EFFECT OF ENKEPHALIN AND CIMETIDINE ON THE ONSET AND COURSE OF DUODENAL ULCERS IN RATS

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Substances of peptide nature which are ligands of opiate receptors, namely enkephalins and endorphins, previously detected in the CNS [2], have recently been found in the gastrointestinal tract. Their wide distribution in the antral portion of the stomach and in the proximal part of the duodenum, where enkephalins are located in nerve fibers of the submucosa and in specific endocrine cells of the antral portion of the stomach, very reminiscent of the G-cells which produced gastrin [9], has been demonstrated. The role of enkephalins in the physiology of the digestive tract is being studied intensively, but most research in this field has dealt with their motor function [3]. Data on the effect of enkephalins on the secretory function of the stomach are contradictory [1, 7]. It is accordingly interesting to study the effect of enkephalins on ulcer formation in the duodenum, in the development of which the role of the acid-peptic factor is generally recognized.

The aim of the present investigation was to compare the effect of enkephalin and cimetidine, which specifically blocks histamine H_2-receptors, on the development of experimental duodenal ulcers in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 180 male Wistar rats weighing 180-200 g in which duodenal ulcer formation was induced by administration of cysteamine by a modified Szabo's method [11, 12]. Cysteamine hydrochloride (from Serva, West Germany) was given as a single subcutaneous injection in a dose of 350 mg/kg, dissolved in 0.3 ml physiological saline, to 170 animals. The animals were divided into three groups. The 60 rats of group 1 received a synthetic analog of enkephalins, [synthesized in the Laboratory of Peptide Synthesis (Head M. I. Titov), All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR], (Tyr-D-Ala-Gly-Phe-NH_2) in a dose of 125 nmole/kg subcutaneously, and this was repeated every 12 h thereafter. The animals were killed in groups of 10 rats 3 h and 1, 2, 3, 7, and 14 days after the injection of cysteamine. The 40 rats of group 2 received subcutaneous injections of cimetidine (from Smith, Kline, and French, USA) in a dose of 17 μmoles/kg immediately after the injection of cysteamine, and thereafter repeatedly according to the same scheme. These animals also were killed in groups of 10 rats at a time, 1, 2, 7, and 14 days after the injection of cysteamine. The 70 rats of group 3 served as the control; they were given injections of physiological saline every 12 h after the injection of cysteamine. The animals of this group were killed 3 h and 1, 2, 7, 14, and 28 days later (10 rats at a time). In the animals of all three groups the state of the gastric and duodenal mucosa was assessed visually. No change in the mucosa or the presence of simple duodenitis was rated at 0 points, the presence of duodenitis + erosions as 1 point, single ulcers as 2 points, multiple (usually pairs) — 3 points, penetrating and perforating ulcers — 4 points. The severity of the lesion for each group of 10 rats was expressed by the mean number of points. The frequency of ulcer formation was estimated as the ratio between the number of animals with duodenal ulcers and the total number of animals in the group; the multiplicity of ulcer formation was estimated as the ratio of the total number of ulcers in the group to the total number of animals. By adding all three parameters together, an ulcer index (UI) was obtained for each group:

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TABLE 1. Changes in UI under the Influence of Cimetidine, Cysteamine, and Enkephalin Analog

Substances injected	Time after beginning of injection of cysteamine							
	3 h	24 h	48 h	3 days	7 days	14 days	21 days	28 days
Cysteamine + physiological saline	0,3	3,2	3,4	—	1,8	1,2	0,4	0
Cysteamine + cimetidine	—	1,6	1,6	—	0,8	0	—	—
Cysteamine + enkephalin analog	0	1,4	0,4	0,8	0	0	—	—

TABLE 2. Changes in Gastrin Level (in pg/ml) under the Influence of Cysteamine and Enkephalin Analog

Substances injected	Time after beginning of injection of cysteamine								
	0	3 h	24 h	48h	3 days	7 days	14 days	21 days	28 days
Cysteamine + physiological saline	70±12,7	138±20,5	115±7,1	90±10,5	—	68±5,1	74±4,8	78±4,5	85±6,2
Cysteamine + enkephalin analog	70±12,7	75±11,2	67±9,2	73±6,8	80±4,5	69±3,0	—	—	—

Legend. Difference from control group significant at 95% Level ($P < 0.05$).

$$UI = \frac{\text{total number of points + number of rats with ulcers + total number of ulcers in group}}{\text{Number of animals in group}}$$

In the animals of groups 1 and 3 blood serum was obtained after sacrifice in order to determine the gastrin level by a radioimmunologic method using standard kits from Cea-Ire-Sorin (France). The values obtained for the serum gastrin level were compared with those in the control group of 10 rats. The results were subjected to statistical analysis by Student's t-test with a level of significance of 95% ($P < 0.05$).

EXPERIMENTAL RESULTS

As Table 1 shows, UI for rats of the control group rose gradually and reached a maximum 48 h after injection of cysteamine. Later, UI fell gradually. No ulcers could be found in the animals after 28 days. Under the influence of cimetidine a significant decrease in UI was observed after 24 and 48 h. The ulcers disappeared completely after 14 days. An even greater fall in UI took place after injection of enkephalin: No ulcers could be found after only 7 days.

The gastrin level (Table 2) rose sharply 3 h after injection of cysteamine in the control animals. Later it began to fall gradually, and by the end of the first week it was back to normal. The gastrin level then rose slowly, to reach 85 pg/ml by the end of the 4th week. This rise was significant compared with the level on the 7th day. Injection of enkephalin prevented the rise in the gastrin level induced by cysteamine practically completely.

One mechanism of the development of cysteamine ulcers is a sharp increase in acid production by the stomach, with a simultaneous increase in the blood gastrin concentration [8]. The appearance of ulcers can be largely prevented by removal of the pituitary, adrenal, or thyroid glands. Ulcer formation also is sharply inhibited by vagotomy, sympathectomy, and injection of atropine and of histamine H_2-receptor blockers [12].

The results of the present investigation confirmed some of these findings. For instance, a marked increase in the serum gastrin level was observed in response to injection of cysteamine, and cimetidine had an antiulcerative action. The fact that deserves attention from our point of view is that the gastrin concentration increased before ulcer formation increased (Fig. 1). The gastrin level, which rose sharply 3 h after injection of cysteamine, fell significantly after 48 h, when UI reached its maximum. Later, as UI declined, a second increase was observed in the gastrin level, to reach a maximum when the ulcers had completely disappeared. It can be tentatively suggested that the earlier sharp increase in the gastrin

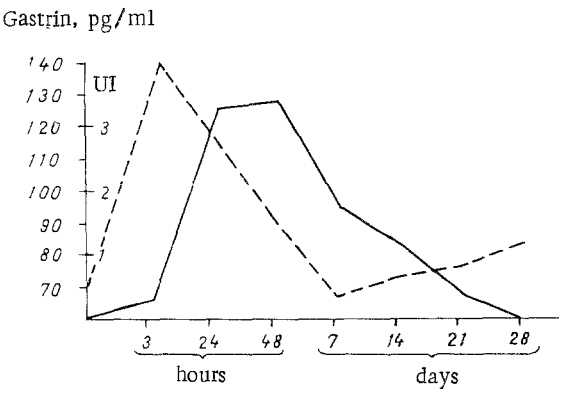

Fig. 1. Time course of changes in gastrin level and UI in rats (control group). Broken line shows serum gastrin concentration (in pg/ml), continuous line shows UI.

concentration determines the first, "acid-peptic" stage in the development of the cysteamine ulcer, and this is succeeded by disturbances of other systems, especially somatostatin [13, 14] and dopamine [5]. The possibility cannot be ruled out that in the development of duodenal ulcer in man there is also a stage of a sharp rise in the gastrin level, but because of its short duration, it has eluded the attention of investigators. The secondary, moderate rise in the gastrin concentration which coincides with complete healing of the ulcers is probably connected with the protective action of gastrin [4]. Injection of enkephalin caused the cysteamine ulcers to heal 4 times faster. To some degree this can probably be attributed to blocking of the release of gastrin and to a decrease in hydrochloric acid production by the stomach [1]. Considering the discovery of gastrin and enkephalin in the same endocrine cells [9], it can be postulated that these substances possess definite antagonistic properties. Whereas this follows from the results of the investigations cited above, in the experimental model now used enkephalin had a stronger antiulcerative effect than cimetidine, a member of the class of histamine H_2-receptor blockers. These substances have a powerful antisecretory action on the stomach, being stronger in this respect than enkephalins, and at the present time they are the most effective antiulcer preparations. It thus follows from the above account that the action of enkephalin in the experimental model used cannot be explained purely by its antisecretory effect, and this confirms once again that the acid-peptic factor is not the only mechanism of formation of cysteamine ulcers [6]. The possibility cannot be ruled out that enkephalins have a direct protective action on the duodenal mucosa or an indirect action through other mediator systems.

The results of this investigation may perhaps provide a basis for the discovery of fundamentally new drugs for the treatment of duodenal ulcer in man.

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