ROLE OF THE ADENYLATE CYCLASE SYSTEM IN INHIBITION OF GASTRIC SECRETION BY LIMITED PROTEOLYSIS PRODUCTS OF κ-CASEIN

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It was shown previously that during limited proteolysis of κ-casein by pepsin or renin in vivo or in vitro peptides with marked inhibitory activity on acid secretion in the dog and rat stomach are formed [6, 7, 9]. The biochemical mechanisms lying at the basis of this phenomenon have not yet been discovered. We know that the cyclase system of the cell is one component regulating the secretory process [3, 4]. Close correlation exists between the concentration of cyclic nucleotides in the gastric mucosa (GM) and the rate of acid secretion in the stomach [11]. Inhibitors of gastric secretion (secretin, cholecystokinin–pancreozymin, prostaglandins, adrenalin, glucagon) activate the adenylate cyclase of mucosal cells [13]. Other inhibitors, such as somatostatin and opioid peptides, depress adenylate cyclase activity [3, 5].

The aim of this investigation was to determine the role of the cyclase system of GM cells, namely the enzyme adenylate cyclase, cAMP, and cGMP, in the inhibition of gastric secretion of acid by peptides formed during limited proteolysis of bovine κ-casein by pepsin.

EXPERIMENTAL METHOD

A preparation of peptides from κ-casein obtained by gel-filtration of the original peptic digest of κ-casein on columns with Sephadexes, which we have conventionally called peptide fraction B_{12} [1], was used. Experiments to determine cAMP and cGMP levels in GM were conducted on male Wistar rats weighing 180-200 g. The B_{12} fraction, in a dose of 4 mg per animal in 1 ml of physiological saline, was injected into the caudal vein, and the same volume of physiological saline was injected into control rats. The animals were killed, the fundal or antral portion of the stomach was excised, and scrapings of the mucosa of each part of the stomach, in a quantity weighing 60-80 mg, were transferred to a test tube containing 1 ml of acidified ethanol (1 ml of 1 N HCl to 100 ml rectified spirit) to extract cyclic nucleotides. The mucosal tissue was then homogenized in a glass-glass homogenizer and centrifuged at 1000g for 10 min; the supernatant was transferred into test tubes and the residue resuspended in 1.5 ml of a mixture of alcohol and water in the ratio of 2:1 and recentrifuged. The supernatants were pooled and evaporated on a rotary evaporator at 55°C under a vacuum. The cAMP and cGMP levels were determined by means of kits from Amersham International (England). Adenylate cyclase activity in the mucosal homogenate from the fundal or antral part of the rat stomach was determined by the method in [15], following method [8]. Female Wistar rats weighing 180-200 g had free access to water and food. The procedures of obtaining the mucosa and homogenizing the tissue were described previously [13]. Scrapings of mucosa were homogenized manually in medium containing 0.32 M sucrose, 50 mM Tris-HCl buffer, pH 7.4, at 4°C in a Potter–Elvehjem homogenizer. For each gram of tissue 10 ml of medium was used. The adenylate cyclase incubation medium (final volume 50 µl) contained 50 mM Tris-HCl buffer (pH 7.5 at 37°C), 5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM cAMP, 0.25 mM ATP, 10 mM theophylline, 20 mM creatine phosphate, 0.5 µg/ml creatine kinase, and α-^{32}P-ATP (10^6-$1.2 \cdot 10^6$ cpm). The peptide preparation was added to the incubation medium in a volume of 10 µl. In the experiments to study the role of calcium in the inhibition of adenylate cyclase by the B_{12} peptide fraction, the peptide was preincubated with EGTA in a concentration of 2 mM. Adenylate cyclase activity was determined at 37°C; the reaction was started by the addition (with careful mixing) of 10 µl of homogenate (100-120 µg

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TABLE 1. cAMP and cGMP Concentrations in Fundal and Antral Portions of Rat Stomach after Intravenous Injection of 4 mg of B_{12} (M ± m, n = 10)

Nucleotide determined	Conditions of determination	Concentration of cyclic nucleotides, pmoles/mg tissue	
		part of stomach	
		fundal	antral
cAMP	Control	0.37±0.05	0.33±0.04
	Experiment	0.42±0.03	0.30±0.03
cGMP	Control	0.020±0.004	0.035±0.0016
	Experiment	0.016±0.005	0.023±0.0010

TABLE 2. Effect of Preparation of Peptide Fraction B_{12} on Adenylate Cyclase Activity of Mucosal Homogenate from Fundal and Antral Portions of Rat Stomach

Effector and its concentration in sample	Part of stomach			
	fundal		antral	
	enzyme activity, pmoles cAMP/mg protein/min	effect, %	enzyme activity, pmoles cAMP/mg protein/min	effect, %
Without effector	6.3	100	2.4	100
NaF, 10 mM	9.5	432	16.0	667
B_{12}:				
0.2 mg/ml	4.5	72	1.9	80
2 mg/ml	3.0	47	0.4	17

protein). The yield of cAMP was determined by the method in [8]. The yield for the batch of alumina used in these experiments was 81%. All values given in Table 1 are mean values of five parallel measurements. The calcium concentration in the B_{12} preparation was determined by complexometric titration [2]. A weighed sample of the preparation (about 1 mg) was dissolved in 0.2 ml of deionized water and titrated with 0.001 M EDTA solution. The exact $MgCl_2$ concentration was determined on a Beckman 495 atomic absorption spectrometer. The protein concentration in the homogenate was determined by Lowry's method [12].

EXPERIMENTAL RESULTS

To reduce the effect of heterogeneity of the GM cell population levels of cyclic nucleotides and adenylate cyclase activity were measured separately in the mucosa from the fundal part of the stomach, which consists mainly of chief and parietal cells, and in the mucosa of the antral part of the stomach, which contains the maximal concentration of gastrin-producing G cells.

Table 1 shows that intravenous injection of peptide fraction B_{12} caused no significant change in the cyclic nucleotide concentration in the mucosa of both fundal and antral portions of the rat stomach. An increase in the dose of the peptide to 10 mg likewise did not lead to the appearance of statistically significant changes in the cAMP level in the mucosa of the fundal portion of the stomach. The cAMP level rose from 0.66 ± 0.07 to 0.82 ± 0.55 pmoles/mg tissue ($p > 0.05$). The writers showed previously that after intravenous injection of peptide inhibitor from $\varkappa$-casein into rats the basal secretion of acid was inhibited by a lesser de- than pentagastrin-stimulated secretion [7]. Intravenous injection of B_{12} in a dose of 4 mg into an anesthetized rat inhibited pentagastrin-stimulated acid secretion in the stomach on average by 50% [1].

In the next series of experiments the effect of peptide fraction B_{12} on adenylate cyclase activity was studied in a mucosal homogenate from the fundal and antral portions of the rat stomach in vitro. It was found that the B_{12} preparation in a dose of between 10 and 100 μg inhibited adenylate cyclase activity in both parts of the stomach (Table 2).

It will be clear from Table 2 that the B_{12} preparation caused dose-dependent inhibition of adenylate cyclase activity of homogenate from both parts of the stomach in all experiments.

We know that adenylate cyclase of the rat GM is very sensitive to inhibition by calcium. In a concentration of 0.15 mM calcium inhibits the enzyme by 50%, and in a concentration of 1 mM inhibition amounts to 85% [14]. Determination of the calcium concentration in the B_{12} preparation showed that it was 2.8% by weight. Calculation shows that the calcium concentration in the adenylate cyclase incubation medium after addition of 100 µg of the B_{12} peptide preparation may reach 1.5 mM. This calcium level is perfectly able to cause the depression of adenylate cyclase activity observed in the experiments described above.

To bind calcium present in the peptide preparation, the solution of the B_{12} fraction was preincubated with EGTA in a concentration of 2 mM; on the addition of 10 µl of peptide solution to the incubation medium the EGTA concentration could not exceed 0.4 mM. Under these experimental conditions inhibition of adenylate cyclase activity by the peptide fraction B_{12} preparation was completely abolished. Activation of adenylate cyclase by NaF was preserved in the presence of EGTA in concentrations up to 1 mM, evidence of the normal functioning of the catalytic subunit of the enzyme. It can thus be tentatively suggested that the inhibitory effect observed is due to the presence of calcium in the B_{12} preparation.

It can be concluded that the mechanism of inhibition of acid secretion in the stomach by the peptide preparation under examination evidently does not require direct participation of the cyclase system of the mucosal cells. The structure of regulation of cell mechanisms of acid secretion in the stomach is in general a multicomponent and complex system. Besides the cyclase system, an important role in regulation is played by gastrin [3, 4]. These two components of the regulating system are evidently separate. Gastrin does not affect the cAMP level in parietal cells or adenylate cyclase activity of isolated parietal cells. Acid secretion is modified by gastrin through direct stimulation of its release [10]. Neither gastrin nor histamine affects adenylate cyclase activity of rat GM homogenate [13]. It is possible that the decisive role in the mechanism of action of the peptide fraction studied in these experiments is played by the gastrin stage of regulation of secretion.

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