GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Stress-Protective and Stress-Potentiating Effects of Antibodies to Glutamate and γ-Aminobutyric Acid after Intranasal Administration in C57Bl/6 Mice

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We studied the effects of antibodies to glutamate and GABA administered intranasally 1 h before and immediately after stress on the development of stress response in C57Bl/6 mice. Antibodies to glutamate administered 1 h before stress prevented the development of the stress-reaction judging from behavioral parameters in the open field test and hyperalgesia test, while antibodies to GABA potentiated the severity of stress-induced behavioral disorders: total behavioral activity in the open field decreased significantly. When administered after stress, antibodies to glutamate did not reduce, but exacerbated stress reaction; antibodies to GABA also exhibited a stress-potentiating effect.

Key Words: *antibodies; glutamate; γ-aminobutyric acid; stress; intranasal administration*

Many central aspects of the stress responses include activation of the hypothalamic—pituitary—adrenal (HPA) axis. Glutamate (Glu) and GABA play a key role in central integration of HPA stress responses [11,12]. We previously showed the effects of antibodies (Ab) to Glu and GABA during active immunization with Glu—BSA and GABA—BSA conjugates on the development of convulsive reaction to pentilentetrazole, neurogenic pain syndrome, and combined water immersion stress [2,4,6]. A possible mechanism underlying the effect of Ab to neurotransmitters during active immunization is a direct influence on the brain Glu- and GABA-ergic systems. However, Ab penetration into the brain is limited by the blood-brain barrier, which can reduce activity of Ab produced during active immunization. After intranasal administration, γ-globulin can directly penetrate into the brain by-passing the blood-brain barrier [15]. This administration route provides more pronounced effects of anti-Glu and anti-GABA Ab. Moreover, their effects depend on the term of administration (before or after stress): because preliminary administration modulates initial activity of the Glu- and GABA-ergic systems, while Ab administered after stress modulate activity of systems activated by stress.

Here we evaluated the effects of anti-Glu and anti-GABA Ab on the development of the studied reactions after intranasal administration 1 h before stress and immediately after stress.

MATERIALS AND METHODS

Experiments were carried out on 90 C57Bl/6 male mice weighing 24-26 g. The animals were kept in a vivarium under standard conditions with water and food *ad libitum*.

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Antibodies to Glu and GABA were obtained in the form of γ-globulin fraction from the serum of rabbits hyperimmunized with Glu-BSA and GABA-BSA conjugates [1]. γ-Globulin obtained from the serum of intact animals served as the control.

Two experimental series were performed. In series I, anti-Glu and anti-GABA Ab were administered intranasally 1 h before stress, and in series II, anti-Glu and anti-GABA Ab were administered intranasally immediately after stress modeling. In each series, the mice were divided into 5 groups: intranasal administration of physiological saline (group 1, control); stress+intranasal administration of physiological saline (group 2); stress+intranasal administration of Ab to Glu (group 3); stress+intranasal administration of Ab to GABA (group 4); stress+intranasal administration of rabbit γ-globulin (group 5).

Combined water immersion stress adapted for mice was used as the stress model in both experimental series [7]. The mice of groups 2, 3, 4, and 5 were placed into tight hollow plastic cylinders (3 cm in diameter, height 12 cm); the cylinders were submerged into water 20-21°C to the level of mouse neck for 1 h. After the procedure the animals were placed in individual cages.

Intranasal administration of Ab was performed as follows: the experimenter held the mouse by withers and skin fold on the back in the supine position. This posture is recommended for intranasal administration of compounds [14]. It provides more complete penetration of the solution into nasal passages and sinuses. Ab solution was dropped with a pipette with thin spout, 5 μl in each nostril. Ab dose was chosen on the bases of previous studies [3] and was 30 μg/kg.

One hour after stress, open field activity of mice from all groups was evaluated over 3 min. The latency of the first movement, latency of visiting the field center, number of crossed squares, number of rearing episodes, and number of explored objects were recorded. The integral activity score was calculated as the sum of crossed squares, rearing episodes, and explored objects. Pain sensitivity was assessed 10 min after open field testing. The latency of hindlimb licking in response to nociceptive thermal irritation in the hot plate test (56°C) was assessed using Ugo Basile apparatus. The maximal testing time was 60 sec. After completion of the experiment, the mice were decapitated. Stress target-organs (thymus, spleen, and adrenals) were weighed and gastric mucosa was examined for ulcerations.

The results were statistically processed using Statistica software. The significance of differences was evaluated using Student's *t* test and Fisher's test. Intergroup differences were significant at $p \leq 0.05$. Numerical data are presented as means±standard errors.

RESULTS

In experimental series I, combined stress exposure significantly decreased total behavioral activity (Table 1): the integral activity score decreased to 78.3±7.8. In mice of the main experimental group (intranasal administration of Ab to Glu 1 h before stress), a trend to shortening the latency of visiting the field center entrance was noted, which attested to anxiolytic effect of the administered Ab [8]. Moreover, the integral activity score in this group was 101.9±5.8, *i.e.* did not significantly differ from that in the control group (without stress) and was higher than in mice exposed to the same stress stimulus. Intranasal administration of Ab to GABA significantly decreased the integral activity score to 39.2±11.3, and this parameter became lower than in other groups. The latency of visiting the field center after administration of Ab to GABA was significantly longer, which also attested to more pronounced stress response [5]. Behavioral activity in mice receiving γ-globuline 1 h before stress was similar by all parameters to that in mice immunized with Ab to GABA.

TABLE 1. Effects of Intranasal Administration of Anti-Glu and Anti-GABA Ab 1 h before Stress on Open Field Behavior of C57Bl/6 Mice

Group	*n*	Latency		Number of			Integral activity score
		first movement, sec	visiting field centre, sec	crossed squares	rearings	explored objects	
1 (intact control)	11	8.7±1.4	68.7±15.2	85.2±4.2	15.5±0.7	16.5±2.2	117.3±5.0
2 (stressed control)	13	9.5±5.5	59.4±12.8	60.8±5.8**	7.9±1.9**	9.5±1.2**	78.3±7.8**
3 (Ab to Glu+stress)	8	6.1±1.8	40.6±8.8^{xx}	82.12±5.00^{+xx}	11.6±1.7*^{xx}	8.12±2.12*	101.9±5.8^{+xx}
4 (Ab to GABA+stress)	9	8.6±3.3	157.1±16.1**$^{++}$	30.3±8.4**$^{++}$	2.44±1.32**$^{+}$	6.44±1.91**	39.2±11.3**$^{++}$
5 (γ-globulin+stress)	8	6.1±2.8	133.5±21.0*$^{++}$	35.5±11.8**$^{+}$	4.10±1.85**	6.87±2.43**	46.5±15.6**

Note. Here and in Table 2: * $p<0.05$, ** $p<0.01$ compared to intact control; $^{+}p<0.05$, $^{++}p<0.01$ compared to stressed control; $^{x}p<0.05$, $^{xx}p<0.01$ compared to animals receiving γ-globulin.

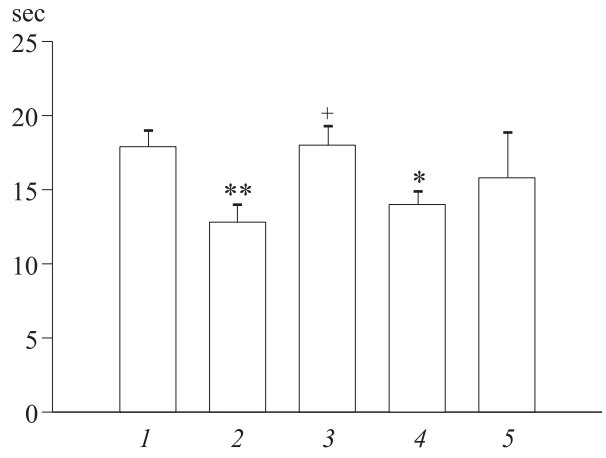

Fig. 1. Effects of intranasal administration of anti-Glu and anti-GABA Ab to C57Bl/6 mice 1 h before stress on latency of hindlimb licking in the hot plate test. Here and on Figs. 2 and 3: *1*) intact control; *2*) stressed control; *3*) administration of Ab to Glu; *4*) administration of Ab to GABA; *5*) administration of γ-globulin. *$p<0.05$, **$p<0.01$ compared to intact control; $^{+}p<0.01$ compared to stressed control.

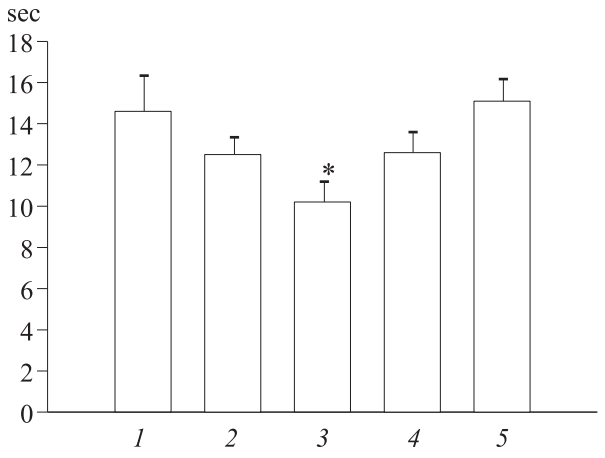

Fig. 2. Effects of intranasal administration of anti-Glu and anti-GABA Ab to C57Bl/6 mice immediately after stress on the latency of hindlimb licking in the hot plate test. *$p<0.05$ compared to intact control.

Evaluation of pain sensitivity in the hot plate test showed that combined water immersion test induced hyperalgesia (Fig. 1): the latency of hindlimb licking significantly decreased to 12.8±1.2 sec *vs.* 17.9±1.1 in the control group. In mice receiving Ab to Glu, the latency of hindlimb licking after exposure to stress was similar to that in control mice and was 18.0±1.3 sec. In mice receiving Ab to GABA, this latency was shorter than in intact mice and was similar to that in the stressed control group. In mice receiving γ-globulin, this latency did not significantly differ from that in intact control and a trend to its increase in comparison with the latency in the stressed control group was noted.

No significant differences in the weights of stress target organs (spleen, thymus, adrenals) between the control and stressed animals were revealed in this experimental series. Stress exposure was associated with ulceration of the gastric mucosa in mice. However, the incidence of ulceration did not significantly differ in stressed immunized and non-immunized animals, which reflects complex neuroimmunoendocrine mechanism of the studied process. In control mice, no changes in the stomach mucosa were noted.

In series II, Ab to neurotransmitters were administered immediately after stress exposure. In intact mice, exposure to combined water immersion stress reduced total behavioral activity in the open field test (Table 2). Moreover, the latency of visiting the field center increased and the integral activity score decreased in comparison with the corresponding parameters in non-stressed animals. In mice receiving Ab to Glu after stress, the integral activity score significantly decreased in comparison with that in intact and stressed control groups. At the same time, the latency of visiting the field center tended to increase. The integral activity score in mice receiving Ab to GABA was significantly shorter than in intact and stressed groups. Behavioral activity in mice receiving γ-globulin was significantly lower than in stressed control group by the number of rearing episodes and explored objects; in other parameters did not significantly differ.

TABLE 2. Effects of Intranasal Administration of Anti-Glu and Anti-GABA Ab Immediately after Stress on Open Field Behavior in C57Bl/6 Mice

Group	*n*	Latency		Number of			Integral activity score
		first movement, sec	visiting field centre, sec	crossed squares	rearings	explored objects	
1 (intact control)	8	11.6±2.7	32.0±5.1	76.4±4.1	11.8±0.9	13.9±0.6	100.5±4.2
2 (stressed control)	10	10.1±4.6	53.6±17.0	73.2±9.2	8.9±1.4	10.4±2.1	92.5±11.2
3 (Ab to Glu+stress)	8	7.1±3.2	63.9±18.9	36.7±7.1^{**++}	2.3±0.7^{**++xx}	5.1±1.1^{**+}	45.5±9.1^{**++x}
4 (Ab to GABA+stress)	8	6.8±2.5	43.3±10.3	53.3±7.7*	10.7±3.2	9.8±2.1	71.1±12.0^{*+}
5 (γ-globulin+stress)	7	3.1±2.6*	47.3±14.9	65.4±10.5	7.2±1.0**	7.1±1.6**	79.6±13.1

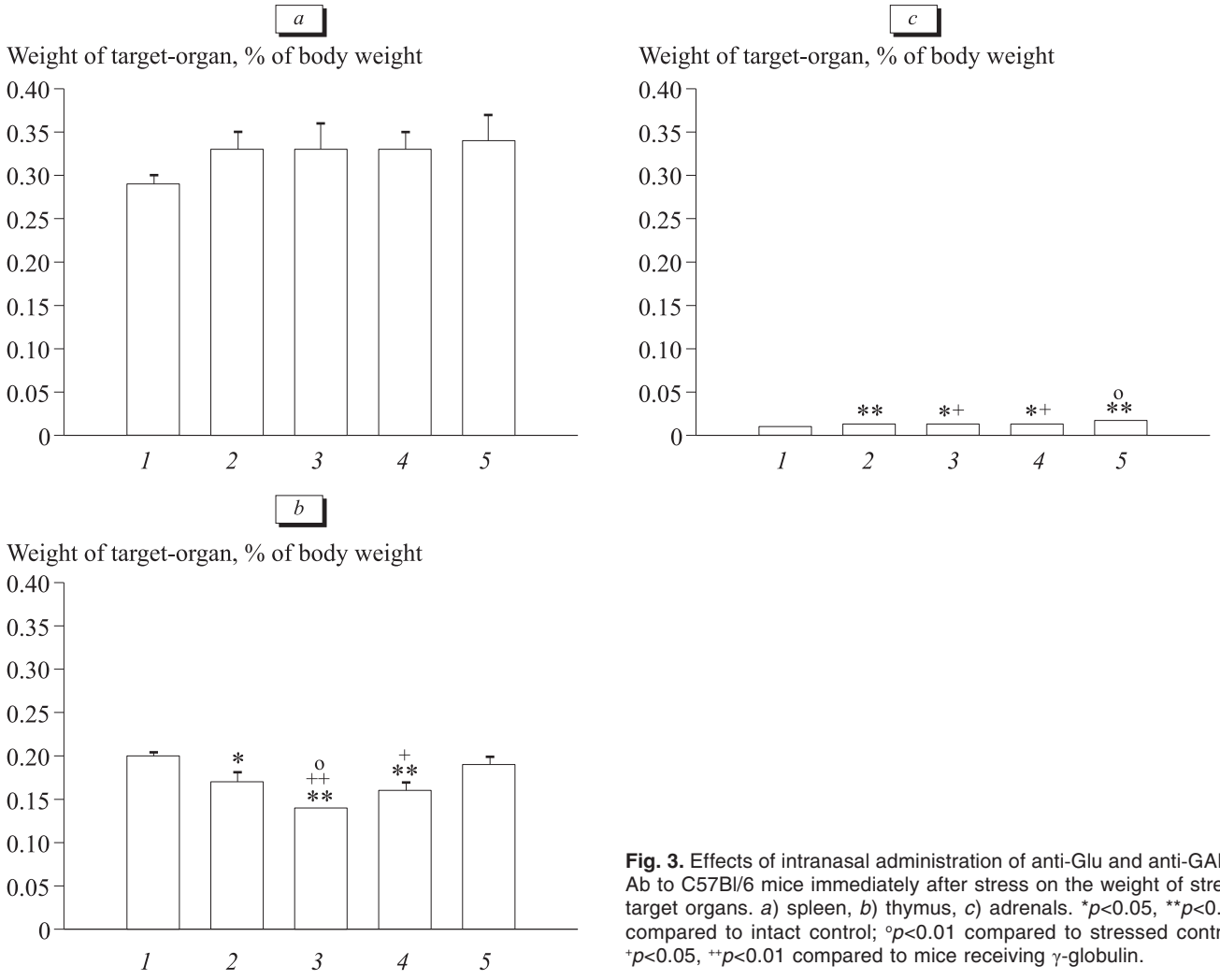

Fig. 3. Effects of intranasal administration of anti-Glu and anti-GABA Ab to C57Bl/6 mice immediately after stress on the weight of stress target organs. *a*) spleen, *b*) thymus, *c*) adrenals. $^{*}p<0.05$, $^{**}p<0.01$ compared to intact control; $^{\circ}p<0.01$ compared to stressed control; $^{+}p<0.05$, $^{++}p<0.01$ compared to mice receiving γ-globulin.

Hot plate testing, like the previous experimental series, showed that stress exposure leads to hyperalgesia (Fig. 2). The mice receiving Ab to Glu demonstrated significantly shorter latency of the reaction compared to stressed animals. Ab to GABA did not affect pain sensitivity: the latency was similar to that in the stress control group. In mice receiving γ-globulin, the latency was longer than in stressed control and was similar to that in intact mice.

Examination of stress target-organ revealed adrenal hypertrophy in stressed mice (Fig. 3). Thymus involution was observed in stressed mice and in mice receiving anti-Glu and anti-GABA Ab, these changes were more pronounced after administration of Ab to Glu. Examination of the gastric mucosa revealed ulceration in all stressed groups. The incidence of ulcerations in this experimental series was similar to that in experimental series I and did not significantly differ between the groups of stressed animals receiving and not receiving Ab. No changes in the stomach mucosa were noted in the control group.

Thus, paradoxical effects of Ab to Glu were established. In experimental series I, intranasal administration of these Ab 1 h before stress inhibited the development of the stress response judging from behavioral parameters and prevented the development of hyperalgesia induced by exposure to stress. Ab to GABA aggravate stress-induced behavioral disorders (significantly decrease the integral behavioral activity in the open field test), but did not significantly affect the intensity of hyperalgesia. Rabbit γ-globulin potentiated the stress response in terms of behavioral parameters and slightly reduced hyperalgesia severity, which can be explained by proportion of anti-Glu and anti-GABA Ab in the serum of intact rabbits. In experimental series II, the stress response was not alleviated, but even strengthened in animals receiving Ab to Glu.

The observed phenomenon of differently directed effects of Ab to Glu administered before and after stress requires special analysis. This can be explained by initial preferential influence of the Glu-ergic system

on the stress response [11]. Under conditions of moderate psychoemotional stress, Ab to Glu can reduce the role of activator of the immune- and stress-limiting hypothalamic—pituitary—adrenal axis. At the same time, Ab to GABA, in contrast to experiments with their preliminary penetration into CNS, reciprocally activated already stimulated Glu-ergic system, thus potentiating manifestation of emotional stress. Moreover, we can hypothesize that the poststress effects of Ab to Glu require higher dose suppressing activation of NO-synthase, which takes place in response to low doses of Glu not bound to Ab [9,10,13].

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