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EFFECT OF BRAIN EXTRACTS FROM RATS SUBJECTED TO METRAZOL KINDLING ON GENERALIZED EPILEPTIC ACTIVITY

G. N. Kryzhanovskii,* M. Yu. Karganov,
A. A. Shandra, L. S. Godlevskii,
V. K. Lutsenko, R. F. Makul'kin, and
A. M. Mazarati

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It can be postulated that not only neuronal connections between formations of the CNS, but also certain substances, products of neuronal activity, can participate in the formation of the pathological systems (PS) lying at the basis of corresponding neuropathological syndromes [2]. Appearing together with PS as a result of its activity, they contribute to the further formation of the PS, to intensification of its activity, and to the formation of new, similar PS. One type of PS is the epileptic system [1, 2]. It has been shown that during chronic daily injection of metrazol in subconvulsive doses (metrazol kindling) animals develop increased predisposition to convulsions, expressed by the fact that to each subsequent injection of metrazol, seizures of increasing intensity arise [4]. This form of epileptogenesis is based on the formation of an epileptic system, the primary pathological determinant of which is a hyperactivated hippocampus [3]. The aim of the present investigation was to discover substances in the brain of animals subjected to metrazol kindling capable of giving rise to proepileptogenic effects, i.e., facilitating the formation and/or activity of the epileptic system.

EXPERIMENTAL METHOD

Donor Animals. To obtain brain extracts for the investigations, 40 male Wistar rats weighing 180-250 g were used. A convulsive kindling syndrome was induced in 20 rats by the method described previously [4]. Repeated daily injections of metrazol in a subconvulsive dose (30 mg/kg) led to a gradual increase in predisposition to convulsions, so that the same doses of metrazol induced seizure responses of increasing severity — from single twitches to generalized clonicotonic fits, with the animals falling on their side, with autonomic disorders, and with postictal depression. Before brain tissue was taken, the average severity of the seizure manifestations in animals of the experimental group was 3.5 ± 0.2 points. In the control group of animals (20 rats), which received the same volume of physiological saline every day, no seizure responses occurred. The rats were decapitated 24 h after the last injection, i.e,. 24 h after a seizure, and the brain together with the cerebellum and brain stem

*Academician of the Academy of Medical Sciences of the USSR.

Laboratory of General Pathology of the Nervous System, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Department of Pathophysiology, N. I. Pirogov Odessa Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 271-274, March, 1989. Original article submitted June 30, 1988.

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TABLE 1. Effect of Brain Extracts from "Kindling" Animals on Generalized Seizures Induced in Mice by Injection of Metrazol (M ± m)

Substances injected	Number of animals	Latent period of first seizures, sec	Latent period of clonicotonic seizures, sec	Number of animals with clonitonic seizures	Mortality, %	Mean severity of seizures, points
1. Metrazol (60 mg/kg)	15	107,3±8,4	180,6±33,4	8	13,3	2,7±2
2. Brain extract from control animals (50 mg/kg)	10	123,9±7,8 ($p_1-p_2>0,05$)	150,0±18,9	6	0 ($p_1-p_2>0,025$)	2,6±0,2
3. Brain extract from "kindling" animals (50 mg/kg)	22	68,3±4,8 ($p_2-p_3<0,01$)	168,8±29,5 ($p_2-p_3>0,05$)	21 ($p_2-p_3<0,025$)	40,9 ($p_2-p_3<0,025$)	3,5±0,1 ($p_2-p_3<0,01$)
4. Brain extract from "kindling" animals (50 mg/kg) + naloxone (1.0 mg/kg)	12	109,4±12,7 ($p_3-p_4<0,01$)	150,0±19,9 ($p_3-p_4>0,05$)	9 ($p_3-p_4>0,025$)	0 ($p_3-p_4<0,025$)	2,8±0,1 ($p_3-p_4<0,01$)
5. Brain extract from control animals, treated with pronase (50 mg/kg)	15	92,0±7,1 ($p_2-p_5<0,05$)	125,4±13,5 ($p_2-p_5>0,05$)	13 ($p_2-p_5>0,025$)	26,7 ($p_2-p_5>0,025$)	3,1±0,2 ($p_2-p_5>0,05$)
6. Brain extract from "kindling" animals, treated with pronase (50 mg/kg)	14	105,9±10,4 ($p_3-p_6<0,01$)	135,2±11,7 ($p_3-p_6>0,05$)	13 ($p_3-p_6>0,025$)	7,7 ($p_3-p_6>0,025$)	3,1±0,1 ($p_3-p_6<0,05$)

was removed, frozen in liquid nitrogen, and kept at a temperature of −20°C. The frozen tissue was placed in a homogenizer containing 1 M acetic acid, heated to 90°C, the homogenizer was kept for 5 min in a boiling water bath, after which the tissue was dispersed by means of a glass pestle for 10 min [5]. The suspension was cooled and centrifuged at 10,000 rpm for 15 min (K-24, East Germany). The supernatant was sampled and the pH adjusted to 7.0 with concentrated ammonia solution. The residue was separated by filtration and the resulting solution freeze-dried. The dry form of the extracts was used in the subsequent investigations and dissolved before injection in physiological saline.

Recipient Animals. Effects of the extracts were studied on (CBA × C57BL/6)F_1 mice weighing 18-24 g (acute seizures) and on Wistar rats weighing 180-250 g, in which a metrazol kindling syndrome had been induced. Generalized seizures were caused by intraperitoneal injection of metrazol (60 mg/kg) or picrotoxin (5.0 mg/kg). The extracts were injected intraperitoneally in a volume of 0.1-0.3 ml of physiological saline and in a dose of 50 mg/kg body weight 30 min before injection of the testing dose of the convulsants, and also into the lateral cerebral ventricles in a volume of 10 µl of physiological saline and in doses of 20 and 100 µg per rat. Naloxone (1.0 mg/kg, from "Sigma," USA) was injected subcutaneously 30 min before the brain extracts. For treatment of the extracts with pronase, samples with a volume of 1.0 ml, containing 1.2-1.6 mg protein of extract, were treated with 100 µg of pronase ("Calbiochem," USA; specific activity 45,000 U/g), $5 \cdot 10^{-4}$ M $CaCl_2$ in 0.05 M borate buffer, and incubated at 37°C for 2 h; the reaction was stopped by immersing the sample in a boiling water bath for 10 min. The results were subjected to statistical analysis [6].

EXPERIMENTAL RESULTS

The aim of the experiments of series I was to study the effect of brain extracts from "kindling" animals and rats of the control group on generalized seizures induced in mice by a single injection of metrazol in a convulsive dose (60 mg/kg). An additional aim was to study the effects of extracts in conjunction with administration of naloxone, and after treatment of the extracts with pronase. When the mice were given intraperitoneal injections of brain extracts from "kindling" animals, seizures induced by metrazol occurred much sooner (significant reduction of the latent period of the first seizure responses), the number of animals with generalized clonicotonic seizures and with a lethal outcome increased, as also did the average severity of the seizures compared with the seizure response induced by the same dose of metrazol both independently and when brain extracts from animals of the control group were injected (Table 1). Preliminary administration of naloxone or treatment of brain extracts from the "kindling" animals with pronase abolished these proepileptic effects.

The effect of intraventricular injection of extracts (20 µg per animal) on seizure activity induced by injection of metrazol (35 mg/kg) and on seizure effects arising after injection of brain extracts alone in a dose of 100 µg per animal was studied in the experiments of series

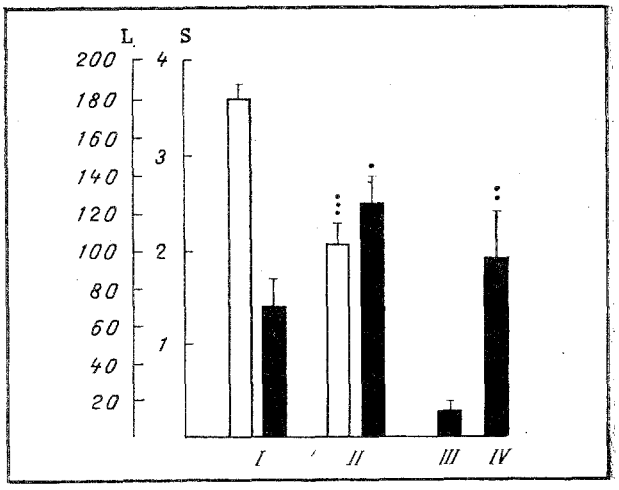

Fig. 1

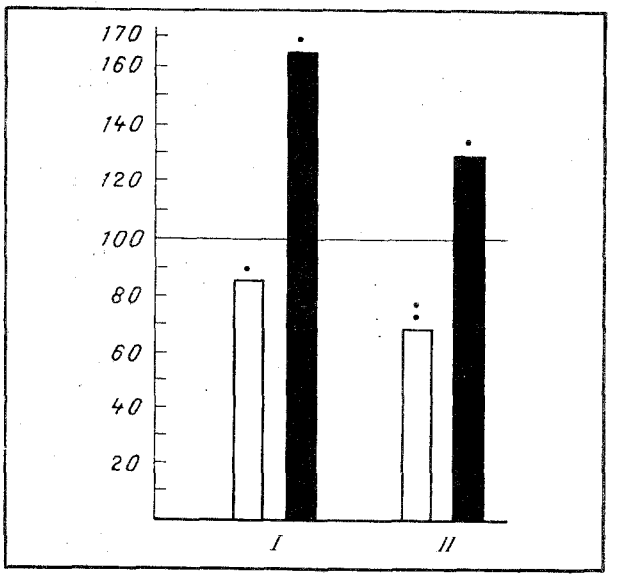

Fig. 2

Fig. 1. Effect of intraventricular injection of brain extracts on seizure activity induced in rats by intraperitoneal injection of metrazol. Abscissa: I) parameters of seizure activity induced by metrazol against the background of action of brain extracts (20 μg/animal) of control group of animals; II) parameters of seizure activity induced by metrazol, against the background of action of brain extracts (20 μg/animal) from "kindling" animals. III and IV) seizure responses to injection of brain extracts from animals of control and "kindling" groups respectively (100 μg/animal). Ordinate: L) latent period of first seizures (sec), S) severity of seizures (in points). Unshaded columns – latent period of first seizures, black columns – severity of seizures. *$p < 0.02$; **$p < 0.01$; ***$p < 0.001$.

Fig. 2. Effect of intraperitoneal injection of brain extracts on generalized seizure activity of varied nature: I) parameters of generalized seizures induced in mice by intraperitoneal injection of picrotoxin, under influence of brain extracts from "kindling" animals; II) parameters of generalized seizures in rats during metrazol kindling, under the influence of brain extracts from "kindling" animals. Unshaded columns – latent period of first seizure responses, black columns – severity of seizures in % of control., taken as 100%, and indicated by a horizontal line. *$p < 0.05$, **$p < 0.01$ compared with control.

II. Under the influence of brain extracts from "kindling" animals, the latent period of onset of the first seizure manifestations was reduced highly significantly, and the severity of the convulsions was increased, also significantly, compared with effects of brain extract from animals of the control group (Fig. 1). Motor excitation, with rapid runs, was observed 2-4 min after intraventricular injection of extract of "kindling" animals in a dose of 100 μg per animal in nine of ten rats. In six of ten animals individual clonic seizures, changing into clonic spasms of the forelimbs, were observed 8-12 min after injection of the extract. The average severity of the seizures was 1.9 ± 0.5 points. In the group of animals receiving brain extract from rats of the control group, the seizures were significantly weaker: in three of 12 animals individual convulsive twitches were oberved (Fig. 1). Persistent seizure manifestations (clonic contractions of separate groups of muscles) occurred in the animals during 20-40 min after their appearance, after which the frequency and intensity of the clonic contractions diminished and they disappeared after a further 10-20 min.

In the experiments of series III effects of brain extract from "kindling" animals and animals of the control group on generalized convulsions induced in mice (25 animals) by injection of picrotoxin (5.0 mg/kg) were studied. Injection of picrotoxin 30 min after brain extract from animals of the control group caused the appearance of the first seizure manifestations on average after 11.1 ± 0.3 min. The average severity of the seizures was 1.8 ± 0.3 points. Under the influence of brain extract of "kindling" animals the latent period of the first seizure responses was shortened and their severity increased by 14.4 and 67%, respectively, compared with the corresponding parameters in animals receiving brain extract from rats of the control group (Fig. 2, I).

In the experiments of series IV on rats (20 animals) receiving daily injections of metrazol in subconvulsive doses (19 injections), the effect of brain extracts on the severity of the seizure responses was studied during successive testing injections of metrazol. On intraperitoneal injection of brain extract from animals of the control group and 30 min before a routine (the 20th) injection of metrazol, the first seizure manifestations appeared in the rats 110 ± 9.5 sec after the injection of metrazol; the average severity of the seizures was 2.4 ± 0.2 points. Seizures induced by metrazol after injection of brain extract from "kindling" animals were characterized by a significantly shorter (by 33%) latent period of the first seizures and also by a significant increase (by 30%) in their severity compared with the corresponding parametes in the group of animals with injection of brain extracts from animals of the control group (Fig. 1, II).

The experiments thus show that intraperitoneal injection of brain extracts from animals subjected to metrazol kindling leads to enhancement of epileptic activity in the recipient animals, induced by injection of metrazol and picrotoxin, facilitates metrazol seizures after intraventricular injection, and also independently induces seizures when given by intraventricular injection. The observed enhancement of the epileptogenic effects of convulsants under the influence of brain extract from epileptized animals was considerably weakened by treatment of the extract with pronase. These findings suggest a peptide nature for the active substance of the extract. The fact that naloxone weakens the proepileptogenic effect of the extract suggests that opioid peptides are involved in the mechanism of this effect. During the action of extract of epileptized animals, the seizure response induced not only by acute (in mice) and chronic (in rats) administration of metrazol, but also by injection of picrotoxin, was enhanced. These observations are evidence of the nonspecific nature of the neurochemical mechanisms of facilitation of epileptogenesis under the influence of brain extract of epileptized rats, and also of the species-nonspecific nature of the "proepileptogenic" substances in the extract.

It can be tentatively suggested that during the development of a chronic (on a model of metrazol kindling) form of epilepsy, due to the formation of an epileptic system [2, 4], activity of the latter gives rise to metabolic disturbances in the brain tissue, affecting peptide metabolism also. The results of the present investigation are evidence that substances of peptide nature may be both indicators and inducers of activity of a pathological system.

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