Effects of Antibodies to Glutamate on Focal Penicillin-Induced Epileptic Activity


The effects of intranasal pretreatment with antibodies against glutamate on focal penicillin-induced epileptic activity were studied by recording electrocorticogram in non-anesthetized freely moving male Wistar rats. Anticonvulsant effects of intranasal administration of anti-glutamate antibodies (300 μg/kg) 1 h before application of penicillin (30,000 U/ml) on the sensorimotor cortex was demonstrated: the latency of ictal discharges increased and their frequency decreased.

Key Words: focal epileptic activity; penicillin; antibodies; glutamate

We previously showed that antibodies against glutamate (anti-Glu Ab) produce immunocorrector effects on acute generalized epileptiform activity (EA) after active immunization of mice of various strains with glutamate-BSA conjugate (Glu-BSA) [2] and after systemic administration of anti-Glu Ab [1]. It was shown that anti-Glu Ab produce antiepileptic effects, increase the thresholds of clonic seizures and tonic phase of seizures with fatal outcome, and lengthen the latency of these seizures. This effect was also observed in kindled animals [3]. Anticonvulsant effects of anti-Glu Ab are related to suppression of the glutamatergic system due to their interaction with Glu in CNS.

Here we studied the effects of anti-Glu Ab on focal EA induced by convulsant penicillin. This model of focal EA is adequate to clinical forms of human focal epilepsy.

MATERIALS AND METHODS

Experiments were performed on 27 male Wistar rats weighting 200-220 g. For modeling focal EA, a hole (2×4 mm) was drilled in animal skull above the left sensorimotor cortex 1 day before the experiment under narcosis (325 mg/kg chloral hydrate, intraperitoneally) and local novocaine anesthesia, the dura mater was removed, and a monopolar silver ball electrode (d=0.5 mm) was implanted for recording electrical activity in this cortical area. Silent electrode was implanted in the nasal bones of the skull. The external contacts of electrodes were fixed to the skull surface using dental paste and a capsule was formed around the trephine opening. The capsule was filled with 0.9% NaCl and covered with waterproof film fixed with dental paste to prevent drying of open brain area. On the next day, the film was removed from the capsule and EA focus was modeled by application of filter paper soaked in sodium benzylpenicillin solution (30,000 U/ml) on the cortical surface. Electrococtigogram was recorded using an electroencephalograph EEG 8S in non-anesthetized freely moving animals. The following parameters were recorded: latent period (LP) of the first interictal discharge (IID), LP of the first ictal discharge (ID), mean frequency of IID generating during each 30-min interval, mean frequency of ID generating during each 30-minute interval, mean and total duration of ID during EA episode, number of ID during the EA episode, and the duration of EA episode (the time from the appearance of the first IID to their disappearance).

Anti-Glu Ab were obtained by hyperimmunization of Chinchilla rabbits with a Glu-BSA conjugate synthesized using a bifunctional reagent glutaraldehyde.
The concentration of anti-Glu Ab in blood serum of immunized rabbits was measured by ELISA using a conjugate on a heterologous protein carrier (equine γ-globulin) as the test antigen (Ab titer was 1:1024). Ab (γ-globulin fraction) were isolated from plasma samples of immunized rabbits by ammonium sulfate precipitation and purified by dialysis. γ-Globulin fraction was purified from Ab against carrier protein by affinity chromatography on BrCN-activated Sepharose 4B with immobilized BSA as the sorbent. Then this fraction was lyophilized and stored at 4°C. γ-Globulin fraction of blood plasma from intact non-immunized rabbits was isolated similarly. Anti-Glu Ab were absent in plasma samples from control rabbits.

Animals were randomized into three groups. Control rats received 0.9% NaCl or intact (non-immunized) γ-globulin (group 1 and 2, respectively). Purified anti-Glu Ab were administered to group 3 animals.

Anti-Glu Ab and γ-globulin were administered in a dose of 300 μg/kg. All solutions were administered intranasally for bypassing the blood-brain barrier [8]. Anti-Glu Ab and γ-globulin were dissolved in 10 μl 0.9% NaCl, and put into each nostril (5 μl) 1 h before penicillin application using a pipette with thin tip. Group 1 (control) animals received 0.9% NaCl in the same way.

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

RESULTS

In group 1 animals, penicillin application induced EA development in 3-5 minutes: single IID peaks were observed against the background of spontaneous electrocorticogram, and their frequency and amplitude gradually increased. Seizure ID (bursts of high-frequency and high-amplitude hypersynchronous discharges) appeared in 7-9 min after the application. The phase of significant seizure activity with regular ID appearance started 15-20 minutes after the application and lasted for 30-40 minutes; then, the frequencies of ID and IID decreased (Table 1; Fig. 1). The mean duration of EA episodes (from the appearance to total refusal of EA) was 100 minutes. About 34 ID were recorded during this period.

Administration of intact (non-immunized) γ-globulin to group 2 control animals was followed by a decrease in LP of the first IID in comparison with γ-globulin.

| TABLE 1. Effects of Intranasal Administration of Anti-Glu Ab (300 μg/kg) on Focal EA Induced by Application of Penicillin on Brain Cortex of Rats (M±m) |
|-----------------|----------------|----------------|-------------------|----------------|
| Group           | LP 1, min      | LP 2, min      | Number of ID during EA episode | Mean duration of ID, sec | DFE, min     |
| PS (n=9)        | 4.09±0.54      | 8.35±0.81      | 33.67±3.35          | 14.97±1.46     | 102.50±2.95  |
| γ-Globulin (n=9)| 2.65±0.30*     | 5.87±1.02      | 25.56±3.15          | 15.07±1.03     | 150.11±9.06*** |
| Anti-Glu Ab (n=8)| 4.49±0.64*   | 25.00±5.92***  | 5.00±1.37***        | 15.09±2.15     | 109.38±7.44++ |

Note. n, animal number. LP 1 and LP 2, The LP of the first IID and ID, respectively. DFE, the duration of focus episode. PS, 0.9% NaCl. *p<0.05, **p<0.02, ***p<0.001 in comparison with animals received PS. *p<0.05, **p<0.01, ***p<0.001 in comparison with animals received γ-globulin.