EFFECT OF DESTRUCTION OF THE HIPPOCAMPUS AND CAUDATE NUCLEUS ON DEVELOPMENT OF EPILEPTIC ACTIVITY ASSOCIATED WITH METRAZOL KINDLING

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Systematic injection of metrazol into animals in a subthreshold (not inducing convulsions) dose has been shown to lead to the progressive development of epileptic activity and the formation of pharmacologic (metrazol) kindling [5]. The first signs of epileptic activity with which generalization began were observed in the hippocampus. It has been suggested [3] that the hippocampus plays the role of pathological determinant [2], which is connected with the formation of a pathological epileptic system, that lies at the basis of metrazol kindling. We also know that the caudate nucleus is a structure of the antiepleptic system [2] and that its activation causes inhibition of epileptic activity [1, 4, 9, 12, 13]. In this investigation the effect of destruction of the hippocampus and caudate nucleus on the development of metrazol kindling was studied.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 250-350 g. The preliminary operation (insertion of electrodes, destruction of the hippocampus and caudate nucleus) was performed under hexobarbital anesthesia (100 mg/kg). Bilateral electrolytic destruction of the above-mentioned structures was carried out with silver electrodes 0.3 mm in diameter, inserted in accordance with data in the stereotaxic atlas [8]. Coordinates of the hippocampus were: AP = 2.0, L = 1.0, H = 4.5; of the caudate nucleus: AP = −1.0, L = 2.5, H = 4.0-5.5. A direct current of 4.5-5.0 mA was applied for 60 sec. After the operation the animals were kept in individual cages with alternation of light and darkness every 12 h. Daily for 3 days, starting 14 days after the operation, the animals were given an intraperitoneal injection of metrazol in a subthreshold dose (30 mg/kg), and their behavior was observed for 30 min after the injection. The intensity of the seizure responses was expressed in points [5]. Latent periods of the first manifest seizures, of marked convulsions (when the animal fell onto its side), and maximal convulsions, the frequency of tonic convlusions, of tonic extensions, and mortality, also were determined. After the end of the experiments the location of the destroyed brain structures was determined by comparing frontal brain sections with maps in the stereotaxic atlas [8]. The seizure threshold (SD_{50}) was determined by the method in [10]. The experimental results were processed by method of analysis of variance and nonparametric statistical tests. The location and volume of the lesion in the hippocampus and caudate nucleus are illustrated in Fig. 1, A, B.

EXPERIMENTAL RESULTS

After bilateral destruction of the hippocampus no change was observed in the seizure threshold compared with the control (Table 1). Systematic injection of metrazol caused an increase in sensitivity to the action of the convulsant and an increase in the intensity of the seizure reactions in animals of both groups (Fig. 2A). Myoclonic spasms and clonic convul-

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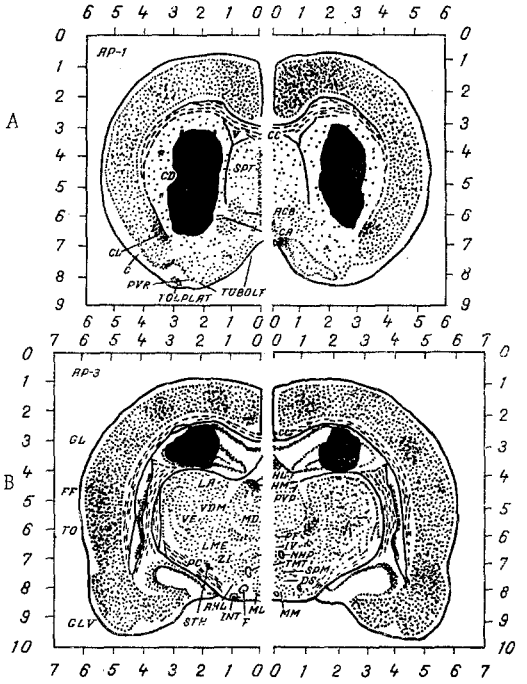

Fig. 1. Location of bilateral destruction lesions in the caudate nucleus (A) and hippocampus (B). Black areas denote zones of destruction (one typical case). Coronal sections through rat brain at AP = −1 (A), AP = +3 (B), according to the atlas [8].

TABLE 1. Effect of Destruction of Hippocampus and Caudate Nucleus on Changes in Seizure Threshold during Development of Kindling

Experimental conditions	SD_{50}, mg/kg	
	Before development of seizures	After development of seizures
1. Control	20,0 (17,1—23.4) n=24	12,0* (10,6—13,6) n=22
2. Destruction of hippocampus	20,0 (16,7—24,0) n=18 $P_{1-2}>0,05$	16,1 (14,2—18,2) n=14 $P_{1-2}<0,001$
3. Control	19.9 (17,2—23.1) n=22	11,8* (10,0—13,9) n=21
4. Destruction of caudate nucleus	15.0 (7,1—31.9) n=24 $P_{3-4}<0.05$	9,8* (8,6—11,0) n=18 $P_{3-4}>0,05$

Legend. Asterisk indicates significant differences between SD_{50} in each group before and after development of kindling seizures at the $P < 0.05$ level. n) Number of experiments.

sions appeared in the animals of both groups without any significant differences. After eight to 10 injections of metrazol rats of the control group developed generalized seizures, whereas in animals of the experimental group the first generalized seizures did not develop until after 12 to 14 injections of the drug. The average intensity of the seizure reactions in rats with destruction of the hippocampus remained less throughout the experiments than in intact animals. Starting with the 15th day of the experiments differences in the intensity of the seizures were statistically significant ($P < 0.001$).

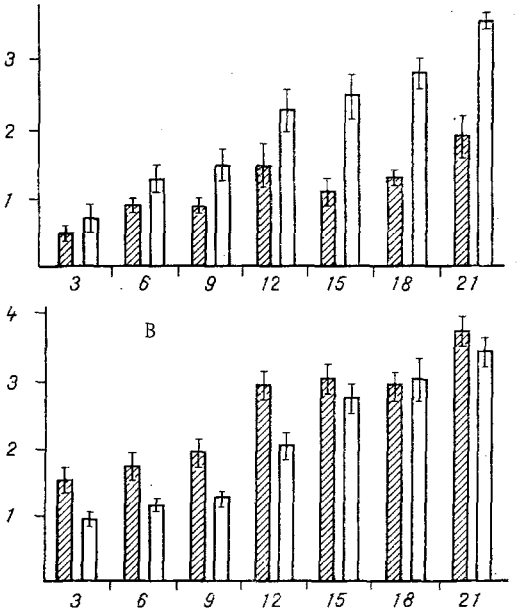

Fig. 2. Effect of destruction of hippocampus (A) and caudate nucleus (B) on development of metrazol kindling. Abscissa, duration of observations with daily injection of metrazol (in days); ordinate, intensity of seizure reactions (in points). Shaded columns indicate experiment, unshaded — control.

After 3 weeks of administration of metrazol most animals with destruction of the hippocampus showed myoclonic spasms and clonic convulsions; the mean intensitv of the seizure reactions in this case did not exceed 2 points. Generalized seizures (intensity 3-4 points) were observed in only four of the 14 rats. Seizures with an intensity of not less than 3 points were observed in all animals of the control group during this period. The mean duration of the generalized seizures in animals with destruction of the hippocampus was 28.0 ± 0.8 sec, and in animals of the control group it was 60.0 ± 0.5 sec ($P < 0.001$). Determination of the seizure threshold 2 weeks after the last injection of metrazol showed a significant fall in SD_{50} in animals of the control group, but no significant change in SD_{50} in animals with destruction of the hippocampus (Table 1). Investigation of the duration of the state of increased proneness to seizures revealed that the mean intensity of the seizure reactions in rats of the control and experimental groups 1, 2, and 3 months after the end of metrazol administration was 3.7 ± 0.1, 3.5 ± 0.2, and 3.7 ± 0.2 points respectively in the control, and 2.0 ± 0.4, 1.3 ± 0.2, and 1.0 ± 0.2 points in the experiments.

After bilateral destruction of the caudate nucleus, a significant lowering of the seizure threshold was observed in the animals (Table 1). Even after the first injections of metrazol, rats with destruction of the caudate nucleus exhibited marked starting movements and clonic convulsions involving fore- and hind limbs, and rhythmic spasms. From the 3rd through the 14th injections of metrazol the seizure reactions of animals with destruction of the caudate nucleus were significantly stronger than in the control. During subsequent injections of metrazol, with the appearance of generalized seizures, the mean intensity of the convulsions in animals of the two groups did not differ significantly (Fig. 2, B). However, the duration of the seizures in animals with destruction of the caudate nucleus averaged 72.0 ± 0.9 sec, significantly ($P < 0.001$) longer than the seizures in intact animals (57.0 ± 1.2 sec). Repeated seizures mainly of clonic convulsions, with strong myoclonic spasms, frequently appeared. In most animals, after the end of the generalized seizures, myoclonic shaking and starting movements were observed for a period of between 20-30 min and 1-2 h. Determination of seizure threshold after the development of kindling had ended demonstrated a significant fall in animals of both groups (Table 1), with no significant differences between experiment and control ($P < 0.05$).

Bilateral destruction of the hippocampus thus significantly retards the development of epileptic activity under metrazol kindling conditions. These findings confirm the conclusion drawn from previous investigations, that the development of epileptic activity during kindling is largely associated with the formation of a generator of pathologically enhanced excitation

in the hippocampus, where it plays the role of hyperactive determinant [3]. Mason and Cooper [11], who studied the effect of a hippocampal lesion on the development of metrazol kindling, found that destruction of the hippocampus increases the severity of seizures in rats and increases their sensitivity to the action of metrazol. The disagreement between our own results and those obtained by Mason and Cooper can be explained not only by the different technical conditions (dose of metrazol, frequency of injections, etc.), but also by the fact that those workers used unilateral destruction of the hippocampus. Such a lesion may perhaps lead to reciprocal and compensatory potentiation of activity of the contralateral hippocampus. The phenomenon of reciprocal inhibition of limbic structures of the right and left hemispheres when subjected to alternate electrical stimulation has been described during kindling induced by electrical stimulation [6, 7]. The fact that the state of increased proneness to seizures persists in animals with destruction of the hippocampus for a much shorter period of time after the end of metrazol administration compared with the time observed in animals of the control group, confirms the view that the hippocampus is the structure which maintains activity of the pathological epileptic system during kindling. Yoshida [14], who studied the effect of bilateral destruction of the hippocampus on the course of seizures induced by systematic electrical stimulation of the amygdala in rats, showed that destruction of the hippocampus causes a marked decrease in the intensity of seizures or their complete cessation in different animals. Yoshida also concluded that the hippocampus has a facilitatory and maintaining effect on kindling.

The results of the experiments with destruction of the caudate nucleus suggests that two mechanisms of its activation as an antiepileptic structure exist: activation by the epileptogen itself and activation by the seizure process. Destruction of the caudate nucleus evidently leads to enhancement of epileptic activity under the influence of metrazol, which was manifested by a considerable fall of the seizure threshold in the animals before systematic administration of metrazol began and by facilitation of the seizure reactions on the first few days of injection of the drug. At later stages of development of epileptic activity, however, when a state of increased proneness to seizures has already been formed, the direct effect of metrazol on the caudate nucleus has largely lost its triggering role. Formation of a state of increased proneness to seizures is evidence that the process of epileptization of the brain has overcome the antiepileptic mechanism effected by the caudate nuclei. Their role on this stage of the process was therefore diminished. Meanwhile even at this stage, in animals with destruction of the caudate nuclei more prolonged and often reaped seizures were observed, evidence of the inhibitory effect of the caudate nuclei on the seizure process.

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