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LOCALIZATION OF HISTAMINASE (DIAMINE OXIDASE) IN RAT SMALL INTESTINAL MUCOSA: SITE OF RELEASE BY HEPARIN

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Abstract—Previous studies have suggested that in the rat, small intestine is the source for rise in plasma histaminase levels seen after heparin administration. The cellular location of histaminase in intestine and the mechanism of heparin release have not been previously investigated. The present study identifies intestinal villus cells rather than crypt cells as the location of intestinal histaminase; at this site, the enzyme is not associated with brush border. Heparin added to incubations containing isolated intestinal cells did not release histaminase into the medium. Perfusion of intestinal vasculature with heparin caused a prompt release of this enzyme into venous effluent. The present investigation, therefore, suggests that heparin releases histaminase from vascular binding sites rather than directly from parenchymal cells. The use of isolated intestine with perfusion of the vasculature could serve as a useful tool for further defining the relationship between the sites of synthesis and the binding sites involved with heparin releasable enzymes such as histaminase.

In humans and many other animal species heparin administration releases a variety of enzymes into the plasma. These include lipases, such as lipoprotein lipase [1, 2] (glycerol-ester hydrolase EC 3.1.1.3), monoglyceride hydrolase [2] and phospholipase A (EC 3.1.1.4) [3, 4]. Although the mechanisms for heparin release of these enzymes are not precisely known, the suggestion that heparin displaces lipoprotein lipase from blood vessels [5] was strengthened by a recent study which demonstrated a binding site for lipoprotein lipase at the capillary wall [6].

Heparin also induces a rise in plasma activity of a non-lipid metabolizing enzyme, histaminase (diamine oxidase EC 1.4.3.6) in humans, guinea pig, rats, rabbit and other vertebrate species [7–11]. In humans, kidney and intestine contain appreciable histaminase activity [12] and are likely sources of the enzyme. The major site for the heparin release of this enzyme is probably the liver in guinea pig [13] and the intestine in rats and rabbits [14, 15]. Extirpation of the intestine or occlusion of mesenteric arteries drastically reduces the histaminase response to heparin in rats and rabbits [14–16] and the intestinal histaminase activity in these animals falls considerably after large doses of heparin [14, 15].

Histaminase readily diffuses from everted rat intestinal sacs into the incubating medium; but heparin does not enhance this process [17]. Although some limited data suggest that heparin releases histaminase from vascular binding sites [13, 18] rather than from parenchymal cells there is no direct evidence to confirm this hypothesis. Moreover, prior studies have not defined the precise cellular location of histaminase in the rat intestine or the exact source of the histaminase released by heparin from the intestinal tract. The present report describes the cellular location of histaminase in rat intestinal mucosa; the effect of heparin on release of histaminase directly from mucosal cells and from the intestinal vasculature is investigated.

MATERIALS AND METHODS

The lithium salt of heparin, p-nitrophenol, *p*-nitrophenyl phosphate, sucrose-grade I, glucose oxidase, dianisidine, and dithiothreitol were purchased from Sigma; horseradish peroxidase, grade D, from Worthington Chemicals; and the tetrasodium salt of ethylene diamine tetracetic acid (EDTA) from J. T. Baker Chemicals. β-[^{3}H]histamine (1.4 Ci per mmole), a gift from Dr. Michael Beaven (National Heart and Lung Institute, Bethesda, Maryland) was prepared as previously described [19]. Dowex, AG-50 W-X2, 200–400 mesh, was purchased from BioRad and Instabray scintillation fluid from Yorktown Research.

Male Sprague-Dawley albino rats, 200–300 g, were used for all experiments; the animals were anesthetized by intraperitoneal injection of pentobarbital sodium.

Intestinal cell isolation. Isolated intestinal epithelial cells were prepared from rat intestine according to the method of Stern [20] as modified by Weiser [21]. After the entire small intestine was removed and thoroughly rinsed with normal saline containing 1 mM dithiothreitol, the intestinal lumen was filled for 15 min with buffer containing NaCl, 96 mM; KCl, 1.5 mM; sodium citrate, 27 mM; Na_2HPO_4, 5.6 mM and KH_2PO_4, 8 mM, pH 7.3. This solution was discarded and the intestine was filled with phosphate buffered saline (NaCl, 154 mM; KCl, 2.168 mM; Na_2HPO_4, 8.1 mM; KH_2PO_4, 1.47 mM; 1.5 mM EDTA; and 0.5 mM dithiothreitol, pH 7.3) for various periods of time to allow the sequential isolation of nine fractions of intestinal cells. During the entire procedure the intestine was kept at 37° with saline soaked compresses and over manipulation of the gut

was carefully avoided. Isolated cell fractions were washed twice with the phosphate buffered saline and then resuspended in this buffer. The cells were counted in a hemocytometer.

Cell incubation. The upper villus, middle villus and crypt cells were incubated separately in phosphate buffered saline with and without heparin. After 30 min of incubation the medium was separated from the cells by centrifugation and assayed for histaminase activity. In other experiments each cell type (2.8 to 3.3 × 10^6 cells per flask) was incubated separately in 2 ml of phosphate buffered saline at 4°. After 24 hr the medium was separated from the cells and assayed for histaminase activity as described previously.

Enzyme assay methods. Histaminase (diamine oxidase) activity was measured by the method of Beaven and Jacobsen [19] as later modified [22]. The procedures were performed exactly as we previously reported [23] and results were expressed as units of histaminase activity per ml, where 1 unit equals 1 pmole of β-[^{3}H]histamine deaminated per hr. (The term histaminase is used in this paper since histamine was always used as the substrate.)

Sucrase was determined by method of Dahlqvist [24]. Alkaline phosphatase was measured as described by Weiser [21] with *p*-nitrophenyl phosphate as a substrate.

Perfusion experiments. In anesthetized rats the abdomen was opened by a midline incision. A 3 to 5 mm incision was made in the abdominal aorta after placing two 3/0 silk ligatures immediately above and below the origin of the superior mesenteric artery. Through the aortic incision, a 12 cm length of polyethylene tubing (internal diameter 3.75 mm) was introduced into the superior mesenteric artery for about 1 cm, and was held in place with a silk tie. The superior mesenteric vein was similarly cannulated with polyethylene tubing and was tied off above the site of cannulation. The duodenal vein and artery, and the inferior mesenteric artery and vein were also tied off. The superior mesenteric artery was perfused using a flexible Volutrole bag (100 ml capacity) and a plastic tubing with an adjustable clamp, with phosphate buffered saline at a pressure of 80 cm water at a rate of 0.3 ml/min/200 g wt of the rat. The effects of heparin were assessed in paired experiments using litter mates, one of which served as a control. Control animals were infused with phosphate buffered saline for 37.5 min, while the litter mate was perfused with phosphate buffered saline for the first 7.5 min and then for 30 min with phosphate buffered saline containing heparin, 2000 U/ml. The effluent from the superior mesenteric vein was collected in ice cooled test tubes at timed intervals. The samples were kept at 4° and analyzed within 24 hr for histaminase activity. During the entire experiment the intestine was kept at 37° with saline-soaked cotton gauze compresses.

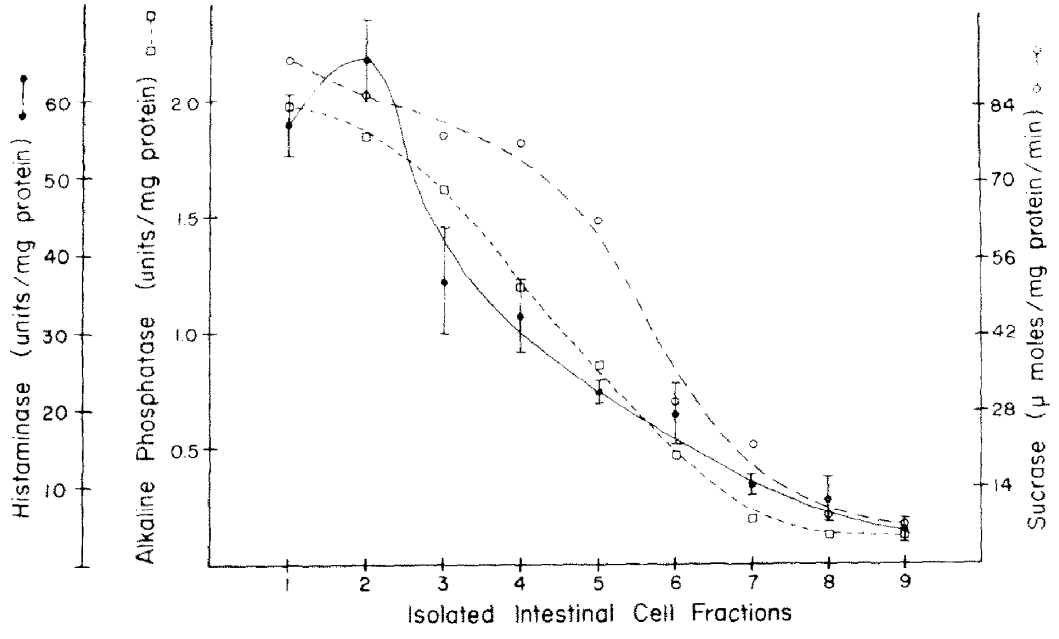

Fig. 1. Distribution of histaminase activity in rat small intestinal epithelial cells. The intestinal cells, prepared as described in Methods, were disrupted with a Sonifier Cell Disruptor (Model W 185D Heat Systems, Ultrasonics, Inc., Plain View, NY) for 0.5 min at an output control of 3. Completeness of cell disruption was evaluated by microscopic examination of the suspension. The disrupted cell suspension was centrifuged at 1500 *g* for 10 min at 4° in an International Clinical Centrifuge. The clear supernatant was used to assay the activities of sucrase, alkaline phosphatase and histaminase; protein concentration was determined by the Lowry method [26]. Each point represents the mean of 3 experiments done in duplicate. The values for S.E.M. for sucrase in upper villus cell fractions (Fraction 1) were ±11.9, middle villus (Fraction 5) ± 4.08 and crypt cells (Fraction 9) ±0.9; for alkaline phosphatase the S.E.M. in Fraction 1 was ±0.27, in Fraction 5, ±0.14 and in Fraction 9 ±0.05. Histaminase activity is expressed in the Figure as the mean ±S.E.M.

Table 1. Histaminase purified intestinal brush border*

Preparation	Alkaline phosphatase			Histaminase		
	Mean† units/mg protein	Range	Fold purification	Mean† units/mg cell protein	Range	Fold purification
Homogenate	0.24	0.20–0.29	1	82.4	71.7–97.9	1
Crude brush border	1.52	1.27–1.92	6.3	64.2	59.2–82.3	0.8
Purified brush border	2.45	1.63–3.81	10.2	44.0	40.0–46.0	0.5

* Rat intestinal brush border was prepared as described by Forstner *et al.* [25]. Intestinal mucosa was homogenized at slow speed in a Waring Blender for 25 sec (Homogenate). Crude brush border was prepared by centrifugation of the homogenate followed by three washes with cold EDTA buffer. Crude brush border was further purified by saline sedimentation and filtration through glass wool (Purified brush border). Protein determinations [26] and enzyme assays were done in duplicate after disrupting each preparation for 0.5 min in a Sonifier Cell Disruptor at an output control of 3 (Model W 185D, Ultrasonics Inc. Plain View, NY).

† The values represent the mean of 3 separate experiments done in duplicate.

RESULTS

Histaminase activity in the small intestinal mucosa showed a distinct gradient from the upper villus to the crypt region (Fig. 1); crypt cells contained only 7.3 per cent of the histaminase found in upper villus cells. This distribution for histaminase paralleled that for sucrase and alkaline phosphatase although there was a steeper fall in histaminase activity between the upper and middle villus cells. The low histaminase activity in the crypt cells did not result from leakage of this enzyme into the buffers and cell washes during the cell isolation procedure; only 4 per cent of the total activity in all the cells appeared in the pooled wash prior to sonication of the cells. Histaminase was found to be stable for 24 hr when kept at 4° and at the end of 24 hr there was only 3–4 per cent loss in the activity of this enzyme in each cell fraction. When the isolated intestinal cells were incubated at 4° in buffer the enzyme leakage was only 19.5 per cent for upper villus cells and 9.4 per cent and 10.5 per cent for middle villus and crypt cells respectively.

The similarity in cell distribution between histaminase and alkaline phosphatase and sucrase, two upper villus enzymes localized to the brush border [25] raised the possibility that histaminase might also be a brush border enzyme. However, the specific activity of histaminase in purified brush borders (Table 1) fell to 51.3 per cent of the value for the initial whole cell homogenates, while the specific activity of alkaline phosphatase was 10-fold higher in the brush borders than in the initial homogenates.

We investigated whether heparin added into the incubation medium can directly release histaminase from the intestinal epithelial cells (Table 2); no differences were seen in the release of histaminase into the media with and without heparin.

The effects of heparin infusion on histaminase release in the rat intestinal vascular system is shown in Fig. 2. In control animals, during infusion of buffer into the superior mesenteric artery the venous effluent contained 0.6 ± 0.1 units/ml (mean ± S.E.M.) over the entire 37.5 min perfusion period. A similar level was seen in test animals during the first 7.5 min of perfusion with buffer alone, (0.5 ± 0.2 units/ml); after heparin infusion was begun there was a rapid rise in histaminase activity in the venous effluent, which could be easily detected within 2.5 to 5 min of starting the heparin. Mean histaminase activity remained elevated throughout heparin infusion as compared with controls (10.6 ± 1.9 units/ml—$P > 0.005$). As seen from the values for the S.E.M. in Fig. 2 there was considerable variation in the maximum increase in histaminase levels achieved with heparin in the 3 experiments; also, there was considerable variation in the pattern of histaminase release between experiments. In one experiment histaminase levels increased steadily over the 30 min of infusion. In the other 2 experiments peak histaminase levels were reached after 2.5 to 7.5 min of heparin infusion; histaminase

Table 2. Effect of heparin on histaminase release from isolated intestinal epithelial cells*

Cell type	Histaminase release†		Histaminase content of cells†
	Without heparin units/ml	With heparin	
Upper villus	5.7 ± 0.3	6.6 ± 0.4	131.0 ± 24.7
Middle villus	3.6 ± 0.2	3.7 ± 0.2	44.0 ± 8.3
Crypt	1.4 ± 0.2	1.4 ± 0.6	10.5 ± 4.0

* Upper villus, middle villus or crypt cells (2.8 to 3.3 × 10^6 cells in 2 ml of phosphate buffered saline) were incubated separately with or without heparin (2000 units/ml) in stoppered plastic flasks at 37° in a water bath shaker at 125 oscillations/min. After 30 min the samples were transferred to ice-cooled plastic centrifuge tubes and were centrifuged at 900 *g* for 10 min at 4°. The supernatant fluid, carefully removed with a Pasteur pipette, was used for histaminase assay. Histaminase activity was also determined after disrupting the intestinal cells. Each value represents the mean ± S.E.M. of 3 separate experiments done in duplicate.

† Histaminase release and cellular content of histaminase are calculated for 3 × 10^6 cells.

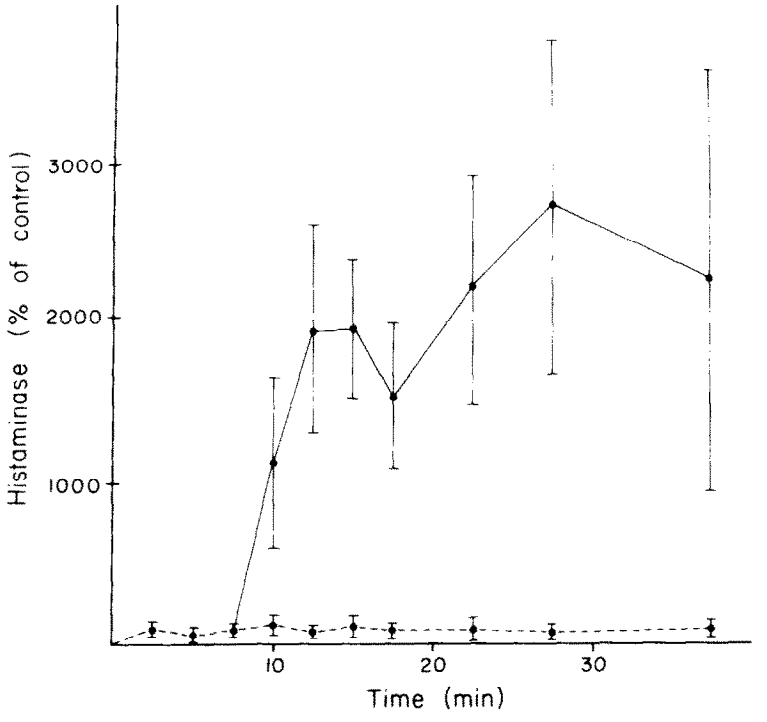

Fig. 2. Effect of heparin infusion on histaminase release into the rat intestinal vascular system. Phosphate buffered saline with or without heparin was infused as described in Methods. A total of 6 experiments was done, 3 controls and 3 with heparin infusion. The post-heparin histaminase levels (mean ± S.E.M.) are expressed as percentage of control at each time period and the control histaminase levels (mean ± S.E.M.) between 7.5 and 37.5 min are calculated as percentage of the mean histaminase levels during the first 7.5 min control period.

levels then declined gradually over the next 7.5 to 25 min. In all studies, the histaminase levels were still 2–3-fold higher than the control at the end of the infusion period.

DISCUSSION

Although the presence of high amounts of histaminase in the rat small intestine has long been recognized, to our knowledge the cellular location of this enzyme has not been studied. The present investigation shows that histaminase is located in the mature, well differentiated cells of the villus region, rather than with the proliferating cells of the crypt area. This observation is of considerable interest for two reasons. Firstly, the synthesis of putrescine, a polyamine which is an excellent substrate for histaminase, has been associated with rapidly growing cells [27] such as those found in the crypt region. Investigations of the sites of polyamine synthesis in rat intestinal mucosa and comparison to the present data could perhaps provide information regarding the role of histaminase in polyamine metabolism and of polyamines in cell growth and differentiation. Secondly, unlike the other enzymes located in the villus tip region of intestinal mucosa [25], histaminase activity does not appear to be confined to the brush border. This finding suggests that histaminase may play a role other than that of providing a barrier against absorption of bacterial amines from the gut lumen as has been previously suggested [28].

In most species studied, including rat, the release of histaminase by heparin is thought to occur from the intestine. The present study shows that this release is probably not due to a direct action of heparin on the intestinal cells. The small fraction of histaminase activity which leaked from isolated cells into the incubating medium was not increased when heparin was added to the incubates. With heparin infusion into the superior mesenteric artery there was a marked increase in the histaminase activity in the superior mesenteric venous effluent. In these experiments the histaminase levels in the venous effluent from the superior mesenteric vein was found to be considerably lower than the plasma levels of histaminase seen after heparin administration in rats [17]. The use of isolated intestinal vascular system perfused with phosphate buffered saline rather than whole blood as occurs *in vivo*, could explain these observed differences. The present data are consistent with earlier observations by Schmutzler *et al.* [18] who demonstrated that addition of heparin to guinea pig liver tissue slices did not promote histaminase release into the incubation medium. However, perfusion of the liver with heparin solution produced a considerable release of histaminase activity into the venous effluent [13]. Shaff and Beaven showed that the addition of heparin to incubates of everted rat intestinal sacs did not enhance release of histaminase into the incubation medium [17]. These earlier findings coupled with the present observations, strongly suggest that heparin releases histaminase from binding sites in the rat intestinal vasculature. The location of intestinal

mucosal histaminase activity intracellularly rather than in the brush border, is consistent with the movement of the enzyme from the mucosal cell to vascular binding sites.

Although the present data suggest that histaminase is released from the intestinal vasculature rather than directly from the synthetic sites within the intestinal cells, the mechanism of transport of this enzyme from the epithelial cells to the vascular binding sites remains to be investigated. Our present findings suggest that future investigations of rat intestinal mucosal cells and intestinal vasculature could provide a useful model for defining the nature of such a transport system and of the binding sites involved with heparin releasable enzymes.

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