Increasing the Efficacy of Antiepileptic Effect of Diazepam by Cerebellar Cortex Electrostimulation

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Diazepam effects on epileptic foci induced by benzylpenicillin application to the posterior sigmoid gyrus were studied in acute experiments on cats. Diazepam more effectively decreased the activity of the foci after preliminary electrostimulation of the IX and X lobules of the vermis than without such a pretreatment.

Key Words: *diazepam; cerebellum; electrostimulation; epileptic activity*

Electrical stimulation (ES) of caudate portions of the vermis during regular use of the benzodiazepines diazepam or phenazepam potentiates the anticonvulsive effect of the drugs both during and after ES and eliminates the factors promoting epileptogenesis [1,4].

Our purpose was to assess the opposite effect: stimulation of antiepileptic action of diazepam by preliminary ES of the cerebellar cortex.

MATERIALS AND METHODS

The study was performed on 27 cats of both sexes weighing 2.5-3.5 kg. Tracheostomy, trephination, implantation of constantan bipolar electrodes (0.12-0.15 μ, distance between the electrodes 0.25 mm) in the cerebellar cortex (IX-X lobules of the vermis according to [5]), and catheterization of the femoral vein were carried out under ether rausch narcosis. Electrodes were fixed to the skull bones by rapidly hardened Noracryl. The animals were transferred to forced respiration after intraperitoneal injection of 0.25 mg/kg d-tubocurarine (Orion). Follow-up started from 22.5 h after the end of ether narcosis. Twenty sessions of ES of cerebellar cortex (rectangular pulses, 100-300 Hz, 300-400 μA, pulse duration 0.25 msec, ES duration 5-7 sec) were performed every 3 min with an ESU-2 electrostimulator. To control animals, electrodes were similarly implanted, but no ES was performed.

After opening the dura mater, a focus of epileptic activity (EpA) was created by applying a filter paper (2x2 mm) moistened with fresh-prepared benzylpenicillin sodium solution (10,000 U/ml) onto the posterior sigmoid gyrus. Activity of EpA was recorded through monopolar electrodes by an electroencephalograph (Medicor) with indifferent electrodes fixed in the nasal bones.

EpA of the foci was expressed in arbitrary units: a mean amplitude of 1 mV at 1 charge/min generation was taken for one unit. A 1-min Bpoque of charge generation was taken for estimating the level of EpA, and the life span of foci was determined as the period from the first to the last spike [3]. Diazepam (Gedeon Richter) was injected intravenously in doses 0.5 and 1.5 mg/kg during generation of stable activity in the foci. Controls were injected with 0.5 ml normal saline.

Results were statistically processed using ANOVA tests with subsequent processing by the Neumann-Keuls test. Differences were considered significant at $p<0.05$.

RESULTS

After application of benzylpenicillin (in 3-7 min), the first spike charges were generated in the focus. The amplitude and frequency of these charges in-

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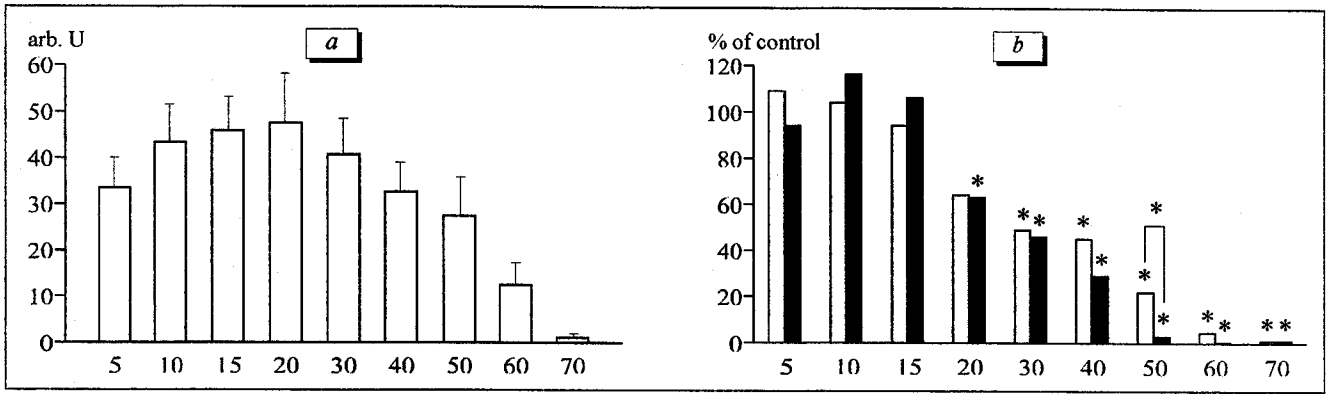

Fig. 1. Effects of diazepam on the foci of epileptic activity created by application of penicillin (10,000 U/ml) to the cerebral cortex of cats. *a*) time course of power of foci in the control (0.5 ml normal saline); *b*) effects of diazepam in doses 0.5 (light bars) and 1.5 mg/kg (dark bars). Here and in Fig. 2: abscissa: time since generation of first epileptic charges in focus, min; ordinate: power of foci; *$p<0.05$ *vs.* the control (100%).

creased during subsequent 10-15 min (with the application on-going), reaching 1.2-1.9 mV and 25-43 charge/min, respectively. Twenty minutes after application of the epileptogen, the power of foci was 47.5±9.6 arb. units (Fig. 1, *a*). Stable EpA was observed during the next 10-15 min, after which the frequency and amplitude of charges gradually subsided during 20-35 min. Total life span of foci was 65.7±10.7 min.

Injection of 0.5 mg/kg diazepam 15 min after the potentials had been evoked in the focus was associated with a decrease in the frequency and amplitude of charges 5 min after the injection (Fig. 1, *b*). After 15 min, the power of foci was 20.29±5.8 arb. units, which was significantly lower than in the control (normal saline, $p<0.05$, Fig. 1, *b*). The differences persisted until the end of observation, and the total life span of foci was the same as in the control ($p<0.05$). Injection of 1.5 mg/kg diazepam decreased the power of foci in 5 min by 46.1% *vs.* the control ($p<0.05$, Fig. 1, *b*). Significant differences persisted until the end of observation, and total life span of foci was the same as in the control.

Application of benzylpenicillin to the cerebral cortex of cats exposed to 20 sessions of ES of the cerebellar cortex was associated with appearance of the first spike potentials 5-15 min after the epileptogen was applied. During subsequent 10-15 min, EpA power gradually increased, and 15 min after generation of the first potentials it was 37.2±7.2 arb. units (Fig. 2, *a*). Stable EpA was observed for 10-15 min, after which the frequency and amplitude of spike potentials decreased (Fig. 2, *a*). The total life span of EpA foci under these conditions was 52.7±9.8 min, which was virtually the same as in the group without ES of cerebellum (65.7±10.7 min, $p<0.05$).

Injection of 0.5 mg/kg diazepam 15 min after generation of the first spike charges decreased in 5 min the power of foci, which was 57.3% lower than the control (cerebellar ES without diazepam, $p<0.05$, Fig. 2, *b*). These differences remained significant until the end of observation (Fig. 2, *b*). On the other

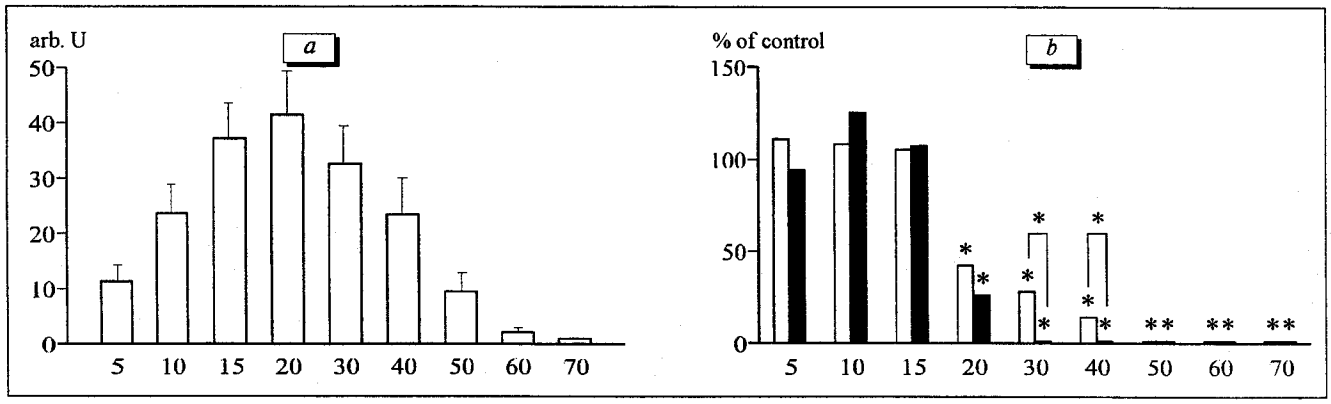

Fig. 2. Effects of diazepam on foci of epileptic activity created by application of penicillin (10,000 U/ml) to the cerebral cortex of cats after preliminary electrostimulation of cerebellar cortex. *a*) time course of power of foci during 20 sessions of preliminary electric stimulation of cerebellar cortex and injection of 0.5 ml normal saline; *b*) effect of diazepam in a dose of 0.5 (light bars) and 1.5 mg/kg (dark bars) after 20 sessions of electric stimulation of the cerebellar cortex.

hand, 25 min after injection of diazepam, the power of foci was significantly lower (3.5±0.6 arb. units) than in animals injected with the same diazepam dose without cerebellar ES (14.7±3.2 arb. units, p=0.007). The life span of foci was 35.2±7.2 min, which was the same as in the control (p<0.05). It is noteworthy that the power of foci 40 and 50 min after diazepam injection differed significantly from that in animals injected diazepam in the same doses without ES of the cerebellum (Fig. 2, *b*).

Five minutes after injection of diazepam in a dose of 1.5 mg/kg, the power of EpA foci decreased 3.9 times in comparison with the control (cerebellar ES without diazepam) (p<0.05, Fig. 2, *b*). The differences were significant till the end of observation. Fifteen minutes after diazepam injection, the power of the foci was significantly lower (9.4±2.1 arb. units) than in animals injected with the same dose of diazepam without preliminary ES (18.6±4.4 arb. units, p=0.002). The life span of foci under these conditions was 27.6±5.6 min, which was shorter than in cats exposed to ES without diazepam (p=0.046) and as long as in cats injected with the same diazepam dose (p>0.05).

These findings indicate that during the formation of EpA foci generating interictal (spike) activity in the cerebral cortex of cats, diazepam exerts a dose-dependent antiepileptic effect; this agrees with published data [4]. The drug promotes a decrease in the frequency and amplitude of spike charges and in the life span of foci.

Exposure of the cerebellum to ES (20 sessions) impeded further induction of EpA foci by penicillin applications. This indicates a long-term antiepileptic effect of ES of the cerebellum, due to the formation of a generator of increased excitation at the site of stimulation [2,4]. Aantiepileptic effect of diazepam under these conditions was more potent than that of diazepam alone. Interactions between diazepam and peptides in the liquor that mediate the antiepileptic action of cerebellar ES may underlie this effect.

From these results we conclude that activation of the cerebellar cortex potentiates the antiepileptic efficiency of benzodiazepines and, probably, of other antiepileptic drugs. This means that drug effects can be modified by preliminary ES of brain structures and can explain the resistance of ES-kindling to antiepileptic agents [6,7].

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