AGGRESSIVENESS OF GASTRIC JUICE

N. Sh. Amirov and D. V. Antonov

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Much evidence has now been obtained to show that gastric proteases take an active part in ulcer formation in the gastroduodenal system [3, 14, 15]. The gastric juice of dogs with an atophan-induced gastric ulcer, if introduced into the stomach of rats, has been shown to cause ulcer formation; the ulcerogenic effect was most marked at pH 4.0-5.0 [3].

Different stimulators of secretion stimulate protease liberation differently [7, 11-13]. Among the widely used clinical stimulators of gastric secretion, histamine and pentagastrin deserve priority of mention. It has been shown [8, 13] that the mechanisms of stimulation of secretion of acid and pepsin by these substances differ, and this accounts for differences in the protease composition of the gastric juice.

With these considerations in mind it was decided to study the ulcerogenic activity of human gastric juice in relation to the gastric mucosa of rats, using for this purpose the gastric juice of healthy subjects and of patients with peptic ulcer, obtained in response to stimulation by histamine and pentagastrin. The results of such an investigation should help to shed light on the problem of which stimulator can realize to the fullest extent the ulcerogenic properties of the gastric juice and what mechanisms of secretion are more severely disturbed during ulcerogenesis.

EXPERIMENTAL METHOD

To determine the aggressive properties of the gastric juice of a patient with peptic ulcer relative to the gastric mucosa of rats, samples of test juice, adjusted with 0.2 M CH_3COONa-HCl-buffer with pH values from 1.0 to 5.0, at intervals of 1 unit, to the corresponding pH values [1], were introduced into the stomach of rats. The final dilution of gastric juice was 1:4. After exposure for 24 h the rats were killed and their stomachs, divided along the greater curvature, were examined under a binocular microscope. Data showing the type of lesion of the mucosa are given in Table 1. The final results were expressed as the percentage of animals with a given type of lesion compared with the total number of rats used in the experiment. Gastric juice was obtained from three healthy subjects and six patients with peptic ulcer in response to histamine stimulation and from five healthy subjects and six patients with duodenal ulcer in response to stimulation by pentagastrin.

The series of experiments to determine what proteases are present in the gastric juice of dogs in response to stimulation of secretion by histamine and pentagastrin, under normal conditions and during ulcer formation, was carried out on eight mongrel dogs with a gastric fistula. Experimental ulcer formation was induced by administration of atophan [9]. Samples of gastric juice were subjected to disc electrophoresis, after which points of proteolysis was identified at pH 1.0-5.0 [2]. By comparing the R_f values of the protein fraction and the points at which hydrolysis of hemoglobin was observed, the presence of proteases in the samples was determined. By pooling the data obtained in the experiments, the number of cases in which each of the fractions was found when histamine or pentagastrin was used for stimulation was obtained. This number, expressed as a percentage of the number of experiments, gave the frequency of detection of proteases.

RESULTS AND DISCUSSION

Data on the aggressiveness of the gastric juice of healthy subjects and patients with

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TABLE 1. Aggressiveness of Gastric Juice from Healthy Subjects and Patients with Gastroduodenal Ulcers Relative to Rat Gastric Mucosa

Type of lesion	Normal or ulcer	Number (in %) of affected animals after treatment with "histamine" (A) and "pentagastrin" (B) gastric juice with different pH values											
		A						B					
		pH 1,0	pH 2,0	pH 3,0	pH 4,0	pH 5,0	total	pH 1,0	pH 2,0	pH 3,0	pH 4,0	pH 5,0	total
Hemorrhage	Normal	6,67	6,67	6,67	20,0	20,0	60,0	16,0	8,0	16,0	12,0	16,0	68,0
	Ulcer	20,0	16,7	16,7	16,7	16,7	86,8	10,0	3,3	—	6,7	6,7	26,7
Edema	Normal	—	3,33	3,33	3,33	—	10,0	4,0	4,0	—	12,0	—	20,0
	Ulcer	16,7	10,0	13,3	13,3	6,7	60,0	—	3,33	3,33	3,33	3,33	13,3
Maceration	Normal	6,66	6,66	—	13,4	—	26,7	4,0	8,0	8,0	8,0	—	28,0
	Ulcer	16,7	13,3	13,3	10,0	6,7	60,0	—	3,33	3,33	—	6,66	13,3
Erosion	Normal	6,66	6,66	—	6,66	6,66	26,7	4,0	—	8,0	4,0	12,0	28,0
	Ulcer	3,33	3,33	—	—	3,33	10,0	6,6	3,33	10,9	6,66	13,32	40,0
Ulceration	Normal	6,66	—	—	—	6,66	13,3	8,0	—	12,0	—	8,0	28,0
	Ulcer	13,3	16,7	16,7	9,9	6,67	63,3	—	—	—	—	6,6	6,6

peptic ulcers are given in Table 1. Gastric juice obtained in response to histamine from healthy subjects exhibited moderate aggressiveness relative to the rat gastric mucosa at pH 1.0, 2.0, and 3.0 and somewhat higher aggressiveness at pH 4.0 and 5.0. An ulcerogenic effect was observed at pH 1.0 and 5.0.

Gastric juice obtained from patients with peptic ulcer in response to stimulation by histamine induced ulcer formation at all pH values, including pH 2.0, 3.0, and 4.0, at which healthy human gastric juice did not induce ulcer formation. The ulcerogenic action of the gastric juice of the patients in response to histamine stimulation was almost 5 times stronger than that of healthy gastric juice (63.3 and 13.3%, respectively). This fact is evidence of increased aggressiveness of the gastric juice. With respect to other types of lesions than erosions, the gastric juice of peptic ulcer patients obtained in response to histamine also was much more aggressive than healthy human gastric juice. With regard to erosions, the percentage of rats with this type of lesion was probably reduced somewhat because ulcers were formed at the site of erosions.

Somewhat different results were obtained when indices of aggressiveness of gastric juice from healthy subjects and patients with peptic ulcer were compared during stimulation of gastric juice secretion by pentagastrin. Gastric juice secreted in response to pentagastrin in patients with peptic ulcer was less aggressive than healthy human gastric juice. Data on aggressiveness of healthy human gastric juice during stimulation of secretion by histamine and pentagastrin were very similar. Only in its ability to induce erosions was the gastric juice secreted in response to pentagastrin in patients with peptic ulcer more aggressive than in normal subjects. With respect to the other indices, aggressiveness was reduced almost by half, whereas with respect to ulcerogenic effect it was reduced fourfold.

It will also be noted that ulcer formation in rats in response to administration of patients' gastric juice was observed at pH 5.0; this is further evidence of the unimportant role of the acid factor alone in the production of lesions of the gastric mucosa.

It can be tentatively suggested that the cause of the difference in aggressiveness of "histamine" and "pentagastrin" gastric juice from peptic ulcer patients could be differences in its protease spectrum.

To study this problem a series of experiments was carried out on eight dogs under normal conditions and during experimental atophan-induced gastric ulcer.

The composition of the protease fractions of gastric juice in normal dogs during stimulation of secretion by histamine and pentagastrin was characterized by absence of proteases with electrophoretic mobility of between 0.60 and 0.54, and also of fraction with $R_f = 0.73$, when histamine was used and the fraction with $R_f = 0.66$ when pentagastrin was used (Table 2).

Ulcer formation was accompanied by the appearance of proteolytic fractions in the "histamine" and "pentagastrin" gastric juice not found under normal conditions (Table 2). In juice secreted in response to histamine these were fractions with R_f values of 0.73, 0.60, 0.57, and 0.54, and in juice secreted in response to pentagastrin, the fraction with $R_f = 0.66$.

The frequency of occurrence of only four fractions was reduced in "histamine" juice from

TABLE 2. Frequency of Occurrence (in %) of Proteases in Gastric Juice of Dogs under Normal Conditions and with Atophan-Induced Gastric Ulcer, in Response to Stimulation of Secretion by Histamine and Pentagastrin

Stimulus under normal conditions and during ulcer	Frequency of occurrence of proteases with different R_f values																
	0,98	0,96	0,93	0,91	0,89	0,87	0,85	0,83	0,79	0,77	0,75	0,73	0,70	0,66	0,60	0,57	0,54
Normal																	
Histamine	60,3	83,3	79,2	39,8	62,5	41,7	81,3	43,7	56,3	45,8	37,5	0	37,5	18,8	0	0	0
Pentagastrin	85,3	70,8	56,3	39,8	70,8	39,8	43,7	100,0	68,8	56,3	75,0	58,3	56,3	0	0	0	0
Ulcer																	
Histamine	75,5	62,5	77,3	26,4	73,5	50,9	50,9	77,3	81,0	47,2	37,7	54,6	49,0	24,6	11,3	11,3	11,3
Pentagastrin	56,0	60,0	46,0	34,0	48,0	32,0	60,0	60,0	70,0	24,0	42,0	30,0	36,0	26,0	0	0	0
$\frac{\text{Ulcer}}{\text{Normal}} \times 100\%$																	
Histamine	125,2	75,0	97,6	66,3	117,6	120,0	62,6	176,7	143,7	103,1	100,5	—	130,7	131,0	—	—	—
Pentagastrin	65,7	84,8	81,8	85,4	67,8	80,4	137,3	60,0	101,7	42,6	56,0	51,4	64,0	—	—	—	—

dogs with ulcer formation — those with R_f values of 0.96, 0.93, 0.91, and 0.85. The frequency of occurrence of most other protease fractions increased, most of all the fraction with R_f = 0.83 (by 76.7%), and least of all the fractions with R_f = 0.77 (by 3.1%) and with R_f = 0.75 (by 0.5%).

Unlike histamine, during ulcer formation pentagastrin caused mainly a decrease in the frequency of occurrence of proteases down to 42.6% of normal. Only two fractions — those with R_f = 0.85 and R_f = 0.79 — were secreted during ulcer formation in response to pentagastrin rather more frequently than in healthy dogs. The fraction with R_f = 0.66 was never found in samples of gastric juice from healthy dogs, whereas in dogs with ulcer formation it was found in 26% of samples.

Certain differences thus exist in the protease composition of the gastric juice of dogs secreted in response to histamine and pentagastrin under normal conditions, and these differences increase considerably during ulcer formation.

The possibility cannot be ruled out that such disturbances of the protease spectrum, leading to changes in the mechanisms of regulation of secretion, also take place during peptic ulcer in man. In that case, the results now obtained are further evidence not only of the possible ulcerogenic role of gastric juice in ulcer formation in general, but also that this role belongs mainly to proteases.

Another possible explanation of the low aggressiveness of the "pentagastrin" juice of peptic ulcer patients may be the very low secretion or absence of secretion of pepsin in response to pentagastrin [8]. This explanation also confirms the writers' hypothesis regarding the role of the gastric juice proteases in ulcerogenesis.

Attention is drawn to a publication [16] the author of which, while accepting the aggressive role of proteases relative to the gastric mucosa, does not consider that those proteases which give rise to proteolysis at pH 5.0-7.0 play the role of "pathological" proteases. This view is based on the results of an investigation of the proteolytic activity of gastric juice of patients with achlorhydric gastritis, in whom, evidently, these proteases are responsible for biological adaptation. Meanwhile such observations, in our view, do not contradict the conclusions of the present experiments, for, and this is also the opinion of the author cited above [16], "hydrochloric acid without the presence of proteolytic enzymes, and the latter without the aid of hydrochloric acid, are unlikely to affect the formation of gastric or duodenal ulcers."

The view is held [6] that the gastric juice of patients with peptic ulcer has no specific enzyme activity. However, this conclusion is based on the results of a study of the proteolytic activity of gastric juice at two pH values only (2.0 and 5.0), without the use of healthy human gastric juice as a control. With regard to specific enzyme activity, "tyrosine" methods can give information only about total gastric juice protease activity and do not allow any differential estimation of the role of each protease in protein hydrolysis. The method of biological testing used by the present writers reveals largely the specific changes

in protease activity (aggressiveness), associated with ulcer formation. However, the method of biological testing must be used fully at different pH values. In that case it can be established that the hypothetical "ulcerogenic factor" [4, 5] possesses the distinguishing properties of enzymes. The data on enzyme secretion in dogs in response to histamine and pentagastrin, cited above, indicate that ulcer formation is accompanied by disturbances in the protease spectrum, and the results of the study of aggressiveness of the gastric juice of patients with peptic ulcer demonstrate a connection between the stimulator of the secretion of gastric juice (and proteases!) used and the ulcerogenic effect. In the present writers' view it can be concluded from these observations that the aggressiveness of the gastric juice of patients with peptic ulcer is protease in nature.

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