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Intestinal Na^+/H^+ Exchanger Activity is Up-Regulated by Bowel Resection in the Weanling Rat

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ABSTRACT. Increased Na^+/H^+ exchanger activity is associated with cellular hyperplasia. Cellular hyperplasia is an adaptive response to small-intestinal resection. Therefore, we hypothesized that the small-intestinal Na^+/H^+ exchanger activity increases in response to small-intestinal resection. Twenty-one-d-old, male Sprague-Dawley rats were randomly divided to receive either a 70% small intestinal resection ($n = 59$), or a mid–small intestinal transection ($n = 49$). Seven d postoperatively, the animals were killed and the Na^+/H^+ exchanger activity of the intestinal remnants was studied by a well validated brush border membrane vesicle technique. The initial rate of Na^+ uptake in the presence of an outwardly directed pH gradient and the V_{max} of the amiloride-sensitive Na^+ uptake were significantly increased ($p < 0.01$ and $p < 0.001$, respectively) in the resection as compared with the transection remnants and to a greater magnitude in the distal as compared with the proximal remnants. Km values were not significantly different. The amiloride-sensitive Na^+ uptake in the setting of various intravesicular pH was significantly greater ($p < 0.001$) in the distal resection as compared with the distal transection remnants, with points of enhanced Na^+/H^+ exchanger activity of intravesicular pH 6.62 and 6.87, respectively. The presence and activation of the Na^+/H^+ exchanger's internal modifier site was confirmed by demonstrating the effect of intravesicular pH on Na^+ efflux. The present study demonstrates an up-regulation of intestinal Na^+/H^+ exchange activity in a small-bowel resection model in the weanling rat. This adaptive increase in Na^+/H^+ exchange activity is secondary to an increase in the V_{max} of the intestinal Na^+/H^+ exchanger and is associated with a shift in the sensitivity of its internal modifier site. This adaptive response may play a role in the cellular hyperplasia in small-bowel resection. (*Pediatr Res* 33: 215–220, 1993)

Abbreviations

BLM, basolateral membrane
BBMV, brush border membrane vesicle
DR, distal resection
DT, distal transection
HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid
K_i, inhibitory constant
pH_i, intravesicular pH
pH_o, extravesicular pH
PR, proximal resection
PT, proximal transection
SBS, short-bowel syndrome
TMA, tetramethylammonium

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SBS occurs after extensive small intestinal resection and is found in the pediatric population. Today, the most common causes are midgut volvulus and necrotizing enterocolitis, but other causes include multiple intestinal atresias and abdominal wall defects (1). Morbidity and mortality can result from SBS due to the inability of the remaining small bowel to absorb an adequate amount of nutrients. However, it is known that the small intestine has the adaptive capacity to morphologically and functionally compensate after intestinal resection. Cellular hyperplasia is one of the known adaptive responses to intestinal resection (2).

The Na^+/H^+ exchanger is a component of the plasma membrane in virtually all mammalian cells and catalyzes the influx of extracellular Na^+ in exchange for the efflux of intracellular H^+ with a stoichiometry of 1:1. It appears to be involved in multiple cellular functions, such as regulation of the pH_i, and cellular hyperplasia. The Na^+/H^+ exchanger is also believed to have an internal modifier site that determines its pH_i sensitivity (3).

Enhanced knowledge concerning the adaptive response of the small intestine to resection would help improve the management of patients with SBS. Increased Na^+/H^+ exchanger activity is associated with cellular hyperplasia (3). Because cellular hyperplasia is an adaptive response to small intestinal resection (4), we hypothesized that the small intestinal Na^+/H^+ exchanger activity increases in response to intestinal resection. Previous *in vitro* studies have demonstrated an increase in the Na^+/H^+ exchanger activity in response to mitogens; however, no *in vivo* model has demonstrated this point (3). Therefore, we used an SBS model in the weanling rat to demonstrate an up-regulation of the intestinal Na^+/H^+ exchanger. This up-regulation may play a role in the cellular hyperplasia in small-bowel resection.

MATERIALS AND METHODS

Materials. ^{22}Na was obtained from New England Nuclear, Boston, MA. Amiloride was provided by Merck Research Laboratories, West Point, PA. TMA gluconate was made by titrating solutions of TMA hydroxide with gluconic acid. Cellulose nitrate filters, 0.45-μm pore size, were obtained from Sartorius Filters, Hayward, CA. Six-0 prolene, 5-0 silk, and 5-0 Ethilon were obtained from Ethicon, Inc., Somerville, NJ. All other chemicals were of the highest purity available.

Intestinal resection/transection. Weanling (21-d-old) male albino Sprague-Dawley rats (Sasco, Omaha, NB) weighing an average of 48 g were caged and allowed *ad libitum* access to tap water and Purina rat chow (Rodent Laboratory Chow 5001, Ralston Purina, Richmond, IN). The rats were randomly divided into two groups: small intestinal resection and sham-operated mid–small intestinal transection serving as controls. Approval was obtained from the Vanderbilt University Animal Care Committee. All rats were given Pedialyte (Ross Laboratories, Columbus, OH) exclusively 18 to 24 h before surgery. For 1 h before the procedure, the animals were given nothing by mouth. Access to Pedialyte was provided 1 h postoperatively, and then *ad libitum* access to tap water and Purina rat chow was again provided 18 to 24 h later. Each rat's weight change and amount of chow consumed were recorded daily.

The rats assigned for resection underwent approximately 70% small intestinal resection as follows. After anesthesia with halothane, the fur was shaved from the abdominal wall, alcohol skin prep was applied, and then a midline incision was made. In animals this size, the length of the combined jejunum and ileum was approximately 50 cm. The intestine was exposed, and a 35-cm segment was excised, beginning 5 cm distal to the ligament of Treitz and terminating 10 cm proximal to the ileocecal junction. The segments were measured by gently straightening contiguous short portions of *in vivo* intestine and measuring them with premeasured umbilical tape. Continuity of the intestinal lumen was reestablished by joining the cut ends of the remaining small intestine by end-to-end anastomosis with the use of 6-0 prolene. After hemostasis was insured, the shortened intestine was replaced into the peritoneal cavity. The peritoneum was closed with 5-0 silk, and the skin was closed with 5-0 Ethilon. Subsequently, the rat was returned to its cage. No antibiotics were administered. Sham-operated rats underwent mid–small intestinal transection, 25 cm proximal to the ileocecal junction, and end-to-end reanastomosis but without removal of intestinal tissue. In all other respects, the procedure was identical to that used with the resected rats.

Seven d postoperatively, all rats were killed by cervical dislocation, and intestinal remnants were removed for investigation. The segments labeled "proximal" consisted of intestine from the ligament of Treitz to the surgical reanastomosis. The segment labeled "distal" consisted of intestine from the ileocecal junction to the surgical reanastomosis. Operative details were similar to those used by Urban and Pena (5).

Preparation of BBMV. BBMV were prepared from scraped rat intestinal mucosa using an Mg^{2+}/EGTA precipitation method (6). The respective small intestinal segments were flushed with normal saline, everted, and scraped with a glass slide. All steps in this preparation were conducted on ice. The mucosal scrapings were homogenized in a Waring blender at maximal speed for 3 min in 30 mL of 300 mM mannitol, 5 mM EGTA, 12 mM Tris HCl (pH 7.1), and 120 mL of ice-cold distilled water. The homogenate was treated with 1.5 mL of 1 mM $MgCl_2$ and centrifuged at 5 000 rpm for 15 min in a Beckman rotor model J2–21 (Beckman Instruments, Fullerton, CA). The supernatant was then centrifuged at 15 000 rpm for 30 min. The resulting pellet was resuspended in 30 mL of 60 mM mannitol, 5 mM EGTA, and 12 mM Tris HCl (pH 7.1) and homogenized in a Potter-Elvehjem tube for 10 strokes at the highest speed. The homogenate was treated with 0.3 mL of 1 mM $MgCl_2$ and centrifuged at 5 000 rpm for 15 min. The supernatant was centrifuged at 15 000 rpm for 30 min. The pellet was resuspended in 15 mL of preincubation solution and homogenized in a Potter-Elvehjem tube for 10 strokes at the highest speed. The preincubation solution composition is described in each figure legend. The homogenate was centrifuged at 20 000 rpm for 30 min. The pellet was resuspended in the desired volume of preincubation solution using a tuberculin syringe with a 25-gauge needle. In addition, to prevent the possibility of differential samplings along the crypt/villus axis influencing the results, the respective samples of intestinal tissue from both transection and resection rats were obtained before and after the mucosa was scraped for BBMV preparation. These tissue samples were fixed in 4% paraformaldehyde and subsequently mounted in cross-section and stained with hematoxylin and eosin for microscopic histologic examination. All scraped tissues revealed complete scraping of the crypt/villus axis. According to Hanson *et al.* (13), the crypt/villus axis increases proportionately within the first week after intestinal resection. Therefore, because complete scraping of the crypt/villus axis was documented, there should be no sampling effect influencing the results.

Transport measurement. Transport measurements were made from the uptake of radiolabeled sodium that was measured by a rapid filtration technique (6). All incubations were done at room temperature and were initiated by addition of 20 μL of vesicle suspension to 80 μL of incubation solution. The composition of the incubation medium is noted in the legend of each figure. At each desired incubation time interval, 1 mL of ice-cold stop solution, which consisted of 185 mM K gluconate, 10 mM Tris, 16 mM HEPES, and 0.1 mM amiloride, was added to the reaction mixture. The cold, diluted reaction mixture was immediately pipetted onto a prewetted filter (cellulose nitrate, 0.45-μm pore size; Sartorius Filters) and kept under suction. The filter was rinsed with 5 mL of ice-cold stop solution and dissolved in Ready Protein liquid scintillation cocktail (Beckman Instruments, Palo Alto, CA). Radioactivity was counted in a scintillation counter (model LS 400: Beckman Instruments). Radioactivity remaining in the filters after pipetting incubation medium into the radioactive substrate in the absence of vesicles was considered as background and was accounted for in the calculations.

Purity of BBMV preparation. Purity of the BBMV preparations was validated by the measurement of the activity of specific marker intestinal disaccharidases (sucrase, maltase) by the method of A. Dahlqvist (7). In addition, the sp act of Na^+-K^+-ATPase, a marker of BLM, was measured by the method of Scharschmidt *et al.* (8). This was performed to rule out contamination by BLM Na^+/H^+ exchangers.

Protein concentration measurement. The protein concentration was measured by the method of Lowry *et al.* (9), using BSA as a standard.

Dissipation of pH gradient in BBMV. Dissipation of the pH across BBMV was measured using the fluorescence quenching of acridine orange as described by Kleinman *et al.* (10). BBMV from DT and DR rats were preincubated in 100 mM TMA gluconate, 50 mM K gluconate, 40 mM HEPES, and 90 mM 2(*N*-morpholino)ethane sulfonic acid (pH 5.2). Vesicles were then diluted in a medium containing: 100 mM TMA gluconate, 50 mM K gluconate, 85 mM HEPES, 45 mM Tris, and 6 μM acridine orange (pH 7.5). Acridine orange fluorescence quenching occurred with spontaneous dissipation of the pH gradient. Monensin (an ionophore) 10^{-2} M was added as a positive control, which totally collapsed the pH gradient. Changes in the fluorescence quenching were monitored at room temperature using the SPEX (Edison, NJ) Flurolog spectrometer.

Analysis of data. All values were expressed as nmol of sodium uptake/mg of vesicle protein. The *t* test was used to evaluate the statistical significance of differences between groups. A probability value of <0.05 was considered statistically significant.

RESULTS

Rat postoperative weight and chow consumption. All rats lost weight immediately postoperation; however, both transection and resection rats regained at least their preoperative weight within 3 to 5 d. The average daily weight gain per rat was similar between the two groups (4.5 *versus* 4.4 g/d for transection *versus* resection, respectively). The average amount of chow consumed daily per rat was not significantly different between the groups (26 *versus* 25 g/d for transection *versus* resection, respectively).

The mortality rates among the rats were three (6%) of 49 for the transection group and five (8%) of 59 for the resection group. All surviving rats were healthy and did not have diarrhea.

Mucosal hyperplasia. The average values for mg of mucosal protein/cm length of intestinal remnant were significantly increased ($p < 0.001$) in the resection groups as compared with the transection groups (Table 1). This indicates cellular hyperplasia as an adaptive response to small intestinal resection.

Purity of BBMV preparation. The average activities of both BBMV marker enzymes, sucrase and maltase, were enriched approximately 9-fold in the BBMV preparation (11.8 ± 3.3 and 5.9 ± 1.4 $\mu M \cdot min^{-1} \cdot mg\ protein^{-1}$, respectively) as compared with the total mucosal homogenate (1.43 ± 0.27 and 0.65 ± 0.16 $\mu M \cdot min^{-1} \cdot mg\ protein^{-1}$, respectively). In addition, the average sp act of Na^+-K^+-ATPase, a marker for BLM, was impoverished by 0.2-fold in the BBMV preparation as compared with the total mucosal homogenate (1.7 ± 0.6 *versus* 8.4 ± 0.43 $\mu M \cdot$inorganic phosphate$\cdot h^{-1} \cdot mg\ protein^{-1}$, respectively). Similar results were found in all remnants tested (Table 2). This indicates purity of the BBMV preparation without contamination by BLM Na^+/H^+ exchangers.

Initial rate of uptake of Na^+/H^+ exchanger. The initial rate of Na^+ uptake at a 1-mM Na^+ concentration was determined under an outwardly directed pH gradient (pH_i/pH_o = 5.7/7.5) for each intestinal remnant (PT, PR, DT, and DR). Na^+ uptake was measured at 3, 5, 7, 10, 12, 15, and 30 s and was linear up to 12 s in all remnants as depicted in Figure 1. The nonzero intercept on the ordinate indicates a minimal binding component. The slope of the initial rate of uptake of the Na^+/H^+ exchanger was 2.6-fold greater ($p < 0.01$) in the distal resection as compared with the DT remnants and 1.5-fold greater ($p < 0.01$) in the PR as compared with the PT remnants.

Kinetics of Na^+/H^+ exchanger. The kinetics of the Na^+ transport was determined after 5 s of uptake in the presence of an outwardly directed pH gradient (pH_i/pH_o = 5.2/7.5), using varying Na^+ concentrations between 1 and 50 mM in the presence and absence of 1 mM of amiloride. The amiloride-sensitive component (total uptake − uptake in the presence of 1 mM of amiloride) was analyzed using a computerized model of Michaelis-Menten kinetics (Enzfitter, Biosoft, Cambridge, UK). Table 3 depicts the kinetic data. The V_{max} for amiloride-sensitive Na^+ uptake of the Na^+/H^+ exchanger was 2.1-fold greater ($p < 0.001$) in the DR as compared with DT remnants, and 1.6-fold greater ($p < 0.001$) in the PR as compared with the PT remnants. Km values were not significantly different between any remnant pair.

Table 1. *Values of mg of mucosal protein per cm length of intestinal remnant*

Intestinal remnant	mg protein/cm length ± SEM	Statistical significance
PR	0.0149 ± 0.0014	
PT	0.0038 ± 0.0003	$p < 0.001$
DR	0.0082 ± 0.0007	
DT	0.0034 ± 0.0006	$p < 0.001$

* Values represent the mean ± SEM of six experiments on different membrane preparations.

Effect of varying amiloride concentrations on Na^+/H^+ exchanger. The effect of varying concentrations of amiloride (0.1 to 10 mM) on 1 mM Na^+ uptake under an outwardly directed pH gradient (pH_i/pH_o = 5.2/7.5) was studied on the DR and DT intestinal remnants as previously described (10). Linear regression analysis of amiloride concentration *versus* $1/Na^+$ uptake showed a linear relationship as expressed by the formulas $y = 6.2x + 0.34$, $r = 0.99$, and $y = 5.3x + 0.26$, $r = 0.99$, for DR and DT remnants, respectively. The K_i for amiloride, calculated by Dixon plot, was 0.05 mM for both intestinal remnants.

Influence of pH_i on Na^+/H^+ exchanger. The pH of the intravesicular compartment was varied from 5.2 to 7.5, whereas the incubation media pH was maintained at 7.5. The amiloride-sensitive Na^+ uptake was determined at 5 s well within the linear line of uptake. Due to the greater magnitude of difference between the DT and DR kinetics, these remnants were chosen to enhance detection of any changes under these experimental conditions. Figure 2 depicts the effect of varying the pH_i on the amiloride-sensitive Na^+ uptake of the two remnants studied. Both remnants demonstrated increasing Na^+ uptake with decreasing pH_i. However, at each point except pH 7.5, sodium uptake was significantly greater in the resection as compared with the transection remnants ($p < 0.001$). The point of enhanced Na^+/H^+ exchanger activity for the DR as compared with the DT remnants was pH_i 6.62 and 6.87, respectively. To further confirm the findings in Figure 2, the effect of pH_i on Na^+ efflux was examined by methods used previously (11). BBMV prepared from both DR and DT intestinal remnants were preincubated in ^{22}Na media at either pH_i 7.4 or 6.5. The intravesicular ^{22}Na content was then assayed before and after dilution in a media with and without amiloride. As seen in Figure 3, the amiloride-sensitive Na^+ efflux was greater from BBMV with a pH_i of 6.5 as compared with a pH_i of 7.4. This indicates the presence and activation of the Na^+/H^+ exchanger's internal modifier site by the intravesicular H^+. Moreover, the Na^+ efflux with a pH_i of 6.5 in the resection remnant was greater as compared with the transection remnant. This indicates a greater magnitude of activation of the Na^+/H^+ exchanger's internal modifier site by resection.

Dissipation of pH gradient in BBMV. Figure 4 depicts the rate of dissipation of the pH gradient as measured by the fluorescence quenching of acridine orange in the DT and DR BBMV. No differences in the rate of dissipation of the H^+ gradient were noted.

DISCUSSION

The present study was designed to examine the effect of intestinal resection on the Na^+/H^+ exchanger. Compensatory morphologic and functional changes occur that allow for adaptation to shortened intestine after extensive small-bowel resection. Morphologic changes are characterized by villus cellular hyperplasia and increased villus height and crypt depth along with dilation and lengthening of the intestinal remnant (2). Morphologic adaptation develops rapidly within 48 h and reaches a peak 7 to 12 d after intestinal resection (2). The adaptive changes are much more striking in the distal intestinal remnant

Table 2. *Sp act of marker enzymes in BBMV and total mucosal homogenate**

	PR		PT		DR		DT	
	BBMV	Mucosa	BBMV	Mucosa	BBMV	Mucosa	BBMV	Mucosa
Sucrase ($\mu M \cdot min^{-1} \cdot mg\ protein^{-1}$)	13.5	1.47 (9.1)	22	2.42 (9)	6.26	0.69 (9)	5.6	0.61 (9.1)
Maltase ($\mu M \cdot min^{-1} \cdot mg\ protein^{-1}$)	6.6	0.72 (9.1)	10.3	1.1 (9.3)	4.16	0.46 (9)	2.9	0.31 (9.3)
Na^+-K^+-ATPase ($\mu M \cdot P_i \cdot h^{-1} \cdot mg\ protein^{-1}$)	2.2	14.2 (0.15)	1.3	9.2 (0.14)	2.4	6.2 (0.38)	1.2	4.3 (0.27)

* Number in parentheses indicates enrichment or impoverishment factor. P_i, inorganic phosphate.

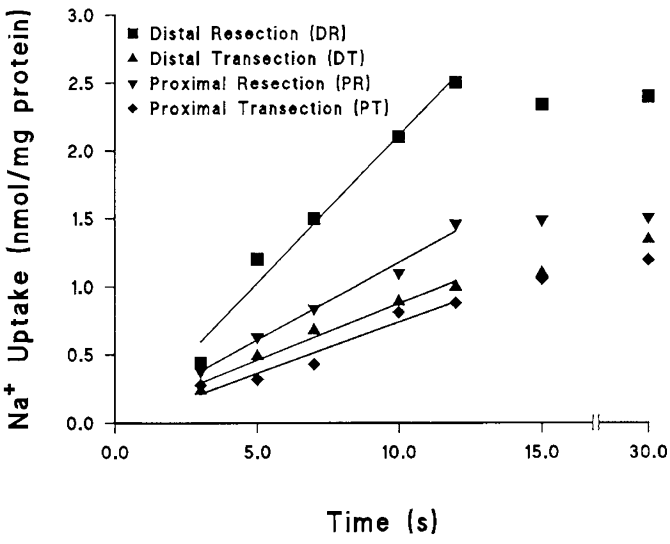

Fig. 1. Effect of resection on the initial rate of ^{22}Na uptake under an outwardly directed pH gradient (pH_i/pH_o = 5.2/7.5). BBMV were prepared in 100 mM TMA gluconate, 40 mM HEPES, and 90 mM 2(*N*-morpholino)ethane sulfonic acid buffer (pH 5.2). The reaction was started by the addition of 20 μL of vesicles to a medium containing: 100 mM TMA gluconate, 85 mM HEPES, 45 mM Tris buffer (pH 7.5), 1 mM Na^+ gluconate, and tracer ^{22}Na. The reaction was stopped at the desired time intervals. Each *point* represents the mean of three separate experiments on different membrane preparations. Linear regression analysis revealed the following: PR: $y = 0.114x + 0.039$, $r = 0.94$; PT: $y = 0.075x - 0.009$, $r = 0.99$; DR: $y = 0.217x - 0.057$, $r = 0.98$; and DT: $y = 0.083x + 0.046$, $r = 0.98$. Slope of DR is > DT by 2.6-fold ($p < 0.01$); slope of PR is > PT by 1.5-fold ($p < 0.01$).

as compared with the proximal intestinal remnant (4). The degree of hyperplastic response is directly proportional to the percentage of intestinal resection (15). Functionally, *in vivo* perfusion techniques in laboratory animals with proximal enterectomy show increased intestinal uptake of water, electrolytes, sugars, and amino acids by the intestinal remnant (14). Studies of the residual small bowel in man, after small intestinal resection, also demonstrate morphologic changes such as mucosal hyperplasia (13) and functional changes such as increased water, sodium, and glucose absorption (16, 17).

Evidence for the Na^+/H^+ exchanger participating in cellular proliferation comes from studies that demonstrate mitogens increasing its activity. The activation of the Na^+/H^+ exchanger leads to an increase in the influx of Na^+ and the efflux of H^+, which subsequently results in cytoplasmic alkalinization. Mitogens such as serum, epidermal growth factor, and platelet-derived growth factor have been shown to activate the Na^+/H^+ exchanger in *in vitro* models; however, no *in vivo* model has demonstrated this point (3).

The mechanism of activation of the Na^+/H^+ exchanger has been studied and is believed to involve an alkaline shift in its pH_i dependence. This shift apparently reflects an altered behavior of the exchanger's internal modifier site, inasmuch as this site largely determines its pH_i sensitivity. According to this proposed model, the internal modifier's set point is shifted upward (by 0.15 to 0.3 pH units) toward a more alkaline environment. As a result of this set point shift, the exchanger is temporarily activated and then returns to near quiescence when pH_i attains the new set point value. Although regulated, pH_i is not invariant; the internal modifier's set point can be shifted both downward, *e.g.* by depletion of ATP, and upward, *e.g.* by growth factors. Therefore, the internal modifier site operates as a variable pH-stat. The molecular events involved in mitogenic activation of the Na^+/H^+ exchanger are not fully known; however, it has been proposed that phosphorylation of the exchanger or an ancillary protein is responsible for the alteration in the set point. The importance of mitogen-induced cellular alkalinization can be fully appreciated when the pH_i sensitivity of cell growth is taken into consideration. DNA synthesis in proliferating cells has been found to be dependent on intracellular pH, with increasing rates of synthesis at more alkaline levels. Therefore, it is evident that stimulation of the Na^+/H^+ exchanger, with a subsequent increase in pH_i, could be involved in the development of the proliferative response (3).

In this study, the changes in the Na^+/H^+ exchanger activity were evaluated 7 d postoperatively because morphologic adaptation peaks 7 to 12 d after intestinal resection (12). The length of intestinal tissue used for BBMV preparation was greater in the transected group. However, this does not alter the validity of the results, because the vesicle uptake is reported per mg of vesicle protein, and this has been shown to be an appropriate method of normalizing determinations in vesicles obtained from different amounts of tissue (18).

The initial rate of Na^+ uptake was performed to examine the difference in the activity of the Na^+/H^+ exchanger in each intestinal remnant. The activity of the Na^+/H^+ exchanger was significantly increased ($p < 0.01$) in the resection as compared with the transection remnants and to a greater magnitude in the distal as compared with the proximal remnants (Fig. 1). These results concur with previous studies that have demonstrated that intestinal resection results in an increased morphologic and functional adaptive response (2, 14). In addition, this adaptive response is more pronounced in the distal as compared with the proximal remnants (4). Because the Na^+/H^+ exchanger activity was significantly increased in the resected group of this study and hyperplasia is known to occur secondarily to resection, this suggests that increased Na^+/H^+ exchanger activity and intestinal cellular hyperplasia may be related.

To elucidate whether the increase in the initial rate uptake of the Na^+/H^+ exchanger activity was due to an increase in the affinity (Km) or the capacity (V_{max}) of the exchanger, kinetic studies were performed. The V_{max} for the amiloride-sensitive Na^+

Table 3. *Kinetics of Na^+/H^+ Exchangers of Intestinal BBMV**

Intestinal remnant	V_{max} (nmol/mg protein/5 s)	Magnitude of difference	Statistical significance	Km (mM)	Statistical significance
PR	4.92 ± 1.07			27.5 ± 11.8	
PT	2.92 ± 0.19	1.6-fold	$p < 0.001$	23.7 ± 3.26	NS
DR	6.86 ± 0.94			33.2 ± 8.45	
DT	3.32 ± 0.32	2.1-fold	$p < 0.001$	21.6 ± 4.47	NS

* The effect of resection on the kinetics of the Na^+/H^+ exchanger of BBMV from each intestinal remnant. BBMV were prepared in 100 mM TMA gluconate, 40 mM HEPES, and 90 mM 2(*N*-morpholino)ethane sulfonic acid buffer (pH 5.2). The reaction was started by the addition of 20 μL of vesicles to a medium containing 100 mM TMA gluconate, 85 mM HEPES, 45 mM Tris buffer (pH 7.5), and various concentrations of Na^+ gluconate (1–50 mM) and tracer ^{22}Na in the presence and absence of amiloride. TMA gluconate concentration was decreased with increasing Na^+ to maintain equal osmolality across the membranes. The reaction was stopped at 5 s. Values represent the mean ± SEM of three experiments on different membrane preparations. V_{max} and Km values were analyzed using a computerized model of the Michaelis-Menten kinetics. V_{max} of DR is > DT ($p < 0.001$); V_{max} of PR is > PT ($p < 0.001$); Km values demonstrated no significant differences between any remnant pair.

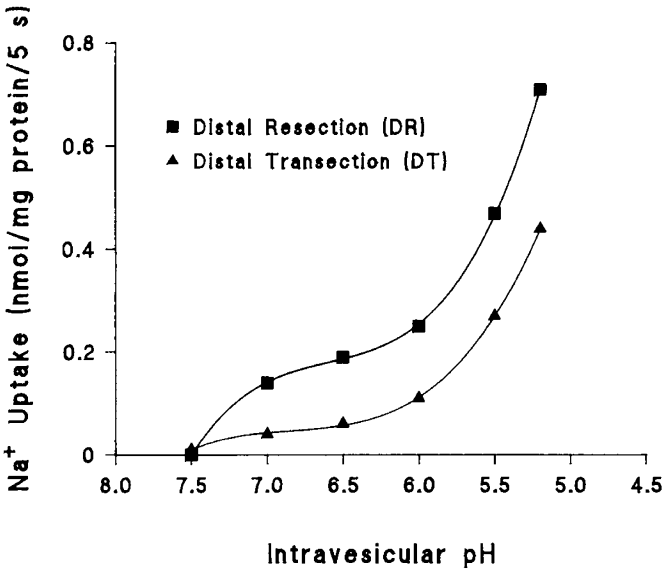

Fig. 2. Effect of varying pH_i on intestinal BBMV Na^+/H^+ exchange activity. BBMV were prepared in 100 mM TMA gluconate and various combinations of Tris, HEPES, and 2(*N*-morpholino)ethane sulfonic acid (130 mM total) to bring the pH_i from 7.5 to 5.2. The reaction was started by the addition of 20 μL of vesicles to a medium containing 100 mM TMA gluconate, 85 mM HEPES, 45 mM Tris buffer (pH 7.5), 1 mM Na^+ gluconate, and tracer ^{22}Na. The reaction was stopped at 5 s. Each *point* represents the mean of three separate experiments on different membrane preparations. At each point, except pH 7.5, Na^+ uptake of DR is > DT ($p < 0.001$). The deflection point of enhanced Na^+/H^+ exchanger activity revealed the following: DR $pH_i = 6.62$ *vs* DT $pH_i = 6.87$ ($p < 0.001$).

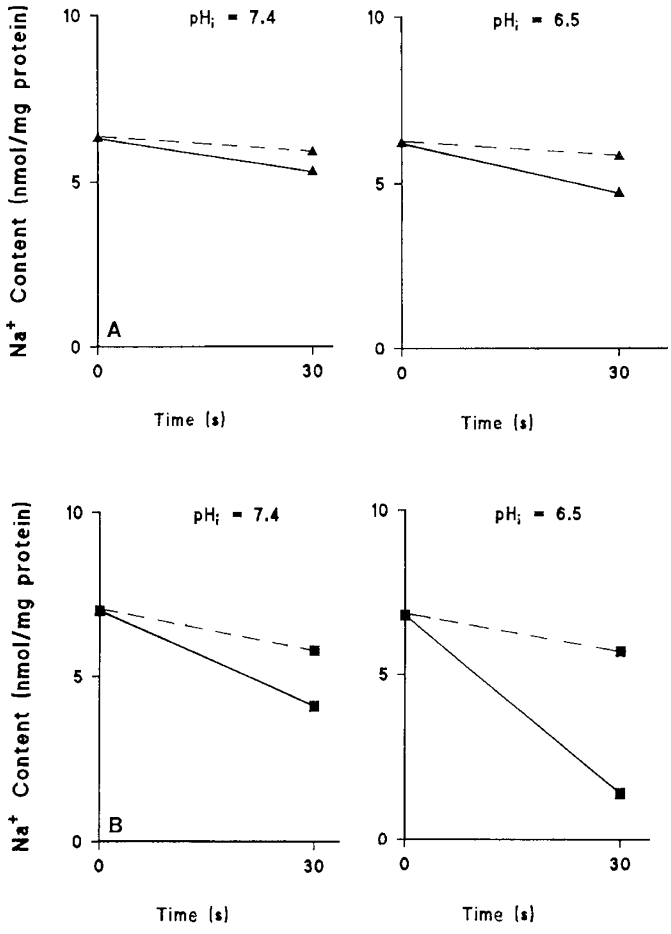

Fig. 3. Effect of pH_i on Na^+ efflux. BBMV were preincubated in a media containing 52 mM NaCl, ^{22}Na, 22 mM K gluconate, 42 mM HEPES, and either 157 mM mannitol (pH 7.4) or 140 mM mannitol and 17 mM 2(*N*-morpholino)ethane sulfonic acid (pH 6.5) for 120 min at room temperature. Intravesicular ^{22}Na content was determined before and after 1:100 dilution in media containing 200 mM mannitol, 41 mM K gluconate, and 80 mM HEPES (pH 7.5) with or without 1 mM amiloride. *A*, DT (▲); *B*, DR (■). — — — with amiloride; ———, without amiloride.

uptake of the Na^+/H^+ exchanger was significantly increased ($p < 0.001$) in the resection as compared with the transection remnants and to a greater degree in the distal as compared with the proximal remnants (Table 3). However, Km values were not significantly different between any remnant pair. Again, these results correlate with previous studies that show an increased adaptive response in resection and distal remnants (4, 14). The kinetic studies demonstrate that the increase in the initial rate of uptake of the Na^+/H^+ exchanger activity was due to an increase in the capacity (V_{max}) of the exchanger. However, whether this increase is due to an increase in the number of transporters or represents an increase in the turnover rate of each transporter cannot be determined from this study. To evaluate the specificity of these findings, the slope of the amiloride-insensitive Na^+ uptake was calculated from the above kinetics for each intestinal remnant. There is no significant difference between PR and PT or DR and DT. Therefore, the findings noted in this study are specific for the amiloride-sensitive Na^+ uptake or, in other words, the Na^+/H^+ exchanger. The nutritional and biochemical factor(s) responsible for the adaptive response in the resection model are not known but may include oral nutrients, pancreatic biliary secretions, and hormones such as enteroglucagon, epidermal growth factor, and prostaglandin E_2 (19).

To examine whether there is a different isoform of the Na^+/H^+ exchanger in the intestinal resection remnant, the K_i for amiloride was determined for both DT and DR intestinal remnants. This was accomplished by examining the effect of varying amiloride concentrations on the Na^+/H^+ exchanger. The K_i for amiloride, calculated by Dixon plot, was 0.05 mM for both intestinal remnants. Therefore, on the basis of functional characteristics, this suggests that the Na^+/H^+ exchanger is the same isoform in both intestinal remnants.

To examine whether intestinal resection had an effect on the set point of the Na^+/H^+ exchanger's internal modifier site, the amiloride-sensitive Na^+ uptake in the BBMV prepared from the DT and DR remnants was measured under varying pH_i. Both remnants demonstrated increasing Na^+ uptake with decreasing pH_i. However, at each pH studied except 7.5, sodium uptake was significantly greater ($p < 0.001$) in the resection as compared with the transection remnants (Fig. 2). The point of enhanced Na^+/H^+ exchanger activity was calculated by expressing the data from Figure 2 in a 3rd-degree polynomial equation (using Fig Perfect computer software, Durham, NC) and then determining the value of the pH at which the second derivative of this equation was zero. This resulted in a point that was found to be more acidic in the resection as compared with the transection BBMV (pH_i 6.62 *versus* 6.87, respectively, $p < 0.001$). This suggests that resection shifts the threshold of sensitivity of the Na^+/H^+ exchanger's internal modifier site. The Na^+/H^+ exchanger can mediate net Na^+ transport in either direction (3); therefore, this principle was used to further confirm the validity of the internal modifier site as suggested by the findings in Figure 2. The effect of pH_i on Na^+ efflux was examined by methods used previously (11). Na^+ efflux was determined from BBMV prepared from both DR and DT intestinal remnants. The vesicles were preincubated with ^{22}Na at either pH_i 7.4 or 6.5. As seen in Figure 3, the amiloride-sensitive Na^+ efflux was greater from the BBMV with a pH_i of 6.5 as compared with a pH_i of 7.4. This indicates the presence and activation of the Na^+/H^+ exchanger's internal modifier site by the intravesicular H^+, because thermo-

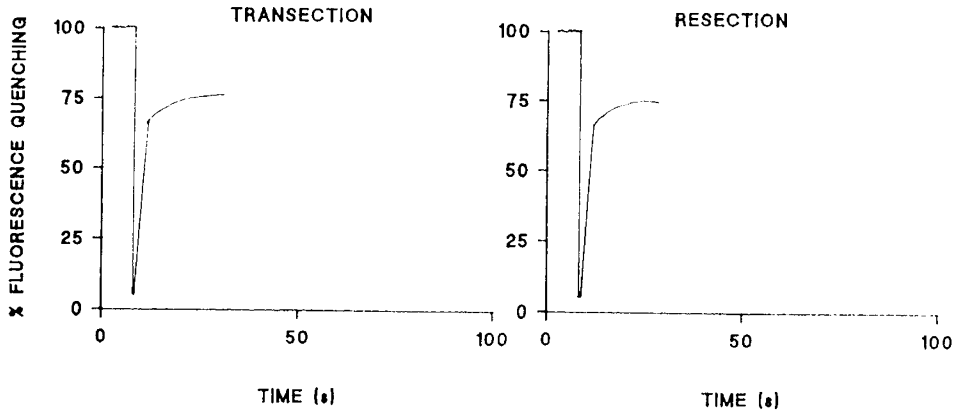

Fig. 4. The effect of surgery on the dissipation of the pH gradient in intestinal BBMV from DT and DR remnants. BBMV were prepared in 100 mM TMA gluconate, 50 mM K gluconate, 40 mM HEPES, and 90 mM 2(*N*-morpholino)ethane sulfonic acid (pH 5.2). Vesicles were then diluted in a medium containing 100 mM TMA gluconate, 50 mM K gluconate, 85 mM HEPES, 45 mM Tris buffer (pH 7.5), and 6 μM acridine orange. Monesin 10^{-2} M was added as a positive control, which totally collapsed the pH gradient (not shown). Changes in fluorescence quenching were monitored at room temperature using the SPEX Fluorolog spectrometer. No differences were noted between DT and DR BBMV.

dynamically the driving force for Na^+ efflux would be lower with a pH_i of 6.5 if there was not an internal modifier site. Moreover, the Na^+ efflux with a pH_i of 6.5 in the resection remnant was greater as compared with the transection remnant. This indicates a greater magnitude of activation of the Na^+/H^+ exchanger's internal modifier site by resection.

To determine whether the BBMV from the DT and DR remnants were affected by the different surgical techniques and whether they continued to maintain similar pH gradients, dissipation of the pH gradient was studied using the fluorescence quenching of acridine orange as described previously (10). No differences were noted between the DT and DR BBMV (Fig. 4). Therefore, these results demonstrate that the differences in the kinetic parameters between the DT and DR BBMV were due to actual differences in the exchanger activity and not differences in the rate of dissipation of the pH gradients across the membranes of each group.

In consideration of the relationship between the Na^+/H^+ exchanger activity and cellular hyperplasia, one may hypothesize that intestinal resection initiates a sequence of events that stimulate the expression or release the inhibition of certain growth factor(s). This, in turn, may shift the Na^+/H^+ exchanger's internal modifier set point toward a more alkaline pH_i. Subsequently, the Na^+/H^+ exchanger's activity is increased and leads to an intracellular alkalinization that enhances the environment for cellular DNA synthesis and subsequent cellular hyperplasia.

In summary, the present study demonstrates an up-regulation of intestinal Na^+/H^+ exchange activity in a small-bowel resection model in the weanling rat. This adaptive increase in Na^+/H^+ exchange activity is secondary to an increase in the V_{max} of the intestinal Na^+/H^+ exchanger and is associated with a shift in the sensitivity of its internal modifier site. This adaptive response may play a role in the cellular hyperplasia in small-bowel resection.

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