MORPHOMETRIC EVALUATION OF THE EFFECT OF OPIOID PEPTIDES ON CYSTEAMINE-INDUCED ULCERS IN RATS

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Opioid peptides (enkephalins and endorphins) are found not only in the CNS, but also in the gastrointestinal tract [6]. The writers previously demonstrated the protective action of these substances on the duodenal mucosa during the experimental production of duodenal ulcers induced by cysteamine [1] and showed that they inhibit gastric secretion [2].

The aim of this investigation was to study the effect of opioid peptides on the duodenal mucosa during induction of ulcers with cysteamine in rats.

EXPERIMENTAL METHOD

The action of a representative of the enkephalin class, the stable analog Tyr-D-Ala-Gly-Phe-NH_2, which has marked analgesic activity [5, 8], and also of β-endorphin, an opioid peptide with a possible endocrine role [7], was studied.

Experiments were carried out on 58 male Wistar rats weighing 150-200 g, divided into five groups: Group 1) 18 animals were given a single subcutaneous injection of cysteamine hydrochloride (from Fluka, Switzerland) in a dose of 350 mg/kg, inducing duodenal ulcer formation in 50-60% of rats [1]; group 2) after receiving an injection of cysteamine, 10 animals were injected with the enkephalin in a dose of 125 nmoles/kg twice a day subcutaneously; group 3) 10 rats received naloxone (from Endo Laboratories, USA) in a dose of 500 nmoles/kg simultaneously with the enkephalin; group 4) instead of cysteamine, 10 rats were given an injection of β-endorphin in a dose of 300 nmoles/kg; group 5) simultaneously with β-endorphin, 10 animals were given an injection of naloxone in a dose of 500 nmoles/kg. The rats were killed 48 h after the injection of cysteamine, the duodenum was removed and divided into 3-5 parts, which were fixed in a mixture of 75% saturated picric acid and 25% neutral formalin. Paraffin blocks

TABLE 1. Morphometric Parameters of Duodenal Mucosa in Rats with Experimental Ulcer

Experimental conditions	Thickness of mucosa	Height of villi, μ	Depth of crypts, μ	Lymphocytes in epithelium of villi, %	Cell density	Lymphocytes	Monocytes	Tissue macrophages	Histiocytes
Control	718,0±17,5	454,0±12,7	263,0±14,5	12,5±0,6	24 476±316 / 15 608±161	6765±155 / 1717±58	534±33 / 0	235±42 / 0	1318±67 / 446±24
Cysteamine	658,0±25,0*	387,0±17,0*	278,0±14,5	19,5±1,8*	19 315±233* / 17 205±271*	6306±332 / 1798±132*	243±25* / 70±24*	569±28* / 66±32	1385±156 / 636±38*
Enkephalin + cysteamine	742,0±27,1	501,0±20,7	247,0±12,6	18,3±1,3*	20 503±313* / 13 703±303	7012±515 / 2742±191*	484±57 / 0	361±19* / 0	1006±93* / 535±83
Enkephalin + naloxone + cysteamine	701,0±25,1	400,0±18,0*	286,0±7,2	18,5±2,4*	18 836±406* / 17 153±251*	6178±245 / 3516±329*	226±35* / 60±21*	551±37* / 70±45	1581±127 / 1063±110*
β-endorphin + cysteamine	685,0±31,0	410,0±23,4	250,0±9,3	21,3±1,3*	22 333±281* / 14 892±337	8004±254* / 3016±85*	526±35 / 0	260±66 / 0	1400±60 / 502±31
β-endorphin + naloxone + cysteamine	646,0±33,0*	392,0±18,7*	244,0±16,0	12,1±0,4	19 464±386* / 18 814±288*	6388±132 / 3673±56*	334±32* / 54±31	474±37* / 56±31	1003±93* / 733±123*

Legend. Numerator gives number of cells per square millimeter stroma in intervillous space, denominator — number in intercryptal space; *P < 0.05 compared with control.

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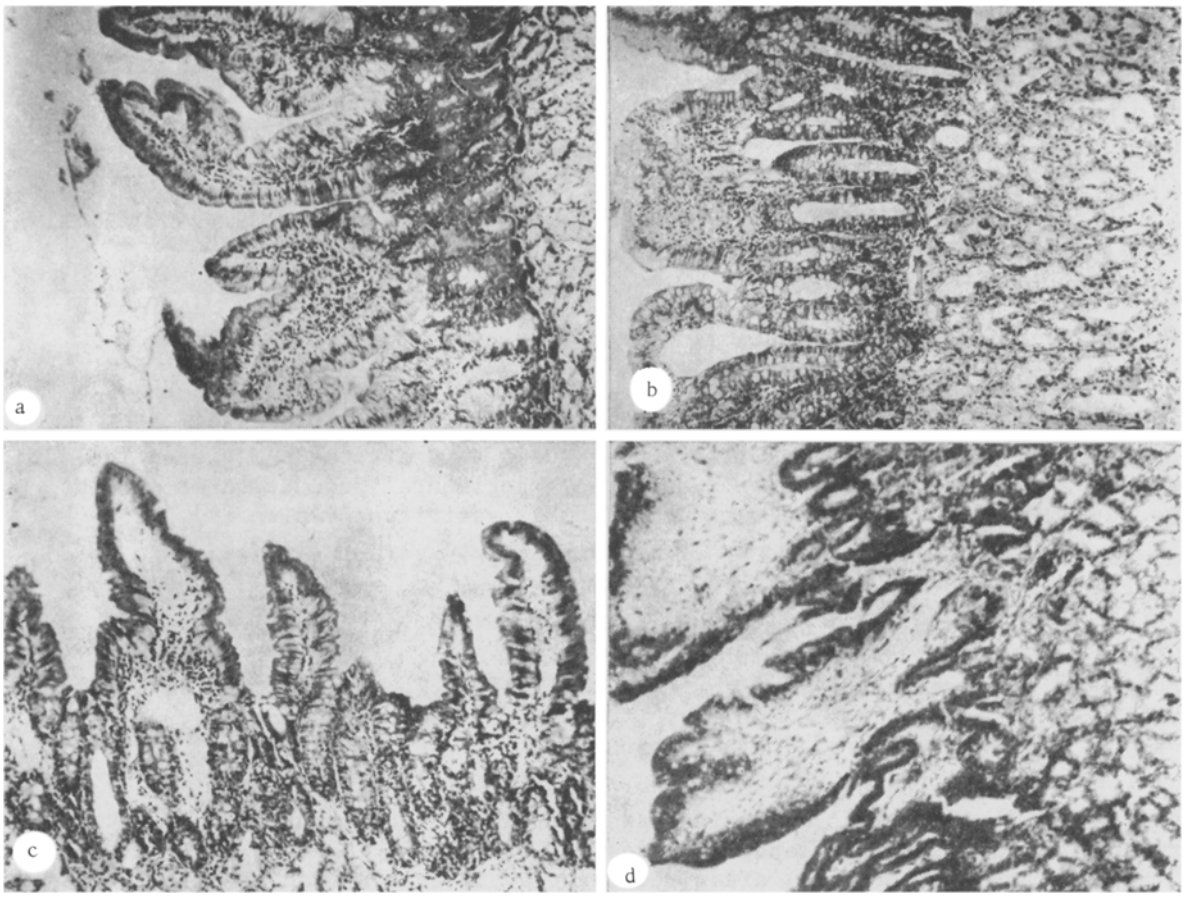

Fig. 1. Morphology of duodenal mucosa in rats. a) Control; b) 48 h after injection of cysteamine; c) after injection of enkephalin analog; d) after simultaneous injection of enkephalin analog and naloxone. Hematoxylin–eosin.

were oriented to obtain longitudinal sections 3-5 μ thick, which were stained with hematoxylic and eosin.

To assess changes in the duodenal mucosa morphometric parameters [3] were used. The cell density of the zone of infiltration per square millimeter of mucosa was determined, by investigating three to five fields of vision in each preparation. The peptides used were synthesized in the Laboratory of Peptide Synthesis (Head M. I. Titov), All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, by the classical methods of peptide chemistry. The numerical results were subjected to statistical analysis by Student's test with 95% level of significance ($P < 0.05$). The results are given in Table 1.

EXPERIMENTAL RESULTS

Injection of cysteamine was accompanied by moderate changes in the general architectonics of the intestine: the thickness of the mucosa decreased on account of shortening and flattening of the villi (Fig. 1b). Changes in the form of cloudy swelling with vacuolation of the apical portion of the cytoplasm and an increase in the relative proportion of mucocytes (Fig. 2) and lymphocytes were observed in the epithelium of the villi. The number of mucocytes was increased in the epithelial layer of the crypts (Fig. 2). The density of cellular infiltration was reduced in the layer of the villi proper, and increased between the crypts. The number of monocytes and of young and mature fibroblasts was reduced, and the number of tissue macrophages simultaneously increased among the cells of the stroma of the villi. Cysteamine thus induces degenerative changes in the villi and proliferative changes in the duodenal mucosa proper.

In the rats of group 2 many effects of cysteamine were abolished after injection of the enkephalin analog: The villi were digitiform in shape (Fig. 1c), the epithelium showed degenerative changes only in isolated cases, and the thickness of the mucosa and the number of

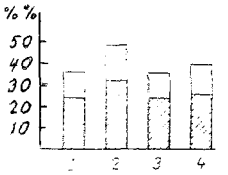

Fig. 2

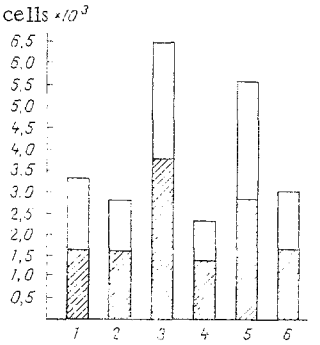

Fig. 3

Fig. 2. Number of mucocytes in duodenal epithelium. Ordinate, number of mucocytes (in % of total number of epithelial cells). Shaded part of columns represents mucocytes in epithelium of crypts, unshaded part of columns — mucocytes in epithelium of villi. 1) Control; 2) cysteamine ulcer; 3) cysteamine combined with enkephalin analog; 4) cysteamine combined with β-endorphin.

Fig. 3. Number of eosinophils in stroma of duodenal mucosa. Ordinate, number of eosinophils in 1 mm^2 mucosa. Shaded part of column represents eosinophils in intercryptal space, unshaded part of columns — eosinophils in stroma of villi. 1) Control; 2) cysteamine-induced ulcer; 3) cysteamine combined with enkephalin analog; 4) abolition of effect of enkephalin by naloxone; 5) cysteamine combined with β-endorphin; 6) abolition of effect of β-endorphin by naloxone.

mucocytes in the epithelium of the villi and crypts were restored to normal (Fig. 2). The number of tissue macrophages was reduced. The density of infiltration in the stroma between the crypts corresponded to the normal values, whereas an increase was observed in the cell density in the stroma of the villi (significant compared with the control). The increase in the number of eosinophilic granulocytes in the epithelium of the villi and crypts and also in the mucosa proper was somewhat unexpected (Fig. 3).

Naloxone abolished the antidystrophic action of the enkephalin analog: In the rats of this group marked cloudy swelling again was observed in the enterocytes, often with vacuolation of the apical part of the cytoplasm, and the villi were often flattened. The morphometric parameters in the animals of this group were similar to those in rats receiving cysteamine only. Injection of naloxone reduced the density of cellular infiltration in the intervillous space with an increase in its density in the intercryptal space, it blocked the increase in the number of eosinophils both in the epithelium of the villi and crypts and in the stroma of the duodenal mucosa, and increased the number of histiocytes and tissue macrophages. No effect of enkephalin and naloxone on the increased number of intraepithelial lymphocytes due to injection of cysteamine or on the number of plasma cells in the stroma of the mucosa was noted.

β-Endorphin abolished the effects of cysteamine on the duodenal mucosa by a lesser degree than the enkephalin analog (Table 1): Single flattened and shortened villi with degenerative changes in the epithelial layer, in the form of cloudy swelling or, less frequently, with vacuolation of the cytoplasm of the enterocytes, were observed in the rats. Injection of β-endorphin restored the normal density of cellular infiltration in the intercryptal space and increased it considerably in the intervillous space of the duodenal mucosa. The number of mucocytes in the epithelium of the crypts was restored to normal and their number in the epithelium of the villi was reduced compared with the control. The number of young fibroblasts in the stroma of the villi and between the crypts and also the number of monocytes, histiocytes, and tissue macrophages in the stroma were indistinguishable from normal, whereas neutrophilic granulocytes became less numerous in the villous part of the mucosa. Meanwhile, the

number of eosinophils in the epithelial layer of the villi and among the cells of the duodenal mucosa proper was increased (Fig. 3).

In rats receiving naloxone together with β-endorphin the morphological picture was similar to that observed after administration of cysteamine: Flattening and shortening of the villi with marked degenerative changes, in the form of cloudy swelling and vacuolation of the cytoplasm of the epitheliocytes and redistribution of the density of cellular infiltration in the stroma of the mucosa were observed. In addition naloxone reduced the number of eosinophilic granulocytes both in the epithelium of the villi and in the stroma, and also reduced the number of plasma cells in the villous part of the duodenal mucosa and increased the number of histiocytes and tissue macrophages in the duodenal mucosa compared with these parameters in the rats of group 4.

This investigation confirmed the protective action of endogenous opioid peptides on the duodenal mucosa under conditions of formation of an experimental cysteamine-induced ulcer. Meanwhile, the increase in the number of eosinophils in the duodenal mucosa was unexpected. Similar changes, incidentally, were found in rats after space flights on satellites [4]. Blocking of this effect by naloxone clearly demonstrated its connection with activation of opiate receptors.

The increase in the number of tissue eosinophils may be one mechanism of the antiulcerative activity of opioid peptides, for these cells can block the effects of endogenous histamine [10].

The results are evidence of the greater importance of enkephalins than of endorphins in their protective action on the duodenal mucosa. Considering information on the role of enkephalins as neurotransmitters [8], it can be postulated that the enkephalinergic innervation of organs of the digestive system plays an essential role in maintaining the structural integrity of the duodenal mucosa. Synthetic analogs of enkephalins can accordingly be looked upon as potential substances for use in the treatment of peptic ulcer.

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