Induction of epithelial Na\textsuperscript{+} channel in rat ileum after proctocolectomy

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Koyama, Kaori, Iwao Sasaki, Hiroo Naito, Yuji Funayama, Kouhei Fukushima, Michiaki Unno, Seiki Matsuno, Hisayoshi Hayashi, and Yuichi Suzuki. Induction of epithelial Na\textsuperscript{+} channel in rat ileum after proctocolectomy. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G975–G984, 1999.—In patients with colectomy, epithelial transport function in the remnant small intestine can be regulated in response to the increased fecal electrolyte and fluid loss. Using a rat colectomy model, we investigated the Na\textsuperscript{+} and K\textsuperscript{+} transport mechanisms underlying the intestinal response. Proctocolectomy with ileoanal anastomosis was performed on rats. The small intestinal mucosa was mounted in Ussing chambers; then short-circuit currents and \textsuperscript{22}Na\textsuperscript{+} fluxes were measured. mRNA expression of the epithelial Na\textsuperscript{+} channel (ENaC) was determined by Northern blotting. Amiloride-sensitive, electrogenic Na\textsuperscript{+} absorption appeared in the ileum after proctocolectomy. This functional change was accompanied by the chronological induction of mRNAs for \(\alpha\), \(\beta\), and \(\gamma\)-subunits of the ENaC in the ileum. Tetraethylammonium-sensitive short-circuit current was also activated. We conclude that electrogenic Na\textsuperscript{+} absorption and probably K\textsuperscript{+} secretion are induced in the ileum after proctocolectomy. This induction of electrogenic Na\textsuperscript{+} absorption is probably mediated by the increase in the mRNA levels for all three types of subunits of the ENaC and may contribute to the recovery from the increased fecal Na\textsuperscript{+} loss.

\begin{itemize}
  \item aldosterone; potassium secretion; sodium absorption; intestinal adaptation; intestinal absorption
\end{itemize}

EVIDENCE IS ACCUMULATING that intestinal transport of fluid and electrolytes is regulated not only to cope with intestinal functions but also for the purpose of maintenance of fluid and electrolyte balance in the whole body (27, 29). This can be clearly seen in patients undergoing colectomy whose fluid and electrolyte balance is disturbed because of loss of the absorptive function of the colon (37, 41). Episodes of fluid and electrolyte depletion in such patients are associated with a decrease in the volume and the Na\textsuperscript{+} concentration of the effluent and an increase in K\textsuperscript{+} concentration (10, 19, 32, 37). Some evidence suggests that this “intestinal adaptation” is a result of the regulation of epithelial Na\textsuperscript{+} and K\textsuperscript{+} transport in the remnant small intestine, particularly in the ileum (10, 25, 28, 37, 50, 51), but the precise mechanism involved in this regulation remains to be clarified. It is speculated that increases in the aldosterone level may mediate this regulation (10, 21, 33). Detailed characterization of this pathophysiological regulation of intestinal transport after colectomy may result in further insight into the role of the small intestine in control of the overall fluid and electrolyte balance of the organism. Also, it may result in improvement of the clinical treatment of patients requiring colectomy.

Aldosterone has been known to activate amiloride-sensitive, electrogenic Na\textsuperscript{+} absorption as well as K\textsuperscript{+} secretion in the colon and distal nephron (4, 7, 40). The regulation of electrogenic Na\textsuperscript{+} absorption by aldosterone involves the activation of the amiloride-sensitive, epithelial Na\textsuperscript{+} channel (ENaC), which mediates Na\textsuperscript{+} entry through the apical membrane, due to induction of the ENaC and other regulatory proteins (2, 3, 8, 9, 11, 13, 20, 34, 35, 39, 44). The ENaC consists of three homologous subunits, \(\alpha\), \(\beta\), and \(\gamma\), and it has been shown that the simultaneous expression of these three subunits causes a large Na\textsuperscript{+} current (5, 9, 36, 38a).

Reports have shown that an amiloride-sensitive, electrogenic Na\textsuperscript{+} absorption can be evoked in the lower small intestine of rats and chickens when the animals are salt restricted or treated with aldosterone (22, 48). Evidence for the presence of a mineralocorticoid receptor, as well as 11\textbeta-hydroxysteroid dehydrogenase type II, has implicated the small intestine, particularly the lower small intestine, as a mineralocorticoid-targeted tissue (17, 18, 38, 43). These findings suggest that both amiloride-sensitive, electrogenic Na\textsuperscript{+} absorption and possibly K\textsuperscript{+} secretion can be induced by aldosterone in the remnant ileum and contribute to the intestinal adaptation in colectomized patients.

The purpose of this study was to verify these possibilities. Using a rat model, in which proctocolectomy with ileoanal anastomosis was performed, both Na\textsuperscript{+} absorption and K\textsuperscript{+} secretion in the remnant small intestine were examined in vitro using Ussing chambers. Changes in mRNA levels of the three ENaC subunits in the remnant small intestine were also examined.

MATERIALS AND METHODS

Animals and surgical procedures. Male Sprague-Dawley rats were housed in a temperature-controlled room on a 12:12-h light-dark cycle and fed with standard laboratory chow (Funabashi F11, Funabashi Farm, Funabashi, Jap) and water ad libitum. When rats reached 8 wk old (300–350 g), proctocolectomy was performed under pentobarbital anesthesia (pentobarbital sodium, 50 mg/kg body wt, intraperito-
na samples were dried in an oven at 105°C for 24 h. To determine concentrations in the fecal water. The water content of feces group of rats was fed a low-Na diet for 7–10 days. Experimen
tal protocols used in this study were strictly followed according to the guidelines of the Committee for the Care and Use of Laboratory Animals of Tohoku University and the University of Shizuoka. Intestinal and urinary excretions and serum corticosteroid levels. Freshly discharged feces were collected between 9:00 AM and 11:00 AM. Part of the sample was used for determining water content and the rest for determining Na⁺ and K⁺ concentrations in the fecal water. The water content of feces was determined from the decrease in the fecal weight after samples were dried in an oven at 105°C for 24 h. To determine Na⁺ and K⁺ concentrations, feces were diluted with H₂O by fourfold (wt/wt), agitated on a Vortex mixer, and centrifuged (for 5 min at 1,000 rpm). With the resultant supernatants, Na⁺ and K⁺ concentrations were determined by ion chromato
tography using an ion-exchange column (Shim-pack IC-C1, Shimadzu, Tokyo, Japan), and a conductivity detector (CDD6-A, Shimadzu). Urine was collected for 24 h, and its volume was determined. Na⁺ and K⁺ concentrations were determined as mentioned above.

To determine the plasma corticosteroid level, the animals were anesthetized with ether and blood was collected from the abdominal aorta between 12:00 AM and 2:00 PM (to avoid diurnal variation). Concentrations of plasma aldosterone and corticosterone were determined by RIA kits obtained from Dainabot (Tokyo, Japan).

Short-circuit current and ²²Na⁺ flux measurements. The short-circuit current (Isc) was measured in vitro in Ussing chambers. The rats were killed by a blow to the head followed by exsanguination. Segments (2 cm) were isolated from areas 1–3 cm (designated as the terminal ileum), 14–16 cm, and 24–26 cm from the end of the ileum (i.e., from the ileocecal junction in the nonoperated group and from the ileocolonic anastomosis in the proctocolectomy group, respectively). The last segment was the area approximately two-thirds of the distance from the ligament of Treitz to the end of the ileum. The isolated segment was opened and rinsed free of intestinal contents, and the external muscular layer was removed by blunt dissection. The tissue was then mounted vertically between acrylic resin chambers with an internal surface area of 0.5 cm². Bathing solution in each chamber was 10 ml and was kept at 37°C in a water-jacketed reservoir. The mucosal solution contained (in mM) 119 NaCl, 21 NaHCO₃, 2.4 K₂HPO₄, 0.6 K₂HPO₄, 1.2 CaCl₂, 1.2 MgCl₂, and 8.5 mannanse. The serosal solution had the same composition as that of the mucosal solution, except that it contained 2.5 mM glutamine, 5 mM glucose, and 1 mM β-hydroxybutyrate (Na⁺ salt) instead of mannanse. Each solution was gassed with 95% O₂ and 5% CO₂ (pH 7.4).

Tissues were continuously short-circuited, with a compensation for fluid resistance between the two potential-sensing bridges, using a voltage-clamping amplifier (CEZ9100, Nihon Kohden, Tokyo, Japan). The transepithelial potential was measured through 1 M KCl-agar bridges connected to a pair of calomel half-cells, with the transepithelial current applied across the tissue through a pair of Ag-AgCl electrodes kept in contact with the mucosal and serosal bathing solutions using a pair of 1 M NaCl-agar bridges. The asymmetric potential from the pair of calomel half-cells used for the potential measurement was less than ±3 mV and changed by less than ±0.2 mV during each experiment. The Isc value was expressed as positive when the current flowed from the mucosa to serosa. Transmural tissue resistance (Gt) was calculated from the change in current in response to voltage pulses according to Ohm’s law.

Unidirectional transmural ²²Na⁺ fluxes were measured in Ussing chambers under short-circuit conditions. The mucosal-to-serosal (Jₘ→s) and serosal-to-mucosal (Jₛ→m) fluxes were measured in the adjacent tissues that had Gt values differing by <±30%. Thirty minutes were allowed for isotopic steady state to be reached after labeling either the serosal or mucosal side of the bathing solution with 100 kBq of ²²Na⁺.

Six samples (0.5 ml each) were taken from the unlabeled side at 15-min intervals and replaced with an equal volume of the unlabeled solution. Medium samples were assayed for ²²Na⁺ in a gamma-well spectrometer. ²²Na⁺ was purchased from DuPont NEN (Boston, MA).

Complementary DNA probes were prepared by using reverse-transcribed cDNA of rat colon as the PCR template. Primer sets specific for the α-subunit [sense: 5'-ATGGTAGCGATGTC-CCGTCAGAAAG-3' (1327–1351), antisense: 5'-AGCAGACGT- GTAGCCCCGTCTCCT-3' (2330–2306)], β-subunit [sense: 5'-CTTGGCTGTCGGGAGAAATCTG-3' (1286–1310), antisense: 5'-GGAGACTATAGGGTAGGTGGATG-3' (1932–1908)], and γ-subunit [sense: 5'-CTCTCTATCATCGCGCCGGCTAGT-3' (2373–2397), antisense: 5'-GTCAAAATGATCCCCAGGCTCT-3' (2573–2549)] of the rat epithelial Na⁺ channel (rENaC) were synthesized on the basis of each cDNA sequence. Each pair of primers was used for PCR amplification. The PCR products were electrophoresed, purified, and subcloned into pBluescript (Stratagene, La Jolla, CA). Finally, we confirmed the sequences of the PCR products using a sequenc
ing kit (United States Biochemical, Cleveland, OH). Complementary DNA probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was prepared as described previously (1).

Forty micrograms of total RNA were electrophoresed on 1% agarose gel and transferred onto nylon membrane (Hy
bond-N, Amersham). Complementary DNA probes for α-, β-, γ-rENaC and GAPDH were labeled with ³²P using the Megaprime DNA labeling system (Amersham). After a 2-h prehybridization, the blot was hybridized overnight with labeled cDNA probes at 42°C in hybridization buffer containing 50% formamide, 5× sodium chloride-sodium phosphate-EDTA (SSPE), and 200 µg/ml denatured herring DNA. The blot was subjected to the following procedures: 1) washed with 2× sodium chloride-sodium citrate (SSC) and 0.1% SDS at room temperature for 15 min (twice), 2) washed with 2× SSPE and 0.1% SDS or 0.1× SSC and 0.1% SDS at 42°C for 15 min, and 3) rinsed with 0.1× SSC and 0.1% SDS at room temperature. The blot was exposed to autoradiography at −70°C or visualized using a BAS imaging analyzer (Fuji Film, Kanagawa, Japan). The same blot was washed in 0.02× SSC-1% SDS solution at 100°C and rehybridized with different probes.
Statistics. Values are presented as means ± SE, with n representing the number of animals. Statistical comparisons between two means were made with the Student’s t-test (paired or unpaired, as appropriate), whereas multiple comparisons were made with one-way ANOVA followed by Fisher’s test. Significance was accepted at P < 0.05.

RESULTS

Body weight, food and water intake, and Na⁺, K⁺, and water excretion in urine and feces after proctocolectomy. The body weight of proctocolectomized rats was decreased to 68 ± 5% of preoperative levels at 2 wk (P < 0.05) and was maintained until 8 wk after surgery (72 ± 5% of preoperative weight, n = 8). The food intake during this postoperative period was not significantly different from that at the preoperative level (preoperative = 19.1 ± 0.4, 2 wk = 17.6 ± 0.9, 8 wk = 17.8 ± 0.5 g/day). The water intake was markedly increased after proctocolectomy (preoperative = 24 ± 3, 2 wk = 48 ± 4 ml/day; P < 0.05) and sustained at this increased level until 8 wk after surgery (50 ± 4 ml/day). On the other hand, nonoperated 12-wk-old rats (age matched to the proctocolectomized animals at the 4-wk time point) exhibited a weight gain of 48 ± 2% during the 4-wk period, a food intake of 18.5 ± 1.6 g/day, and a water intake of 23 ± 1 ml/day (n = 4). Thus the proctocolectomy did not change the food intake but reduced the efficiency of the weight gain per gram of food intake and increased the water intake.

Urine volume was slightly but significantly decreased after the proctocolectomy (Fig. 1A). Both urine Na⁺ and K⁺ concentrations were decreased considerably and showed little recovery by 8 wk after surgery (Fig. 1B).

Fecal water content 2 wk after proctocolectomy was increased by approximately threefold compared with that at preoperative levels (Fig. 2A). The increased fecal water content was not significantly altered until 8 wk. The concentrations of Na⁺ and K⁺ in the fecal water were increased considerably 2 wk after surgery (Fig. 2B). However, at 4 wk, the increase in fecal Na⁺ concentration was significantly attenuated, and, at 8 wk, the increase was not statistically significant compared with that at preoperative levels. In contrast, the K⁺ concentration in the fecal water remained elevated until 8 wk after surgery.

Plasma corticosteroid level. The plasma aldosterone level was elevated by 30-fold 1 wk after surgery (Fig. 3A). It continued rising thereafter, achieving a level 80-fold higher than that at the preoperative level, 8 wk after surgery. In contrast, the plasma corticosterone level had not changed after the surgery (Fig. 3B).

Isc measurements in the small intestine. We examined whether an amiloride-sensitive, electrogenic Na⁺ absorption was functionally activated in the ileum after proctocolectomy. No significant change in transmural Gₑ could be detected in association with the Isc increase by 10.220.32.247 on April 1, 2017 http://ajpgi.physiology.org/ Downloaded from G977

suggesting that an amiloride-sensitive, electrogenic Na⁺ absorption was functionally activated in the ileum after proctocolectomy. No significant change in transmural Gₑ could be detected in association with the Isc increase by 10.220.32.247 on April 1, 2017 http://ajpgi.physiology.org/ Downloaded from G977

Isc increase by TEA (see Table 1 for the values of the electrical parameters). The concentration dependency of the effect of amiloride on Isc showed an IC₅₀ of 0.45 μM (Fig. 5). The inhibitory effect of amiloride on Isc in the ileum of proctocolectomized rats was similar to that observed in the distal colon of unoperated rats treated with a low-Na⁺ diet (Fig. 5).

When tetraethylammonium (TEA), a K⁺ channel inhibitor, was added to the mucosal side (2 mM, Cl⁻ salt) after amiloride treatment, Isc increased in the terminal ileum of proctocolectomized, but not control, rats (Fig. 4). A change in Gₑ was not detected with the Isc increase by TEA (see Table 1 for the values of the electrical parameters). The TEA-induced Isc increase was completely abolished when the tissue was pre-treated with serosal bumetanide (0.1 mM), a Na⁺-K⁺-2Cl⁻ cotransport inhibitor (data not shown, 4 proctocolectomized rats). Bumetanide alone increased Isc (17.1 ± 12.1 μA/cm²). These results suggest that the bumetanide-inhibitable, TEA-sensitive, electrogenic K⁺...
secretion documented in the mammalian distal colon (7, 45) was also activated in the terminal ileum of proctocolectomized rats.

Table 1 summarizes the time course of changes in electrical parameters in the terminal ileum (1–3 cm from ileoanal anastomosis) of proctocolectomized rats. The basal $I_{sc}$ was slightly, but not significantly, increased after proctocolectomy (the increase in basal $I_{sc}$ reached a significant level in another series of experiments, as shown in Table 2). Amiloride-sensitive $I_{sc}$ was induced after surgery. The values of $I_{sc}$ were comparable among 2, 4, and 8 wk. Similarly, the $I_{sc}$ increase caused by the 2 mM TEA was not significantly altered during this period. Noticeably, the $G_0$ was gradually reduced after proctocolectomy.

We next examined the distribution of the amiloride-sensitive $I_{sc}$ and TEA-sensitive $I_{sc}$ in the ileum after proctocolectomy (Fig. 6). In the terminal ileum, the values of the $I_{sc}$ decrease at 2 and 4 wk after proctocolectomy caused by the 0.1 mM amiloride treatment were not different. In contrast, in the region 14–16 cm from the anastomosis, the value of the amiloride-sensitive $I_{sc}$ was rather low at 2 wk, but it was significantly increased by 4 wk after surgery. In the region 24–26 cm from the anastomosis (intestinal segment located approximately at two-thirds from the ligament of Treitz to the end of the ileum), an amiloride-sensitive $I_{sc}$ was not observed at either 2 or 4 wk. A similar time-dependent change in distribution pattern was also observed for the value of the $I_{sc}$ increase induced by 2 mM TEA (Fig. 6B). Thus amiloride-sensitive $I_{sc}$ and TEA-sensitive $I_{sc}$ are activated apparently in parallel, and they emerge first in the terminal ileum region and then spread to the more proximal part of the ileum, thereby probably increasing their capacities.

A transport study on the more proximal segments of the small intestine was impossible to accomplish because the integrity of these tissues appeared to decline in Ussing chambers, as judged from the absence of glucose-induced $I_{sc}$. This is in contrast to the brisk and reproducible $I_{sc}$ response to luminal glucose application in the ileum; the reason for this differing viability among the different intestinal segments is not known.

We determined bidirectional $^{22}\text{Na}^+$ flux measurement in the terminal ileum. We determined bidirectional $^{22}\text{Na}^+$ fluxes in the terminal ileum of control and proctocolectomized rats using benzamil (10 µM), a more specific inhibitor for the
ENaC than amiloride (3, 20) (Table 2). Neither $^{22}\text{Na}^+$ absorption nor $I_{sc}$ was affected by mucosal benzamil in control rats. In contrast, in proctocolectomized rats, $^{22}\text{Na}^+$ absorption (and $I_{sc}$) was significantly decreased by mucosal benzamil, mainly due to a decrease in $J_m$. Therefore, the amiloride (benzamil)-sensitive, electrogenic Na$^+$ absorption is actually activated after proctocolectomy. A dosage of 10 µM benzamil is probably the maximum necessary, since benzamil is more potent than amiloride and TEA, values were not significantly different at 2, 4, and 8 wk after surgery. ANOVA. In the proctocolectomized rats, for both $\alpha$- and $\gamma$-subunit rENaC mRNAs were all increased in a parallel manner and almost reached a maximum at 4 wk after proctocolectomy. Thus these increases in rENaC mRNA levels apparently corresponded well with the increase in the capacity of electrogenic Na$^+$ absorption.

**DISCUSSION**

The present study investigated the regulation of Na$^+$ and K$^+$ transport in the small intestine after proctocolectomy in rats. The results have shown de novo induction of an amiloride-sensitive, electrogenic Na$^+$ absorption.
in the lower small intestine after proctocolectomy in a time-dependent fashion, demonstrated by both molecular and electrophysiological methods. The increases in the amount of mRNAs of the α-, β- and γ-ENaC subunits and in the capacity of the amiloride-sensitive, electrolytic Na⁺ absorption occurred in a parallel fashion, indicating that electrolytic Na⁺ absorption can be activated in the remnant ileum through the induction of the rENaC mRNA. The significant recovery from the elevation of the fecal Na⁺ concentration, particularly during the period between 2 and 4 wk after proctocolectomy (Fig. 2B), is possibly explained by this molecular and functional regulation of Na⁺ absorption in the terminal ileum.

The plasma aldosterone level, but not the corticosterone level, markedly increased after proctocolectomy. The increased aldosterone levels observed in this study could be one of the factors responsible for the induction of rENaC mRNA and the activation of electrolytic Na⁺ absorption in the ileum after proctocolectomy. The presence of a mineralocorticoid receptor (17, 18) and 11β-hydroxysteroid dehydrogenase type II (38, 43) has been demonstrated in the ileum, supporting the hypothesis that the lower small intestine is a target organ for aldosterone. Actually, it has been reported that an amiloride-sensitive, electrolytic Na⁺ absorption can be observed in the lower small intestine of rats and chickens when the animals are fed a salt-depleted diet or when administered aldosterone (22, 48). The plasma aldosterone level in these Na⁺-depleted rats was reported to be 13 ng/ml, which is comparable to the levels observed in the proctocolectomized rats of the present study (48). We have recently demonstrated in the rat ileum in vitro in Ussing chambers that the application of aldosterone to the bathing solution can induce amiloride-sensitive Iₛₑ (31). It remains to be demonstrated, however, whether aldosterone may also upregulate all three rENaC subunits in the ileal mucosa. The amiloride-sensitive Iₛₑ was observed only in the terminal ileum at 2 wk after proctocolectomy but still was detected in further proximal parts of the ileum at 4 wk. The plasma aldosterone level was nearly doubled during the period between 2 and 4 wk after proctocolectomy, suggesting that there is a distal-to-proximal gradient of aldosterone sensitivity for induction of electrolytic Na⁺ absorption in the ileum. A similar distal-to-proximal gradient of aldosterone sensitivity has been demonstrated in the distal colon (12).

Regulation of apical ENaC activity through induction of mRNA is one of the mechanisms of activation of electrolytic Na⁺ absorption in epithelial tissues by aldosterone (3, 20, 40). We have demonstrated that all α-, β- and γ-ENaC subunit mRNAs increased in the remnant ileal mucosa after proctocolectomy. In the rat colon, the α-subunit gene is constitutively expressed, whereas preferential gene induction of β- and γ-subunits has been demonstrated under secondary hyperaldosteronism (2, 13, 34, 39, 44). Aldosterone enhanced α-subunit gene expression in the kidney but had a minimal effect on the expression of β- and γ-subunits. Thus what occurs in the ileum after proctocolectomy is not similar to what occurs in either the kidney or the colon.

The excellent temporal correlation demonstrated in the present study suggests that the activation of electrolytic Na⁺ absorption in the ileum results in the decrease of fecal Na⁺ concentration and plays a crucial role in the prevention of fecal Na⁺ loss after proctocolectomy. Hill et al. (25) have also reported the major contribution of the terminal ileum in reducing fecal Na⁺ concentration in patients who underwent colectomy, but the underlying mechanism has not been determined. It has been demonstrated previously that the electrolytic Na⁺ absorption can decrease the Na⁺ concentration of the intestinal content in the small and large intestine (15, 16, 48). In contrast to the dramatic
change in the Na⁺ concentration, the fecal water volume was not markedly changed during the 2–8 wk after surgery (Fig. 2). This suggests that the activated electrogenic Na⁺ absorption had a minimal effect on the fecal water volume, although several previous studies have shown that water absorption in the small and large intestine increased in association with the activation of electrogenic Na⁺ absorption (15, 16, 48).

Several problems, however, remain to be solved before concluding that the activation of electrogenic Na⁺ absorption in the ileum is mainly responsible for the recovery from the elevation of fecal Na⁺ concentration after proctocolectomy. First, the electrogenic Na⁺ absorption probably constitutes only a minor component of total Na⁺ absorption in the ileum of proctocolectomized rats. In the Ussing chamber experiments, benzamil-insensitive 22Na⁺ absorption contributed to more than half of the total 22Na⁺ absorption (Table 2). Unfortunately, it was not clear from the present results whether the benzamil-insensitive 22Na⁺ absorption in the ileum was also regulated after proctocolectomy. In addition, other types of Na⁺ absorption mechanisms including nutrient-coupled ones may participate in the in vivo Na⁺ absorption (14). Second, regulation of 22Na⁺ transport in the more proximal part of the small intestine should also be determined. We could not perform the electrophysiological and 22Na⁺ flux experiments because of the poor viability of proximal segments in Ussing chambers. Finally, digestive and absorptive processes of foods in the intestine may well have been disturbed after proctocolectomy. If so, this may produce the alteration in the ion and water absorption by the intestine. Consequently, many factors other than electrogenic Na⁺ absorption can influence the Na⁺ and water content in the ileal effluent. Thus further studies are required to determine the underlying mechanism of time-dependent changes in ion and water composition of ileal effluent after proctocolectomy.

This study has also shown that the TEA- and bumetanide-sensitive Isc, which are absent in normal intestine, were enhanced in the rat ileum after proctocolectomy. This Isc component probably is a result of an electrogenic K⁺ secretion, which has been demonstrated in the distal colon but not in the small intestine (7, 45). In the colon, a bumetanide-sensitive Na⁺-K⁺-2Cl⁻ cotransport has been shown to be the primary mechanism of K⁺ uptake across the basolateral membrane, although K⁺ uptake via Na⁺-K⁺-ATPase occurs when Na⁺-K⁺-2Cl⁻ cotransport is impaired (45). The electrogenic K⁺ secretion in the distal colon can be activated by aldosterone (4, 23, 45). It is therefore possible that aldosterone is also responsible for the

![Fig. 6](http://ajpgi.physiology.org/)

![Fig. 7](http://ajpgi.physiology.org/)
Our results have shown that the G\textsubscript{12} as well as the serosal-to-luminal \(^{22}\text{Na}^+\) flux significantly decreased in the ileum after proctocolectomy (Tables 1 and 2), suggesting that permeability of the paracellular pathway was diminished. The previous studies on the regulation of epithelial permeability by aldosterone have provided conflicting results: both the increase (47, 49) and the decrease (4, 26) of G\textsubscript{12} and passive Na\textsuperscript{+} permeability have been demonstrated. Interestingly, it has been reported that G\textsubscript{12} and serosal-to-luminal \(^{22}\text{Na}^+\) flux are smaller in ileal biopsies taken from patients with ileostomy than those in control ileal mucosa (24), consistent with the results of our present rat model. The decrease in paracellular Na\textsuperscript{+} permeability of the lower small intestine after proctocolectomy may contribute to the low concentration of fecal Na\textsuperscript{+} by reducing the amount of Na\textsuperscript{+} leakage into the lumen.

In conclusion, the present results have characterized the possible mechanisms by which the small intestine participates in long-term regulation of electrolyte and water balance in the whole body. Using our rat model, we have demonstrated that amiloride-sensitive, electrogenic Na\textsuperscript{+} absorption and probably also TEA-sensitive, electrogenic K\textsuperscript{+} secretion were induced in the ileum after proctocolectomy. We also indicated that the induction of the electrogenic Na\textsuperscript{+} absorption depended on the increase in the amount of all three subunits of the ENaC mRNAs (\(\alpha\), \(\beta\), and \(\gamma\)), probably due to activation at the transcription level. Hyperaldosteronemia, which was observed after surgery, may play an important role in the induction of ENaC and the functional activation of electrogenic Na\textsuperscript{+} absorption. Thus the present results confirmed and extended the previous observations in Na\textsuperscript{+}-deficient or aldosterone-treated animals. It should be noted, however, that rats in the present proctocolectomy model are different from Na\textsuperscript{+}-deficient or aldosterone-treated animals in several respects, such as in the absence of the large intestine and possible changes in small intestinal transit time of the contents, compared with normal rats. Thus future study using the present rat model may provide unique and significant information concerning the regulation of small intestinal functions. One of the interesting questions to be addressed may be whether the proximal or middle part of the ileum expresses more amiloride-sensitive \(I_{sc}\) when, in addition to the colon, the distal part of the ileum is removed. The results of such a study may be useful for clinicians to help determine if the distal part of the ileum should be conserved in colectomy patients to prevent excessive fecal Na\textsuperscript{+} loss. It remains to be investigated, however, whether similar regulation processes (demonstrated here in rats) can be induced in the human ileum.

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**Fig. 8. Time course of the increase in rENaC \(\alpha\)-, \(\beta\)-, and \(\gamma\)-subunit mRNA levels in the lower third of the small intestine after proctocolectomy.** A and B: Northern blotting of control rat (lane 1) and rats 1 wk (lanes 2 and 3), 2 wk (lanes 4 and 5), 4 wk (lanes 6 and 7) and 8 wk (lanes 8 and 9) after proctocolectomy. C: signal of each band was quantified by an image analyzer. Results (means of 2 animals) are expressed, after being normalized with GAPDH mRNA levels, as the ratio to the mRNA level at 8 wk after proctocolectomy for each subunit.

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activation of electrogenic K\textsuperscript{+} secretion in the ileum in our study. The electrogenic K\textsuperscript{+} secretion in the ileum may be responsible, at least in part, for the elevation of fecal K\textsuperscript{+} concentration after proctocolectomy (Fig. 2). It has been shown that in patients who have undergone colectomy the K\textsuperscript{+} concentration in the ileal effluent is elevated in association with the increase in aldosterone levels (19, 21, 30, 33, 42). The activation of K\textsuperscript{+} secretion seems to be an unfavorable response to proctocolectomy because it precipitates K\textsuperscript{+} deficiency (Fig. 1). However, the electrogenic K\textsuperscript{+} secretion might conceivably provide an electrical driving force for electrogenic Na\textsuperscript{+} absorption, thereby increasing Na\textsuperscript{+}-retaining activity indirectly. In the present study, we used 2 mM TEA, which inhibits only 50\% of K\textsuperscript{+} secretion in the rat distal colon (Suzuki, unpublished observations). The potency of the inhibitory effect of mucosal TEA on ileal K\textsuperscript{+} secretion remains unknown. Therefore, the actual amount of K\textsuperscript{+} secretion enhanced after proctocolectomy cannot yet be determined. For the same reason, it is also difficult to evaluate whether \(I_{sc}\) components other than electrogenic Na\textsuperscript{+} absorption and K\textsuperscript{+} secretion are also changed after proctocolectomy.
REFERENCES


