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POLYPEPTIDES ISOLATED FROM THE GASTRIC MUCOSA AND THEIR ACTION ON PEPSIN BIOSYNTHESIS BY GASTRIC GLANDS

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Administration of natural and artificial gastric juice is based on the principle of replacement therapy, in which a patient is prescribed a remedy containing pepsin [5, 6, 11]. However, it has recently been noted that preparations of the pepsidil type and new artificial gastric juice (NAGJ) contain a hydrochloric acid solution of products of enzymic hydrolysis of gastric mucosal tissue [4, 11], which has the power to excite gastric secretion [1, 12]. It has also been shown that a mixture of polypeptides, formed during the production of pepsin preparations, the technology of which is based on the principle of autolysis [13], possesses a similar property. These polypeptides have been isolated by the writers from the autolyzate by chromatography with Sephadex G-100, after which they were separated by repeated chromatography on Sephadex G-25 columns into three fractions, conventionally termed A (mol. wt. ∿2800), B (mol. wt. ∿2500), and C (mol. wt. ∿2200).

The object of the present investigation was to study which of these polypeptides has the greatest ability to stimulate pepsin biosynthesis in the gastric mucosa and whether this property of the peptides is modified through the action of proteinases (pepsin and trypsin) on them in the digestive tract.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male albino rats weighing 120-150 g. A food stimulus (1 ml milk) was introduced simultaneously with one of the test substances into the stomach of all the rats through a tube. After 1 h the stomach was removed quickly from the peritoneal cavity and opened, after which it was washed twice with cold distilled water. A homogenate was prepared from the gastric mucosa and 0.01 N HCl in the ratio of 1:10. To convert the whole of the pepsinogen into pepsin, the homogenate was incubated for 1 h at 37°C. Pepsin activity in the homogenate was determined as its proteolytic action at pH 3.0 [15], for it is in such a medium that pepsin mainly exhibits its action.

The milk-curdling ability of pepsin is due to rupture of peptide bonds in the caseinogen molecule, which is converted into the unstable form – casein [3]. This test is widely used to determine the activity of the enzyme at pH 5.0, on the basis of its milk-curdling action [8].

A mixture of the food stimulus and 1 ml of 0.01 N HCl, into which 30 mg of polypeptide B, denatured at 100°C, had first been dissolved, was introduced into the stomach of the rats of group 1 (control). Besides the food stimulus, the animals of groups 2, 3, and 4 each received 1 ml of 0.01 N HCl in which 30 mg of polypeptides A, B, and C, respectively, had been dissolved. The rats of group 5 received the food stimulus alone: 0.75 ml 0.01 N HCl and 0.25 ml NAGJ (containing 2 mg/ml pepsin and 106 mg/ml of a mixture of polypeptides). The results of these series were subjected to statistical analysis [2].

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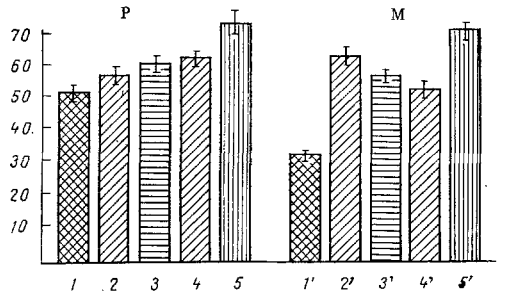

Fig. 1

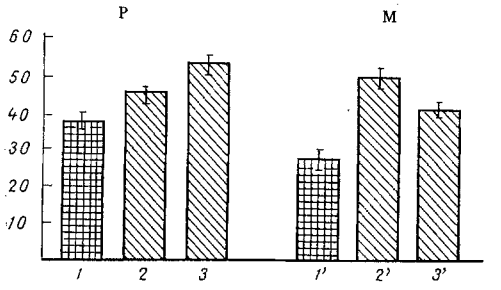

Fig. 2

Fig. 1. Proteolytic (P) and milk-curdling (M) activity of pepsin in gastric mucosa of rats after introduction of milk and hydrochloric acid solution, with the addition of denatured polypeptide B (control) (1), polypeptide A (2), polypeptide B (3), and polypeptide C (4), and also after introduction of milk, HCl, and NAGJ (5), into the stomach. Abscissa, series of experiments; ordinate, pepsin activity (in units/g wet weight of tissue).

Fig. 2. Proteplytic (P) and milk curdling (M) activity of pepsin in gastric mucosa of rats after introduction of milk into the stomach and intraperitoneal injection of a solution of enzymically untreated polypeptide B (control) (1), of polypeptide B hydrolyzed by pepsin (2), and polypeptide B hydrolyzed by trypsin (3). Legend for abscissa and ordinate as in Fig. 1.

The food stimulus was introduced into the stomach of the animals of group 6 and 1.2 ml of buffer solution, pH 1.5 (consisting of 0.2 M HCl and 0.2 M CH_3COONa), in which 20 mg polypeptide B and 5 mg pepsin (products of the Olaine Factory) had been incubated beforehand for 1 h at 37°C, was injected into them intraperitoneally. Before intraperitoneal injection, the acid mixture was neutralized with 0.1 N NaOH. The rats of group 7 received the same food stimulus and the same mixture for intraperitoneal injection, but it was boiled immediately after addition of the pepsin (control). The food stimulus was introduced into the stomach of the rats of group 8, but 1.2 ml phosphate buffer solution (pH 7.6), in which 20 ml polypeptide B and 5 mg trypsin (from SPOFA, Czechoslovakia) had previously been incubated, was injected intraperitoneally. The rats of group 9 received milk and the same mixture for intraperitoneal injection, but after preliminary boiling (control).

EXPERIMENTAL RESULTS

It will be clear from Table 1 that polypeptides A, B, and C can increase both the proteolytic and the milk-curdling activity of pepsin.

It has recently been shown that under the influence of factors in the external medium changes taking place in the milk-curdling and proteolytic activity of pepsin are not parallel [10]. Experiments on dogs with a Pavlov gastric pouch have shown that in response to a change of food stimuli gastric juice is stimulated in which these properties of the pepsin also have undergone different changes [1]. The explanation probably lies in the presence of pepsin isozymes [14].

In the present investigation the proteolytic and milk-curdling activities of the pepsin likewise increased independently. For instance, under the influence of polypeptide A proteolytic activity increased by 10% whereas milk-curdling activity increased by 88%; under the influence of polypeptide C the increases were 21 and 61% respectively. Polypeptide B possessed similar properties. Differences in the response of the glandular apparatus of the gastric mucosa were evidently linked not only with the different molecular weight of the polypeptide, but also with differences in their primary structure.

It will also be clear from Table 1 that the highest response of the gastric glands secreting pepsin occurred when NAGJ containing all three polypeptides was administered, and that the milk-curdling activity of pepsin showed more marked changes than its proteolytic activity. This was evidently connected with the specificity of the food stimulus used (milk).

Polypeptides isolated from NAGJ evidently possess hormone-like properties similar to gastrin, which excites gastric secretion [7]. The question accordingly arises whether the polypeptides studied may not be precursors of smaller peptides that are formed from them under natural conditions in the gastrointestinal tract.

The effects of products of peptic hydrolysis of polypeptide B on pepsin biosynthesis was investigated in the rats of groups 6 and 7, and the effects of products of tryptic hydrolysis of polypeptide B were studied on rats of groups 8 and 9 (Table 2).

Table 2 shows that after intraperitoneal injection of a peptic digest of polypeptide B the proteolytic activity of pepsin in the rats' gastric mucosa was increased by 19.5%, whereas the milk-curdling activity was increased by 82%; under the influence of the tryptic digest the corresponding increases were 41.6 and 60%.

TABLE 1. Pepsin Activity (in units/g tissue) in Gastric Mucosa of Rats 1 h after Introduction of Food Stimulus and Hydrochloric Acid Solutions of Polypeptides Isolated from NAGJ into the Stomach (M ± m)

Additional stimulators of gastric secretion	Pepsin	
	relative to proteolytic action	relative to milk-curdling action
Hydrochloric acid solution of denatured polypeptide B (control) (n = 10)	52,0±1,6	35,5±3,1
Hydrochloric acid solution of polypeptide:		
A (*n*=10)	57,0±2,8	60,4±7,3*
B (*n*=10)	58,6±2,1*	53,6±7,4*
C (*n*=10)	63,1±6,0*	51,0±5,0*
NAGJ + HCl (n = 10)	71,2±4,2*	62,9±4,6*

TABLE 2. Pepsin Activity (in units/g tissue) in Gastric Mucosa of Rats 1 h after Introduction of Food Stimulus into Stomach and Intraperitoneal Injection of Hydrolysis Products of Polypeptide B (M ± m)

Stimulus for intraperitoneal injection	Pepsin	
	relative to proteolytic action	relative to milk-curdling action
Hydrochloric acid solution of polypeptide B and denatured pepsin (control) (*n*=10)	38,0±3,0	13,5±0,9
Pepsin hydrolysate of polypeptide B (n = 10)	45,4±2,4*	24,6±1,3*
Phosphate buffer solution of polypeptide B and denatured trypsin (control) (*n*=10)	37,5±2,75	13,3±1,1
Trypsin hydrolysate of polypeptide B (n = 10)	53,8±2,7*	21,1±3,1*

Legend. Here and in Table 2, asterisk indicates statistically significant difference compared with control ($P < 0.01$).

The pepsin preparation pepsidil, marketed by the Medical Industry, and the new artificial gastric juice (NAGJ), contain not only pepsin, but also biologically active substances, namely polypeptides A, B, and C, which stimulate pepsin biosynthesis in the gastric mucosa. They differ in their activity, and each one of them separately has a weaker action than the combined action of NAGJ. Meanwhile it can be postulated that in the gastrointestinal tract a number of smaller peptides, which also participate in the stimulation of pepsin biosynthesis, are formed from them under the influence of pepsin and trypsin.

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