Effect of Antibodies to Glutamate and GABA on the Stress Response in C57Bl/6 Mice

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We studied the effect of antibodies to glutamate and GABA (active immunization with conjugates of glutamate—bovine serum albumin and GABA—bovine serum albumin) on the course of combined water-immersion stress in C57Bl/6 mice. Preimmunization of animals with the conjugate of glutamate—bovine serum albumin was accompanied by strong production of antibodies to glutamate, which reduced the majority of signs of the stress response. Antibodies to GABA had no effect on the development of stress.

Key Words: *antibodies to neurotransmitters; glutamate; GABA; restraint stress*

The stress response is accompanied by significant changes in neurotransmitter metabolism in the central nervous system (CNS) [5]. Besides the dopaminergic and adrenergic systems, the stress syndrome is realized via glutamatergic (glutamate, GLU) and GABAergic processes. Experiments on rats and mice and clinical observations showed that various disorders of CNS are associated with the production of autoantibodies to neurotransmitters [2]. Previous studies revealed that antibodies to dopamine and serotonin play a role in immune modulation of brain functions that are related to the behavior and emotional state [2,6]. Antibodies to GLU and GABA had a modulatory effect on the symptoms of experimental epileptogenesis and central pain syndrome [1,3]. However, little is known about the influence of antibodies to GLU and GABA on the behavioral stress reaction in animals. Here we studied the effect of antibodies to GLU and GABA (active immunization) on the stress response in mice.

MATERIALS AND METHODS

Experiments were performed on 88 C57Bl/6 mice weighing 22-24 g. Two series were conducted with active immunization of mice by conjugated antigens GLU—BSA (bovine serum albumin) and GABA—BSA. This treatment was followed by stress exposure. In each series, the mice were divided into the following four groups: group 1, control; group 2, stressed control; group 3, immunization with GLU—BSA and stress exposure; and group 4, immunization with GABA—BSA and stress exposure. The scheme of immunization and stress exposure was similar in all series. Group 3 and 4 mice were immunized 3 times with the corresponding conjugated antigens at 2-week intervals. The scheme of immunization appeared as follows: 1st immunization, 2 mg/kg conjugate in 0.1 ml 0.9% NaCl and 0.1 ml complete Freund's adjuvant (subcutaneously into the back); 2nd immunization, 5 mg/kg conjugate in 0.1 ml 0.9% NaCl and 0.1 ml incomplete Freund's adjuvant (subcutaneously into the back); and 3rd immunization, 10 mg/kg conjugate in 0.2 ml 0.9% NaCl with no adjuvant (intraperitoneally). Control animals were treated with 0.2 ml physiological saline by the same scheme.

Conjugated antigens GLU—BSA and GABA—BSA were synthesized using glutaraldehyde [9]. The mice were exposed to combined unavoidable water-immersion stress on day 8 after the last immunization [4,7]. Group 2, 3, and 4 mice were placed in narrow plastic cylinders with holes. The animals were immersed in cold water (17.5-18°C)

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up to the level of the neck for 1 h. After stress, the mice were maintained in home cages for 1 h. The behavioral test was conducted in the follow-up period. The stress response was evaluated from behavioral activity (open-field test and dark-light chamber), pain sensitivity (hot-plate test), weight of stress-marker organs (spleen, thymus, and adrenal glands), and injuries of the gastric mucosa (ulcers and erosions).

In series I, the behavior of mice was studied in the open-field test for 3 min. We recorded the latency of the first movement and entry into the center of the open field, as well as the number of crossed squares, vertical rearing postures, and explored objects. The total score of activity (TSA) was calculated as the sum of crossed squares, vertical rearing postures, and explored objects.

In series II, behavioral parameters of mice were evaluated in the dark-light chamber test [7,8]. We recorded the latency to leave the dark compartment, number of looking-out episodes, incidence of entries into the light compartment, and total time spent in the light compartment. The pain threshold was determined on a hot plate (56°C) 10 min after the open-field test or light-dark chamber test. The latency of hind-paw licking was measured.

The mice were decapitated after the study. The blood was sampled and examined for GLU and GABA. The production of antibodies to GLU—BSA and GABA—BSA was studied by means of solid-phase enzyme immunoassay on a Mini-Reader device (Dynateck) using polystyrene plates. Conjugates of GLU and GABA were synthesized on a heterologous protein carrier (horse γ-globulin) and used as the test antigens. Blood plasma from intact mice served as the control.

The results were analyzed by Student's *t* test and Fischer's test.

RESULTS

Immunization of mice with antigens of GLU—BSA and GABA—BSA was followed by the production of antibodies to GLU (titer 1:256) and GABA (titer 1:32-1:64).

No differences were found in the open-field behavior of mice under baseline conditions. Combined restraint stress was accompanied by the decrease in open-field TSA in mice of groups 2, 3, and 4 (Fig. 1). These changes were particularly pronounced in stressed control mice (53.1%) and animals immunized with a GABA—BSA conjugate (56.9%). Stress exposure was followed by a 39.1% decrease in TSA of mice immunized with a GLU—BSA conjugate. The observed changes were less

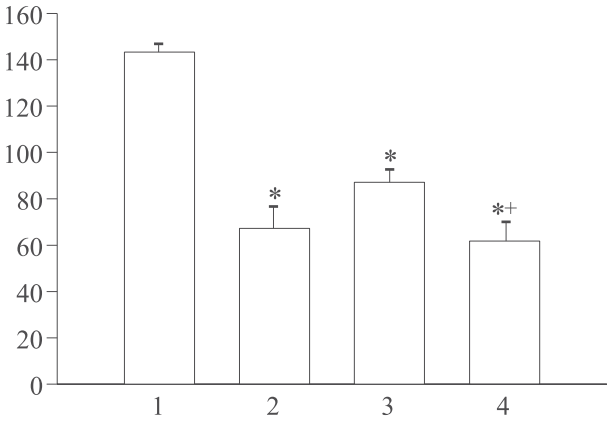

Fig. 1. Effect of active immunization of C57Bl/6 mice with GLU—BSA and GABA—BSA conjugates on the total score of activity in the open field after combined restraint stress. Ordinate: total score of activity of mice. Abscissa: groups of mice. *$p<0.01$ compared to group 1; $^{+}p<0.05$ compared to group 3.

significant compared to those in stressed control mice and animals immunized with a GABA—BSA conjugate. Immunization of mice with GLU—BSA and GABA—BSA caused the opposite changes in the dark-light chamber behavior after stress exposure (Table 1). In control mice, the latency to leave the dark compartment was 12.5±2.6 sec. The total time spent in the light compartment was 156.4±7.9 sec. Stress exposure in group 2 mice was followed by a significant increase in the latency to leave the dark compartment (23.1±3.9 sec) and decrease in the time spent in the light compartment (126.2±10 sec). The latency to leave the dark compartment in group 3 mice (GLU—BSA immunization and stress) was much lower than in stressed control animals (group 2), but did not differ from that in control specimens (group 1). The total time spent in the light compartment was higher in group 3 mice than in group 2 animals. However, this parameter did not differ in group 3 mice and control specimens. The latency to leave the dark compartment and total time spent in the light compartment for the group of mice immunized with GABA—BSA and exposed to stress were similar to those for stressed control animals.

The mean values of pain sensitivity in the hot plate test (2 series) are shown in Fig. 2. Combined restraint stress was followed by a significant decrease in the pain threshold in group 2 mice (as compared to the control). The latency of hind-paw licking after stress exposure did not differ in mice immunized with GLU—BSA and control specimens (15.5±0.9 and 14.8±0.6 sec, respectively). Therefore, antibodies to GLU had the antinociceptive effect. Immunization with GABA-BSA had no effect on pain sensitivity in stressed mice. The la-

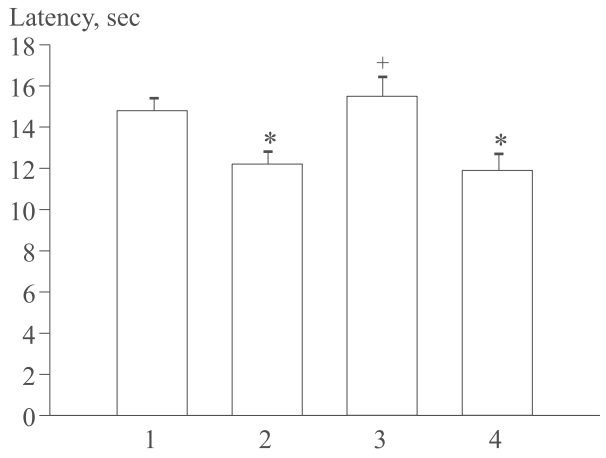

Fig. 2. Effect of active immunization of C57Bl/6 mice with GLU—BSA and GABA—BSA conjugates on the latency of hind-paw licking in the hot plate test after combined restraint stress. Abscissa: groups of mice. $p<0.01$: *compared to group 1; +compared to group 2.

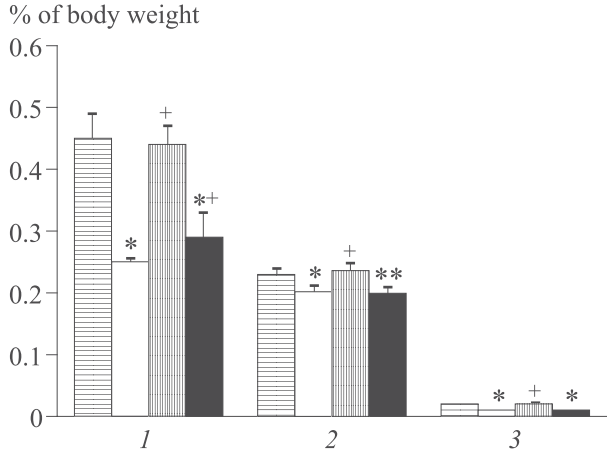

Fig. 3. Weight of stress-marker organs in C57Bl/6 mice after combined restraint stress. Ordinate: weight of stress-marker organs. Spleen (*1*), thymus (*2*), and adrenal glands (*3*). Horizontal shading, group 1; light bars, group 2; vertical shading, group 3; dark bars, group 4. *$p<0.01$ and **$p<0.05$ compared to the control; +$p<0.01$ compared to group 2.

tency of paw-licking in immunized animals did not differ from that in group 2 mice.

The weight of stress-marker organs (spleen, thymus, and adrenal glands) decreased in group 2 mice (compared to the control), which illustrates the stress response of these animals (Fig. 3). Immunization with GLU—BSA prevented a change in the weight of stress-marker organs after stress exposure. No differences were found in the weight of stress-marker organs in immunized mice and control specimens. Antibodies to GABA had no effect on these parameters. The weight of stress-marker organs in mice immunized with GABA—BSA and exposed to stress was similar to that in stressed control specimens. No between-group differences were revealed in the severity of gastric mucosal injury in experimental animals. Ulceration was found in group 2 mice (46.6% specimens) and animals immunized with GLU—BSA (50% specimens) or GABA—BSA (38.7% specimens). Gastric mucosal injury was not typical of group 1 mice.

It could be suggested that repeated administration of physiological saline modulates the stress response in control mice. To test this hypothesis, the stress response was evaluated in animals not receiving physiological saline. The following parameters were studied: open-field behavior of mice; TSA; pain sensitivity; weight of stress-marker organs; and state of the gastric mucosa. Injection of physiological saline had little effect on the stress response. TSA of mice receiving and not receiving physiological saline was 67.0±9.5 and 60.3±8.5, respectively, after combined restrain stress. Repeated treatment with physiological saline did not modulate the degree of stress-induced hyperalgesia. After stress exposure the latency of hind-paw licking in mice receiving and not receiving physiological saline was 12.6±0.6 and 12.8±1.7, respectively. No significant differences were found in the weight of stress-marker organs. The incidence of gastric mucosal injury tended to increase in mice not receiving physiological saline and exposed to stress (by 1.6 times compared to animals of the physiological saline group, $p=0.08$).

Our results show that preimmunization of mice with a GLU—BSA conjugate reduces the majority of signs of the stress response and, therefore, has the antistress effect. Immunization of mice with a GABA—BSA conjugate did not modulate the stress response during combined restraint stress. Stress symptoms in mice immunized with GABA—BSA were similar to those in nonimmunized stressed animals.

Stress exposure caused hyperalgesia in mice, which was manifested in a decrease in the latency of hind-paw licking in the hot plate test. Preimmunization with GLU—BSA prevented the development of hyperalgesia under stress conditions. Previous experiments on the model of central neurogenic pain syndrome showed that intrathecal administration of antibodies to GLU has the antinociceptive effect [3].

Preimmunization wit a GLU—BSA conjugate prevented a change in the weight of stress-marker organs, but had no effect on gastric ulceration in mice. Probably, the level of antibodies to GLU after immunization with a GLU—BSA conjugate is not sufficient to prevent ulceration as one of the most severe complications of stress. These data require further investigations. Moreover, the mechanism for action of antibodies to GLU and GABA remains

TABLE 1. Effect of Active Immunization with GLU—BSA and GABA—BSA Conjugates on the Behavior of C57Bl/6 Mice in a Dark-Light Chamber after Combined Restraint Stress

Group	Latency to leave, sec	Number of looking-out episodes	Number of exits	Total time spent in the light compartment, sec
1	12.5±2.6	7.8±0.7	7.3±0.5	156.4±7.9
2	23.1±3.9**	4.8±0.5*	6.8±0.5	126.5±10.0**
3	12.8±3.5+	3.75±0.60*	5.1±1.2	156.3±27.0+
4	32.5±7.9*	2.7±0.4*+	5.3±0.6**	129.4±19.5

Note. *$p<0.01$ and **$p<0.05$ compared to group 1; +$p<0.01$ compared to group 2.

unknown. It may be suggested that these antibodies enter CNS during stress exposure. Immunization with a GABA—BSA conjugate had no effect under these conditions, which was probably related to low production of anti-GABA antibodies. The titer of antibodies to GLU and GABA was 1:256 and 1:64, respectively. These features are probably associated with low immunogenicity of the conjugate.

We conclude that antibodies to GLU and, probably, to GABA are involved in the pathogenetic mechanisms of psychoemotional stress.

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