Neuroimmunomodulatory Effect of Antibodies Against GABA on Acute Generalized and Chronic Epileptiform Activity

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 11, pp. 505-508, November, 2006
Original article submitted January 13, 2006

We studied the possibility of induction of anti-GABA autoantibodies during chronic epileptization of the brain and the effect of systemic intraperitoneal administration of anti-GABA antibodies to C57Bl/6 mice on acute generalized and chronic epileptiform activity. It was found for the first time that chronic epileptization is accompanied by induction of anti-GABA autoantibody synthesis. Antibodies against GABA produced a proepileptic effect.

Key Words: *epilepsy; kindling, autoantibodies; antibodies against GABA*

The important role of immunological mechanisms in the pathogenesis of epilepsy was demonstrated in numerous studies [1,2]. The imbalance between glutamatergic and GABAergic processes are the key elements in the pathogenesis of epilepsy [8,9, 14], which is confirmed by the presence of autoantibodies (AAB) against NMDA and AMPA receptors for glutamate in the serum and cerebrospinal fluid of patients with epilepsy [11,12,14]. However, it remains unclear whether epilepsy is accompanied by the appearance of AAB against inhibitory amino acids and their receptors.

Here we studied the possibility of induction of anti-GABA AAB synthesis during chronic epileptization of the brain (pharmacological kindling). The effect of systemic intraperitoneal treatment with antibodies (AB) against GABA on acute generalized and chronic epileptiform activity was studied in C57Bl/6 mice.

MATERIALS AND METHODS

Experiments were performed on 227 male C57Bl/6 mice. Series I was performed on 34 mice weighing 11-15 g. Serum concentration of anti-GABA AAB was measured during chronic epileptization of the brain (pharmacological kindling). The process of kindling suggests that repeated administration of a convulsant in subconvulsive doses is followed by a progressive increase in seizure readiness of the brain. These changes include the development and increase in the severity of seizures after subsequent administration of the convulsant. Phenomenologically, pharmacological kindling manifests in a decrease of seizure threshold in response to the convulsant in the test dose. 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 to PTZ in each animal was evaluated daily and expressed in points using the standard scale. The animals were decapitated to take blood samples on days 14 and 24 of kindling. Serum concentration of anti-GABA AAB was measured by ELISA using 96-well polystyrene plates sensitized with the test antigen GABA-bovine serum albumin (BSA) synthesized using bifunctional reagent glutaraldehyde [12]. AAB concentration was estimated by optical density of the serum measured on a Mini-Reader reader (Dynateck) at 495 nm. AAB concentration was expressed in arbitrary units of activity (arb. units) and the ratio of optical

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0007-4888/06/1425-0562

density of the plasma from each treated mouse to the mean optical density of control samples was calculated (K). Plasma dilution was 1:30. The presence of AAB was verified by K>1.

In series II (133 mice, body weight 23-27 g) we studied the effect of intraperitoneal pretreatment with anti-GABA AB in doses of 10 and 25 mg/kg on acute generalized epileptiform activity. The thresholds for clonic seizures and tonic phase of seizures with lethal outcome were determined. PTZ (1%) was infused intravenously at a rate of 0.01 ml/sec. The seizure-inducing dose of PTZ was estimated individually for each animal (mg/kg). Control mice received physiological saline and γ-globulin from intact animals.

The drugs were administered 1.5 h before PTZ injection. AB against GABA were obtained by hyperimmunization of rabbits with the GABA-BSA conjugate. Serum AB titer in immunized animals was measured by ELISA (1:2000-1:4000). γ-Globulin fractions of AB isolated from blood serum by precipitation with ammonium sulfate were dialyzed, lyophilized, and stored at 4°C. The γ-globulin fraction was also isolated from intact nonimmunized rabbits.

Series III was performed on 60 mice weighing 11-15 g. The effect of systemic intraperitoneal treatment with AB against GABA was studied on the model of chronic epileptization of the brain (kindling). The drugs in a dose of 25 mg/kg were administered 1.5 h before the 1st and 15th injections of PTZ (before kindling and during epileptiform activity).

The results were analyzed by parametric and nonparametric tests (Student's test and Fischer test).

RESULTS

In series I, the synthesis of anti-GABA AAB was studied during chronic epileptization of the brain. Anti-GABA AAB in 60% mice were detected during the initial increase in seizure readiness of the brain (daily injections of PTZ for 14 days). The concentration of AAB was 1.46±0.16 arb. units. By the end of kindling (24-day treatment with PTZ), anti-GABA AAB were found in 40% animals (1.41± 0.06 arb. units). No correlation was revealed between the concentration of anti-GABA AAB and severity of seizures under these experimental conditions.

Series II showed that intraperitoneal injection of AB against GABA in a dose of 10 mg/kg decreased the threshold of clonic and tonic seizures with lethal outcome (by 13.55 and 21.24%, respectively, compared to control animals receiving physiological saline, Fig. 1). Administration of AB against GABA in a dose of 25 mg/kg did not potentiate a decrease in the seizure threshold. The threshold of clonic and tonic seizures in these mice was lower than in control animals by 19.42 and 18.59%, respectively. Pretreatment with γ-globulin in doses of 10 and 25 mg/kg had no effect on the seizure threshold. Hence, AB against GABA have a proepileptic effect on acute generalized PTZ-induced seizures. These AB decrease the threshold of clonic and tonic seizures with lethal outcome.

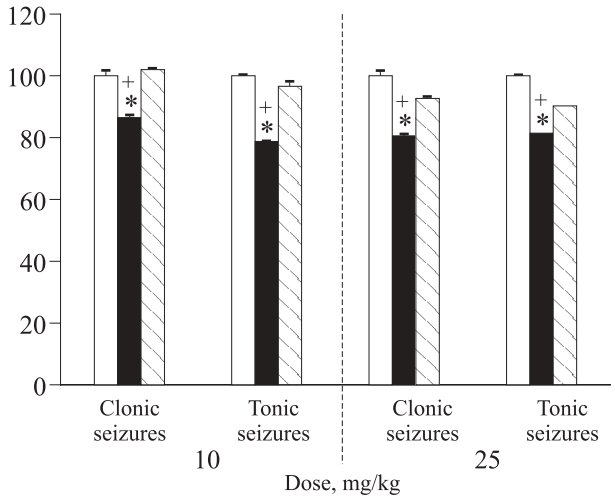

Fig. 1. Seizure threshold in mice 1.5 h after administration of anti-GABA AB and normal γ-globulin. Ordinate: dose of PTZ inducing clonic and tonic seizures (% of the control). Light bars, control; dark bars, AB against GABA; shaded bars, γ-globulin. $p<0.001$: *compared to the control (physiological saline); +compared to γ-globulin.

Series III was performed to study the increase in seizure readiness of the brain during kindling. Pretreatment with AB against GABA (1.5 h before administration of PTZ in a test dose) accelerated the development of 1-point seizures by 1-2 days. Under these conditions the number of animals with seizures was 2-fold higher compared to the control (Fig. 2). A similar effect was observed in mice receiving γ-globulin. The severity of seizures and number of animals with seizures progressively increased during kindling. After 14-day treatment with PTZ in the test dose, seizures developed in 90% mice receiving AB against GABA, 46% control animals, and 50% specimens of the γ-globulin group (Fig. 2).

After repeated administration of anti-GABA AB during epileptiform activity (day 15 of kindling), the number of animals with seizures was higher compared to the control (Fig. 2). Five-point seizures in 20% mice were detected after repeated treatment with anti-GABA AB. These seizures were not found in animals of other groups. Kindling induced the development of seizures in all animals

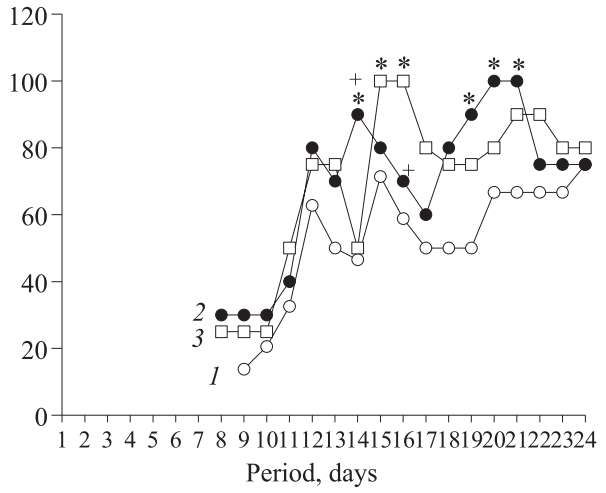

Fig. 2. Effect of AB against GABA on increase in seizure readiness of the brain. Ordinate: number of animals with seizures (%). Control animals (*1*); animals receiving AB against GABA (*2*); animals of the normal γ-globulin group. $p<0.05$: *compared to the control (physiological saline); +compared to γ-globulin.

receiving AB or γ-globulin, as well as in 75% control mice. Therefore, AB against GABA modulate chronic epileptiform activity. The influence of γ-globulin on the increase in seizure readiness of the brain in some animals was similar but less significant compared to that of AB. Desynchronization and single short-term high-amplitude discharges on the electrocorticogram were revealed after intracortical administration of 10 μg γ-globulin. This effect of γ-globulin is probably associated with the ability of immunoglobulins to bind nonspecifically some neurotransmitters [5].

We showed for the first time that chronic epileptization of the brain is accompanied by induction of anti-GABA AAB synthesis. The absence of correlation between the concentration of anti-GABA AAB and severity of seizures in animals can be explained by experimental conditions and individual features of antibody production and epileptogenesis.

Systemic administration of AB against GABA has a proepileptic effect on acute and generalized epileptiform activity. The convulsant effect of PTZ is associated with a decrease in Cl^- conductance of the $GABA_A$-receptor complex (*i.e.*, impairment of inhibitory GABAergic mechanisms). After systemic administration, AB against neurotransmitters can cross the blood-brain barrier. The amount of these antibodies is small but sufficient to modulate functional activity [3]. AB permeate the central nervous system and bind GABA, which results in inactivation of the GABAergic system. These changes probably contribute to the proepileptic effect of AB against GABA. Previous studies on the model of pathological pain showed that intrathecal application of AB against GABA to the dorsal surface of the lumbar region in the spinal cord has a modulatory effect on GABAergic mechanisms in rats [6]. Our results and published data [4,7] indicate that the neuroimmunopathogenesis of epilepsy should be studied in details.

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