ROLE OF MU- AND DELTA-OPIATE RECEPTORS IN THE ACTION OF ENKEPHALINS ON THE COURSE OF HYPOXIC HYPOXIA

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There is evidence of the antihypoxic action of opioids. Opioid peptides and some opiate analgesics increase the resistance of the myocardium to hypoxia, as is confirmed by the results of both experimental [5] and clinical [7] investigations; they lengthen life of experimental animals exposed to hypoxic hypoxia [1, 2] and prevent tissue damage caused by stress or ischemia [3, 6]. It is quite evident that a further study of the mechanisms of action of opioid peptides (OP), including the role of different types of receptors in the realization of the antihypoxic effect of opioids, is interesting.

The aim of the present investigation was to study the role of mu- and delta-opiate receptors in the action of enkephalin analogs on the course of normobaric hypoxic hypoxia, which is an adequate and convenient experimental model with which to study the antihypoxic effect of OP [1].

EXPERIMENTAL METHODS

Enkephalin analogs with linear, cyclic, and branched structure of their peptide chain, of the following composition were synthesized by classical methods of peptide chemistry:

Tyr—D—Ala—Gly—Phe$(NO)_2$—NH_2 (A)

Tyr—D—Orn—Gly—Phe (B)

Tyr—D—Orn—Gly—Phe—Asp (C)

Tyr—D—Orn—Gly—Phe
Ac—Leu (D)

We also used a natural Leu^5-enkephalin (LE; from "Fluka"): the selective delta-receptor agonist Tyr–D–Ala–Gly–Phe–D–Leu (DADLE), the mu-receptor agonist Tyr–D–Ala–Gly–MePhe–Gly–Ol (DAGO), and the opiate receptor blocker naloxone (obtained at the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Russian Academy of Medical Sciences), also were used. The effect of the peptides on opiate receptors was determined from their ability to inhibit contractions of a segment of the longitudinal muscle of the guinea pig ileum (GPI) and of the isolated mouse vas deferens (VD), evoked by electrical stimulation (single pulses 1 msec in duration, frequency 0.1 Hz, by means of platinum electrodes). Interaction of the peptides with mu-receptors was assessed on preparations of GPI, with delta-receptors on VD. Isometric contractions were recorded by means of a TB-611T transducer, connected to a polygraph ("Nihon Kohden", Japan). Activity of the substances was expressed in terms of IC_{50} (the concentration causing inhibition of contractions by 50%, in moles/liter). The antihypoxic activity of the opioid peptides was studied on 250 CBwH mice weighing 20-22 g. Normobaric hypoxic hypoxia was produced by placing the animals in an airtight chamber containing soda-lime to absorb the excess of carbon dioxide [1]. The survival time of the mice was determined with a stopwatch, after cessation of respiratory movements

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TABLE 1. Effect of Enkephalins on Peripheral Opiate Receptors and Survival Time of Mice Exposed to Hypoxia

Preparation	IC_{50}, moles/liter		Length of survival of exposed mice			
	GPI	VD	number of animals	length of survival, in min	change in per cent compared with control	
Control	—	—	20	10,9±0,3		
LE	$(2,85\pm0,78)\cdot10^{-7}$	$(2,00\pm0,1)\cdot10^{-8}$	20	12,4±0,5	+13	<0,05
Control	—	—	20	11,3±0,3		
A	$(1,54\pm0,55)\cdot10^{-8}$	$(3,15\pm0,66)\cdot10^{-8}$	36	16,0±0,7	+41,6	<0,01
B	$(9,20\pm1,76)\cdot10^{-8}$	$(5,80\pm0,8)\cdot10^{-7}$	12	14,5±0,8	+28,3	<0,01
C	$(2,00\pm0,40)\cdot10^{-7}$	$(1,60\pm0,23)\cdot10^{-7}$	12	13,8±0,8	+22,1	<0,05
D	$(6,47\pm2,62)\cdot10^{-7}$	$(6,59\pm1,00)\cdot10^{-7}$	10	11,5±1,7	+1,8	>0,10
Control	—	—	24	11,1±0,4		
DAGO	—	—	24	15,7±0,5	+41,1	<0,01
DADLE	—	—	24	12,2±0,4	+9,9	>0,05
DAGO+DADLE	—	—	24	15,2±0,5	+36,9	<0,01
Naloxone + DAGO	—	—	24	13,9±0,6	+28,3	<0,01

[1]. The peptides for study were injected intraperitoneally in a dose of 1.0 mg/kg in 0.1 ml of isotonic sodium chloride solution immediately before the animals were placed in the airtight chamber. This dose is optimal for the study of antihypoxic activity of opioid peptides [1, 2]. Mice of the control group were given an injection of isotonic sodium chloride solution. The results were subjected to statistical analysis (X ± m).

EXPERIMENTAL RESULTS

Investigation of interaction of compounds A, B, C, D, and LE with opiate receptors on preparations of GPI and VD revealed high affinity of peptide A for mu- and delta-receptors. Compound B had its predominant effect on mu-, but LE on delta-receptors. Substance C interacted with both mu- and delta-receptors, but from this point of view it was virtually one order of magnitude weaker than peptide A. Preparation D had the lowest affinity for both types of receptors (Table 1). With respect to efficiency of interaction with mu-receptors the test substances could be arranged in the following descending order of magnitude: A > B > C > LE > D, and by affinity for delta-receptors, in the order LE > A > C > B > D.

These substances likewise did not equally affect the parameters of resistance of the animals to hypoxia.

As the data in Table 1 show, the length of survival of the mice in the airtight chamber was most effectively increased by compound A, whereas D had virtually no effect on this parameter. As regards the intensity of the antihypoxic action of the preparations, they were arranged in the following order: A > B > C > LE > D, i.e., like the distribution of affinity for mu-receptors.

The characteristics of the opioid activity of DAGO and DADLE have been well studied and described in the literature [8-10]. In particular, DAGO is a selective agonist of mu-, DADLE of delta-receptors.

In our investigations injection of DAGO significantly increased the length of survival of the mice during exposure to hypoxic hypoxia, whereas DADLE caused no statistically significant change in this parameter. If the two peptides were given simultaneously, DADLE did not change the antihypoxic effect of DAGO (Table 1). Preliminary (5 min before DAGO) administration of naloxone in a dose of 0.1 mg/kg considerably prevented the effect of the mu-agonist on the duration of survival of the mice exposed to hypoxia (Table 1).

The data given above are evidence of correlation between the antihypoxic activity of opioid peptides and the characteristics of their interaction with opiate receptors. Compounds interacting effectively with mu-receptors, independently of the degree of their affinity for delta-receptors, led to increased duration of survival of the mice exposed to hypoxia. The intensity of the antihypoxic effect of the opioid peptides in this case was proportional to their specificity for mu-receptors. The role of mu-receptors in the mechanism of the antihypoxic action of enkephalins is clearly demonstrated by the results of experiments in which selective agonists of mu- (DAGO) and delta- (DADLE) receptors were used. These substances are widely used for the pharmacologic study of the role of opiate receptors in regulation of various physiological and biochemical processes [4, 8, 9]. In our experiments DAGO considerably increased, whereas DADLE did not change the duration of survival of mice exposed to hypoxia. In the

case of combined administration, the delta-agonist did not change the character of the antihypoxic effect of the mu-agonist. Conversely, preliminary injection of the opiate receptor blocker naloxone in a dose of 0.1 mg/kg, which inhibits selectively mu-receptors [9, 10], significantly prevented the effect of opioids on the duration of survival of the mice during hypoxia.

Enkephalins and their synthetic analogs effectively interact with mu- and delta-receptors, but have no significant effect on other types of opiate receptors [8, 9]; we therefore did not investigate the role of kappa-, sigma-, and epsilon-receptors.

The results are thus evidence that enkephalins, when exerting their influence through mu-receptors, increase the resistance of the recipient to the action of hypoxic hypoxia, whereas delta-receptors are not involved in the realization of the antihypoxic effect of opioid peptides.

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