EFFECT OF BRAIN EXTRACTS FROM KINDLED ANIMALS ON SEIZURE ACTIVITY OF RECIPIENT RATS

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During the formation of neuropathological syndromes substances which are effectors of the corresponding pathological systems begin to appear in the animal brain [1, 3]. During the establishment of chronic epilepsy in animals subjected to kindling by repeated injections of a subthreshold dose of metrazol, the formation of substances of peptide nature, which exert their functional effects through enhancement of the activity of the pathological epileptic system, also is intensified in the brain [3]. It is interesting to study the role of individual brain formations in the formation of endogenous proepileptic factors. For this purpose, in the investigation described below the effect of extracts of the hippocampus, the zone of formation of a hyperactive determinant structure during pharmacological kindling [2], and of the ventral portions of the mesencephalon (VM), with an important role in the development of motor seizure disturbances during kindling [8, 9], was studied on generalized seizure activity provoked by metrazol in intact recipient rats.

EXPERIMENTAL METHOD

To obtain brain extracts in these investigations 40 male Wistar rats weighing 200-300 g were used. A seizure kindling syndrome was induced in 20 rats by the method described in [2, 5]. Before the brain tissue was taken (24 h after the last convulsion) the average severity of the seizure manifestations was 3.7 ± 0.2 point. In animals of the control group (20 rats), which received daily injections of the corresponding volume of physiological saline, no seizure reactions occurred. The rats were decapitated 24 h after the last injection, the brain was removed with the brain stem, and the hippocampal tissue excised bilaterally within the range from AP 2.0 to AP 3.2, including VM (Fig. 1). The tissue samples thus obtained were frozen in liquid nitrogen and kept at −20°C. Later, the extracts were obtained with the aid of glacial acetic acid, by the method described previously [3, 7]. A dry form of extracts was used in the investigations and dissolved in physiological saline immediately before injection.

The effects of the extracts were studied in Wistar rats weighing 250-320 g, into which cannulas were implanted under hexobarbital anesthesia (100 mg/kg), taking coordinates from the atlas [10], into the lateral ventricles (AP − 0.8, L 1.2, H 3.5), the hippocampus (AP − 2.8, L 1.5, H 2.5), and the amygdala (AP − 2.8, L 4.5, H 7.0). The test extracts were injected: intraventricularly in a volume of 5.0 μl (0.2 and 20 μg per animal) and into the parenchyma of the brain, in a volume of 1-2 μl (10 μg per animal). The rate of injection of the solution of the extracts was 1.0 μl/min. The animals were given an intraperitoneal injection of metrazol (35 and 45 mg/kg) 20 min after the intracerebral injection. Observation on the seizure reactions continued for 30 min. The latent period of the first seizure manifestations and the severity of the seizures, assessed in points on the accepted scale [2], were taken into account. The results were subjected to statistical analysis [4].

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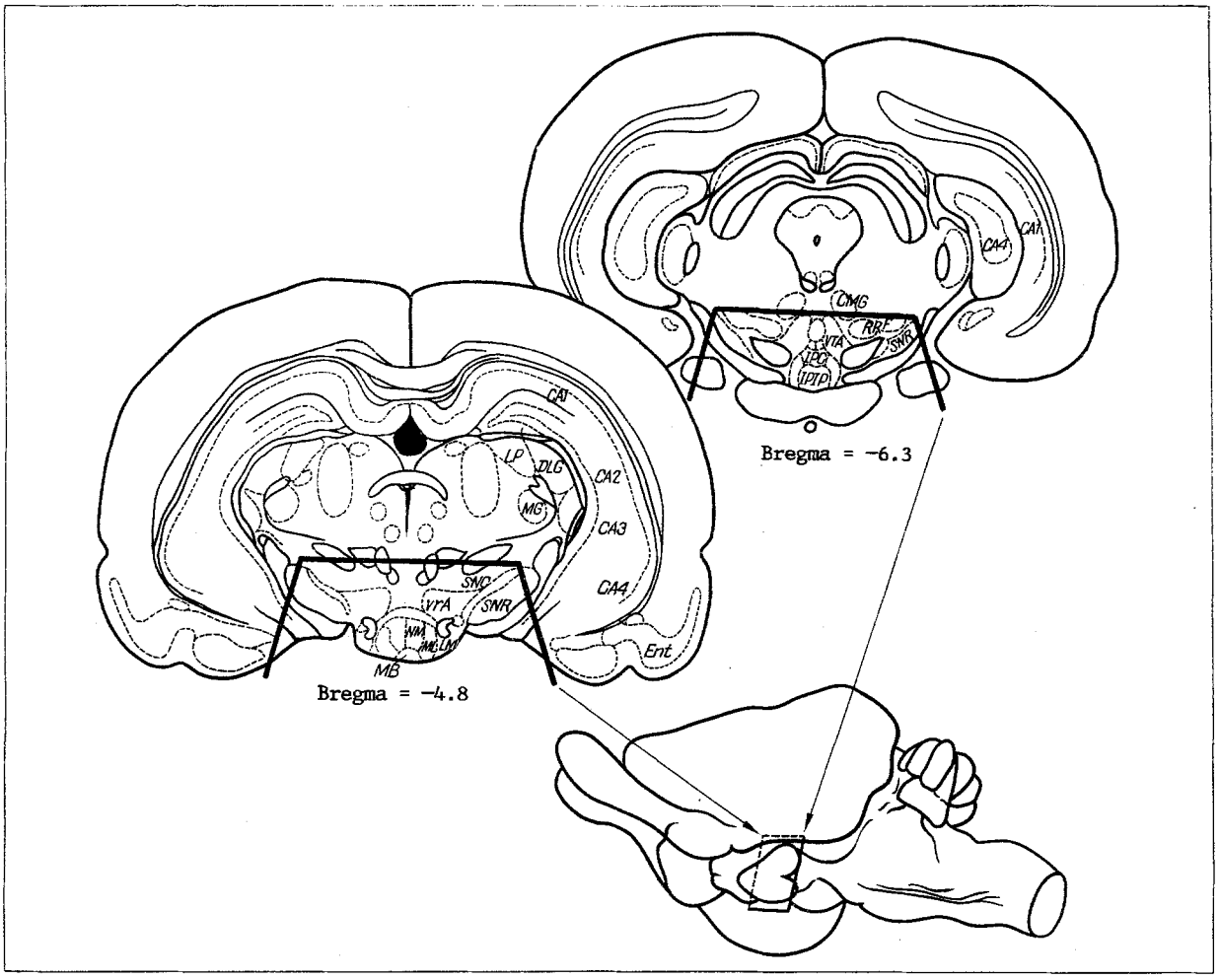

Fig. 1. Diagram showing method of obtaining tissue from VM.

EXPERIMENTAL RESULTS

The aim of the experiments of series I was to study the effects of intracerebral injections of extracts of the hippocampus and the "rest of the brain" from animals subjected to metrazol kindling, on the intensity of generalized convulsions induced by intraperitoneal injection of metrazol in a dose of 35 mg/kg. Neither intraventricular injection of the extracts of the rest of the brain of the kindled rats, nor their injection into the hippocampus and amygdala caused any significant change in the latent period of the first seizure manifestations and caused a significant increase in the severity of the seizure manifestations induced in the recipient animals by intraperitoneal injection of metrazol, compared with that observed in animals of the corresponding control groups (Fig. 2a, b). Generalized clonicotonic convulsions and marked clonic spasms of muscles of the whole body were observed in half of the animals. In the groups of animals receiving intraventricular and intracerebral (intrahippocampal and intraamygdalar) injections of hippocampal extracts from kindled rats and animals of the control group there were no differences with respect to the latent period and severity of the seizures (Fig. 2c, d).

The aim of the experiments of series II was to study the effects of intraventricular injection of extracts of VM and of the rest of the brain from kindled animals to seizures induced by metrazol (35 mg/kg) in intact recipient rats. Injection of metrazol after intraventricular injection of extract of VM (20 μg) from animals subjected to kindling was accompanied by the development of seizures, whose latent period was significantly shorter but whose average severity was greater than those of recipient animals receiving an intraventricular injection of extract of VM from animals of the control group (Fig. 3, I). Clonicotonic seizures were observed under these circumstances in three of the five rats. After intraventricular injection of extracts of the rest of the brain from kindled animals, the latent period of seizures induced by injection of metrazol and their severity did not differ from those in the group of animals receiving the injection of metrazol after intraventricular injection of extract of the rest of the brain from rats of the control group.

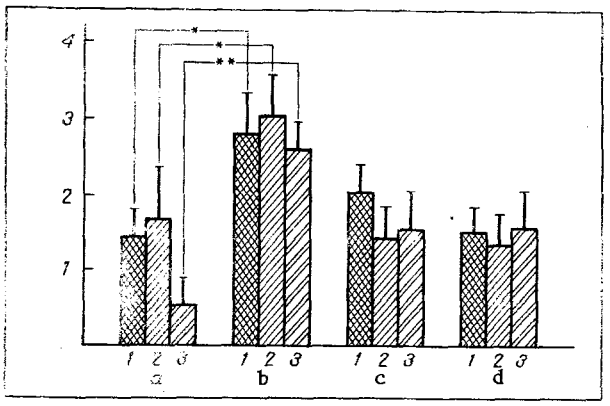

Fig. 2. Effect of intracerebral injections of brain extracts of seizure activity induced by metrazol in rats: a) effect of injection of extract of rest of brain from animals of control group, b) from kindled animals; c and d) effects of injections of hippocampal extracts from animals of control group and kindled animals respectively. 1) Intraventricular injection (20 μg per animal); 2, 3) injections into hippocampus and into amygdala (10 μg/animal) respectively. In all groups metrazol was used in a dose of 35 mg/kg 20 min after intracerebral injection of extract. $*p < 0.05$, $**p < 0.001$.

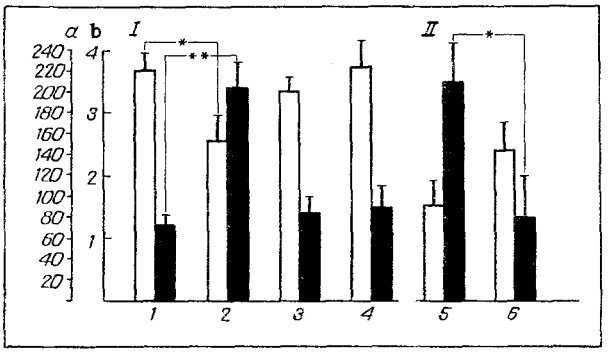

Fig. 3. Effect of intraventricular injection of extracts of VM on seizure activity induced by metrazol in rats. I) In all groups injection of metrazol in a dose of 35 mg/kg was given 20 min after injections of extracts of VM (20 μg/animal); 1, 2) injection of extracts of VM into rats of control and kindling groups; 3, 4) injections of extracts of rest of brain of animals of control group and of rats subjected to kindling. II) Metrazol injected in a dose of 45 mg/kg 20 min after injection of extracts of VM (0.2 μg/animal) from animals of control group (five) and rats subjected to kindling (six) respectively. Ordinate: a) latent period of first seizures (s) corresponds to unshaded columns, b) severity of seizures (points) corresponds to black columns. Remainder of legend as to Fig. 2.

The effects of intraventricular injection of extracts of VM from kindled animals in a dose of 0.2 μg on seizure reactions provoked by injection of metrazol in a dose of 45 mg/kg into the recipient animals were studied separately. The use of extract of VM from the brain of the kindled rats was accompanied by a significant decrease in the severity of the seizures compared with that in animals of the control group, receiving extract of VM from the brain of rats of the control group (Fig. 3, II).

The results thus demonstrate that substances which are inducers and activators of activity of the epileptic system and which lie at the basis of the chronic epileptic syndrome associated with kindling, accumulate in VM of the brain of animals subjected to metrazol kindling [1-3]. It was shown previously that the active substance of these extracts is peptide in nature, for its

effect is abolished by treating the extracts with pronase, and it is not observed when naloxone is administered. Probably activation of opiatergic mechanisms takes place in the structures of VM during kindling.

It was shown previously that the pathological determinant of the epileptic syndrome during kindling is formed in structures of the hippocampus [2]. Since proepileptic factors do not accumulate in the zone of the determinant structure, it can be tentatively suggested that the epileptic system associated with metrazol kindling develops through the involvement in the pathological system of structures of the brain stem and, in particular, of the substantia nigra, which plays an important role in the spread of epileptic activity, and in whose activity factors of peptide nature are of great importance [9, 11].

It was also shown that injection of extracts of VM in a dose 100 times less than that potentiating epileptic activity had an antiepileptic effect. These data suggest that in the initial stages of formation of epileptic activity, activation of the peptidergic systems of the brain may be a defensive and compensatory mechanism preventing the formation of the pathological system and the development of the epileptic syndrome. In the later stages, however, prolonged activity of the pathological determinant causing a disturbance of the mechanisms of inhibitory control and forming multiple vicious circles, increases the rate of production of endogenous factors and their accumulation in large quantities, thus promoting development of the pathological system. Dose-dependent pharmacological effects of this kind are characteristic of substances of peptide nature [6].

These investigations showed that involvement of structures of VM in the pathological system on account of hyperactivation of opiatergic brain systems is an essential mechanism of formation of the pathological epileptic system.

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