weakened by cycloheximide [1]. This suggests that experiments on identified neurons of Helix lucorum is an adequate model for the pharmacological analysis of trace processes in the nervous system.

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EFFECT OF VERAPAMIL ON FOCAL EPILEPTIC ACTIVITY IN THE RAT CEREBRAL CORTEX

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KEY WORDS: penicillin; Ca^{++} ions; epileptic activity; generator of pathologically enhanced excitation; verapamil

An important role in the hyperactivation of neurons which collectively constitute a generator of pathologically enhanced excitation (GPEE) [3] is played by a number of factors, including Ca^{++} ions. During the development of epileptic activity (EA), produced by different methods, the inflow of Ca^{++} into neurons from the extracellular medium and its release into the cytoplasm from the internal depots are intensified [1, 2]. Under these conditions blockers of intracellular calcium inflow may have a protective action.

The writers showed previously [4] that the development of generalized EA is accompanied by inactivation of the Ca pump of the synaptic membranes, with which an increase in the intracellular Ca^{++} concentration and the development of pathological hyperreactivity of the neurons may be connected. It has been suggested that inhibitors of Ca channels may be anticonvulsants.

The aim of this investigation was to study the effect of verapamil (izoptin), a blocker of voltage-dependent Ca channels, on focal EA induced by application of penicillin to the rat cerebral cortex.

EXPERIMENTAL METHOD

Experiments were carried out on 32 control and 32 experimental male Wistar rats weighing 200-220 g. Under hexobarbital (150 mg/kg, intraperitoneally) and local procaine anesthesia, burr-holes measuring 2 × 4 mm were drilled in the animal's skull in symmetrical regions of the sensomotor cortex of both cerebral hemispheres 24 h before the experiment, the dura was removed from these regions, and monopolar silver cortical electrodes were applied to record

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TABLE 1. Effect of Verapamil on Focal EA Induced by Penicillin Application in Concentration of 12,000 IU/ml to the Rat Cerebral Cortex (Series I; M ± m)

Group of animals	No. of animals	Before injection			After injection			Duration of existence of focus, min
		number of discharges per minute	amplitude of discharges, μV	power of focus, relative units	number of discharges per minute	amplitude of discharges, μV	power of focus, relative units	
1- (Control — physiological saline)	15	11,6±0,7	810±27	9296±415	11,7±0,4	812±21	9307±379	95±5,6
2- (Verapamil, 5 mg/kg)	7/11	16,1±1,3	610±23	9644±476	10,9±1,2**	443±54**	5689±606*4	75±7,2*
3- (Verapamil, 10 mg/kg)	9/11	11,4±0,9	840±30	9467±388	6,9±1,2***	481±59*4	4837±625*4	70±6,7*

Legend. Here and in Table 2: numerator gives number of animals in which verapamil caused suppression of EA; denominator gives total number of animals in the group. *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001 compared with corresponding parameters before injection.

TABLE 2. Effect of Verapamil in a Dose of 10 mg/kg on Focal EA Induced by Penicillin Application in Concentration of 20,000 IU/ml to Cerebral Cortex (Series II; M ± m)

Group of animals	No. of animals	Before injection			After injection			Duration of existence of focus, min
		number of SD in 1 min	amplitude of SD, μV	number of PD in 1 min	number of SD in 1 min	amplitude of SD, μV	number of PD in 1 min	
1-(Control	10	12,8±1,3	956±41	0,56±0,06	12,9±1,3	952±45	0,57±0,07	150±16
5-(Experiment)	8/10	13,3±0,8	934±37	0,51±0,05	10,0±1,3*	806±67	0,030±0,07**	105±17*

Legend. *p < 0.05, **p < 0.02 compared with corresponding parameter before injection.

electrical activity from those cortical regions (ECoG). The reference electrode was implanted into the nasal bones. The sternal leads of the electrodes were secured to the surface of the skull by means of "Noracryl" dental paste and a capsule formed around the burr-holes. To prevent the exposed areas of the brain from drying the capsule was filled with physiological saline and covered with waterproof film, which was fixed around its edges with Noracryl. Next day, to create foci of EA, the film was removed from the capsule and a filter paper soaked with a solution of the sodium salt of benyzlpenicillin in a concentration 12,000 IU/ml (series I) and 20,000 IU/ml (series II) was applied to the exposed areas of cerebral cortex. The ECoG was recorded on an RM-86 polygraph (Nihon Kohden, Japan) simultaneously from two unrestrained animals with foci of EA: verapamil (izoptin, from LEK, Yugoslavia) was injected into one of them, physiological saline into the other. Verapamil was injected in doses of 5 and 10 mg/kg, intraperitoneally, when stable EA had developed in the focus.

The experimental results were analyzed on an M-44 computer complex (Olivetti, Italy). The amplitude-frequency characteristics and duration of existence of the foci were determined. The power of the epileptogenic focus was calculated by multiplying the discharge frequency and amplitude, and expressed in relative units. For statistical analysis of the data, only those animals in which verapamil suppressed EA were taken into account.

EXPERIMENTAL RESULTS

As the preliminary experiments showed, application of penicillin (12,000 IU/ml) led to the appearance of EA after 5-7 min: against the background of the spontaneous ECoG separate epileptic spike discharges (SD) appeared, and their amplitude gradually increased. After 15-20 min, EA stable in frequency and amplitude was generated in the newly formed epileptogenic focus, and this continued for 25-35 min, after which the amplitude and frequency of the discharges declined. The mean duration of existence of the epileptogenic foci after the appearance of the first SD until their complete disappearance was 95 min.

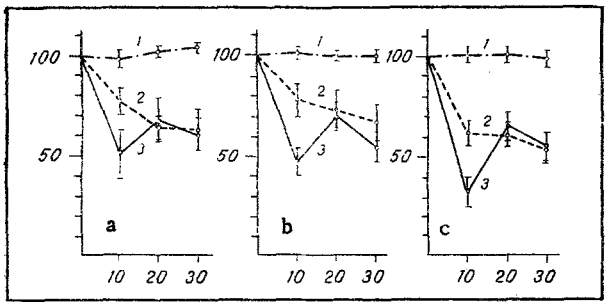

Fig. 1. Effect of verapamil on number (a) and amplitude (b) of SD, and power (c) of epileptogenic focus, induced by application of penicillin in a concentration of 12,000 IU/ml. Abscissa, time after injection of verapamil (in min); ordinate, parameters tested (in %, initial values before injection of verapamil taken as 100). 1) Control; 2, 3) verapamil in doses of 5 and 10 mg/kg respectively.

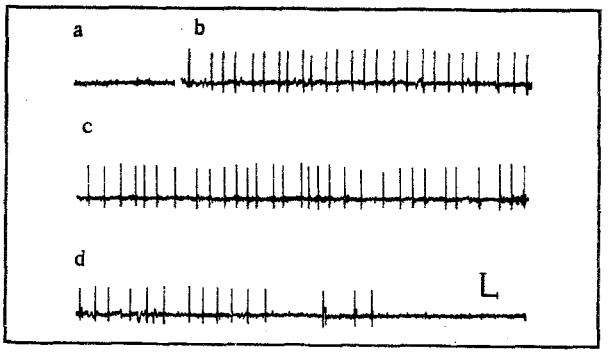

Fig. 2. Changes in electrical activity in epileptogenic focus induced by application of penicillin (12,000 IU/ml) to cerebral cortex of rats after intraperitoneal injection of physiological saline and verapamil. a) Background ECoG; b) 15 min after application of penicillin (stage of stable generation of SD); c) 2 min after injection of physiological saline; d) 2 min after injection of verapamil (10 mg/kg). Calibration: 500 μV, 5 sec.

The character of EA after intraperitoneal injection of physiological saline into animals of the control group (Table 1) did not change significantly. In some animals (32%) a very small decrease in frequency of generation and amplitude of SD could be observed during the first 3 min after the injection.

Injection of verapamil in a dose of 5 mg/kg against the background of stable EA in the focus (15-20 min after penicillin application) caused suppression of EA in 64% of cases, while in four rats (36%) it had no effect. Suppression of EA was expressed as a decrease in the amplitude and frequency of discharge generation and also the power of the epileptogenic focus (Table 1). The effect of suppression of EA after injection of verapamil in the dose mentioned was most marked 20-30 min after its injection (Fig. 1). Complete suppression of EA was observed in one animal 26 min after injection of verapamil. The duration of existence of the epileptogenic foci was shorter ($p < 0.05$) than in animals of the control group.

Verapamil in a dose of 10 mg/kg (Table 1) caused more marked suppression of EA (in 82% of cases). The inhibitory effect of verapamil in animals of this group was most marked in the first few minutes after injection of the drug (compare Fig. 1 and Fig. 2). For instance, the frequency of generation and amplitude of discharges in the focus were reduced by 49 and 52% respectively and the power of the epileptogenic focus was reduced by 68%. The duration of existence of the epileptogenic foci in the animals of this group was less than in the control ($p < 0.05$).

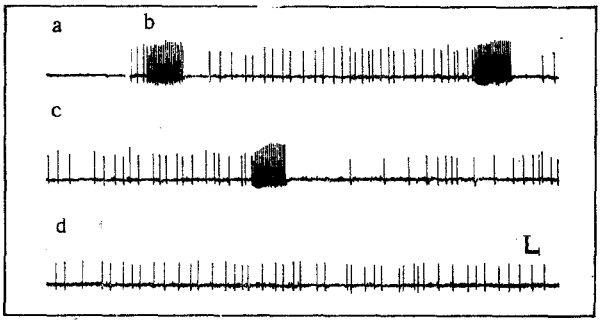

Fig. 3. Changes in electrical activity in epileptogenic focus caused by application of penicillin (20,000 IU/ml) to the cerebral cortex of rats receiving an intraperitoneal injection of verapamil. a) Background ECoG; b) 25 min after application of penicillin (stage of well defined PD); c, d) 10 and 25 min after injection of verapamil (10 mg/kg). Calibration: 500 μV, 10 sec.

Experiments of series II were carried out on animals with more powerful foci of EA, induced by application of penicillin in a concentration of 20,000 IU/ml. In these animals, the appearance of the first SD was recorded after 3-7 min on the ECoG, and after 6-15 min paroxysmal discharges (PD) appeared — bursts of high-frequency and high-amplitude hypersynchronized discharges. PD appeared regularly in the focus. The total duration of existence of the epileptogenic focus was 150 min. In animals of the control group, intraperitoneal injection of physiological saline did not affect the character of EA in the focus 25-30 min after application of penicillin at the height of development of paroxysmal activity (Table 2).

Injection of verapamil in a dose of 10 mg/kg at the height of development of paroxysmal activity reduced the frequency of appearance of PD in 80% of the animals (Table 2; Fig. 3), while in 30% of rats PD were completely suppressed. In all cases the frequency of generation and the amplitude of SD were reduced. The mean duration of existence of the epileptogenic focus was shortened ($p < 0.05$).

The results of these investigations on weak (series I) and strong (series II) epileptogenic foci caused by application of different concentrations of penicillin, with a different character of their EA, are evidence that in a large majority of animals verapamil had an antiepileptic effect, expressed as a decrease in the frequency of generation and the amplitude of SD and a decrease in the frequency of appearance of PD. The results showing the antiepileptic action of the calcium antagonists are in agreement with results obtained by other workers who used different calcium antagonists and different methods to administer them [9, 13].

Although the effects of verapamil may not be limited to blocking of Ca channels, nevertheless it can be tentatively suggested that the antiepileptic effect of this drug is mainly attributable to its ability to block Ca^{++} inflow into the neuron. This conclusion is in agreement with the results of a previous study [4], which showed that with the onset of EA the Ca-pump of the synaptic membranes is inactivated. According to data in the literature [8], verapamil passes with difficulty through the blood-brain barrier (BBB). However, we know [5] that during EA the permeability of the BBB is increased. This may be connected with the rapid appearance of the antiepileptic effect of verapamil after its intraperitoneal injection.

Verapamil and other blockers of Ca channels are widely used in cardiologic practice for the treatment of disturbances of cardiac rhythm (supraventricular tachyarrhythmias), and to limit ischemic lesions associated with myocardial infarction [6, 10-12], etc. Ectopic foci of excitation in the myocardium are similar in their molecular-membrane mechanisms to foci of hyperactivity of brain neurons. Both are GPEE [3], in whose mechanisms of origin an important role is played by an excess of free Ca^{++} in the cells. Verapamil therefore suppresses not only disturbance of the cardiac rhythm, but also EA in the cerebral cortex. In this connection it must be noted that certain classical antiepileptic drugs such as phenytoin, carbamazepine, and phenobarbital, can also block the Ca-channels of neuronal membranes [7, 14].

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PHARMACOKINETICS OF ETHANOL WHEN INJECTED INTRAPERITONEALLY AND INTRAVENOUSLY INTO RATS DIFFERING IN INITIAL ALCOHOL MOTIVATION

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KEY WORDS: ethanol; predisposition; pharmacokinetics

It was shown previously that animals differing in their initial level of alcohol motivation differ in their rate of elimination of ethanol when injected intraperitoneally [2, 3]. A distinguishing feature of these groups of animals was found to be differences in their ethanol absorption constant [3].

To determine the importance of absorption of ethanol for its elimination, the pharmacokinetics of ethanol was studied in the blood of rats differing in their initial predisposition to the formation of alcohol motivation.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male laboratory albino rats weighing 180-200 g, divided initially into two groups depending on the duration of ethanol narcosis (dose of ethanol 4.5 g/kg of a 25% solution, intraperitoneally), and possessing different levels of initial alcohol addiction [1]. Two groups of rats were selected for the experiments: short sleepers — predisposed to alcohol consumption with a mean duration of ethanol narcosis of 72.8 ± 13.8 min (group 1) and long-sleepers — not predisposed to alcohol consumption, with a mean duration of ethanol narcosis of 141 ± 7.5 min (group 2). After the study of the duration of ethanol narcosis, the animals were kept for 3 days in communal animal house cages so that their metabolism could become normalized, after which the pharmacokinetics of their blood ethanol was determined. The kinetics of ethanol was studied 15 and 30 min and

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