these compounds was not abolished by injection of the benzodiazepine antagonist Ro-15 1788 (10 mg/kg intraperitoneally, 15 min before testing). In this case the mean number of "punishable" approaches to the drinking bowl was 2.8 ± 0.6 in the control and 6.2 ± 0.6 and 10.2 ± 1.4 in groups of animals receiving pyracetam and mebicar respectively, compared with 2.4 ± 0.5 in rats receiving diazepam (5 g/kg 40 min before testing).

Thus pyracetam and mebicar in experiments *in vivo* on normal animals can exert their anxiolytic action without the participation of benzodiazepine receptors. The possibility remains that either the interaction of pyracetam and mebicar with benzodiazepine receptors which we observed *in vitro* has a different interpretation than competition of these compounds with specific binding sites of ^{3}H-flunitrazepam, or in experiments on normal animals *in vivo* GABA-benzodiazepine receptor complex does not accept pyracetam and mebicar, for it contains endogenous inhibitors of GABA-modulating action. Whatever the case, the results are evidence that the anxiolytic action of pyracetam (and mebicar) may be realized with the participation of this supramolecular compex.

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CEREBROVASCULAR EFFECTS OF MET- AND LEU-ENKEPHALINS

R. S. Mirzoyan, Kh. S. Ragimov, and T. S. Gan'shina

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KEY WORDS: cerebral circulation; Met- and Leu-enkephalins; naloxone.

The important role of central adrenergic and GABA-ergic mechanisms in the control of the cerebral circulation has recently been discovered [3]. Considering the extensive data in the literature on interaction of neuropeptides with adrenergic and GABA-ergic brain systems it is evidently important to study the effect of opioid peptides on the cerebral hemodynamics.

We found no information in the literature on the cerebrovascular effects of enkephalins. Meanwhile the effects of naloxone, an antagonist of opiate receptors, on the cerebral circulation has been reported in patients with neurological disturbances [5-10]. The possible role of opioid peptides in the development of cerebrovascular disorders is discussed in these publications.

The aim of the present investigation was to study the effect of Met- and Leu-enkephalins on the cerebral circulation and on neurogenic cerebrovascular reactions. The effects of Leu-enkephalin also were studied when GABA-receptors were blocked by bicuculline and opiate receptors by naloxone.

Department of Neuropharmacology, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow.

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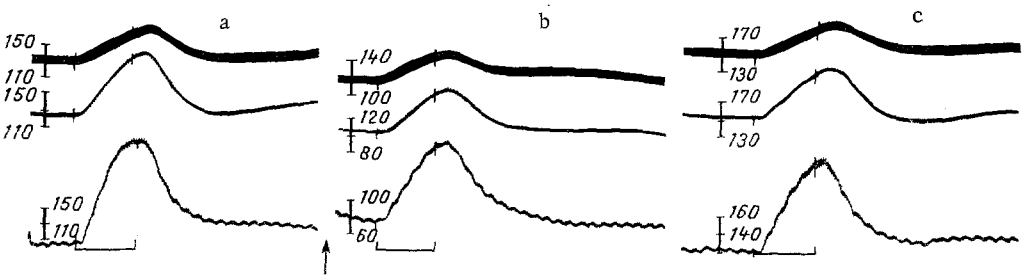

Fig. 1. Effect of Leu-enkephalin (0.5 mg/kg, intravenously) on constrictor reactions of cerebral vessels to stimulation of afferent fibers of n. tibialis. a) Control; b,c) 2 and 35 min respectively after injection of peptides. From top to bottom: perfusion pressure in systems of carotid and vertebrobasilar arteries, BP (in mm Hg), marker of stimulation (20 V, 40 Hz, 2 msec, for 15 sec).

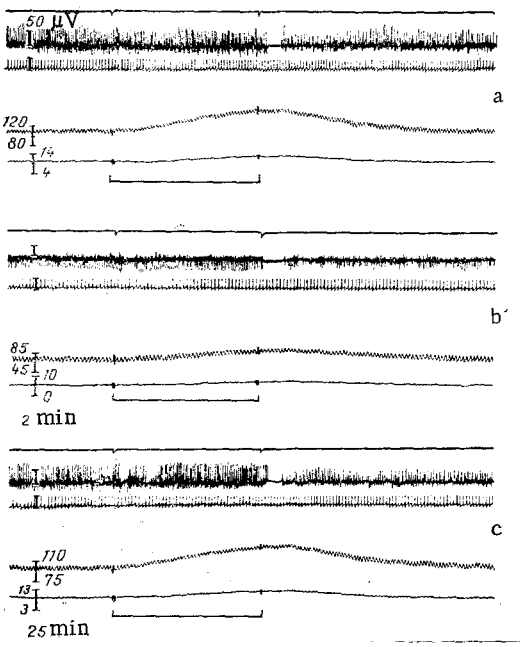

Fig. 2. Effect of Met-enkephalin (0.5 mg/kg, intravenously) on somatosympathetic and vasomotor reflexes: a) control; b, c) 2 and 35 min respectively after injection of peptide. From top to bottom: time marker (1 sec), tonic and reflex activity in sympathetic nerves of kidney, ECG, BP (in mm Hg), blood flow in carotid artery (in ml/min), marker of stimulation (20 V, 40 Hz, 2 msec, for 15 sec).

EXPERIMENTAL METHOD

Experiments were carried out on 58 cats weighing 3-4 kg under general anesthesia (urethane, chloralose) with artificial vertilation of the lungs, and on five waking rabbits.

The inflow of blood into the brain through the carotid artery was measured, after careful ligation of the extracranial branches, by means of an electromagnetic flowmeter (Nihon Kohden, Japan). At the same time the ECG in lead II and the blood pressure (BP) in the femoral artery were recorded. Tonic activity and reflex discharges were recorded in sympathetic nerves of the renal plexus [1]. The vascular component of the action of the substances on the cerebral hemodynamics was differentiated by a technique of separate perfusion of the carotid and vertebrobasilar arteries on both sides [2]. The parameters were recorded on a Mingograf-81 instrument. The cerebral blood flow also was recorded in the cortex of waking rabbits by the hydrogen clearance method, after preliminary implantation of a platinum electrode [4]. The partial pressure of CO_2 (PCO_2) was determined in samples of arterial blood by

the micro-Astrup method, and maintained within limits of the control values (30-35 mm Hg). Reflex responses of the cerebral vessels and BP were evoked by electrical stimulation of the central end of the divided tibial nerve (20-40 V, 20-40 Hz, 2 msec, for 15 msec).

The test substances were injected intravenously and into the lateral ventricle: Met- and Leu-enkephalins (from Serva, West Germany) 0.5 and 0.1 mg/kg respectively, naloxone (Narcan, USA) 0.2-0.4 and 0.1 mg/kg. Bicuculline (Serva) was injected intravenously only and in a dose of 0.2 mg/kg. The animals were killed with a mixture of urethane and chloralose.

EXPERIMENTAL RESULTS

The study of the effect of Met- and Leu-enkephalins (0.5 mg/kg, intravenously) on the cerebral circulation showed that the peptides induced an initial, transient (2 min) increase in the cerebral blood flow by 14.0 ± 3.3 and 16.0 ± 2.8% respectively. The blood supply to the brain then fell to 29.0 ± 2.8 and 28.0 ± 5.3% below its initial level. Restoration of the control values was observed 10-15 min after injection of the opioid peptides. Under the influence of Met- and Leu-enkephalins BP fell in most experiments by 32.0 ± 2.9 and 36.0 ± 1.9% respectively. The opioid peptides reduced the heart rate.

In experiments with direct recording of cerebravascular tone Met- and Leu-enkephalins reduced vascular resistance in the carotid (by 14.0 ± 2.2 and 16.0 ± 1.6% respectively) and vertebrobasilar (by 17.0 ± 3.8 and 13.0 ± 2.8%) systems.

Met- and Leu- enkephalins had a marked depressant effect on neurogenic constrictor responses of the cerebral vessels. In experiments in which reflex cerebrovascular responses were recorded, Met- and Leu-enkephalins depressed the responses of vessels of the carotid system by 60.0 ± 9.4 and 44.0 ± 7.3%, and of the vertebrobasilar system by 56.0 ± 10.2 and 44.0 ± 6.5% respectively (Fig. 1). At the same time, pressor reflex responses of BP were weakened by the action of the peptides by 49.0 ± 7.4 and 45.0 ± 8.0% respectively.

These opioid peptides significantly inhibited tonic activity and reflex discharges in the sympathetic nerves (Fig. 2).

Similar changes in the cerebral blood flow, discharges in the sympathetic nerves, and BP were observed after injection of Met- and Leu-enkephalins (0.1 mg/kg) into the lateral ventricles.

The aim of the subsequent investigation was to study the neurotransmitter mechanism of these effects of the opioid peptides. Considering that the cerebrovascular effects of Met- and Leu-enkephalins were identical, the mechanism of action of only one of them, namely Leu-enkephalin, was analyzed. In a separate series of experiments the effect of Leu-enkephalin on the cerebral circulation and cerebrovascular reflexes was studied after preliminary blocking of GABA-receptors by bicuculline. The cerebrovascular effects of Leu-enkephalin were found not to be mediated through bicuculline-sensitive GABA receptors.

A different picture was found when the effect of Leu-enkephalin on the blood supply of the brain and on neurogenic constrictor respsonses was studied after blocking of opiate receptors by naloxone. Against the background of naloxone, cerebrovascular tone was depressed by a much lesser degree and the effect of the peptide on cerebrovascular and vasomotor reflexes was weakened or completely abolished.

The results thus indicate that Met- and Leu-enkephalins can exert a biphasic action on the blood supply to the brain. An initial increase in blood flow is followed by a decrease in the blood flow into the brain.

These results indicate a marked depressant effect of enekephalins on neurogenic constrictor responses of the cerebral vessels and on somatosympathetic and vasomotor reflexes. The effect of the enkephalins observed in these experiments also are exhibited when they are injected into the lateral ventricles. This indicates that the inhibitory effect of opioid peptides is due to their influence on central processes of regulation of the cerebral circulation and BP.

Bicuculline does not affect, but naloxone abolishes or weakens the effects of Leu-enkephalin on the cerebral circulation and its nervous control.

Consequently, opiate receptors evidently participate in realization of the cerebrovascular effects of the opioid peptides. The results also indicate that the opioid system of the brain participates in regulation of the cerebral circulation.

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EFFECT OF CHLORGYLINE ON THE GABA LEVEL IN HYPEROXIA

I. A. Goroshinskaya and K. B. Sherstnev

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A fall in the γ-aminobutyric acid (GABA) concentration, discovered in the brain of rats, mice, rabbits, chickens, and hamsters at different stages of hyperoxia, is an important step in the mechanism of development of oxygen poisoning during exposure to hyperbaric oxygen [3, 6, 8, 9]. GABA performs the function of inhibitory mediator [5]. Lowering of its level in the brain is regarded as one cause of the development of oxygen convulsions [10]. The writers showed previously that in hyperoxia qualitative changes take place in the catalytic properties of mitochondrial monoamine oxidase (MAO) [amine: oxygen-oxidoreductase (deaminating), flavin-containing, EC 1.4.3.4], as a result of which the enzyme becomes capable of deaminating several substances that normally are not MAO sustrates, including GABA [2]. Ability to modify substrate specificity during exposure to various factors and, in particular, to hyperoxia, is a feature of type A MAO (whose natural substrates are serotonin and noradrenalin) [1]. Chlorgyline, a selectively acting inhibitor of type A MAO, prevents transformation of the enzyme under the influence of hyperoxia and has a protective effect on the body, delaying the onset of oxygen convulsions and increasing the survival rate of the animals [7].

To study the mechanism of the protective action of chlorgyline, its effect on the GABA level was studied in the brain of animals exposed to hyperoxia.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred adult rats of both sexes weighing 150-180 g. The effect of oxygen under a pressure of 0.7 MPa. An intact animal and an animal receiving an intraperitoneal injection of clorgyline in a dose of 5 mg/kg 30 min before the session of hyperoxia, were placed in a pressure chamber simultaneously. The experiments ended in one series with the onset of convulsions in the intact rat (on average after 30 min, limits of variations 19-42 min). Animals protected with chlorgyline did not develop convulsions at this time. In the other series of experiments decompression was carried out after convulsions had begun in the rat protected with chlorgyline. Intact animals and animals taken 60 min after injection of clorgyline served as the control. An alcohol (10%) homogenate of the brain was centrifuged for 10 min at 400g, the residue was discarded, and the supernatant was evaporated on a rotary evaporator and its GABA content was determined on an AAA-881 automatic amino acid analyzer (Microtechna, Czechoslovakia) on a small column in 0.2M Na-citrate buffer, pH 4.25. Chlorgyline (M and B 9302), obtained from Dr. G. D. Barber (May and Baker, England), was generously supplied by Professor V. Z. Gorkin.

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