Unique regulation of anion/HCO$_3^-$ exchangers by constitutive nitric oxide in rabbit small intestine

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Coon, Steven, and Uma Sundaram. Unique regulation of anion/HCO$_3^-$ exchangers by constitutive nitric oxide in rabbit small intestine. Am J Physiol Gastrointest Liver Physiol 285: G1084–G1090, 2003; 10.1152/ajpgi.00013.2003.—In the rabbit small intestine, there are three functionally different brush-border membrane (BBM) anion/HCO$_3^-$ exchangers: 1) Cl/HCO$_3^-$ exchange on the BBM of villus cells responsible for coupled NaCl absorption; 2) Cl/HCO$_3^-$ exchange on the BBM of crypt cells possibly involved in HCO$_3^-$ secretion; and 3) short-chain fatty acid (SCFA)/HCO$_3^-$ exchange on the BBM of villus cells, which facilitates SCFA absorption. Although constitutive nitric oxide (cNO) has been postulated to alter many gastrointestinal tract functions, how cNO may specifically alter these three transporters is unknown. Inhibition of cNO synthase with N$^G$-nitro-L-arginine methyl ester (L-NAME) 1) did not affect villus cell BBM Cl/HCO$_3^-$ exchange, 2) stimulated crypt cell BBM Cl/HCO$_3^-$ exchange, and 3) inhibited villus cell BBM SCFA/HCO$_3^-$ exchange. d-NAME, an inactive analog of l-NAME, and 1-NAME (1-iminoethyl)lysine, a more selective inhibitor of inducible NO, did not affect these transport processes. Kinetic studies demonstrated that 1) the mechanism of inhibition of crypt cell BBM Cl/HCO$_3^-$ exchange is secondary to a decrease in the maximal rate of uptake of Cl, without an alteration in the affinity of the transporter for Cl, and 2) the mechanism of stimulation of villus cell BBM SCFA/HCO$_3^-$ exchange is secondary to an increase in the affinity of the transporter for SCFA without an alteration in the maximal rate of uptake of SCFA. These results indicate that cNO uniquely regulates the three BBM anion/HCO$_3^-$ transporters in the rabbit small intestine.

IN THE RABBIT SMALL INTESTINE, three different brush-border membrane (BBM) anion/HCO$_3^-$ exchangers have been demonstrated, which have unique functions: 1) Cl/HCO$_3^-$ exchange on the BBM of villus cells, which coupled by intracellular pH with Na/H exchange, is responsible for coupled NaCl absorption (25); 2) Cl/HCO$_3^-$ exchange on the BBM of crypt cells possibly involved in HCO$_3^-$ secretion (25); and 3) short-chain fatty acid (SCFA)/HCO$_3^-$ exchange on the BBM of villus cells (11). Although the molecular identity of these three anion exchangers has yet to be elucidated, it has been well established that in the normal intestine and in intestinal pathophysiology, these three exchangers are uniquely regulated. For example, in the normal rabbit intestine, a secretogogue such as serotonin has been demonstrated to inhibit coupled NaCl absorption by inhibiting Cl/HCO$_3^-$ but not Na/H exchange on the BBM of villus cells (26). In contrast, serotonin stimulates Cl/HCO$_3^-$ exchange on the BBM of crypt cells, which may result in HCO$_3^-$ secretion by these cells (26).

Similarly, during chronic intestinal inflammation all three of these anion exchangers are again uniquely regulated as well (4, 7, 27). For example, although both villus cell BBM Cl/HCO$_3^-$ and SCFA/HCO$_3^-$ exchange are inhibited, the mechanism of inhibition of each transporter is unique (4, 7). Cl/HCO$_3^-$ exchange is inhibited secondary to an alteration in the affinity of the transporter for Cl without a change in transporter numbers (4). In contrast, SCFA/HCO$_3^-$ exchange is inhibited secondary to a decrease in transporter numbers without an change in the affinity of the transporter (7). Unlike both of these villus cell BBM anion exchangers, the crypt cell BBM Cl/HCO$_3^-$ exchange is secondarily stimulated to secrete HCO$_3^-$ during chronic intestinal inflammation (27). Thus studies clearly indicate that there are at least three functionally different anion/HCO$_3^-$ exchangers on the BBM of villus and crypt cells in the rabbit intestine.

NO has been demonstrated to be produced by multiple cell types in the intestine. Nitric oxide (NO) produced by constitutive nitric oxide (cNO) synthase in the normal intestine and by inducible NO (iNO) synthase in pathophysiological states has been demonstrated to regulate gastrointestinal functions (2, 39). In general, the small quantities of NO produced by cNO synthase in the normal mammalian intestine has generally been thought to be beneficial (2, 3, 7, 14, 19, 21, 36, 39). In contrast, during pathophysiological states (e.g., Crohn’s disease and ulcerative colitis), the larger quantities of NO produced by iNO are thought to be deleterious (2, 5, 7, 8, 22, 39).

The role of NO on intestinal electrolyte transport in general is poorly understood (9). Whether NO has no effect, promotes absorption, or secretion appears to depend, at least in part, on 1) whether the condition of the intestine is physiological or pathophysiological, 2) the part of the intestinal tract studied, and 3) the...
species studied (1, 10, 11, 21, 34, 40). Further inhibition of NO vs. stimulation of NO production has not necessarily yielded the expected corollary response on electrolyte absorption and secretion from study to study (16, 23, 38). At least some of the conflicting 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 (1, 11, 21).

Another potential concern with intact tissue studies that were used to determine the role of NO on electrolyte transport is that the epithelium is composed of functionally different villus and crypt cells (25). The former is absorptive, and the latter is secretory, thus any observed effect will be a compilation of these two properties and therefore is less likely to be mechanistic. So, it is important to separate these two cell types to best determine the effect of an agent on electrolyte transport in the intestine, as was done in this study. Also in studies to date, whether cNO can uniquely regulate a related family of BBM transporters and between absorptive villus and secretory crypt cells has not been deciphered. Thus, given this background, the aims of this study were to determine whether cNO may indeed regulate anion/HCO3 exchange.

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tine. As shown on Fig. 2, $^{36}$Cl uptake was significantly reduced in L-NAME-treated intestine at all early time points measured. These data indicated that cNO stimulates crypt cell BBM Cl/HCO$_3$ exchange under physiological conditions.

To ensure that the effect of L-NAME is indeed specific to diminishing the production of cNO, the effect of the inactive analog d-NAME was then studied. d-NAME had no effect on DIDS-sensitive and HCO$_3$-gradient-stimulated $^{36}$Cl uptake in crypt cell BBMV (Fig. 3). Similarly, whereas one would not expect a role for iNO in the normal mammalian intestine, the effect of L-NIL, a more specific inhibitor of iNO synthase was nevertheless studied. As demonstrated in Fig. 4, L-NIL also had no effect on Cl/HCO$_3$ exchange in crypt cell BBMV. These data indicate that the L-NAME treatment inhibits crypt cell BBM Cl/HCO$_3$ exchange and that the effect is specific to its ability to reduce cNO.

Villus cell BBM SCFA/HCO$_3$ exchange. Because cNO had no effect on villus cell BBM Cl/HCO$_3$ exchange, to determine whether a different villus cell BBM anion/HCO$_3$ exchanger may be affected differently by cNO, we looked at SCFA/HCO$_3$ exchange. This exchanger is known to be present only on the BBM of villus cells in the rabbit small intestine (12). We studied the effect of inhibition of cNO production with L-NAME on pH and HCO$_3$-gradient-stimulated $[^{14}$C]butyrate uptake in villus cell BBMV prepared from control and L-NAME-treated rabbit small intestine. As shown in Fig. 5, butyrate uptake was significantly enhanced in villus cell BBM from L-NAME-treated intestine at all early time points measured. These data indicated that cNO inhibits villus cell BBM SCFA/HCO$_3$ exchange under physiological conditions.

To determine whether the effects of L-NAME on SCFA/HCO$_3$ exchange is indeed specific to diminishing the production of cNO, the effect of the inactive analog D-NAME was then studied. D-NAME had no effect on pH and HCO$_3$-gradient-stimulated $[^{14}$C]butyrate uptake in villus cell BBMV (Fig. 6). Also, whereas one would not expect any substantial amount of iNO in the normal mammalian intestine, the effect of L-NIL, a more specific inhibitor of iNO synthase, was nevertheless studied on SCFA/HCO$_3$ exchange. As demonstrated in Fig. 7, L-NIL also had no effect on SCFA/HCO$_3$ exchange in villus cell BBMV. These data indicate that L-NAME-mediated inhibition of SCFA/HCO$_3$ exchange in villus cell BBM is via its ability to reduce cNO.

Kinetic studies. To determine the mechanism of diminished cNO-mediated inhibition of crypt cell BBM Cl/HCO$_3$ exchange, kinetic studies were performed.
Uptake for all the concentrations was carried out at 6 s, because the initial uptake studies for pH and HCO$_3^-$ gradient-stimulated $^{36}$Cl uptake in crypt cell BBMV were linear for at least 10 s. Figure 8 demonstrates the kinetics of $^{36}$Cl uptake in crypt cell BBMV from the rabbit small intestine. Figure 8 shows the uptake of $^{36}$Cl as a function of varying concentration of extravesicular Cl. As the concentration of extravesicular Cl was increased, the uptake of $^{36}$Cl was simulated and subsequently became saturated in all conditions. With the use of Enzfitter, kinetic parameters derived from these data demonstrated that $V_{\text{max}}$ was markedly reduced by the inhibition of cNO production (Fig. 8A; $V_{\text{max}}$ was 54.6 ± 0.3 nmol-mg protein$^{-1}$·s$^{-1}$ in control and 25.8 ± 1.0 nmol-mg protein$^{-1}$·s$^{-1}$ in L-NAME treated, $n = 4; P < 0.05$). However, the affinity ($K_m$) for Cl uptake was not altered by L-NAME treatment (Fig. 8B; $K_m$ was 6.6 ± 0.2 mM in control and 6.0 ± 0.1 mM in L-NAME treated, $n = 4$). These data demonstrated that inhibition of cNO production inhibits Cl/HCO$_3^-$ exchange in crypt cells by likely reducing transporter numbers.

Next, to determine the mechanism of reduced cNO production-mediated stimulation of villus cell SCFA/HCO$_3^-$ exchange, kinetic studies were performed. Uptake for all of the concentrations was carried out at 3 s, because the initial uptake studies for pH and HCO$_3^-$ gradient-stimulated $[^{14}]$Cbutyrate uptake in villus cell BBMV were linear for at least 6 s. Figure 9 demonstrates the kinetics of $[^{14}]$Cbutyrate uptake in villus cell BBMV from the rabbit small intestine. Figure 9 shows the uptake of $[^{14}]$Cbutyrate as a function of varying concentration of extravesicular butyrate. As the concentration of extravesicular butyrate was increased, the uptake of $[^{14}]$Cbutyrate was simulated and subsequently became saturated in all conditions. With the use of Enzfitter, kinetic parameters derived from these data demonstrated that $V_{\text{max}}$ was not affected by the inhibition of cNO production (Fig. 9A; $V_{\text{max}}$ was 15.1 ± 0.2 nmol-mg protein$^{-1}$·s$^{-1}$ in control and 14.5 ± 0.3 nmol-mg protein$^{-1}$·s$^{-1}$ in L-NAME treated, $n = 4$). However, $K_m$ for butyrate uptake was markedly reduced by L-NAME treatment (Fig. 9B; $K_m$ was 39.6 ± 0.8 mM in control and 12.4 ± 0.4 mM in L-NAME treated, $n = 4; P < 0.05$). These data indicated that inhibition of cNO production stimulates SCFA/HCO$_3^-$ exchange in villus cells by likely increasing the affinity of the transporter for butyrate.

**DISCUSSION**

This study, for the first time, demonstrates directly that cNO uniquely regulates the three functionally different anion/HCO$_3^-$ exchangers on the BBM mammalian small intestinal villus and crypt cells. On the villus cell BBM, Cl/HCO$_3^-$ exchange, thought to be important for coupled NaCl absorption, is not altered by cNO. However, Cl/HCO$_3^-$ exchange on the BBM of crypt cells, thought to be involved in intestinal HCO$_3^-$ secretion, is inhibited when cNO production is diminished. This indicates that in physiological states, cNO exerts a positive tone on HCO$_3^-$ secretion in the small intestine. Unlike these two anion/HCO$_3^-$ exchangers, SCFA/HCO$_3^-$, which is only found on the BBM of villus cells, is stimulated when cNO production is reduced. This indicates that under physiological conditions, cNO exerts a negative tone on SCFA/HCO$_3^-$ exchange.

Several anion exchanger families have been identified and cloned. Anion exchangers 1–4 (AE 1–4), down-regulated adenoma (DRA), pendrin (PDS), and putative anion transporter (PAT 1) have all been proposed as candidates for BBM anion/HCO$_3^-$ exchange activity (13, 20, 24, 37). Melvin et al. (13) suggested that DRA is the main candidate for Cl/HCO$_3^-$ exchange in these cells. However, other studies would point to a member of the AE family, specifically AE1–3, because all have been proposed as the BBM Cl/HCO$_3^-$ exchanger by some, whereas others have demonstrated them on the BBM (13, 15, 20, 24, 37). Thus, whereas the molecular identity of the three BBM anion/HCO$_3^-$ exchangers studied here has yet to be resolved, they without a doubt function uniquely in the normal intestine and are also altered uniquely in the chronically inflamed intestine. This suggests that they may indeed be different proteins and thus lend themselves to unique regulation by an agent. Indeed, as demonstrated in this study, NO, a 30-kDa biological mediator known to be present in the intestine, uniquely regulates these...
three BBM anion/HCO\textsubscript{3}\textsuperscript{-} exchangers. DIDS-sensitive and HCO\textsubscript{3}\textsuperscript{-}-dependent Cl uptake, defined as Cl/HCO\textsubscript{3} exchange, was not affected in the villus cell BBM, however, it was significantly reduced when NO production was diminished in crypt cells. In contrast, HCO\textsubscript{3}\textsuperscript{-} and pH-dependent butyrate uptake, defined as SCFA/HCO\textsubscript{3} exchange, known only to be present on the BBM of villus cells, was markedly enhanced when NO production was reduced. Whereas the molecular identity of this anion exchange is also not known, monocarboxylate-type transport proteins (MCT1 and -2) are possible candidates (15). In summary, these data indicate that cNO, under normal physiological conditions, stimulates crypt cell Cl/HCO\textsubscript{3} exchange and inhibits villus cell SCFA/HCO\textsubscript{3} exchange, whereas it has no effect on villus cell Cl/HCO\textsubscript{3} exchange.

To date, specifically how NO may alter intestinal electrolyte transport has been unclear, partly due to species variation, segment of the gut studied, and the condition of the intestine studied. This last is especially important, because it is known that there are three NO synthases: neuronal or nNOS or NOS1; endothelial or eNOS or NOS3; and inducible or iNOS or NOS2. NOS1 and 3 are considered constitutive and produce nanomolar quantities of NO. On the other hand, NOS2 predominantly occurs during pathophysiological conditions and produces micromolar quantities of NO. Thus the inconclusive results seen to date regarding NO regulation of electrolyte and fluid transport in the intestine (9, 10, 14, 29, 35, 38) may be related to the levels of NO generated in these studies. Variable NO levels could have led to physiological and/or pathophysiological responses in electrolyte transport in these studies. To allay these concerns, cNO was inhibited in vivo in these studies.

To determine the mechanism of inhibition of crypt cell BBM Cl/HCO\textsubscript{3} exchange when NO production was diminished with l-NAME, kinetic studies were then
performed. The studies demonstrated that although the affinity of the transporter was not affected, there was a substantial decrease in the $V_{\text{max}}$ for Cl/HCO$_3^-$ exchange. This indicated that the mechanism of inhibition of crypt cell Cl/HCO$_3^-$ exchange when cNO is reduced is secondary to a decrease in transporter numbers. Kinetic studies were also performed to determine the mechanism of stimulation of villus cell BBM SCFA/HCO$_3^-$ exchange when NO production was diminished. These studies demonstrated that although the $V_{\text{max}}$ was not affected, there was a substantial decrease in $K_m$. This indicated that the mechanism of stimulation of villus cell SCFA/HCO$_3^-$ exchange when cNO is reduced is secondary to an increase in the affinity of the transporter for SCFA.

Determination of the effect of cNO on electrolyte transport 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 these functionally different villus cells and crypt cells. Current evidence would suggest that villus cells are primarily absorptive, because Na-dependent solute cotransport processes, SCFA/HCO$_3^-$ exchange, and coupled NaCl absorption, which occurs via the dual operation of Na/H and Cl/HCO$_3^-$ exchange, are localized to the BBM of villus cells. In contrast, the crypt cells, at least functionally, only have Cl/HCO$_3^-$ exchange. This indicated that the mechanism of stimulation of crypt cell Cl/HCO$_3^-$ exchange. This indicated that the mechanism of stimulation of crypt cell Cl/HCO$_3^-$ exchange when cNO is inhibited is secondary to an increase in the affinity of the transporter for SCFA.

In conclusion, this study, for the first time, demonstrates that cNO uniquely regulates anion/HCO$_3^-$ exchange in the mammalian small intestine. Inhibition of cNO does not alter the villus cell BBM Cl/HCO$_3^-$ exchange; it inhibits crypt cell BBM Cl/HCO$_3^-$ exchange, whereas villus cell BBM SCFA/HCO$_3^-$ exchange is stimulated. Thus cNO imposes an excitatory tone on crypt cell BBM Cl/HCO$_3^-$ exchange and an inhibitory tone on villus cell BBM SCFA/HCO$_3^-$ exchange in the normal mammalian small intestine. Finally, the mechanism of cNO-mediated alterations of anion/HCO$_3^-$ exchangers is also different. cNO regulates crypt cell BBM Cl/HCO$_3^-$ exchange by altering transporter numbers, whereas it regulates villus cell BBM SCFA/HCO$_3^-$ exchange by altering the affinity of the transporter.

DISCLOSURES

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REFERENCES

24. Soleimani M, Greetley T, Petrovic S, Wang Z, Amial H, and Kopp Burnham CE. Pendrin: an apical Cl$^-$/OH/HCO$_3^-$ exchanger; it inhibits crypt cell BBM Cl/HCO$_3^-$ exchange. This indicated that the mechanism of inhibition of crypt cell Cl/HCO$_3^-$ exchange when cNO is reduced is secondary to a decrease in transporter numbers. Kinetic studies were also performed to determine the mechanism of stimulation of villus cell BBM SCFA/HCO$_3^-$ exchange when NO production was diminished. These studies demonstrated that although the $V_{\text{max}}$ was not affected, there was a substantial decrease in $K_m$. This indicated that the mechanism of stimulation of villus cell SCFA/HCO$_3^-$ exchange when cNO is reduced is secondary to an increase in the affinity of the transporter for SCFA. Determination of the effect of cNO on electrolyte transport 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 these functionally different villus cells and crypt cells. Current evidence would suggest that villus cells are primarily absorptive, because Na-dependent solute cotransport processes, SCFA/HCO$_3^-$ exchange, and coupled NaCl absorption, which occurs via the dual operation of Na/H and Cl/HCO$_3^-$ exchange, are localized to the BBM of villus cells. In contrast, the crypt cells, at least functionally, only have Cl/HCO$_3^-$ exchange. This indicated that the mechanism of stimulation of crypt cell Cl/HCO$_3^-$ exchange when cNO is inhibited is secondary to an increase in the affinity of the transporter for SCFA. In conclusion, this study, for the first time, demonstrates that cNO uniquely regulates anion/HCO$_3^-$ exchange in the mammalian small intestine. Inhibition of cNO does not alter the villus cell BBM Cl/HCO$_3^-$ exchange; it inhibits crypt cell BBM Cl/HCO$_3^-$ exchange, whereas villus cell BBM SCFA/HCO$_3^-$ exchange is stimulated. Thus cNO imposes an excitatory tone on crypt cell BBM Cl/HCO$_3^-$ exchange and an inhibitory tone on villus cell BBM SCFA/HCO$_3^-$ exchange in the normal mammalian small intestine. Finally, the mechanism of cNO-mediated alterations of anion/HCO$_3^-$ exchangers is also different. cNO regulates crypt cell BBM Cl/HCO$_3^-$ exchange by altering transporter numbers, whereas it regulates villus cell BBM SCFA/HCO$_3^-$ exchange by altering the affinity of the transporter.

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