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ROLE OF OPIATE MECHANISMS OF THE HIPPOCAMPUS AND SUBSTANTIA NIGRA IN BEHAVIORAL AND SEIZURE DISTURBANCES DURING PICROTOXIN-INDUCED KINDLING

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Kindling is a method of forming epileptic activity (EA) by means of repeated, initially subliminal, epileptogenic procedures [1-5, 13]. Our previous investigations [1, 2, 4, 5] showed that the development of the seizure syndrome during kindling induced by repeated injections of metrazol or picrotoxin, is based on the formation of an epileptic pathological system with determinant structure, in the formations of the hippocampus. It has also been shown that during the formation of EA during kindling, endogenous opioid peptides, playing the role of stabilizers and effectors of activity of the epileptic pathological system, and also of a specific antiepileptic system of the brain in different stages of the pathological process, accumulate in the animals' CNS [3, 6]. The important role of the opiatergic mechanisms of the reticular part of the substantia nigra (RSN) in the appearance and disappearance of EA in the course of kindling induced by electrical stimulation of the amygdala [8], and also by repeated injections of picrotoxin [6], has recently been established. However, the role of the different subtypes of opiate receptors in the mechanisms of kindling is not yet clear. Great importance has recently been attached to the mu- and kappa-opiate systems of the brain in the formation and disappearance of EA during kindling [10]. The aim of the present investigation

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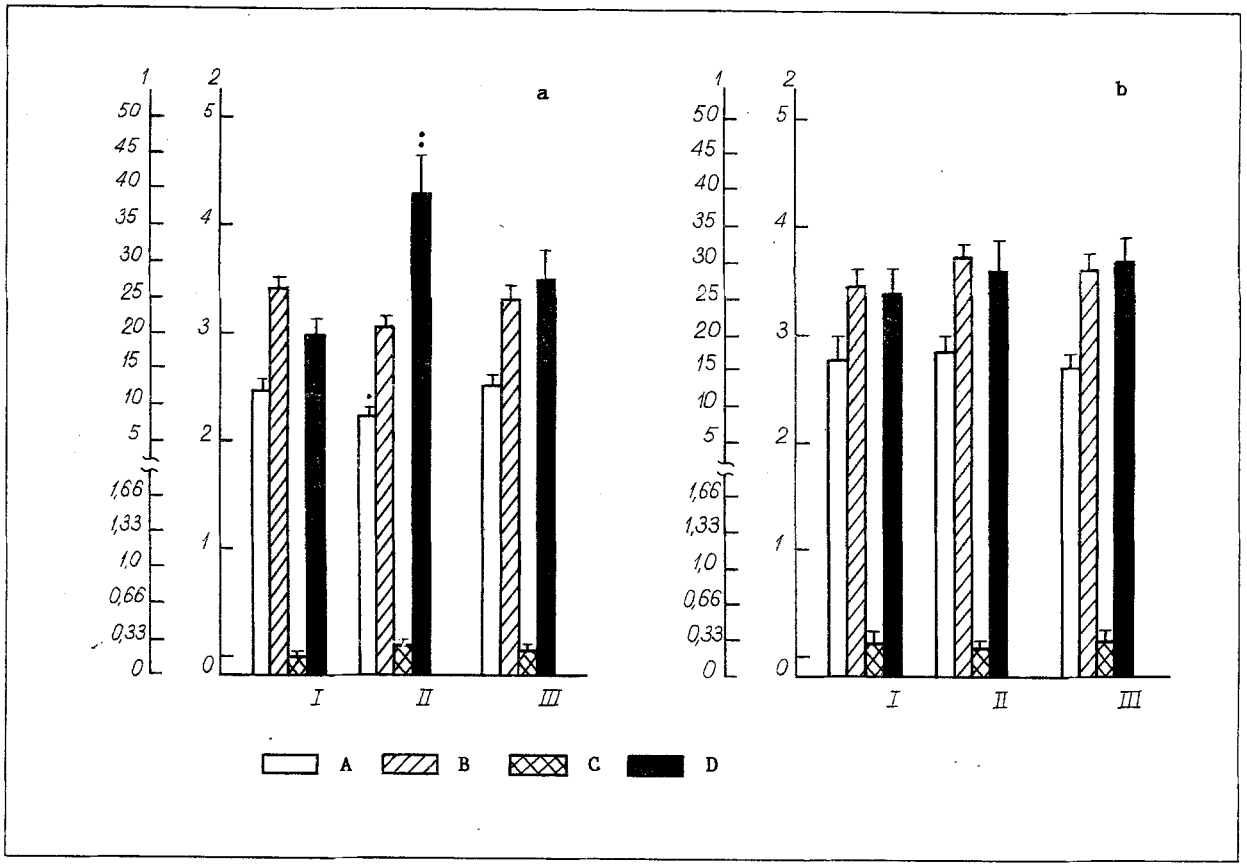

Fig. 1. Effect of intracerebral injections of Met-enkephalin on intensity of seizures induced by a testing injection of picrotoxin (1 mg/kg) in rats subjected to kindling. a) Intensity of seizures induced by injection of picrotoxin following injection of Met-enkephalin into hippocampus. Abscissa: I and II) injection of Met-enkephalin in doses of 1 and 10 μg respectively, III) control (injection of physiological saline). Ordinate: 1) time, in min; 2) intensity of seizures, in points. Legend: A) latent period of first seizure manifestations, B) latent period of seizures, C) duration of tonic phase of seizures, D) intensity of seizure reactions (points). $^{*}p < 0.05$, $^{:}p < 0.01$ compared with control; b) intensity of seizures induced by picrotoxin after injection of Met-enkephalin into reticular part of substantia nigra. Legend as to fragment "a."

was to study changes in behavioral and seizure reactions caused by injection of selective agonists of mu- and kappa-opiate receptors into the hippocampus and RSN of rats subjected to picrotoxin-induced kindling. Met-enkephalin was chosen as the mu-agonist and Dynorphin-A-1-13 as the kappa-agonist [10].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. Each group consisted of 10 animals. Pharmacological kindling was induced by the method described in [4] by daily single intraperitoneal injections of picrotoxin ("Serva," West Germany) in an initially subconvulsive dose (1 mg/kg). The intensity of the seizures was assessed on a six-point scale [1]. The latent period of the first seizures and of generalized fits, and the duration of the tonic phase of the seizure episodes also were determined. When kindling had been established for 1-5 days the rats were anesthetized with ether, placed in a stereotaxic apparatus, and, using a microsyringe ("Scientific Glass Engineering," Australia), Met-enkephalin or Dynorphin-A-1-13 (from the "Vektor" Research and Production Combine, Novosibirsk) was injected bilaterally into structures of the hippocampus (AP = –4.8, L = 4.5, H = 7.0) or of RSN (AP = 4.8, L = 2.5, H = 8.0) bilaterally, taking coordinates from the stereotaxic atlas [16]. The preparations were injected in doses of 1 and 10 μg in 1 μl of 0.9% sodium chloride solution at the rate of 0.5 μl/min. Animals of the control group received the solvent only under similar conditions. The testing injection of picrotoxin (1 mg/kg) was given 20-30 min after the microinjection of the peptides. The animals remained under observation for 60 min after injection

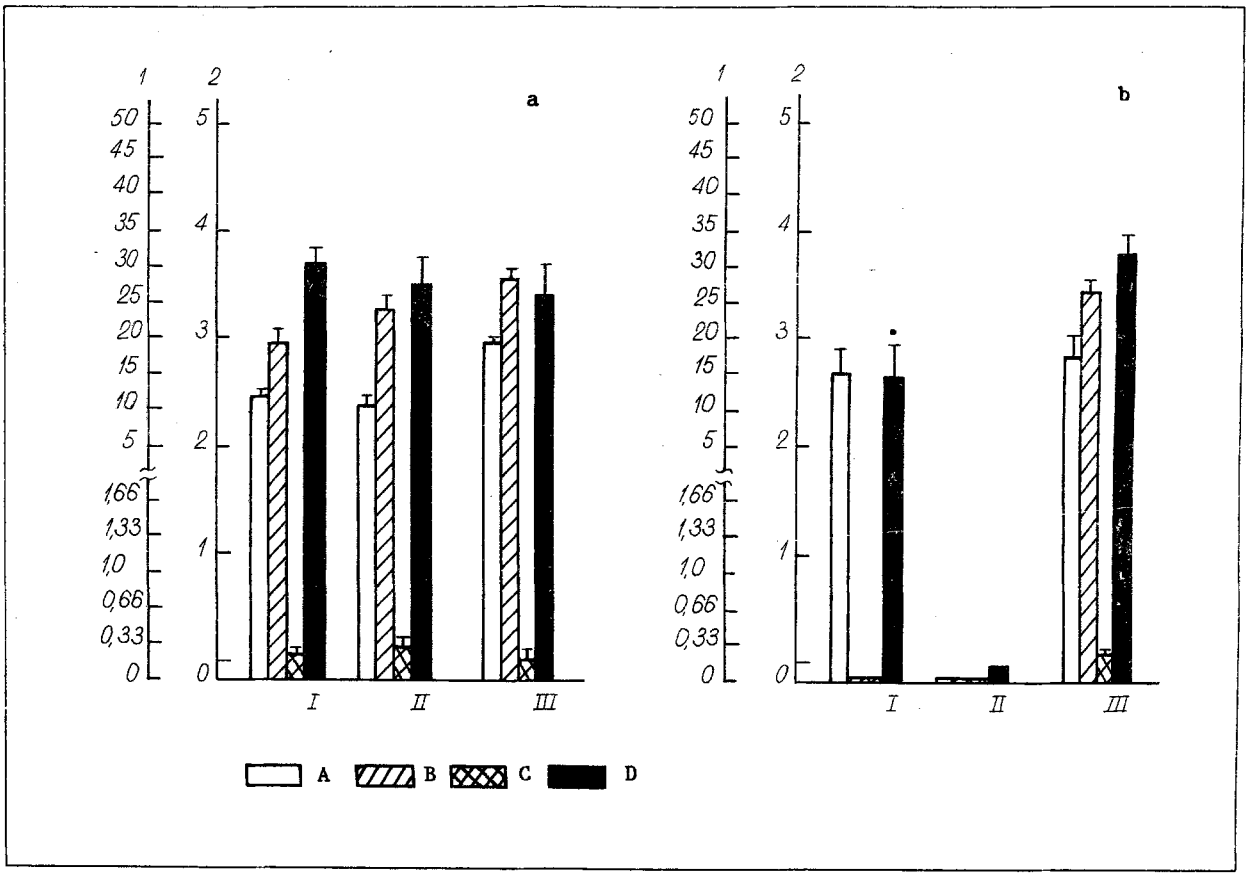

Fig. 2. Effect of intracerebral injections of Dynorphin-A-1-13 on intensity of seizures induced by testing injection of picrotoxin (1 mg/kg) in rats subjected to kindling. a, b) Intensity of seizures induced by picrotoxin after injection of Dynorphin into hippocampus and substantia nigra respectively. Legend as to Fig. 1.

of the convulsant. The intensity of the seizure responses was estimated by the method described above. Particular features of the animals' behavior, both after injection of opioids and after the occurrence of the generalized clonicotonic fits also were studied. The following behavioral reactions were tested: muscle relaxation — absence of resistance of the fore- and hind limbs when abducted, in combination with the characteristic position of the animal, i.e., lying in the prone position with the limbs outstretched, the overturning reflex, which was considered to be disturbed if the rat assumed the original position after being turned over onto its side after a delay of more than 5 sec, and explosive behavior, namely high jumping, fast running, fighting with other animals, biting and chewing its own tail [10]. At the end of the experiments, the regions of injection were verified histologically by demonstrating the track of the needle of the microsyringe. The results were subjected to statistical analysis [7].

EXPERIMENTAL RESULTS

The Effects of Picrotoxin. Repeated injection of picrotoxin (1 mg/kg) led to the appearance and progressive increase in the intensity of seizure manifestations, from single myoclonic twitches to generalized clonicotonic fits, with falling onto the side. At the end of the kindling procedure (after the 20th injection of the convulsant), generalized seizure episodes were observed in 76 of 100 animals, and the remaining rats showed repeated clonic convulsions of the whole trunk and/or the forelimbs. The average intensity of the seizures was 3.6 ± 0.3 point. After the end of the generalized seizure episodes, the animals exhibited postictal motor disorders: the majority of the rats developed depression — motor inhibition, muscle relaxation, disturbance of the overturning reflex. Meanwhile, 16 of the 100 animals exhibited explosive behavior in the postictal period. Disappearance of the postictal behavioral disturbances took place 1-1.5 min after the clonicotonic seizures.

Effects of Met-enkephalin. Injection of Met-enkephalin (1 μg) into the hippocampus caused no disturbances of behavior in the animals and had no significant effect on the latent period of the first seizure manifestations and generalized seizures, or on the duration of the tonic phase of the fit and on the average intensity of seizure reactions induced by a testing injection of

picrotoxin (Fig. 1a). At the end of the seizures the rats developed motor inhibition with muscle relaxation and disturbance of the overturning reflex. After injection of Met-enkephalin into the hippocampus in a dose of 10 μg, explosive disturbances of behavior occurred in six of the 10 rats, but no such reactions were observed in animals of the control group (none of 10, $p < 0.025$). A testing injection of picrotoxin induced the development of generalized seizures in all the animals, with falling into the side position, and four of the 10 rats developed a second episode of seizures. The latent period of the first seizure manifestations was significantly shorter, whereas the average intensity of the seizures was significantly greater than in rats of the control group (Fig. 1a). After the end of the seizures, explosive behavior was observed in eight of the 10 animals (in none of the 10 in the control group, $p < 0.025$).

Injection of Met-enkephalin into RSN (1 and 10 μg) caused no changes in the animals' behavior. The latent period of the first seizure manifestations and fits, the duration of the tonic phase, and the intensity of the seizures induced by picrotoxin were the same as in rats of the control group (Fig. 1b). At the end of the fits, motor inhibition and disturbance of the overturning reflex were observed in the animals receiving Met-enkephalin, just as in rats of the control group.

Effects of Dynorphin-A-1-13. Injection of Dynorphin into the hippocampus (1 and 10 μg) did not cause behavioral disturbances. After injection of picrotoxin (1 mg/kg) seizure reactions with a mean intensity the same as that in animals of the control group were observed (Fig. 2a). At the end of the generalized seizures, all the animals developed postictal depression.

After injection of Dynorphin into RSN (1 and 10 μg) motor disturbances were observed in all the rats. The animals lay in the prone position or on they did not move, and the overturning reflex was disturbed. The duration of these disturbances after injection of Dynorphin in doses of 1 and 10 μg was 25-35 and 55-65 min respectively. The testing injection of picrotoxin after previous intranigral injection of Dynorphin (1 μg) led to the appearance of myoclonic spasms or clonic convulsions of the whole trunk. The intensity of the seizure reactions was significantly lower than in animals of the control group. Injection of picrotoxin after previous injection of Dynorphin in a dose of 10 μg did not cause seizures (Fig. 2b).

The investigations thus showed that injection of Met-enkephalin into the hippocampus of rats subjected to kindling led to an increase in the intensity of the seizure reactions caused by a testing injection of the convulsant; intranigral injection of Dynorphin had an anticonvulsant action. These effects of endogenous opioids were specific for the structures studied. The results confirm data on the important role of hyperactivation of the hippocampus and the formation of a generator of pathologically enhanced excitation (GPEE) in it in the genesis of the seizure syndrome associated with pharmacological (metrazol, picrotoxin) kindling [1, 2, 4, 5]. An important stage in the mechanisms of formation of the GPEE in the hippocampus may perhaps be activation of the mu-opiate system of this structure, taking place in this investigation after local injection of Met-enkephalin. In investigations by other workers [9, 18] involvement of the hippocampal opiate systems in the formation of EA was demonstrated in kindling produced by ES of the amygdala: injection of opiate agonists Met-enkephalin, beta-endorphin, and morphine into the hippocampus increased the intensity of the seizure reactions induced by testing ES. Repeated injection of Met-enkephalin into the hippocampus also led to the appearance and potentiation of seizure manifestations [9]. It can thus be tentatively suggested that activation of the hippocampal mu-opiate system is the mechanism of formation of EA associated with different modifications of kindling.

The investigations showed that explosive behavior is observed in some animals in the course of kindling, at the end of seizures. It was also shown that injection of Met-enkephalin into the hippocampus of such animals itself induces explosive reactions in rats and increases the number of animals with these behavioral disturbances in the postictal period. On this basis it can be concluded that explosiveness during pharmacological kindling is due to preservation of enhanced activity of the hippocampal mu-opiate system after the end of clonicotonic seizures. Other investigations [10] showed that postseizure explosiveness during ES-induced kindling of the amygdala was combined with more intensive metabolism in the hippocampus, as reflected in the degree of utilization of 2-deoxyglucose. Postictal explosiveness, just as the intensification of metabolism, was blocked by the mu-opiate antagonist naloxone. To sum up the results it can be concluded that activation of the hippocampal mu-opiate system is one of the mechanisms of formation of the GPEE in that structure and in the activity of the pathological epileptic system during kindling, that is manifested as generalized seizures and also as postictal explosiveness in a certain proportion of the animals.

It can be concluded from data obtained in relation to the anticonvulsant action of intranigral injection of Dynorphin during kindling that the kappaopiate system of RSN is involved in the control and suppression of EA during kindling. Other investigations [8] showed a decrease in the intensity of seizures when Dynorphin was injected into the RSN of rats subjected to ES-induced kindling of the amygdala. A decrease in the intensity of the seizure manifestations also was found after injection of other preparations into RSN and, in particular, GABA agonists [12, 15]. These findings suggest that RSN and, in particular, its kappa-opiate mechanisms, are involved in function of the antiepileptic system of the brain, which suppresses activity of the deter-

minant structure of the seizure syndrome [5]. It is a noteworthy fact that injection of Dynorphin into RSN induced behavioral disturbances in rats subjected to kindling similar to those in the majority of animals in the postictal period: motor inhibition and muscle relaxation. Other investigations [10, 11, 14] showed that postictal depression in ES-induced kindling of the amygdala, hippocampus, and globus pallidus is accompanied by a decrease in sensitivity to epileptogenic agents, and that endogenous opiates play an important role in the development of these disturbances. It is considered [17, 18] that the opiatergic mechanisms of the brain, activated during kindling, are aimed at abolishing seizures and suppressing EA. It can be concluded that an important role in the mechanisms of termination of seizures and the development of postictal depression is played by the kappa-opiate system of RSN — structures of the antiepileptic system.

At different stages of EA, during picrotoxin-induced kindling, opiatergic mechanisms of the hippocampus and RSN are activated successively. At this stage of EA formation and of activity of the pathological epileptic system, mu-opiate mechanisms of the hippocampus are activated; the kappa-opiate system of RSN is involved in the mechanisms of termination of EA and suppression of activity in the hippocampus. Evidently in cases when the degree of activation of the antiepileptic system and, in particular, of RSN, is insufficient to suppress EA, termination of seizures is brought about by means of other mechanisms, and preservation of hippocampal hyperactivity is manifested as postictal explosive behavior.

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