INHIBITORY ACTION OF METHIONINE-ENKEPHALIN AND MORPHINE ON PENTAGASTRIN-STIMULATED GASTRIC SECRETION IN DOGS

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Endogenous opium-like peptides (enkephalins), discovered in 1975, interact with opiate receptors and are present in the body as leucine-enkephalin and methionine-enkephalin (ME), which differ structurally in one amino acid [2]. These peptides are found in large quantities in the brain and intestine. They are also abundant in the antral portion of the stomach and in the duodenum, where they are located in nerve fibers and endocrine cells [4]. The writers previously demonstrated the inhibitory action of leucine-enkephalin on gastric secretion in dogs [1].

The object of this investigation was to study the effect of ME and morphine on hydrochloric acid secretion in dogs stimulated by pentagastrin (PG), and when opiate receptors were blocked by the specific antagonist, naloxone.

EXPERIMENTAL METHOD

Experiments were carried out on six dogs weighing 18-22 kg. A Basov's fistula was formed beforehand in three dogs and a Heidenhain's gastric pouch in the other three. The dogs were deprived of food for 18 h before the experiments. Gastric secretion was stimulated by continuous intravenous infusion of PG solution (1 μg/kg/h) throughout the experiment (5 h). Preliminary investigations showed that injection of this dose stimulates secretion of hydrochloric acid at 50% of the maximum level. To obtain a constant volume of secreted gastric juice during perfusion with PG, administration of ME was started, and its dose was increased tenfold after each hour of perfusion (1, 10, and 100 μg/kg/h, respectively); during the last hour of the experiment PG alone continued to be administered. The PG and ME used in the experiments were dissolved beforehand in physiological saline. The rate of perfusion of the solutions was constant at 20 ml/h. In individual series of experiments on dogs with a gastric fistula, the same scheme was used but during perfusion with PG morphine was injected in successive doses of 6 and 60 μg/kg/h. In one additional series of experiments ME was used in a dose of 500 μg/kg/h. Any further increase in the dose of the peptide was impossible because of the onset of vomiting. In some experiments, after stable secretion had been attained during administration of PG to the dogs, a single intravenous injection of naloxone (100 μg/kg) was given, and during the next hour perfusion with ME (10 μg/kg/h) was carried out together with administration of PG, which continued during the 3rd hour of the experiment. During the investigation gastric juice was collected at intervals of 15 min, the volume of each sample was measured, and its acidity was determined by titration with 0.1 M NaOH solution to pH 7.0. When the results were analyzed, hydrochloric acid production during the last 30 min of each hour of perfusion was calculated in milliequivalents. Me (tyr–gly–gly–phe–met), synthesized by classical methods of peptide chemistry in the Laboratory of Peptide Synthesis (Head, M. I. Titov), All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, was used. The physicochemical characteristics of the products were: specific optical rotation $[\alpha]_D$ = +20.7 (C = 1, menthol); amino-acid analysis after hydrolysis with HCl at 110°C for 20 h: gly 2.08, phe 1.00, met 0.93, tyr 0.97 (theoretical values gly 2, phe 1, met 1, tyr 1). Melting point 189-191°C. Chromatographic mobility (R_f) = 0.51, butyl alcohol–acetic acid–H_2O (3:1:1). The PG used in the experiments was from Serva, West Germany, and naloxone from Endo Laboratories, USA. The results were subjected to statistical analysis by Student's two-sample t test. Differences were considered to be significant at the 95% level ($P < 0.05$).

EXPERIMENTAL RESULTS

Injection of ME during perfusion with PG (1 μg/kg/h) caused a marked decrease in hydrochloric acid production in dogs with a gastric fistula (Fig. 1). This decrease depended on the dose of ME: With an increase in dose (1, 10, and 100 μg/kg/h) it gradually increased. The maximal inhibitory effect of ME was achieved with a dose of 100 μg/kg/h, manifested as a fall in the secretion of acid by 67.7% from the initial level (1.75 ± 0.46 meq/30 min compared with 5.44 ± 0.50 meq/30 min on perfusion with PG alone; $P < 0.05$). If ME was given in doses of 1 and 10 μg/kg/h, the rate of secretion was 3.70 ± 0.53 and 2.57 ± 0.64 meq/30 min, i.e., a decrease of 32 and 53%, respectively, from the initial values (in both

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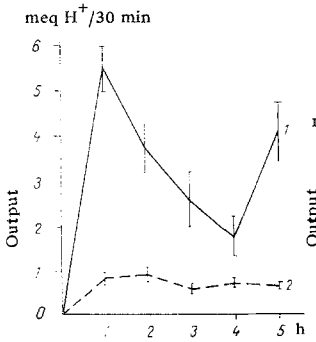

Fig. 1

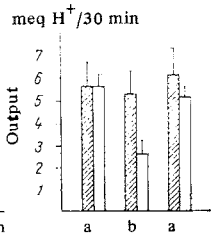

Fig. 2

Fig. 1. Effect of ME on hydrochloric acid secretion in dogs. 1) HCl secretion in dogs with Basov's gastric fistula; 2) HCl output in dogs with isolated Heidenhain's gastric pouch.

Fig. 2. Abolition by naloxone of the action of ME on PG-stimulated HCl secretion. Shaded columns represent HCl secretion after naloxone (100 μg/kg), unshaded columns show HCl secretion in the absence of naloxone; a) administration of PG, b) administration of PG and ME in a dose of 10 μg/kg.

cases $P < 0.05$). After the end of administration of ME acid secretion increased to reach 4.05 ± 0.67 meq/30 min (75% of the initial value). An increase in the dose of ME to the maximal tolerated value (500 μg/kg/h), while reducing the secretion of acid by 35.8% (to 4.0 ± 3.17 meq/30 min), nevertheless had a weaker effect than smaller doses of the peptide (10 and 100 μg/kg/h). Inhibition of acid formation in the stomach by ME was thus exhibited only within a certain dose range, and with any considerable increase in dose the effect diminished.

In the isolated stomach, hydrochloric acid secretion was virtually unchanged by the action of ME (Fig. 1): During perfusion with PG acid output was 0.80 ± 0.12 meq/30 min, and when ME was given in doses of 1, 10, and 100 μg/kg/h it was 0.85 ± 0.14, 0.57 ± 0.1, and 0.71 ± 0.08 meq/30 min, respectively.

Under the influence of morphine, acid secretion also decreased, to 3.85 ± 0.33 meq/30 min during administration of PG alone and 2.50 ± 0.15 meq/30 min (64.8%; $P < 0.05$) and 2.58 ± 0.95 meq/30 min (67%) when morphine was given in doses of 6 and 60 μg/kg/h, respectively (the doses of morphine was equimolar to doses of ME of 10 and 100 μg/kg/h).

A single injection of naloxone (100 μg/kg) before the beginning of infusion completely abolished the action of ME on gastric secretion (Fig. 2) in dogs with a gastric fistula but did not affect the output of acid in dogs with an isolated gastric pouch. The difference between the output of acid in animals receiving ME in a dose of 10 μg/kg/h (2.57 ± 0.64 meq/30 min) in the absence of naloxone and after administration of naloxone (5.2 ± 0.97 meq/30 min) is statistically significant ($P < 0.05$).

The results thus indicate that ME inhibits gastric secretion of hydrochloric acid stimulated by PG. The inhibitory effect of the enkephalins, incidentally, was exhibited only in dogs with a gastric fistula, i.e., with their nervous connections intact, and it was not found in dogs with an isolated, denervated gastric pouch. It can be concluded from these results that the inhibitory effect of enkephalins on gastric secretion is mediated by the nervous system. The writers obtained similar results previously when studying the action of leucine-enkephalin [1]. The absence of a direct effect of enkephalins on the acid-forming cells of the stomach also is confirmed by the absence of any action of enkephalins and some of their synthetic analogs on H^+ secretion by the isolated frog's gastric mucosa [1]. It has been suggested that ME and morphine stimulate hydrochloric acid secretion in dogs [3]. It is difficult to explain the disagreements between the experimental data obtained by different workers, especially because the experimental conditions which we ourselves used and in [3] were practically identical. However, the impression is created that endogenous opium-like peptides exert their action on gastric secretion within a definite dose range, and that variation of the dose may lead to disappearance or, indeed, reversal of the effect. Disappearance of the inhibitory action of ME when its dose was increased to 500 μg/kg/h, observed in the present investigation, is evidence in support of this hypothesis.

To sum up, endogenous opium-like peptides and, in particular, enkephalins possess marked activity on the digestive tract and may play a role in the regulation of the secretory function of the stomach.

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