Regional Distribution of Sodium–Potassium–Activated Adenosine Triphosphatase in the Rat Gastrointestinal Tract

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Several solutes are preferentially absorbed by specific segments of the gastrointestinal tract(1). This variation in the distribution of sites for particular absorptive mechanisms suggests that the enzymes related to these processes may also be asymmetrically distributed. The observation that ouabain, an inhibitor of sodium–potassium-activated adenosine triphosphatase (Na–K–ATPase) in vitro, inhibits intestinal transport systems(2,3) suggests that this enzyme may be involved in the active absorption of sodium by the gut. The present study was designed to test the hypothesis that the regional distribution of Na–K–ATPase in the gut will correlate with physiological information on the sites of preferential absorption of sodium and water.

METHODS

Eight female Sprague–Dawley rats weighing 200–230 g were fed a standard pellet diet. Each animal was killed by concussion, and the gut was removed without its mesentery from the ileocecal junction to the ligament of Treitz. The gut was washed with saline and divided into proximal, midgut, and distal segments of equal length. The segments were opened and the mucosa was scraped gently from the seromuscular coat into an ice-cold Petri dish. The activity of Na–K–ATPase was determined by the method of Katz and Epstein(4) with minor modifications. The mucosal scraping was weighed and homogenized in a 20:1 (v/w) ratio in a solution containing 0.25 mole sucrose, 5 mmoles Na₂EDTA, 30 mmoles histidine buffer/liter, and 0.1% sodium deoxycholate at pH 6.8. Duplicate aliquots of 0.05 ml of tissue suspension were used for the assays. Total ATPase activity was determined in 5 ml of a reaction mixture prewarmed at
37° for 5 min containing 100 mmoles NaCl, 20 mmoles KCl, 10 mmoles imidazole buffer, 5 mmoles MgCl₂, and 5 mmoles ATP/liter at pH 7.8. The reaction was carried out for 15 min in a shaking water bath at 37° and terminated by the addition of 1 ml ice-cold 35% (w/v) trichloroacetic acid. The precipitated protein was discarded after centrifugation, and the inorganic phosphate was determined in duplicate by the method of Fiske and Subbarow(5). The protein content of the tissue suspensions was determined in duplicate by the method of Lowry and coworkers(6) and was in the range of 7–9 mg/ml. ATPase activity is expressed in micromoles of inorganic phosphate liberated per milligram of protein per hour (µmoles P₁/mg/hr). The Na–K–activated portion of the Total ATPase is defined as the difference between the inorganic phosphate liberated in the presence and in the absence of potassium. Magnesium-dependent ATPase (Mg–ATPase) is the ATPase activity measured when potassium is absent from the reaction mixture.

RESULTS

The mean activities of Na–K–ATPase in the jejunal and midgut segments were increased 42% and 52%, respectively, over that of the ileal segment. The mean activities in µmoles P₁/mg/hr (± SEM) for jejunum, midgut, and ileum were 8.75 ± 1.30, 9.36 ± 0.81, and 6.16 ± 1.24, respectively (Fig. 1). The difference in midgut and ileal levels was statistically significant (p < .05), while the difference between ileum and jejunum was of borderline statistical significance. No difference was observed in the levels of Mg–ATPase, which were 16.15 ± 3.11, 16.05 ± 2.58, and 15.95 ± 2.43 for jejunum, midgut, and ileum, respectively.

DISCUSSION

Several investigators have studied the absorptive capacities of different intestinal segments of the rat. Clarkson and Rothstein(7) found that maximum sodium

![Fig. 1. Regional distribution of Na–K–ATPase activity in the gastrointestinal tract in micromoles of inorganic phosphate liberated per milligram of tissue protein per hour (± SEM).]
and water transport occurred in everted sacs made from the second quarter of the intestine. The lowest rate of transport occurred in sacs made from the most distal quarter. Similarly, Barry and coworkers (8) found that in the presence of glucose maximum transport of water was observed in everted sacs made from the middle fifth of the gut. Again, the lowest level of transport occurred in sacs made from the distal fifth. Using the method of perfusion in vivo, McHardy and Parsons (9) found that the jejunum was superior to the ileum in its capacity to transport sodium when the lumen was perfused with normal saline. Therefore, the finding in this study that the activity of Na–K–ATPase is greater in midgut and jejunum than in the ileum is in agreement with physiological data on the site of preferential transport of sodium and water. This correlation of the site of maximum enzymatic activity with the site of greatest transport capacity suggests that Na–K–ATPase may be important in supplying the energy for the active transport of sodium by the gut.

**SUMMARY**

The activity of Na–K–ATPase in mucosal homogenates was found to be greater in jejunum and midgut than in ileum. This distribution is consistent with information implying that the proximal gut is superior to the ileum in its absorptive capacity for sodium and water. The correlation of the activity of this enzyme with the sites of preferential sodium and water absorption is compatible with the hypothesis that Na–K–ATPase may play a role in the "sodium pump" mechanism of the gastrointestinal tract.

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