Region-specific adaptation of apical Na/H exchangers after extensive proximal small bowel resection

MARK W. MUSCH,1 CRESC BOOKSTEIN,1 FLAVIO ROCHA,1 ALVARO LUCIONI,1 HONGYU REN,1 JANET DANIEL,1 YUE XIE,1 REBECCA L. MCSWINE,1 MRINALINI C. RAO,2 JOHN ALVERDY,3 AND EUGENE B. CHANG1

1The Martin Boyer Laboratories, 2Department of Surgery, University of Chicago, Chicago 60637; and 3Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, Illinois 60612

Received 18 December 2001; accepted in final form 21 June 2002

After massive small bowel resection (MSBR), the remnant small intestine adapts to restore Na absorptive function. The possibility that this occurs through increases in cellular Na absorptive capacity was examined by assessing the regional effects of 50% proximal MSBR on the function and expression of the apical membrane Na/H exchangers (NHEs) NHE2 and NHE3. Morphometric analysis confirmed adaptive changes consistent with villus hypertrophy, particularly distal to the anastomosis. Villus epithelium prepared by light mucosal scrapings from 2-wk-postresected and -posttransected control rats exhibited comparable brush-border hydrolase activities, total cell protein per DNA, and villin expression but increased basolateral Na-K-ATPase activity. Parallel increases of two- to threefold in protein and mRNA abundance of NHE2 and NHE3 were observed only in ileal regions distal to the anastomosis of resected rats. Basolateral NHE1 expression was unchanged. After 80% resection, increases in NHE2 and NHE3 became evident in proximal colon. We conclude that increased enterocyte expression and function of apical membrane NHEs in regions distal to the anastomosis play a role in the adaptive process after MSBR. The increased luminal Na load to distal bowel regions after proximal resection may stimulate increases in apical membrane Na gene transcription and protein expression. Sodium transport; sodium/hydrogen exchange; intestinal surgery; intestinal adaptation; diarrhea; malabsorption; epithelial cell; intestinal physiology

SODIUM/HYDROGEN EXCHANGERS (NHEs) comprise a family of highly related proteins that mediate the electroneutral 1:1 exchange for extracellular Na with intracellular H (1, 5–7, 11, 24, 46). However, the various NHE isoforms of the family exhibit considerable cell- and tissue-specific differences in their expression and regulation of function. The mammalian small and large intestine, for instance, express three NHE isoforms. NHE1 is present in virtually all cells but, in epithelial cells, is selectively expressed in the basolateral membrane where it is believed to have a role in maintaining pH and volume homeostasis. NHE2 and NHE3, in contrast, exhibit epithelial-specific expression and are predominantly found in the apical membrane where they are believed to have an important role in non-nutrient-dependent, vectorial absorption of Na. These two apical isoforms differ in a number of ways. Although both are amiloride-inhibitable, NHE2 is significantly more sensitive to pharmacological inhibition by HOE-642 than NHE3, a functional property used to distinguish them (24). NHE3 expression, in contrast to NHE2, is also sensitive to hormonal and metabolic signals. Glucocorticoids, for instance, increase ileal NHE3 expression (6, 41, 46), whereas mineralocorticoids or hypokalemia increase NHE3 in rat proximal colon (5, 41). Although NHE2 expression is ontologically regulated (7), few humoral or luminal signals have not been identified that regulate NHE2 expression in the adult intestine except for epidermal growth factor (EGF; see Ref. 47).

After massive small bowel resection (MSBR), the remnant small intestinal mucosa rapidly adapts to restore normal nutrient, water, and electrolyte absorptive capacity (11, 29, 32, 34, 43–45). Alterations in the smooth muscle have also been noted after bowel resection (39). In addition, a number of studies have demonstrated alterations in monosaccharide transporters after bowel resection (1, 19, 36, 42). Increased overall electrolyte absorption has also been observed, which has been attributed to increases in mucosal mass and surface area (9, 25). However, these results were based on extrapolated multipliers from morphometric data, which are imprecise. Although a major component of this adaptive process does involve villus hypertrophy (increase in villus length) and increased absorptive surface area, the possibility that increased cellular Na

Address for reprint requests and other correspondence: E. B. Chang, The Martin Boyer Laboratories, Univ. of Chicago, MC 6084, 5841 S. Maryland Ave., Chicago, IL 60637 (E-mail: echang@medicine.bsd.uchicago.edu).
absorptive capacity contributed to this process remains controversial (2, 27, 43–45).

Because a large portion of intestinal Na absorption is mediated by the apical NHEs NHE2 and NHE3 (24, 46), we examined this possibility by assessing the regional effects of 50% proximal small bowel resection on their function and expression. It should be noted that Sacks et al. (32) demonstrated increased brush-border membrane NHE activity in weaning rats after bowel resection but did not determine at that time whether it was because of NHE2 or NHE3. More recently, Falcone et al. (11) have demonstrated increased NHE3 mRNA and protein levels in mice that had MSBR. No differences in NHE2 were observed, and changes in NHE1 were not investigated. However, this study did not determine whether these changes were a result of mucosal hypertrophy (increase in villus length), cellular adaptation (increased NHE activity/cell), or increased apical NHE expression along the villus-crypt axis (recruitment). In this study, we report increased expression and function of these apical membrane NHEs per villus cell in regions downstream of the anastomosis as one mechanism of intestinal adaptation after MSBR. The extent of downstream cellular adaptation was proportional to the extent of MSBR. These conclusions were based on data normalized to functional and biochemical parameters found not to be changed in villus cell preparations of either resected or transected animals. Moreover, we found no evidence of apical NHE recruitment along the villus-crypt axis.

MATERIALS AND METHODS

Surgical resection of the proximal 50% of rat small intestine. All procedures were approved by the Institutional Animal Care Use Committee of the University of Chicago. Rats (200–255 g) were anesthetized, and a 40-cm segment of the small bowel was removed beginning 15 cm distal to the ligament of Treitz. Great care was taken to maintain blood supply to the remnant bowel. To control for the effects of surgical manipulation of the bowel, paired rats underwent intestinal transection and anastomosis at 15 cm distal to the ligament of Treitz. The weight of the rats was monitored every day for the first 4 days and subsequently every 2 days. For the first 2 days, most rats lost 8–12 g of their body weight, probably from postoperative fluid losses and diminished oral intake. Thereafter, all rats began to consume chow at comparable levels (20–25 g/day) and gained weight in a similar fashion (5–7 g/day). With the use of the weight data from a number of groups (since only certain experimental data could be analyzed in consideration of the length of the region of the small intestine proximal to the anastomosis), the presurgical weights were 244 ± 5 and 246 ± 7 in the transected and resected groups, and 14 days after surgery the weights were 308 ± 14 and 301 ± 17 in the respective groups (n = 12). After 14 days, the rats were killed, and the entire length of the small intestine was measured, generally 85–95 cm in transected rats and 37–42 cm in resected rats. The ligament of Treitz was noted, and 8-cm sections of the small intestine were taken beginning either 5 cm proximal or 15 cm distal to the anastomosis (i.e., away from the anastomosis). A section of the proximal large intestine was taken just after the cecum. The sections of the intestine were processed as appropriate for the analysis.

For analysis of sucrase, villin, NHE2, and NHE3, brush-border membranes were isolated from villus epithelial samples prepared from light scrapings of the intestinal mucosa using glass slides (6). Shearing at the villus-crypt junction by this technique was confirmed by histological staining of the remaining gut segment from transected and resected animals (see Fig. 1). In using this approach, direct comparisons of the villus cell compartment between resected and transected animals could be compared directly without confounding variables such as admixture of crypt, muscle, or mesenchymal cells. It is important to note that, when the epithelial-cell-specific protein villin was analyzed in homogenates from the scrapings, the amount of villin expressed per microgram protein was not different in the corresponding section of the transected vs. the resected rats, suggesting a similar proportion of villus cells. Because DNA content per cell can be used to estimate cell number (assuming that the amount of DNA/cell does not change between conditions), these data suggest that the protein content per cell was not significantly different between the resected and transected animals, i.e., there was no evidence for cellular hypertrophy (increased cell protein expression while the cell remains the

Fig. 1. Histological confirmation of villus cell population removed by light scraping in rats that underwent transection and resection. The small intestine was removed, and a section was removed for fixation in formalin and hematoxylin and eosin staining. Section immediately adjacent was lightly scraped, and the remaining mucosa and muscle were fixed and also processed for staining. The images were obtained on a Leitz microscope using Pixar software. The images shown are representative of those on 3 occasions.

RESECTED

TRANSECTED

INTACT

SCRAPPED

AJP-Gastrointest Liver Physiol • VOL 283 • OCTOBER 2002 • www.ajpgi.org
same size). Cellular hypertrophy can also be interpreted to mean increased cell size. In this case, one may not predict the amount of protein expressed per cell, and this information must still be determined by measuring cell protein and then preferably cell number (which is not possible in cells such as intestinal enterocytes, which tend to clump) or by an indirect measure of cell number (such as DNA). This does not discount the development of villus hypertrophy (lengthening of villi), where there is an increased number of enterocytes per villus, but argues against a generalized increase in protein content of individual enterocytes. As will be discussed later, no differences in villin content, brush-border hydrolase-specific activities, and basolateral NHE1 expression were observed between villus preparations of resected and transected animals. These data strongly suggested the comparability of villus cell preparations of the two groups, making any observed differences in NHE2 and NHE3 meaningful and reflective of specific cellular adaptive changes. Additionally, any increases observed in Na transport function would then be considered somewhat specific, since we know that other sorts of hypertrophy (e.g., Na-K-ATPase) are also increased by proximal bowel resection.

Tissues for morphometric analysis were fixed in neutral buffered formalin and stained with hematoxylin and eosin by the surgical pathology laboratories of the University of Chicago Hospitals. Morphometric analyses were collected from fixed sections of three separate rats, and the averages from each animal (n = 3 from each animal were first averaged) were used to determine the means and SE. Protein was measured in the light mucosal scrapings by the bicinchoninic acid procedure (35) and DNA using Hoechst 33258 reagent (4).

For immunohistochemical analysis of NHE2, paraffin sections were heated to 65°C for 30 min, paraffin was removed by xylene, and slides were rehydrated through graded ethanol washes. After a saline wash, antigen recapture was performed by microwaving in 0.1 M citrate (pH 6.0) four times. Slides were then treated using the Vector Elite staining procedure (Vector Laboratories, Burlingame, CA). Non-specific binding was blocked using goat serum and both avidin and biotin blocking solutions. Slides were incubated overnight with a 1:50 dilution of anti-NHE2 polyclonal antiserum that was developed and characterized by our laboratory (24). Slides were washed and developed with the ABC Elite Vector kit.

Brush-border membranes were prepared as previously described (6). A sample of both the crude homogenate and the purified brush-border membranes was saved for the analysis of succrase, alkaline phosphatase, and villin to determine enrichment factors. Succrase and alkaline phosphatase were measured as previously described (5, 6), and villin was determined by Western blotting using a specific monoclonal antibody (Transduction Laboratories, Lexington, KY). An aliquot of the basolateral membranes was saved for analysis of K-stimulated phosphatase activity. K-stimulated phosphatase was measured as previously described (15) in crude homogenates and the basolateral membrane fraction, which also contained endoplasmic reticulum and Golgi.

In a limited number of experiments, 80% of the small intestine was resected where 60 cm of intestine was removed compared with 40 cm for the 50% resections. These rats lost weight for the first 7 days (generally 40–50 g of 225 g body wt) but by day 10 were gaining 5–7 g/day and eating Chow at a comparable rate to 50% resected rats. For these studies, only NHE activities and protein levels were measured.

Measurement of intestinal NHE2 and NHE3 activities. The activity of NHE2 and NHE3 in the brush-border membranes was measured as previously described (6). For the present experiments, the 22Na uptakes were always performed with both HOE-694 (30 μM) and dimethylamiloride (DMA, 500 μM) so that NHE2 and NHE3 activities could be distinguished. Both exchangers are sensitive to DMA. At 1 mM Na, NHE2 is completely inhibited by the HOE amiloride analog at 30 μM, whereas NHE3 is inhibited <5%. Thus the former was defined as the HOE-694-sensitive and the latter as the HOE-694-insensitive component of the DMA-inhibitable uni-directional 22Na influx. Fluxes were measured under acid-loaded conditions as previously described (6).

Measurement of NHE2 and NHE3 protein expression. An aliquot of the brush-border membranes was analyzed using Western blots. The brush-border proteins were solubilized in Laemml stop solution and resolved on a 7.5% SDS-PAGE. Proteins were immediately transferred to a polyvinylidene fluoride membrane and blocked with 5% wt/vol nonfat milk in Blotto [composition in mmol/l: 150 NaCl, 5 KCl, and 10 Tris (pH 7.4) with 0.05% Tween 20]. Blots were incubated with specific polyclonal antibodies developed and characterized in our laboratory to NHE2 and NHE3 (1, 24). Blots were visualized using an enhanced chemiluminescence system. Because NHE1 is a basolateral and not a brush-border membrane protein (1), a different membrane fraction was used. Scraped mucosa was homogenized and spun at 500 g (5 min at 4°C) to remove nuclei and unbroken cells. The supernatant was spun at 10,000 g (10 min at 4°C) to pellet mitochondria, and the resulting supernatant was spun at 100,000 g (20 min at 4°C) to obtain a membrane fraction that contained the plasma membranes (both apical and basolateral) and the endoplasmic reticulum and Golgi. Because of the less purified nature of these membranes, 50 μg were generally analyzed for Western blots.

Quantification of NHE mRNA expression. In some experiments, the intestinal scrapings were immediately placed into Trizol and homogenized using an Ultra-Turrax at maximum speed for 20 s. RNA was isolated from Trizol according to the manufacturer’s instructions and extracted one additional time using acid phenol-chloroform to remove any remaining DNA and protein. Immediately before analysis, the RNA was repelleted and quantitated by the absorbance at 260 nm. RNA (20 μg) was size-separated on a formaldehyde-denaturating agarose gel using a MOPS buffer system described previously (1). The RNA was transferred to a positively charged nylon membrane overnight, and the RNA was cross-linked to the membrane by ultraviolet irradiation. The blots were analyzed for NHE2, NHE3, and the constitutive probe glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using an XOTCH hybridization solution (1). For NHE2, the probe consisted of a section of a coding region (bases 1564–2540), and, for NHE3, a full-length probe of the coding region was used. The cDNA probes used for NHE2 and NHE3 have been validated previously by our laboratory to be specific for their respective isoform (1). GAPDH mRNA abundance was used to normalize data, using a full-length murine GAPDH cDNA probe obtained from American Tissue Culture Collection. Blots were always hybridized overnight and washed up to a stringency of 0.5× saline-sodium citrate and 0.5% SDS at 55°C.

RESULTS

Regional brush-border hydrolase and villin expression after resection or transection. Because MSBR is reported to cause hypertrophy of the intestine at and around the anastomotic area (29, 30), samples were always taken well away from this area and from the
Effect of 50% proximal small bowel resection

Accurate cell counts could not be determined, since intestinal enterocytes scraped off as sheets tend to clump. Therefore, DNA was measured in the homogenates to assess cell number. As shown in Table 2, both protein and DNA per length increased in the portion of the remnant small intestine corresponding to the ileum (Table 2). No difference was observed in the protein-to-DNA ratio from villus specimens harvested from corresponding segments (jejunum, ileum, and proximal colon). Because cellular hypertrophy would be associated with an increased protein-to-DNA ratio, these data argue against this possibility. On the other hand, the results presented in Table 1 (increased villus length) and Table 2 (increased protein and DNA/length of distal small intestine remnant) are consistent with the mucosal hypertrophy, particularly in villus regions.

Contributions caused by mucosal hypertrophy, such as altered numbers of crypt cells, smooth muscle, and other stromal elements, increased mucosal surface area, and changes in bowel length can be eliminated by performing subsequent analyses only on villus cell preparations. Observed changes in apical NHE function or activity will therefore reflect specific changes in intestinal epithelial (villus cell) adaptation and not increases in total function resulting from generalized mucosal hypertrophy. Activities of the brush-border enzymes sucrase and alkaline phosphatase and the protein expression of the microvillus protein villin were then determined in crude homogenates and purified brush borders prepared from ileum of both transected and resected rats. When these results are normalized to milligram protein, no differences in the specific activities of sucrase or alkaline phosphatase activities of villus scrapings were observed in any regions of the small bowel of either group. Of note, the degrees of enrichment of brush-border membranes prepared from both groups were similar (presented below each brush-border membrane value for alkaline phosphatase, sucrase, or villin in Fig. 2). The only enzyme marker that changed significantly was Na-K-ATPase, a basolateral protein, which occurred only in sections distal to the anastomosis of resected animals. These data demonstrate the comparability of brush-border membrane preparations between the two experimental groups. Thus the observed changes corroborate many of those previously reported and indicate that intestinal adaptation did indeed take place after MSBR. However, when villus specimens prepared from light mucosal scrapings from the two groups were compared, no differences in the specific activities of epithelial cell markers (brush-border hydrolase, villin expression) could be observed (Fig. 2C).

MSBR selectively increases brush-border membrane NHE2 and NHE3 activities and expression distal to the anastomosis. To determine the effects of bowel resection on regional intestinal mucosal NHE2 or NHE3 activity, measurements of brush-border NHE activity

same position in all rats. Morphometric analysis revealed adaptive changes similar to those reported previously by other groups (43, 45), characterized by villus hypertrophy distal to the anastomosis (see Table 1). To further characterize the adaptive changes, length and weight of the intact intestine and protein and DNA of the mucosal scrapings (a predominantly villus cell preparation) were analyzed. As shown in Table 2, there were no changes in length per weight or protein per DNA in the proximal small intestine or in the colon from rats that underwent 50% MSBR. In the small intestine distal to the anastomosis, there was a small increase in the weight/length that was not statistically significant. Accurate cell counts could not be determined, since intestinal enterocytes scraped off as sheets tend to clump. Therefore, DNA was measured in the homogenates to assess cell number. As shown in Table 2, both protein and DNA per length increased in the portion of the remnant small intestine corresponding to the ileum (Table 2). No difference was observed in the protein-to-DNA ratio from villus specimens harvested from corresponding segments (jejunum, ileum, and proximal colon). Because cellular hypertrophy

Table 1 Effect of resection on mucosal morphology

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Transected</th>
<th>50% Resected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Circumference</td>
<td>1.25 ± 0.09</td>
<td>1.41 ± 0.16</td>
</tr>
<tr>
<td>Villus Length</td>
<td>0.51 ± 0.04</td>
<td>0.64 ± 0.09*</td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crypt Length</td>
<td>0.10 ± 0.02</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Muscle Thickness</td>
<td>0.06 ± 0.02</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Villus Width</td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Circumference</td>
<td>0.91 ± 0.11</td>
<td>1.29 ± 0.16*</td>
</tr>
<tr>
<td>Villus Length</td>
<td>0.37 ± 0.09</td>
<td>0.56 ± 0.10*</td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crypt Length</td>
<td>0.15 ± 0.02</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>Muscle Thickness</td>
<td>0.09 ± 0.02</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Villus Width</td>
<td>0.13 ± 0.02</td>
<td>0.15 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE for separate determinations in 3 rats in each group. *P < 0.05 compared with same segment in transected group by paired Student’s t-test.

Table 2 Effect of 50% proximal small bowel resection on protein/DNA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Transected</th>
<th>50% Resected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length/weight of intact tissue, (mg/cm)</td>
<td>8.1 ± 0.6</td>
<td>8.6 ± 1.1</td>
</tr>
<tr>
<td>Ileum</td>
<td>7.6 ± 0.8</td>
<td>8.8 ± 1.5</td>
</tr>
<tr>
<td>Colon</td>
<td>4.7 ± 0.5</td>
<td>4.9 ± 0.7</td>
</tr>
<tr>
<td>Protein/length in mucosal scrapings, (µg/cm)</td>
<td>494.9 ± 53.1</td>
<td>613.1 ± 67.0</td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>422.6 ± 41.1</td>
<td>742.7 ± 83.7*</td>
</tr>
<tr>
<td>Colon</td>
<td>205.4 ± 24.4</td>
<td>236.2 ± 26.9</td>
</tr>
<tr>
<td>DNA/length in mucosal scrapings, (µg/cm)</td>
<td>4.26 ± 0.51</td>
<td>5.09 ± 0.46</td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>3.66 ± 0.38</td>
<td>6.81 ± 0.44*</td>
</tr>
<tr>
<td>Colon</td>
<td>1.97 ± 0.44</td>
<td>2.17 ± 0.46</td>
</tr>
<tr>
<td>Protein/DNA in mucosal scrapings, (µg protein/ µg DNA)</td>
<td>116 ± 8</td>
<td>120 ± 6</td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>115 ± 8</td>
<td>109 ± 8</td>
</tr>
<tr>
<td>Colon</td>
<td>103 ± 8</td>
<td>108 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE for 3 separate determinations from 3 rats in each group. Protein and DNA were measured in mucosal homogenates as described in MATERIALS AND METHODS. *P < 0.05 compared with same segment in transected group by paired Student’s t-test.
were performed in brush-border membrane vesicles under conditions of a transmembrane acid gradient. Extravesicular and intravesicular pH values were maintained at 7.4 and 6.1, respectively. Fluxes were measured as previously described, where NHE2 and NHE3 activities were defined as the HOE-694-sensitive and -insensitive components of DMA-inhibitable $^{22}$Na influx. Resection of the proximal 50% of bowel caused a large increase in the activities of both NHE2 (Fig. 3A) and NHE3 (Fig. 3B) in the distal portion of the remnant small intestine. NHE2 activity increased 92 ± 11%, whereas NHE3 activity increased 89 ± 14%. These changes occurred in distal ileal segments, since great care was taken to avoid inclusion of segments immediately distal to the anastomosis (i.e., samples were always taken 5 cm away from the anastomosis). Similarly, the proximal small intestinal mucosal samples excluded regions immediately adjacent to the anastomosis. No changes in NHE2 or NHE3 activities were observed in the most proximal portion of the small intestine or in the colon.

As shown in Fig. 4, the region-specific increases in brush-border NHE2 and NHE3 activity were paralleled by increases in brush-border membrane NHE2 and NHE3 protein expression. Again, significant changes of greater than twofold were observed only in the ileal regions distal to the anastomosis ($P < 0.05$, $n = 4$, densitometric analysis is shown in Fig. 4, right).
These changes were specific, since the villin content of loaded samples was essentially no different in corresponding regional segments of resected and transected groups (Fig. 2). Furthermore, as mentioned previously, brush-border hydrolase activities of corresponding segments from resected and transected rats were not significantly different. In contrast to NHE2 and NHE3, no significant changes in mucosal NHE1 protein expression were noted, as shown in the representative blot shown in Fig. 4.

To determine if proximal resection altered the expression of apical NHEs along the villus-to-crypt axis (recruitment phenomenon), immunohistochemical analyses using an affinity-purified anti-NHE2 polyclonal antiserum were performed. As can be observed in Fig. 5, NHE2 is specifically expressed at the luminal and subapical membranes of villus cells but not by crypt cells of transected and resected rat ileum, i.e., expression increasing abruptly at the villus-crypt junctions. Because affinity-purified anti-NHE3 was not available at the time of these studies, NHE3 immunohistochemistry was not performed.

To determine if larger resection of the small intestine would upregulate NHE2 and NHE3 to a greater extent or more distally, an 80% bowel resection was performed. Brush-border NHE2 and NHE3 activities and protein levels increased in the small intestine distal to the anastomosis. The increases observed in the distal small intestine after 80% resection were slightly but not significantly greater compared with the increases after 50% resection (increased ileal NHE2 105 ± 32% and NHE3 142 ± 28%, n = 3). However, NHE2 and NHE3 activities were increased in the proximal colon after 80% resection (Fig. 6), whereas no increases were observed in this region after 50% resection (increase in the colon for NHE2 112 ± 52% and NHE3 161 ± 42%, n = 3). Western blots of brush-border membranes from rats that had 80% resection are shown in Fig. 7. Increases in both NHE2 and NHE3 were noted in both ileal (117 ± 33%, n = 3) and colonic (134 ± 39, n = 3) values in corresponding portions of the resected rats.

Fifty percent small bowel resection upregulates NHE2 and NHE3 mRNA abundance. Northern blots for NHE2 and NHE3 mRNA were performed to determine if changes corresponding to altered protein and activity of the respective isoforms occurred. As shown in Fig. 8, the mRNA for both NHE2 and NHE3 increased only in the distal portion of the remnant small intestine, corresponding to the region where activity and protein expression of these isoforms were observed. However, densitometric quantitation of these changes revealed a slightly greater degree of increase in NHE2 and NHE3 mRNA increased only in the distal portion of the remnant small intestine, corresponding to the region where activity and protein expression of these isoforms were observed. These data thus suggest that increased mRNA, possibly through transcription activation of the NHE2 and NHE3 genes, plays a role in the NHE adaptive process after MSBR.

DISCUSSION

The gastrointestinal tract has a remarkable adaptive capacity to restore digestive and absorptive functions after injury or MSBR. This process is in part mediated by increased mucosal hypertrophy, typified by greater villus height and size and enlargement of crypt depth (2, 27, 43, 44). In addition, studies have reported increases in luminal circumference, size of intestinal folds, and cells per unit length of bowel. All these findings would contribute to an overall increase
in absorptive surface area and capacity (2, 27, 44). However, controversy continues as to whether specific cellular functions for nutrient and electrolyte transport are upregulated as an additional mechanism of mucosal adaptation after intestinal resection. Several studies have suggested that certain cellular function may be decreased despite an overall increase in intestinal absorptive capacity. For instance, one study reported decreased glucose and amino acid transport when data were normalized to intestinal dry weight (25). Brush-border disaccharidase abundance and activity have also been noted to be variably changed after resection because of the normalization protocol used (3, 21). It has been speculated that the decrease in overall villus cell function is a consequence of a hyperproliferative response of the mucosa after resection, resulting in a greater proportion of relatively less well-differentiated villus enterocytes, and this may relate to the expression of proteins considered to be markers of the mature enterocyte.

Our studies, as well as those of others, would suggest that this hypothesis that resection leads to enterocytes with diminished function is incorrect. The present studies demonstrate upregulation of the two apical exchangers required for small intestinal Na absorption. Several investigators have reported increased cellular uptake of glucose in the remnant small bowel, believed to be because of a specific upregulation of glucose transporters, namely the apical Na-dependent transporter SGLT1 (14) and the basolateral glucose transporter GLUT2 (36). In fact, the upregulation of GLUT2 has been hypothesized to serve a dual function, facilitating glucose absorption and enhancing the ability of the enterocyte to obtain glucose from the blood. Furthermore, the specific activity of the Na-K-ATPase pump in the remnant ileum after small bowel resection is significantly increased compared with Mg-dependent ATPase, which was essentially unchanged (40). This observation was later confirmed by Hines et al. (18) and the present studies.

We believe that the apparent discrepancies in defining a role of the enterocyte in intestinal adaptation may be attributed to differences in methodology and experimental conditions. For instance, considerable differences exist among studies attributable in part to the way data were normalized. In some cases, conclusions were based on rough extrapolations or estimates of highly variable reference data, including measurements of crypt depth, villus length, and circumference. Data outcome can also be influenced by timing of observations after resection, choice of controls, and differences in the types or length of intestinal resection. For these reasons, we took great care to define experimental conditions to provide a definitive answer regarding the effects of proximal intestinal resection on enterocyte expression and function of apical Na/H exchange. Because of the known effects of surgical manipulation of bowel on intestinal mucosal function, rats undergoing intestinal transection were felt to be the most appropriate controls for assessing changes after intestinal resection. We also confined our studies to villus preparations made from mucosal scrapings, a technique that is commonly employed to yield a predominant villus cell harvest. Using this approach, we documented that the enrichment of brush-border membrane preparations was identical between resected and transected intestinal preparations and that the villin content and brush-border hydrolase activities of brush-border membranes from both groups were equivalent. Thus these findings provide justification for dependable comparative analysis of mucosal NHE function and expression between these two groups.
Fig. 6. NHE activities in brush borders from 80% resected and transected rats. $^{22}$Na uptakes were measured as described in MATERIALS AND METHODS under maximally stimulating conditions. $^{22}$Na influx for NHE2 was defined as that flux which was inhibitable by 30 μM HOE-694, whereas NHE3 was defined as the flux further inhibited by 500 μM dimethylamiloride. Data shown are means ± SE for 4 rats. For each rat, each value was determined in triplicate. BBMV, brush-border membrane vesicles. *P < 0.05 and **P < 0.01 compared with comparable segment by paired Student’s t-test.

Our studies demonstrate several important findings. First, they show that part of the intestinal adaptation after MSBR does involve selective and region-specific upregulation of enterocyte NHE3 and NHE2, but not NHE1, function and expression. No concomitant changes in brush-border hydrolase, villin expression, or DNA or protein content were noted. The increases in activity and protein levels for NHE2 and NHE3 agreed well; however, larger changes in mRNA levels were observed for both NHE isoforms. The changes in mRNA suggest that increased enterocyte NHE2 and NHE3 result from increased gene activation, but the possibility that additional posttranscriptional mechanisms are involved in regulating NHE3 expression cannot be ruled out.

The immunohistochemical analysis of NHE2 expression along the crypt-villus axis also argues against recruitment of NHE2-expressing enterocytes in crypt regions. Such a mechanism has been suggested for the increased SGLT1 expression observed in chronically diabetic rats that demonstrate villus hypertrophy (12). Our studies fail to show a similar phenomenon for apical NHEs in MSBR. Moreover, these changes occurred in the absence of changes in specific activities of brush-border sucrase and alkaline phosphatase, NHE1 expression, and villin content. We therefore feel that the specific upregulation of cellular apical NHE expression represents one form of intestinal adaptation after MSBR.

Previous studies in weanling rats have noted increased NHE activity in the small intestine after bowel resection (32), and more recent studies using a mouse model noted increased NHE3 protein and mRNA without a change in NHE2 (11). Neither of these studies investigated changes in the colon, which our studies would suggest do not occur until >50% resection. After 50% resection, it is unknown why the mouse did not upregulate NHE2 as we observed in the rat. The differences could be species dependent and may relate to the length of the bowel, the precise nature and placement of the resection, or differences in hormonal regulation of NHE2 between the species. EGF has been demonstrated to be a potent modulator of the response to resection and in the rat potently upregulates NHE2 (47). The studies in the mouse model did not report a detailed investigation of morphological or biochemical changes; thus, our studies may be useful, since NHE upregulation may be viewed as specific and since other brush-border enzymes like sucrase and alkaline phosphatase and the structural protein villin did not
change. Our studies suggest that there is a specific upregulation of apical NHE activity per cell (since brush-border protein was used as a denominator) and also that intestinal Na absorptive capacity is increased because of an increase in the numbers of villus enterocytes in the distal remnant of the small intestine (because of the increase in villus length).

The region-specific increases in apical membrane NHE expression and function after resection are particularly noteworthy and deserve comment. Increases were only observed in ileal segments distal to and distant from the site of anastomosis. The latter is an important distinction to make, since it is well known that persistent functional changes occur in mucosal regions at the site of anastomosis of transected or resected bowel. The increases we observed are therefore not part of this phenomenon. The increases in ileal NHE2 and NHE3 function and expression are also not characteristic of hyperaldosteronism, which can occur in response to prolonged volume depletion. Aldosterone stimulates increased NHE3 expression only in the proximal colon (5, 41) and has no effects on intestinal NHE2. Furthermore, serum aldosterone levels were measured in both transected and resected animals and were not found to be different (data not shown). Likewise, chronic increases in serum glucocorticoids can stimulate increases in ileal NHE3 expression but have no effects on intestinal NHE2. Thus these changes are not likely to be secondary to increased corticosteroid stimulation. In fact, intestinal NHE2 expression and function have been remarkably insensitive to most physiological perturbations and have only been reported to change during ontogeny (7). Therefore, the stimuli causing these postresection changes in NHE2 appear to be unique, albeit not understood presently.

One possibility for causing this phenomenon is the increased luminal Na load to the distal segment resulting from massive proximal bowel resection serving as a luminal cue. More distal, i.e., colonic NHE, expression did not occur because the ileal adaptation adequately compensated for the additional luminal Na load. Consistent with this notion is that, in a few animals that underwent 80% resection, increases in apical NHE2 and NHE3 were observed more distally, i.e., in colonic segments distal to the small bowel anastomosis (Fig. 6). These studies were not pursued because of the increased morbidity and mortality attended with more extensive bowel resection. Substrate-induced enhancement of membrane transporters has been observed previously, most notably for sugar transporters (13, 16, 26, 28, 29, 33, 42); there may be such an effect for electrolyte transporters. It should be noted that Dowling and Booth (9) proposed in 1967 that the adaptive hyperfunction response of the remnant ileum after proximal resection of the jejunum might be in large part the result of the presence of a nutrient-rich chyme never before seen by the ileum. The importance of humoral factors in the upregulation of apical NHEs cannot be dismissed and may also play an important role. A number of growth factors, including EGF (10, 17), insulin-like growth factors (22, 48, 49), and glucagon-like peptides (36), have all been demonstrated to be potent modulators of intestinal enterocyte growth and gene expression. Additionally, many genes are turned on in the enterocyte shortly after bowel resection (8, 31). These genes may be required in the generation of humoral factors involved in the adaptive response, or these genes may result in changes in transcription factors involved in the adaptive response. The nutritional status of the animal may also play a
role in the adaptive response, since modulation of glu-
tamine or short-chain fatty acids in the diet has also
been demonstrated to modulate the adaptive response
(29, 30, 33–34, 36–38, 48). It cannot be determined
from these studies whether these effects are direct or
indirect (e.g., through a stimulated increase of growth
factors). Growth factors could contribute to upregula-
tion of the apical NHEs and increased basolateral
Na-K-ATPase. Our studies, and previous studies (18),
have noted increased Na-K-ATPase after bowel resec-
tion. Because the Na-K-ATPase is required to maintain
low cell Na and therefore is essential for secondary
active transporters that use the Na gradient, we spec-
ulate whether increases in total Na pump activity can
induce apical NHE activity and protein expression.

In summary, we believe these data provide strong
support for a role of selective functional adaptation of
the enterocyte after MSBR. The region-specific changes
in apical membrane NHE2 and NHE3 are unique and
implicate a role for increased luminal Na or
nutrient-rich chyme in promoting this response in re-
gions distal to the anastomosis. These changes are
likely to be important in allowing the intestinal mu-
cosa to compensate for decreased absorptive surface
area resulting from proximal resection or disease.

This work was supported by National Institute of Diabetes
and Digestive and Kidney Diseases Grants DK-38510 and
DK-47722 (E. B. Chang) and DK-42086, the Gastrointestinal Research
Foundation of Chicago, and a grant from the Crohn’s and Colitis
Foundation of America.

REFERENCES

   MC, and Chang EB. Na/H exchangers NHE1 and NHE3, of rat
2. Bristol JB and Williamson RCN. Nutrition, operations, and
   intestinal adaptation. J Parenteral Enteral Nutr 12: 299–309,
3. Buts JP, DeKeyser N, and Dive C. Contribution of the
   rat small intestine after proximal enterectomy: changes in
   microvillus enzyme and in the secretory component of immuno-
4. Cesaroni CF, Bolognesi C, and Santi L. Improved microflu-
   orometric DNA determination in biological material using 33258
   K, and Chang EB. Aldosterone stimulates intestinal Na absorp-
   tion in rats by increasing NHE3 expression of the proximal colon.
6. Cho JH, Musch MW, DePaoli AM, Bookstein C, Xie Y,
   Burant CF, Rao MC, and Chang EB. Glucocorticoids regulate
   Na/H exchange activity and expression in region- and tissue-
specific manner. Am J Physiol Cell Physiol 267: C796–C803,
   1994.
7. Collins JF, Kiela PR, Xu H, Zheng J, and Ghishan FK.
   Increased NHE2 in rat intestinal epithelium during ontogeny is
   transcriptionally mediated. Am J Physiol Cell Physiol 275:
   MS. Analysis of cloned cDNAs differentially expressed in adapt-
   ing remnant small intestine after partial resection. Am J Physiol
9. Dowling RH and Booth CC. Structural and functional changes
   following small intestinal resection in the rat. Clin Sci 32:
   139–149, 1967.
10. Dunn JG, Parungo CP, Fonkalsrud EW, McFadden DW,
    and Ashley SW. Epidermal growth factor selectively enhances
    functional enterocyte adaptation after massive small bowel re-
    Soleimani M, and Warner BW. Differential expression of ileal
    Na+/H(+) exchanger isoforms after enterectomy. J Surg Res
12. Fedorak RN, Cheesman CI, Thomson AB, and Porter VM.
    Altered glucose carrier expression: mechanism of intestinal ad-
    aptation during streptozotocin-induced diabetes in rats. Am J
13. Ferraris RP and Diamond J. Regulation of intestinal sugar
14. Freeman HH, Ellis ST, Johnston GA, Kwan WA, and
    Quammo GA. Sodium-dependent D-glucose transport after
    proximal small intestinal resection in rat. Am J Physiol Gastro-
15. Garaefji PJ, Pouchan MI, and Rega AF. Potassium acti-
    vated phosphatase from human red blood cells. J Physiol
16. Hammond KA, Lam M, Lloyd KCK, and Diamond J. Simul-
    taneous manipulation of intestinal capacities and nutrient loads
    in mice. Am J Physiol Gastrointest Liver Physiol 271: G969–
    G979, 1996.
17. Holmworth MA, Shin CE, Erwin CR, and Warner BW. Intes-
    tinal adaptation is enhanced by epidermal growth factor inde-
    pendent of increased ileal epidermal growth factor receptor
    EE, Zinner MJ, and Ashley SW. Upregulation of Na-K adena-
    sine triphosphatase after massive small bowel resection. Sur-
    McFadden DW, and Ashley SW. Adaptation of the Na/glucose
cotransporter following intestinal resection. J Surg Res 57:
20. Hoogewerf WA. NHE2 and NHE3 are human and rabbit intes-
    tinal brush border proteins. Am J Physiol Gastrointest Liver
    Physiol 270: C94–C96, 1996.
    Small bowel disaccharidase activity in the rat as affected by
    intestinal resection and pectin feeding. Am J Clin Nutr 47:
22. Lund PK. Molecular basis of intestinal adaptation: the role
    of insulin-like growth factor system. Ann NY Acad Sci
23. Mather MM, Gontarek JD, Bess RS, Donowitz M, and Yeo
    CJ. The Na/H exchange isoform NHE3 regulates basal canine
24. McSweeney RL, Musch MW, Bookstein C, Xie Y, Rao MC,
    and Chang EB. Regulation of apical membrane Na/H exchangers
    NHE2 and NHE3 in intestinal epithelial cell line C2/bbe. Am
25. Menge H and Robinson JW. The relationship between the
    functional and structural alterations in the rat small intestine
    following proximal resection of varying extents. Res Med (Berl)
    and Brot-Laroche E. Sugar-dependent expression of the fruc-
    tose transporter GLUT5 in Caco-2 cells. Biochem J 312:
    outcome of massive small bowel resection. Am J Gastroenterol
28. Miyamoto KI, Hase K, Takagi T, Fuji T, Takekata Y, Mi-
    nami H, Oka T, and Nakabow Y. Differential responses of
    intestinal glucose transporter mRNA transcripts to levels of
29. O’Connor TP, Lam MB, and Diamond J. Magnitude of func-
    tional adaptation after intestinal resection. Am J Physiol Regul
30. Robinson MK, Ziegler TR, and Wilmore DW. Overview of
    intestinal adaptation and its stimulation. Eur J Ped Surg


