Na-H Exchanger Isoform-2 (NHE2) Mediates Butyrate-dependent Na⁺ Absorption in Dextran Sulfate Sodium (DSS)-induced Colitis*

Vazhaikkurichi M. Rajendran[‡1], Navalpur S. Nanda Kumar[‡2], Chung M. Tse[§], and Henry J. Binder[¶]

From the [‡]Department of Biochemistry and Molecular Biology, West Virginia University School of Medicine, Morgantown, West Virginia 26506, the [§]Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, and the [¶]Department of Internal Medicine, Yale University, New Haven, Connecticut 06520

Background: Defective Na⁺ absorption is the primary cause of diarrhea in inflamed colon such as ulcerative colitis (UC).
Results: Butyrate stimulates Na⁺ and water absorption in dextran sulfate sodium-induced inflamed colon.
Conclusion: Na-H exchanger-2 (NHE2) is activated and mediates butyrate-dependent Na⁺ absorption in inflamed colon.
Significance: Butyrate-dependent Na⁺ absorption would be a potential target to control UC-associated diarrhea.

Diarrhea associated with ulcerative colitis (UC) occurs primarily as a result of reduced Na⁺ absorption. Although colonic Na⁺ absorption is mediated by both epithelial Na⁺ channels (ENaC) and Na-H exchangers (NHE), inhibition of NHE-mediated Na⁺ absorption is the primary cause of diarrhea in UC. As there are conflicting observations reported on NHE expression in human UC, the present study was initiated to identify whether NHE isoforms (NHE2 and NHE3) expression is altered and how Na⁺ absorption is regulated in DSS-induced inflammation in rat colon, a model that has been used to study UC. Western blot analyses indicate that neither NHE2 nor NHE3 expression is altered in apical membranes of inflamed colon. Na⁺ fluxes measured *in vitro* under voltage clamp conditions in controls demonstrate that both HCO_3^--dependent and butyrate-dependent Na⁺ absorption are inhibited by S3226 (NHE3-inhibitor), but not by HOE694 (NHE2-inhibitor) in normal animals. In contrast, in DSS-induced inflammation, butyrate-, but not HCO_3^--dependent Na⁺ absorption is present and is inhibited by HOE694, but not by S3226. These observations indicate that in normal colon NHE3 mediates both HCO_3^--dependent and butyrate-dependent Na⁺ absorption, whereas DSS-induced inflammation activates NHE2, which mediates butyrate-dependent (but not HCO_3^--dependent) Na⁺ absorption. In *in vivo* loop studies HCO_3^--Ringer and butyrate-Ringer exhibit similar rates of water absorption in normal rats, whereas in DSS-induced inflammation luminal butyrate-Ringer reversed water secretion observed with HCO_3^--Ringer to fluid absorption. Lumen butyrate-Ringer incubation activated NHE3-mediated Na⁺ absorption in DSS-induced colitis. These observations suggest that the butyrate activation of NHE2 would be a potential target to control UC-associated diarrhea.

Diarrhea is a common symptom in patients with inflammatory bowel diseases (1, 2). Inflammatory bowel diseases include Crohn disease and ulcerative colitis (UC).[3] Crohn disease is the chronic inflammatory disease that affects any part of the gastrointestinal tract, whereas UC affects only the colon (3, 4). Secretory diarrhea occurs as a result of reduced Na⁺ absorption and/or increased Cl⁻ secretion (3). Decreased Na⁺ absorption has been shown as the primary mechanism of diarrhea in UC (6, 7). Both electroneutral Cl⁻-dependent (*e.g.* Na-Cl) and Cl⁻-independent electrogenic Na⁺ absorption are present in healthy human colon. Epithelial Na⁺ channels (ENaC) mediate electrogenic Na⁺ absorption, whereas Na-H exchanger (NHE) and Cl⁻-HCO_3^- exchanger (an anion exchanger; AE) that are coupled via intracellular pH mediate electroneutral Na⁺ absorption (8). Because cAMP has been shown to inhibit NHE and activate ENaC by endocytosis and exocytosis tracking regulation, respectively, it is likely that although both Na-Cl and ENaC-mediated Na⁺ absorption are present, only Na-Cl mediated Na⁺ absorption is affected in infectious diarrhea (9–11).

Both HCO_3^- and butyrate (a short chain fatty acid) independently regulate the Cl⁻-dependent NHE-mediated Na⁺ absorption in colon. Parallel function of NHE and Cl⁻-HCO_3^--exchanger, and NHE, butyrate-HCO_3^- exchanger and Cl⁻-butyrate exchanger represent the mechanism of HCO_3^-- and butyrate-dependent Na-Cl absorption, respectively (Fig. 1) (12–14). Thus, both HCO_3^--dependent and butyrate-dependent Na⁺ absorptive processes require NHE. Molecular studies have identified nine different NHE isoforms (NHE1–9) that exhibit cell and organ-specific expression (15). Of these nine NHEs, NHE1 has been localized on the basolateral membranes, whereas NHE2 and NHE3 have been localized on the apical membranes of small intestine and colon epithelial cells (15, 16). Under basal conditions, both HCO_3^- and butyrate utilize NHE3 to mediate Na⁺ absorption, whereas in the absence of NHE3 (*i.e.* inhibition of NHE3 by cholera toxin/cAMP) butyrate, but

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[1] To whom correspondence should be addressed: One Medical Center Dr., P. O. Box 9142, West Virginia University School of Medicine, Morgantown, WV 26506. Tel.: 304-293-0510; Fax: 304-293-4896; E-mail: vrajendran@hsc.wvu.edu.
[2] Present address: Massachusetts General Hospital and Harvard Medical School, Boston, MA 02129.

[3] The abbreviations used are: UC, ulcerative colitis; APM, apical membranes; DSS, dextran sulfate sodium; NHE, Na-H exchanger; m-s, mucosal to serosal; s-m, serosal to mucosal; EIPA, ethylisopropyl amiloride; EPEC, enteropathogenic *E. coli*.
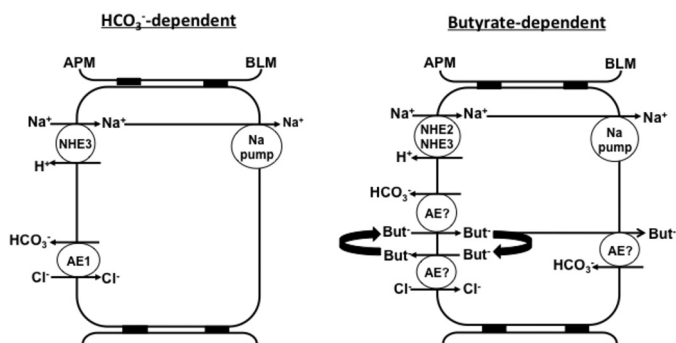
FIGURE 1. **Cellular models of HCO$_3^-$-dependent and butyrate (But$^-$)-dependent electroneutral Na-Cl absorption in colonic epithelial cells.** Coordinated regulation of NHE3 and anion exchanger-1 (*AE*; Cl$^-$-HCO$_3^-$ exchanger) mediate HCO$_3^-$-dependent Na$^+$ absorption, whereas the coordinated regulation of NHE isoform-2/-3 (NHE2/NHE3), But$^-$-HCO$_3^-$ exchanger, and But$^-$-Cl$^-$ exchanger mediate butyrate-dependent Na$^+$ absorption. *AE?*, unknown anion exchanger; *BLM*, basolateral membranes.

not HCO$_3^-$ regulates Na$^+$ absorption through NHE2 in rat distal colon (17).

Although both NHE2 and NHE3 are present on the apical membranes, NHE3 is the primary transporter that mediates both butyrate and HCO$_3^-$-dependent Na$^+$ absorption in normal rat distal colon (17). In addition, phosphorylation and steroid hormones have also been shown to regulate the NHE3 isoform (9, 16, 18, 19). Recent studies have reported a conflicting regulatory pattern of NHE3 in UC (20, 21). Sullivan *et al.* (20) have reported decreased mRNA abundance and protein expression of NHE3 in patients with inflammatory bowel disease. In addition, this study has also reported decreased NHE3 expression in both trinitrobenzene sulfonate and dextran sulfate sodium (DSS)-induced colitis in mouse colon (20). In contrast, Yeruva *et al.* (21) have reported decreased NHE3 activity, but not NHE3 expression (*i.e.* mRNA and protein) in patients with mild, moderate, and severe UC. Both studies have reported that NHE2 expression is not altered either in experimental or human colonic inflammation (20, 21). As mentioned, in the absence of NHE3, butyrate regulated NHE2-mediated Na$^+$ absorption in the colon (17). Thus, this present study was initiated to determine whether NHE2 function is present and mediates butyrate-dependent Na$^+$ absorption in the DSS-induced inflamed rat distal colon. This study demonstrates that neither NHE2- nor NHE3-specific protein expression is altered in inflamed colon. However, HCO$_3^-$-dependent Na$^+$ absorption is inhibited, whereas butyrate-dependent Na$^+$ absorption remains unchanged in DSS-colitis. In addition, colonic loops instilled with butyrate-Ringer significantly enhanced fluid absorption and stimulated HCO$_3^-$-dependent Na$^+$ absorption in inflamed colon. These observations indicate that inflammation-activated NHE2 mediates butyrate-dependent Na$^+$ absorption, whereas butyrate incubation restores NHE3 function to mediate HCO$_3^-$-dependent Na$^+$ absorption in inflamed colon.

Experimental Procedures

Model of Colonic Inflammation—Colonic inflammation was developed in male Sprague-Dawley rats (Charles River, Raleigh, NC; 125–150 g) by giving 5% DSS/water *ad libitum* for five alternate weeks. In recent studies, loss and distortion of crypts,

crypt abscess, and presence of excess inflammatory cells within the lamina propria were histologically noticed as features of DSS-induced inflammation (22). All experimental protocols used in this study were approved by the Yale University and West Virginia University Institutional Animal Care and Use Committees.

Na$^+$ Flux Studies—^{22}Na$^+$ (PerkinElmer Life Sciences) fluxes were measured across the mucosal layer of the distal colon mounted under voltage-clamp conditions using the EasyMount Ussing chamber system (Physiological Instruments, San Diego, CA), as previously described (22, 23). In brief, colon excised from anesthetized rats was flushed with ice-cold saline. Mucosal layers were gently separated from serosal muscle layers from colon opened along the mesenteric border. Two distal (1 cm proximal to rectum) segments obtained from each animal were mounted in Lucite chambers with an exposed surface opening of 1.12 cm^2, and both sides were bathed with an equal volume of either HCO$_3^-$-Ringer or butyrate-Ringer. The HCO$_3$-Ringer solution contains (in mM): 115 NaCl, 25 NaHCO$_3$, 2.4 K$_2$HPO$_4$, 0.4 KH$_2$PO$_4$, 1.2 CaCl$_2$, 1.2 MgCl$_2$, and 10 glucose, pH 7.4. In butyrate-Ringer solution 25 mM NaHCO$_3$ was substituted with 25 mM Na-butyrate. The bathing solutions were maintained at 37 °C. HCO$_3^-$-Ringer was gassed with 5% CO$_2$, 95% O$_2$, whereas butyrate-Ringer was gassed with 100% O$_2$. Short-circuit currents (I_{SC}) and conductance (G) were recorded every 20 s (Multichannel Voltage/Current Clamp Instrument, Physiological Instruments). For flux studies, a trace of ^{22}Na (0.25 μCi) was added to either mucosal or serosal bath. Following a 15-min equilibration, mucosal-to-serosal (m-s) and serosal-to-mucosal (s-m) Na$^+$ fluxes were measured under voltage-clamp conditions. Net fluxes were calculated from the difference between m-s and s-m fluxes of tissue pairs that were matched with transepithelial tissue conductance of <10%. Fluxes were measured for 15 min. In additional experiments, following basal measurements, Isc and Na$^+$ fluxes were also measured following mucosal addition of either 50 μM HOE694 ((3-methylsulfonyl-4-pieridinobenzoyl)guanidine methanesulfonate), a guanidine derivative and/or 3 μM S3226 (3-[2-(3-guanidino-2-methyl-3-oxopropenyl)-5-methyl-phenyl]-N-isopropylidene-2-methyl-acrylamide dichloride), a bismethylacryloyl guanidine derivative that inhibits NHE2- and NHE3-mediated Na$^+$ absorption, respectively (17, 24, 25). HOE694, a potent basolateral NHE1 inhibitor inhibits NHE2 with a half-maximal inhibitory constant (K_i) of 5 μM, whereas NHE3 is not affected by concentrations <100 μM (24). S3226 inhibits NHE3 and NHE2 with apparent K_i values of 0.2 and 80 μM, respectively (17, 25). Thus, 50 μM HOE694- and 3 μM S3226-sensitive Na$^+$ absorption represent mediated NHE2 and NHE3 isoforms, respectively, with negligible cross-reactivity.

In Vivo Determination of Water Movement—In vivo water movement (*i.e.* fluid absorption and secretion) was measured in distal colon loops, as previously described for ileum (26). At laparotomy the distal colon loop (5–6 cm) of the anesthetized rat was constructed between 1 cm distal to the proximal colon and the rectum. The colon loop flushed with warm Ringer solution was instilled with 5 ml of either HCO$_3^-$-Ringer or butyrate-Ringer. The abdomen was closed and the anesthetized rat was maintained at 37 °C by placing on the warm plate. Following a
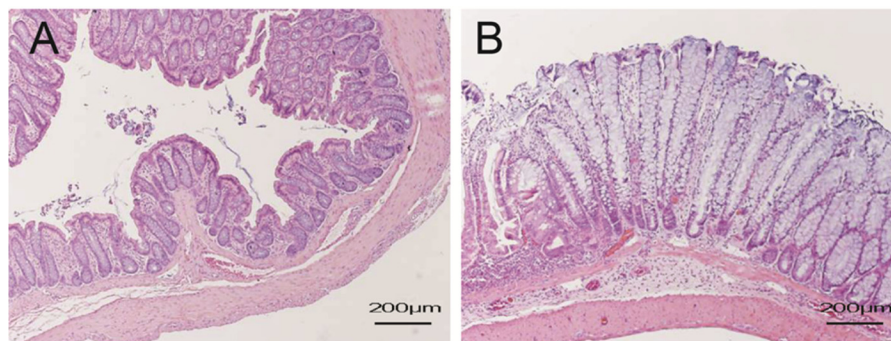
FIGURE 2. **H&E staining of normal and DSS-induced inflamed rat distal colon.** *A,* shorter and less densely packed colonic crypts with well preserved surface epithelium, and minimal edema and lymphocytes, rare eosinophils are present in the interstitium of normal rat distal colon. *B,* in DSS-induced inflamed colon, the crypt architecture is preserved and surface absorptive epithelium is intact. There is marked diffuse edema with numerous mononuclear cells in the interstitium in inflamed colon. Magnification ×5.

3-h incubation, the remaining luminal water content and the length of the colon loop were measured to calculate the rate of fluid movement. Net water movement ($V_i - V_f$) was calculated by subtracting the water volume collected at the end of the experiment (V_f) from that of the initially instilled water volume (V_i). The rate of water movement is expressed as ml/3 h·cm of colon. Positive and negative water movement represents net absorption and net secretion, respectively. At the end of the *in vivo* fluid movement experiments, the colon removed from the euthanized animal was then used for ion flux studies.

Apical Membrane Isolation and Western Blot Analyses—Western blot analyses of protein extracted from apical membranes (APM) of normal and DSS-induced inflamed colonic epithelial cells were performed using NHE2 and NHE3 specific antibodies, as described previously (26). The APM were purified from isolated colonic epithelial cells by the method of Stieger *et al.* (27), as described previously (28). The APM purity of normal (homogenate *versus* APM: 10.2 ± 0.8 *versus* 115.3 ± 9.2 nmol of P$_i$ liberated/mg of protein·min) and DSS-induced inflamed (homogenate *versus* APM: 4.8 ± 1.6 *versus* 45.9 ± 7.9 nmol of P$_i$ liberated/mg of protein·min) colon were validated by 11.3- and 9.6-fold enrichment of H,K-ATPase activity over homogenate, respectively. H,K-ATPase was assayed by the method of Forbush *et al.* (29), as described earlier (30). Protein was measured by the method of Lowry *et al.* (31). Three separate APM preparations were used for H,K-ATPase assay and Western blot analyses. Each APM preparation utilized distal colon from 8 rats.

For Western blot, 30 μg of apical membrane proteins resolved by polyacrylamide gel electrophoresis were transferred onto nitrocellulose membrane (Hybond ECL, Amersham Biosciences, Piscataway, NJ). The membrane blot incubated with primary antibody (NHE3 (1:3000) or NHE2 (1:2000)) were incubated with horseradish peroxidase-conjugated anti-mouse (NHE3) and anti-rabbit (NHE2) IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Immune complexes were detected on film using enhanced chemiluminescence (Amersham Biosciences).

Histology—Normal and DSS-induced inflamed distal colons removed from anesthetized rats were washed with ice-cold saline and fixed overnight in 10% neutral formalin. The paraffin-embedded tissue sections (5 μm) mounted on glass slides

were stained with hematoxylin and eosin (H&E) for histological evaluation. Similar to the earlier observations (22), the normal rat distal colon exhibits shorter and less densely packed colonic crypts with minimal edema and lymphocytes (Fig. 2*A*), whereas DSS-induced inflamed colon exhibits, preserved surface absorptive epithelium with mucosal as well as submucosal layers edematous with marked thickening of the mucosal layer (Fig. 2*B*).

Immunofluorescence Studies—The 5-μm tissue sections were deparafinized and heat fixed. Slides were microwaved for antigen recovery in 10 mM sodium citrate buffer, pH 6.0, for 2–5 min. Following 30 min cooling, sections were washed in phosphate-buffered saline (PBS) and blocked with 5% normal goat serum in PBS for 45 min. Sections were incubated overnight with rabbit polyclonal antibodies against NHE2 (Ab5697) or mouse NHE3 monoclonal antibody (generously provided by Dr. Daniel Biemesderfer, Yale University, New Haven, CT) at 1:100 dilution (33). After 2 washes with PBS, sections were incubated with Alexa Fluor 488 anti-rabbit or Alexa Fluor 568 anti-mouse secondary antibodies (Invitrogen). Sections were washed, counterstained with Hoechst 33342 (Invitrogen) for nuclei, and mounted with glass coverslips mounting medium. Immunofluorescence images were obtained with a LSM 510 Meta confocal microscope.

Statistics—Results are presented as mean ± S.E. Statistical analyses were performed using paired *t* test and Bonferroni's one-way analysis of variance post hoc test with SPSS software. $p < 0.05$ was considered statistically significant.

Results

Immunofluorescence studies were performed to identify whether NHE2- or NHE3-specific protein expression and/or cell (*i.e.* surface *versus* crypt cell) specific expression patterns had been altered in the DSS-induced inflamed colon. As shown in Fig. 3, *A* and *C*, NHE2 and NHE3 proteins were localized on the APM of normal rat distal colon. Both NHE2 and NHE3 protein expression were substantially increased in epithelial cells with insignificant alteration in APM of DSS-induced inflamed colon (Fig. 3, *B* and *D*). To establish that APM expression of NHE2 and NHE3 was not altered, Western blot analyses were performed. Neither NHE2 (Fig. 4, *A* and *B*) nor NHE3 (Fig. 4, *C* and *D*) specific protein expression was significantly altered
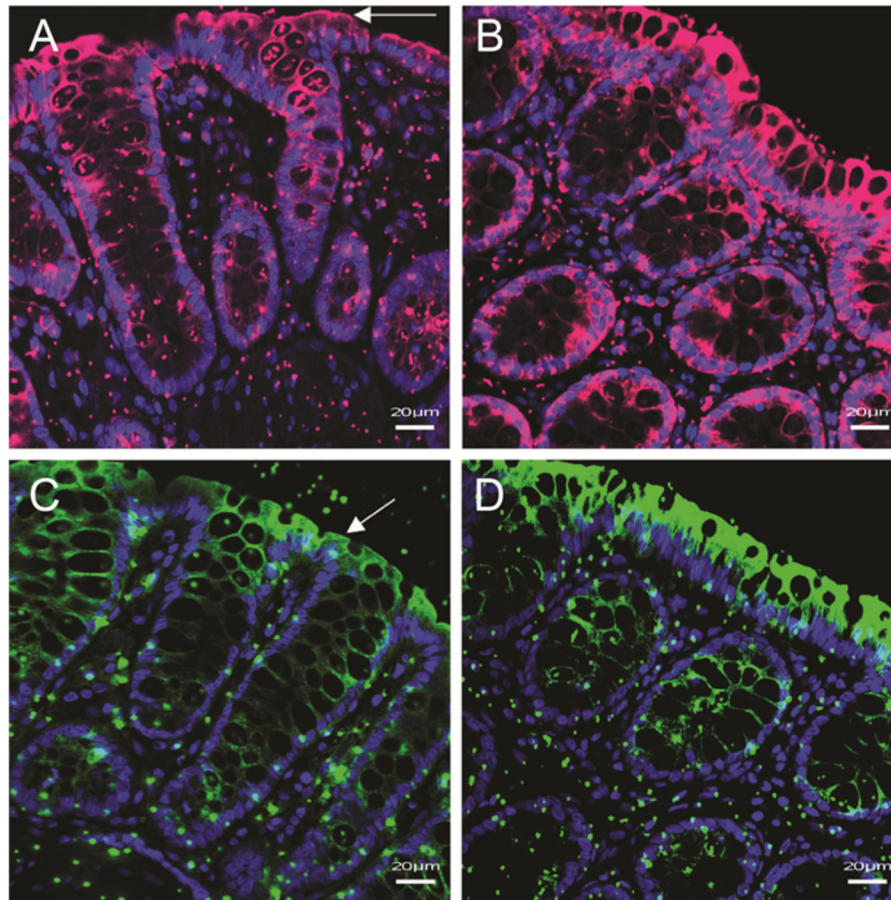
FIGURE 3. Immunofluorescence localization of NHE3 and NHE2 isoforms in normal and DSS-induced inflamed rat distal colon. *Arrows* indicate strong staining of NHE2 (*A*) and NHE3 (*C*) specific proteins on apical membranes of normal colons. In inflamed colons, epithelial cells were strongly stained with both NHE2 (*B*) and NHE3 (*D*) with no distinct apical membrane staining. Magnification ×40.

in APM of experimental colonic inflammation. The present demonstration of unaltered NHE2 expression is consistent with earlier observations that had been performed with human and animal models of colonic inflammation (20, 21). In contrast, this observation of unaltered NHE3 expression is consistent with one of the earlier observations with human UC (21), but differs from another study, which reported decreased NHE3 expression in both human and experimental colonic inflammation (20).

Na⁺ flux measurements were performed in two different experimental conditions. 1) In one series of studies determination of Na⁺ fluxes was made on both normal and DSS-inflamed colon immediately following sacrifice, which provided an assessment of the direct effect of inflammation on NHE2- and NHE3-mediated Na⁺ absorption. 2) A second set of experiments was designed to evaluate Na⁺ absorption following a 3-h *in vivo* incubation with either butyrate or saline. These experiments were designed to determine whether *in vivo* incubation with a short chain fatty acids would modify NHE2 and/or NHE3 function (or expression).

To establish whether, similar to colonic NHE2 and NHE3 expression, Na⁺ absorption was also preserved, Na⁺ fluxes were measured in mucosa with colonic inflammation compared with normal colon (Table 1 and Fig. 5). Similar to the earlier observations (22), net Na⁺ absorption was present in the
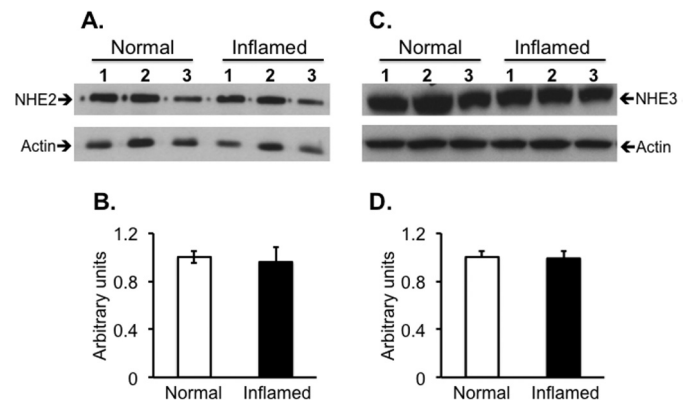

FIGURE 4. Western blot analyses of NHE2 (*A*) and NHE3 (*B*) isoform-specific protein expression in apical membranes from normal and DSS-induced inflamed rat distal colon. *A*, anti-NHE2 antibody detects a protein in apical membranes from both normal and inflamed colon. *B*, densitometric analyses of the Western blot shown in *A* indicate comparable levels of NHE2-specific protein expression in apical membranes of normal (*open bar*) and inflamed (*closed bar*) colon. *C*, anti-NHE3 antibody detects a protein in apical membranes of both normal and inflamed colon. *D*, densitometric analyses of the Western blot shown in *C* indicate comparable levels of NHE3-specific protein expression in apical membranes of normal (*open bar*) and inflamed (*closed bar*) colon. Three different apical membrane preparations were used for Western blot analyses. Six rats were used for each apical membrane preparation. Different blots probed with anti-NHE2 and anti-NHE3 antibodies were stripped and probed with anti-actin antibody for internal control. Quantitation of NHE2 and NHE3 expression was performed using personal densitometer SI ImageQuant software and was normalized to actin.

TABLE 1

Effect of HCO_3^- and butyrate on Na^+ fluxes and electrical parameters in normal and DSS-induced inflamed rat distal colon

Mucosal to serosal (m-s), serosal to mucosal (s-m), and net Na^+ fluxes, and short circuit current (Isc) and trans-epithelial tissue conductance (G) were measured in the presence of HCO_3^--Ringer and butyrate-Ringer as described under "Experimental Procedures."

| | Na^+ fluxes | | | | | |
	m-s	s-m	net	Isc	G	n
	$\mu Eq/h\,cm^2$					
HCO_3^--Ringer						
Normal	10.5 ± 0.6	5.2 ± 0.4	5.5 ± 0.6	2.15 ± 0.07	8.48 ± 0.74	6
Inflamed	6.8 ± 1.4	5.9 ± 0.5	0.9 ± 1.1	-0.48 ± 0.23	7.77 ± 0.83	6
	$*p < 0.001$		$*p < 0.001$	$*p < 0.05$		
Butyrate-Ringer						
Normal	8.6 ± 0.5	3.8 ± 0.3	4.8 ± 0.5	$0.19 \pm 0.12*$	7.72 ± 0.57	6
Inflamed	$13.2 \pm 1.0*$	5.3 ± 0.8	$7.9 \pm 0.9*$	$0.14 \pm 0.13*$	8.39 ± 1.83	6
	$*p < 0.001$		$*p < 0.001$	$*p < 0.05$		

presence of both HCO_3^--Ringer and butyrate-Ringer in normal colon (Table 1 and Fig. 5A). In contrast, in colonic mucosa with inflammation, net Na^+ absorption was present only in the presence of butyrate-Ringer, and not in the presence of HCO_3^--Ringer (Table 1 and Fig. 5B). Net Na^+ absorption that was observed in the presence of butyrate-Ringer was a result of increased m-s fluxes in inflamed colon (Fig. 5B). The s-m Na^+ fluxes were not altered in the butyrate-Ringer studies in either normal or colonic inflammation (Table 1 and Fig. 5). Similar to earlier observations, a positive residual Isc (that represents either cation absorption or anion secretion) was observed in the presence of HCO_3^--Ringer in normal colon (Table 1) (34). In colon with inflammation the residual Isc reversed to serosal negative Isc that might represent cation (possibly K^+) secretion (Table 1). Butyrate-Ringer inhibited the residual Isc present in the presence of HCO_3^--Ringer in both normal and inflamed colon (Table 1). Although Na^+ absorption (*i.e.* decreased Na^+ absorption) and Isc (*i.e.* negative Isc) were altered, trans-epithelial tissue conductance (G) was not significantly changed either in HCO_3^--Ringer or butyrate-Ringer in normal and inflamed colon (Table 1). These observations indicate that 1) DSS-induced inflammation inhibited HCO_3^--dependent Na^+ absorption, but not butyrate-dependent Na^+ absorption; and 2) increased fluid secretion might have occurred as a result of decreased Na^+ absorption, but not as a consequence of increased anion secretion in DSS-induced inflammation.

Both HCO_3^-- and butyrate-dependent Na^+ absorptions require mucosal NHE in normal colon (17). Therefore, to examine whether the butyrate-dependent Na^+ absorption present in colonic inflammation was also mediated via NHE, the effect of ethyl isopropyl amiloride (EIPA; a nonspecific NHE inhibitor that inhibits both NHE2 and NHE3) on net Na^+ absorption was studied. Mucosal addition of EIPA (50 μM) completely inhibited both HCO_3^- and butyrate-dependent Na^+ absorptions in normal colon, as well as inhibiting butyrate-dependent Na^+ absorption in colonic inflammation (Fig. 6, A and B). These observations indicate that butyrate-dependent Na^+ absorption is also mediated via NHE during inflammation.

Because both NHE2 and NHE3 isoforms are present on the APM (16), the effect of S3226 (3 μM; a NHE3 isoform specific inhibitor) and HOE694 (a NHE2 isoform-specific inhibitor) was examined on HCO_3^-- and butyrate-dependent Na^+ absorption (Figs. 7 and 8). The inhibitor S3226 completely inhibited both HCO_3^--dependent and butyrate-dependent Na^+
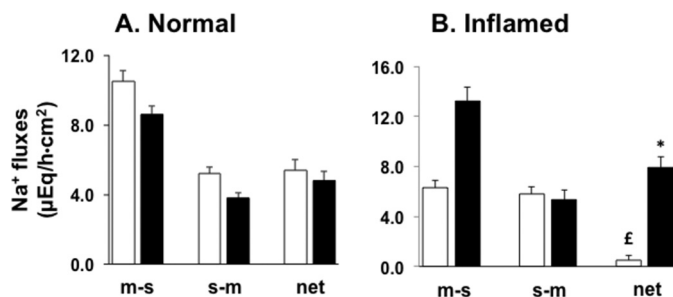

FIGURE 5. **Effect of HCO_3^--Ringer and butyrate-Ringer on Na^+ fluxes in normal and DSS-induced inflamed rat distal colon.** Mucosal to serosal (*m-s*), serosal to mucosal (*s-m*), net Na^+ fluxes and Isc were measured in the presence of HCO_3^--Ringer (*open bars*) and butyrate-Ringer (*closed bars*) as described under "Experimental Procedures." The results presented are derived from Table 1. *A*, net Na^+ absorption is present in the presence of both HCO_3^--Ringer and butyrate-Ringer in normal colon. *B*, net Na^+ absorption is present only in the presence butyrate-Ringer, but not in the presence of HCO_3^--Ringer in inflamed colon. £, $p < 0.001$ compared with respective HCO_3^--Ringer; *, $p < 0.001$ compared with respective HCO_3^--Ringer in normal.
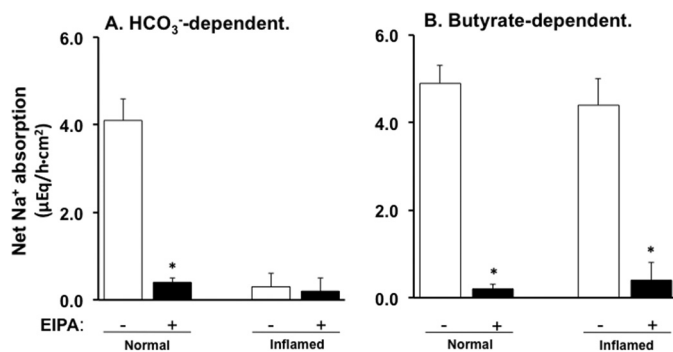

FIGURE 6. **Effect of mucosal EIPA (a nonspecific Na-H exchange inhibitor) on net Na^+ absorption in normal and DSS-induced inflamed colon.** Net Na^+ absorption was measured in the presence of HCO_3^--Ringer (*A*) and butyrate-Ringer (*B*) in normal and inflamed colon. Net Na^+ absorption was also measured in the mucosal presence (*closed bars*) and absence (*open bars*) of 100 μM EIPA. EIPA inhibits the HCO_3^--dependent Na^+ absorption in normal colon. Butyrate-dependent Na^+ absorption is present in both normal and inflamed colon. Mucosal EIPA inhibits the butyrate-dependent Na^+ absorption in both normal and inflamed colon. *, $p < 0.001$, compared with the absence of EIPA.

absorption in normal colon (Fig. 7, A and B). This observation is consistent with our earlier observations that S3226-sensitive NHE3 mediates both HCO_3^-- and butyrate-dependent Na^+ absorption in normal colon (16). In contrast, in DSS-induced colonic inflammation, S3226 did not inhibit butyrate-dependent Na^+ absorption (Fig. 7B). This observation suggests that the S3226-insensitive NHE2 isoform
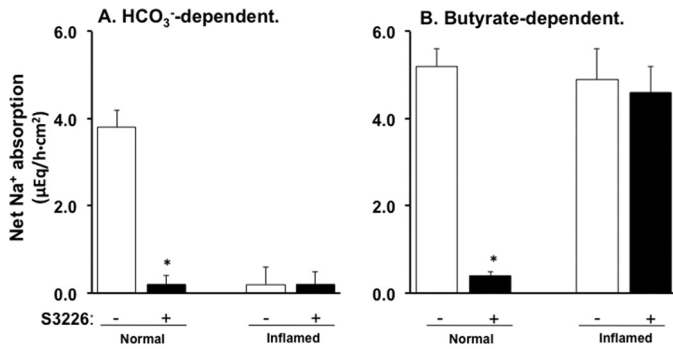
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FIGURE 7. Effect of S3226 (NHE3 specific inhibitor) on HCO$_3^-$-dependent and butyrate-dependent Na$^+$ absorption in normal DSS-induced inflamed distal colon. *A*, HCO$_3^-$-dependent Na$^+$ absorption is present in normal, but not in inflamed colon. Mucosal addition of S3226 (3 μM) inhibits the HCO$_3^-$-dependent Na$^+$ absorption in normal colon. *B*, butyrate-dependent Na$^+$ absorption is present in both normal and inflamed colon. Mucosal S3226 inhibits the butyrate-dependent Na$^+$ absorption in normal, but not in inflamed colon. *, $p < 0.001$, compared with the absence of S3226.
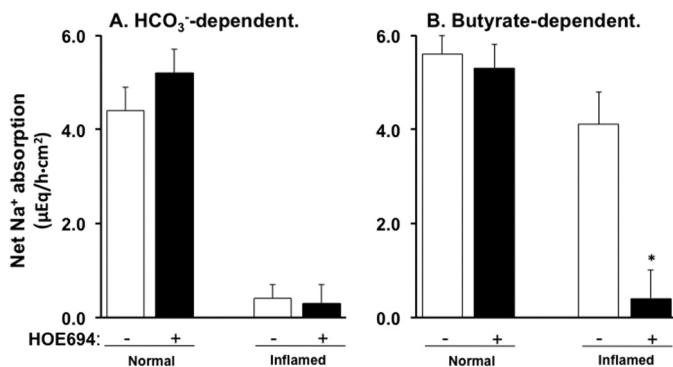

FIGURE 8. Effect of HOE694 (NHE2 specific inhibitor) on HCO$_3^-$-dependent and butyrate-dependent Na$^+$ absorption in normal and DSS-induced inflamed rat distal colitis (*UC*). *A*, HCO$_3^-$-dependent Na$^+$ absorption is present in normal, inflamed colon. Mucosal addition of HOE694 (50 μM) inhibits the HCO$_3^-$-dependent Na$^+$ absorption in normal colon. *B*, butyrate-dependent Na$^+$ absorption is present in both normal and inflamed colon. Mucosal HOE694 inhibits the butyrate-dependent Na$^+$ absorption in inflamed, but not in normal colon. *, $p < 0.001$, compared with in the absence of HOE694.

might mediate butyrate-dependent Na$^+$ absorption in colonic inflammation.

To confirm that the NHE2 isoform mediates butyrate-dependent Na$^+$ absorption, the effect of mucosal HOE694 (50 μM; a NHE2 isoform-specific inhibitor) was examined on butyrate-dependent Na$^+$ absorption in DSS-inflamed colon. Mucosal addition of HOE694 did not inhibit either HCO$_3^-$-dependent or butyrate-dependent Na$^+$ absorption in normal colon (Fig. 8, *A* and *B*). In contrast, however, mucosal HOE694 completely inhibited butyrate-dependent Na$^+$ absorption in inflamed colon (Fig. 8*B*). These observations establish that both HCO$_3^-$-dependent and butyrate-dependent Na$^+$ absorption are mediated by NHE3 in normal, non-inflamed colon, whereas colonic inflammation induced butyrate-dependent Na$^+$ absorption via NHE2.

Because fluid absorption follows Na$^+$ absorption, studies were also designed to establish whether butyrate-stimulated NHE2-mediated Na$^+$ absorption was also associated with altered water movement in colonic inflammation. In this study, the rate of water movement during instillation with HCO$_3^-$-Ringer and butyrate-Ringer was determined *in vivo* in colon
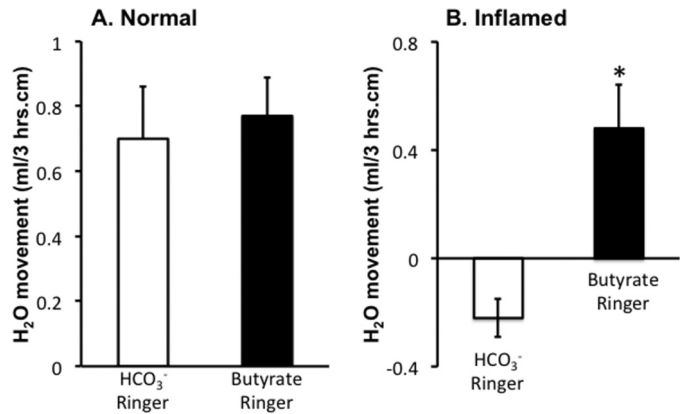

FIGURE 9. Effect of butyrate on *in vivo* water movement in normal and DSS-induced inflamed rat distal colon. Colon loop constructs instilled with 5 ml of either HCO$_3^-$-Ringer (*open bars*) or butyrate-Ringer (*closed bars*) solution were incubated for 3 h. The rate of water movement was calculated as described under "Experimental Procedures." *A*, colon loops instilled with HCO$_3^-$-Ringer and butyrate-Ringer solution had similar rates of water absorption in normal rat. *B*, colon loops instilled with HCO$_3^-$-Ringer had water secretion, whereas colon loops instilled with butyrate-Ringer had water absorption in inflamed colon. Results presented represent mean $\pm$ S.E. of three normal and nine DSS-induced inflamed rat distal colon loops. *, $p < 0.001$, compared with HCO$_3^-$-Ringer.

loops of DSS-treated and normal rats (Fig. 9). Similar rates of water absorption were observed in both HCO$_3^-$-Ringer and butyrate-Ringer instilled normal colon loops (Fig. 9*A*). In contrast, loops instilled with HCO$_3^-$-Ringer manifested net fluid secretion in DSS-treated colonic loops (Fig. 9*B*). In contrast, during experiments with butyrate-Ringer net water absorption was observed in the colonic loops with inflammation (Fig. 9*B*). These observations indicate that in colonic inflammation net fluid secretion is induced, whereas butyrate inhibits water secretion and/or stimulates water absorption.

Butyrate has been shown to induce NHE3 expression and increase Na$^+$ absorption in rat colon, as well as blocking cholera toxin-induced fluid secretion by enhancing apical NHE3 expression in rat ileum (26, 35). Thus, studies were designed to identify whether butyrate incubation altered NHE3 function and/or expression in inflamed colon. In this study, Na$^+$ fluxes were measured in the presence of HCO$_3^-$-Ringer in colon that had been instilled with HCO$_3^-$-Ringer (HCO$_3^-$ incubated) or with butyrate-Ringer (butyrate incubated) for 3 h. The results of these studies are presented in Fig. 10. Neither m-s nor net Na$^+$ fluxes are altered in DSS-inflamed colon incubated with HCO$_3^-$-Ringer (Fig. 10*A*). In contrast, however, both m-s and net Na$^+$ fluxes are significantly enhanced in DSS-inflamed colon incubated with butyrate-Ringer (Fig. 10*B*). The effect of S3226 (a specific NHE3 inhibitor) was also examined, as earlier studies had shown that HCO$_3^-$-dependent Na$^+$ absorption is mediated only by NHE3 (Figs. 7 and 8). As shown in Fig. 10*B*, mucosal S3226, but not HOE694 completely inhibited both m-s and net Na$^+$ fluxes in butyrate-incubated inflamed colon. Colonic loops incubated with HCO$_3^-$-Ringer and butyrate-Ringer did not alter either NHE2 or NHE3 protein expression in inflamed colon (data not shown). This observation indicates that the 3-h butyrate incubation partially activates NHE3-mediated Na$^+$ absorption in DSS-inflamed colon.

Because butyrate incubation activated NHE3-mediated (*i.e.* HCO$_3^-$-dependent) Na$^+$ absorption (Fig. 10*B*), studies were
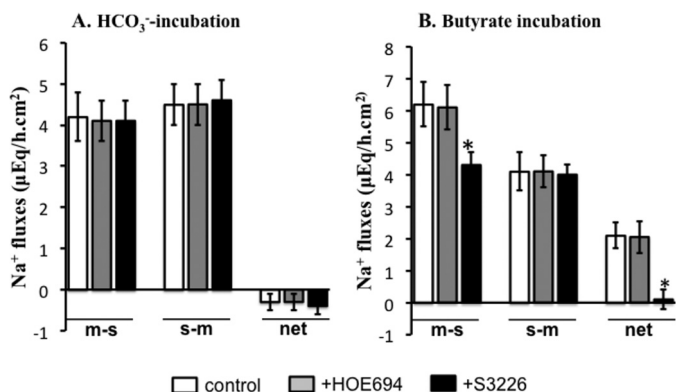
FIGURE 10. **Effect of HCO$_3^-$ and butyrate incubation on HCO$_3^-$-dependent Na$^+$ fluxes in DSS-induced inflamed rat distal colon.** Mucosal to serosal (*m-s*), serosal to mucosal (*s-m*), and net Na$^+$ fluxes were measured in the presence of HCO$_3^-$-Ringer as described under "Experimental Procedures." Na$^+$ fluxes were measured in mucosal sheets separated from loops with colonic inflammation, which were incubated with either HCO$_3^-$-Ringer (*A*) or butyrate-Ringer (*B*) for the fluid movement measurement as described in the legend to Fig. 6. Three consecutive Na$^+$ fluxes were also measured. The first flux period was measured in the absence of inhibitor (*white bars*), whereas the second and third periods were measured in the presence of 50 μM HOE694 (*gray bars*) and 3 μM S3226 (*black bars*), respectively. Results presented represent mean ± S.E. from 3 experiments. *, $p < 0.001$, compared with control.

designed to identify whether butyrate incubation also altered the NHE2-mediated Na$^+$ absorption. In this study, the effect of butyrate on Na$^+$ fluxes was measured in DSS-inflamed colon that had been incubated for 3 h in butyrate-Ringer. As shown in Fig. 11, butyrate-dependent Na$^+$ absorption is present in the butyrate-incubated inflamed colon. The rate of butyrate-dependent Na$^+$ absorption in butyrate-incubated inflamed colon is almost similar to that present in inflamed colon that was not incubated with butyrate (Fig. 5*B*). In contrast, HOE694 only partially inhibited the butyrate-dependent Na$^+$ absorption, whereas HOE693-insensitve Na$^+$ absorption was inhibited by S3226 in the butyrate-incubated inflamed colon (Fig. 11). These observations indicate that butyrate incubation (3 h) partially activated the NHE3-mediated (*i.e.* HCO$_3^-$-dependent and S3226-sensitive) Na$^+$ absorption, whereas NHE2 (HOE694-sensitive)-mediated butyrate-dependent Na$^+$ absorption is not inhibited in inflamed colon.

Discussion

The present observations emphasize the differential regulation of NHE2 and NHE3 isoforms in DSS-inflamed rat colon and are qualitatively similar to prior studies that have examined the effect of a enteropathogenic *Escherichia coli* (EPEC) strain on NHE function (36–38). EPEC that causes diarrhea in newborns and infants has been shown to differentially regulate NHE2 and NHE3 in human colon cell lines (CaCo2, HT29, and T84) (37). Thus, an EPEC effector protein EspF inhibits NHE3 activity, whereas EPEC-mediated protein kinase C (PKC) regulates the up-regulation of NHE2 function (39, 40). Similarly, cAMP and cholera toxin have also been shown to differentially regulate NHE isoforms in the rat colon and ileum, respectively (17, 26). Although NHE2 function is increased in cell lines, diarrhea is present in EPEC-infected patients. Thus, Hecht *et al.* (37) have concluded that increased NHE2 may not compensate for the fluid losses caused by defective NHE3 function during
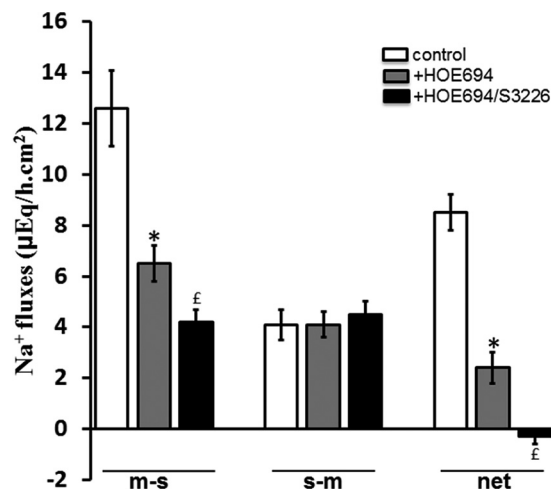

FIGURE 11. **Effect of HCO$_3^-$ and butyrate incubation on butyrate-dependent Na$^+$ fluxes in DSS-induced inflamed rat distal colon.** Mucosal to serosal (*m-s*), serosal to mucosal (*s-m*), and net Na$^+$ fluxes were measured in the presence of butyrate-Ringer as described under "Experimental Procedures." Na$^+$ fluxes were measured in mucosal sheets obtained from loops with colonic inflammation, which were incubated with butyrate-Ringer for the fluid movement measurement in Fig. 6*B*. Three consecutive Na$^+$ fluxes were also measured. The first flux periods were measured in the absence of inhibitor (*white bars*), whereas the second and third periods were measured in the presence of 50 μM HOE694 (*gray bars*), and 50 μM HOE694 and 3 μM S3226 (*black bars*), respectively. Results presented represent mean ± S.E. from 3 experiments. *, $p < 0.001$, compared with respective control; £, $p < 0.001$, compared with HOE694.

EPEC infection. In recent studies, however, we have shown that butyrate stimulates NHE2-mediated Na$^+$ absorption, whereas NHE3 is inhibited by dibutyryl-cAMP in colon and cholera toxin in ileum (17, 26). The present study demonstrates that butyrate stimulates water absorption by activating NHE2-mediated Na$^+$ absorption in the DSS-inflamed rat distal colon.

Both NHE3 and NHE2 mediate pH-dependent, amiloride-sensitive Na$^+$ absorption in both the small and large intestine, but functionally are distinct. Under normal physiological conditions, NHE3 mediates both HCO$_3^-$-dependent and butyrate-dependent Na$^+$ absorption; in contrast, NHE2 is linked only to butyrate-dependent Na$^+$ absorption (Fig. 1). Prior studies had demonstrated that cholera toxin and cyclic AMP inhibit HCO$_3^-$-dependent Na$^+$ absorption via NHE3 but did not affect butyrate-stimulated Na$^+$ absorption that is mediated by NHE2 (17, 26). Our present results demonstrate that DSS-induced inflammation down-regulates NHE3 but not NHE2 (Figs. 4). Because NHE2 is not associated with HCO$_3^-$-dependent Na$^+$ absorption, HCO$_3^-$-dependent Na$^+$ absorption was not observed in the present studies with DSS-induced inflammation.

Both NHE2 and NHE3 isoforms are localized on the apical membranes of intestinal epithelial cells (15, 16). Two recent studies have reported conflicting observations on NHE3 expression in the human colon with UC (20, 21). However, both studies observed NHE2 expression is intact in both active and inactive UC (20, 21). Under basal conditions NHE3 mediates both HCO$_3^-$-dependent and butyrate-dependent Na$^+$ absorption, whereas in the absence of NHE3 (*i.e.* which can be inhibited by cAMP) NHE2 catalyzed butyrate-dependent Na$^+$ absorption in distal colon (17, 26). These present experiments were, therefore, initiated to identify whether NHE2 and/or NHE3 expression is altered and whether active Na$^+$ absorption

is inhibited in colonic inflammation induced by DSS in rat distal colon.

The results presented in this study demonstrate that under the basal condition butyrate activation of NHE2-mediated Na$^+$ absorption reverses fluid secretion to fluid absorption, whereas butyrate incubation activated NHE3-mediated, HCO$_3^-$-dependent Na$^+$ absorption in DSS-induced colonic inflammation. This conclusion is supported by the following observations. 1) Neither NHE2 nor NHE3 protein expression on the APM were altered in inflamed colon (Figs. 3 and 4); 2) although HCO$_3^-$-dependent Na$^+$ absorption was inhibited, butyrate-dependent Na$^+$ absorption was present in the DSS-treated colon (Table 1 and Fig. 5); 3) the NHE isoform-specific inhibitor studies revealed that NHE2-mediated butyrate-dependent Na$^+$ absorption was activated in colonic inflammation (Figs. 6 and 7); 4) fluid movement studies established that butyrate reversed water secretion that was demonstrated in the presence of HCO$_3^-$ to water absorption in colonic inflammation (Fig. 9); and 5) butyrate, but not HCO$_3^-$ incubation activated HCO$_3^-$-dependent, Na$^+$ absorption mediated via NHE3 (*i.e.* S3226-sensitive and HOE694-insensitive Na$^+$ absorption) in colonic inflammation (Fig. 10).

In DSS-induced inflammation NHE2 mediates butyrate-dependent, but not HCO$_3^-$-dependent Na$^+$ absorption (Fig. 5*B*), whereas butyrate activates the NHE3-mediated (*i.e.* S3226-sensitive) HCO$_3^-$-dependent Na$^+$ absorption (Fig. 10*B*). Assuming that similar findings of enhanced NHE2 function are present in patients with UC the diarrhea in UC persists and might be a result of insufficient availability of butyrate. This conclusion is further supported by the observation that incubation of colonic loops with butyrate-Ringer activated HCO$_3^-$-dependent Na$^+$ absorption that is mediated via a S3226-sensitive NHE3 in DSS-induced inflammation (Fig. 10). Consistent with this suggestion it should be noted that butyrate administration has been shown to diminish colonic inflammation and alleviate diarrhea associated with both diversion colitis and UC (41–43). Although NHE3 function is diminished, NHE2 that can mediate butyrate-dependent Na$^+$ absorption is activated in inflamed colon. Butyrate, the primary nutrient of colonocytes, is produced during microbial fermentation of non-absorbed carbohydrate in colon (44). Although regulating Na-Cl absorption, butyrate also inhibits cAMP-activated Cl$^-$ secretion (45). In addition, butyrate has also been shown to increase the anti-inflammatory cytokine IL-10 and decrease proinflammatory cytokine IFN-γ secretion in human lymphocytes *in vitro* (46, 47). It is not unlikely that butyrate availability might have been reduced and as a result, decreased microbial colonization in an inflamed colon. Thus, increased supply of butyrate would possibly control diarrhea and improve inflammation in colon.

Although apical membrane expression is not altered, NHE3 function is entirely absent in inflamed colon (Figs. 4 and 5). These observations are consistent with the data shown in human UC (21). Membrane mobility and second messenger interaction of NHE3 are regulated by a family of Na-H exchanger regulatory factors (NHERF1, NHERF2, and NHERF3) (32, 48, 49). Cyclic nucleotides (cAMP and cGMP) reduce and/or prevent plasma membrane expression of NHE3 by endocytosis/exocytosis pathways (5, 9). Thus, because apical NHE3 expression is not altered, it is likely that NHERF-associated regulatory pathways might have affected NHE3 function in inflamed colon. NHERF1, NHERF2, and NHERF3 expressions have been shown decreased in experimental colitis and human UC (20, 21). A recent study that used a knock-out mouse model has identified NHERF3 as the primary candidate responsible for NHE3 dysfunction in UC (21). Although this present study did not evaluate the NHERF expression pattern, it suggests that butyrate might restore the decreased NHERF level and/or the defective NHE3/NHERF interaction in DSS-induced inflamed colon. This conclusion is further supported by the observation that butyrate incubation partially restored HCO$_3^-$-dependent, S3226-sensitive Na$^+$ absorption in inflamed colon (Fig. 10). However, the mechanism of NHERF3 dysregulation and its restoration by butyrate is not known.

This study demonstrates that although NHE2 and NHE3 expression is not altered, their functions are differentially regulated in inflamed colon. NHE3, which regulates both HCO$_3^-$- and butyrate-dependent Na$^+$ absorption in normal colon, is down-regulated in inflamed colon (Fig. 5). In contrast, NHE2, which is nonfunctional in normal, is activated to regulate butyrate-dependent Na$^+$ absorption in inflamed colon (Fig. 5). We have previously shown that cAMP inhibited NHE3-mediated Na$^+$ absorption, while activating NHE2 that mediated butyrate-dependent Na$^+$ absorption in normal rat distal colon (17). cAMP-mediated phosphorylation has been shown to reduce apical membrane NHE3 expression via exocytosis (9). Thus, it is unlikely that phosphorylation activates NHE2, as apical membrane NHE3 expression is not altered in inflamed colon. It is possible that in the inflamed colon activation of NHE2 might be associated with proinflammatory cytokine-regulated pathways.

In summary, in DSS-induced colonic inflammation: 1) despite unaltered NHE2 and NHE3 expression, butyrate-dependent, but not HCO$_3^-$-dependent Na$^+$ absorption is present; 2) NHE2 mediates butyrate-dependent Na$^+$ absorption; 3) luminal presence of butyrate reverses water secretion to water absorption; and 4) lumen butyrate incubation activates NHE3-mediated HCO$_3^-$-dependent Na$^+$ absorption. These observations suggest that activation of NHE2 function could be a potentially critical target for the control of diarrhea during colonic inflammation, especially in patients with UC.

References

1. Greig, E., and Sandle, G. I. (2000) Diarrhea in ulcerative colitis: the role of altered colonic sodium transport. *Ann. N.Y. Acad. Sci.* **915,** 327–332

2. Binder, H. J. (2009) Mechanisms of diarrhea in inflammatory bowel diseases. *Ann. N.Y. Acad. Sci.* **1165,** 285–293

3. Binder, H. J. (2010) Role of colonic short-chain fatty acid transport in diarrhea. *Annu. Rev. Physiol.* **72,** 297–313

4. Podolsky, D. K. (2002) Inflammatory bowel disease. *N. Engl. J. Med.* **347,** 417–429

5. Yip, R. G., and Wolfe, M. M. (2000) GIP biology and fat metabolism. *Life Sci.* **66,** 91–103

6. Sandle, G. I., Higgs, N., Crowe, P., Marsh, M. N., Venkatesan, S., and Peters, T. J. (1990) Cellular basis for defective electrolyte transport in inflamed human colon. *Gastroenterology* **99,** 97–105

7. Amasheh, S., Barmeyer, C., Koch, C. S., Tavalali, S., Mankertz, J., Epple, H. J., Gehring, M. M., Florian, P., Kroesen, A. J., Zeitz, M., Fromm, M., and Schulzke, J. D. (2004) Cytokine-dependent transcriptional down-regulation of epithelial sodium channel in ulcerative colitis. *Gastroenterology* **126,** 1711–1720

8. Binder, H. J., Sandle, G. I., and Rajendran, V. M. (1991) Colonic fluid and electrolyte transport in health and disease. in *The Large Intestine* (Phillips, S. F., Shorter, R., and Pemberton, W., ed) pp. 141–168, Raven Press, New York

9. Moe, O. W. (1999) Acute regulation of proximal tubule apical membrane Na/H exchanger NHE-3: role of phosphorylation, protein trafficking, and regulatory factors. *J. Am. Soc. Nephrol.* **10,** 2412–2425

10. Butterworth, M. B., Edinger, R. S., Johnson, J. P., and Frizzell, R. A. (2005) Acute ENaC stimulation by cAMP in a kidney cell line is mediated by exocytic insertion from a recycling channel pool. *J. Gen. Physiol.* **125,** 81–101

11. Yang, L. M., Rinke, R., and Korbmacher, C. (2006) Stimulation of the epithelial sodium channel (ENaC) by cAMP involves putative ERK phosphorylation sites in the C termini of the channel's β- and γ-subunit. *J. Biol. Chem.* **281,** 9859–9868

12. Rajendran, V. M., and Binder, H. J. (1994) Apical membrane Cl-butyrate exchange: mechanism of short chain fatty acid stimulation of active chloride absorption in rat distal colon. *J. Membr. Biol.* **141,** 51–58

13. Knickelbein, R., Aronson, P. S., Atherton, W., and Dobbins, J. W. (1983) Sodium and chloride transport across rabbit ileal brush border: I. evidence for Na-H exchange. *Am. J. Physiol.* **245,** G504–510

14. Knickelbein, R., Aronson, P. S., Schron, C. M., Seifter, J., and Dobbins, J. W. (1985) Sodium and chloride transport across rabbit ileal brush border: II. evidence for Cl-HCO3 exchange and mechanism of coupling. *Am. J. Physiol.* **249,** G236–245

15. Donowitz, M., Ming Tse, C., and Fuster, D. (2013) SLC9/NHE gene family, a plasma membrane and organellar family of Na⁺/H⁺ exchangers. *Mol. Aspects Med.* **34,** 236–251

16. Ikuma, M., Kashgarian, M., Binder, H. J., and Rajendran, V. M. (1999) Differential regulation of NHE isoforms by sodium depletion in proximal and distal segments of rat colon. *Am. J. Physiol.* **276,** G539–549

17. Krishnan, S., Rajendran, V. M., and Binder, H. J. (2003) Apical NHE isoforms differentially regulate butyrate-stimulated Na absorption in rat distal colon. *Am. J. Physiol. Cell Physiol* **285,** C1246–1254

18. Chen, T., Kocinsky, H. S., Cha, B., Murtazina, R., Yang, J., Tse, C. M., Singh, V., Cole, R., Aronson, P. S., de Jonge, H., Sarker, R., and Donowitz, M. (2015) Cyclic GMP kinase II (cGKII) Inhibits NHE3 by altering its trafficking and phosphorylating NHE3 at three required sites: identification of a multifunctional phosphorylation site. *J. Biol. Chem.* **290,** 1952–1965

19. Cho, J. H., Musch, M. W., DePaoli, A. M., Bookstein, C. M., Xie, Y., Burant, C. F., Rao, M. C., and Chang, E. B. (1994) Glucocorticoids regulate Na⁺/H⁺ exchange expression and activity in region- and tissue-specific manner. *Am. J. Physiol.* **267,** C796–803

20. Sullivan, S., Alex, P., Dassopoulos, T., Zachos, N. C., Iacobuzio-Donahue, C., Donowitz, M., Brant, S. R., Cuffari, C., Harris, M. L., Datta, L. W., Conklin, L., Chen, Y., and Li, X. (2009) Downregulation of sodium transporters and NHERF proteins in IBD patients and mouse colitis models: potential contributors to IBD-associated diarrhea. *Inflamm. Bowel Dis.* **15,** 261–274

21. Yeruva, S., Farkas, K., Hubricht, J., Rode, K., Riederer, B., Bachmann, O., Cinar, A., Rakonczay, Z., Molnár, T., Nagy, F., Wedemeyer, J., Manns, M.,

22. Raddatz, D., Musch, M. W., Chang, E. B., Hegyi, P., and Seidler, U. (2010) Preserved Na⁺/H⁺ exchanger isoform 3 expression and localization, but decreased NHE3 function indicate regulatory sodium transport defect in ulcerative colitis. *Inflamm. Bowel Dis.* **16,** 1149–1161

22. Kanthesh, B. M., Sandle, G. I., and Rajendran, V. M. (2013) Enhanced K⁺ secretion in dextran sulfate-induced colitis reflects up-regulation of large conductance apical K⁺ channels (BK; Kcnma1). *Am. J. Physiol. Cell Physiol.* **305,** C972–980

23. Sandle, G. I., and Rajendran, V. M. (2012) Cyclic AMP-induced K⁺ secretion occurs independently of Cl⁻ secretion in rat distal colon. *Am. J. Physiol. Cell Physiol.* **303,** C328–333

24. Counillon, L., Scholz, W., Lang, H. J., and Pouysségur, J. (1993) Pharmacological characterization of stably transfected Na⁺/H⁺ antiporter isoforms using amiloride analogs and a new inhibitor exhibiting anti-ischemic properties. *Mol. Pharmacol.* **44,** 1041–1045

25. Schwark, J. R., Jansen, H. W., Lang, H. J., Krick, W., Burckhardt, G., and Hropot, M. (1998) S3226, a novel inhibitor of Na⁺/H⁺ exchanger subtype 3 in various cell types. *Pflugers Arch* **436,** 797–800

26. Subramanya, S. B., Rajendran, V. M., Srinivasan, P., Nanda Kumar, N. S., Ramakrishna, B. S., and Binder, H. J. (2007) Differential regulation of cholera toxin-inhibited Na-H exchange isoforms by butyrate in rat ileum. *Am. J. Physiol. Gastrointest. Liver Physiol.* **293,** G857-G863

27. Stieger, B., Marxer, A., and Hauri, H. P. (1986) Isolation of brush-border membranes from rat and rabbit colonocytes: is alkaline phosphatase a marker enzyme? *J. Membr Biol.* **91,** 19–31

28. Rajendran, V. M., and Binder, H. J. (1990) Characterization of Na-H exchange in apical membrane vesicles of rat colon. *J. Biol. Chem.* **265,** 8408–8414

29. Forbush, B., 3rd. (1983) Assay of Na,K-ATPase in plasma membrane preparations: increasing the permeability of membrane vesicles using sodium dodecyl sulfate buffered with bovine serum albumin. *Anal. Biochem.* **128,** 159–163

30. Del Castillo, J. R., Rajendran, V. M., and Binder, H. J. (1991) Apical membrane localization of ouabain-sensitive K⁺-activated ATPase activities in rat distal colon. *Am. J. Physiol.* **261,** G1005–1011

31. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275

32. Sarker, R., Valkhoff, V. E., Zachos, N. C., Lin, R., Cha, B., Chen, T. E., Guggino, S., Zizak, M., de Jonge, H., Hogema, B., and Donowitz, M. (2011) NHERF1 and NHERF2 are necessary for multiple but usually separate aspects of basal and acute regulation of NHE3 activity. *Am. J. Physiol. Cell Physiol.* **300,** C771–782

33. Biemesderfer, D., Rutherford, P. A., Nagy, T., Pizzonia, J. H., Abu-Alfa, A. K., and Aronson, P. S. (1997) Monoclonal antibodies for high-resolution localization of NHE3 in adult and neonatal rat kidney. *Am. J. Physiol.* **273,** F289–299

34. Binder, H. J., Rajendran, V., Sadasivan, V., and Geibel, J. P. (2005) Bicarbonate secretion: a neglected aspect of colonic ion transport. *J. Clin. Gastroenterol.* **39,** S53–58

35. Musch, M. W., Bookstein, C., Xie, Y., Sellin, J. H., and Chang, E. B. (2001) SCFA increase intestinal Na absorption by induction of NHE3 in rat colon and human intestinal C2/bbe cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **280,** G687–693

36. Nataro, J. P., and Kaper, J. B. (1998) Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* **11,** 142–201

37. Hecht, G., Hodges, K., Gill, R. K., Kear, F., Tyagi, S., Malakooti, J., Ramaswamy, K., and Dudeja, P. K. (2004) Differential regulation of Na⁺/H⁺ exchange isoform activities by enteropathogenic *E. coli* in human intestinal epithelial cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **287,** G370–378

38. McNamara, B. P., Koutsouris, A., O'Connell, C. B., Nougayréde, J. P., Donnenberg, M. S., and Hecht, G. (2001) Translocated EspF protein from enteropathogenic *Escherichia coli* disrupts host intestinal barrier function. *J. Clin. Invest.* **107,** 621–629

39. Hodges, K., Alto, N. M., Ramaswamy, K., Dudeja, P. K., and Hecht, G. (2008) The enteropathogenic *Escherichia coli* effector protein EspF decreases sodium hydrogen exchanger 3 activity. *Cell Microbiol.* **10,**

1735–1745

40. Hodges, K., Gill, R., Ramaswamy, K., Dudeja, P. K., and Hecht, G. (2006) Rapid activation of Na$^+$/H$^+$ exchange by EPEC is PKC mediated. *Am. J. Physiol. Gastrointest. Liver Physiol.* **291,** G959–968

41. Breuer, R. I., Soergel, K. H., Lashner, B. A., Christ, M. L., Hanauer, S. B., Vanagunas, A., Harig, J. M., Keshavarzian, A., Robinson, M., Sellin, J. H., Weinberg, D., Vidican, D. E., Flemal, K. L., and Rademaker, A. W. (1997) Short chain fatty acid rectal irrigation for left-sided ulcerative colitis: a randomised, placebo controlled trial. *Gut* **40,** 485–491

42. Harig, J. M., Soergel, K. H., Komorowski, R. A., and Wood, C. M. (1989) Treatment of diversion colitis with short-chain-fatty acid irrigation. *N. Engl. J. Med.* **320,** 23–28

43. Soergel, K. H., Harig, J. M., Loo, F. D., Ramaswamy, K., and Wood, C. M. (1989) Colonic fermentation and absorption of SCFA in man. *Acta Vet. Scand. Suppl.* **86,** 107–115

44. Bugaut, M. (1987) Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comp. Biochem. Physiol. B* **86,** 439–472

45. Vidyasagar, S., and Ramakrishna, B. S. (2002) Effects of butyrate on active sodium and chloride transport in rat and rabbit distal colon. *J. Physiol.* **539,** 163–173

46. Säemann, M. D., Böhmig, G. A., Osterreicher, C. H., Burtscher, H., Parolini, O., Diakos, C., Stöckl, J., Hörl, W. H., and Zlabinger, G. J. (2000) Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. *FASEB J.* **14,** 2380–2382

47. Weber, T. E., and Kerr, B. J. (2006) Butyrate differentially regulates cytokines and proliferation in porcine peripheral blood mononuclear cells. *Vet. Immunol. Immunopathol.* **113,** 139–147

48. Donowitz, M., and Li, X. (2007) Regulatory binding partners and complexes of NHE3. *Physiol. Rev.* **87,** 825–872

49. Donowitz, M., Mohan, S., Zhu, C. X., Chen, T. E., Lin, R., Cha, B., Zachos, N. C., Murtazina, R., Sarker, R., and Li, X. (2009) NHE3 regulatory complexes. *J. Exp. Biol.* **212,** 1638–1646