Elevation of Corazol-Induced Seizure Threshold after Active Immunization of Mice of Various Genetic Strains with Glutamate-Bovine Serum Albumin Conjugate

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

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 9, pp. 287-289, September, 2003
Original article submitted March 21, 2003

We studied the effect of active immunization with glutamate-bovine serum albumin conjugate on acute generalized epileptiform activity evoked by single administration of corazol. Antibodies to glutamate produced an antiepileptic effect and elevated the threshold for clonic seizures and tonic phase of lethal seizures. In BALB/c mice this effect was more pronounced than in C57BL/6 mice.

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

Glutamate (L-glutamic acid, Glu) is one of the major excitatory neurotransmitters in CNS. Excitatory neurotransmitter amino acids and their receptors play a role in the pathogenesis of epilepsy, seizures, and paroxysmal activity [4,8,9,11]. P. Seguela *et al*. [10] synthesized conjugates of Glu and protein carriers, obtained specific antibodies (AB) to the linear amino acid, and used these AB in immunocytochemical experiments. However, the effect of anti-Glu AB on the development of epileptiform activity was not studied.

Here we studied the effect of active immunization of mice with the Glu-protein conjugate on acute generalized epileptiform activity evoked by corazol.

MATERIALS AND METHODS

The conjugated antigen of Glu and bovine serum albumin (BSA) was synthesized by a modified method using bifunctional reagent glutaraldehyde [10]. Immunogenicity of the conjugate was determined by active immunization of rabbits. Plasma titer of anti-Glu AB in immunized rabbits was 1:1024.

Two series of experiments were performed on C57BL/6 (n=100) and BALB/c mice (n=35) weighing 20-23 g. Series I was conducted on 4 groups of C57BL/6 mice. Control animals of groups 1 and 2 received physiological saline and complete Freund's adjuvant (CFA), respectively. Control mice of group 3 were immunized with BSA. Group 4 animals were immunized with Glu-BSA conjugate. Group 1 mice received 1 subcutaneous and 2 intraperitoneal injections of physiological saline (0.2 ml). Group 2 animals received single subcutaneous injection of 0.1 ml CFA and 0.1 ml physiological saline and 2 intraperitoneal injections of 0.2 ml physiological saline. The mice of groups 3 and 4 were immunized 3 times with conjugated antigen Glu-BSA and BSA in increasing doses, respectively, at 2-week intervals. During the first treatment the substance (2 mg/kg) dissolved in 0.1 ml physiological saline and 0.1 ml CFA was injected subcutaneously on the back. During the second and third treatments the substance in doses of 5 and 10 mg/kg, respectively, was dissolved in 0.2 ml physiological saline and injected intraperitoneally.

Laboratory of Functional Neurochemistry, Laboratory of Neuroimmunopathology, Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

0007-4888/03/1363-0250$25.00

TABLE 1. Effect of Active Immunization of C57BL/6 Mice with Glu-BSA Conjugate on Corazol-Induced Generalized Seizures (Series I, *M*±*m*)

Group	Dose of corazol			
	clonic seizures		lethal outcome	
	mg/kg	%	mg/kg	%
1 (*n*=12)	24.83±1.03	100	39.60±1.33	100
2 (*n*=11)	24.74±0.40	99.64±1.61	44.98±1.29**°	113.59±3.26
3 (*n*=13)	22.26±0.44***++	89.65±1.77	38.22±1.05	96.52±2.65
4 (*n*=14)	29.36±0.74***+°	118.24±2.98	53.94±1.33*+°	136.21±3.36

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to group 1; +p<0.001 and ++p<0.01 compared to group 2; °p<0.05 compared to group 3.

Series II was conducted on mice of various genetic strains. C57BL/6 and BALB/c mice were divided into 2 groups. Experimental animals were immunized with Glu-BSA conjugate. Control mice received physiological saline in an equivalent volume.

Plasma level of anti-Glu AB in control and immunized mice was measured by solid-phase enzyme immunoassay. Glu was conjugated with horse γ-globulin as described previously and this conjugate served as the test antigen [10]. Immunological control for the level of anti-Glu AB was performed in both series with 5 immunized and 5 control animals.

The threshold of clonic seizures and tonic phase of lethal seizures was determined on the model of acute generalized epileptiform activity 1 week after the last injection [7]. The solution of corazol (1%) was infused intravenously at a flow rate of 0.01 ml/sec. The threshold dose of corazol that induced seizures was estimated individually and expressed in mg/kg.

The results were analyzed by Student's *t* test.

RESULTS

Active immunization of mice with Glu-BSA conjugate induced synthesis of anti-Glu AB (titer 1:16).

In series I active immunization of C57BL/6 mice with Glu-BSA conjugate elevated thresholds for clonic seizures and tonic phase of lethal seizures by 18.24 and 36.21%, respectively (Table 1). Administration of BSA decreased clonic seizure threshold by 10.35%. CFA elevated the threshold for the tonic phase of seizures by 13.59%.

In series II we determined the threshold for seizures evoked by corazol. Table 2 shows that thresholds for clonic seizures and tonic phase of lethal seizures in BALB/c mice were lower than in C57BL/6 mice by 22.22 (p<0.001) and 18.75% (p<0.01), respectively. Therefore, BALB/c mice were less resistant to the proconvulsant effect of corazol than C57BL/6 mice. Active immunization of mice with the Glu-BSA conjugate elevated seizure thresholds (Table 2). Im-

TABLE 2. Effect of Active Immunization with Glu-BSA Conjugate on Corazol-Induced Generalized Seizures in C57BL/6 and BALB/c Mice (*M*±*m*)

Group	Dose of corazol			
	clonic seizures		lethal outcome	
	mg/kg	%	mg/kg	%
C57BL/6 (*n*=9)				
control, physiological saline	23.40±0.97	100	43.20±1.19*	100
C57BL/6 (*n*=18)				
treatment, immunization	28.18±0.77*	120.43±3.29	49.26±1.05*	114.03±3.47
BALB/c (*n*=10)				
control, physiological saline	18.20±0.59+	100	35.10±2.23*++	100
BALB/c (*n*=15)				
treatment, immunization	24.15±0.68*	132.69±2.87++	49.81±1.43*	141.91±4.07+

Note. *p<0.001 compared to the control; +p<0.001 and ++p<0.01: interstrain differences.

munized BALB/c mice were more resistant to the development of corazol-induced seizures than C57BL/6 mice. In BALB/c mice the threshold dose of corazol inducing clonic seizures and tonic phase of lethal seizures was higher than in C57BL/6 mice by 12.22 ($p<0.01$) and 27.88% ($p<0.001$), respectively.

Our results indicate that anti-Glu AB produce an antiepileptic effect on acute generalized seizures induced by corazol and elevate the thresholds for clonic seizures and tonic phase of lethal seizures. This effect was observed in mice of various genetic strains. Clonic and tonic seizure thresholds in BALB/c mice increased more significantly than in C57BL/6 mice. It should be emphasized that before immunization seizure thresholds in BALB/c mice were lower than in C57BL/6 mice. Our previous studies revealed considerable differences in the behavioral response of animals to treatment with anti-Glu AB. Active immunization mainly affected the state of BALB/c mice, which was manifested in enhanced behavioral activity, reduction of anxiety, and improved retention of conditioned passive avoidance response [1,5]. It can be explained by genetically determined differences in activity of brain neurotransmitter systems (*e.g.*, glutamatergic system) in mice of different strains.

The development of epileptiform activity is related to an imbalance between the inhibitory and excitatory mechanisms and predominance of excitation [3,4,11]. These changes result from the impairment of GABAergic inhibition. The proconvulsant effect of carazol is associated with the inhibition of chlorine channels in the $GABA_A$-receptor complex and impairment of the GABAergic inhibitory mechanisms [3,6]. During active immunization and systemic treatment of animals the amount of AB to neurotransmitters entering CNS via the brain-blood barrier is low, but sufficient to modulate functional activity of the nervous system [2]. AB entering CNS bind Glu and suppress the glutamatergic system, which probably contributes to the antiepileptic effect of anti-Glu AB.

REFERENCES

1. L. A. Vetrile, L. A. Basharova, O. I. Mikovskaya, *et al*., *Byull. Eksp. Biol. Med.*, **133**, No. 3, 274-277 (2002).
2. V. A. Evseev, T. V. Davydova, and O. I. Mikovskaya, *Dysregulation Pathology* [in Russian], Ed. G. N. Kryzhanovskii, Moscow (2002), pp. 420-428.
3. M. N. Karpova and I. G. Rebrov, *Ibid*., pp. 596-604.
4. K. S. Raevskii, *Patofiziologiya*, No. 1, 3-9 (1990).
5. N. A. Trekova, O. I. Mikovskaya, L. A. Basharova, *et al*., *Byull. Eksp. Biol. Med*., **133**, No. 2, 140-143 (2002).
6. A. S. Bazyan, V. V. Zhulin, M. N. Karpova, *et al*., *Brain Res*., **888**, 212-220 (2001).
7. R. S. Fisher, *Brain Res. Rev*., **14**, 245-278 (1989).
8. B. S. Meldrum, *J. Nutr*., **130**, Suppl. 4, 1007S-1015S (2000).
9. I. M. Najm, *Neurol. Clin*., **19**, No. 2, 237-250 (2001).
10. P. Seguela, M. Geffard, R. Buijs, *et al*., *Proc. Natl. Acad. Sci. USA*, **81**, No. 12, 3888-3892 (1984).
11. K. Tanaka, *Adv. Neurol. Sci*., **44**, 13-22 (2000).