EFFECT OF AGONISTS AND ANTAGONISTS OF OPIATE RECEPTORS ON RESISTANCE OF ANIMALS TO HYPOXIC HYPOXIA

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We know that morphine increases the resistance of animals to hypoxic hypoxia [1, 4, 5]. However, the mechanism of this effect and, in particular, the question of the possible role of opiate receptors in its realization, has not been studied.

It was accordingly decided to undertake a comparative study of the effect of both agonists and antagonists of opiate receptors on the resistance of animals to hypoxic hypoxia. As agonists of opiate receptors were used morphine and synthetic analogs of endogenous morphine-like substances (enkephalins): Try-D-Ala-Gly-Phe-(NO_2)-NH_2, Tyr-D-Ala-Gly-Phe-D-Leu, Tyr-D-Ala-Gly-(Me)Phe-Gly-ol**, FK 33-824-Try-D-Ala-Gly-MePhe-Met-(O)-ol (Peninsula Laboratories, Inc.).

Peptides marked with two asterisks were synthesized at the All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, under the direction of Doctor of Chemical Science M. I. Titov. Opiate receptors were blocked by naloxone (a "pure" antagonist) and nalorphine (an agonist-antagonist). Thyrotrophin releasing hormone (TRH) — Pyr-His-Pro-NH_2 (from Peninsula Laboratories, Inc.), which has no ability to bind with opiate receptors, although it abolishes many of the effects of opiates and of endogenous opiate peptides, also was used [3, 10].

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice (1200 animals), nine cats, and 11 rabbits. The conditions of normobaric hypoxic hypoxia in mice were simulated by keeping them one at a time in 250-ml containers, in which part of the air had previously been replaced by nitrogen, after which the chambers were closed airtightly [4]. The initial O_2 concentration in the inspired air was 8 vols. %. CO_2 was absorbed by soda lime. The length of survival of the mice (until respiratory arrest) under conditions of hypoxic hypoxia was recorded. All drugs were injected intraperitoneally (except bicuculline, which was injected subcutaneously) in a volume of 0.05 ml/mg body weight. Naloxone and TRH were injected 5 min, and the remaining drugs 10 min before the mice were placed in the airtight chamber. Animals of the control groups received isotonic NaCl solution. The analgesic effect was assessed by Haffner's method. Acute hypoxic hypoxia was induced in totally curarized cats and rabbits (diplacin, 5 mg/kg, intravenously) by switching off the artificial respiration apparatus for 5 min. The duration of preservation of cortical biopotentials (the EEG of the sensomotor cortex was recorded) was determined. Naloxone was injected intravenously in a dose of 0.1-0.3 mg/kg 5 min before the artificial respiration apparatus was turned off.

EXPERIMENTAL RESULTS

Depending on the dose morphine increased the period of survival of the animals during hypoxic hypoxia (Table 1). Of the four synthetic analogs of enkephalins used in the work,

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TABLE 1. Effect of Agonists and Antagonists of Opiate Receptors on Resistance of Mice to Hypoxic Hypoxia (M ± m)

Compound and dose, mg/kg	No. of mice	Wt. of mice, g	Duration of survival, min
Agonists			
Control	12	20±1	18±1
Morphine 0.1	8	19±1	20±1
Control	22	20±1	17±1
Morphine 1.0	15	20±1	23±1 ‡
Control	15	20±1	16±1
Morphine 5.0	16	22±1	23±1 ‡
Control	22	20±1	17±1
Morphine 10.0	16	20±1	30±2 ‡
Control	16	18±1	19±1
Tyr-D-Ala-Gly-(Me)Phe-Gly-ol, 0,1	16	19±1	19±1
Control	16	18±1	19±1
Tyr-D-Ala-Gly-(Me)Phe-Gly-ol, 10,0	15	19±1	23±1 †
FK 33-824, 1,0	22	21±1	25±1 ‡
Control	15	22±1	16±1
FK 33-824, 5,0	8	21±1	24±1 ‡
Control	8	20±1	16±1
FK 33-824, 10,0	8	20±1	23±1 ‡
Control	14	18±1	20±1
Tyr-D-Ala-Phe-(NO_2)-NH_2, 1,0	16	19±1	24±1 †
Control	14	18±1	20±1
Tyr-D-Ala-Gly-Phe-(NO_2)-NH_2, 5,0	16	18±1	23±1*
Control	14	18±1	20±1
Tyr-D-Ala-Gly-Phe-(NO_2)-NH_2, 10,1			
10,0	13	19±1	26±1 ‡
Control	24	19±1	20±1
Tyr-D-Ala-Gly-Phe-D-Leu, 1,0	16	19±1	19±1
Control	16	20±1	17±1
Tyr-D-Ala-Gly-Phe-D-Leu, 10,0	16	19±1	18±1
Antagonists			
Control	16	19±1	20±1
Naloxone, 0.1	16	19±1	14±1 ‡
Control	32	19±1	19±1
Naloxone, 1.0	32	19±1	16±1*
Control	24	19±1	20±1
Naloxone, 10.0	16	19±1	20±1
Control	24	19±1	19±1
Nalorphine, 1.0	16	19±1	16±1*
Control	32	18±1	19±1
Nalorphine, 5.0	16	19±1	16±1*
Control	32	18±1	19±1
Nalorphine, 10.0	16	19±1	17±1
Control	15	19±1	19±1
TRH, 1.0	16	19±1	10±1 ‡
Control	15	19±1	19±1
TRH, 5.0	14	19±1	7±1 ‡
Control	15	19±1	19±1
TRH, 10.0	16	19±1	7±1 ‡

Legend. *P < 0.05, † P < 0.01, ‡ P < 0.001 compared with control.

three also were found to have an antihypoxic action: FK 33-824, Tyr-D-Ala-Gly-Phe-(NO_2)-NH_2, Tyr-D-Ala-Gly-MePhe-Gly-ol. Compound FK 33-824 which, in a dose of 1.0 mg/kg, had a stronger action than morphine in the same dose, is particularly interesting from this standpoint. The antihypoxic effect of morphine and enkephalin analogs was blocked by naloxone in doses of 0.1-1.0 mg/kg (Fig. 1), which suggests that it is connected with stimulation of opiate receptors. The fact will be noted that the enkephalin analog Tyr-D-Ala-Gly-Phe-D-Leu (unlike the other peptides tested) has no antihypoxic properties whatever. This is not due to rapid inactivation of the peptide by enzymes, for all the enkephalin analogs studied have D-alanine in position 2, which protects them against the action of peptidases. To solve the problem of whether poor permeability of the blood-brain barrier for this peptide is the reason

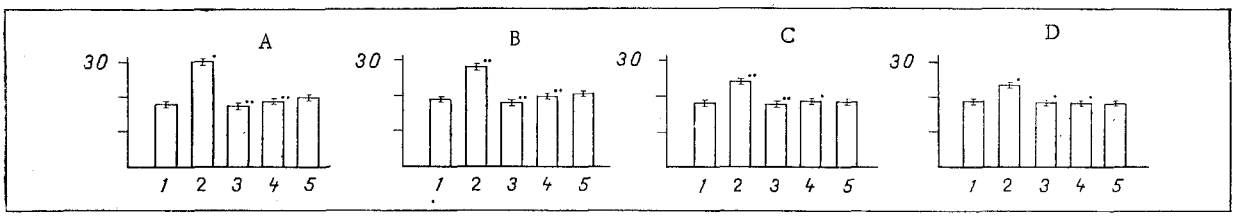

Fig. 1. Effect of morphine and enkephalin on duration of survival of mice in airtight chamber initially containing 8 vols. % oxygen in the inspired air. A: 1) Control, 2) morphine (10 mg/kg), 3) morphine (10 mg/kg) + naloxone (0.1 mg/kg), 4) morphine (10 mg/kg) + bicuculline (1 mg/kg), 5) bicuculline (1 mg/kg). B: 1) Control, 2) FK 33-823 (1 mg/kg), 3) FK 33-824 (1 mg/kg) + naloxone (0.1 mg/kg), 4) FK 33-824 (1 mg/kg) + bicuculline (1 mg/kg), 5) bicuculline (1 mg/kg). C: 1) Control, 2) Tyr-D-Ala-Gly-Phe-(NO_2)-NH_2 (10 mg/kg), 3) Tyr-D-Ala-Gly-Phe-(NO_2)-NH_2 (10 mg/kg) + naloxone (0.1 mg/kg), 4) Tyr-D-Ala-Gly-Phe-(NO_2)-NH_2 (10 mg/kg) + bicuculline (1 mg/kg), 5) bicuculline (1 mg/kg). D: 1) Control, 2) Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (10 mg/kg), 3) Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (10 mg/kg) + naloxone (0.1 mg/kg), 4) Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (10 mg/kg) + bicuculline (1 mg/kg), 5) bicuculline (1 mg/kg).

for the absence of an antihyposic effect, the analgesic activity of this compound was estimated and compared with that of the other enkephalin analogs tested. All the synthetic opioid peptides, like morphine itself, were found to have analgesic activity when injected intraperitoneally in a dose of 10 mg/kg. According to the strength of its analgesic effect, the highest activity was exhibited by FK 33-824, followed by Tyr-D-Ala-Gly-Phe-(NO_2)-NH_2, then morphine, and the remaining enkephalin analogs. Naloxone (1.0 mg/kg) blocked the analgesic effects of the compounds. Consequently, all synthetic opioid peptides used in these experiments passed through the blood-brain barrier, and the absence of antihypoxid properties of Tyr-D-Ala-Gly-Phe-D-Leu cannot be attributed to poor penetration into the brain. The reason for the absence of antihypoxic activity in this last peptide may perhaps be that, unlike morphine, FK 33-824, and other enkephalin analogs, which are agonists of μ-opiate receptors [8, 9, 13], it exhibits selective affinity for δ-opiate receptors [7, 8, 11].

Unlike morphine and opioid peptides, their antagonists — naloxone (0.1-1.0 mg/kg), nalorphine (1.0-5.0 mg/kg), and TRH (1.0-10.0 mg/kg) — shortened the survival of the mice during exposure to hypoxic hypoxia (Table 1). A similar effect also was observed in experiments on rabbits and cats on the other model of acute hypoxic hypoxia. In cats, for instance, naloxone in doses of 0.1-0.3 mg/kg shortened the duration of preservation of cortical potentials to 2.3 ± 0.4 min compared with 3.4 ± 0.3 min in the control ($P < 0.05$). Consequently, it can be postulated that endogenous opioid peptides play a protective role *in vivo* in hypoxic hypoxia.

Considering data obtained previously on the ability of bicuculline to exert a naloxone-like action in analgesia induced by morphine and synthetic analogs of enkephalins [2], it was decided to investigate the effect of bicuculline on the antihypoxic effect of morphine and enkephalin analogs. In this case also, bicuculline was found to exhibit naloxone-like properties (Fig. 1).

The results of this investigation showing that morphine and some synthetic enkephalin analogs increase the resistance of animals to exposure to hypoxia and that this effect is blocked by naloxone thus indicate that the effect is mediated through interaction with opiate receptors. The presence of antagonism with bicuculline suggests the possibility of GABA-ergic modulation of this interaction.

In conjunction with previous data on the protective role of endogenous opioid peptides in traumatic shock [6] the results of the present investigation indicate that opiate receptors and their endogenous ligands (opioid peptides) participate in the protective responses of the body during exposure to these extremal conditions.

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MECHANISM OF THE FALL IN INTRACRANIAL PRESSURE DUE TO THE ACTION OF FUROSEMIDE

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Furosemide is widely used in clinical practice for the treatment of cerebral edema and to lower the intracranial pressure. The mechanism of its action is attributed to its direct effect on water and electrolyte metabolism in brain tissue [4], to a decrease in CSF formation [6, 8], and to dehydration of brain tissue caused by a rise in the osmotic pressure of the blood as a result of loss of salts and water by the kidney [1, 7]. The writers showed previously that after injection of polyethyleneglycol-400 dehydration of brain tissues is accompanied by an increase in the blood volume of the brain, maintaining a stable fluid level in the airtight cranial cavity [3]. It could accordingly be postulated that the cause of the fall in CSF pressure after administration of furosemide was a reduction in the intracranial blood volume due to loss of extracellular fluid as a result of intensive diuresis. The aim of the present investigation was to study this possibility.

EXPERIMENTAL METHOD

Experiments were carried out on 70 mongrel dogs weighing 5-15 kg and 17 female albino rats weighing 150-200 g. Under pentobarbital anesthesia (25-35 mg/kg) siliconized catheters were introduced into the femoral vein and artery and jugular vein of the dogs; the cisterna magna was punctured and the urinary bladder catheterized at the same time. The arterial blood pressure was measured by the direct method with a mercury manometer, and the pressure in the inferior vena cava and jugular vein and the cisternal CSF pressure were measured with a water manometer. Furosemide was injected intravenously in a dose of 10 mg/kg.

Under pentobarbital anesthesia the rats' skulls were trephined above the right cerebral hemisphere and brain trauma was inflicted by a mechanical method. On the 3rd day after trauma, when cerebral edema was most marked, furosemide was injected intraperitoneally into the animals; 60 min later they were decapitated, blood and the cerebral hemispheres were removed for investigation, dried at 105°C to constant weight to determine the water content, after which concentrated HNO_3 was added and, after the organic matter had dissolved, the Na^+ and K^+ concentrations were determined by flame photometry, Fe by atomic absorption spectrophotometry, the serum protein concentration on a refractometer, and osmolarity by a cyroscopic method.

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