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ANTICONVULSANT PROPERTIES OF THE CEREBROSPINAL FLUID DUE TO ANTIEPILEPTIC SYSTEM ACTIVATION

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KEY WORDS: seizures; antiepileptic system; electrical stimulation; cerebrospinal fluid

Electrical stimulation of structures of the antiepileptic system [1] is known to inhibit epileptic activity (SpA) of separate foci [2] and of epileptic complexes [4] and also generalized EpA [5]. It can be tentatively suggested that endogenous substances formed or secreted intensively in response to activation of the antiepileptic system are involved in the realization of antiepileptic effects arising during electrical stimulation of the structures of that brain system.

The aim of this investigation was to study the effect of the cerebrospinal fluid (CSF) of animals subjected to chronic electrical stimulation of the cerebellar cortex, which plays an important role in suppression of EpA [1, 4] and also of the CSF of animals subjected to electroshock stimulation (seizures) of focal and generalized EpA.

EXPERIMENTAL METHOD

Acute experiments were carried out on cats of both sexes weighing 2.5-3.5 kg and on male Wistar rats weighing 250-300 g.

Donor cats were anesthetized with pentabarbital (40 mg/kg) and stimulating electrodes were implanted into the cortex of the vermis cerebelli (lobes V-VII). Single daily electrical stimulations (ES) began 5-7 days after the operation (100 Hz, 0.5 msec, 5-10 V, duration of stimulation 5 sec; total number of sessions 30-40). Under ether anesthesia, tracheotomy was performed on cats of another group, and stimulating electrodes were implanted in the frontal zones of the cerebral cortex. When 2.5-3 h had elapsed after the end of ether administration electroshock stimulation began (60 Hz, 5.0 mA, duration 2 sec). After 3 to 10 seizures and 0.5-1.5 min after the next stimulation, CSF was withdrawn. Stimulating electrodes were implanted into the cerebellum and cerebral cortex of the animals of the control group, but an electric current was not applied. The CSF of the experimental and control cats was obtained by suboccipital puncture, and treated with gordox (Gedeon Richter, Hungary) in a dose of 1000 U/ml or with bacitracin in a dose of 1 mg/ml to inhibit proteolysis.

Under hexobarbital anesthesia (100 mg/kg) cannulas were inserted into the lateral ventricle of the recipient rats, at coordinates (AP = −0.8; L = 1.2; H = 3.5) taken from the atlas [6]. The animals were used in the experiments 7-10 days after the operation. CSF was injected in a dose of 10 µl by means of a microinjector over a period of 1.5-2 min. Metrazol in a dose of 40 mg/kg was injected intraperitoneally 10 min after injection of the CSF. The animals were kept under observation for 10 min after the injection of metrazol. The seizure response was assessed in points of the following scale: 0) no seizure response; 1 point) paroxysmal twitching; 2 points) clonic spasms of the trunk; 2 points) clonic spasms of the forelimbs, the animals rising on their hind limbs (kangaroo posture); 4 points) marked clonicotonic convulsions with the animal falling on its side; 5 points) repeated

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TABLE 1. Effect of Intraventricular Injection of CSF on Seizures Induced in Rats by Metrazol (M ± m)

Experimental conditions	Number of animals	Latent period, sec	Severity of seizure response, points (number of animals)						Average severity of seizures, points
			0	1	2	3	4	5	
Injection of CSF from cats undergoing mock operation	8	102,0±32,2	—	2	—	—	5	1	3,4±0,4
Injection of CSF from cats subjected to ES of cerebellum	9	137,3±32,4	3	3	—	2	1*	—	1,4±0,5***
Injection of CSF (10 µl) from ESh cats	13	126,4±17,6	—	4	4	3	2*	—	2,2±0,3**

Legend. *p < 0.025 compared with number of animals with generalized seizures in control group; **p < 0.05, ***p < 0.01 compared with corresponding parameters in control groups.

clonicotonic convulsions. The latent period of the first seizure manifestations and of the manifest convulsions and their duration also were determined.

Under ether anesthesia tracheotomy was performed on the recipient cats and a cannula implanted in the lateral ventricle at coordinates (AP = 13; L = 3.0; H = 17.0) taken from the atlas [7], the skull was trephined, and the dura opened in the region of the frontal cortex. Tubocurarine (0.12-0.28 mg/kg) was injected into the animals, which were artificially ventilated. A focus of EpA was created in the posterior signoid gyrus 2.5-3 h after administration of the ether had ceased, by application of a piece of filter paper (2 × 2 mm) soaked in a 0.1% solution of strychine nitrate. Application of the convulsant ceased after EpA appeared. By means of a microinjector CSF was injected in a volume of 250 µl over a period of 3 min. To characterize the EpA a power index of the foci was used, calculated by multiplying the mean amplitude of the discharges (in mV) by the number of potentials during 1 min of observation. Changes in power of the foci were studied after injection of CSF, compared with the initial power level taken as 100%. Electrical activity was recorded by a monopolar technique, the reference electrode being fixed in the nasal bones. The EcoG was recorded on a 4-ÉÉG-3 ink-writing electroencephalograph.

The experimental results were subjected to statistical analysis by variance and nonparametric methods [3].

EXPERIMENTAL RESULTS

Injection of the CSF of cats subjected to ES of the cortex of the vermis cerebelli into rats led to lengthening of the latent period of the first seizure manifestations by 34.6% compared with the control; a decrease in the number of animals with generalized seizures was observed in this group ($p < 0.025$) and the average severity of the seizures was reduced by 58.8% ($p < 0.01$) compared with the corresponding control (Table 1).

Injection of CSF from cats subjected to electroshock stimulation (ESh cats) into rats caused lengthening of the latent period of the first seizure manifestations by 23.9% compared with the control, a decrease in the number of animals with generalized seizures ($p < 0.025$), and a decrease in the average severity of the seizures by 35.3% ($p < 0.05$) compared with the corresponding parameters in the control animals (Table 1).

In the next series of experiments 17 recipient cats were used. Spike potentials with an amplitude of 400-800 µV began to appear 6-12 min after application of strychnine solution (0.1%) to the posterior sigmoid gyrus, and during the next 5-8 min they reached an amplitude of 1.7-2.3 mV. The frequency of discharge generation under these circumstances varied from 18-20 to 35-52 per minute. The average power of the foci in the period of generation of stable EpA was 61.1 ± 6.5 relative units.

A decrease in power of the epileptic foci by 27% compared with the initial power was observed 1 min after injection of CSF into these animals from ESh cats during the period of stable EpA, which was 15% less than the relative power of the foci in animals of the control group ($p > 0.05$; Fig. 1). The power of the foci in the animals of the experimental group 1 min later remained 22% lower than initially and 25% lower than the relative power of the foci in the animals of the experimental group was greater than in the control animals ($p < 0.05$).

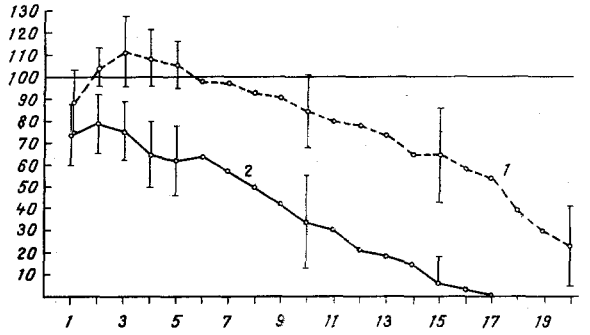

Fig. 1

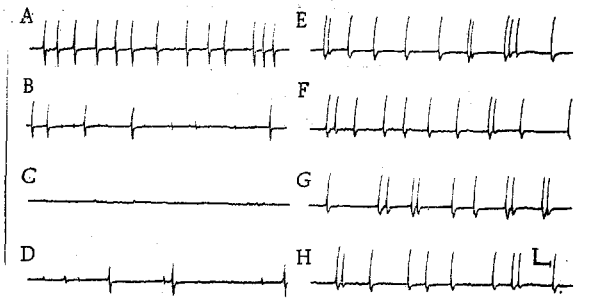

Fig. 2

Fig. 1. Effect of intraventricular injection of CSF on foci of EpA in cat cerebral cortex. Abscissa, time (in min) after injection of CSF; ordinate, relative power of foci (in % of initial level of power of foci, taken as 100%). 1) Relative power of foci after injection of CSF fron cats undergoing mock operation; 2) relative power of foci after injection of CSF from cats with electroshock stimulation.

Fig. 2. Effect of CSF application on activity of foci in cat creebral cortex. A) (Cat No. 1) 5 min after cessation of application of 0.1% strychnine solution to posterior sigmoid gyrus; B, C) 1.5 and 2.5 min, respectively, after beginning of application of CSF from ESh cats to zone of focus; D) 2.5 min after cessation of CSF application; E) (cat No. 2) 5 min after cessation of application of 0.1% struchine solution to posterior sigmoid gyrus; F, G, H) 1, 2, and 5 min, respectively, after beginning of application of CSF from cats undergoing mock operation to zone of focus. Calibration: 500 μV, 2 sec.

During the next 15 min of observation (Fig. 1) the decrease in the relative power of the foci in the animals of the exerpimental group was greater than in the control ($p < 0.05$). In animals of the experimental group, complete suppression of activity of the foci was observed in six of 11 cases, whereas in the control similar suppression of EpA was observed in only one of six cases ($p > 0.025$).

The effect of application of CSF on activity of the foci of EpA also was studied in experiments on cats. Application of a piece of filter paper soaked in CSF from an ESh cat to the zone of the focus during generation of stable EpA in it (initial power of the foci 60.0 ± 2.9 relative units) led to reduction of the power of the foci 1-2 min after the beginning of application to 27.5 ± 5.2 relative units (Fig. 2b), which was less than in the control ($p < 0.01$; Fig. 2e). Another 1-2 min later complete suppression of activity of the foci was observed compared with the control ($p < 0.001$; Fig. 2c, g). Cessation of application was accompanied by recovery of activity of the foci up to 25.8 ± 6.1 relative units (Fig. 2d), significantly greater than before application of the CSF ($p < 0.01$).

The investigations thus show that the CSF from cats subjected to chronic ES of the cortex of the vermis cerebelli of kindling type caused suppression of EpA in the recipient animals. The anticonvulsant effects of the CSF were discovered both against the focal form of EpA, induced by application of strychnine to the cerebral cortex, and against generalized EpA induced by injection of metrazol.

It must be emphasized that seizures were never observed in the donor animals during stimulation of the cortex of the vermis cerebelli. These results are evidence that seizures are not an essential condition for the appearance of antiepileptic substances in the CSF. Activation of structures of the antiepileptic substances in the CSF. Activation of structures of the antiepileptic system and, in particular, structures of the cerebellum, which causes suppression of EpA [1, 2], at the same time leads to the formation or stimulates the release of substances which also exert an antiepileptic action. It can be postulated that the influence of these endogenous antiepileptic substances is one of the mechanisms whereby the antiepileptic system realizes its effects, i.e., preventing the onset of and suppressing existing EpA. This conclusion is confirmed by the fact that application of "antiepileptic" CSF directly to a focus of EpA in the cerebral cortex causes total suppression of that focus.

The fact that an antiepileptogenic substance (or substances) also appears in CSF taken from cats which have developed seizures as a result of electroshock stimulation (ESh cats) is evidence that it may begin to be produced actually during the seizure period. From this point of view our results agree with those of a recently published study [8] in which seizures induced by Fluorotyl were found to be weakened by CSF taken from rats exhibiting single generalized seizures. However, the appearance of antiepileptic properties of the CSF is not linked with the seizure process itself, as the authors cited above suggest. Our investigations show that the anticonvulsant properties of the CSF are due to activity of the antiepileptic system, which is activated during the seizure process and participates in its suppression, or even causes this suppression. This conclusion is supported by the fact that the antiepileptic properties of the CSF in cats subjected to electrical stimulation, of kindling type, of an antiepileptic structure (the cortex of the vermis cerebelli) are more marked than in ESh cats with a seizure syndrome.

The results given above show that the antiepileptic substance discovered does not possess species specificity. Whatever its nature, the important point is that it is endogenous in origin and is the result of activity of the antiepileptic system and is one of the mechanisms whereby its effects are realized.

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CHANGES IN CYCLIC NUCLEOTIDE LEVELS AND LYSOSOMAL ENZYME ACTIVITY IN THE GASTRIC MUCOSA OF RATS WITH EXPERIMENTAL ULCERATION

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KEY WORDS: ulceration; lysosomes; cyclic nucleotides.

The development of ulcers of the gastric mucosa may be linked with the damaging effect of hydrolases [2], released from lysosomes into the cytosol after labilization of their membranes [3, 10, 12], on cellular structures. Meanwhile the stability of the lysosomal membranes largely depends on cyclic nucleotide levels [13]. In ulceration both elevation of the cAMP level in the gastric mucosa [5] or its lowering may be observed [6].

The aim of this investigation was to study changes in levels of cyclic nucleotides (cAMP and cGMP) and their ratio during the development of ulcers in the gastric mucosa under the influence of catecholamines. Acid phosphatase and proteinase activity also was investigated in the lysosomal fraction and the fraction of enzymes remaining in the supernatant after ultracentrifugation of mucosal homogenates. The choice of model of ulceration was determined by the fact that hyperactivation of adrenergic processes by an important factor in the development of ulcers of the gastric mucosa [1, 7].

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