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CHANGES IN ACID PHOSPHATASE IN THE GASTRIC MUCOSA DURING ULCER FORMATION

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UDC 616.33-002.44-07:616.33-018.73-008.931:577.152.361

KEY WORDS: acid phosphatase; cathepsins; ulcer formation; acid proteases.

Much information has accumulated in the recent literature on a possible role of proteolytic enzymes in the mechanisms of ulcer formation in the stomach and duodenum [1-3, 5, 8-11, 13]. Besides characteristic changes in the spectrum of proteolytic enzymes exhibiting activity at pH values from 1.0 to 5.0 in the gastric mucosa during the period of ulcer formation, others active also at pH values from 6.5 to 7.0 may also appear. It has been suggested that the source of this activity is the intracellular lysosomal enzymes, which exert their lytic action on the surrounding autologous substrate as a result of liberation of these enzymes from intracellular lysosomes [3].

This paper describes a further study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 192 albino rats of both sexes weighing 160-200 g. An acetate model of gastric and duodenal ulcer was used [12]. An ulcer was produced on the anterior wall of the forestomach, the glandular part of the body of the stomach, the antrum, the duodenum, and the terminal portion of the ileum. The animals were killed 10, 60, and 90 min after ulcer formation. Washings from the region of the ulcer and from an area of the intact mucosa of similar size, the gastric or intestinal contents, and tissue from the floor of the ulcer, the edges of the ulcer, and intact areas of mucosa were investigated. A piece of mucosa weighing 100 mg was excised and homogenized in 2.0 ml of distilled water. Acid phosphatase, a marker of liberation of lysosomal enzymes [7], was determined in the material thus obtained, and the proteolytic activity of extracts of the mucosa at pH 6.5-7.0 was determined by the method described previously [6].

EXPERIMENTAL RESULTS

No acid phosphatase was found in the gastric contents of intact (control) rats, but comparison of data obtained 10, 60, and 90 min after the formation of an experimental ulcer in the body of the stomach showed that the acid phosphatase concentration in the gastric contents reached a maximum after 60 min (Table 1). The maximal content of acid phosphatase in washings from the zone of the ulcer also was found after the same interval of 60 min. These observations evidently indicate that this is the time when destructive processes take place most intensively in the tissues of the gastric mucosa after application of acetic acid to the serous membrane.

In view of these results, the next tests were carried out at hourly intervals after formation of the ulcer. Acid phosphatase, which was absent in the control, was found to appear in the gastric contents after formation of an ulcer in the forestomach, while at the same time its level fell sharply in the tissue of the mucosa in the zone of the ulcer (1.0 ± 0.2 μmole p-nitrophenol/g dry weight of tissue compared with 6.4 ± 0.6 in the control — Table 1). Ulcer formation in the antral portion was accompanied by the appearance of considerable quantities of acid phosphatase in washings from the zone of the ulcer (0.165 μmole p-nitrophenol/ml compared with 0). The acid phosphatase content also was significantly in-

Central Research Institute of Gastroenterology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 9, pp. 55-57, September, 1982. Original article submitted May 7, 1982.

 0007-4888/82/9409-1226$07.50

TABLE 1. Acid Phosphatase Activity in Washings (in μmoles p-nitrophenol/ml), and in Contents of Stomach and Forestomach, and also in Test Tissues (in μmoles p-nitrophenol/g wet weight of mucosal tissue) 10, 60, and 90 min after Formation of "Acetate" Ulcer in Different Locations

Test object	Control ($n=10$)	10 min ($n=6$)	60 min ($n=7$)	90 min ($n=6$)	P_{10}	P^{60}_{0}	P_{90}
Body of stomach:							
contents	0	0,024±0,05	0,57±0,04	0,345±0,02	—	—	—
washings	0,023±0,002	0,018±0,001	0,14±0,01	0,03±0,002	<0,05	<0,001	<0,05
stomach	6,6±0,6	4,6±0,4	7,3±0,6	6,6±0,6	<0,05	<0,8	
Forestomach:							
contents	0	—	0,02±0,002	—	—	—	—
washings	0,015±0,01	—	0,12±0,001	—	—	<0,1	—
stomach	6,4±0,6	—	1,0±0,2	—	—	<0,001	—
Antrum:							
washings	0	—	0,165±0,02	—	—	—	—
stomach	3,6±0,4	—	5,3±0,5	—	—	<0,05	—
Duodenum:							
washings	0,015±0,001	—	0,29±0,03	—	—	<0,001	—
stomach	7,0±0,7	—	8,33±0,8	—	—	<0,3	—
Ileum:							
washings	0,105±0,01	—	0,255±0,03	—	—	<0,05	—
stomach	7,0±0,7	—	5,33±0,5	—	—	<0,1	—

TABLE 2. Proteolytic Activity of Extracts of Affected Area of Mucosa at pH 6.5-7.0 Depending on Location of "Acetate" Ulcer (in extinction units at 280 nm) 60 min after Its Formation

Test object	Control ($n=6$)	Ulcer ($n=7$)	P
Forestomach	0,15±0,02	0,23±0,02	<0,02
Body of stomach	0,18±0,02	0,40±0,04	<0,005
Antrum	0,4 ±0,05	0,5 ±0,05	<0,3
Duodenum	0,6 ±0,06	1,12±0,1	<0,005
Ileum	0,37±0,04	0,65±0,06	<0,01

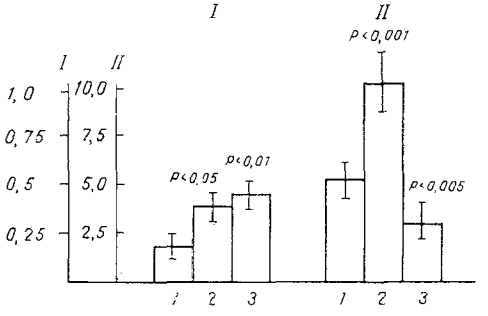

Fig. 1. Proteolytic activity (I) at pH 6.5-7.0 (in extinction units at 280 nm) and acid phosphatase activity (II) in tissues of floor and edges of "acetate" ulcer (in moles p-nitrophenol/g dry weight of tissue) located in body of stomach, 60 min after its formation. 1) Control, 2) edges of ulcer, 3) floor of ulcer.

creased in washings from the zone of the ulcer when this was formed in the duodenum (0.29 μmole p-nitrophenol/ml in the experiment, 0.015 in the control). Similar results also were obtained when an ulcer was formed in the ileum.

Comparison of the acid phosphatase content in tissue of the floor of the ulcer and its edges showed a sharp decrease in the level of this enzyme in the necrotic masses in the floor of the ulcer and a significant increase in its content in tissues in the immediate vicinity of the crater (Fig. 1).

A parallel investigation of proteolytic activity at pH 6.5-7.0 showed a marked increase in the zone of the ulcer in the forestomach, the body of the stomach, duodenum, and ileum (Table 2). A separate investigation of proteolytic activity at pH 6.5 in material obtained from the floor and edges of an ulcer formed in the body of the stomach revealed a significant increase in this activity in both regions compared with its value in the corresponding area of mucosa of intact rats (Fig. 1).

The results thus indicate a regular increase in acid phosphatase secretion at the height of destruction of the mucosal tissue (60 min after the beginning of ulcer formation). This process as a rule was accompanied by a considerable increase in proteolytic activity at pH 6.5-7.0 in tissue extract obtained from the zone of the lesion. It is well known that pepsinogens of the stomach are activated at pH 5.2-5.4 and below, and that at the above-

mentioned gastric pH values they are practically completely inactivated [12]. Consequently, activity detected in extracts of mucosa in which an ulcer is present cannot be attributed to the secreted enzymes. This conclusion is confirmed by the fact that proteolytic activity at pH 6.5-7.0 was found in the present experiments not only in the secretory zone of the stomach affected by an ulcer, but also in zones where chief cells producing pepsinogens are absent: the forestomach, the duodenum, and ileum. With these facts in mind, as well as the parallel increase in acid phosphatase activity, it can be tentatively suggested that the increase in proteolytic activity observed at pH 6.5-7.0 in tissue homogenate from the zone of the ulcer is most probably due to liberation of lysosomal proteases at the height of tissue destruction. It is, in fact, known that lysosomes of epithelial cells of the gastric mucosa contain four cathepsins, whose activity is manifested at different pH values, ranging from 2.5 to 7.5, including cathepsin G (active at pH values of about 7.5) [4]. It can thus be postulated that ulcer formation in the mucosa produced by application, in this case, of acetic acid, is associated with the manifestation of proteolytic activity inside the tissue by cathepsins liberated as a result of destruction of the lysosomal membranes of the corresponding cells.

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