Composition of Glycoproteins of the Supraepithelial Mucous Layer of the Intestinal Tract for Administration of Pentagastrin and Carbacholine

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The composition of structural glycoproteins from the supraepithelial mucous layer of the stomach and intestine was determined by the concentration of the protein part and monosaccharides of the oligosaccharide part in dogs. It is shown that after administration of pentagastrin at 10 μg/kg and carbacholine at 6 μg/kg the release of structural glycoproteins increases in the stomach (the degree of glycosylation as well as the partial composition of monosaccharides does not markedly change). After stimulation of gastric secretion, the release of structural glycoproteins in the intestine increases insignificantly, whereas the glycosylation of glycoproteins from the supraepithelial mucous layer of the stomach is intensified.

Key Words: *supraepithelial mucous layer; glycoproteins; pentagastrin; carbacholine*

The mucous lining of the digestive tract is covered by a continuous supraepithelial mucous layer (SML). Its main functions are protection (mechanical and chemical), barrier, digestion, and transport, all of which are determined by the presence of structural glycoproteins (GP), bicarbonate, enzymes, and food particles from the cavity of the digestive tract [1]. Structural GP, characterized by a polymer structure of molecules, form the basis of the SML. It is now thought that the process of mucus GP secretion includes several stages, namely, the generation of a polypeptide chain, its glycosylation, polymerization of GP subunits and accretion of them in secretory granules, discharge of the finished glycoprotein secretion in SML, and its degradation in the layer [4-6,9]. The regulatory mechanisms of this process are still not clear.

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This study attempts to determine the composition of structural GP of the SML of the upper parts of the digestive tract in dogs for the administration of pentagastin and carbacholine.

MATERIALS AND METHODS

Acute experiments were carried out on mongrel male dogs aged 2-3 years weighing 12-19 kg. Prior to the experiment all dogs were receiving the standard vivarium diet. One day before the experiment at 16:00 h all food was removed while access to water remained free. All experiments began at 09:00 h. Intact dogs (n=6) served as controls. Test dogs (n=10) were s.c. injected pentagastrin at 10 μg/kg and carbacholine at 6 μg/kg. Heart arrest was induced by the administration of a saturated KCl solution 60 min after drug injection. The initial parts of the digestive tract were removed, the cavity was washed under running water, and the lesser and greater curvature and pyloric part of the stom-

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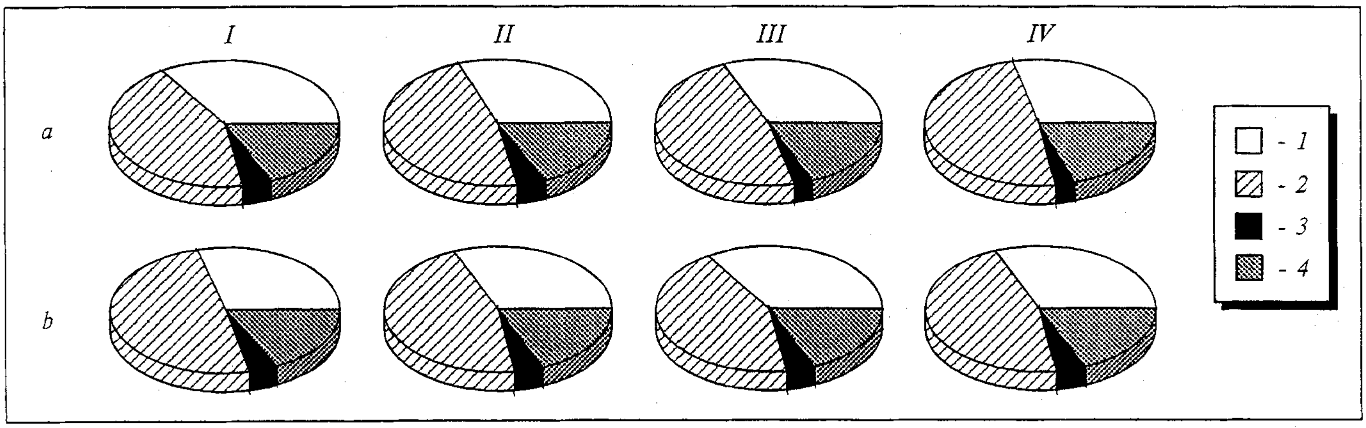

Fig. 1. Partial composition of monosaccharides of SML GP in stomach parts in the control (*a*) and after administration of pentagastrin and carbacholinee (10 and 6 μg/kg, respectively) (*b*). *I*) lesser curvature of stomach; *II*) greater curvature of stomach; *III*) pyloric part of stomach. Here and in Fig. 2: the area of the circle equals 100%; percent content of hexosamines(*1*), galactose (*2*), fucose (*3*), and sialic acid (*4*).

ach, and the proximal, medial, and distal thirds of the duodenum and the proximal part of the jejunum were then isolated. All parts were washed in 0.9% NaCl solution (+4°C) and then spread out on tracing paper. Removal of the SML was performed by careful collection mucus using a blunt metal spatula. The mucus samples from each part underwent a clearing procedure [2] and, finally, mucous structural GP and extrastructural components, including products of structural GP degradation, were obtained. The amount of the latter was assessed according to the sum of monosaccharides for the determination of the total pool of GP in the SML. Obtained samples of GP were subjected to step-by-step hydrolysis in 2 N HCl at 100°C in test tubes with reflux condensers. A sample for the determination of the galactose [10] and fucose [8] concentration was taken after 1 h of hydrolysis and after 5 h the concentration of hexosamines was determined in the hydrolysate [7]. The concentration of sialic acid was determined according to the method described in [11] and the protein part of GP by precipitation of amido black. The sum content of all monosaccharides in 1 ml of mucus and its ratio per mg of protein in the same volume was calculated, which makes it possible to determine the degree of GP glycosylation. The ratio of concentrations of individual monosaccharides was presented as their partial composition, where the sum of concentrations of all monosaccharides was taken to be 100%. Results were processed statistically using calculation of means, their errors, and the Student test ($p<0.05$).

RESULTS

It was found that the ratio of the sum of monosaccharides/protein in mucous structural GP in intact dogs is significantly higher in stomach parts then in intestine parts. This is especially evident in two neighboring parts namely, in the pyloric part of the stomach and in the proximal third of the duodenum ($p<0.05$) (Table 1). The same correlation is noted for injection of pentagastrin and

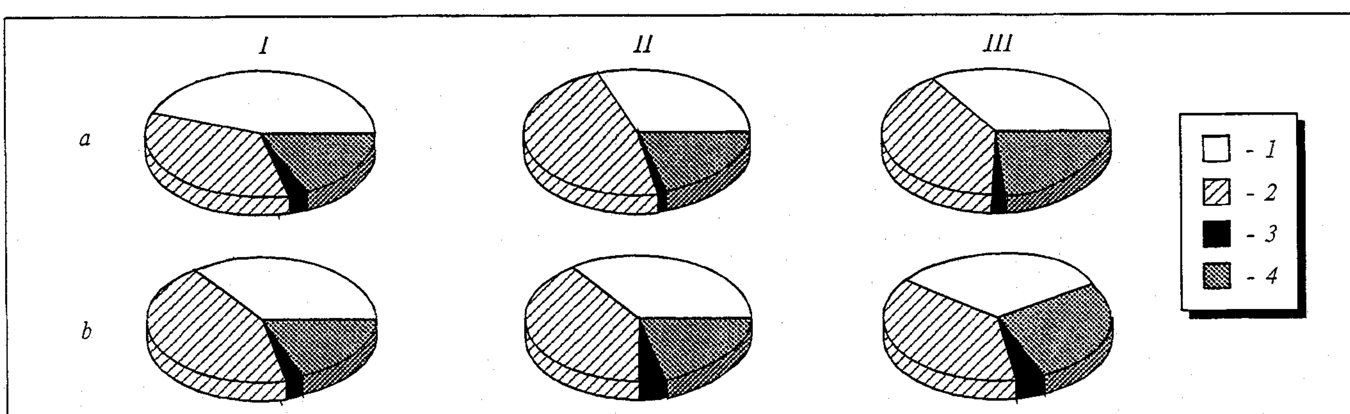

Fig. 2. Partial composition of monosaccharides of SML GP in stomach parts in the control (*a*) and after administration of pentagastrin and carbacholine (10 and 6 μg/kg, respectively) (*b*). *I*) proximal third of duodenum; *II*) medial third of duodenum; *III*) distal third of duodenum; *IV*) proximal part of jejunum.

carbacholine. Therefore, a higher degree of glycosylation is characteristic for gastric GP. The partial composition of individual monosaccharides may characterize the ratio of activities of transferases responsible for the inclusion of one or another monosaccharide in the oligosaccharide chain of GP. The partial composition of monosaccharides is different in GP of stomach and intestine parts in intact dogs: in the intestine the sum of galactose and fucose is similar to the sum of hexosamines and sialic acid, while in the stomach parts the sum of galactose and fucose always exceeds the sum of hexosamines and sialic acid (Fig. 1, *a* and 2, *a*). The lesser curvature of the stomach is characterized by a relatively lesser content of hexosamines and a greater content of galactose then in other parts of the stomach.

After pentagastrin and carbacholine administration the percentage of structural GP in the total pool of SML GP of all parts of the stomach increased in the lesser curvature before and after stimulation by 49%, in the greater curvature by 27 and 51%, respectively, and in the pyloric part by 35 and 58%. In intestinal parts this index changed to a lesser degree, namely in the proximal third of the duodenum by 23 and 26%, in the medial third by 16 and 23%, in the distal third by 19 and 26%, and in the proximal part of the jejunum by 17 and 25%, respectively. Therefore, the administration of pentagastrin and carbacholine steps up the release of mucus GP in the stomach and affects this process to a lesser degree in the intestine.

After pentagastrin and carbacholine administration the concentration of the protein part of GP increased in the stomach. The concentration of individual monosaccharides in GP of the stomach mucus also rose proportionally, resulting in no changes in the ration of the monosaccharides/protein sum (Table 1). The partial composition of individual monosaccharides in the stomach mucous GP did not change in the greater curvature but did change in the lesser, as well as in the pyloric part: the relative content of hexosamines increased in the lesser curvature and decreased in the pyloric part (Fig. 1). It is assumed that the ratio of transferase activities in these parts is less stable than in the greater curvature. In GP of the intestine mucus the protein concentration tended to drop, while the concentration of monosaccharides tended to rise or remained at the control level, resulting in a significant (as compared to the control) increase of the degree of glycosylation of GP in the proximal and medial parts of the duodenum, while in the other two parts of the intestine it tended to increase (Table 1). The partial composition of individual monosaccharides in the

TABLE 1. Composition of Structural GP of the SML of the Digestive Tract

Experimental series	Lesser curvature of stomach	Greater curvature of stomach	Pyloric part of stomach	Proximal duodenum	Medial duodenum	Distal duodenum	Proximal jejunum
			Protein, mg/ml				
a	0.82±0.16	1.78±0.49	1.71±0.55	4.38±0.86	4.01±0.51	4.61±0.36	3.24±0.65
b	4.12±0.57*	3.69±0.46*	4.63±0.71*	3.17±0.43	2.54±0.49*	3.49±0.48	3.15±0.45
			Hexosamines, μM/ml				
a	5.18±0.68	10.33±2.40	11.05±2.78	12.00±2.37	7.50±1.14	9.43±1.85	8.22±1.80
b	22.89±3.89*	22.31±2.68*	19.30±2.99*	11.99±1.71	8.61±1.17	8.20±2.11	8.87±2.68
			Galactose, μM/ml				
a	6.89±1.68	9.11±1.54	10.09±1.66	9.62±1.07	4.51±0.85	6.70±1.48	5.11±0.99
b	22.34±3.73*	20.19±5.18*	25.67±6.46*	7.58±0.72	6.13±1.45	6.66±1.43	6.15±1.35
			Fucose, μM/ml				
a	3.29±0.64	6.11±1.85	6.42±1.12	5.41±0.98	3.40±0.75	4.16±0.65	3.68±0.77
b	15.59±3.77*	12.42±1.30*	22.84±5.92*	5.35±0.54	4.20±1.22	4.73±1.42	4.09±0.91
			Sialic acid, μM/ml				
a	0.12±0.03	0.35±0.09	0.36±0.07	0.56±0.07	0.29±0.03	0.34±0.04	0.54±0.08
b	0.92±0.32*	0.93±0.16*	0.87±0.15*	0.59±0.12	0.53±0.14	0.46±0.16	0.43±0.15
			Sum of monosaccharides/protein, μM/mg				
a	16.28±4.38	15.87±2.37	16.97±4.43	5.59±0.81	4.16±0.55	4.17±0.56	4.72±0.52
b	14.54±2.54	16.50±2.55	16.30±3.86	9.10±1.08*	7.71±1.01*	6.54±1.40	5.65±1.54

Note. *a*) control; *b*) administration of pentagastrin (10 mg/kg) and carbacholine (6 mg/kg); asterisk signifies the reliability of differences as compared to the control ($p<0.05$).

intestine mucus GP did not change (Fig. 2). This probably means that all glycosylation transferases function more actively but in the same order as in basal secretion.

Simultaneous administration of pentagastrin and carbacholine induces a secretory response of the stomach similar to the response to appropriate food stimulation. Synchronization of the activity of mucous cells of the stomach, probably occurs, just as was shown for other secretory cells [3], resulting in an overall increase of GP release from secretory granules and in the activation of biosynthetic processes. As this take place, there are no marked changes in the makeup of structural GP, except for modulation of the partial composition of monosaccharides noted in the pyloric part and in the lesser curvature. Another mechanism of underlying the protective function of mucous GP is found in the intestine after stimulation of stomach secretion: the level of their release increases insignificantly, but the degree of glycosylating GP rises, especially in the initial parts of the duodenum. The characteristic features of oligosaccharide chain structure are thereby preserved. The formation of the SML in the stomach and intestine is realized by different cell elements. The findings attest that the reaction of the covering epithelium, glands of Brunner, and goblet cells is different for the administration of pentagastrin and carbacholine.

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