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EFFECT OF PN 200-110 ON PENICILLIN-INDUCED EPILEPTIC ACTIVITY IN THE RAT CEREBRAL CORTEX

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Since entry of Ca^{2+} into the neuron plays an important role in the mechanisms of hyperactivation of neurons and the genesis of epileptic activity (EA) [7, 8, 13], interest in Ca-channel blockers as possible antiepileptic agents has increased [4, 9-11].

The aim of this investigation was to study the effect of one such blocker, namely PN 200-110 (the preparation was generously provided for testing by the firm of "Sandoz," Switzerland), a substance belonging to the 1,4-dihydropyridine class, on activity of a penicillin-induced epileptic focus in the cerebral cortex of unanesthetized, unrestrained animals.

EXPERIMENTAL METHOD

Experiments were carried out on 65 male Wistar rats weighing 250-300 g. Under hexobarbital anesthesia (150 mg/kg, intraperitoneally) and local procaine anesthesia, 24 h before the experiment a burr-hole measuring 2 × 4 mm was drilled in the animal's skull above the sensomotor cortex of the left cerebral hemisphere, the dura was removed from that part, and monopolar cortical silver electrodes were applied to record electrical activity (electrocorticogram — ECoG). The reference electrode was inserted into the nasal bones. The external leads of the electrodes were fixed to the surface of the skull with "Noracryl" dental paste and a capsule was formed around the burr-hole. To prevent exposed parts of the brain from drying the capsule was filled with physiological saline and covered above by a waterproof film which was fixed around the edges with Noracryl. Next day, to create foci of EA the film was removed from the capsule and filter paper soaked with a solution of the sodium salt of benzyl penicillin in a concentration of 20,000 IU/ml was applied to the exposed area of the cortex. The ECoG was recorded on an EEG8S electroencephalograph (Hungary) from two unrestrained animals with foci of EA simultaneously: one animal had been given PN 200-110, the other the solvent, dimethyl sulfoxide (DMSO). The solution of PN 200-110 was made up immediately before injection in a dark room illuminated with red light.

There were three series of experiments. In the experiments of Series I the preparation was injected intraperitoneally in a dose of 2 mg/kg against a background of stable EA in the focus (25-35 min after penicillin application). In the experiments of Series II the preparation also was injected intraperitoneally in a dose of 5 mg/kg, 25 min before penicillin application. The control animals received DMSO in the same volume (0.1 ml) under similar experimental conditions. In the experiments of Series III the preparation was injected into the cerebral ventricles in a dose of 1 and 10 nmoles in 2 μl of a 50% solution of DMSO. The injection was given by means of a steel cannula (concentric configuration, external diameter 0.82 mm), implanted into the lateral ventricle, taking coordinates from the atlas [12], 20 min before penicillin application. The control animals received 50% DMSO solution in the same volume. The location of the cannula was verified after each experiment.

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TABLE 1. Effect of PN 200-110 (2 mg/kg, intraperitoneally) on Focal EA (M ± m)

Group of animals	Before injection			20-30 min after injection			No. of ID during existence of focus	Duration of existence of focus, min
	No. of ID in 1 min	amplitude of IID, μV	No. of ID in 1 min	No. of IID in 1 min	Amplitude of IID, μV	No. of ID in 1 min		
Control (n = 6)	13,28±2,21	966±135	0,44±0,13	14,46±4,19	834±195	0,36±0,11	34±4,25	152±12
Experimental (n=7/6)	13,88±1,55	840±116	0,47±0,10	19,78±5,90	646±25	0,08±0,03**	21±3,71*	112±10*

Legend. Here and in Tables 2 and 3, number of animals given in parentheses. Numerator — total number of animals in group, denominator — number of animals in which preparation caused appearance of EA. *p < 0.05, **p < 0.02.

TABLE 2. Effect of Preliminary Injection of PN 200-110 (5 mg/kg, intraperitoneally) on Focal EA (M ± m)

Group of animals	Latent period 1, sec	Latent period 2, sec	Number of animals with ID	No. of ID during existence of focus	Duration of ID, sec	Duration of existence of focus, min
Control (n = 10)	4,70±0,62	12,14±0,76	9	19,00±1,78	19,53±1,14	123,9±5,8
Experimental (n = 10)	6,43±0,58*	16,16±2,66	6	11,33±2,58*	17,78±1,78	79,7±7,4**

Legend. Here and in Table 3: latent period 1) time from moment of application of penicillin to appearance of first IID, latent period 2) time from moment of application of penicillin to appearance of first ID. *p < 0.05, **p < 0.001 compared with corresponding values in control.

TABLE 3. Effect of Preliminary Intraventricular Injection of PN 200-110 on Focal EA (M ± m)

Group of animals	Latent period 1, sec	Latent period 2, sec	No. of ID during existence of focus	Duration of existence of focus, min
Control (n = 8)	4,25±0,62	15,28±2,73	15,17±3,40	113,00±5,47
1 nmole PN 200-110 (n=10)	4,63±0,64	14,50±2,25	24,88±6,94	105,33±6,60
10 nmoles PN 200-110 (n=8)	6,79±0,72**	19,36±3,98	5,00±1,18*	86,17±6,77**

Legend. *p < 0.05, **p < 0.001.

The data were processed by M-44 computer complex ("Olivetti," Italy). Amplitude–frequency characteristic curves and the duration of existence of the foci of EA were determined.

EXPERIMENTAL RESULTS

Preliminary experiments (6 rats) showed that application of penicillin to the sensomotor cortex led to the appearance of EA after 3-7 min: against the background of the spontaneous ECoG, discrete interictal discharges (IID) appeared, their amplitude increasing gradually, so that after 10-15 min ictal discharges (ID) appeared, and 25-35 min later a stage of marked seizure activity supervened, and was characterized by the regular appearance of ID and continued for 30-40 min, after which the ID appeared less frequently, and the frequency of generation and amplitude of the IID also were reduced. The average duration of existence of the EA foci from the time of application of penicillin until complete disappearance of EA was 150 ± 30 min.

Intraperitoneal injection of PN 200-110 in a dose of 2 mg/kg against a background of developed EA with stable generation of ID in the focus caused suppression of EA in the absolute majority (in six of seven) of animals (Table 1). This effect was observed in three animals 20 min after injection of the preparation, in two animals after 10 min, and in one rat complete suppression of ID and virtually complete suppression of IID were observed. The total number of ID in the animals of this group during the period of existence of the focus was 38% less than in animals of the control group. In three rats a decrease in the number of ID was accompanied by an increase in the number of IID, whose amplitude was lower (Fig. 1). The duration of existence of the focus of EA in the experimental animals was reduced, and the frequency of generation and the amplitude of IID

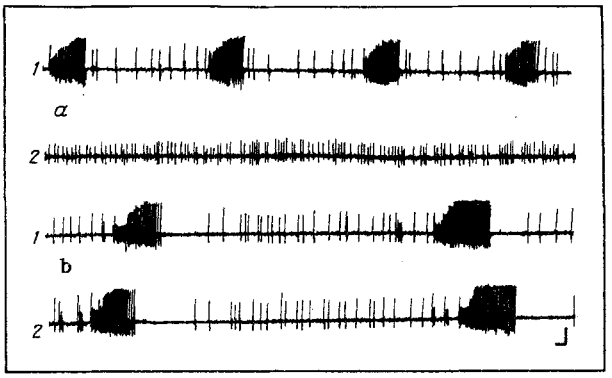

Fig. 1. Changes in electrical activity in penicillin-induced focus after intraperitoneal injection of PN 200-110 (a) and DMSO (b). 1) Before injection (stage of stable EA with regular appearance of ID), 2) 35 min after injection. Here and in Fig. 2, calibration: 500 μV, 5 sec.

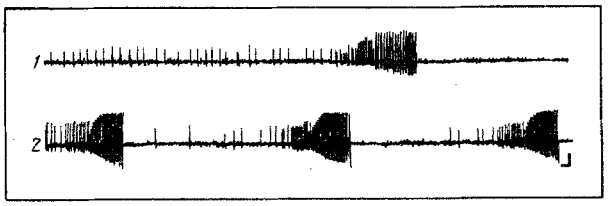

Fig. 2. Effect of preliminary injection of PN 200-110 (5 mg/kg, intraperitoneally) on change in electrical activity in penicillin-induced epileptogenic focus. 1) 40 min after penicillin application with preliminary injection of PN 200-110, 2) 90 min after penicillin application preceded by injection of DMSO.

on average for the whole group showed no significant change (Table 1). Under analogous experimental conditions intraperitoneal injection of DMSO into animals of the control group had no effect on the character of EA.

Preliminary injection of the preparation (5 mg/kg, intraperitoneally) completely prevented the development of EA in two of the 10 animals (absence of IID and ID). Meanwhile, in those animals in which EA appeared, the preparation caused a significant increase in the latent period of the first IID (Table 2). As a result of this, the frequency of generation and amplitude of IID were less in the first 15-20 min after penicillin application in the animals of this group than in rats of the control group, but later these parameters did not differ significantly in the animals of the two groups. Two of the animals developed ID. In six rats a tendency was noted for the latent period of appearance of the first ID to increase. The total number of ID in animals receiving PN 200-110 was significantly less than the control rats (Table 2). In four of six rats a pattern of ID was distinguished by a lower discharge generation frequency in the burst: from 2-3/sec in the control animals to 1/sec in the experimental animals (Fig. 2). The preparation significantly shortened the duration of existence of the focus of EA to 80 min. The effects of the compound observed on the pattern of ID also was preserved if the dose was reduced to 1 mg/kg. Preliminary injection of DMSO did not affect the character of EA: in nine of 10 control animals application of penicillin induced the characteristic pattern of EA for this particular epileptogen, and one rat did not develop ID.

Preliminary intraventricular injection of PN 200-110 in a dose of 1 nmole had no effect on the development and character of EA (Table 3). In a dose of 10 nmoles the compound caused a significant increase in the latent period of appearance of the first IID, significantly reduced the number of ID, and shortened the duration of existence of the focus (Table 3). Injection of the compound in this dose also reduced the amplitude of IID but had no effect on the frequency of their generation (Fig. 3).

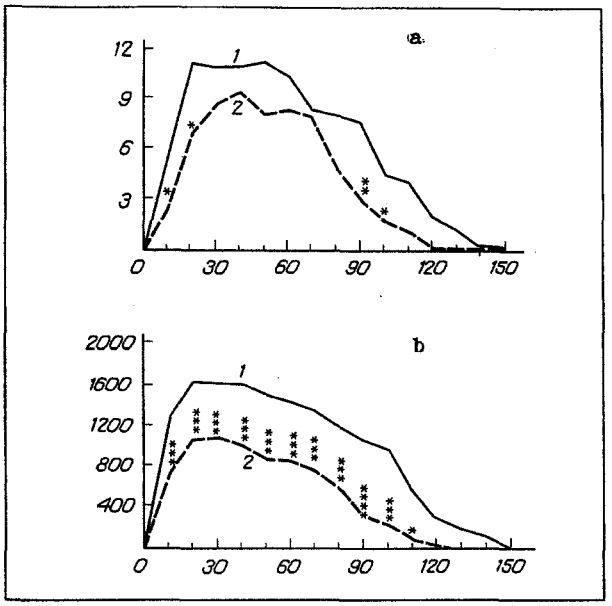

Fig. 3. Effect of preliminary intraventricular injection of PN 200-110 (10 nmoles) on number (a) and amplitude (b) of IID in penicillin-induced epileptogenic focus. Abscissa, time after penicillin application (in min); ordinate, number of IID (a) and their amplitude (in μV) (b). 1) Control, 2) PN 200-110. *p < 0.05; **p < 0.02; ***p < 0.01.

Thus the results of this investigation are evidence that intraperitoneal injection of PN 200-110 in a dose of 2 mg/kg, against the background of stable EA, led to its suppression in most animals. The antiepileptic effect was expressed as a lower frequency of generation of ID and shortening of the time of existence of the focus of EA. Preliminary intraperitoneal injection of PN 200-110 in a dose of 5 mg/kg and intraventricular injection of 10 nmoles of this substance 20-25 min before creation of the focus of EA, caused an increase in the latent period of appearance of IID, a decrease in the number of ID, and shortening of the duration of existence of the focus.

These results showing the antiepileptic effect of PN 200-110 are in agreement with those obtained by other workers [6], who showed that preliminary (15 min beforehand) intraventricular injection of 1 nmole PN 200-110 prevented seizures induced by potassium channel blockers (mast cell degranulating peptide, dendrotoxin, 4-aminopyridine), if injected intraventricularly.

In the present investigation PN 200-110 was ineffective in a dose of 1 nmole, but in a dose of 10 nmoles it caused suppression of the penicin-induced focal EA in the rat cerebral cortex. These differences are probably linked with the different models of EA used to study PN 200-110.

The-most marked antiepileptic effect of PN 200-110 was weakening of ID generation. These distinguishing features of the antiepileptic effect were observed when we also used other anticonvulsants with calcium channel blockers [1, 2]. Incidentally, the genesis of ID is accompanied by a significantly greater fall of the extracellular Ca^{2+} concentration than the appearance of IID [7, 13]. This may perhaps be the reason why Ca^{2+} antagonists prevent the appearance primarily of ID. The predominant suppression of ID also was found in a study of the anticonvulsant action of anticonvulsants such as diazepam and diphenylhydantoin [3, 5], in whose complex mechanism of action, blockade of the calcium channels of neuron membranes is a component [14, 15].

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Zn^{2+}-, Cu^{2+}-CONTAINING SUPEROXIDE DISMUTASE IN BRAIN TISSUE OF RAT OFFSPRING EXPOSED ANTENATALLY TO ALCOHOL

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Exposure to alcohol and its metabolites in the antenatal period of development leads to various neurochemical disturbances in the brain tissue of the offspring in postnatal ontogeny [1, 4, 6]. We have suggested that one possible pathogenetic mechanism of the metabolic disturbances developing in the brain tissue of the offspring after antenatal exposure to alcohol may be increased production of free radicals, which exert a teratogenic action on the developing brain [3, 5]. An increase in free radical production in adult animals under the influence of alcohol has been demonstrated [7, 12]. Manifestation of the damaging effect of free radicals may be facilitated by exhaustion of the antioxidant protection reserves following exposure to alcohol [7, 13].

Zn^{2+}-, Cu^{2+}-containing (cytoplasmic) and Mn^{2+}-containing (mitochondrial) isozymes of superoxide dismutase (SOD) have been discovered in mammalian tissues. It has been shown [10, 14] that the greatest specific activity of SOD in brain tissue is due to the Zn^{2+}-, Cu^{2+}-containing isozyme. Changes in SOD activity in the brain tissue of the offspring, in cases of antenatal pathology, have been studied extremely inadequately. However, such research would be very promising in connection with the establishment of the pathogenetic mechanisms of development of inborn disturbances of the CNS and the development of corrective methods.

The aim of this investigation was to study activity of Zn^{2+}, Cu^{2+}-containing SOD in the brain tissue of the offspring of rats exposed antenatally to alcohol, during postnatal development.

EXPERIMENTAL METHOD

Experiments were carried out on 32 young Wistar rats aged 14 and 30 days. The mothers of the experimental animals consumed 12% ethanol (on average 8 g/kg body weight/day) as the sole source of fluid throughout pregnancy. Control and experimental animals were kept on the standard animal house diet. The rats were decapitated at the appropriate age, the brain was removed, and the cerebral cortex, hippocampus, and brain stem (mesencephalon plus diencephalon together) were separated. All operations with the brain were carried out at 0°C.

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