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Effect of Systemic Treatment with Antibodies against Glutamate on the Seizure Response of C57Bl/6 Mice after Pentylenetetrazole Kindling

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We studied the effect of single intraperitoneal injection of antibodies against glutamate on acute generalized epileptiform activity induced by intravenous injection of pentylenetetrazole in kindled C57Bl/6 mice. We found that in animals at various stages of kindling, administration of antibodies against glutamate in a dose of 25 mg/kg 1.5 h before testing increases the threshold of clonic seizures and tonic phase of seizures with lethal outcome and latency of seizure development, *i.e.* produces an antiepileptic effect.

Key Words: *glutamate; antibodies; kindling; pentylenetetrazole; seizures*

The pathogenesis of epilepsy is characterized by multifactor nature and involvement of various organs and systems into dysregulation pathology. Epileptogenesis is associated with dysregulation of the inhibitory and excitatory systems in the brain (*e.g.* glutamatergic system), which results in predominance of excitation over inhibition [1,7,10]. Neuroimmune processes also play a role in epileptization of neurons [1-3,8]. Our previous experiments showed that antibodies (AB) against glutamate have an immunocorrecting effect on acute epileptiform activity after immunization with glutamate conjugated with bovine serum albumin (BSA) and systemic administration of AB [5,6]. AB against glutamate had an antiepileptic effect and increased the threshold for clonic seizures and tonic phase of seizures with lethal outcome. Here we studied the effect of systemic treatment with AB against glutamate on chronic epileptiform activity. Pharmacological kindling was used as an adequate model of progressive chronic epileptization of the brain [7,10].

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MATERIALS AND METHODS

Experiments were performed on 110 male C57Bl/6 mice weighing 18-24 g.

AB against glutamate were obtained by hyperimmunization of Chinchilla rabbits with glutamate-BSA conjugate synthesized using a bifunctional reagent glutaraldehyde [11]. Plasma concentration of antiglutamate AB in immunized rabbits was measured by ELISA using a conjugate on a heterologous protein carrier (equine γ-globulin) as the test antigen. The titer of AB was 1:1024. AB (γ-globulin fraction) were isolated from plasma samples by ammonium sulfate precipitation, purified from AB against BSA (protein carrier) by affinity chromatography on BrCN-activated Sepharose 4B with immobilized BSA, lyophilized, and stored at 4°C.

The γ-globulin fraction of blood plasma from intact nonimmunized rabbits was isolated similarly. AB against glutamate were absent in plasma samples of control rabbits.

Pharmacological kindling was induced by intraperitoneal injection of pentylenetetrazole (PTZ) in a daily subconvulsive dose of 30 mg/kg for 28 days. The process of kindling suggests that repeated administration of the 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. Kindling manifests in a decrease in seizure threshold in response to the testing dose of the convulsant. The seizure response to PTZ in each animal was evaluated daily and scored using a standard 5-point scale.

Seizure threshold in PTZ-kindled mice was evaluated on the model of acute generalized epileptiform activity. The solution of PTZ (1%) was infused intravenously at a flow rate of 0.01 ml/sec. We measured the threshold for clonic seizures and tonic phase of seizures with lethal outcome. The threshold seizure-inducing dose of PTZ was estimated individually for each animal (mg/kg).

The study was performed in 2 series. Seizure thresholds were measured 1 day after the 14th (series I) and 28th injection of PTZ (series II). Each series involved 3 groups of animals. Control animals of groups 1 and 2 received physiological saline and intact γ-globulin, respectively. AB against glutamate were administered to group 3 animals. AB and γ-globulin (25 mg/kg by protein content) were dissolved in physiological saline and injected intraperitoneally in a single dose 0.1 ml/10 g body weight 1.5 h before the study. The dose of the preparations and time of treatment were selected on the basis of our previous results [5]. Control animals of group 1 received an equivalent volume of physiological saline.

The significance of differences was estimated by Student's *t* test.

RESULTS

In series I, daily administration of the convulsant PTZ induces seizures (severity score 2-3). In group 3 animals, pretreatment with AB against glutamate increased the threshold doze of PTZ for the development of clonic seizures (by 50.53 and 40.63%) and tonic phase of seizures with lethal outcome (by 51.47 and 52.13%) compared to controls receiving physiological saline and γ-globulin, respectively (Table 1). γ-Globulin had no effect on the threshold of clonic and tonic seizures: this parameter was similar in groups 1 and 2.

In series II, daily injections of PTZ led to a progressive increase in the severity of seizures (severity score 5); 50% animals with 5-point seizures died. Seizure threshold was measured in animals with 4-5-point seizures. Similarly to series I, administration of AB against glutamate was followed by an increase in seizure threshold: the threshold of clonic seizure in treated mice was higher that that in group 1 and 2 animals by 20.66 and 13.37%, respectively, and the threshold for tonic phase of seizures by 30.51 and 24.90%, respectively (Table

TABLE 1. Seizure Threshold in Mice Subjected to 14 Day-Kindling after Administration of Preparations (*M*±*m*)

Group	Number of animals	Seizure-inducing dose of PTZ, mg/kg	
		clonic	tonic
1 (physiological saline)	11	27.11±0.95	48.49±1.51
2 (γ-globulin)	9	29.02±2.07	48.29±3.99
3 (AB against glutamate)	9	40.81±0.85*+	73.45±5.44*+

Note. Here and in Table 2: $p<0.001$: *compared to group 1; +compared to group 2.

TABLE 2. Seizure Threshold in Mice Subjected to Kindling over 28 Days after Administration of Preparations (*M*±*m*)

Group	Number of animals	Seizure-inducing dose of PTZ, mg/kg	
		clonic	tonic
1 (physiological saline)	16	29.87±0.95	49.19±1.89
2 (γ-globulin)	16	31.79±1.02	51.40±2.32
3 (AB against glutamate)	22	36.04±1.08*+	64.20±1.82*+

2). Similarly to series I, γ-globulin had no effect on the threshold of clonic and tonic phases of seizures (compared to group 1 animals).

The elevation of seizure threshold means the increase in the doze of PTZ inducing clonic and tonic seizures. It can be suggested that the latency of seizures also increases, because the convulsant was administered at a constant flow rate. The latency of seizures in group 1 and 2 animals was 4.61±0.16 and 4.50± 70.19 sec, respectively. This parameter in group 3 mice was 5.73±0.23 sec ($p<0.001$ compared to control groups). The latency of tonic seizures with lethal outcome in group 1 and 2 animals was 7.83± 0.54 and 7.69±0.42 sec, respectively. This parameter in group 3 mice was 11.18±0.80 sec ($p<0.001$ compared to control groups).

Our results suggest that AB against glutamate have an antiepileptic effect under conditions of chronic epileptization of the brain. Kindling, a process of progressive increase in seizure readiness of the brain, proceeds in several stages. These stages are arbitrary, but each of them has specific features due to changes that develop at the previous stage. At the early stage of kindling (in the absence of seizures) the pathological process of epileptogenesis is preceded by activation of protective sanogenetic mechanisms (antiepileptic processes). Seizures do not develop when these processes are in the active state [7,9]. Antiepileptic mechanisms are suppressed, while the intensity of proepileptic processes increases during the intermediate and final stage of kindling. This period is characterized by the development and increase in seizure severity [7]. The results of our study show that systemic pretreatment with AB against glutamate has an antiepileptic effect at the intermediate and final stage of chronic epileptization of the brain. They increase the threshold for clonic seizures and tonic phase of seizures with lethal outcome and latency of seizure development. Our previous experiments demonstrated that AB against neurotransmitters after systemic administration cross the blood-brain barrier and enter the central nervous system (CNS) in amounts sufficient for modulation of functional activity of the nervous system [4]. Hence, AB against glutamate after systemic administration can cross the blood-brain barrier that is damaged during kindling. The amount of these AB is sufficient to decrease activity of the glutamatergic system due to binding of glutamate with antiglutamate AB in the brain and action of the antigen-antibody complex on glutamate receptors. The latter assumption requires further investigations.

We conclude that neuroimmunomodulation with AB against neurotransmitters involved in the pathogenesis of the disease can modulate activity of the pathological epileptic system. The role of neuroimmune processes in the pathogenesis of epilepsy should be studied in details.

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