blood vessels, and in the form of lines above the capsule of the organ, repeating their dimensions and arrangement. Whereas 10 min after injection of the preparation the picture above the large vessels had the appearance of confluent stains, after 1 and 3 h small translucencies appeared in the center of these stains, the density of which weakened at each successive time of investigation, and the stains themselves assumed the appearance of steering wheels.

Measurement of the optical density of the autoradiographs by means of a microdensitometer showed that whereas density above blood vessels of the testes became maximal 10 min after injection of the pertechnetate-^{99m}Tc, and subsequently fell gradually, optical density above the parenchyma of the organ reached a maximum after 1 h (Fig. 3).

Comparison of curve II in Fig. 1, reflecting the concentration of the preparation in the isolated testis as a whole, and curves I and II in Fig. 3, showing indirectly the concentration of radionuclide separately in the vascular system of the testis and in its parenchyma, shows that curve II in Fig. 1 and curve I in Fig. 3 repeat one another to a large extent, which is not the case with curve II in Fig. 1. Analysis of these results confirms once again that the integral index of radioactivity in the testis obtained by scintigraphy is determined primarily by the concentration of pertechnetate-^{99m}Tc in the blood vessels of the organ studied.

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INHIBITORY EFFECT OF LEU-ENKEPHALIN ON GASTRIC SECRETION IN DOGS

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The attention of research workers in recent years has been drawn to the regulatory role of a new class of biologically active substances; the neuropeptides. The importance of leu- and met-enkephalins, morphine-like pentapeptides of endogeneous origin, has been particularly widely discussed [4, 5, 8].

The object of this investigation was to study the action of leu-enkephalin (EK) on gastric secretion and to elucidate some of its mechanisms. The EK used was synthesized in the laboratory of peptide synthesis (Head, M. I. Titov), All-Union Clinical Scientific Center, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD

Several experimental models were used, namely: two groups of mongrel dogs (four dogs in each group) with different types of fistulas; the isolated gastric mucosa of the frog Rana temporaria; the effect of EK on the blood gastrin concentration in dogs also was studied.

During a preliminary operation on the dogs of group 1 a large Basov's fistula was formed; denervated gastric pouches were created by Heidenhain's method in the animals of group 2. Before the experiments, the

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TABLE 1. Changes in Gastric Secretion in Dogs after Intravenous Injection of Leu-Enkephalin (M ± m)

Experimental conditions	Volume of gastric juice, ml		Total acidity of gastric juice, meq.	
	after intravenous injection of pentagastrin (5 μg/kg/h)	after intravenous injection of leu-enkephalin (+5 μg/kg/h)	after intravenous injection of pentagastrin (5 μg/kg/h)	after intravenous injection of leu-enkephalin (+5 μg/kg/h)
Dogs with Basov's gastric fistula	18.84 ± 3.09	10.47 ± 2.25	2.72 ± 0.44	1.67 ± 0.29
	$P \leq 0.05$		$P \leq 0.01$	
Dogs with Heidenhain's denervated gastric pouch	2.37 ± 0.40	1.58 ± 0.22	0.20 ± 0.03	0.14 ± 0.02
	$P > 0.05$		$P > 0.05$	

dogs were deprived of food for 24 h. Gastric secretion was stimulated by intravenous injection of pentagastrin solution in a dose of 5 μg/kg/h by means of a perfusion pump. The doses of EK given in different experiments were 1, 5, 10, and 25 μg/kg/h. Perfusion of the EK solution began when the volume of gastric secretion had reached a constant level and it continued for 60-90 min. At the end of injection of EK, administration of the initial pentagastrin solution was resumed for 30 min. Throughout the investigation the secreted gastric juice was collected at intervals of 15 min. The volume of juice in each sample was measured and its acidity determined by titration with 0.1M NaOH solution.

Secretion of H^+ ions by the isolated frog gastric mucosa was studied in a two-chamber model [1] by means of continuous titration of the secreted acid with 0.01M NaOH solution, with maintenance of a constant pH of 7.0 (with a pH-stat from Radiometer, Denmark). Hydrochloric acid secretion was expressed in μeq/h/cm^2 gastric mucosa.

The effect of EK on the gastric concentration in blood serum was investigated in 16 dogs, from which blood was taken from a vein after starvation for 18 h; EK solution was then injected into the vein in a dose of 25 μg/kg/h through a catheter by means of a perfusion pump. Blood was taken 15, 30, 60, and 90 min after the beginning of the investigation. One sample of blood was taken 30 min after the end of perfusion. Gastrin was determined by a radioimmunologic method using standard kits from CEA-IRE-Sorin (France, Italy, Belgium). The gastric concentration was expressed in picograms/ml.

EXPERIMENTAL RESULTS

The largest number of experiments on dogs in both groups was carried out with perfusion with EK in a dose of 5 μg/kg/h. The results showed (Table 1) that injection of EK in this dose causes a marked decrease in the volume and total quantity of hydrochloric acid in dogs both with a Basov's gastric fistula and with Heidenhaim's denervated gastric pouches. However, the inhibitory effect of EK on hydrochloric acid secretion in dogs with Heidenhain's gastric pouches was much weaker (almost 10% less) than in dogs with the innervation of their stomach preserved. The maximal decrease in acidity in the dogs of both groups took place 60-75 min after the beginning of EK injection. Whereas in dogs with Basov's fistulas inhibition of hydrochloric acid secretion was observed in 100% of cases and with effect from the 15th minute after injection of EK (test 1), in dogs with denervation of the stomach this effect could be detected in only 70% of cases, and in the case of a positive action, this began to be manifested after 30-45 min. On resumption of pentagastrin perfusion, the volume and acidity of the gastric juice again rose appreciably, but did not regain their initial values. With an increase in the doses of EK to 10 and 25 μg/kg/h, the character of the decrease in acidity did not change significantly, but the effect was observed more clearly. General changes in behavior also were observed in some dogs receiving the doses: The dogs had a desire to vomit and were visibly restless.

The investigation showed that secretion of H^+ ions by the isolated frog gastric mucosa, both under basal conditions and after stimulation with histamine and pentagastrin, was unchanged by EK.

The results obtained by determination of the blood serum gastrin level of the dogs (Table 2) showed a tendency for the gastrin concentration to fall following administration of EK.

The results of previous experiments on dogs with isolated Pavlov's gastric pouches also showed that EK inhibits acid secretion [2]. The new data now described enabled suggestions to be made regarding the mechanism of the inhibitory effect arising under the influence of EK; they show that maximal depression of

TABLE 2. Changes in Blood Gastrin Concentration of Dogs following Intravenous Injection of Leu-Enkephalin (M ± m)

Hormone	Basal level (0 min)	Perfusion with leu-enkephalin (25 μg/kg/h)				30 min after perfusion
		15 min	30 min	60 min	90 min	
Gastrin level, pg/ml	44,0±13,2	33,8±4,1	34,3±5,8	33,6±4,8	41,7±5,8	49,1±5,8

Legend. In all tests $P > 0.05$.

hydrochloric acid secretion is attained in dogs with a Basov's gastric fistula, i.e., with the nerve and humoral connections of the stomach completely intact. Weaker inhibition of acid secretion was found in dogs with Heidenhain's denervated gastric pouches, which retained their humoral connections with the rest of the body. Finally, no inhibition of secretion of H^+ ions was observed in the preparation of isolated frog's gastric mucosa under the influence of this neuropeptide. The effect of EK thus clearly depends on the degree of integrity of functional connections between the acid-producing zone of the stomach and the rest of the body. It can be tentatively suggested that the inhibitory effect of EK is mediated through both nervous and humoral connections. Considering that EK is found in the endocrine cells of the antral portion of the stomach, which are morphologically similar to G-cells that produce gastrin [3, 7], it can be postulated that the enkephalins are functional endogeneous antagonists of gastrin.

In the writer's view it seems probable that EK inhibits gastric secretion mainly by presynaptic blockade of cholinergic fibers, for enkephalins and morphine are known to have the property of lowering the acetylcholine concentration in the tissues [6]. The intensity of cholinergic influences both on the parietal cells of the stomach and on the G-cells is thus reduced, with a consequent decrease in gastrin secretion and in the activity of the principal humoral component stimulating gastric secretion. On the basis of the results of the present investigation a direct inhibitory influence of EK on gastrin secretion likewise cannot be ruled out.

At the present time there is no question about the urgency of the study of the effects of neuropeptides on function of the gastrointestinal tract. Further combined studies of the effects of neuropeptides on gastric secretion will be of both theoretical and practical importance in the quest for new approaches to the treatment of diseases of the stomach and duodenum and, in particular, of peptic ulcers.

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