DISTURBANCE OF REGULATION OF OPHTHALMOTONUS BY A GENERATOR OF PATHOLOGICALLY ENHANCED EXCITATION CREATED IN THE HIPPOCAMPUS

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The creation of a generator of pathologically enhanced excitation (GPEE) in certain structures of the CNS induces hyperactivation of those structures and converts the corresponding physiological system into a pathological system [8]. This approach has been used to develop methods of neuropathological syndromes relating to different spheres of CNS activity [8]. Since an important role in the development of regulation of ophthalmotonus is played by changes in the functional state of the limbic system [3, 10, 12, 14, 17, 18], it has been suggested that disturbances of the regulation of intraocular pressure (IOP) can be reproduced by creating a GPEE in structures of the limbic system. It was shown previously that electrical stimulation (ES) of the hippocampus causes changes in ophthalmotonus [9] during the period of stimulation.

The object of this investigation was to study the state of IOP during creation of long-term GPEE in the hippocampus and other structures of the limbic system.

EXPERIMENTAL METHOD

Experiments were carried out on 155 Chinchilla rabbits weighing 2-3 kg and aged from 8 months to 2 years. The GPEE was created by two methods. The first method was based on the fact that tetanus toxin (TT) disturbs various kinds of inhibition in the CNS and may have a distinctive kind of depolarizing effect on the membrane, as a result of which a neuron population with insufficiency of its inhibitory mechanisms will be converted into a GPEE [5, 6, 8]. The second method is by ES of the limbic structures. Creation of a GPEE by ES is based on Kindling's [16] phenomenon.

In the first case a needle electrode was filled with TT, introduced stereotaxically in accordance with assigned coordinates, and fixed with acrylic glue. TT was injected slowly over a period of 30 min in a dose of between 25 and 80 mouse MLD in a volume of 4×10^{-4} ml. A dose of under 25 MLD did not disturb the regulation of ophthalmotonus whereas a dose of over 80 MLD caused death of the rabbit from convulsions. In control experiments inactivated TT was injected.

ES of the limbic structures was carried out daily for 2-5 months. The parameters of ES were: duration of stimulus 0.5 msec, strength 2-3 V, frequency 60 Hz, duration of stimulation 1 min. Electrodes were inserted under hexobarbital anesthesia (50-70 mg/kg, intraperitoneally) into the dorsal (H 6 mm, α 6 mm, P 6 mm) and ventral (H 15-17 mm, α 6 mm, P 9 mm) hippocampus. These electrodes were used for monopolar recording of action potentials. To record electrical activity (EA) a Soviet EEG-P-02 and Hungarian MB-52-02 electroencephalograph and a Hungarian MB-52-04 analyzer were used. The location of the electrodes was verified histologically.

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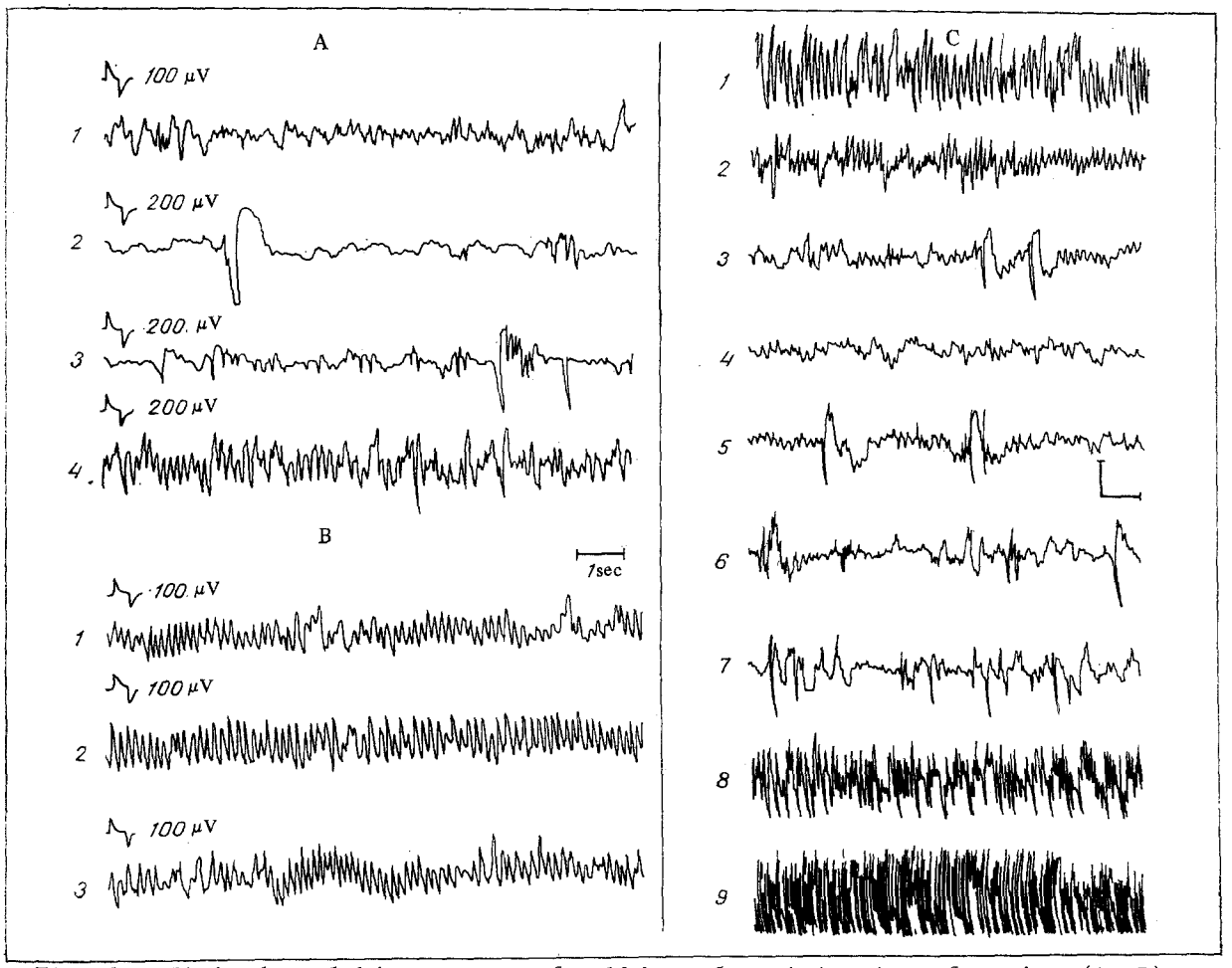

Fig. 1. EA in dorsal hippocampus of rabbits after injection of native (A, B) and inactivated (C) TT into it. A) EA in dorsal hippocampus 7 (1), 12 (2), 20 (3), and 43 days (4) after injection of TT; B) EA in dorsal hippocampus 7 (1), 15 (2), and 30 (3) days after injection of inactivated TT. C) Various types of recorded activity of GPEE created by injection of TT into dorsal hippocampus. Calibration: amplitude 300 μV, time 1 sec.

To evaluate the state of ophthalmotonus the following methods were used: tonometry (using Maklakov's tonometer weighing 7.5 g), observing the recommendations in [7, 15], elastotonometry by the method in [12], investigation of the ocular hydrodynamics by the method in [1, 5], and tests involving the use of drugs acting on the autonomic nervous system.

EXPERIMENTAL RESULTS

A GPEE was created by means of TT in 65 animals. The first changes in the hippocampus were observed on the 2nd-3rd day after injection of TT in this dose. They were characterized by disorganization of the natural theta-rhythm. Starting with the 7th-10th day, potentials of spike-wave type began to appear against the background of the disorganized theta rhythm, and their amplitude reached 1 mV (Fig. 1A). Activity was inhibited between spikes. After 30-40 days a relative regular theta-rhythm was restored. Against its background spikes or potentials of the spike-wave type still continued to appear; these epileptiform discharges disappeared after 60-80 days.

In experiments with inactivated toxin no abnormality was found in hippocampal EA at the same times as after injection of native TT (Fig. 1B).

Activity of the GPEE could be different in different cases (Fig. 1C) depending on the dose of TT and individual reactivity of the animals. Bursts of hippocampal electrical activity were accompanied as a rule by seizures. Such animals usually died in status epilepticus.

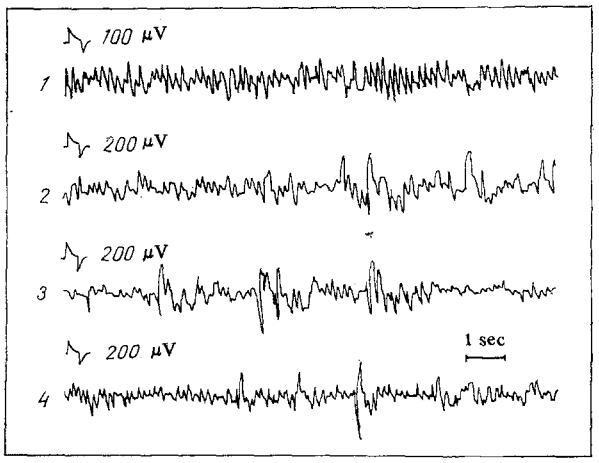

Fig. 2. EA in dorsal hippocampus during chronic ES in a rabbit. 1) Before ES, 2-4) on 8th, 17th, and 35th days of ES respectively.

A GPEE was created by means of ES in 25 animals. Changes in EA appeared in the zone of stimulation starting from the 7th-8th day after stimulation, the amplitude of the theta-rhythm was reduced, its frequency was increased, and against the background of these changes in the theta-rhythm single potentials of the spike-wave type, with an amplitude twice as great as that of the basic rhythm, appeared (Fig. 2). The theta-rhythm was disorganized 12-15 days after the beginning of stimulation, potentials of the spike-wave type were more clearly marked and occurred at a higher frequency, and the amplitude of the spike potentials was increased to 1 mV. Gradual normalization of EA usually began 7-10 days after cessation of daily stimulation.

Similar changes in EA were observed when a GPEE was created in other structures of the limbic system (subiculum, presubiculum, parasubiculum, entorhinal cortex), but the intensity of activity of GPEE was much less. When EA was recorded in the sensomotor and visual cortex, asynchronous potentials of spike and spike-wave types were observed. By the 15th-17th day these potentials appeared synchronously with spike potentials in the hippocampus. During decline of the GPEE in the hippocampus, toward the 40th-50th day, spike potentials in the cortex were extinguished. If powerful GPEE developed rapidly in the hippocampus, epileptiform activity occurred in the cortex as early as on the 2nd-3rd day. In these cases rapid generalization of the process took place and clonic and tonic convulsions appeared. Characteristic changes in the animals' behavior were straining, excessive motor activity, reactions of fear, aggressiveness, and so on.

Disturbance of the regulation of ophthalmotonus was similar in character during the action of all the factors described above on the hippocampus and on other structures of the limbic system. However, they were most marked when a GPEE was created unilaterally in the dorsal hippocampus.

Tonometry on 92 eyes (46 rabbits) showed a significant change of pressure in both eyes after creation of a GPEE by means of TT in the right dorsal hippocampus.

Three phases of disturbance of regulation of IOP were observed: the first was characterized by some decrease in IOP and it lasted 5-12 days, the second by an increase in IOP and fluctuations thereof for 4-28 weeks, the third by gradual normalization of IOP in the course of 5-8 weeks. The initial level of ophthalmotonus was 18.2 ± 0.04 mm Hg for the right eye and 18.8 ± 0.4 mm Hg for the left eye. In the first phase it was 16.9 ± 0.23 and 17.4 ± 0.25 mm Hg respectively. The decrease in IOP in the first phase was significant in both eyes, but its fluctuations were statistically significant only in the right eye (on the side of the GPEE). In the first phase the character of the elastotonometric curve (ETC) was modified. Statistically significant shortening of the rise of ETC (by 2.4 mm Hg) and lowering of its initial part (from 15.8 ± 0.25 to 14.3 ± 0.5 mm Hg, $P < 0.01$) were observed in the left eye; in the right eye there was only a tendency for the ETC to fall. In the first phase an increase in production of aqueous humor (by 80%) and an increase in the coefficient of ease of drainage (by 110%) were observed. Becker's coefficient was almost halved (reduced by 47.4%), whereas Mertens' coefficient was increased by one-third (33.3%).

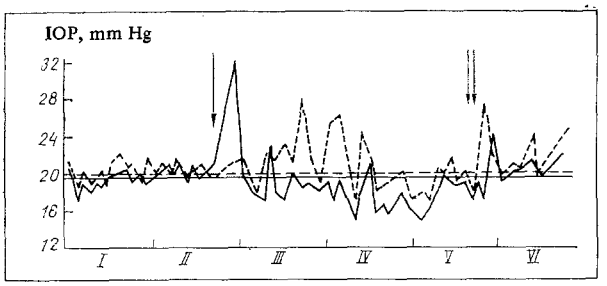

Fig. 3. IOP before and after creation of GPEE with the aid of TT and after ES of the right dorsal hippocampus. Single arrow — time of creation of GPEE by injection of TT, double arrow — ES of hippocampus in which a GPEE had been created previously, after return of IOP to normal.

The second phase of change in IOP was characterized by a statistically significant increase in the IOP level in the right eye by 0.55 mm Hg ($P < 0.05$) and in the left eye by 1.44 mm Hg ($P < 0.01$), i.e., the rise in the level of ophthalmotonus was greater in the contralateral eye. Fluctuations in IOP in the right eye increased from 12.8 $\pm$ 0.88% to 26.0 $\pm$ 3.7% and in the left eye from 11.5 $\pm$ 0.7 to 23.5 $\pm$ 3.04%; the beginning of the ETC was raised by 1.19 mm Hg in the right eye ($P < 0.01$) and by 2.15 mm Hg in the left eye ($P < 0.01$). Shortening of the rise of the ETC for the right eye was observed, by 1.9 and 2.7 mm Hg respectively, i.e., from 10.0 $\pm$ 0.19 to 8.1 $\pm$ 0.68 mm Hg and from 10.3 $\pm$ 0.22 to 8.1 $\pm$ 0.95 mm Hg. In the second phase an increase in the production of aqueous humor by 174.5% was observed, the coefficient of ease of drainage increased by 86.4%, Mertens' coefficient increased by 123.3%, and Becker's coefficient decreased by 55.2%.

During the period of clinical normalization of ophthalmotonus, additional ES was applied to the hippocampus in which a GPEE had been created previously (through the needle electrode which remained *in situ*). In six rabbits the level of IOP rose again (Fig. 3), its fluctuations increased, and the ETC and the results of the tests with drugs acting on the automatic nervous system became pathological in character. These disturbances of IOP regulation occurred as early as on the 2nd-3rd day after the beginning of ES (i.e., much sooner than in intact animals), and their latent period was shortened by 3-5 times.

Creation of a GPEE in structures of the limbic system thus leads to disturbance of the regulation of ophthalmotonus. Particularly marked changes were observed when the GPEE was created in the hippocampus.

These results confirm, under conditions of the model, the pathogenetic role of changes in activity of the limbic system in the disturbance of regulation of ophthalmotonus.

The results of these investigations on this new model confirm the general concept of the role of hyperactive determinant structures (the GPEE) in CNS pathology [8]. In this case it is a question of the pathology of regulation of an autonomic function. The disturbance of ophthalmotonus which, as we know, frequently precedes development of glaucoma, can thus be classed as one of the diseases of regulation [8].

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EFFECT OF CHRONIC STRESS ON ULTRASTRUCTURE OF THE MYOCARDIUM AND HYPOTHALAMUS IN EMOTIONAL AND UNEMOTIONAL RATS

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Emotional-stress states often lie at the basis of development of many cardiovascular, nervous, and mental diseases. The severity of the pathological processes depends on individual characteristics and on differences in the type of response to stress. Different species and lines of experimental animals differ considerably in their response to a stress situation and in their sensitivity to psychotropic drugs [2, 3]. The study of the pathological manifestations of emotional stress in vitally important organs (myocardium, nervous system) and their dependence on individual characteristics is of great importance for the oriented pharmacological correction of these states.

In the investigation described below ultrastructural changes in the myocardium and hypothalamus of rats with different levels of emotional-behavioral reactivity, induced by chronic stress, were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male rats weighing 200 ± 26 g. A state of chronic emotional stress was produced in the animals by prolonged (7 days) selective deprivation of their rapid phase of sleep by Jouvet's "small platform" method [13]. Somatic responses to stress were assessed by counting the number of ulcers in the gastric mucosa and the change in body weight and in weight of the adrenals and thymus. Emotional-behavioral responses after the end of stress were studied in versions of the "open field" method, a dark chamber with holes, and the response to a moving object, and were estimated quantitatively and on a scale of points. Tissue from the left ventricle and anterior lobe of the hypothalamus (the region of the supraoptic and paraventricular nuclei) for electron-microscopy were fixed in 1% OsO_4 solution, dehydrated, and embedded in Araldite. Electron micrographs were obtained with the IEM-100B microscope. The number of glycogen granules was

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