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EFFECT OF DESTRUCTION AND ACTIVATION OF SOME LIMBIC STRUCTURES ON DEVELOPMENT OF SEIZURES AND EMOTIONAL DISTURBANCES IN PICROTOXIN KINDLING

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Pharmacologic kindling, caused by repeated injections of picrotoxin in a subconvulsive dose, and characterized by an increase in severity of seizure manifestations, also has been shown to lead to the formation of a syndrome of pathologically enhanced defensive behavior [6]. The hippocampus has been shown to play the role of determinant structure of the epileptic pathological system (PS) lying at the basis of development of the seizure syndrome during pharmacologic kindling [3, 4]. Structures of the amygdala also play an important role in realization of aggressive-defensive forms of behavior and in the development of the enhanced emotional response during kindling evoked by electrical stimulation of this structure [8].

The aim of this investigation was to study the effect of destruction of the dorsal hippocampus and the basomedial amygdala and also their activation on the development of seizures and emotional disorders associated with picrotoxin kindling.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 220-250 g. Each group consisted of at least 10 animals. The rats were anesthetized with pentobarbital (40 mg/kg), fixed in a stereotaxic apparatus, and taking coordinates from the atlas [11], kainic acid [7], in a dose of 1 μg in 1 μl of phosphate buffer (pH 7.4), was injected by means of a "TOP" microsyringe (India) bilaterally into structures of the hippocampus (AP = −2.8, I = 1.5, H = 3.5) or amygdala (AP = −2.8, I = 4.5, H = 9.0) in order to destroy them. Activity of the hippocampus and amygdala was enhanced by microinjection of 5 μl of homologous blood [9], diluted 4:1 with distilled water, into these structures. Animals of the control groups received 1 μl respectively of phosphate buffer solution pH 7.4 or 5 μl of isotonic NaCl solution, diluted 4:1 with distilled water, under similar conditions. Daily for 3 weeks, 24 h after the injections of blood and 15 days after the microinjections of kainic acid, the animals received a single intraperitoneal injection of picrotoxin in a below-threshold dose of 1.0 mg/kg body weight [6]. The animals' behavior was observed for 90 min after the injections. The intensity of the seizures and of aggressive-defensive behavior was expressed in

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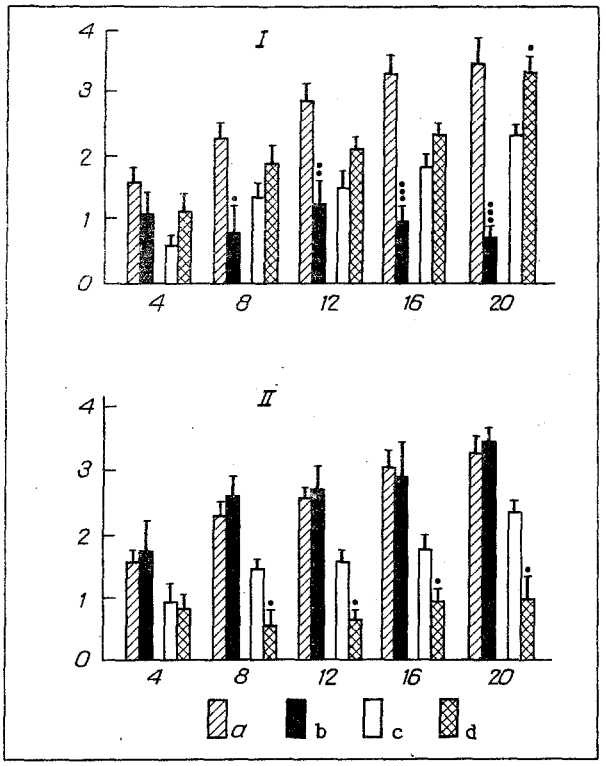

Fig. 1. Effect of destruction of hippocampus and amygdala on formation of seizures and emotional disorders in picrotoxin kindling. I) Effect of bilateral hippocampal injury on formation of seizures and emotional disorders. Abscissa, days of injection of picrotoxin; ordinate, intensity of seizures or of defensive reactions (in points). a) Intensity of seizures in animals of control group, b) of experimental group, c) intensity of defensive reactions in animals of control group, d) of experimental group. $*p < 0.05$ compared with control. II) Effect of bilateral injury to amygdala on formation of seizures and emotional disorders. Legend as to Fig. 1, I.

points [3, 12]. The location of the cannulas and of destruction of the brain structures was verified histologically at the end of the experiments. The results were subjected to statistical analysis [13].

EXPERIMENTAL RESULTS

Repeated injections of picrotoxin into the rats after destruction of the hippocampus and into rats of the control group led to the appearance, after the 3rd or 4th injections, of single myoclonic spasms in the majority of animals (Fig. 1, I). In the course of further injections of the epileptogen, rats of the experimental group showed only weak convulsive spasms, whereas in animals of the control group there was a progressive increase in the severity of the seizure manifestations: the appearance of clonic convulsions of the whole trunk after the 5th to 8th injections and of generalized clonico-tonic convulsions after the 8th or 9th injections. Starting with the 8th injection of picrotoxin, in animals with destructive lesions of the hippocampus the seizure responses were weaker than in the control. After administration of picrotoxin for 3 weeks the intensity of the seizures in the experimental and control groups was 0.6 ± 0.3 and 3.3 ± 0.4 points ($p < 0.001$; Fig. 1, I), respectively.

Starting with the 3rd or 4th injection of picrotoxin, animals of both groups showed enhanced passive-defensive reactions in the form of avoidance during attempted handling and resistance to capture. After the 8th injection of the drug these emotional disorders were observed in all animals of the experimental and control groups. After the 10th to the 12th injection of picrotoxin, rats of both groups also exhibited active defensive reactions (assuming a defensive posture, biting when caught). After the 20th injection of picrotoxin, eight of the 12 rats in the experimental group but only two of the 12 rats in the control group exhibited active defensive reactions. The mean intensity of the emotional disorders in animals with destructive lesions of the hip-

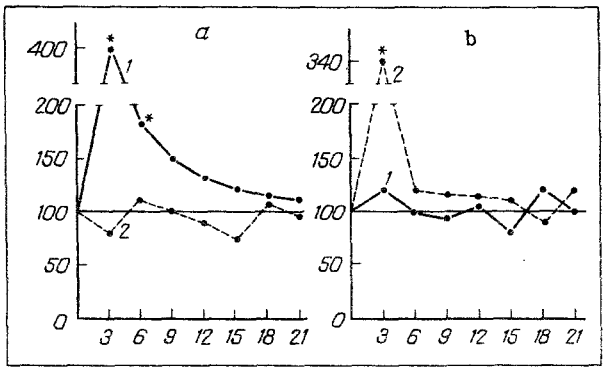

Fig. 2. Effect of injection of blood into hippocampus and amygdala on formation of seizures and emotional disorders during picrotoxin kindling. a) Effect of injection of blood into hippocampus on formation of seizures and emotional disorders. Abscissa, days of injections of picrotoxin; ordinate, intensity of seizures and of defensive reactions (in % of control). 1) Intensity of seizures, 2) intensity of defensive reactions. Asterisk indicates significant differences compared with control ($p < 0.05$); b) effect of injection of blood into amygdala on formation of seizures and emotional disorders. Legend as to Fig. 2a.

pocampus was significantly greater than in rats of the control group (3.2 ± 0.3 and 2.2 ± 0.2 respectively, $p < 0.05$, See Fig. 1, I). After bilateral injury to the amygdala, no change was observed in the development of seizure reactions during kindling compared with that in animals of the control group (Fig. 1, II). After the 20th injection of picrotoxin the mean intensity of the seizures in rats of the experimental and control groups was 3.4 ± 0.3 and 3.3 ± 0.2 points respectively ($p < 0.05$, see Fig. 1, II). Meanwhile, in the animals of this group repeated injections of picrotoxin led to the appearance of only minimal passive-defensive reactions (1 point). Starting with the 8th injection of the convulsant, the intensity of the defensive reactions in rats with destructive lesions of the amygdala was significantly less than in animals of the control group: after the 20th injection of picrotoxin it was 0.9 ± 0.5 and 2.3 ± 0.3 points respectively ($p < 0.05$, see Fig. 1, II).

After bilateral injection of blood into the hippocampus the animals showed increased sensitivity to picrotoxin. After the first few injections of the drug marked convulsive spasms and clonic convulsions were observed. After the 6th injection of picrotoxin, seizure reactions with an intensity of 2 points were observed in all animals of the experimental group; in addition, in some animals repeated clonic convulsions of the forelimbs were observed (average intensity of seizures 2.6 ± 0.3 points). During the first six injections of the drug the intensity of the seizure reactions in rats of the experimental group was significantly higher than in the control ($p < 0.05$, Fig. 2a). During subsequent injection of picrotoxin, with the appearance of generalized convulsive fits, the average severity of the seizures in the animals of the two groups did not differ significantly (Fig. 2a).

Besides the formation of a seizure syndrome, the appearance of defensive reactions de novo, or an increase in their intensity was observed in animals receiving an injection of blood into the hippocampus and in rats of the control group, in the form of avoidance during attempted handling. During kindling, no differences were observed in the intensity of the defensive reactions in animals of the experimental and control groups (Fig. 2b).

Bilateral injection of blood into the amygdala had no significant effect on the development of seizure reactions during picrotoxin kindling (Fig. 2b). After the 21st injection of picrotoxin the average intensity of the seizures in the experimental and control groups was 3.2 ± 0.3 and 3.2 ± 0.1 points, respectively.

Starting with the 2nd or 3rd injection of the drug, passive-defensive reactions were observed in animals of the experimental and control groups (1-2 points). After the 3rd injection of the epileptogen, these emotional disorders were observed in all animals receiving an injection of blood into the amygdala (the average intensity of this parameter was 1.7 ± 0.2 points) in half (five of the ten) of the animals of the control group (0.5 ± 0.2 point; $p < 0.05$, see Fig. 2b). With an increase in the intensity of the passive-defensive reactions, differences in the intensity of the emotional disorders in rats of the experimental and control groups disappeared (Fig. 2b). After the 21st injection of picrotoxin this parameter had a value of 2.5 ± 0.2 in the experimental and 2.2 ± 0.2 points in the control group.

Thus injection of blood, which has a pro-oxidant action [9] and promotes the formation of a generator of pathologically enhanced excitation (GPEE) [2], into the hippocampus facilitates, whereas destruction of the hippocampus with kainic acid prevents, the development of a seizure syndrome in picrotoxin kindling, confirming the view that the hippocampus plays the role of determinant structure of an epileptic PS [3, 4]. The fact that injection of blood into the hippocampus does not facilitate, and its destruction does not prevent, the formation of a syndrome of pathologically enhanced defensive behavior suggests that the development of that syndrome is unconnected with hyperactivation of hippocampal structures. Moreover, after destruction of the hippocampus, the intensity of the emotional disorders increases.

The results of these experiments show that neither destruction nor stimulation of the amygdala affects the intensity of seizure disorders. They are in agreement with data showing that hyperactivation of the amygdala is not essential for the development of the seizure syndrome during kindling [10]. Meanwhile, injection of blood into the amygdala accelerates, whereas its destruction prevents, the formation of a syndrome of pathologically enhanced defensive behavior. This suggests that this particular limbic structure is a pathological determinant of the PS which lies at the basis of development of emotional behavioral disorders. The results are in agreement with data on the role of the amygdala as a determinant structure of emotional-behavioral neuropathological syndromes arising when a GPEE is created actually in this structure [5], and also in its involvement in the realization of aggressive-defensive behavior [1].

Pharmacological kindling thus leads to the formation of two neuropathological syndromes, each of which is based on its own PS, with determinants in different structures, namely the hippocampus and amygdala.

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