Immunobiological Specificity of Antibodies against Glutamate and γ-Aminobutyric Acid

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Experiments on C57Bl/6 mice showed that chronic epileptization of the brain (pharmacological pentylenetetrazole kindling) is accompanied by induction of autoantibodies against glutamate and GABA. Immunohistochemical similarity of antibodies against glutamate and GABA was demonstrated. Study on the model of pentylenetetrazole-induced acute generalized epileptiform activity showed high immunobiological specificity of intraperitoneally injected antibodies against GABA and glutamate: antiepileptic effect of antiglutamate antibodies and proepileptic effect of antibodies against GABA.

Key Words: *antibodies; glutamate; GABA; acute epileptiform activity; kindling*

Numerous studies proved a crucial role of glutamate (Glu) and the imbalance between glutamatergic and GABAergic processes in the pathogenesis of epilepsy [4,7,8,14]. Study of experimental epilepsy and observation of epileptic patients revealed production of autoantibodies (autoAB) against glutamate NMDA and AMPA receptors [10,14] and glutamate decarboxylase [11]. However, production and role of autoAB against Glu and GABA in the development of neuropathology remain unknown. AB against linear amino acids Glu and GABA were first obtained by immunization of rabbits with antigens Glu and GABA conjugated to a protein carrier (BSA). They were used in a variety of immunocytochemical studies, including visualization of Glu and GABA in the brain and intestine [9,12].

Here we studied the synthesis of autoAB against Glu and GABA during chronic epileptization of the brain (pharmacological kindling). Immunochemical and immunobiological specificity of these autoAB was evaluated.

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MATERIALS ABD METHODS

Two series of experiments were performed on male C57Bl/6 mice. Series I involved 44 mice weighing 11-15 g. The synthesis and immunospecificity of autoAB against Glu, GABA, and protein carrier (BSA) were studied during kindling. Kindling was induced by intraperitoneal injection of pentylene tetrazole (PTZ) in a daily subconvulsive dose of 30 mg/kg for 24 days. The seizure response of each animal to the convulsant was evaluated daily and scored using the standard scale. Ten animals were decapitated on day 14 of kindling and others on day 24. Blood samples were taken to measure the concentration of autoAB against neurotransmitters. Control mice (n=10) received intraperitoneal injections of physiological saline. The concentration of autoAB to Glu and GABA in blood plasma was measured by enzyme-linked immunosorbent assay (ELISA). The test antigens Glu-BSA and GABA-BSA were synthesized using glutaraldehyde [12]. The concentration of autoAB was estimated from optical density of blood plasma. The measurements were performed on a Mini-Reader device (Dynateck) at 495 nm. The concentration of autoAB was expressed in arbitrary units of activity (K) cal-

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culated as the ratio of optical density of the plasma from each treated mouse to the mean optical density of control samples. Plasma dilution was 1:30. The presence of autoAB was verified by K>1.

Immunochemical specificity of autoAB was studied in the reaction of competitive inhibition. AB were inhibited using Glu, GABA, Glu-BSA, and GABA-BSA in a concentration of 1 mg/ml.

Series II was performed on 133 mice weighing 23-27 g. The effect of anti-GABA AB was studied on the model of acute generalized epileptiform activity induced by intravenous injection of PTZ. AB against GABA were obtained by hyperimmunization of Chinchilla rabbits with GABA-BSA conjugate. The concentration of AB against GABA in blood plasma from immunized rabbits was measured by ELISA. The conjugate on a heterologous protein carrier (horse γ-globulin) served as the test antigen. The mean titer of AB against GABA was 1:1024. The γ-globulin fraction of AB was isolated from plasma samples of immunized rabbits by reprecipitation with ammonium sulfate. They were purified from AB against BSA by affinity chromatography on BrCN-activated Sepharose-4B with immobilized BSA (immunosorbent), lyophilized, and stored at 4°C. The γ-globulin fraction from blood plasma of intact rabbits was isolated similarly. AB in doses of 10 and 25 mg/kg were injected intraperitoneally 1.5 h before the induction of acute generalized epileptiform activity. We evaluated the threshold dose of PTZ inducings clonic seizures and tonic phase of seizures with lethal outcome. Control animals received intraperitoneal injections of physiological saline.

The results were analyzed by Student's *t* test (SWP4 and Primer software) and Fischer test.

RESULTS

Series I with chronic epileptization of the brain (kindling) showed that repeated injection of PTZ induces seizures in mice. The severity of seizures progressively increased. After 14 days, seizures of 3.08±0.08 points were detected in 46.51% animals. The production of autoAB against neurotransmitters increased in this period. Anti-Glu autoAB were found in all treated mice (Fig. 1, *a*); mean content 1.46±0.09 arb. units (Fig. 1, *b*). Anti-GABA autoAB were revealed in 60% mice; mean content 1.46± 0.16 arb. units (Fig. 1, *a*, *b*). It can be hypothesized that during this period, Glu (one of the seizure-inducing agents) intensively formed endogenous Glu—protein conjugates activating the synthesis of anti-Glu AB.

By the end of kindling (after 24-days PTZ treatment), the number of animals with convulsant-induced seizures increased to 75%. The severity of seizures scored 3.07±0.35. The incidence of autoAB against Glu decreased to 57.8% ($p<0.001$), but the incidence and concentration of autoAB against GABA remained unchanged. These specific features probably contributed to an increase in the signs of experimental epilepsy. We hypothesized that kindling is accompanied by increased synthesis of anti-albumin autoAB (mouse serum albumin). These autoAB cross-react with the protein carrier in conjugates (BSA) during ELISA. Plasma samples from mice with 24-day corasol kindling were tested for the presence of autoAB against BSA. In plasma samples from treated and control mice K was 1.08±0.07 and 1.00±0.08 arb. units, respectively. These data illustrate the absence of autoAB against BSA. No correlation was found between the concentration of autoAB against Glu and GABA and

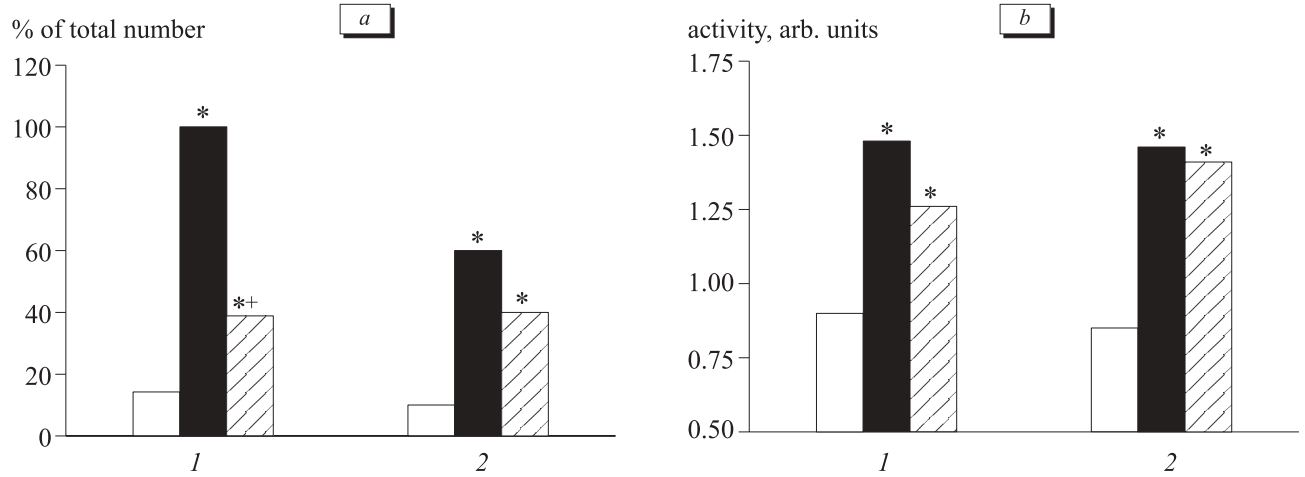

Fig. 1. Incidence of detection (*a*) and concentration (*b*) or autoAB against GABA and Glu in the dynamics of chronic epileptization of the brain (corasol kindling). autoAB against Glu (*1*), autoAB against GABA (*2*). Light bars, control; dark bars, 14 days after kindling; shaded bars, after 24 days. $^{*}p<0.001$ compared to the control; $^{+}p<0.01$ compared to the previous period.

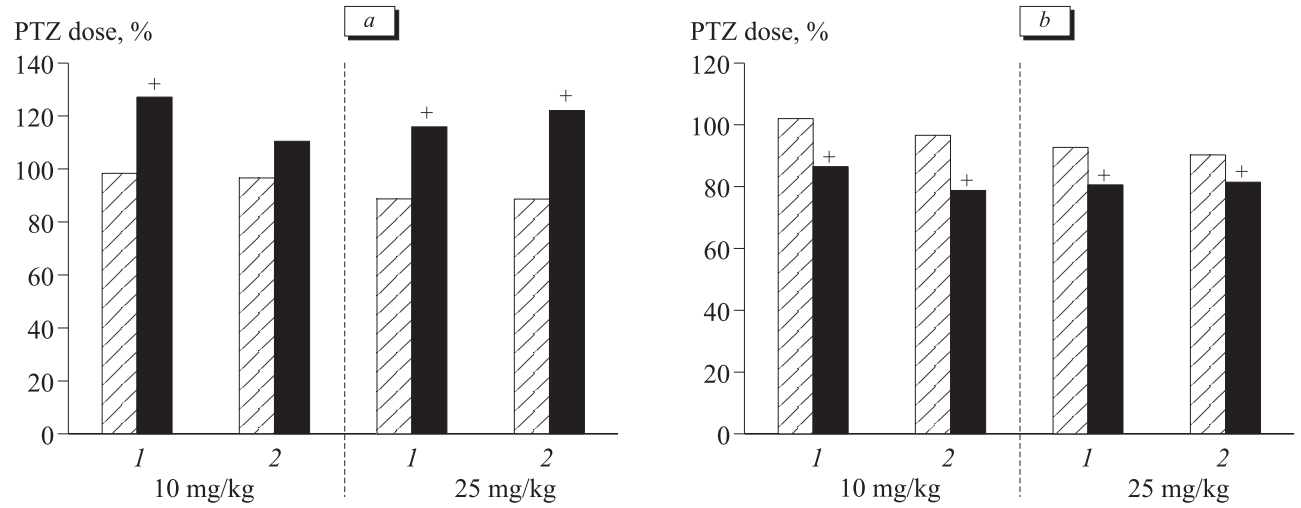

Fig. 2. Seizure thresholds in C57Bl/6 mice 1.5 h after administration of AB against Glu (*a*) and GABA (*b*). PTZ-induced clonic seizures (*1*); tonic seizures with lethal outcome (*2*). 100%, PTZ dose in control animals. Dark bars, administration of AB against Glu (*a*) and GABA (*b*); shaded bars, γ-globulin from intact rabbit. $^{+}p<0.01$ compared to γ-globulin from intact rabbit.

severity of seizures. We studied whether autoAB against Glu and GABA play a role in the mechanisms of kindling. Immunochemical and immunobiological specificity of AB against neurotransmitters was estimated. The study for immunochemical specificity of autoAB against Glu and GABA showed that AB against Glu and GABA do not bind free neurotransmitter during competitive inhibition. However, they were suppressed by Glu and GABA bound to the protein carrier (Glu-BSA and GABA-BSA). It should be emphasized that AB against Glu cross-reacted with GABA, while AB against GABA bound Glu (Table 1).

Our previous studies showed that AB against Glu induced by active immunization of mice with the Glu-BSA antigen conjugate have an antiepileptic effect under conditions of PTZ-induced acute generalized seizures. It was manifested in an increase in the threshold for clonic seizures and tonic phase of seizures with lethal outcome [3]. Single intraperitoneal injection of AB against Glu had a similar effect on acute generalized epileptiform activity in mice (Fig. 2, *a*) [5].

TABLE 1. Specificity of autoAB against Glu and GABA in Kindled C57Bl/6 Mice

Inhibitory substance, 1 mg/ml	AutoAB against Glu	AutoAB against GABA
Glu	0	0
Glu-BSA	49.2	28.6
GABA	0	0
GABA-BSA	36.1	44.7

In series II we studied the effect of AB against GABA on acute generalized seizures. Pretreatment with AB against GABA in doses of 10 and 25 mg/kg decreased the seizure threshold for clonic seizures and tonic seizures with lethal outcome (Fig. 2, *b*). Hence, AB against GABA produce a proepileptic effect. Previous studies on the model of pathological pain showed that intrathecal application of AB against GABA (lumbar spinal cord, L_{IV}-L_{VI}) has a similar reciprocal effect. AB against GABA caused mechanical allodynia in rats, while AB against Glu significantly decreased the severity of spontaneous attacks of central pain syndrome induced by application of a penicillin plate to the dorsal surface of the spinal cord [4].

Our results indicate that chronic epileptization of the brain is accompanied by increased synthesis of autoAB to Glu and GABA. The reaction of competitive inhibition in ELISA showed that autoAB against Glu and GABA cross-react with the competitive neurotransmitter. Despite immunochemical similarity of AB, experiments on the model of acute generalized epileptiform activity demonstrated high immunobiological specificity of these AB. AB against Glu and GABA had the antiepileptic and proepileptic effects, respectively. AB against Glu and GABA differ in high selectivity in biological tests and may be considered as neurotransmitter antagonists. This phenomenon is probably associated with molecular features of ligand-receptor interactions.

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