CONTENT OF SOME INTESTINAL PEPTIDASES IN THE MUCOUS COATING OF THE DOG'S SMALL INTESTINE

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UDC 612.332.84.612.332

KEY WORDS: small intestine; luminal digestion; content of intestinal peptidases.

The macroscopic layer of the mucous coating is known to perform several functions. The protective functions of the mucous coating have been widely discussed in the literature [4, 7]. It has been stated, for instance, that this layer protects the mucosa against the harmful action of some components of the intestinal contents (proteolytic enzymes, hydrochloric acid, etc.), interacts with bacteria, and possesses water-retaining and "lubricating" properties [8].

These protective functions of the layer are determined both by its chemical composition (the presence of mucopolysaccharide macromolecules), and by its continuity [9]. Transport of food substances, i.e., diffusion through the mucous coating, is thus evidently an essential stage which preceeds juxtamural contact digestion. Reports have recently appeared of the discovery of enzymes in the mucous coating [5, 12] and hydrolysis of food substrates in this layer has been postulated [1].

Previously the writers determined the content of trypsin and chymotrypsin in the mucous coating [2]. In the present investigation the aim was to assess the activity of some intestinal peptidases, namely leucine-aminopeptidase and cathepsins B and D, in the mucous coating of the small intestine of adult dogs.

EXPERIMENTAL METHOD

Experiments were carried out on normal unanesthetized mongrel dogs with a fistula into the duodenum. Samples of duodenal contents were withdrawn and biopsy specimens of the mucosa taken from dogs deprived of food for 18 h. The contents were freed from mechanical impurities by centrifugation for 30 min at 3000 rpm. Under the control of a binocular loupe, the mucous coating was separated from the mucosa without damage to the villi or structures of the glycocalyx [1]. Electron-microscopic investigations (I. A. Morozov) confirmed that separation of the mucous coating did not cause damage to the glycocalyx. The coating was weighed, transferred to a bottle containing 0.5 ml of a solution of electrolytes, isotonic and isoionic with the duodenal contents, and the pH of the solution was adjusted to be equal to the pH at which enzyme activity was determined. The mucous coating was then homogenized in a homogenizer with glass pestle for 3 min at 3000 rpm. Activity of leucine-aminopeptidase was determined by the method in [11] in the writers' modification. Into the cuvette of a Specord VV-Vis spectrophotometer were introduced 0.1 ml of 0.125 M $MgCl_2$ solution, 1.8 ml 0.5 M Tris-buffer, pH 8.5, 0.1 ml of 2.5 mM solution of the substrate (leucine paranitroanilide), and 0.5 ml of the test preparation. The reaction velocity was recorded by the increase in optical density at 405 nm. For selective inhibition of leucine-aminopeptidase activity, 0.025 ml of a 0.01 M solution of phenanthroline was added to the cuvette in a parallel series of determinations.

Cathepsin B activity was determined by the method in [10]. Iodoacetamide, added to the tube in a volume of 0.1 ml of a 0.001 M solution of this compound, was used as selective inhibitor of cathepsin B.

Cathepsin D activity was determined by the method in [6] in the writers' modification. A 6% solution of hemoglobin (Olaine Chemical Reagents Factory, purified beforehand on Sephadex G-25) was used as the substrate. Into a microtube were added 40 µl of 1 M formate buffer

Laboratory of Physiology and Biochemistry of Digestion, Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. G. Gazenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 10, pp. 387-389, October, 1984. Original article submitted November 2, 1983.

0007-4888/84/9810-1301$08.50

TABLE 1. Activity of Intestinal Peptidases in Mucous Coating and Duodenal Contents from Dogs (M ± m)

Enzyme	Substrate	Specific activity			
		mucous coating		duodenal contents	
		without inhibition	with selective inhibition	without inhibition	with selective inhibition
Leucine-aminopeptidase	Leucine paranitroanilide	0,206±0,035	0,067±0.011* (13)	0,009±0,002	Trace levels* (7)
Cathepsin B	Benzoylarginine β-naphthylamide	3,173±0,560	2,570±0,453† (12)	0,784±0,153	0,784±0,153 (9)
Cathepsin D	Hemoglobin	2,616±0,517	1,792±0,354† (11)	0,163±0,043	0,114±0,030† (13)

Legend. *Inhibitor phenanthroline, †inhibitor iodoacetamide, preliminary incubation at pH 8.5 and 4°C. Number of experiments given in parentheses.

(pH 3.0), 40 µl of hemoglobin solution, 20 µl of 0.45% solution of Trition X-100, and 80 µl of the test preparation. The samples were incubated for 30 min at 37°C, treated with 180 µl TCA, and centrifuged for 15 min at 3000 rpm. To 100 µl of supernatant 2 ml of K_2HPO_4 and 50 µl of a solution of fluorescamine were added. Fluorescence was measured at an excitation wavelength of 486 nm and absorption wavelength of 388 nm on a Hitachi MPF-2A spectrofluorometer. In parallel experiments pepsin activity was inhibited by the method in [3], by alkalification of the samples for analysis with 0.5 M Tris-buffer to pH 8.5, followed by incubation for 2 h at 4°C.

Enzyme activity was expressed in micromoles of hydrolysis products formed per minute per gram wet weight of mucous coating or per milliliter of duodenal juice for leucine-aminopeptidase, and in conventional units for the cathepsins.

EXPERIMENTAL RESULTS

Results of determination of the velocities of hydrolysis of the substrates used by homogenates of the mucous coating and by the duodenal contents are given in Table 1. The results showed that selective inhibition of leucine-aminopeptidase and of cathepsin B reduces hydrolysis of leucine paranitroanilide and of benzoylarginine naphthlyamide by homogenates of the mucous coating by 70 and 20% on average respectively. The velocity of hydrolysis of these same substrates by the duodenal contents was so low that it could not be reliably determined by the methods used.

Pepsin, passing from the stomach into the lumen of the duodenum, may evidently be partly adsorbed into the mucous coating. The possibility therefore cannot be ruled out that under the conditions of determination used in this investigation, proteolysis of hemoglobin was effected by both cathepsin D and pepsin. Alkaline denaturation of pepsin, unlike cathepsin D, is known to be largely irreversible [3] (preliminary incubation of the pepsin preparation for 2 h at pH 8.5 and 4°C reduced the activity of pepsin by 30 times). We made use of this fact to determine cathepsin D and pepsin separately. Hydrolysis of hemoglobin in the mucous coating under the conditions of determination is attributable by 70% to the action of cathepsin D and by 30% to that of pepsin. The same ratio of 70 to 30% is also characteristic of the duodenal contents. Thus leucine-aminopeptidase and cathepsins B and D, besides trypsin and chymotrypsin [2], were reliably identified in the juxtamural mucous coating of the adult dog duodenum.

The diffusion resitance of the juxtamural mucous coating for high-molecular-weight compounds is very high [8], and it therefore seems very probable that proteins and relatively large oligopeptides will undergo hydrolysis as they diffuse into the layer. Since the pH gradient of the mucous coating in the duodenum lies between limits of pH 6.0-7.0 hydrolysis of this kind may be effected by trypsin, chymotrypsin, and leucine-aminopeptidase. The arrival of proteolytic enzymes both of intrinsic intestinal origin and of pancreatic origin, and also of pepsin in the juxtamural layer of the mucous coating evidently takes place during its formation.

Since the mucous coating in fact fills the space between the villi and covers their apical part, epitheliocytes and separate components, especially the enzymes leucine-aminopeptidase and cathepsins B and D, may enter it from the mucosa. Meanwhile the mucous coating is bounded by the intraluminal medium of the intestine, and parts of the enteric contents,

including enzymes such as trypsin, chymotrypsin, and pepsin, must be adsorbed onto its surface and incorporated into its gel. In turn, the mucous coating, if detached into the intraluminal medium, constitutes a supplier of the solid part of the intestinal juice.

The authors are grateful to L. D. Rumsh, Senior Scientifc Assistant in the Laboratory of Enzyme Chemistry, Institute of Bioorganic Chemistry, and to Yu. M. Gal'perin, Head of the Laboratory of Experimental Pathology, N. V. Sklifosovskii Moscow Emergency Aid Research Institute, for discussing the results of this investigation.

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EFFECT OF SUBSTANCE P ON MONOAMINE-CONTAINING TASTE BUD CELLS

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UDC 612.87.015.2:547.233].014.46:615.357:577.175.82

KEY WORDS: Substance P; serotonin; taste buds; fluorescence microscopy.

The presence of nerve fibers, immunoreactive toward substance P, has recently been demonstrated in taste buds of the tongue of some species of mammals by indirect immunohistochemical methods [3-5]. These fibers, running through the basement membrane of the papilla, form a dense perigemmal plexus immediately beneath the epithelium. It has been shown that some immunoreactive fibers running from this plexus penetrate into the taste buds and spread there as far as the gustatory pore. The distribution of substance P in the taste buds may indicate a connection and possible interaction of this peptide with the population of serotonin-containing cells located here, as is the case, for example, in certain structures of the CNS [6].

To confirm this hypothesis, a fluorescence-histochemical study was made of the effect of exogenous substance P on serotonin-containing cells of frog taste buds. The frog was chosen as test object because of the possibility of obtaining total stretch preparations from the layer of gustatory epithelium, so that whole populations of monoamine-containing cells can be studied.

EXPERIMENTAL METHOD

Experiments were carried out on frogs *(Rana temporaria)* kept under standard laboratory conditions at 10°C. In the experiments of series I synthetic substance P (from Serva, West Germany) was injected intraperitoneally into normal animals in a dose of 6-7 μg. The peptide was made up in Ringer's solution for cold-blooded animals immediately before injection. The

Laboratory of Physiology of Reception, P. K. Anokhin Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 10, pp. 389-390, October, 1984. Original article submitted January 5, 1984.

