Mechanism of regulation of rabbit intestinal villus cell brush border membrane Na/H exchange by nitric oxide

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Coon S, Shao G, Wisel S, Vulaupalli R, Sundaram U. Mechanism of regulation of rabbit intestinal villus cell brush border membrane Na/H exchange by nitric oxide. Am J Physiol Gastrointest Liver Physiol 292: G475–G481, 2007; doi:10.1152/ajpgi.00263.2005.—In the mammalian small intestine, coupled NaCl absorption occurs via the dual operation of Na/H and Cl/HCO3 exchange on the villus cell brush border membrane (BBM). Although constitutive nitric oxide (cNO) has been demonstrated to alter gastrointestinal tract functions, how cNO may specifically alter these two transporters to regulate coupled NaCl absorption is unknown. In villus cells, inhibition of cNO synthase (cNOS) with L-NAME, Na/H exchange was also stimulated. D-NAME, an inactive analog of L-NAME, and Nω-(1-imonoethyl)-L-lysine dithyrolide, a more selective inhibitor of inducible NO synthase, did not affect Na/H exchange. Kinetic studies demonstrated that the mechanism of stimulation is secondary to an increase in the maximal rate of uptake of Na, without an alteration in the affinity of the transporter for Na. Northern blot studies demonstrated an increase in the message for the BBM Na/H exchanger NHE3, and Western blot studies showed that the immunoreactive protein levels of NHE3 was increased when cNOS was inhibited. Thus these results indicate that cNO under nominal physiological states most likely maintains an inhibitory tone on small intestinal coupled NaCl absorption by specifically inhibiting BBM Na/H expression.

NITRIC OXIDE (NO) has been demonstrated to regulate gastrointestinal functions in normal and pathophysiological states (2, 41). NO is produced at multiple levels in the intestine. The epithelial cells, endothelial cells, myenteric neurons, and leukocytes (neutrophils, mast cells, and macrophages) have been demonstrated to produce NO (8, 13, 22, 23, 38). The small quantities of NO produced by constitutive nitric oxide (cNO) synthase (cNOS) in the normal mammalian intestine has generally been thought to be beneficial (2, 10, 29, 41). However, in pathophysiological states (e.g., Crohn’s disease and ulcerative colitis) the larger quantities of NO produced by inducible nitric oxide (iNO) synthase (iNOS) is thought to be deleterious (2, 10, 29, 41). For example, in the normal gastrointestinal tract cNO has been demonstrated to regulate motility, mucosal blood flow and mucus secretion (3, 9, 25, 39). However, in inflammatory bowel disease iNO is thought to contribute to and perpetuate the intestinal inflammation (7, 11, 16).

The exact mechanism of regulation by cNO of an important mammalian small intestinal function, specifically coupled NaCl absorption, is poorly understood. cNOS has been suggested to have no effect on rat small intestinal and colonic electrolyte transport, although other studies have suggested that inhibition of cNO in the rat small intestine promotes secretion (15, 16, 21, 30, 40). Differences in response to NO may be species specific. For example, NO-donating compounds have been shown to be proabsorptive in dog, mouse, and rabbit ileum (1, 18, 28) but prosecretory in rat and guinea pig ileum (17, 37, 42). The literature is equally unclear when it comes to the effect of inhibition of NO production vs. stimulation of NO production and the resultant effect on electrolyte transport. For example, in the rat jejunum both stimulation of NO production and inhibition of NO production have been shown to result in secretion (21, 30, 40). At least some of the contradictory information pertaining to the effect of NO on electrolyte transport in the intestine may be owing to the inability to reproduce the NO levels similar to cNO of the normal mammalian small intestine in these studies. Thus, in studies in which inhibition of coupled NaCl absorption was observed (guinea pig and the rat intestine and colon and pig colon), pathophysiological levels of NO may have been generated, whereas in studies in which stimulation of coupled NaCl absorption was observed (mouse, dog, and rabbit small intestine) lesser levels of NO may have been achieved (1, 9, 18, 28). Perhaps a better strategy would be to inhibit cNO in vivo in the normal mammalian intestine to determine its role is in the regulation of coupled NaCl absorption.

Also, in studies to date the specific mechanism of NO-mediated regulation of coupled NaCl absorption has not been fully deciphered. In the mammalian small intestine coupled NaCl absorption occurs via the dual operation of Na/H and Cl/HCO3 exchange on the brush border membrane (BBM) of villus, but not crypt cells. These transporters are functionally coupled by intracellular pH (pHi) (31). Thus cNO may regulate coupled NaCl absorption by altering Na/H and/or Cl/HCO3 exchange. Of the nine isoforms of Na/H exchange that have been identified to date (43), Na/H exchange (NHE3) is thought to be the isoform responsible for coupled NaCl absorption in the mammalian small intestine (10). In studies to date, specifi-
ically how Na/H and Cl/HCO₃ exchange may be regulated by cNO is not known.

Given this background, the aims of this study were to determine whether cNO may in fact regulate coupled NaCl absorption and to decipher the mechanism of this regulation in the mammalian small intestine.

METHODS

Drug treatment. New Zealand White male rabbits (2.5–2.7 kg) were treated as previously reported from this laboratory (5, 6). Upon review of the literature, in a variety of studies a dose range from 0.1 to 1.3 mM of t-NOS nitroarginine methyl ester (t-NNAME) has been used (13–15, 18, 21, 24). We studied a dose range from 0.01 to 0.5 mM once a day, dissolved in 1 ml of sterile water given intramuscularly for 1–3 days. Sterile water was used a control. Na/H exchange activity in BBMV from villus cells progressively increased from 0.01 to 0.1 mM t-NNAME after 2 days of treatment and then reached a plateau. Cl/HCO₃ exchange was unaffected at all doses and times. On the basis of this information we used a dose of 0.1 mM administered intramuscularly a day for 2 days. Of note, cNOS activity in villus cells from the small intestine also was significantly and maximally inhibited at this dose. Clearly this dose is at the low end of what is used in vivo in the literature; nevertheless, it produced reproducible and unique alterations in Na/H but not Cl/HCO₃ exchange while significantly inhibiting cNOS activity. Therefore, it was chosen as the dose in subsequent studies. Also the inactive analog d-NOS nitroarginine methyl ester (d-NNAME) or N⁶-(1-imonoethyl)-L-lysine dihydrochloride (L-NIL) to inhibit iNOS was used intramuscularly a day for 2 days.

Rabbit model of chronic small intestinal inflammation. Chronic intestinal inflammation was produced in rabbits as previously reported (30). Pathogen-free New Zealand White male rabbits (Prince’s Rabbitry, Oakville, KY) were inoculated with Eimeria magna oocysts or sham inoculated with 0.9% NaCl (control animals). None of these sham inoculations and more than 80% of inoculation with coccidia resulted in chronic small intestinal inflammation during days 13–14. During the chronic phase of ileal inflammation histologically the intestine is free of the coccidia. Only enterocytes from those animals that had chronic small intestinal inflammation were utilized.

Cell isolation. Villus and crypt cells were isolated from the rabbit ileum by a calcium chelation technique as previously described (32). Pathogen-free New Zealand White male rabbits (Prince’s Rabbitry, Oakville, KY) were inoculated with Eimeria magna oocysts or sham inoculated with 0.9% NaCl (control animals). None of these sham inoculations and more than 80% of inoculation with coccidia resulted in chronic small intestinal inflammation during days 13–14. During the chronic phase of ileal inflammation histologically the intestine is free of the coccidia. Only enterocytes from those animals that had chronic small intestinal inflammation were utilized.

BBM vesicle preparation. BBM vesicles (BBMV) from rabbit ileal villus cells were prepared by CaCl₂ precipitation and differential centrifugation as previously reported (31, 34). BBMV were resuspended in a medium appropriate to each experiment. BBMV purity was assayed with marker enzyme enrichment (e.g., alkaline phosphatase).

pH₅.₅ measurements. pH₅.₅ was measured utilizing BCECF as previously described (31, 35, 36). Briefly, the cells were loaded with 10 µM of the acetoxy methylester of BCECF from a 10 mM stock in DMSO for 10 min at 37°C. A coverslip coated with subconfluent monolayer of cells was mounted in a thermostatically controlled cuvette (37°C) in a Perkin-Elmer LS-5 spectrofluorometer with constant perfusion to wash away any leaked dye. The dye was alternatively excited at 450 and 500 nm, and the fluorescence emission was measured at 530 nm. The BCECF fluorescence excitation ratio was calibrated by the high-K₅.₅.₅.₅.₅ technique (31). All experiments were performed in CO₂-HCO₃ or Na-HEPES solutions. The standard CO₂-HCO₃ solution contained (in mM) 115 NaCl, 25 NaHCO₃, 2.4 K₄HPO₄, 0.4 KH₂PO₄, 1.25 MgCl₂, 1.25 CaCl₂ and was gassed with 5% CO₂-95% O₂ (pH 7.4, at 37°C). The Na-HEPES solution contained (in mM) 130 NaCl, 4.5 KCl, 1.2 KH₃PO₄, 1 MgSO₄, 1.25 CaCl₂, 20 HEPES and was gassed with 100% O₂ (pH 7.4 at 37°C). To acid load the villus cells, an NH₄Cl prepulse for 4 min with 30 mM of NH₄Cl replacing 30 mM NaCl in Na-HEPES solutions was used as previously reported (31). To alkaline load the villus cells, a propionate prepulse for 6 min in CO₂-HCO₃ with 50 mM Na-propionate replacing 50 mM NaCl was used also as previously reported (31).

Uptake studies in villus cell BBMV. BBMV uptake studies were performed by the rapid filtration technique as previously described (5, 19). For Na/H exchange experiments BBMV were resuspended in 150 mM mannitol, 100 mM tetramethylammonium (TMA) gluconate, 50 mM MES-Tris (pH 5.5) or 50 mM HEPES-Tris (pH 7.5). The reaction was started by adding 5 µl of vesicles to 95 µl of reaction mix containing 150 mM mannitol, 99 mM TMA gluconate, 50 mM HEPES-Tris (pH 7.5), and 1 mM ²²Na gluconate; 1 mM miloride was added to the reaction mix when relevant. At desired times uptake was arrested by mixing with ice-cold stop solution. The mixture was filtered on a 0.45-µm Millipore (HAWP) filters and washed twice with 5 ml of ice-cold stop solution. Filters with BBMV were dissolved in LiQuisint, and radioactivity was determined (5, 6, 19).

Northern blot studies. Total RNA was extracted from rabbit ileal villus cells by the guanidinium isothiocyanate-cesium chloride method (4). After denaturation, total RNA was electrophoresed on 1.8% agarose-formaldehyde gel, transferred to nylon membrane (Schleicher and Schuell, Keene, NH), and incubated with prehybridization solution. Membranes were hybridized with ³²P labeled NHE3 cDNA. The cDNA was random labeled with [³²P]CTP with Klenow polymerase. β-Actin was used to ensure equal loading of total RNA onto the electrophoresis gels. Hybridized membrane was exposed to autoradiography film (NEN, Boston, MA). The rabbit intestinal specific NHE3 cDNA was generously provided by Drs. Tse and Donowitz.

Western blot studies. BBMV (4 µg) were diluted in SDS buffer, boiled, and electrophoresed on a 12% SDS-PAGE gel. The gel was electrobotted onto a polyvinylidene difluoride membrane and blocked for 2 h in 5% BSA at room temperature. The membrane was incubated at room temperature with 1:3,000 anti-rabbit NHE3 antiserum (Alpha Diagnostics) followed by goat anti-rabbit IgG coupled to horseradish peroxidase (1:10,000, Pierce, Rockford, IL). After each incubation, the membrane was washed extensively with PBS-0.2% Tween 20.

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The signal was developed with the chemiluminescence Western blot kit (NEN Research Products, Boston, MA).

Data presentation. These data are averaged, and means ± SE are shown, except when error bars are inclusive within the symbol. All uptakes were done in triplicate. The number \( n \) for any set of experiments refers to vesicle or isolated cell preparations from different animals. Preparations in which cell viability was <85% were excluded from analysis. Student’s \( t \)-test was used for statistical analysis.

RESULTS

Villus cell studies. To determine whether \( L \)-NAME administered in vivo had an effect solely on cNO production in isolated villus cells from the normal rabbit small intestine, we measured cNOS activity (Ca\(^{2+}\)-dependent NOS activity) in these cells. Treatment with \( L \)-NAME significantly reduced cNOS activity in isolated villus cells from \( L \)-NAME-treated rabbits \( (5.5 ± 0.5 \) pmol citrulline·mg protein\(^{-1} \cdot \text{min}^{-1} \) in control and \( 1.5 ± 0.4 \) in \( L \)-NAME treated, \( n = 5, P < 0.001 \)). These data indicated that \( L \)-NAME inhibits cNO production in villus cells in the rabbit intestine. To ensure that the in vivo effect of \( L \)-NAME on villus cells is not a nonspecific effect secondary to, for example, altered blood flow, etc., we treated nontransformed rat small intestinal cells (IEC-18) in vitro for 2 days with \( 1.0 \) \( \mu \)M \( L \)-NAME. This also inhibited cNOS activity similar to the rabbit \( (4.1 ± 0.6 \) pmol citrulline·mg protein\(^{-1} \cdot \text{min}^{-1} \) in control and \( 0.1 ± 0.1 \) in \( L \)-NAME-treated cells, \( n = 3, P < 0.01 \)). Furthermore, although one would not expect much if any iNO in the normal mammalian intestine, to ensure that iNO was not a significant presence the effect of \( L \)-NIL, a more specific inhibitor of iNOS, was studied. Treatment with \( L \)-NIL did not affect iNOS activity in isolated villus cells from the normal intestine, whereas the expected elevated iNOS activity in villus cells from the chronically inflamed intestine was significantly inhibited by \( L \)-NIL \( (1.7 ± 0.3 \) pmol citrulline·mg protein\(^{-1} \cdot \text{min}^{-1} \) in normal, \( 1.4 ± 0.4 \) in \( L \)-NIL-treated normal, \( 8.3 ± 1.0 \) in inflamed \( (P < 0.05) \), and \( 2.5 ± 0.5 \) in inflamed \( L \)-NIL treated cells \( (P < 0.01) \) \( (n = 3) \). These data indicated that in vivo treatment with \( L \)-NAME does selectively inhibit cNOS in the rabbit intestinal mucosa.

Figure 1A demonstrates the effect of \( L \)-NAME treatment on recovery from an acid load induced by \( \text{NH}_{4}\text{Cl} \) in villus cells. After establishing a steady-state pH \( (\text{pH} 7.12 ± 0.05 \) in control and \( 7.17 ± 0.04 \) in \( L \)-NAME-treated cells, \( n = 5 \) ), cells were briefly pulsed with \( \text{NH}_{4}\text{Cl} \), which results in acidification of the villus cells. Under nominal conditions \( (\text{e.g., Na-HEPES}) \) the cells recover back to basal \( \text{pH} \) \( (\text{pH} 7.10 ± 0.05 \) in control and \( 7.16 ± 0.05 \) in \( L \)-NAME-treated cells, \( n = 5 \) ). However, recovery from an \( \text{NH}_{4}\text{Cl} \)-induced acid load that we have previously demonstrated to be \( \text{Na}^{+} \) and amiloride sensitive \( (\text{e.g., Na/H exchange}) \) in villus cells is accelerated in \( L \)-NAME-treated rabbits \( (\text{Fig. 1A}) \). These data indicated that inhibition of cNOS with \( L \)-NAME resulted in the stimulation of \( \text{Na/H} \) exchange in villus cells. Since coupled \( \text{NaCl} \) absorption occurs via the dual operation of \( \text{Na/H} \) and \( \text{Cl/HCO}_{3} \) exchange \( \) (coupled by \( \text{pH} \)) in rabbit small intestinal villus cells, the effect of cNOS inhibition on \( \text{Cl/HCO}_{3} \) exchange was also determined. Recovery from a propionate-induced alkaline load that we have previously demonstrated to be \( \text{Cl}^{-} \) and DIDS sensitive \( (\text{e.g., Cl/HCO}_{3} \) exchange) in villus cells was not affected in \( L \)-NAME-treated rabbits \( (\text{Fig. 1B}) \). These data indicated that inhibition of cNOS with \( L \)-NAME did not affect \( \text{Cl/HCO}_{3} \) exchange.

Altered intracellular buffering capacity may alter rates of recovery. To ensure that this was not the case in \( L \)-NAME-treated conditions, we measured intracellular buffering capacity in villus cells from control or \( L \)-NAME-treated rabbits \( \) \( (\text{i.e., average intracellular buffering capacity over a 0.2 pH unit \text{ (7.3–7.5)}} \) buffering domain was \( 25.1 ± 3.5 \) in control and \( 22.6 ± 2.9 \) mM/pH unit in \( L \)-NAME-treated villus cells; \( n = 4, P \) not significant). Similarly, it is important to ensure that pH recovery was measured from the same starting pH in both preparations. Indeed, the pH after the \( \text{NH}_{4}\text{Cl} \) prepulse at which the measurement for rates of recovery for \( \text{Na/H} \) exchange were measured started at \( 6.61 ± 0.02 \) for control and \( 6.62 ± 0.02 \) for villus cells from \( L \)-NAME-treated rabbits \( (n = 4, P \) not significant). Similarly, for \( \text{Cl/HCO}_{3} \) exchange, the pH after the propionate prepulse, at which we initiated measurement of rates of recovery was \( 7.56 ± 0.02 \) in control and \( 7.58 ± 0.02 \) in villus cells from \( L \)-NAME-treated rabbits \( (n = 4, P \) not significant).

\( \text{Na/H} \) in BBMV. We then studied \( \text{Na/H} \) exchange, previously defined as amiloride-sensitive and proton gradient-stimulated \( ^{22}\text{Na} \) uptake \( \) (\text{e.g., Na/H exchange}) in villus cell BBMV. As demonstrated in Fig. 2, \( L \)-NAME treatment enhanced \( ^{22}\text{Na} \)
uptake in villus cell BBMV. These data indicate that inhibition of cNOS with L-NAME resulted in the stimulation of Na/H exchange in villus cell BBMV. These data also indicate that the effect of cNO on Na/H is most likely at the level of the transporter.

To ensure that the effect of L-NAME is specific, the effect of the inactive analog, D-NAME, was studied. D-NAME had no effect on $^{22}$Na uptake in villus cell BBMV (Fig. 3A). Similarly, as previously noted although one would not expect a role for iNO in the normal mammalian intestine, the effect of l-NIL, a more specific inhibitor of iNOS, was nevertheless studied on BBMV Na/H exchange. As demonstrated in Fig. 3B, l-NIL also had no effect on Na/H in villus cell BBMV. These data indicate that l-NAME stimulates Na/H and that the effect is specific to its ability to reduce the production of cNO.

**Kinetic studies.** To determine the mechanism of diminished cNO mediated stimulation of Na/H in the small intestine kinetic studies were performed. Uptake for all the concentrations was carried out at 6 s since the initial uptake studies for Na/H exchange in BBMV was linear for at least 10 s. Figure 4 demonstrates the kinetics of $^{22}$Na uptake in villus cells BBMV from the rabbit small intestine. Figure 4 shows amiloride-sensitive and proton gradient-stimulated uptake of $^{22}$Na as a function of varying concentrations of extravesicular Na. As the concentration of extravesicular Na was increased, the uptake of $^{22}$Na was simulated and subsequently became saturated in all conditions (Fig. 4A). Using Enzfitter, kinetic parameters derived from this data demonstrated that the maximal rate of uptake ($V_{\text{max}}$) was markedly increased by l-NAME treatment ($182.8 \pm 10.6 \text{ nmol/mg protein}^{-1}\text{min}^{-1}$ in control and $433.7 \pm 20.3 \text{ in l-NAME treated, } n = 4, P < 0.05$). However, the affinity ($K_{\text{m}}$) for Na uptake was not altered by l-NAME treatment (Fig. 4B; $K_{\text{m}}$ was $21.7 \pm 3.6 \text{ mM}$ in control and $31.3 \pm 7.7 \text{ in l-NAME treated, } n = 4$). These data demonstrated that inhibition of cNO production stimulates Na/H by increasing the BBM transporter numbers or increased turnover rate.

**Molecular studies.** To determine whether indeed the levels of the transporter are altered in villus cells after L-NAME treatment, we looked at the message for NHE3. Northern blot studies demonstrated that the message for NHE3 was indeed increased in villus cells from the l-NAME-treated animals (Fig. 5). Densitometric quantitation demonstrated that although there was no significant difference in $\beta$-actin, NHE3 levels increased four- to fivefold in villus cells from l-NAME-treated rabbits. Because steady-state mRNA levels may not directly correlate with functional protein levels on the BBM, immunoreactive NHE3 levels on the BBM were also determined. Western blot analysis of villus cell BBM showed that the immunoreactive protein levels of NHE3 were also increased in animals treated with l-NAME (Fig. 6). Again, densitometric quantitation demonstrated a three- to fourfold increase in NHE3 protein in villus cell BBM from l-NAME-treated rabbits. These molecular biology studies are consistent with the kinetic studies and indicate that the mechanism of stimulation of NHE3 when cNO production is inhibited is secondary to an alteration in the number of transporters on the BBM without a change in the affinity of the exchanger for Na.

**DISCUSSION**

This study for the first time demonstrates directly that cNO exerts an inhibitory tone on coupled NaCl absorption in the normal mammalian small intestine. cNO affects coupled NaCl absorption via its effect on Na/H, but not Cl/HCO$_3^-$ exchange on the BBM of intestinal villus cells. l-NAME treatment inhibits cNO in villus cells from the rabbit small intestine, and...
in these cells recovery from an acid load was stimulated. These data indicated that cNO normally exerts an inhibitory tone on Na/H exchange in villus cells, which was relieved by its inhibition with L-NAME. In contrast, in villus cells from L-NAME-treated rabbits, recovery from an alkaline load was unaffected. These data indicated that cNO exerts its effect on coupled Na-Cl absorption via its effect on Na/H but not Cl/HCO₃ exchange.

In intact cell studies recovery from an acid or alkaline load could be affected by alterations in the intracellular buffering capacity. However, buffering capacity was not altered in these cells. Nevertheless, studies were performed in BBM vesicles where this is not a concern. Proton gradient-dependent and amiloride-sensitive uptake of ²²Na was enhanced in BBMV prepared from L-NAME-treated rabbit small intestinal villus cells. These data indicated that indeed cNO exerts an inhibitory tone on BBM Na/H exchange, which was relieved after L-NAME treatment. In contrast, HCO₃-dependent and DIDS-sensitive ³⁶Cl uptake was unaffected in villus cell BBMV prepared from the L-NAME-treated rabbits (6). These data indicated that cNO indeed altered coupled NaCl absorption by regulation of BBM Na/H, but not Cl/HCO₃ exchange.

Since only L-NAME but not the inactive analog D-NAME altered Na/H exchange, the effect of L-NAME in terms of inhibition of cNO appears to be specific. Furthermore, since L-NIL, a more selective inhibitor of iNOS, also had no effect on Na/H exchange in the normal small intestine, it is evident that the observed results with L-NAME on Na/H exchange are due to its effect on cNO.

To determine the mechanism of stimulation of Na/H exchange, kinetic studies were then performed. The studies demonstrated that although the affinity of the transporter was not affected, there was a substantial increase in the V_max for Na/H exchange, suggesting an increase in transporter numbers or increased turnover rate. Indeed, subsequent molecular studies demonstrated that the message for NHE3 as well as the immunoreactive protein levels of NHE3 on the BBM of the villus cells from the L-NAME-treated rabbit small intestine increased substantially. Thus the mechanism of stimulation of Na/H exchange when cNO is inhibited with L-NAME is secondary to an increase in the number of NHE3 transporters.

NO has been demonstrated to be produced by multiple cell types in the intestine including epithelial cells themselves (8, 16, 22, 23, 38). Thus it is not surprising that NO has also been demonstrated to alter important gastrointestinal tract functions such as motility, blood flow, and production of mucus. Although NO has been implicated in electrolyte and fluid transport in the intestine, the results of these studies are, if anything, inconclusive (17, 18, 21, 37, 40, 42). The differences may be related to the species. Whether the amount of NO produced by NO generating compounds represents normal or pathophysiological levels is not always known with absolute certainty (10,
17, 29). Small quantities of NO are produced in the normal intestine by cNO and in large amounts during pathophysiological states by iNO (11, 12, 16, 20, 41). Furthermore, since the effect of NO in vivo during chronic intestinal inflammation is a combination of NO release and peroxynitrite formation, studies using NO donors to approximate NO produced by iNO fail to duplicate this scenario and only demonstrate the effects of increased NO alone. Additionally, when NO reaches a higher concentration, as is the case when iNOS produces it, these concentrations also affect the production of inducible cyclooxygenase metabolites such as PGE2 (16). Thus it is more likely that the levels of NO generated in these studies were variable and could have led to physiological and/or pathophysiological responses. Finally, these studies did not decipher the mechanism of alteration at the transporter level. Indeed, the findings of this study for the first time are consistent with the demonstration that mammalian small intestinal NaCl absorption is regulated by cNO by regulating Na/H exchanger numbers and without an effect on Cl/HCO3 exchange.

Determination of the effect of cNO on NaCl absorption in the normal mammalian small intestine is also complicated by the fact that in intact tissue studies it is not possible to separate the effects of a given agent on the primarily absorptive villus cells vs. the primarily secretory crypt cells. This is a significant concern as it has been clearly demonstrated that coupled NaCl absorption takes place via the dual operation of Na/H and Cl/HCO3 exchange on the BBM of villus cells. These two transporters are thought to be linked by pH; thus an effect on one would lead to an overall effect on NaCl transport. In contrast, the crypt cells only have Cl/HCO3 exchange on the BBM; thus it is not capable of NaCl absorption. In view of this, it is important to isolate relatively pure populations of viable villus cells to ideally study the effect of the cNO on coupled NaCl absorption. This was indeed demonstrated in this study, and, as the results demonstrate, coupled NaCl absorption is regulated by cNO by an effect on Na/H but not Cl/HCO3 exchange in villus cells.

In conclusion, this study for the first time demonstrated that in vivo cNO uniquely regulates coupled NaCl absorption in the mammalian small intestine. Inhibition of cNO resulted in the stimulation of Na/H but not Cl/HCO3 exchange. Thus cNO imposes an inhibitory tone on coupled Na-Cl absorption in the normal mammalian small intestine by altering Na/H but not Cl/HCO3 exchange. Finally, the mechanism of cNO-mediated effect on Na/H exchange is by increasing the number of NHE3 isoforms of this transporter on the BBM without an effect on the affinity of the exchanger. (14, 24)

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