THE HIPPOCAMPUS AS A DETERMINANT STRUCTURE GENERATING EPILEPTIC ACTIVITY DURING METRAZOL KINDLING

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Repeated administration of metrazol in a subthreshold dose to animals has been shown to increase their sensitivity to an epileptogen, to enhance epileptic activity (EpA), and to cause the development of pharmacological kindling; administration of a subthreshold dose of metrazol into such animals causes generalized clonicotonic convulsions [7]. Analysis has shown [4] that in developed metrazol kindling the seizure threshold for epileptogens which are GABA antagonists is depressed. Meanwhile, the pathogenetic mechanisms of this form of experimental pathology of the CNS largely remain unexplained. In particular, it is not yet clear whether epileptization has the character of primary generalized activity, spreading simultaneously to all brain structures, or whether a determinant structure is first formed [2], and subsequently leads to generalization of EpA. Data in the literature on the possibility of development of metrazol kindling are contradictory in character [15, 16].

The aim of this investigation was to study changes in electrical activity in the cortex and certain subcortical brain structures in rats during the development of metrazol kindling.

EXPERIMENTAL METHOD

Experiments were carried out on 48 male albino rats weighing 250-350 g. The preparatory operation and insertion of the electrodes were carried out under hexobarbital anesthesia (100 mg/kg, intraperitoneally). Multipolar constant electodes (diameter of tip 0.1 mm) were inserted stereotaxically, taking coordinates from the atlas [14], into the sensomotor cortex and various deep brain structures (dorsal hippocampus, amygdala, rostral part of the caudate nucleus, mesencephalic reticular formation, ventrolateral thalamic nucleus, dentate nucleus of the cerebellum). The reference electrode was fixed in the frontal bond. After the operator, the animals were kept in individual cages with alternation of light and darkness every 12 h. The animals were transferred one week after the operation for a period of testing into a glass box (35 × 30 × 30 cm). After preliminary recording of the EEG, the animals received a subthreshold dose of metrazol (25 mg/kg), intramuscularly daily (between 9 a.m. and noon), and their EEG was recorded under unrestrained conditions. Animals of the control group were given an injection of physiological saline. Convulsions were expressed in points, on the following scale: 0 point) no seizure response, 1 point) myotonic twitches of the head or trunk, 2 points) clonic convulsions of the forelimbs, 3 points) standing up on the hind limbs (kangaroo posture) or powerful repeated clonic convulsions, 4 points) clonicotonic convulsions with the animal falling onto its side, 5 points) lethal convulsions or repeated fallings onto the side. The power of EpA was determined as the product of the mean frequency of epileptic dischargesand their mean amplitude over a period of 15 sec. Diazepam, in a dose of 0.2-1.5 mg/kg, was injected intraperitoneally 30 min before injection of metrazol. Animals of the control group received an injection of dimethylsulfoxide (the solvent of diazepam) in the same volume as diazepam. After the end of the experiments the location of the electrodes was determined histologically.

EXPERIMENTAL RESULTS

After the first injections of metrazol bursts of slow waves and spike-wave complexes were recorded in all leads with a frequency of 3-5 waves/sec for 2-6 sec, as has been described for metrazol and penicillin

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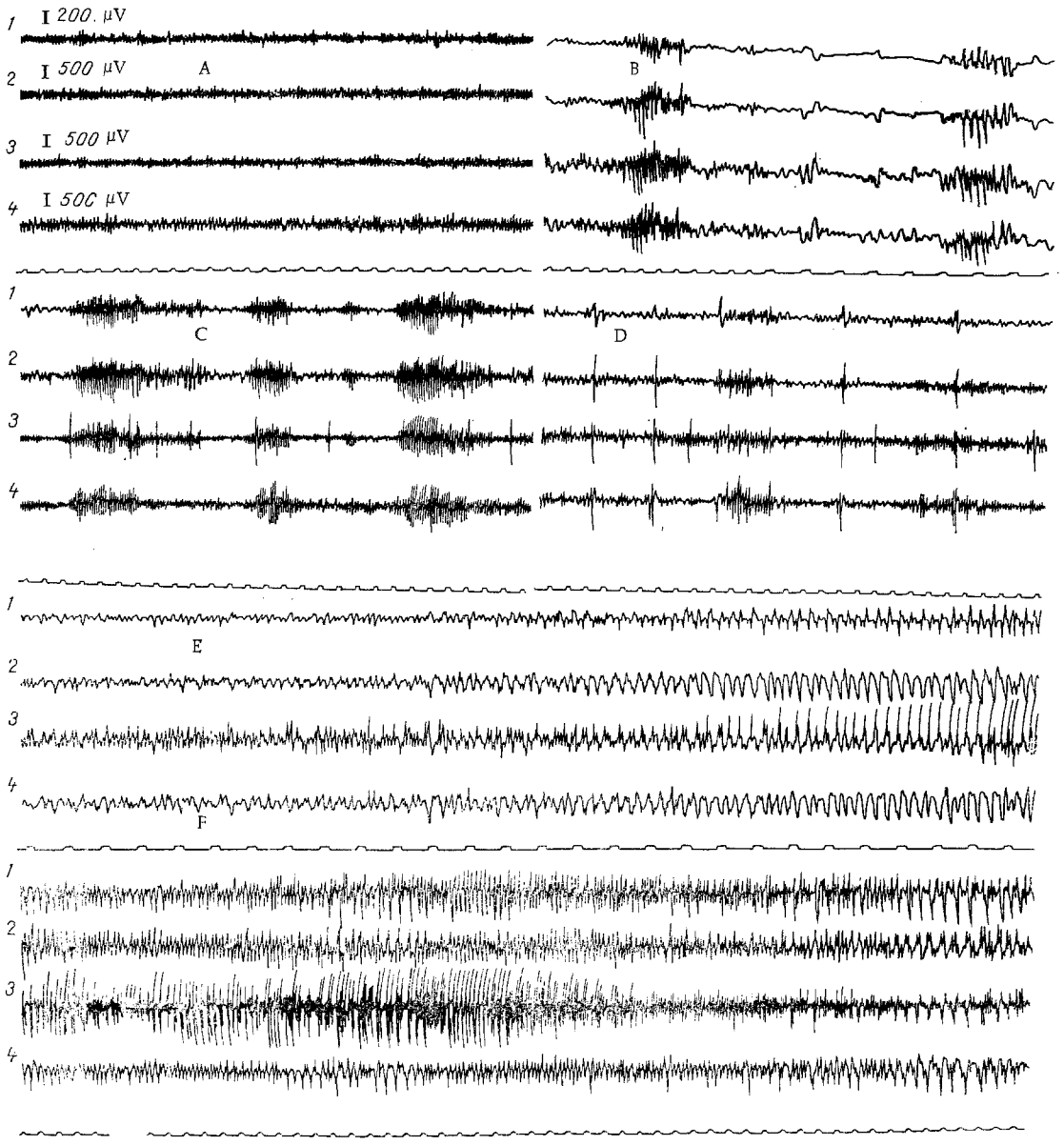

Fig. 1. Changes in brain electrical activity during development of metrazol kindling. A)Spontaneous activity; B-F) after 1, 4, 10, 16, and 20 injections of subthreshold doses of metrazol. 1) Sensomotor cortex, 2) amygdala, 3) dorsal hippocampus, 4) caudate nucleus. Time marker 1 sec.

[9, 16] (Fig. 1B). The changes in electrical activity observed were more marked in the deep brain structures than in the cortex, and they were observed for 15-35 min after injection of metrazol, after which they gradually decreased in amplitude and disappeared. During this period, the animals' behavior was marked by quiescence, accompanied by complete locomotor immobility. In the period between bursts of slow waves the rats sniffed the floor and walls of the chamber and stood up on their hind limbs. On the appearance of the above-mentioned EEG changes locomotor activity again ceased. After 3-5 injections of metrazol an increase in frequency and duration of the bursts of slow waves, combined with spike and spike-wave potentials, was observed (Fig. 1C). In all cases the first spike discharges appeared in the hippocampus (Fig. 1C, zone 3). The behavior of the rats during these EEG changes also was characterized by episodes of locomotor immobility and quiescence, accompanied by weak tremor of the head and lower jaw, chewing, and twitching of the muscles of the mouth and ears. The duration of these behavioral and EEG changes was increased up to several hours. In 6 experiments bursts of slow waves were recorded during 18-24 h after injection of metrazol. Subsequent injections of metrazol (8-12 injections) were accompanied by the appearance of spike potentials and spike-wave

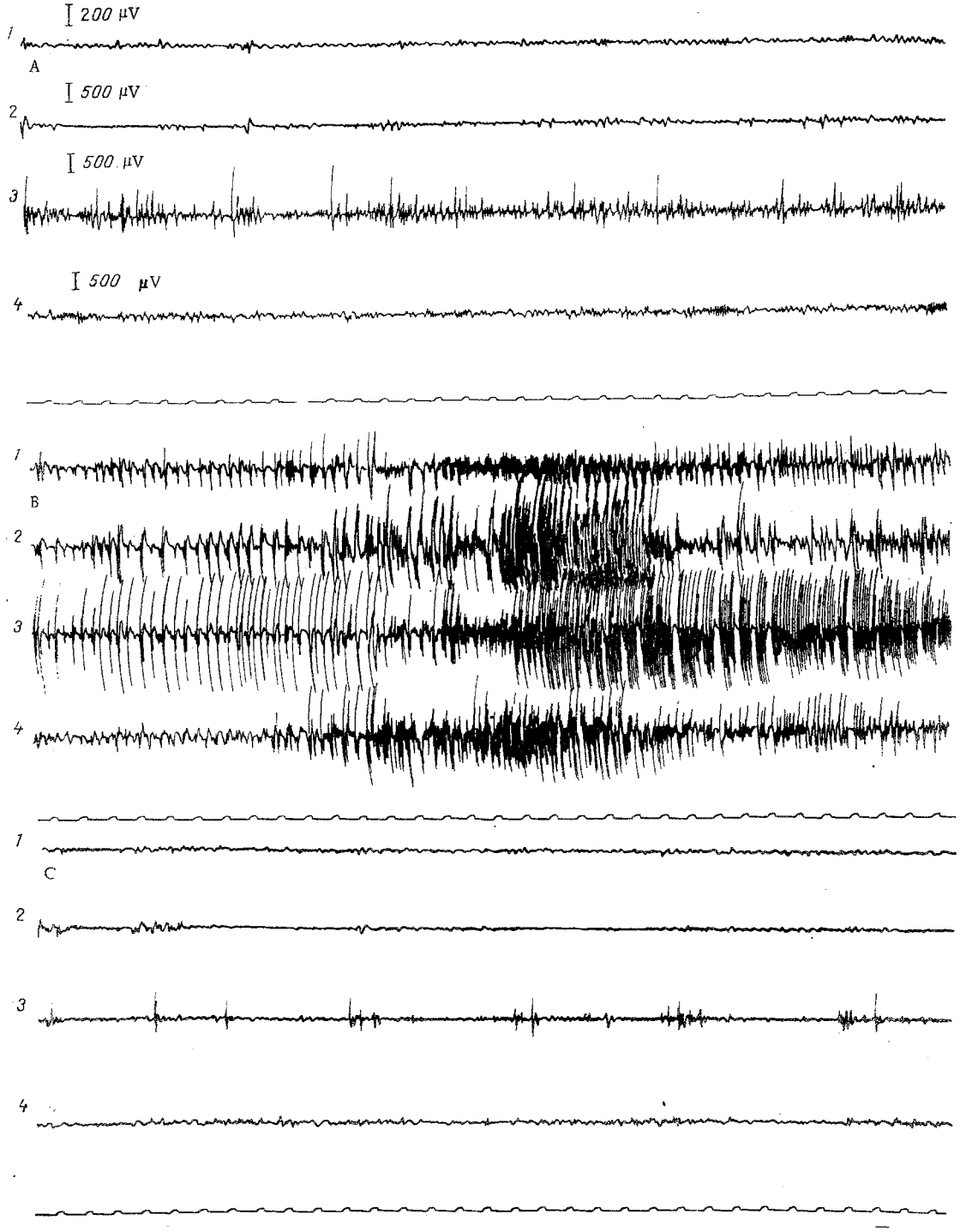

Fig. 2. Changes in brain electrical activity of rats with developed kindling during generalized convulsion. A) Brain electrical activity 24 h after 18th injection of metrazol, B) 4 min after 19th injection of metrazol, C) 1.5 min after B. 1) Sensomotor cortex, 2) caudate nucleus, 3) dorsal hippocampus, 4) dentate nucleus of cerebellum.

complexes on the EEG (Fig. 1D) and a simultaneous decrease in abundance of the bursts of slow waves. The rats' behavior was characterized by myoclonic twitches of the head and individual muscle groups and tonic convulsions of the trunk. As the state of predisposition to seizures progressed, changes were observed in the animals' behavior in the interictal periods: the rats tried to run away, they bit the lead wires, stepped backward, attacked other animals, and performed turning movements with their fore- and hind limbs. On the appearance of grouped high-amplitude spike or spike-wave potentials on the EEG with a frequency of 3 waves/sec

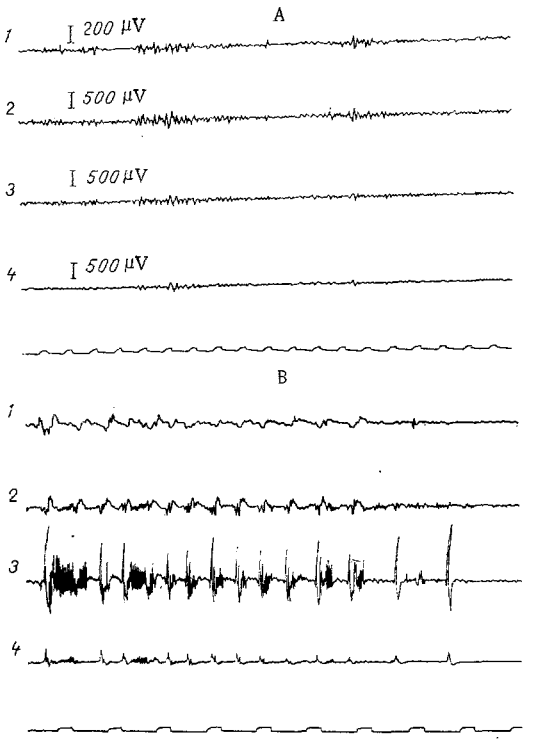

Fig. 3. Effect of diazepam on epileptic activity in rat with developed metrazol kindling. A) Brain electrical activity 30 min after injection of diazepam (0.5 mg/kg), B) 3 min after injection of metrazol (25 mg/kg). 1) Sensomotor cortex, 2) amygdala, 3) dorsal hippocampus, 4) caudate nucleus.

and a duration of between 8 and 25 sec (Fig. 1E), the rats' behavior were marked by clonic seizures of the forelimbs. During the development of these generalized convulsions, as a rule bursts of slow waves were absent on the EEG. An increase of frequency of high-amplitude seizure discharges of the EEG to 6-10 waves/sec (Fig. 1F) was accompanied behaviorally by seizures of the forelimbs, standing up on the hind limbs, and loss of balance and falling on the side. The duration of the generalized convulsions varied in different animals from 8 to 360 sec. After the end of the episode, postictal depression developed and was accompanied by marked inhibition of electrical activity in all brain structures. In 8 experiments repeated convulsions were observed, but as a rule they were shorter in duration than the previous convulsions. Comparison of the time of appearance of EpA in the different brain structures showed that in 14 of 21 cases the first signs of seizure activity, with which the generalization began, were observed in the hippocampus (Fig. 1E). In the remaining experiments generalized EpA was observed to arise virtually simultaneously in all structures, but the amplitude of the spike potentials was always greater in the hippocampus. Investigation of the power of EpA (in conventional units) in different brain structures during the development of generalized convulsions showed that in the neocortex the power was 608.4±27.3, in the dentate nucleus of the cerebellum 871.7±77.5, in the caudate nucleus 1546.6±48.8, in the amygdala 1577.8±54.6, in the mesencephalic reticular formation 1822.1±105.4, in the ventrolateral thalamic nucleus 1901.3±76.4, and in the dorsal hippocampus 3056.1±91.2. Seizure discharges of the greatest power were thus found in the hippocampus.

Single spike potentials remained in the hippocampus of 4 rats after the end of the generalized convulsion. These interictal discharges in the hippocampus also were recorded during the next day (Fig. 2A). EpA was absent in other brain structures. Injection of a subthreshold dose of metrazol into these animals caused the appearance of a hypersynchronous seizure discharge in the hippocampus and in other brain structures (Fig. 2B). Seizure potentials in the hippocampus possessed greater power at the beginning of, and during, the convulsion. After the end of the convulsion seizure discharges remained in the hippocampus (Fig. 2C).

Previous investigations on various models of epilepsy [2, 3] showed that an epileptic focus that plays the role of determinant is most resistant, and under a general inhibitory influence it remains at that stage, when all other foci of EpA had already been suppressed. This technique was used in the present experiments,

also, to detect a determinant structure. Diazapam was used for this purpose and its effect on electroencephalographic and behavioral manifestations of seizure responses was investigated in animals with developed kindling. Diazepam in a dose of 0.5 mg/kg lowered the intensity of convulsions from 3.2±0.2 to 1.8±0.3 points. Parallel with the decrease in intensity of the behavioral seizures, a decrease was observed in the amplitude and frequency of the seizure discharges on the EEG. In all cases, however, marked EpA was recorded in the hippocampus (Fig. 3B).

The investigations thus showed that the first structure in which EpA is formed during metrazol kindling is the hippocampus. It can be tentatively suggested that it plays the role of pathological determinant, with which the subsequent formation of the whole pathological epileptic system is connected. This conclusion is confirmed by experimental data (the results will be published in a later communication), showing that preliminary destruction of the hippocampus leads to considerable delay in the development of kindling, whereas additional stimulation accelerates its formation. The present investigation showed that the highest power of EpA during development of the seizure convulsion was observed in the hippocampus. Spontaneous interictal seizure discharges also arose initially in the hippocampus. These results are in agreement with data in the literature showing that the hippocampus possesses the lowest threshold of excitability in response to the action of various epileptogens of all brain structures [6]. If kindling was induced by electrical stimulation, regardless of which structure of the limbic system was stimulated, the seizure discharges appeared initially in the hippocampus, and attained their maximal development in it after the shortest time [1]. Spontaneous EpA during electrically induced kindling also appeared first in the hippocampus and amygdala [10, 12]. The important role of the hippocampus in the genesis of epileptic seizures in man is confirmed by the results of clinical–physiological and morphological investigations [5]. Confirmation that the hippocampus plays the role of pathological determinant in the development of metrazol kindling was given by the preservations of EpA in the hippocampus when suppressed by diazepam in other brain structures.

The investigations also showed that two periods of behavioral and EEG changes can be distinguished conventionally in the development of metrazol kindling. The first period is characterized by the appearance of bursts of slow waves and spike-wave activity with a frequency of 3–5 waves/sec in the EEG in response to subthreshold doses of metrazol, corresponding behaviorally to periods of quiescence and single episodes of myoclonuc. These changes greatly resemble the picture of a minor epileptic fit in man, for only if the motor and EEG manifestations correspond to those described above is it possible to speak of fits of the petit mal type [13]. During the second period high-frequency polymorphic generalized seizure discharges appear on the EEG and are accompanied by generalized clonicotonic convulsions, hypersalivation, postictal depression, and other autonomic responses which accompany fits of the grand mal type. Clinical observations also show that under certain conditions of development of epilepsy, fits of petit mal type can change into fits of grand mal type [8, 11].

These investigations suggest that the pathogenetic basis of the development of increasing chronic epileptization of the brain during metrazol kindling, just as in kindling of other types, is the formation of a hyperactive determinant structure in the hippocampus [2].

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A MODEL OF MYOCARDIAL LESIONS OF VARIED SEVERITY

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To evaluate the therapeutic effects of drugs it is necessary to study their mechanism of action on models of pathological processes [2]. Both vascular and metabolic disturbances in the myocardium play very important roles in the development of isochemic heart disease [3, 6, 7]. However, experimental models of myocardial lesions combining disorders of the coronary circulation, hemocoagulation, and metabolism, and leading to pathological changes in the heart muscle, are still in an early stage of development and have not yet become widely used in pharmacological or other research.

The aim of this investigation was to develop experimental heart lesions corresponding to various clinical manifestations and suitable for studying the therapeutic efficacy of new cardiotropic drugs.

EXPERIMENTAL METHOD

Experiments were carried out on eight dogs weighing 6-11 kg, 61 rabbits weighing 2.6-3.2 kg, and 30 albino rats of both sexes weighing 200-240 g. The animals were divided into five groups: group 1 (13 rabbits) – myocardial lesions of micronecrosis type produced by subcutaneous injection of isoproterenol (5 mg/kg) and theophylline (20 mg/kg) once only, or twice with an interval of 24 h between them; group 2 (15 rabbits) – toxic myocarditis, produced by subcutaneous injection of isoproterenol (5 mg/kg) and caffeine (50 mg/kg), once only and twice with an interval of 24 h; group 3 (15 rabbits and 20 rats) – focal myocardial dystrophy, produced by subcutaneous injection of thyroxine (0.02 mg/kg) and isoproterenol into rabbits (5 mg/mg) and rats (75 mg/kg), once only and twice with an interval of 24 h; group 4 (10 rabbits) – microfocal myocardial infarction, produced by intravenous injection of 0.5 U/kg body weight of pituitrin, followed after 15 min by subcutaneous injection of isoproterenol (5 mg/kg), which was repeated 5-6 h later; 24 h after injection of pituitrin a repeated injection was given, and isoproterenol was injected in the same doses and in the same order; group 5 (eight dogs, eight rabbits, and 10 albino rats) – a myocardial infarct of macrofocal type was produced in dogs by intravenous injection of 0.3 U/kg of pituitrin, followed 20 min later by 3 mg/kg of isoproterenol; the latter injection was repeated in the same dose 6 h later, and 24 h after the first injection of pituitrin a second injection of 0.15 U/kg was given intravenously, after which isoproterenol was injected in the same dose and order as previously. Under aseptic conditions and under trimeperidine–thiopental anesthesia with controlled respiration, 24 h after the second injection of pituitrin the anterior interventricular branch of the left coronary artery was ligated below the origin of the first collateral. Pituitrin and isoproterenol were injected into the rabbits by the scheme in group 4, and the coronary artery was ligated without artificial respiration, by a strictly midline incision through the sternum without injury to the pleural cavity. Pituitrin was injected intraperitoneally into the rats

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