EFFECT OF OPIOID PEPTIDES ON THE REGIONAL HEMODYNAMICS IN CONSCIOUS RATS

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Opioid peptides are located not only in structures of the CNS but also at the periphery. Endogenous opioid are found in sympathetic ganglia, the adrenal medulla, and sympathetic nerve endings in the heart and blood vessels [6, 9, 13]. In intact animals agonists of opiate receptors may have a variety of effects on the cardiovascular system through their action on central or peripheral receptors. The character of the cardiovascular effects depends on the route of entry of the opioids into the body and on the experimental conditions, especially, whether the animal is anesthetized or conscious [4, 12]. The role of the different types of opiate receptors in realization of the vegetative effects of opioid peptides and the mechanisms of their onset have not been completely elucidated.

The aim of this investigation was to study responses of the systemic and regional hemodynamics to intravenous injection of selective agonists of μ- and δ-opiate receptors in conscious rats. The substance [DAla^2, DLeu^5]-enkephalin (DADL) was used as δ-agonist and [DAla^2, MePhe^4,Gly^5-ol]-enkephalin (DAGO) as the μ-agonist. These peptides have both high affinity and high selectivity for opiate receptors of one type [2]. Both peptides likewise have increased resistance to the degrading action of peptidases [10], and this is particularly important when effects of intravenously injected peptides are studied. Peptides DADL and DAGO were synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, and were generously provided by Professor M. I. Titov.

EXPERIMENTAL METHOD

Male Wistar rats weighing 400-500 g were anesthetized with pentobarbital (30 mg/kg), after which polyethylene catheters, welded from PP10 and PP50 tubes (Portex, England) were introduced into the left ventricle through the right carotid artery, into the abdominal aorta through the femoral artery, and into the left jugular vein. Two steel electrodes were inserted subcutaneously in the region of the thorax to record respiration. The peripheral ends of the catheters and electrodes were taken subcutaneously to the region of the spine and fixed in the interscapular region. The experiments began 24-48 h after the operation. The arterial pressure (BP) was recorded by means of a "Statham P23ID" electromanometer (USA), a type 566 amplifier (Hugo Sachs, West Germany), and a Mark VII WR3101 recorder (Graphtec, Japan). The heart rate (HR) was determined by means of a type 567 (Hugo Sachs) cardiotachometer, and respiration was recorded by means of an PRG2-02 rheoplethysmograph (USSR). The cardiac output and blood flow in 10 different parts of the body were determined with the aid of microspheres 15 μ in diameter, labeled with ^{141}Ce, ^{51}Cr, ^{95}Nb, and ^{46}Sc (New England Nuclear, USA) [3, 5]. About 100,000 microspheres were injected once in physiological saline with 0.05% Tween-80. Immediately before injection the microspheres, placed in polyethylene coils, were thoroughly shaken and sonicated in an ultrasonic bath for 5 min. For 5 sec before injection of the microspheres, blood was withdrawn from the abdominal artery at a speed of 0.6 ml/min. The total sampling time was 90 sec and the suspension of microphores was injected in the course of 30 sec. The volume of blood withdrawn was replaced by injection of 0.54 ml of a 13.4% solution of Ficoll-70. The method of injection of the microspheres was described previously [3]. The experiment included four consecutive injections of microspheres: 1) before intravenous injection of the peptide, 2) 5 min after injection of the peptide, 3) 3 min after intravenous injection of naloxone (Endo Laboratories, USA), 4) 5 min after a second injection of the peptide, preceded by naloxone. The last two injections of microspheres were

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TABLE 1. Values of Hemodynamic Parameters (background and 5 min after injection of peptides) in Conscious Rats Receiving DADL and DAGO

Parameter	DADL (n=11)		DAGO (n=10)	
	background	peptide	background	peptide
BP, mm Hg	102,1±2,9	94,3±3,0*	94,0±3,3	93,3±4,7
HR, beats/min	414±17	397±13*	381±6	387±7
CI, ml/min/100 g	37,9±2,3	40,6±2,4	38,7±7,0	36,4±1,9
TPR, mm Hg/ml/min/100 g	2,76±0,13	2,40±0,15	2,49±0,14	2,60±0,16
Blood flow, ml/min/g				
skin	0,25±0,02	0,28±0,03	0,26±0,03	0,22±0,02*
muscles	0,17±0,02	0,11±0,02*	0,15±0,03	0,12±0,01
stomach	1,19±0,11	1,71±0,27	1,17±0,10	1,03±0,10
pancreas	2,08±0,33	2,01±0,37	1,96±0,22	1,43±0,18*
brain	1,52±0,14	1,51±0,17	1,35±0,06	1,59±0,17
intestine	3,07±0,31	3,78±0,44	2,96±0,23	2,74±0,19
spleen	1,56±0,22	1,85±0,20	2,06±0,19	2,16±0,29
heart	7,41±0,65	8,24±0,75	4,44±1,83	4,53±0,64
kidneys	6,37±0,50	7,33±0,47	6,59±0,42	6,01±0,46
adrenals	6,56±0,70	8,66±0,69*	6,17±1,23	11,31±1,14*

Legend. CI) Cardiac index) TPR) total peripheral resistance. *p < 0.05 compared with background.

given 24 h after the first two in order to avoid any effect of tachyphylaxis to the second injection of the peptides [2]. After the experiment the rats were killed and samples of the organs and tissues were weighed and placed in plastic tubes. All measurements of the number of microspheres were made on the Compugamma 1282 gamma-counter (LKB, Sweden). The cardiac output and regional blood flows were calculated on a Labtam 3015 microcomputer (Australia). The substances were dissolved in physiological saline and injected by the bolus method in a volume of 0.1 ml/kg. The results were subjected to statistical analysis by Student's t test for paired samples and independent series. All data are presented in the form M ± m.

EXPERIMENTAL RESULTS

Background values of the hemodynamic parameters of conscious rats of the two groups are given in Table 1. Intravenous injection of DADL and DAGO in a dose of 1 μmole/kg body weight into the conscious rats caused a fall of BP and the development of bradycardia and apnea. All the effects had a short latent period. The mean duration of apnea was 5-10 sec and it was replaced by rapid and deep respiration. The hypotension and bradycardia reached a maximum in the course of 30-60 sec after injection of the preparation (these effects also were of short duration and lasted not more than 5 min). When the values of the maximal changes in BP and HR evoked by injection of equal doses of DADL and DAGO were compared, no significant differences were found. DAGO lowered BP by 52.3 ± 5.0% and HR by 69.1 ± 5.1%; DADL by 55.7 ± 4.2 and 67.0 ± 4.8%, respectively. The total peripheral resistance and car-

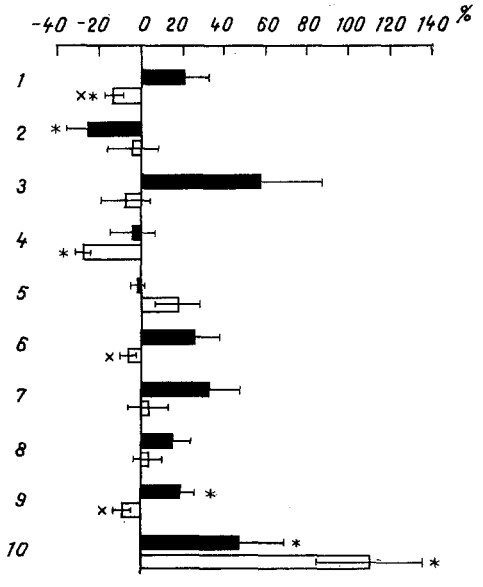

Fig. 1. Changes in regional blood flow (in % of background level) 5 min after intravenous bolus injection of DADL (black columns) and DAGO (unshaded columns) in a dose of 1 μmole/kg body weight in conscious rats. Here and in Figs. 2 and 3: 1) skin; 2) muscles; 3) ventricle; 4) pancreas; 5) brain; 6) intestine; 7) spleen; 8) heart; 9) kidneys; 10) adrenals; *p < 0.05 compared with background, ×p < 0.05 for comparison between groups.

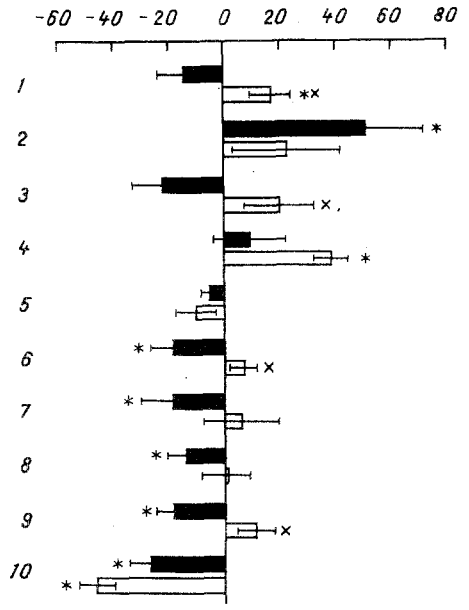

Fig. 2

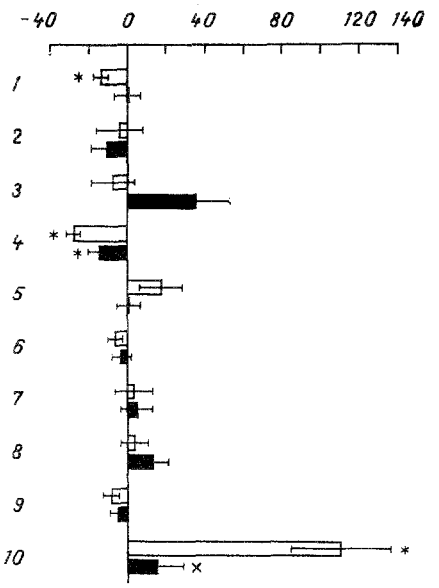

Fig. 3

Fig. 2. Changes in regional peripheral resistance (in % of background level) 5 min after intravenous bolus injection of DADL (black columns) and DAGO (unshaded columns) in a dose of 1 μmole/kg body weight in conscious rats.

Fig. 3. Effect of DAGO (1 μmole/kg) on regional blood flow (change in % relative to background level) in rats not receiving (unshaded columns) and receiving naloxone beforehand in a dose of 1 mg/kg (black columns).

diac output, measured by the radioactive microsphere method 5 min after injection of both peptides did not differ significantly from the background values (Table 1). Intravenous injection of the opiate receptor blocker naloxone in a dose of 1 mg/kg 8 min before the peptides completely prevented development of the effects of DADL and DAGO on the systematic hemodynamics and respiration. Consequently, all these effects owed their origin to the interaction between peptides and opiate receptors. Similar effects in response to systemic administration of DADL and DAGO, and also of other synthetic enkephalin analogs, into anesthetized and decerebrate animals have been described in the literature [2, 14], analyzed, and their reflex character has been demonstrated. These effects in conscious animals also are evidently connected with activation of opiate receptors, associated with vagal afferents of the lungs.

Data on changes in the regional hemodynamics at the 5th minute after injection of the peptides are given in Figs. 1 and 2. Injection of DADL significantly increased the blood flow in the adrenals and reduced it in the skeletal muscles; the vascular resistance fell significantly in the adrenals, kidneys, heart, intestine, and spleen. Injection of DAGO caused a significant increase in the blood flow in the adrenals and a decrease in the skin and pancreas. The vascular resistance under these circumstances was significantly increased in the pancreas and skin and significantly reduced in the adrenals.

Injection of the naloxone increased the blood flow in the heart (by 50.2 ± 20.8%), brain (by 19.8 ± 7.2%), and adrenals (by 73.0 ± 26.8%); $p < 0.05$). The vascular resistance under these circumstances was significantly reduced in the heart, brain, and adrenals (by 31.8 ± 6.3, 20.4 ± 5.5, and 46.9 ± 6.4% respectively). Preliminary injection of naloxone caused significant inhibition only of the response of an increase in the blood flow in the adrenals to injection of DAGO; the response of reduction of the blood flow in the skin and pancreas to DAGO was inhibited by preliminary injection of naloxone (Fig. 3).

The results are evidence of the high degree of differentiation of regional vascular responses to intravenous injection of opioid peptides. Lowering of the vascular resistance in the adrenals in response to injection of both peptides, and in the kidneys, heart, and intestine in response to injection of DADL may be connected with inhibition of background sympa-

thetic activity. For instance, inhibition of sympathetic activity in the renal [1, 11] and splanchnic [15] nerves was observed in anesthetized animals in response to systemic injection of synthetic enkephalin analogs. However, reflex suppression of background sympathetic activity is of short duration and lasts not more than 1 min [11], whereas in the present experiments regional vascular responses were observed 5 min after injection of the peptides. Meanwhile the regional hemodynamic responses could be a reflection of the interaction of peptide agonists directly with peripheral presynaptic opiate receptors of the blood vessels. Data in the literature are evidence that opioid peptides can inhibit noradrenalin release from sympathetic nerve endings and the effector response to sympathetic nervous stimulation; interactions of this kind have been described for the auricular and mesenteric arteries of the rabbit and other vessels [7, 8]. The resulting vasodilatation will lead to an increase in the regional blood flow in the corresponding vascular territories. The differential nature of the regional vascular reactions may be connected with the character of distribution of different opiate receptors in the body.

The greatest changes in the regional blood flow were produced by both peptides in the adrenals, and this may evidently be connected with changes in their secretory activity.

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