Antibodies to Glutamate as Neuromodulators of Behavioral Reactions in Mice with Various Genotypes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 3, pp. 274-277, March, 2002
Original article submitted November 19, 2001

We studied the effects of active immunization with glutamate-protein conjugates on behavioral reactions of C57Bl/6 and BALB/c mice in the open-field test and on animal anxiety in the tail suspension test. Antiglutamate antibodies produced different behavioral changes in mice of these strains.

Key Words: *glutamate antibodies; behavior; anxiety*

Glutamatergic neuromodulation plays an important role in the regulation of higher nervous activity, including behavior, learning, memory and physiological mechanisms of anxiety and aggression, and determination of the convulsion threshold [4,5]. Previous studies demonstrated a relationship between genetically determined behavioral characteristics and activity of the glutamatergic system in experimental animals [1, 4]. It remains unclear whether intensive production of antibodies (AB) to glutamate (GLU) can modulate behavioral reactions in animals. Our previous studies showed that specific antibodies to neurotransmitters serotonin and dopamine can modulate various brain functions associated with behavior and emotional state [2,6]. Experiments on various mouse strains showed that hereditary behavioral parameters can be modulated by immunization with serotonin-protein conjugates [3].

Here we studied the effects of AB against GLU formed after active immunization with GLU-protein conjugates on behavioral reactions of mice with various genotypes.

MATERIALS AND METHODS

Experiments were performed on male mice with various genetically determined behavioral characteristics: highly active and stress-resistant C57Bl/6 mice and low active and stress-predisposed BALB/c mice. The animals of these strains were divided into experimental and control groups (20 mice in each group). Experimental mice were immunized with GLU conjugated with bovine serum albumin (BSA). Control animals were treated with physiological saline. GLU—BSA was synthesized routinely using bifunctional reagent glutaraldehyde [11]. Immunogenicity of the conjugate was estimated by active immunization of rabbits. Chinchilla rabbits were immunized 3 times with the GLU—BSA conjugate in increasing doses. The titer of anti-GLU AB was 1:1024. Anti-GLU AB were not found in control animals.

Experimental mice were immunized 3 times with the GLU—BSA conjugate in increasing doses. The conjugate (2 mg/kg) dissolved in complete Freund's adjuvant (CFA, 0.1 ml) and physiological saline (0.1 ml) was injected subcutaneously. After 2 weeks the mice received intraperitoneal injection of 10 mg/kg conjugate in 0.2 ml physiological saline. The animals were intraperitoneally injected with 15 mg/kg conjugate in 0.2 ml physiological saline after the next 2 weeks. Control mice received 1 subcutaneous and 2 intraperitoneal injections of physiological saline (0.2 ml). Plasma level of anti-GLU AB in immunized animals was measured by enzyme-linked immunosorbent assay. The GLU conjugated with equine γ-globulin was synthesized as described previously and used as the test antigen.

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0007-4888/02/1333-0232$27.00

Behavioral reactions of mice were studied in the open-field (OF) test before immunization and 1 week after each injection of the conjugate. Behavioral parameters were automatically recorded in the Opto-Varimex system (Columbus Instruments) using Auto Track software. The mice were preadapted to OF for 3 days. The distance passed in OF, time of active movements, vertical locomotor activity (rearings), duration of stereotypic activity, number of fine movements, number of clockwise and counterclockwise rotations, latency of exit from the center of OF, and number of crossed central squares were recorded for 3 min. The coefficient of behavioral activity was calculated as the mean sum of deviation (in %) of each behavioral parameter from the corresponding parameter in the control group. One week after each injection of the conjugate the mice were subjected to the tail suspension test [13]. The duration of immobility in this test reflects animal anxiety. The time and number of immobility episodes were recorded for 6 min.

The data are presented as means of 2 series. The results were analyzed by Student's *t* test.

RESULTS

Comparison of the initial (before adaptation) behavioral characteristics of mice confirmed considerable differences in OF behavior between these strains. C57Bl/6 mice differed from BALB/c mice in all behavioral parameters (Table 1). After adaptation to OF, C57Bl/6 mice differed from BALB/c mice in a longer period of stereotypic activity, greater number of fine movements, rearing postures, and crossed central squares, shorter duration of resting episodes, and shorter latency of exit from the center of OF.

After immunization of C57Bl/6 and BALB/c mice with the GLU—BSA conjugate plasma titer of anti-GLU AB was 1:16. In control animals these antibodies were not found.

Active immunization with GLU—BSA conjugates produced various changes in behavioral activity of C57Bl/6 and BALB/c mice. One week after the first injection of GLU—BSA conjugate BALB/c mice, which were initially characterized by low locomotor activity in OF, passed greater distances and displayed longer periods of moving and stereotypic activity and higher number of fine movements, rearing postures, and clockwise and counterclockwise rotations compared to control animals. In these mice the mean activity coefficient was 199.85%. We found no significant differences in behavioral activity of experimental and control animals after the second and third injections of conjugates (Fig. 1).

Anti-GLU AB induced by active immunization with GLU—BSA conjugates did not modulate behavioral activity of C57Bl/6 mice. In these animals the mean activity coefficient tended to increase to 126.4% after the third injection of the conjugate (Fig. 1).

TABLE 1. Behavioral Activity of C57Bl/6 and BALB/c Mice in OF ($M \pm m$)

Parameter	C57Bl/6	BALB/c
Passed distance, m	5.26±0.24	3.98±0.29
Resting time, sec	66.0±2.3	195.0±3.6
Duration of stereotypic activity, sec	71.0±1.2	61.0±2.1
Time of moving, sec	4.10±0.18	2.15±0.19
Number of fine movements	126.0±2.3	99±4
Number of rearing postures	9.5±0.8	3.6±0.6
Latency of exit from the center, sec	1.4±0.2	7.8±0.7
Number of crossed central squares	1.9±0.2	0.20±0.07
Number of grooming episodes	1.7±0.2	1.20±0.15

Note. Significant interstrain differences ($p<0.01$).

In the first tail suspension test we revealed no interstrain differences in the level of anxiety in control C57Bl/6 and BALB/c mice. Control C57Bl/6 mice displayed the same level of anxiety in repeated tests. However, BALB/c mice were characterized by a shorter period of immobility in repeated tail suspension tests (Fig. 2). Active immunization with GLU—BSA conjugates produced various changes in the level of anxiety in C57Bl/6 and BALB/c mice. In C57Bl/6

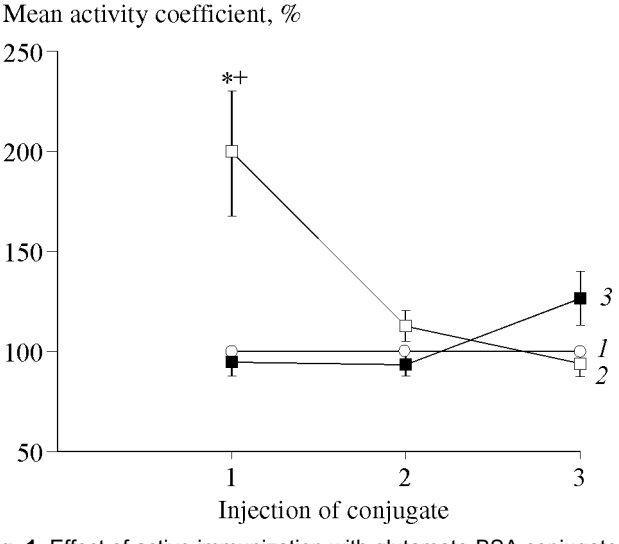

Fig. 1. Effect of active immunization with glutamate-BSA conjugates on the mean activity coefficient for BALB/c and C57Bl/6 mice in open field tests performed 1 week after each injection. Control (*1*), BALB/c (*2*), and C57Bl/6 (*3*). *$p<0.001$ compared to the control; +$p<0.05$ compared to C57Bl/6 mice.

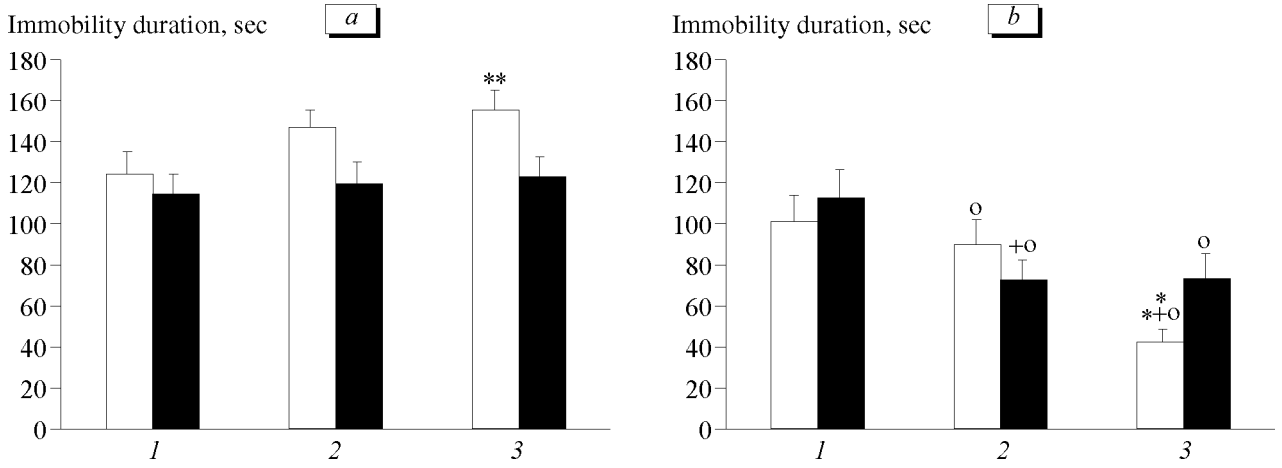

Fig. 2. Effect of active immunization of C57Bl/6 (*a*) and BALB/c mice (*b*) with glutamate—BSA conjugates on the duration of immobility in the tail suspension test. Tests were performed 1 week after the first (*1*), second (*2*), and third injections of conjugates (*3*). Light bars: immunization; dark bars: control (physiological saline). **p*<0.001 and ***p*<0.05 compared to the control; +*p*<0.005 compared to the previous test; °*p*<0.05 compared to C57Bl/6 mice in the same period.

mice the duration of immobility progressively increased and reached 155.5±9.4 sec in the final tail suspension test (*vs*. 122.9±9.1 sec in the control). In BALB/c mice the duration of immobility progressively decreased and was 42.2±6.7 sec in the final tail suspension test (1.7-fold lower than in the control), which markedly differed from the corresponding parameter in immunized C57Bl/6 mice (Fig. 2).

Thus, anti-GLU AB produced various effects on locomotor activity of mice with various genotypes. Active immunization with GLU—BSA conjugate produced most significant changes in stress-predisposed BALB/c mice, which was manifested in an increase in their locomotor activity in the early postimmunization period. However, immunization had no effect on behavioral parameters of active C57Bl/6 mice.

The effects of AB against GLU on behavioral characteristics of BALB/c mice are similar to those produced by NMDA receptor agonists. Previous studies showed that intraperitoneal administration of the noncompetitive NMDA receptor antagonist dizocilpine (MK 801) and microinjections of the competitive NMDA receptor antagonist AP-5 into the ventral or dorsal striatum increase locomotor activity of rats [7]. Our results are consistent with published data that the NMDA receptor antagonist dizocilpine initially increases, but then decreases locomotor activity of intact mice [12].

AB against GLU formed after active immunization with GLU—BSA conjugate probably bind this neuromodulator and, therefore, modulate functional state of its receptors. It can not be excluded that the effects of AB on locomotor activity of mice are mediated by other neuromodulatory systems, including the dopaminergic system. Previous studies demonstrated the existence of close relationships between glutamate- and dopaminergic systems [8-10]. The NMDA receptor antagonist dizocilpine potentiates the stimulatory effect of SKF-38393 (D1 dopamine receptor agonist) on locomotor activity [8].

A more informative test for anxiety revealed different effects of anti-GLU AB on animals of genetically various strains. Induction of anti-GLU AB in stress-resistant C57Bl/6 mice prolonged their immobility, which indicates an increase in animal anxiety [13]. Anti-GLU AB produced an anxiolytic effect on stress-predisposed BALB/c mice, which is consistent with published data. Previous studies showed that NMDA receptor antagonists produce the anxiolytic effect on mice and rats. These compounds increase the time spent by animals in open arms of the plus-maze [14]. High-anxiety animals (*e.g.*, BALB/c mice) are more resistant to antidepressants [13].

Our results indicate that immunization with GLU—BSA conjugate modulates hereditary behavioral characteristics of C57Bl/6 and BALB/c mice. It should be emphasized that BALB/c mice are more sensitive to the effect of AB against GLU than C57Bl/6 mice.

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