GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Effect of Intranasal Administration of Anti-Glutamate Antibodies after Stress Exposure on the Stress Response

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We studied the dose-dependent effect of antibodies to glutamate on the stress response in C57Bl/6 mice. The antibodies were administered immediately after stress exposure. Intranasal administration of antibodies to glutamate in doses of 150 and 250 μg/kg immediately after stress exposure was shown to reduce the stress response under conditions of combined restraint stress. This effect was most pronounced after treatment with antibodies in a dose of 250 μg/kg: we revealed a decrease in the number and severity of erosive and ulcerative lesions in the gastric mucosa, *i.e.* anti-glutamate antibodies have a protective effect.

Key Words: *antibodies; glutamate; stress*

Intranasal administration of antibodies against glutamate (GLU-AB) before and after stress produces various effects. Previous studies showed that intranasal administration of GLU-AB (30 μg/kg) 1 h before stress inhibits the behavioral stress response [2]. Treatment with GLU-AB in the same dose immediately after stress exposure was shown to increase the stress response. It was manifested in a significant decrease in total activity (TA) of animals compared to intact and stressed controls. We hypothesized that the post-stress effect is observed only after administration of GLU-AB in a higher dose. GLU-AB in this dose can suppress the glutamatergic neurotransmitter system, which is excited under stress conditions.

Here we studied the dose-dependent effect of intranasal administration of GLU-AB immediately after stress on the stress response.

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MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice (n=114) weighing 20-22 g. We studied the dose-dependent effect of intranasal administration of GLU-AB immediately after stress on the stress response.

The combined water-immersion stress [5] was adapted for mice [3] and served as the model of stress exposure. The study was conducted in 2 series. In series I (n=48) we studied the effect of intranasal administration of GLU-AB (150 and 250 μg/kg) immediately after stress exposure on the stress syndrome. The mice were divided into the following four groups: group 1 (n=12), intact control; group 2 (n=11), stressed animals (stressed control); group 3 (n=15), intranasal administration of GLU-AB in a dose of 150 μg/kg immediately after stress exposure; and group 4 (n=10), GLU-AB in a dose of 250 μg/kg. GLU-AB were obtained by hyperimmunization of rabbits with the conjugated antigen glutamate-BSA [1]. The γ-globulin fraction of GLU-AB was isolated from blood serum

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of immunized rabbits by precipitation with ammonium sulfate. Admixtures of antibodies to the protein carrier were removed by affinity chromatography. Cyanogen bromide-activated Sepharose 4B with immobilized BSA was used as the sorbent. GLU-AB were isolated, purified, lyophilized, and stored at 4°C.

GLU-AB were dissolved in 10 μl physiological saline. The solution of GLU-AB was administered intranasally (5 μl into each nostril) using a small-tip pipette. Group 1 and 2 mice received 5 μl physiological saline.

Open-field (OF) behavior of animals was studied 1 h after stress exposure and administration of GLU-AB. The following parameters were recorded: latency of the 1st movement; latency of entry into the center of OF; and number of crossed squares, rearing postures, and explored objects. The stress response was evaluated from locomotor activity in OF and TA (sum of crossed squares, rearing postures, and explored objects).

The mice were decapitated. The gastric mucosa was examined for gastric ulcers using a binocular magnifier (×40). The degree of erosive and ulcerative lesions of the gastric mucosa was scored [4]: 0 points, normal state; 1 point, single erosions (not more than 5 erosions); 2 points, numerous erosions (more than 6 erosions) or 1 ulcerative lesion; and 3 points, 2 or more ulcerative lesions.

Series I allowed us to evaluate the most effective dose of GLU-AB (250 μg/kg). Series II (n=66) was conducted to confirm the results of series I. The animals were divided into the following three groups: group 1 (n=10), intact control; group 2 (n=30), stressed mice (stressed control); and group 3 (n=26), intranasal administration of GLU-AB in a dose of 250 μg/kg immediately after stress exposure.

The results were analyzed by nonparametric Mann–Whitney U test and Student's t test.

RESULTS

No between-group differences were found in the initial (baseline) activity of mice (Fig. 1). Stress exposure was followed by a significant decrease in the locomotor activity of animals in OF. We revealed a decrease in the number of crossed squares (by 39.8%, p=0.002, U test), rearing postures (by 38%, p<0.05, t test), and explored objects (by 60.6%, p<0.05, t test). TA decreased from 109.3±5.2 to 64.5±11.3 (p=0.004, U test).

Intranasal administration of GLU-AB in doses of 250 and 150 μg/kg immediately after stress exposure was shown to abolish the decrease in the locomotor activity of mice in OF. The number of crossed squares in group 4 mice was 82.6±6.8, which did not differ from that in group 1 animals (86.6±3.7). The number of crossed squares in group 3 mice tended to decrease under these conditions (66.5±6.6; p=0.09, U test). The number of crossed squares in stressed control animals was 52.1±8.4. TA of group 4 mice was much higher than that of group 2 animals (99.8±8.1 and 64.5±11.3, respectively; p=0.03, U test). No significant differences were found in TA of group 4 and 1 mice (109.3±5.2). TA of group 3 animals was 90.6±6.8 (p=0.052 compared to group 2 specimens, U test).

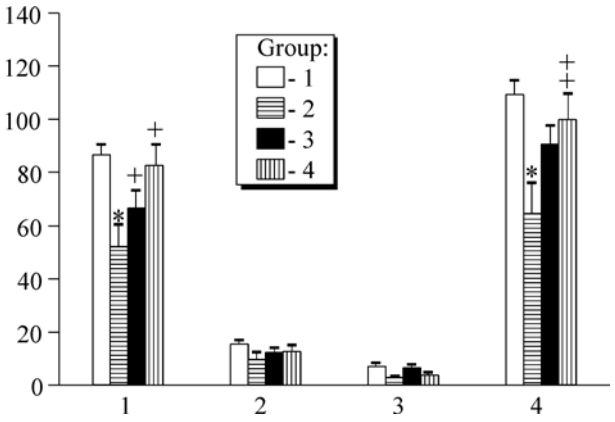

Fig. 1. Dose-dependent effect of GLU-AB on C57Bl/6 mice during combined restraint stress (series I). Here and in Fig. 2: ordinate, locomotor activity. Number of crossed squares (*1*), number of rearing postures (*2*), number of explored objects (*3*), and TA (4). *p<0.01 compared to group 1; +p<0.05 and ++p<0.01 compared to group 2.

Combined restraint stress was followed by the formation of erosive and ulcerative lesions of the gastric mucosa. The severity of ulcerative lesions in 72.7% mice of group 2 was 2.9±0.1 points. GLU-AB in various doses had no effect on ulceration in these animals under stress conditions. The incidence of erosive and ulcerative lesions of the stomach in group 3 and 4 mice was 70% (2.6±0.2 points) and 66.7% (2.7±0.2 points), respectively. Pathological changes in the gastric mucosa were not revealed in group 1 animals.

Therefore, intranasal administration of GLU-AB in doses of 150 and 250 μg/kg immediately after stress exposure reduces the stress response during combined restraint stress (protective effect). This effect was most pronounced after treatment with GLU-AB in a dose of 250 μg/kg. Series II was conducted to confirm the results of series I.

Combined restraint stress was followed by a decrease in locomotor activity of mice in OF (Fig. 2). The number of crossed squares in group 2 mice was lower than in group 1 animals (53.5±4.1 and 69.3±6.7, respectively; p=0.004, U test). TA of group 1 and 2 mice was 86.9±8.2 and 68.9±5.3, respectively (p=0.006, U test). Intranasal administration of GLU-AB in a dose of 250 μg/kg immediately after stress exposure was shown to suppress the stress response. The number of

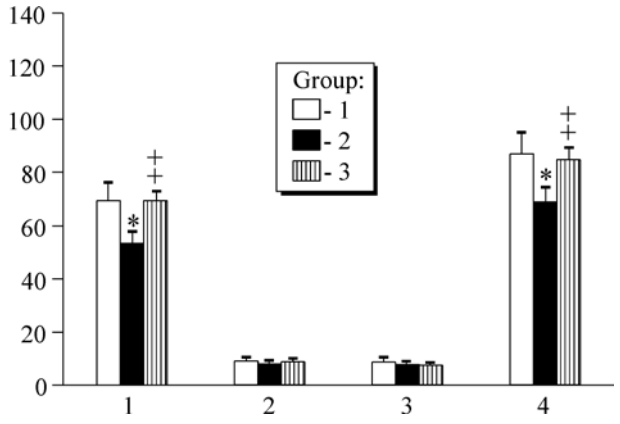

Fig. 2. Effect of GLU-AB in a dose of 250 μg/kg on the stress response of C57Bl/6 mice under conditions of combined restraint stress (series II).

TABLE 1. Effect of Intranasal Administration of GLU-AB in a Dose of 250 μg/kg Immediately after Stress Exposure on the Formation of Lesions of the Gastric Mucosa in C57Bl/6 Mice during Combined Restraint Stress (*M*±*m*)

Group	*n*	Incidence of lesions of the gastric mucosa, %	Severity, score
1	10	0	0
2	30	66.7	2.5±0.2
3	26	34.6*	1.3±0.2*

Note. *$p<0.005$ compared to group 2 (*U* test).

crossed squares in treated mice was much higher than in group 2 animals (69.3±3.5 and 53.5±4.1, respectively; $p=0.01$, *U* test), but did not differ from that in specimens of group 1. TA of group 3 mice was much higher than that of group 2 animals (84.7±4.4 and 68.9±5.3, respectively; $p=0.02$, *U* test). No differences were revealed in TA of group 3 and 1 mice.

Series II showed that GLU-AB have a protective effect on the gastric mucosa in mice (Table 1). Erosive and ulcerative lesions of the gastric mucosa were found in 66.7% animals of group 2. The severity of gastric mucosal lesions in these animals was 2.5±0.2 points. The number of animals with lesions of the gastric mucosa was 2-fold lower in group 3 (34.6% specimens). The severity of pathological changes in these mice was 1.3±0.2 points ($p=0.002$, *U* test).

Good consistency was found between the results of series I and II. We conclude that intranasal administration of GLU-AB in a dose of 250 μg/kg immediately after stress exposure has a protective effect during combined restraint stress.

Previous studies showed that stress is followed by activation of the glutamatergic system [8,10]. This system has a modulatory effect on central mechanisms of the stress response (*e.g.*, hypothalamic-pituitary-adrenal axis), dopamine neurotransmission, and NO synthase activity [7,9]. The effects of GLU-AB are probably related to suppression of the glutamatergic system. GLU-AB enter the central nervous system (CNS) and interact with glutamate. It should be emphasized that the extracellular concentration of glutamate increases during stress exposure [11]. Moreover, intranasal administration of substances (*e.g.*, IFN and γ-globulin) is followed by their transport into CNS over 1.5-4.5 min. The concentration of the test substances in CNS under these conditions is 88-98% higher compared to other routes of administration [6,12].

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