GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Immunomodulation of Inherent Behavior in C57Bl/6 and BALB/c Mice with Antibodies to Glutamate

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Immunization of BALB/c mice with glutamate-BSA conjugate reduced anxiety and improved passive avoidance retention, while in C57Bl/6 mice immunization disturbed passive avoidance retention, but had no effect on anxiety. Interstrain differences in the shuttle box behavior were found between control animals.

Key Words: *glutamate; antibodies; anxiety; memory*

Glutamate (L-glutamic acid, GLU) is the basic excitatory transmitter in CNS. The glutamatergic system was studied in details by neurophysiologists, pharmacologists, and neurochemists [4,5]. Previous studies demonstrated the role of GLU in genetically determined behavior (reaction to stress, aggression, and seizure readiness) and in the development of long-term potentiation underlying neurological memory. P. Seguela *et al*. [13] synthesized GLU-protein conjugates, obtained specific antibodies (AB) to the linear amino acid, and used them in immunocytochemical studies. However, the effect of anti-GLU AB on higher nervous activity was not previously studied.

Previous studies revealed genetically determined differences in higher nervous activity between C57Bl/6 and BALB/c mice. Specifically, these mice differ significantly in the anxiety level, open field behavior, and reaction to stress [6]. We demonstrated the possibility of correcting some inherent behavioral peculiarities of C57Bl/6 and BALB/c mice by active immunization with serotonin-protein conjugates [1,3].

The aim of the present study was immunomodulation of inherent behavioral peculiarities by shifting the balance between some neurotransmitters. We also studied the possibilities of modifying some inherent behavioral peculiarities and memory in mice of contrast strains by active immunization with GLU—BSA (bovine serum albumin) conjugate.

MATERIALS AND METHODS

GLU—BSA conjugate was synthesized using a modified method with glutaraldehyde [13]. This conjugate was characterized by high immunogenicity: the titers of anti-GLU AB in immunized rabbits were 1:1024.

Experiments were carried out on male C57Bl/6 (n=37) and BALB/c (n=38) mice weighing 20-23 g. The animals of each strain were divided into two groups. Experimental mice were immunized with GLU—BSA conjugate according to the following scheme: 1st immunization — 2 mg/kg conjugate in 0.1 ml 0.9% NaCl with 0.1 ml Freund's complete adjuvant subcutaneously, 2nd and 3rd immunizations — 5 and 10 mg/kg conjugate, respectively, in 0.2 ml 0.9% NaCl (intraperitoneally). The controls received 0.2 ml 0.9% NaCl. After the first immunization, the mice of each control and experimental groups were subdivided into 2 equal subgroups. Subgroup 1 mice were tested in a light-dark shuttle box (3 tests) one week after each im-

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munization. Subgroup 2 mice were trained conditioned passive avoidance response (CPAR) 3 days after the last immunization [9].

Anxiety in mice was evaluated in a light-dark shuttle box modified according to A. V. Kaluev [2]. The mouse was placed into the dark compartment and after 2-min adaptation the door to the light compartment was opened. The following parameters were recorded for the next 5 min under bright illumination: latency of the first exit from the dark compartment, the number of peeps and exits from the dark compartment, and the total time spent in the light compartment. CPAR retention was tested on days 1, 7, and 14. During retention test the animals spent in the illuminated compartment at least 180 sec. The time spent in the illuminated compartment during CPAR training and retention tests were recorded. At the end of the experiment, titers of anti-GLU AB were measured in the sera of immunized mice by enzyme linked immunosorbent assay. The test antigen was GLU conjugated with eguine γ-globulin.

The data were analyzed statistically by Student's *t* test using SWP4 software. The experimental data were compared with control values, previous values, or with the corresponding parameter in mice of other strain.

RESULTS

Immunization of control mice with GLU—BSA conjugate induced production of anti-GLU AB (mean titers 1:16).

Shuttle box testing revealed interstrain differences in behavioral parameters between control mice of different strains. The latency of exit from the dark compartment slightly decreased in C57Bl/6 mice and markedly increased in BALB/c mice (from 2.9±0.5 to 8.9±1.9 sec, $p<0.01$). The number of entries and the time spent in the light compartment in C57Bl/6 mice surpassed the corresponding parameters in BALB/c mice throughout the observation period. These parameters remained practically unchanged in C57Bl/6 mice but decreased in BALB/c mice (tests 2 and 3 revealed significant interstrain differences, Fig. 1).

Immunization with GLU—BSA conjugate had no effect on shuttle box behavior in C57Bl/6 mice,

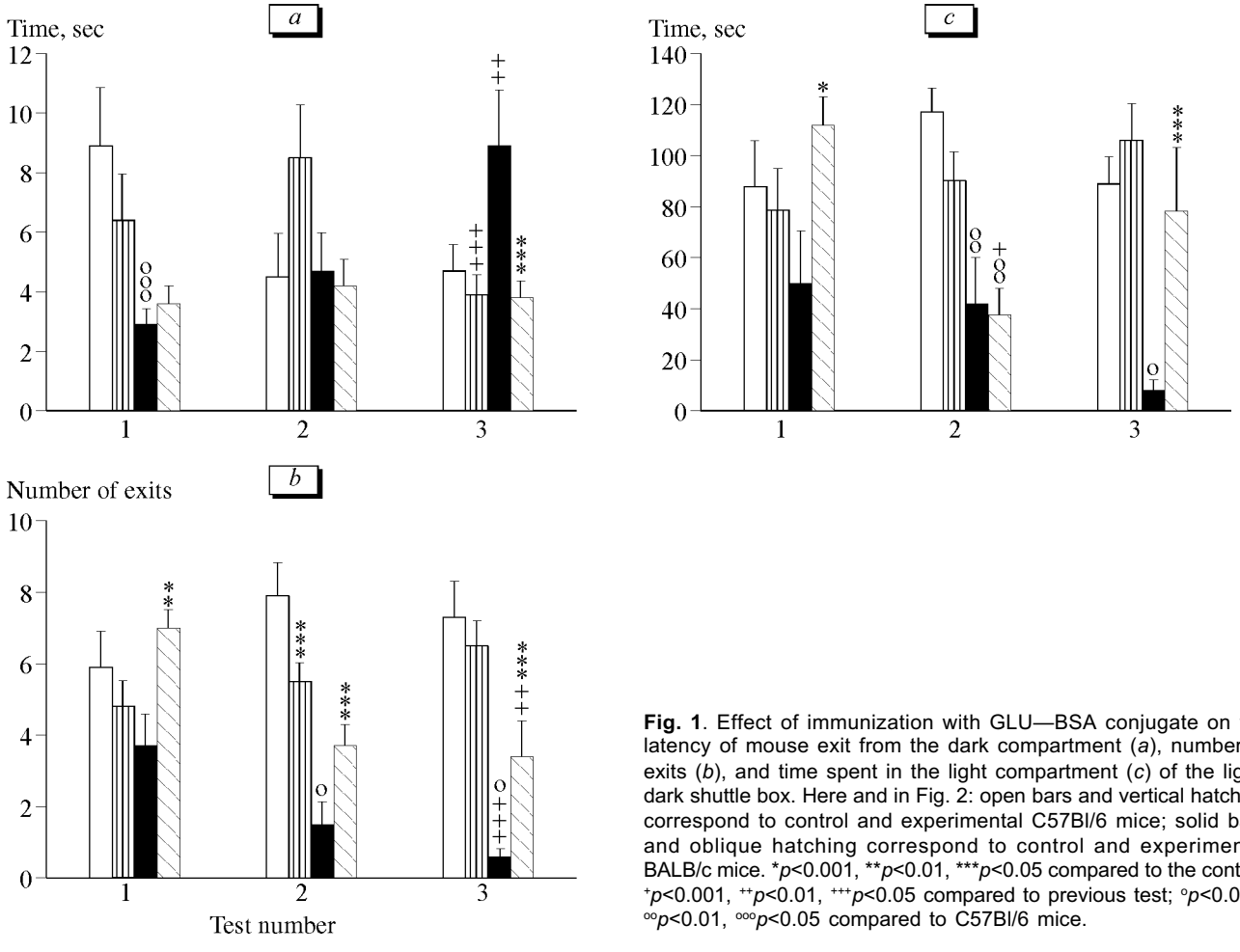

Fig. 1. Effect of immunization with GLU—BSA conjugate on the latency of mouse exit from the dark compartment (*a*), number of exits (*b*), and time spent in the light compartment (*c*) of the light-dark shuttle box. Here and in Fig. 2: open bars and vertical hatching correspond to control and experimental C57Bl/6 mice; solid bars and oblique hatching correspond to control and experimental BALB/c mice. *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared to the control; $^{+}p<0.001$, $^{++}p<0.01$, $^{+++}p<0.05$ compared to previous test; $^{o}p<0.001$, $^{oo}p<0.01$, $^{ooo}p<0.05$ compared to C57Bl/6 mice.

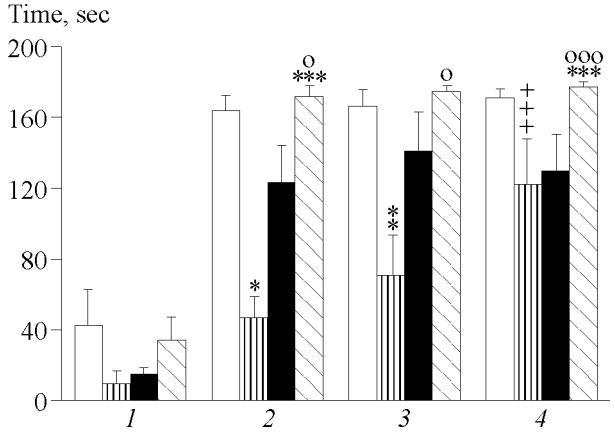

Fig. 2. Effect of immunization with GLU—BSA conjugate on acquisition (*1*) and retention of conditioned passive avoidance response after 1 (*2*), 7 (*3*), and 14 (*4*) days.

while in BALB/c mice it stabilized the latency of exit from the dark compartment: in test 3 this index was significantly lower than in control ($p<0.05$). The number of exits from the dark compartment in immunized BALB/c mice considerably surpassed the control values throughout the experimental period, and the time spent in the light compartment during test 1 and test 3 surpassed the control levels (Fig. 1).

The control and immunized mice of both strains did not significantly differ in CPAR acquisition time. However, this index tended to decrease in immunized C57Bl/6 mice.

Three tests for CPAR retention revealed no significant differences between the control mice of both strains (Fig. 2). In experimental C57Bl/6 mice, testing on days 1 and 7 revealed poorer CPAR retention compared to the control values ($p<0.001$ and $p<0.01$, respectively). On day 14, CPAR retention time in immunized mice increased and approximated the control values at the same term. Immunization of BALB/c mice with GLU—BSA conjugate produced an opposite effects: in all three test they demonstrated better CPAR retention time compared to the control (on days 1 and 14 this difference was significant, $p<0.05$). In all tests, immunized BALB/c mice demonstrated better CPAR retention time than immunized C57Bl mice (Fig. 2). Similar data (decrease of CPAR retention time in C57Bl mice and its increase in BALB/c mice) were previously obtained on these mouse strains after active immunization with serotonin-BSA conjugate [3]. These data attest to possible interaction between the glutamate- and serotoninergic systems similar to previously reported relationships between the glutamate- and dopaminergic systems [5].

Thus, evaluation of anxiety in a light- dark shuttle box showed that control C57Bl/6 and BALB/c mice considerably differ in both behavioral parameters and the effect of GLU—BSA immunization on these parameters. The BALB/c mice are characterized by higher anxiety. Our experiments demonstrated an increase in anxiety level in these mice during repeated tests. Immunization reduced anxiety in BALB/c mice and they demonstrated more active exploration behavior in the light compartment of the shuttle box. In C57Bl/6 mice characterized by low anxiety immunization had no appreciable effects on shuttle box behavior. Similar interstrain differences in the reaction to stimulation were described elsewhere: for example, a synthetic analog of tuftsin produced an anxiolytic effect in BALB/c mice, but was ineffective in C57Bl/6 mice [6].

It was established that excitatory processes in CNS produce an anxiogenic effect, while exogenous substances shifting the balance between excitatory and inhibitory processes in CNS produce pronounced changes in anxiety level and modulate specific behavioral reactions [11]. Inhibition of the excitatory glutamate transmission produced an anxiolytic effect: NMDA-receptor antagonists prolonged the time spent in open arms of elevated plus maze [14]. Our data attest to anxiolytic effect of anti-GLU AB.

Published data suggest that excitatory amino acid receptors in the hippocampus and neocortex participate in learning and memory processes [5,7]. Specifically, acquisition of a conditioned defense reflex was accompanied by an increase in the number of ^{3}H-GLU-labeled hippocampal and neocortical receptors [10]. GLU participates in long-term postsynaptic potentiation, which is directly related to the development of long-term memory [4]. Moreover, GLU plays a key role in the control of memory trace retention [10]. Antagonists of some glutamate receptors (metabotropic, NMDA, and to a lesser degree AMPA receptors) disturb consolidation of memory traces [4,12].

Some high-molecular-weight substances, in particular, antibodies can penetrate through the blood-brain barrier and enter CNS in small amount sufficient to modify its functional activity. Therefore, the revealed disturbance in CPAR retention in C75Bl/6 mice on days 1 and 7 can be related to inhibition of the glutamatergic system caused by GLU binding with anti-GLU AB, interaction of GLU—AB complex with glutamate receptors, and subsequent modulation of their functional activity or density. It can also be related to changes in GLU—GABA balance. The latter hypothesis is based on the fact that GABA and GLU metabolic pathways are closely interconnected; it is also known that GABA and GABAergic substances produce an amnestic effect that can be abolished by GABA antagonists [5].

Restoration of CPAR retention time in immunized C57Bl/6 mice on day 14 probably attests to high adaptive properties and higher stability of nervous processes in this strain. This is also indicated by the following fact found in the detailed individual analysis: repeated tests showed that CPAR retention was more stable in control C57Bl/6 mice (50%) compared to control BALB/c mice (30%).

Thus, immunocorrection decreased anxiety and improved CPAR retention in more "vulnerable" BALB/c mice characterized by passive behavior and high anxiety. It cannot be excluded that anti-GLU AB produce a stress-protective effect in stress-sensitive BALB/c mice, because CPAR acquisition is a stress factor [8].

We previously showed that C57Bl/6 and BALB/c mice differ significantly in monoamine content and functional activity of monoamine receptors in CNS. Immunization with serotonin-BSA conjugate produced different changes in these indices in C57Bl/6 and BALB/c mice [1]. The opposite changes in anxiety and memory indices observed in this study can be related to genetically determined differences in activity of cerebral glutamatergic systems in mice of various strains.

Thus, we demonstrated a possibility of modulating anxiety and memory with anti-GLU AB. Immunization induced opposite changes in mice of various strains, and these changes were more stable in BALB/c mice. In C57Bl/6 mice there was a tendency to restore memory and anxiety indices to the control level.

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