ROLE OF OPIATE RECEPTOR AGONISTS AND ANTAGONIST IN REGULATION OF CONTRACTILE ACTIVITY OF MESENTERIC MICROLYMPHATICS IN RATS

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Activation of contractile activity (CA) of the microlymphatics (ML) is one of the main factors increasing the lymph drainage from the organs and tissues of the body.

Lymphatics are known to have an adrenergic innervation [9, 14, 15] and their CA is stimulated by catecholamines [2-5]. However, catecholamines have a number of side effects (vasoconstriction, disturbance of the microhemocirculation, etc.), so that they cannot be used for lymphatic stimulation.

A powerful lymphatic stimulating action free from side effects on the microcirculation is a property of Leu-enkephalin and some of its analogs [10, 11], which are agonists of opiate receptors. It is possible that ML possess not only adrenergic, but also opiate regulation.

The aim of this investigation was to study the possible role of opiate mechanisms in the regulation of CA of ML.

EXPERIMENTAL METHOD

Experiments were carried out on 60 noninbred male albino rats weighing 250 g, anesthetized with pentobarbital (0.1 g/kg, intramuscularly). Biomicroscopy of the mesentery of the intestine was carried out by the method in [13]. ML with a diameter of 40-200 μ, located near the adipose tissue of the mesentery were studied. CA of the walls and valves of ML was recorded by the method in [1]. An example of a recording is shown in Fig. 1. In six series of experiments (with 10 animals in each series) the effect of a solution of 0.14 M NaCl (control series I), of Leu-enkephalin (II), of naloxone (III), dalargin (IV), Leu-enkephalin followed 10-45 min later by application of naloxone (V), and naloxone followed 10-60 min later by application of Leu-enkephalin (VI) was studied in six series of experiments (10 animals in each series). Since ML contract periodically, all the vessels studied were divided into two groups depending on the initial frequency of contraction of their wall and valve. Group 1 consisted of ML not contracting initially, group 2 of ML with spontaneous rhythmic CA. The following parameters characterizing CA of the walls and valves of ML were estimated: 1) the initial maximal frequency of contraction per minute; 2) the latent period after application of the substance to the beginning of contraction of ML (in min); 3) the maximal frequency of contraction per minute after application of the substance; 4) the time of appearance of the maximal frequency of contraction after application of the substance (in min); 5) the duration of contraction of ML after application of the substance (in min).

Leu-enkephalin ("Serva," West Germany), dalargin (synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR), and naloxone hydrochloride (from "Sigma," USA) were applied to the surface of ML in a dose of 0.001-10 μg in 0.1 ml of 0.14 M NaCl, corresponding to 0.004-40 μg/kg body weight. The numerical results were subjected to statistical analysis by Student's test.

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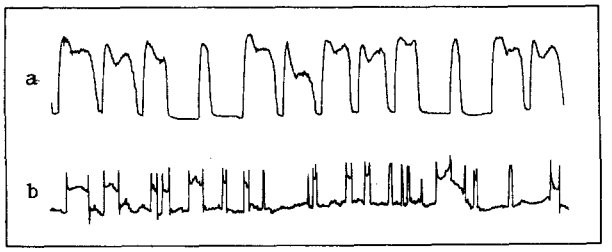

Fig. 1. Intravital recording of contraction of wall (a) and valve (b) of ML in rat mesentery. a) 10 min after application of 10 μg naloxone frequency of contraction of wall was 9/min; b) 25 min after application of 10 μg Leu-enkephalin, frequency of contraction of valve was 15/min. Before application, wall and valve did not contract. Recording speed 2 mm/sec.

EXPERIMENTAL RESULTS

In the control experiments ML of the rat mesentery contracted periodically with a frequency of 3-5 contractions per minute for 5-10 min. Contraction of the wall and valve was accompanied visually by an increase in the velocity of the lymph flow in ML, if it was present beforehand, or the beginning of movement of hitherto immobile lymph.

Leu-enkephalin (0.001-10 μg), when applied to the surface of ML, induced a dose-dependent increase (vessels of group 2) or the appearance de novo (vessels of group 1) of CA of the walls and valves of ML (Fig. 1b; Fig. 2a). In response to application of 10 μg Leu-enkephalin all the ML studied were activated. A further increase in the dose was not accompanied by any increase in CA of ML. Data showing CA of the walls and valves of ML with a different initial background of contraction in response to application of 10 μg Leu-enkephalin are illustrated in Fig. 2a. The results are evidence that the latent period of response of the walls and valves to Leu-enkephalin did not exceed 1 min in all ML. The maximal frequency of contraction of the walls and valves depended on the initial CA of ML: in the vessels of group 1 the increase in frequency of contraction was greater than in the vessels of group 2. However, the frequency of contraction of ML of group 1 after application of Leu-enkephalin was always below the corresponding value for ML of group 2. For example, CA of the walls of ML in group 1, before and after application of Leu-enkephalin, was 0 and 15.15 $\pm$ 1.99 (an increase of 15 contractions per minute). In ML of group 2, CA of the wall was 24.0 $\pm$ 2.3 and 30.5 $\pm$ 5.0 respectively (an increase of only six contractions/min), but 30.5 is twice as great as 15.19. Thus the initial state of CA of ML must be taken into account when the lymphatic-stimulating action of different preparations is compared. The time of appearance of the maximal frequency of contraction was about the same in all groups of ML (from 8 to 12 min). The duration of contraction of the wall of ML in group 2 was 2.5 times longer than the corresponding value for ML in group 1. These data are evidence of activation of CA of ML of group 1 and prolongation of the function of ML of group 2 under the influence of Leu-enkephalin.

Dalargin, a Leu-enkephalin analog, had a similar action on the vessels. However, dalargin in any dose (0.001-10 μg) caused activation of not more than 67% of the ML studied, whereas parameters characterizing CA of ML (maximal frequency of contraction, duration of effect) were lower than after application of Leu-enkephalin [12].

The opiate receptor antagonist naloxone had different effects on CA of ML: in ML of group 1 application of 10 μg naloxone caused contraction of the wall in only 31% (Fig. 1a) and of the valves in 16% of ML. The maximal frequency and duration of contraction of the wall of ML were the same as after treatment with Leu-enkephalin, but for the valves they were significantly less (Fig. 2a). 69% of ML did not respond to application of naloxone. The results suggest possible activation by naloxone of Leu-enkephalin, followed by stimulation of CA of ML (mainly of the wall and not the valves).

In the vessels of group 2 naloxone (10 μg) evoked other responses: in 25% of ML spontaneous contraction of the wall and valves ceased at once; in 75% of ML CA was preserved. Under these circumstances the initial frequency of contraction of the wall was unchanged, whereas that of the valves was reduced (Fig. 2a). Compared with the action of Leu-enkephalin on CA of ML, the maximal frequency and, in particular, duration of contraction were less after application of naloxone. Consequently, naloxone inhibited spontaneous rhythmic CA of some ML, and in functioning ML it shortened the duration of contraction of the wall and valve by two-thirds (incomplete blockade of contraction of ML).

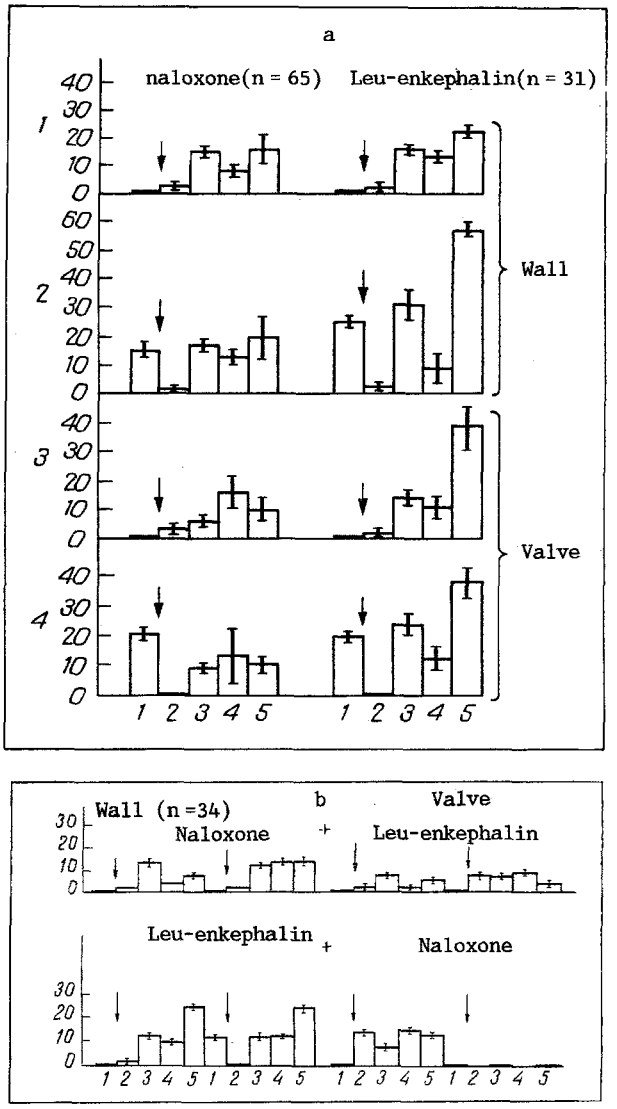

Fig. 2. Effect of Leu-enkephalin, naloxone (a), and a combination of both (b) on contractile activity of wall and valves of ML of rat mesentery. Ordinate, time (in min) and frequency of contraction/min; abscissa: 1) initial maximal frequency of contraction of wall or valve, per minute; 2) latent period to beginning of contraction; 3) maximal frequency of contraction per minute after application of substance; 4) time of occurrence of maximal effect; 5) duration of contraction. Arrows indicate application of naloxone (10 μg) or Leu-enkephalin (10 μg). *n*) Number of ML studied.

In the experiments of series V the effect of naloxone on CA of ML induced by Leu-enkephalin was studied. The investigation was conducted on ML of group 1. Leu-enkephalin (10 μg), after application, caused all ML to contract. Naloxone (10 μg) was applied 10-45 min later. All ML continued to contract with the previous rhythm, i.e., naloxone cannot arrest contraction of ML activated previously by Leu-enkephalin (Fig. 2b).

The effect of Leu-enkephalin (10 μg) on CA of ML of group 1 was studied in group VI after preliminary application of naloxone (10 μg; Fig. 2b). Naloxone was found to prevent subsequent activation of ML by Leu-enkephalin. The inhibitory effect of naloxone was reduced with an increase in the time elapsing after its application (Fig. 3). During the first 30 min after application of naloxone opiate receptors are evidently blocked; after 1 h about 50% of ML can already be activated (the walls in 57% and the valves in 33% of ML). However, the parameters of CA of ML when activated by this scheme were lower than those activated by Leu-enkephalin (Fig. 2).

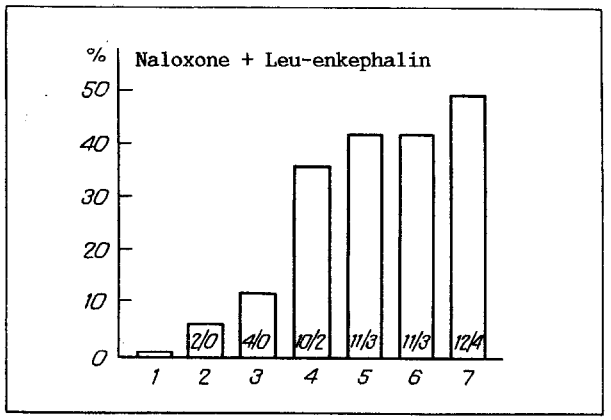

Fig. 3. Activation of CA of ML of rat mesentery by Leu-enkephalin (10 μg) depending on time elapsing after preliminary application of naloxone (10 μg) to ML. Abscissa, time (in min) after application of naloxone to ML: 1) after 1-10 min, 2) 11-20 min, 3) 21-30 min, 4) 31-40 min, 5) 41-50 min, 6) 51-60 min, 7) 61-70 min; ordinate, number of functioning ML (walls and valves; in % of total number of vessels studied). Numbers in columns: numerator, number of functioning walls; denominator, number of valves of ML.

Thus CA of ML depends on what acts first on ML: the agonist or antagonist of opiate receptors. If the antagonist acts first, CA of ML is not subsequently activated; if the agonist acts first, the subsequent action of the antagonist is not manifested. Evidently a response of competitive type takes place in relation to opiate receptors of the mesenteric ML of the rat. The possibility cannot be ruled out that ML in other regions and in other animals, and also in man, possess opiate regulation of ML. This hypothesis can be confirmed by data obtained by various workers who found activation of the lymph flow in rabbits and dogs [7] and that dalargin and Leu-enkephalin are effective in pancreatitis and ulcerative diseases of the gastrointestinal tract in man and animals [6, 8].

Activation of the lymph flow by opioid peptides (Leu-enkephalin and its analogs) and its inhibition by the opiate receptor antagonist naloxone offer wide opportunities for oriented control of the lymph flow.

These results are evidence of the existence of opiate receptors and opiate regulation in mesenteric ML in the rat.

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