ROLE OF MU- AND DELTA-OPIATE RECEPTORS IN REALIZATION OF THE AUTONOMIC EFFECTS OF OPIOID PEPTIDES

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Pharmacological research in recent years has revealed high activity of opioid peptides on the cardiovascular system. Intravenous injection of enkephalins and their synthetic analogs into anesthetized animals causes a transient fall of blood pressure (BP) and heart rate (HR) [1, 11, 12]. The mechanism of development of autonomic responses to systemically injected opioid peptides, and the role of mu- and delta-opiate receptors in their realization still remain unexplained.

To study the mechanism of the cardiovascular action of intravenously injected opioid peptides it was decided to investigate the effect of selective agonists of mu- and delta-opiate receptors on the basic parameters of the hemodynamics and respiratory function in anesthetized animals. To choose the most selective mu- and delta-agonists, we studied the ability of several synthetic analogs of enkephalins to activate opiate receptors *in vitro* — in experiments on the isolated mouse vas deferens (which contains mainly delta-receptors) and the guinea pig ileum (which contains mainly mu-receptors) [6].

EXPERIMENTAL METHOD

Preparations of the longitudinal muscle of the ileum including the enteric plexus were obtained from male noninbred male guinea pigs weighing 500-600 g, and preparations of the mouse vas deferens from (C57BL×CBA)F_1 hybrids weighing 20-25 g. The preparations were placed in a constant-temperature cell, filled with modified Krebs' solution, and stimulated with an electric field [4, 5]. Contractile activity of the preparations was recorded by the use of transducers of isometric contractions. The degree of affinity of the agonist for the receptors was judged from the value of IC_{50} — the concentration of peptide reducing the amplitude of contractile responses of the preparation to electrical stimulation by 50%. Values of IC_{50} were obtained by interpolation on a logarithmic dose–response curve, plotted by the method of least squares, and allowing for all experimental data. Experiments with intravenous injection of the peptides were carried out on male Wistar rats weighing 400-500 g. The animals were anesthetized with 25% urethane solution (1.25 g/kg, intraperitoneally). Polyethylene catheters were introduced into the femoral artery and jugular vein. BP was measured with an electromanometer, HR by a digital cardiotachometer, triggered by the pulse wave of BP. Respiration was recorded plethysmographically. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

All peptides tested in experiments *in vitro* inhibited contractions of preparations of the mouse vas deferens and guinea pig ileum, induced by electrical stimulation, i.e., they possessed agonistic activity relative to both mu- and delta-opiate receptors. The values of IC_{50}, calculated for each compound and reflecting the degree of affinity of the agonist for the receptors, are given in Table 1. The degree of selectivity of the agonist relative to one type of receptor was judged by the value of the ration IC_{50} delta/IC_{50} mu.

To study the effect of the peptides on the hemodynamics and respiration in anesthetized animals, on the basis of the results the peptide [DAla^2, DLeu^5]-enkephalin (DADLE) was chosen as the delta-agonist and DAla^2, [MePhe^4, Gly^5-ol]-enkephalin (DAMPGE) as the mu-agonist. These peptides have both high affinity and high selectivity for opiate receptors of one type. In

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TABLE 1. Effect of Synthetic Enkephalin Analogs on Peripheral Opiate Receptors

Compound	Inhibitory activity of substances on isolated guinea pig ileum (IC_{50} mu), M	Inhibitory activity of substances on isolated mouse vas deferens (IC_{50} delta), M; confidence interval at P = 0.05	Ratio IC_{50} delta / IC_{50} mu
Leucine-enkephalin	$6,15\ (3,51—10,79)\cdot10^{-7}$	$5,01\ (2,65—9,47)\cdot10^{-8}$	0,08
[Arg^6]-leucine-enkephalin	$1,46\ (1,12—1,92)\cdot10^{-6}$	$7,08\ (4,40—11,37)\cdot10^{-7}$	0,48
[$DAla^2$, Arg^6]-leucine-enkephalin	$0,79\ (0,28—2,26)\cdot10^{-7}$	$6,14\ (4,96—7,61)\cdot10^{-8}$	0,77
[$DAla^2$ $MePhe^4$, Arg^6]-leucine-enkephalin	$2,92\ (0,72—11,87)\cdot10^{-8}$	$1,03\ (0,69—1,55)\cdot10^{-7}$	3,53
DADLE	$0,75\ (0,44—1,29)\cdot10^{-7}$	$3,27\ (2,73—3,92)\cdot10^{-9}$	0,04
Methionine-enkephalin	$3,37\ (1,15—9,88)\cdot10^{-7}$	$5,52\ (4,13—7,37)\cdot10^{-8}$	0,16
[Arg^6]-Methionine-enkephalin	$0,75\ (0,18—3,10)\cdot10^{-6}$	$0,78\ (0,48—1,27)\cdot10^{-6}$	1,04
[Arg^6, Phe^7]-Methionine-enkephalin	$1,29\ (0,30—5,59)\cdot10^{-6}$	$1,40\ (0,98—1,98)\cdot10^{-7}$	0,11
[$DAla^2$, $MePhe^4$, $Met(o)^5$-ol]-enkephalin	$3,86\ (1,77—8,43)\cdot10^{-9}$	$6,41\ (4,70—8,75)\cdot10^{-8}$	16,61
DAMPGE	$1,30\ (0,80—2,12)\cdot10^{-8}$	$4,58\ (3,14—6,67)\cdot10^{-7}$	35,23
Morphine	$0,87\ (0,13—5,99)\cdot10^{-7}$	$1,00\ (0,37—2,73)\cdot10^{-5}$	115,07

Legend. The [Arg^6, Phe^7]-methionine-enkephalin was from Serva, West Germany, the [$DAla^2$, $MePhe^4$, $Met(o)^5$-ol]-enkephalin from Sandoz, Sweden. The remaining peptides were synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR.

TABLE 2. Initial Values of Hemodynamics and Respiration for Rats Anesthetized with Urethane: Intact and with Divided Vagus Nerve (M ± m)

Group of animals	Number of animals	BP, mm Hg	HR, beats/min	Respiration rate, cycles/min
Intact rats	12	85,0±7,1	379±10	84,0±3,0
Rats with bilaterally divided vagus nerves	10	79,4±3,7	431±19	60,0±2,7

addition, both peptides have increased resistance to degradation by peptidase [8], which is particularly important when the effects of intravenous injection of opioid peptides are studied.

To analyze autonomic effects of the peptide two series of experiments were conducted *in vivo*: In series I peptides were injected into intact rats, in series II they were injected into rats with the vagus nerves divided in the neck. Values of BP, HR, and respiration rate for rats in both series, before injection of peptides, are given in Table 2.

Intravenous injection of DADLE and DAMPGE in a dose of 10^{-7} mole/kg into intact rats gave rise to a triad of effects: a fall of BP, the development of bradycardia, and expiratory apnea (Fig. 1). All the effects had a short latent period: 2-3 sec after injection of the peptide for hypotension and bradycardia, 5-10 sec for apnea. The hypotension lasted 30-40 sec, and as a rule it was followed by a very small rise of BP. Bradycardia lasted longer than the hypotensive reaction (about 5 min). Apnea lasted on average 10-20 sec and was followed by rapid and deep breathing.

Intravenous injection of naloxone, which blocks opiate receptors, in a dose of 1 mg/kg 5 min before injection of the peptide completely prevented the development of the autonomic effect of DADLE and DAMPGE. This proves that all three effects owe their origin to interaction between peptides and opiate receptors.

It will be noted that a second injection of DADLE and DAMPGE into the animals in the same doses, after an interval of 30-45 min, gave substantially weaker effects on BP, HR, and respiration (Figs. 1 and 2), evidence of the appearance of marked tachyphylaxis, after only a single injection of the peptide. For this reason, it was impossible to obtain dose—effect curves for both peptides in the course of one experiment. When the magnitudes of the effects

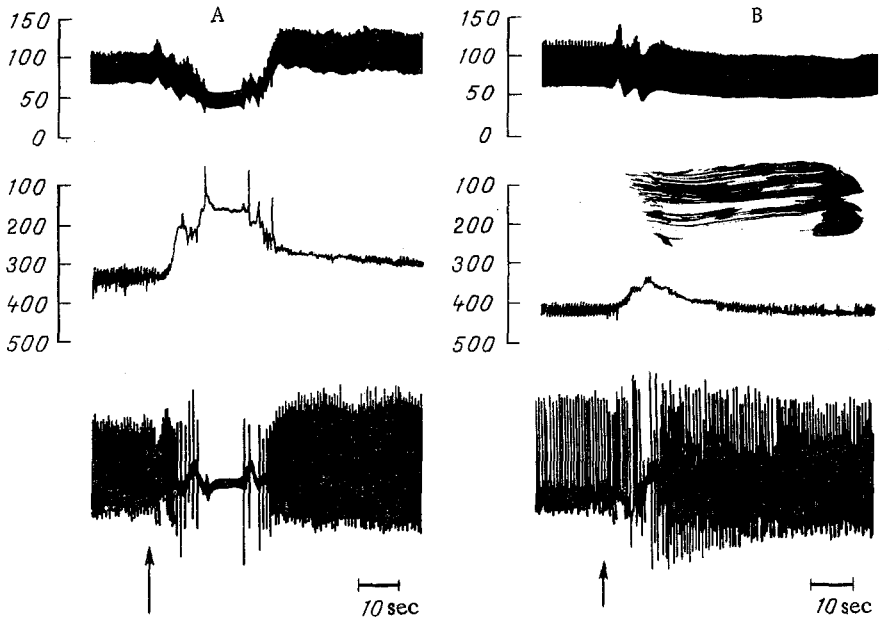

Fig. 1. Changes in BP, HR, and respiration in anesthetized rats during first (left) and second (right) intravenous injection of DADLE in a dose of 10^{-7} mole/kg. From top to bottom: BP (in mm Hg), HR (beats/min), respiration. Arrow indicates time of injection of peptide.

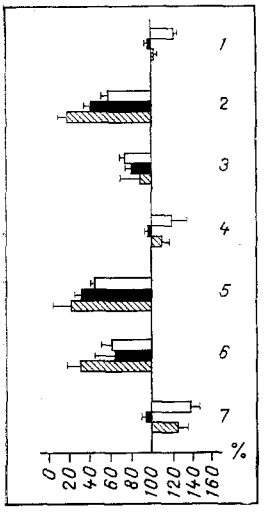

Fig. 2. Changes in BP, HR, and respiration rate (in percent of initial level) in intact rats and rats with bilateral vagotomy, after intravenous injection of DADLE and DAMPGE in a dose of 10^{-7} mole/kg. Unshaded columns — BP, black columns — HR, obliquely shaded columns — respiration rate. 1) Control (0.9% NaCl); 2) DADLE (intact rats, 1st injection); 3) DADLE (2nd injection); 4) DADLE (after vagotomy); 5) DAMPGE (intact rats, 1st injection); 6) DAMPGE (2nd injection); 7) DAMPGE (after vagotomy). Number of experiments 5.

of injection of different doses of DADLE and DAMPGE (10^{-7} mole/kg) were compared no significant differences were found.

Intravenous injection of DADLE and DAMPGE in the same doses into vagotomized rats caused the development of none of the three effects (Fig. 2). The absence of any appreciable effect of intravenously injected DADLE and DAMPGE on the hemodynamics and respiration of the vagotomized animals is evidence that none of the three effects which we observed is the result of the direct action of opioid peptides on the heart, blood vessels, and respiratory center. The results indicate that the main stage in the genesis of cardiovascular responses arising in intact rats to intravenous injection of the peptides is evidently strengthening of vagal influences on the heart on account of the direct action of the peptides on the central nuclei of the vagus nerve, or through their reflex action. The short latent period and duration of the cardiovascular effects observed in intact rats following intravenous injection of the peptides differed sharply from the character of responses caused by opiates which have passed through the blood-brain barrier [7]. In addition, the permeability of the blood-brain barrier for enkephalins and their synthetic analogs is known to be low [3, 9]. The effects we observed cannot therefore be attributed to any primary central action of the peptides.

Thus all three effects developing in response to intravenous injection of DADLE and DAMPGE (bradycardia, hypotension, and apnea) are most probably reflex in nature. The afferent component of this reflex is evidently the vagus nerve. The possibility that the cardiovascular and respiratory effects of opioid peptides may be mediated through the afferent pathways of the vagus nerve is indirectly confirmed by the results of autoradiographic investigations, which demonstrate the presence of opiate receptors capable of being transported to the periphery by the axoplasmic flow, in afferent fibers of the vagus nerve [2, 13]. Sapru et al. [10, 12] found opiate receptors in the lungs and showed that they can be activated by synthetic analogs of enkephalins. The similarity between the effects of DADLE and DAMPGE, observed in the present investigation, and the effects observed by the authors cited above after injection of leucine- and methionine-enkephalinamides into the right atrium of a rat, suggest that under the present experimental conditions the peptides activated opiate receptors associated with vagal afferents from the lungs.

Thus both mu- and delta-opiate receptors are involved in the realization of the autonomic effects of intravenously injected opioid peptides. The physiological importance of opiate receptors, activation of which induces reflex changes in respiration and in the cardiovascular system, still awaits explanation. To understand the role of peripheral opiate receptors in the regulation of autonomic functions, similar experiments must be performed on conscious unanesthetized animals.

LITERATURE CITED

1. O. S. Medvedev and M. I. Titov, in: Transactions of the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR [in Russian], Moscow (1982), pp. 88-101.
2. S. F. Atweh, L. C. Murrin, and M. J. Kuhar, Neuropharmacology, 17, 65 (1978).
3. E. M. Cornford et al., Endocrinology, 103, 1297 (1978).
4. J. Hughes, H. W. Kosterlitz, and F. M. Leslie, Br. J. Pharmacol., 53, 371 (1975).
5. H. W. Kosterlitz, R. J. Lydon, and A. J. Watt, Brit. J. Pharmacol., 39, 398 (1970).
6. H. W. Kosterlitz et al., Br. J. Pharmacol., 68, 333 (1980).
7. M. Laubie et al., Eur. J. Pharmacol., 28, 66 (1974).
8. A. T. McKnight, A. D. Corbett, and H. W. Kosterlitz, Eur. J. Pharmacol., 86, 393 (1983).
9. W. H. Pardridge and L. J. Mietus, Endocrinology, 109, 1138 (1981).
10. H. N. Sapru, S. Gupta, and A. J. Krieger, Fed. Proc., 39, 1017 (1980).
11. E. T. Wei, A. Lee, and J. K. Chang, Life Sci., 26, 1517 (1980).
12. R. N. Willette and H. N. Sapru, Eur. J. Pharmacol., 78, 61 (1982).
13. W. S. Young III et al., Science, 210, 76 (1980).