Systemic Administration of Antibodies to Glutamate Increases Seizure Threshold for Pentylenetetrazole

V. A. Evseev, M. N. Karpova, L. A. Vetrile, N. Yu. Klishina, L. V. Kuznetsova, and N. A. Trekova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 9, pp. 276-278, September, 2005
Original article submitted September 16, 2004

We studied the effect of single intraperitoneal treatment with antibodies to glutamate on pentylenetetrazole-induced acute generalized epileptiform activity in C57Bl/6 mice. The antiepileptic effect was observed 1.5 and 24 h after administration of antibodies to glutamate in doses of 10, 25, and 50 mg/kg. This treatment increased the thresholds of clonic seizures and tonic phase of seizures with lethal outcome.

Key Words: *glutamate; antibodies; epileptiform activity; seizures; pentylenetetrazole*

An immunocorrecting effect of antibodies (AB) to glutamate (Glu) on acute epileptiform activity was revealed during active immunization of mice with Glu-bovine serum albumin (Glu-BSA) conjugate [3]. Immunization increased the thresholds of clonic seizures and tonic phase of seizures with lethal outcome. A principal possibility of modulating mouse behavior with anti-Glu AB during active immunization with Glu-BSA conjugate and single systemic administration was demonstrated [1,4,5].

Here we studied whether passive immunization of mice with anti-Glu AB can prevent the development of pentylenetetrazole-induced (PTZ) acute generalized epileptiform activity in C57Bl/6 mice.

MATERIALS AND METHODS

Experiments were performed on 176 male C57Bl/6 mice weighing 20-28 g. The effect of systemic treatment with anti-Glu AB was studied on the model of acute generalized epileptiform activity [7]. We measured the thresholds of clonic seizures and tonic phase of seizures with lethal outcome. PTZ (0.1%) was infused intravenously (0.01 ml/sec infusion rate). The threshold dose of PTZ producing seizures was determined individually and expressed in mg/kg.

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

Anti-Glu AB were obtained by hyperimmunization of Chinchilla rabbits with Glu-BSA conjugate. It was synthesized using a bifunctional reagent glutaraldehyde [10]. The titer of anti-Glu AB in sera from immunized rabbits was estimated by solid-phase enzyme immunoassay (1:1024). The conjugate bound to heterologous protein carrier (equine γ-globulin) served as a test antigen. The fraction of γ-globulins was isolated from sera of immunized animals by the method of precipitation with ammonium sulfate, purified by dialysis, lyophilized, and stored at 4°C.

Series I was conducted to estimate the optimal dose of AB. The dose-dependent effect of anti-Glu AB on seizure threshold in mice was studied 1.5 h after treatment. AB were injected intraperitoneally (0.2 ml per 20 g body weight) in doses of 10, 25, and 50 mg/kg (by protein content). Control animals received physiological saline in an equivalent dose. In series II epileptogenesis was studied 1.5 and 24 h after administration of anti-Glu AB in the optimal dose. Control animals were divided into 2 groups and received nonimmune γ-globulin in the same dose or physiological saline.

RESULTS

Intraperitoneal injection of anti-Glu AB suppressed PTZ-induced acute generalized epileptiform activity and increased the thresholds of clonic seizures and

0007-4888/05/1403-0295

tonic phase of seizures with lethal outcome (Fig. 1). Administration of anti-Glu AB in a concentration of 10 mg/kg increased the dose of PTZ causing clonic seizures and tonic phase of seizures with lethal outcome by 27.1 ($p<0.001$) and 10.5% ($p<0.05$), respectively, compared to the control. Seizure thresholds increased by 47.4 ($p<0.001$) and 15.4% ($p<0.02$), respectively, after treatment with anti-Glu AB in a dose of 25 mg/kg. However, administration of anti-Glu AB in a dose of 50 mg/kg was not accompanied by a further increase in seizure thresholds. After administration of anti-Glu AB in a dose of 50 mg/kg the threshold of clonic seizures was 19.2% higher than in the control. However, this parameter was lower than in animals receiving anti-Glu AB in doses of 10 and 25 mg/kg. Under these conditions the threshold of tonic seizures did not differ from that in control animals, but was lower than in mice immunized with antibodies in doses of 10 and 25 mg/kg (by 9.8 and 13.6%, respectively). Our results indicate that the optimal dose of anti-Glu AB increasing seizure thresholds is 25 mg/kg. Lower efficiency of anti-Glu AB in a dose of 50 mg/kg is probably associated with physiological characteristics of this neurotransmitter. The effect of Glu is determined by the transmitter/receptor ratio.

Series II showed that the threshold dose of PTZ inducing clonic and tonic seizures increased after 1.5-h exposure to AB (by 15.9 and 22%, respectively, compared to animals receiving physiological saline, Table 1). Treatment with nonimmune γ-globulin in the same dose produced an opposite effect. The threshold of tonic seizures decreased by 11.4% (compared to physiological saline), while the threshold of clonic seizures remained unchanged under these conditions. The effect of anti-Glu AB persisted for 24 h. During this period the thresholds of clonic and tonic seizures remained above the control (by 13.9 and 24.3%, respectively). Nonimmune γ-globulin in a dose of 25 mg/kg decreased the threshold of tonic seizures (by 11.9%), but had no effect on the threshold of clonic seizures.

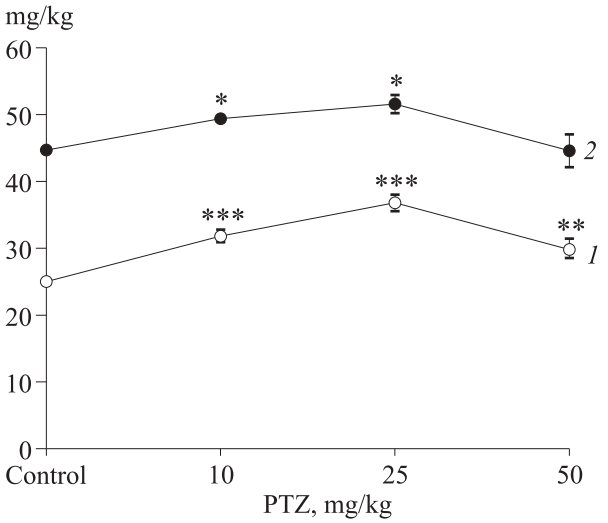

Fig. 1. Dose-dependent effect of antibodies to glutamate on the threshold of pentylenetetrazole-induced (PTZ) seizures in C57Bl/6 mice: clonic seizures (*1*) and tonic seizures with lethal outcome (*2*). *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control.

Our findings suggest that anti-Glu AB produce an antiepileptic effect, prevent PTZ-induced acute generalized seizures, and increase the thresholds of clonic seizures and tonic phase of seizures with lethal outcome. Similar results were obtained during active immunization of mice with Glu-BSA conjugate [3]. Autoantibodies to Glu probably play a protective role in the pathogenesis of epilepsy. There is no direct evidence that anti-Glu AB are synthesized during epilepsy. Clinical, immunological, and experimental observations indicate that AB to the R3-B fragment of NMDA receptors play a role in the pathogenesis of epilepsy [6,8,9]. We hypothesized that production of autoantibodies can mask the presence of anti-Glu AB [2], which probably determines a recurrent course of

TABLE 1. Seizure Thresholds in Mice 1.5 and 24 h after Administration of γ-Globulin and anti-Glu AB in a Dose of 25 mg/kg ($M\pm m$)

Group		Number of animals	PTZ dose			
			clonic seizures		lethal outcome	
			mg/kg	%	mg/kg	%
After 1.5 h	control (physiological saline)	14	25.08±0.96	100	35.69±0.95	100
	control (γ-globulin)	14	22.25±0.74	88.72	31.62±0.86**	88.60
	experiment (anti-Glu AB)	13	29.06±1.27***+	115.87	43.56±2.39**+	122.05
After 24 h	control (physiological saline)	15	26.09±0.94	100	46.31±1.49	100
	control (γ-globulin)	13	25.55±0.92	97.90	40.80±1.40*	88.10
	experiment (anti-Glu AB)	14	29.72±0.62**+	113.91	57.58±1.89***+	124.34

Note. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control (physiological saline); +$p<0.001$ compared to the control (γ-globulin).

this disease. The present study support our assumption. However, this problem requires further clinical studies.

REFERENCES

1. L. A. Vetrile, L. A. Basharova, O. I. Mikoskaya, *et al*., *Byull. Eksp. Biol. Med.*, **133**, No. 3, 274-277 (2002).
2. V. A. Evseev, L. A. Vetrile, and M. A. Karpova, *Neiroimmunologiya*, **2**, No. 1, 9-11 (2004).
3. M. A. Karpova, L. A. Vetrile, N. Yu. Klishina, *et al*., *Byull. Eksp. Biol. Med*., **136**, No. 9, 287-289 (2003).
4. N. A. Trekova and L. A. Vetrile, *Zh. Vyssh. Nervn. Deyat*., **5**, No. 3, 405-409 (2004).
5. N. A. Trekova, O. I. Mikovskaya, L. A. Basharova, *et al*., *Byull. Eksp. Biol. Med*., **133**, No. 2, 140-143 (2002).
6. N. G. Carlson, L. C. Gahring, R. E. Twyman, and S. W. Rogers, *J. Biol. Chem*., **272**, No. 17, 1295-1301 (1997).
7. R. S. Fisher, *Brain Res. Brain Res. Dev*., **14**, 245-278 (1989).
8. M. Levite, I. A. Fliedervish, A. Schwarz, *et al*., *J. Autoimmun*., **13**, No. 1, 61-72 (1999).
9. S. W. Rogers, R. E. Twyman, and L. C. Gahring, *Mol. Med. Today*, **2**, No. 2, 76-81 (1996).
10. P. Seguela, M. Geffard, R. Buijs, and M. Le Moal, *Proc. Natl. Acad. Sci. USA*, **81**, No. 12, 3888-3892 (1984).