Naloxone Effects on Behavior of Inbred Mice with Different Response to Emotional Stress in Open Field Test

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Effects of nonspecific opiate receptor antagonist naloxone in doses of 0.1, 0.5, 1.0, 5.0, 10.0 mg/kg on open field behavior and spontaneous motor activity were studied in male BALB/c and C57Bl/6 mice. Differently directed effects of naloxone on behavioral parameters of emotional-stress reaction in BALB/c and C57Bl/6 mice were observed. Naloxone increased motor activity in the open field test in BALB/c mice, but decreased it in C57Bl/6 mice. In the absence of stress, naloxone in the studied dose range did not affect spontaneous motor activity in C57Bl/6 mice, and significantly reduced activity in BALB/c mice in doses 0.5 and 1.0 mg/kg.

Key Words: *naloxone; inbred mice; open field test; spontaneous motor activity; emotional stress reaction*

Genetic predisposition to different responses to emotional stress was proved in early behavioral genetic studies, in selection of numerous inbred mouse and rat strains [5]. Experimental models were developed demonstrating the dependence of the effects of psychotropic drugs on phenotype of emotional-stress reaction. Certain behavioral phenotypes in open field test (OF) were established to correspond to biochemical characteristics characterizing the stress responses [6]. The latter indicates different organization of neurochemical and neurohormonal systems determining animal behavior during stress. Endogenous opioid system is a stress-limiting system [3]. The effects of naloxone on OF behavior of BALB/c and C57Bl/6 mice were studied to clarify interstrain differences in the role of opioid receptors in the development of emotional stress reaction (ESR).

MATERIALS AND METHODS

Experiments were carried out on 430 male BALB/c mice and 388 male C57Bl/6 mice weighting 22-24 g and obtained from Stolbovaya and Andreevka nurseries. The mice were kept under standard vivarium conditions for 2 weeks in a vivarium of V. V. Zakusov Institute of Pharmacology, 15-20 animals per standard cage (35×20×15 cm), with natural light regimen and water and food *ad libitum*. Twenty-four hours before the experiment, the animals in the home cage were transferred into standard conditions of the experimental room with low noise level, scattered dusky light, and temperature range of 18-22°C for adaptation to experimental conditions. The experiments were performed in fall—winter period from 10 a. m. to 2.30 p. m.

Mouse behavior in OF test (in P. M. Borodin modification) was studied [1]. Spontaneous motor activity (SMA) was measured using Opto-varimex actometer (Colambus Corp.) as described previously [2].

Naloxone (Sigma-Aldrich) was injected intraperitoneally in doses 0.1, 0.5, 1.0, 5.0, 10.0 mg/kg in a volume of 10 ml/kg 5 min before the test. Controls received intraperitoneal injections of distilled water in the equivalent volume.

The data were processed using Mann—Whitney *U* test.

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RESULTS

The results of control series characterizing BALB/c and C57Bl/6 mouse behavior in OF and in animal activity meter Opto-varimex corresponded to previously obtained data (Tables 1, 2, and 3) [4]. Pharmacological tests showed that naloxone in all test doses (from 0.1 to 10.0 mg/kg) affected parameters of OF behavior typical for BALB/c mice (freezing, Table 1). In contrast, after initial startle response the mice demonstrated fast movements across the OF. Thus, transformation of passive ESR phenotype into a new form of behavior with high motor activity was noted against the background of naloxone action.

In C57Bl/6 mice, the effect of naloxone on OF behavior was opposite (Table 2): in doses of 0.1 and 0.5

TABLE 1. Effects of Naloxone on OF Behavior of BALB/c Mice (*M*±*SEM*)

Agent, dose	Motor activity				
	horizontal		visits to the center	vertical	total
	peripheral	central			
Control (*n*=11)	24.4±4.4	2.7±1.3	0.2±0.1	0.0±0.0	27.3±4.0
Naloxone, 0.1 mg/kg (*n*=11)	57.7±7.6**	2.9±1.1	0.0±0.0	0.0±0.0	60.6±7.3**
Control (*n*=16)	11.9±4.4	1.1±0.5	0.0±0.0	0.0±0.0	13.0±4.5
Naloxone, 0.5 mg/kg (*n*=16)	30.3±5.5**	3.2±1.3	0.0±0.0	0.1±0.1	33.6±6.1**
Control (*n*=10)	13.2±3.6	0.0±0.0	0.0±0.0	0.0±0.0	13.2±3.6
Naloxone, 1.0 mg/kg (*n*=12)	49.1±14.3*	3.5±1.7	0.2±0.2	0.3±0.1	53.0±14.6*
Control (*n*=15)	12.5±2.2	1.3±0.6	0.0±0.0	0.0±0.0	13.7±2.0
Naloxone, 5.0 mg/kg (*n*=15)	48.8±13.5**	4.4±2.1	0.2±0.1	0.1±0.1	53.5±13.7**
Control (*n*=10)	11.5±3.5	0.6±0.3	0.0±0.0	0.0±0.0	12.1±3.5
Naloxone, 10.0 mg/kg (*n*=9)	54.3±11.9***	1.8±0.4	0.0±0.0	0.0±0.0	56.1±11.8**

Note. Here and in Table. 2: *p<0.05, **p<0.01, ***p<0.001 compared to the corresponding control.

TABLE 2. Effects of Naloxone on OF Behavior of C57Bl/6 Mice (*M*±*SEM*)

Agent, dose	Motor activity				
	horizontal		visits to the center	vertical	total
	peripheral	central			
Control (*n*=10)	161.0±13.8	40.3±4.2	3.0±0.4	15.2±2.0	219.5±17.8
Naloxone, 0.1 mg/kg (*n*=10)	112.7±12.7*	26.1±4.1**	2.1±0.5	12.1±2.3	153.0±16.7*
Control (*n*=7)	170.9±14.4	41.9±15.4	2.6±0.9	12.0±1.7	227.3±19.5
Naloxone, 0.5 mg/kg (*n*=8)	72.0±10.0**	11.9±2.9*	0.5±0.3	11.4±1.5	95.8±13.8**
Control (*n*=10)	132.1±11.9	24.8±4.4	3.0±0.7	11.9±1.9	171.8±13.3
Naloxone, 1.0 mg/kg (*n*=10)	66.3±5.2**	5.9±1.1***	0.0±0.0**	7.4±1.2	79.6±6.6**
Control (*n*=9)	141.6±13.8	31.4±6.4	2.2±0.7	10.4±1.2	185.7±17.8
Naloxone, 5.0 mg/kg (*n*=9)	52.6±9.4***	5.0±1.2***	0.3±0.2*	8.8±2.2	66.7±12.2***
Control (*n*=7)	179.3±14.6	25.4±6.5	2.1±0.7	14.3±2.3	221.1±17.4
Naloxone, 10.0 mg/kg (*n*=8)	48.8±4.3**	7.0±1.6**	0.3±0.2*	5.3±0.7**	61.3±5.6**

TABLE 3. Effects of Naloxone on SMA of BALB/C and C57Bl/6 Mice in Opto-Varimex Actometer (*M±SEM*)

Mouse strain		Naloxone dose, mg/kg				
		0.1	0.5	1.0	5.0	10.0
BALB/c	control (*n*=6-7)	3531.7±603.3	3954.5±239.3	3825.0±304.1	3551.7±339.3	3381.8±126.2
	naloxone (*n*=6)	3001.8±366.2	2565.0±247.7**	2620.2±361.2*	3205.5±263.0	3209.2±111.8
C57Bl/6	control (*n*=6)	4378.5±198.7	4482.0±406.5	4620.3±260.8	4884.2±230.9	4514.2±188.9
	naloxone (*n*=6)	4254.0±300.8	4326.8±509.6	4137.0±162.7	4273.3±214.4	4485.2±115.5

Note. Overall index was recorded in home cages (5 animals per cage) over 10 min. Each group included 6 home cages (7 for the dose of 5.0 mg/kg in control Balb/c). A total of 305 Balb/c mice and 300 C57Bl/6 were tested. *p<0.05, **p<0.01 compared to the corresponding control.

mg/kg it significant reduced peripheral, central, and total motor activity. Increasing the dose of naloxone (1.0, 5.0 and 10.0 mg/kg) produced further decrease in total motor activity in OF and in its components, peripheral and central activity and visits to the center, characterizing orientation and exploratory behavior of control animals. The number of upright postures specific for C57Bl/6 mouse behavior also decreased after injection of 10.0 mg/kg naloxone.

Naloxone in the studied dose range did not increase SMA in Balb/c and C57Bl/6 mice (Table 3). In BALB/c mice, naloxone in doses of 0.1, 5.0 and 10.0 mg/kg did not affect SMA, and in doses of 0.5 and 1.0 mg/kg significantly decreased motor activity. SMA of C57Bl/6 mice in the actometer was not affected by naloxone in all studied doses (Table 3).

Thus, we demonstrated differently directed effects of naloxone on ESR in OF in BALB/c and C57Bl/6 mice. In BALB/c mice, naloxone in all test doses increased motor activity in OF stress situation and did not change SMA in Opto-varimex actometer without stress. The latter suggests that the effects of naloxone are directed towards ESR formation mechanisms. In C57Bl/6 mice, naloxone in all test doses did not change SMA in Opto-varimex actometer, but reduced parameters of OF behavior, which also suggests that naloxone affects the stress response. These findings suggest that the blockade of opiate receptors with naloxone produces opposite genotype-specific behavioral effects in BALB/c and C57Bl/6 mice. This proves the role of opiate system in ESR formation and demonstrates the existence of different phenotypes of pharmacological effects of naloxone, which requires further experimental and clinical investigations.

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