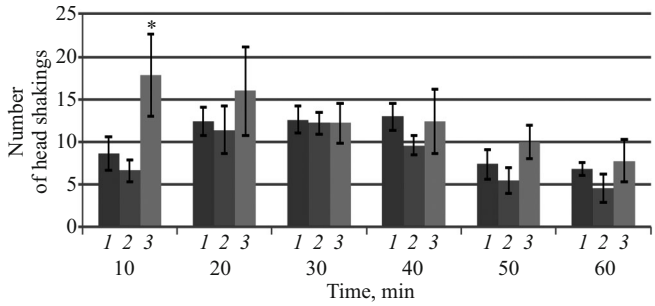

Fig. 3. Influence of M-20 on number of head shakings after 5-hydroxytryptophan (5-HTP) injection. Data are given as $M \pm m$; * $p < 0.05$, differences statistically significant vs. the control group (two-factor dispersion analysis with Bonferroni correction). Control (*1*), M-20 (0.97 mg/kg) + 5-HTP (*2*), M-20 (12 mg/kg) + 5-HTP (*3*).

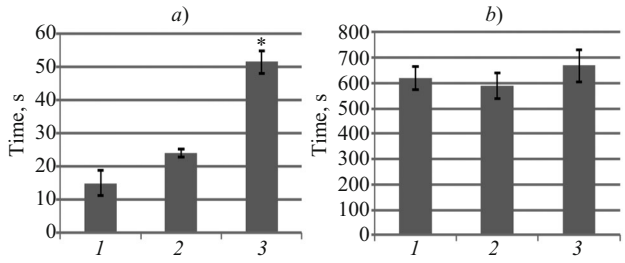

Fig. 4. Influence of M-20 on arecoline effects: latent period of tremor (a), tremor duration (b). Data are given as $M \pm m$; * $p < 0.05$, differences statistically significant vs. the control group (two-factor dispersion analysis with Bonferroni correction). Normal saline + arecoline (*1*), M-20 (0.97 mg/kg) + arecoline (*2*), M-20 (12 mg/kg) + arecoline (*3*).

Tremors developed ~5 min after picrotoxin injection in test mice receiving M-20 at doses of 0.97 and 12 mg/kg ($p < 0.05$).

Convulsions started after 7 min vs. 11 min in the control for the group receiving M-20 at a dose of 12 mg/kg.

Influence of M-20 on hyperkinesis after injection of 5-HTP. The number of head shakings increased gradually in control animals from 10 to 40 min after i.p. injection of 5-HTP and tended to diminish after 60 min (Fig. 3). Preliminary injection of M-20 at a dose of 0.97 mg/kg did not change this although the effects of 5-HTP intensified at a dose of 12 mg/kg. This was manifested as a significant increase of the number of head shakings (at 10 min) as compared to the controls ($p < 0.05$).

Influence of M-20 on arecoline tremors. Tremors developed in the control group 15 sec after s.c. administration of arecoline and lasted 10 min (620 sec) (Fig. 4). The tremor intensity in this group was two balls.

M-20 at a dose of 0.97 mg/kg did not alter the effects of arecoline, lengthened the tremor latent period up to 51 sec, and increased the tremor intensity to three balls at a dose of 12 mg/kg as compared to the controls. M-20 did not affect the duration of the tremors.

Influence of M-20 on cataleptogenic effects induced by haloperidol. Pronounced cataleptogenic effects that reached a maximum by 90 min of observation were observed in control animals receiving i.p. haloperidol at a dose of 1 mg/kg (Fig. 5).

Groups of animals that received preliminarily M-20 at doses of 0.97 and 12 mg/kg remained on a wire during the whole observation period for times that were statistically significantly the same as that of the control group. This probably indicated that M-20 did not affect dopaminergic neurotransmission.

Influence of M-20 on L-DOPA effects. Stereotypic behavior did not develop after i.p. administration of L-DOPA at a dose of 100 mg/kg (Table 1). Stereotypic behavior corresponded to three balls during the whole observation time in animals receiving L-DOPA at a dose of 500 mg/kg.

Test animals receiving M-20 30 min before L-DOPA injection at a dose of 100 mg/kg did not display stereotypic behavior during the whole observation period. This indicated that M-20 did not have MAO-inhibiting action.

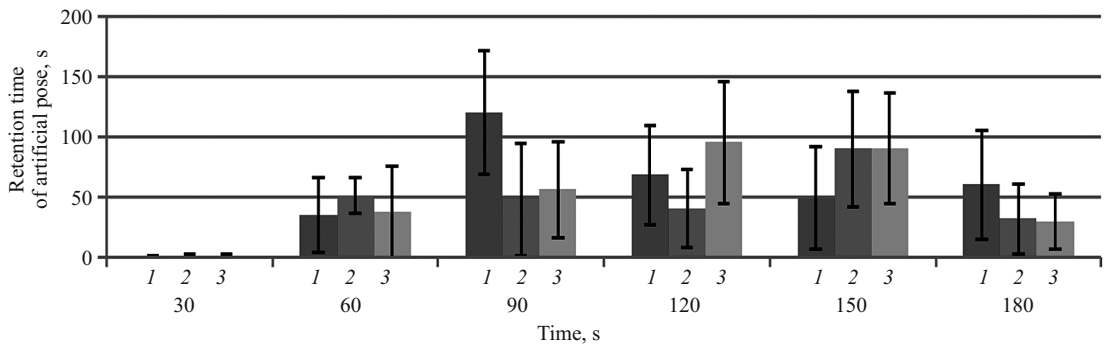

Fig. 5. Influence of M-20 on cataleptogenic effect after haloperidol injection: Normal saline + haloperidol (*1*), M-20 (0.97 mg/kg) + haloperidol (*2*), M-20 (12 mg/kg) + haloperidol (*3*). Data are given as means and interquartile scatter of Me [25%, 75%]; statistically significant differences between control and test groups were not observed (Kruskal—Wallis criterion with Dunn's post-hoc test).

The effects of 5-HTP were enhanced under the influence of M-20 and suggested that it had an agonistic influence on 5-HT_2-serotonin receptors because head shaking in mice induced by 5-HTP injection is mediated through these receptors [14, 15].

Preliminary injection of M-20 weakened the effects of clopheline, indicating that M-20 may have blocked presynaptic α_2-adrenoreceptors, like the antidepressant mianserin, and enhanced serotonin release, a deficiency of which was noted in brains of depression patients [3, 4].

The effects of picrotoxin may have been enhanced under the influence of the new thietanylxanthine derivative because of antagonism of the GABA-ergic mediator system. Considering previous results [16, 17], it could be proposed that M-20 had stimulatory action on 5-HT1A-receptors in the presynaptic membrane of the GABA-ergic synapse that inhibited presynaptic release of GABA. The literature indicates that antidepressants often are associated with antagonism of the GABA-ergic system. Thus, antidepressants were proposed to exert control over the excessive inhibitory influence of GABA, which facilitated the manifestation of an antidepressant effect [18].

The influence of M-20 altered the effects of arecoline by delaying the onset of tremors and enhancing their intensity, indicating that M-20 had multiple influences on central M-choline receptors.

Thus, the neuropharmacological analysis showed that M-20 had a dose-dependent influence one neuromediator system or another. For example, effects indicative of stimulation of adrenergic and inhibition of GABA-ergic neurotransmission were found at a dose of 0.97 mg/kg.

M-20 at a dose of 12 mg/kg influenced adrenergic and GABA-ergic systems and also exhibited effects indicative of stimulatory action on serotoninergic neurotransmission.

M-20 at doses of 0.97 and 12 mg/kg did not alter effects of haloperidol and L-DOPA, which presumably indicated that it did not influence dopaminergic neurotransmission and MAO-inhibitory activity.

The results showed that M-20 (at the low dose) was promising for depression associated with serotoninergic system deficiency and did not have side effects on dopaminergic and cholinergic systems.

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