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(Na^+-K^+)-STIMULATED ADENOSINETRIPHOSPHATASE IN ISOLATED INTESTINAL VILLUS TIP AND CRYPT CELLS*

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SUMMARY

The migration of intestinal epithelial cells from the crypt area to the villus tip is associated with progressive differentiation of these cells. The distribution of (Na^+-K^+)stimulated adenosinetriphosphatase ((Na^+-K^+)-ATPase; EC 3.6.1.3) along the intestinal villus may have functional as well as developmental implications. To define this distribution, rat jejunal and ileal segments were incubated in vitro with a citrate solution that dissociates epithelial cells sequentially from villus tip to crypt area. ATPase activity in cell collections from villus tips and crypt areas were compared. The specific activity of (Na^+-K^+)-ATPase was higher in the villus tip than in the crypt cells of both jejunum and ileum. Crypt cell (Na^+-K^+)-ATPase activity in the jejunum and ileum were similar. Thus, (Na^+-K^+)-ATPase activity of villus tip cells in the jejunum was greater than in the ileum. There was no difference in villus tip and crypt cell Mg^{2+}-ATPase activity in either jejunum or ileum. The steep gradient for (Na^+-K^+)-ATPase along the intestinal villus may signify an important difference in Na^+ transport between the villus tip and crypt area. The higher level of (Na^+-K^+)-ATPase activity in the jejunal villi is consistent with the more important role of the jejunum in Na^+ and substrate-linked Na^+ transport.

INTRODUCTION

The migration of intestinal epithelial cells from the crypt area to the villus tip is associated with two related processes: (1) progressive differentiation of these cells and (2) functional and behavioral alterations. The distribution of several enzymes along the intestinal villus have been reported [1–5]. Although these reports have also emphasized differences in metabolic activities between the villus tip and crypt area, none have suggested a villus tip–crypt difference in ion transport.

* In conducting these studies, the principles of the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences – National Research Council were observed.

The enyzme (Na^+–K^+)-stimulated adenosinetriphosphatase ((Na^+–K^+)-ATPase; EC 3.6.1.3) has been closely linked to Na^+ transport in many tissues [6]. In intestinal mucosa, (Na^+–K^+)-ATPase activity is influenced by diet [7] and perhaps by cholera toxin [8]. The addition of ouabain to the serosal surface of rabbit ileum in vitro, in concentrations known to inhibit (Na^+–K^+)-ATPase ($5 \cdot 10^{-4}$ M), markedly inhibits net Na^+ transport across the mucosa [9]. Thus, a role for this enzyme in intestinal Na^+ transport is likely. In this study, a gradient of (Na^+–K^+)-ATPase activity was found at two anatomical levels of the small intestine: significant enzyme differences were found between jejunal and ileal villus tip cells, and between the villus tip and crypt cells of both jejunal and ileal villi.

METHODS

Normal male albino Walter Reed rats weighing 250–450 g were maintained on a standard diet with free access to water. Under diethyl ether anesthesia the small intestine was removed and sectioned midway between the ligament of Treitz and the ileocolic junction. Epithelial cells were collected from the proximal (jejunum) and distal segment (ileum) by the method of Weiser [1] with minor modifications. Each segment was incubated in a solution containing 27 mM sodium citrate, 96 mM NaCl, 1.5 mM KCl, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4 (pH 7.3) at 37 °C for 15 min. Succeeding incubation periods with a solution containing 130 mM NaCl, 5 mM Na_2EDTA and 30 mM imidazole (pH 7.6, Solution A) separated cells originating from the villus tips in early collections and crypt areas in late collections. Villus tip and crypt cells were washed and centrifuged at $1000 \times g$ for 10 min at 0 °C, and aliquots of each were assayed for alkaline phosphatase (EC 3.1.3.1), thymidine kinase (EC 2.7.1.75) and ATPase activities. Alkaline phosphatase was assayed by the method of Weiser [1], thymidine kinase by the method of Breitman [10] and protein by the method of Lowry et al. [11], using standards of bovine albumin.

Isolated cells assayed for ATPase activity were homogenized 20/1 (v/w) in Solution A containing 2.4 mM sodium deoxycholate with a chilled glass homogenizer and teflon pestle. The homogenate was centrifuged at $770 \times g$ for 10 min at 0 °C. The supernatant was filtered through a double layer of gauze, centrifuged at $10\,000 \times g$ for 10 min at 0 °C, and the pellet resuspended in Solution A. Duplicate samples of the $10\,000 \times g$ pellet were incubated in a medium containing 100 mM NaCl, 20 mM KCl, 10 mM imidazole, 5.6 mM $MgCl_2$ and 5.6 mM ATP (disodium salt; Sigma Chemical Co., Grade II) (pH 7.6) and in a similar medium in which KCl was omitted and the NaCl concentration was 120 mM. The final incubation volume of 5 ml contained 5–30 μg/ml protein. The reaction was begun by addition of $MgCl_2$ and ATP, carried out at 37 °C for 15 min in a shaking water-bath, and terminated by the addition of 1 ml of ice-cold 35 % (w/v) trichloroacetic acid. The samples were centrifuged and the inorganic phosphate liberated was measured by the method of Fiske and SubbaRow [12] and corrected for the non-enzymatic hydrolysis of ATP. Absorbance was read at 660 nm in a Gilford spectrophotometer. (Na^+–K^+)-ATPase activity was defined as the difference between the inorganic phosphate released in the presence and in the absence of K^+ in the incubation mixture. Mg^{2+}-ATPase activity was defined as the inorganic phosphate released in the absence of K^+ in the incubation mixture. Omission of K^+ or addition of ouabain have equivalent effects upon ATPase

in this system as established in our laboratory and in others [13–17].

The specific activities of $(Na^+–K^+)$-ATPase, Mg^{2+}-ATPase, alkaline phosphatase and thymidine kinase in the villus tip and crypt cells of each intestinal segment in each rat were compared, as were villus tip cells (or crypt cells) from adjoining segments. The significance of each comparison was determined using Student's "*t*" test for paired data.

RESULTS

In preliminary experiments, the distribution of $(Na^+–K^+)$-ATPase and Mg^{2+}-ATPase activity among the fractions obtained by centrifugation was determined by measuring both the total (μmoles P_i/h) and specific activity (μmoles P_i/mg protein per h) of each fraction. There was no difference in the $(Na^+–K^+)$-ATPase (Table I)

TABLE I

DISTRIBUTION OF $(Na^+–K^+)$-ATPase ACTIVITY IN ISOLATED INTESTINAL EPITHELIAL CELLS

The percent total activity represents the part of the $(Na^+–K^+)$-ATPase activity (μmoles P_i/h) measured in the whole homogenate, present in each fraction. The relative specific activity compares the specific activity of each fraction (μmoles P_i/mg protein per h) with that of the whole homogenate. All values are the mean of 3–5 experiments.

Fraction	Jejunum				Ileum			
	% total activity		Relative spec. act.		% total activity		Relative spec. act.	
	Villus tip	Crypt	Villus tip	Crypt	Villus tip	Crypt	Villus tip	Crypt
Whole homogenate	100	100	1.0	1.0	100	100	1.0	1.0
770 × *g* pellet	9	16	1.2	1.4	15	16	1.5	1.6
10 000 × *g* pellet	37	37	3.2	3.6	44	45	6.4	4.0
10 000 × *g* supernatant	49	39	0.6	0.6	35	31	0.5	0.5
Recovery of total activity	95	92			94	92		

or Mg^{2+}-ATPase distribution among these fractions between villus tip and crypt cells. Since the 10 000 × *g* pellet contained the highest specific activity, the ATPase activity of this fraction was considered exclusively thereafter.

Since alkaline phosphatase and thymidine kinase are principally present in cells of the villus tip and crypt areas, (refs 1 and 3, respectively), assay of these enzymes documented the site of cell origin in successive cell collections. The specific activities of these enzymes, presented in Table II, demonstrate marked differences between the isolated villus tip and crypt cell collections and between jejunal and ileal segments. These findings have been reported before [1, 3, 5, 18, 19].

The specific activities of $(Na^+–K^+)$-ATPase and Mg^{2+}-ATPase in villus tip and crypt cells of the jejunum and ileum are given in Table III. $(Na^+–K^+$-)ATPase activity was significantly higher in the villus tip than in the crypt cells of both jejunum and ileum. Villus tip cell $(Na^+–K^+)$-ATPase activity was 3-fold greater than the crypt

TABLE II

ALKALINE PHOSPHATASE AND THYMIDINE KINASE ACTIVITIES IN ISOLATED VILLUS TIP AND CRYPT CELLS OF RAT JEJUNUM AND ILEUM

The specific activities of alkaline phosphatase (μmoles *p*-nitrophenol/mg protein per min) and thymidine kinase (10^{-7} μmoles thymidine phosphate/mg protein per min) of villus tip cells were compared to crypt cells obtained from the same segment of intestine. The mean of these comparisons for each enzyme is expressed as the mean difference. All values are mean ± S.E. (number of separate animals).

	Alkaline phosphatase		Thymidine kinase	
	Villus tip	Crypt	Villus tip	Crypt
Jejunum	7.4 ± 1.0 (4)	0.8 ± 0.2 (4)	5.9 ± 1.0 (4)	63.4 ± 19.4 (4)
Mean difference	6.6 ± 0.8		57.5 ± 19.4	
P	< 0.0025		< 0.05	
Ileum	1.3 ± 0.3 (4)	0.2 ± 0.1 (4)	13.7 ± 3.7 (4)	109.0 ± 11.8 (4)
Mean difference	1.1 ± 0.4		95.3 ± 9.9	
P	< 0.05		< 0.0025	

TABLE III

ATPase ACTIVITY IN ISOLATED VILLUS TIP AND CRYPT CELLS OF RAT JEJUNUM AND ILEUM

The specific activities of (Na^+–K^+)-ATPase (μmoles P_i/mg protein per h) and Mg^{2+}-ATPase (μmoles P_i/mg protein per h) of villus tip cells were compared to crypt cells obtained from the same segment of intestine. The mean of these comparisons for each enzyme is expressed as the mean difference. All values are mean ± S.E. (number of separate animals). N.S., not significant.

	(Na^+–K^+)-ATPase		Mg^{2+}-ATPase	
	Villus tip	Crypt	Villus tip	Crypt
Jejunum	86 ± 11 (7)	26 ± 4 (7)	81 ± 10 (6)	89 ± 10 (6)
Mean difference	60 ± 12		8.0 ± 8	
P	< 0.005		N.S.	
Ileum	52 ± 4 (9)	31 ± 3 (9)	75 ± 7 (8)	92 ± 10 (8)
Mean difference	20 ± 3		18 ± 8	
P	< 0.001		N.S.	

cell level in the jejunum and approximately 2-fold greater in the ileum. Crypt cell (Na^+–K^+)-ATPase activity in the jejunum and ileum were similar. Thus, (Na^+–K^+)-ATPase activity of villus tip cells in the jejunum was significantly greater than in the ileum (86 ± 11 μmoles P_i/mg protein per h (7) versus 50 ± 5 μmoles P_i/mg protein per h (7), $P < 0.05$). There was no difference in villus tip and crypt cell Mg^{2+}-ATPase activity in either jejunum or ileum. In addition, no difference in Mg^{2+}-ATPase was found when jejunal and ileal segments were compared.

DISCUSSION

More than 85 % of the (Na^+–K^+)-ATPase activity measured in rat intestinal

epithelial cells is located in a plasma membrane fraction devoid of brush border, nuclei and mitochondria [17]. This polar distribution of (Na^+-K^+)-ATPase along the basal–lateral border of the intestinal epithelial cell, however, is but one level of (Na^+-K^+)-ATPase organization within the small intestine. This study demonstrates a gradient of (Na^+-K^+)-ATPase activity within the intestinal villus, with increasing activity from crypt area to villus tip. A gradient of (Na^+-K^+)-ATPase activity has also been described along the small intestine, with gradually decreasing activity from jejunum to ileum [15]. Our study suggests that this jejunal–ileal gradient, determined in whole scraped mucosa, is a reflection of the difference in the (Na^+-K^+)-ATPase activity of the villus tip cells.

In the kidney, (Na^+-K^+)-ATPase is distributed assymetrically along the tubule, and areas of high (Na^+-K^+)-ATPase activity identify specific Na^+ transport sites [20]. Similarly, the steep gradient for (Na^+-K^+)-ATPase along the intestinal villus may signify important differences in Na^+ transport between the villus tip and and the crypt area. Indeed, this has been proposed by various investigators [21, 22]. The (Na^+-K^+)-ATPase activity in villus tip or crypt cells, then, should reflect the level of ATPase-linked Na^+ transport at this site. Of interest in this regard is the observation in the rat that Na^+ and water absorption is 60 % greater per unit length of jejunum than ileum [23]. This corresponds with the 60 % higher level of (Na^+-K^+)-ATPase activity in the jejunum than in the ileum found in our study. The difference in (Na^+-K^+)-ATPase activity between villus tip and crypt cells may also be important when there is a proportional change in these cell populations. Such a change might be related to the alteration in Na^+ and water transport that accompanies compensatory small intestinal hypertrophy following resection [24, 25] and the many diarrheal disorders characterized by shortened villi and crypt cell hyperplasia [21].

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