Effect of chronic inflammation on ileal short-chain fatty acid/bicarbonate exchange

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Manokas, Tasos, John J. Fromkes, and Uma Sundaram. Effect of chronic inflammation on ileal short-chain fatty acid/bicarbonate exchange. Am J Physiol Gastrointest Liver Physiol 278: G585–G590, 2000.—Short-chain fatty acids (SCFA) have been demonstrated to at least partially ameliorate chronic intestinal inflammation. However, whether and how intestinal SCFA absorption may be altered during chronic intestinal inflammation is unknown. A rabbit model of chronic ileitis produced by coccidia was used to determine the effect of chronic inflammation on ileal SCFA/HCO3− exchange. SCFA/HCO3− exchange was present in the brush-border membrane (BBM) of villus but not crypt cells from normal rabbit ileum. An anion-exchange inhibitor, DIDS, significantly inhibited SCFA/HCO3− exchange. Extravesicular Cl− did not alter the uptake of SCFA, suggesting that SCFA/HCO3− exchange is a transport process distinct from Cl−/HCO3− exchange. In chronically inflamed ileum, SCFA/HCO3− exchange was also present only in BBM of villus cells. The exchanger was sensitive to DIDS and was unaffected by extravesicular Cl−. However, SCFA/HCO3− exchange was significantly reduced in villus cell BBM vesicles (BBMV) from chronically inflamed ileum. Kinetic studies demonstrated that the maximal rate of uptake of SCFA, but not the affinity for SCFA, was reduced in chronically inflamed rabbit ileum. These data demonstrate that a distinct SCFA/HCO3− exchange is present on BBMV of villus but not crypt cells in normal rabbit ileum. SCFA/HCO3− exchange is inhibited in chronically inflamed rabbit ileum. The mechanism of inhibition is most likely secondary to a reduction in transporter numbers rather than altered affinity for SCFA.

Inflammatory bowel disease; short-chain fatty acid transport; immune regulation of nutrient transport; anion exchanger

SHORT-CHAIN FATTY ACIDS (SCFA) are important energy sources for the large intestinal epithelium. SCFA absorption has been described in guinea pig, human, mouse, rat, and rabbit colon (1, 4, 5, 8, 9, 11, 14, 15). These studies suggested two mechanisms of incorporation of SCFA in the mammalian colon: 1) SCFA absorption in its protonated form is passive and the proton is supplied by the brush-border membrane (BBM) Na+−H+ exchanger (14, 15); and/or 2) SCFA is absorbed by SCFA/HCO3− exchange on the BBM of absorptive cells (1, 8, 9). SCFA/HCO3− exchange has also been demonstrated in the human ileum (6, 13). It is of note that this anion exchanger was not inhibitable by stilbene derivatives in these studies (6, 8, 9).

Although there is some controversy about the exact mechanism of SCFA incorporation in the colon, the presence of an active transporter suggests that it may be susceptible to alterations in pathophysiological conditions such as inflammatory bowel disease (e.g., Crohn’s disease). A reduction in the production of intestinal SCFA has been postulated to result in intestinal inflammation (7). Evidence to support this hypothesis largely comes from the observations that local instillation of SCFA in diversion or distal ulcerative colitis ameliorates the intestinal inflammation (2, 7, 12). However, the effect of chronic intestinal inflammation on SCFA absorption is unknown.

Previous studies have demonstrated that during chronic intestinal inflammation unique alterations occur in coupled Na+−Cl− absorption and Na+−dependent nutrient transport (17–20). Given this background the aims of this study were to 1) demonstrate SCFA/HCO3− exchange in the rabbit ileum; 2) determine the villus-extract distribution of SCFA/HCO3− exchange in the rabbit ileum; and 3) delineate the alterations in SCFA/HCO3− exchange during chronic ileal inflammation.

METHODS

Induction of chronic inflammation. Chronic ileal inflammation was produced in rabbits as previously reported (17). Pathogen-free rabbits were intragastrically inoculated with 10,000 Eimeria magna oocytes or sham inoculated with 0.9% NaCl (control animals). None of the sham inoculations and ~80% of inoculations with coccidia resulted in chronic ileal inflammation during days 13–15. Only enterocytes from those animals that had histologically confirmed chronic ileal inflammation were used for experiments.

Cell isolation. Villus and crypt cells were isolated from normal and chronically inflamed rabbit ileum by a calcium chelation technique as previously described (16, 17). Previously established criteria were used to validate good separation and viability of villus and crypt cells (16, 17). Cells used for BBM vesicle (BBMV) preparation were frozen immediately in liquid nitrogen and stored at −70°C until required.

BBMV preparation. BBMV from rabbit ileum villus or crypt cells were prepared by CaCl2 precipitation and differential centrifugation as previously described (18–20). BBMV were resuspended in a medium appropriate to each experiment. BBMV purity was assured with marker enzyme enrichment (18–20).

BBMV uptake studies. Uptake studies were performed by a rapid filtration technique as previously described (18–20). In brief, BBMV were suspended in 100 mM N-methyl-
glucamine (NMG) gluconate, 0.10 mM MgSO4, 50 mM HEPES-Tris (pH 7.5), and 50 mM KHCO3 or K gluconate. The reactions were started by adding 90 µl of reaction mix containing 100 mM NMG gluconate, 0.1 mM MgSO4, 10 µM valinomycin, 50 µM [14C]butyrate, 50 mM HEPES-Tris, pH 7.5 or pH 6.0, and 50 mM KHCO3 or K gluconate. At desired times uptake was arrested by mixing with ice-cold stop solution (50 mM HEPES-Tris buffer, pH 7.5, 0.10 mM MgSO4, 50 mM K gluconate, and 100 mM NMG gluconate). The mixture was filtered on 0.45-µm Millipore (HAWP) filters and washed twice with 3 ml of ice-cold stop solution. Filters with BBMV were dissolved in Optifluor, and radioactivity was determined in a Beckman LS-5 scintillation counter.

Data presentation. When data are averaged, means ± SE are shown except when error bars are inclusive within the symbol. All uptake studies were done in triplicate. The 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

SCFA uptake in rabbit ileal BBMV may be secondary to nonionic diffusion or carrier-mediated transport. To determine whether SCFA uptake could occur via nonionic diffusion, uptake of [14C]butyrate at an alkaline pH (pH 8 where pHin = pHout) and an acidic pH (pH 6 where pHin > pHout) was performed. With a 100-fold increase in proton concentration, an increase in [14C]butyrate uptake was observed (92.5 ± 14.1 vs. 168.4 ± 12.4 pmol/mg protein at 1.5 s; n = 3, P < 0.05).

Next, to determine whether SCFA absorption could occur via a carrier-mediated anion-exchange process such as SCFA/HCO3 exchange, a series of experiments were performed. [14C]butyrate uptake was stimulated by a HCO3 gradient in villus cell BBMV (Fig. 1A). Gradients of pH and HCO3 further stimulated [14C]butyrate uptake in villus cell BBMV. Although a smaller (25 mM) HCO3 gradient did significantly promote [14C]butyrate uptake (340 ± 62 and 95 ± 14 pmol/mg protein at 3 s with and without gradient, respectively), it was significantly less than that seen with a 50 mM HCO3 gradient (750 ± 75 compared with 340 ± 62 pmol/mg protein at 3 s; n = 3, P < 0.01). The distribution of this anion exchanger along the villus-crypt axis of the normal rabbit ileum was then determined. Neither the HCO3 gradient nor the pH and HCO3 gradients stimulated [14C]butyrate uptake in normal ileal crypt cell BBMV (Fig. 1B). Thus SCFA/HCO3 exchange is present in the BBM of villus but not crypt cells in the normal rabbit ileum.

Unlike other anion exchangers (e.g., Cl-/HCO3), the SCFA/HCO3 exchange in human ileum and colon and rat colon has been suggested to be insensitive to anion-exchange inhibitors such as DIDS or SITS. Therefore, the effect of DIDS on rabbit ileal SCFA/HCO3 exchange was studied. DIDS (1 mM) almost completely inhibited HCO3 gradient-stimulated [14C]butyrate uptake in villus cell BBMV (Fig. 2A). DIDS also nearly completely inhibited pH and HCO3 gradient-stimulated [14C]butyrate uptake in villus cell BBMV (Fig. 2A). Thus, unlike SCFA/HCO3 exchangers in human ileum and colon and rat colon, the rabbit ileal SCFA/HCO3 exchanger appears to be sensitive to stilbene derivatives. Figure 2B demonstrates the DIDS dose-response curve for the rabbit ileal SCFA/HCO3 exchange.

To ensure that the observed [14C]butyrate uptake is not via the Cl-/HCO3 exchanger, which is also known to be present on the BBM of rabbit ileal villus cells (16), the effect of extravesicular Cl- on [14C]butyrate uptake was studied. Extravesicular Cl- did not inhibit the uptake of [14C]butyrate in BBMV (Fig. 3). These data indicated that SCFA/HCO3 exchange is distinct from Cl-/HCO3 exchange in normal rabbit ileal villus cells.

Next, to establish whether [14C]butyrate uptake represented electrodiffusional coupling, voltage-clamp experiments were performed. Voltage clamping with equal

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intra- and extravesicular K\textsuperscript+ with its ionophore, valinomycin, did not alter pH and HCO\textsubscript{3}\textsuperscript{-} gradient-stimulated \textsuperscript{14}C\textsuperscript{-}butyrate uptake in villus cell BBMV (Fig. 3). These data indicated that \textsuperscript{14}C\textsuperscript{-}butyrate uptake occurs via an electroneutral process in the villus cell BBMV.

Having demonstrated the presence of a DIDS-sensitive SCFA/HCO\textsubscript{3} exchange in normal ileal villus but not crypt cells, we next looked at the effect of chronic ileal inflammation on this transport process. Both HCO\textsubscript{3}\textsuperscript{-} gradient-stimulated \textsuperscript{14}C\textsuperscript{-}butyrate uptake and pH and HCO\textsubscript{3}\textsuperscript{-} gradient-stimulated \textsuperscript{14}C\textsuperscript{-}butyrate uptakes were present in BBMV prepared from villus cells from the chronically inflamed ileum (Fig. 4A). However, similar to the normal ileum, neither HCO\textsubscript{3}\textsuperscript{-} nor pH and HCO\textsubscript{3}\textsuperscript{-} gradient-stimulated \textsuperscript{14}C\textsuperscript{-}butyrate uptake was present in crypt cell BBMV from the chronically inflamed ileum (Fig. 4B). These data demonstrated that the villus-crypt distribution of SCFA/HCO\textsubscript{3} exchange is not altered during chronic ileal inflammation.

Next, to determine whether the functionality of the SCFA/HCO\textsubscript{3} exchange may be altered in the chronically inflamed ileum, the effect of the anion-exchange inhibitor DIDS was determined. As shown in Fig. 5, similar to the normal rabbit ileum, DIDS also significantly inhibited the SCFA/HCO\textsubscript{3} exchange in the chronically inflamed rabbit ileum.

To ensure that the observed \textsuperscript{14}C\textsuperscript{-}butyrate uptake is not via Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange, which is known to be inhibited in the chronically inflamed rabbit ileum (17), the effect of extravesicular Cl\textsuperscript{-} on \textsuperscript{14}C\textsuperscript{-}butyrate uptake was studied. Similar to the normal ileum, extravesicular Cl\textsuperscript{-} did not inhibit the uptake of \textsuperscript{14}C\textsuperscript{-}butyrate in BBMV (Fig. 6). These data indicated that SCFA/HCO\textsubscript{3} exchange is also distinct from Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange in the chronically inflamed rabbit ileum.

To establish whether \textsuperscript{14}C\textsuperscript{-}butyrate uptake represented electrodiffusional coupling in the chronically inflamed ileum, voltage-clamp experiments were performed. Voltage clamping did not alter the pH and HCO\textsubscript{3}\textsuperscript{-} gradient-stimulated \textsuperscript{14}C\textsuperscript{-}butyrate uptake in villus cell BBMV from the chronically inflamed ileum (Fig. 6). These data indicated that butyrate uptake also occurs via an electroneutral process in the chronically inflamed rabbit ileum.

Next, the effect of chronic ileal inflammation on SCFA/HCO\textsubscript{3} exchange in villus cells from the normal and chronically inflamed ileum was compared. HCO\textsubscript{3}\textsuperscript{-} gradient-stimulated \textsuperscript{14}C\textsuperscript{-}butyrate uptake was reduced in villus cell BBMV from the chronically inflamed ileum (Fig. 7). Furthermore, pH and HCO\textsubscript{3}\textsuperscript{-} gradient-stimulated \textsuperscript{14}C\textsuperscript{-}butyrate uptake was also reduced in villus cell BBMV from the chronically inflamed ileum (Fig. 7). These data indicated that SCFA/HCO\textsubscript{3} exchange is inhibited in the chronically inflamed rabbit ileum.

To decipher the mechanism of inhibition of SCFA/HCO\textsubscript{3} exchange in the chronically inflamed ileum, kinetic studies were performed. Because pH and HCO\textsubscript{3}\textsuperscript{-}-dependent \textsuperscript{14}C\textsuperscript{-}butyrate uptake in BBMV was linear for at least 6 s in the normal as well as the chronically inflamed ileum (data not shown), uptake for all concentrations were carried out at 3 s. Figure 8 demonstrates the kinetics of butyrate uptake in villus cell BBMV from the normal and chronically inflamed.
ileum. The figure shows the uptake of butyrate as a function of varying concentrations of extravesicular butyrate. As the extravesicular concentration of butyrate was increased, the uptake of butyrate was stimulated and subsequently became saturated in the normal as well as the chronically inflamed ileum (Fig. 8A).

With the use of Enzfitter, a Lineweaver-Burk plot of these data was generated, and this is shown in Fig. 8B. Kinetic parameters derived from these data demonstrated that the affinity for [14C]butyrate uptake was not different between the normal and chronically inflamed ileum [Michaelis constant (Km) for butyrate uptake in BBMV was 39.6 mM in normal and 40.9 mM in inflamed ileum]. However, the maximal velocity (Vmax) of [14C]butyrate uptake was reduced severalfold in the chronically inflamed ileum (Vmax for butyrate uptake in BBMV was 16.0 and 3.75 nmol·mg protein−1·s−1 in normal and inflamed, respectively). These data indicated that SCFA/HCO3− exchange was inhibited in the chronically inflamed rabbit ileum secondary to a decrease in the number of anion exchangers rather than altered affinity for butyrate.

**DISCUSSION**

This study demonstrates that SCFA/HCO3− absorption occurs via SCFA/HCO3− exchange in the rabbit ileum. This transporter is present on the BBM of villus but not crypt cells. The rabbit ileal SCFA/HCO3− exchange is DIDS sensitive. This anion exchanger is distinct from Cl−/HCO3− exchange, which is also known to be present on the BBM of villus and crypt cells in the rabbit ileum.

In the chronically inflamed rabbit ileum the SCFA/HCO3− exchanger is also distinct from Cl−/HCO3− exchange. It is only present on the BBM of villus cells and is also DIDS sensitive. However, SCFA/HCO3− exchange is inhibited during chronic ileitis and the mechanism of

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**Fig. 4.** Effect of HCO3− and pH and HCO3− gradients on SCFA uptake in chronically inflamed ileal enterocyte BBMV. A: villus cell. In absence of HCO3− and a pH gradient there is only a minimal uptake of [14C]butyrate. Presence of equal amounts of HCO3− inside and outside the vesicle also does not stimulate [14C]butyrate uptake. A pHin > pHout gradient alone also does not stimulate [14C]butyrate uptake. An HCO3− gradient did stimulate [14C]butyrate uptake in BBMV. A pH and HCO3− gradient further significantly stimulated [14C]butyrate uptake in BBMV. B: crypt cell. HCO3−, pH gradient, HCO3− gradient, or a pH and HCO3− gradient did not stimulate [14C]butyrate uptake in BBMV.

**Fig. 5.** Effect of a competitive anion-exchange inhibitor, DIDS, on SCFA/HCO3− exchange in chronically inflamed ileal villus cell BBMV. pH and HCO3− gradient-stimulated [14C]butyrate uptake was significantly inhibited by DIDS in villus cell BBMV from chronically inflamed ileum.

**Fig. 6.** Effect of extravesicular Cl− and voltage clamping on SCFA/HCO3− exchange in inflamed villus cell BBMV uptake at 1.5 s. Extravesicular Cl− did not alter the pH and HCO3− gradient-stimulated [14C]butyrate uptake in inflamed ileal villus cell BBMV. Voltage clamping with valinomycin also did not alter the pH and HCO3− gradient-stimulated [14C]butyrate uptake in inflamed ileal villus cell BBMV.
inhibition is secondary to a reduction in the number of transporters and not secondary to an alteration in the affinity for SCFA.

Previous studies have demonstrated that SCFA/HCO₃⁻ exchange is present in the BBM in human ileum and rat and human colon. However, the villus-crypt distribution of this anion exchanger was previously unknown. This study demonstrates that SCFA/HCO₃⁻ exchange is localized to the BBM of villus but not crypt cells in the normal rabbit ileum.

The BBM SCFA/HCO₃⁻ exchangers previously demonstrated in the human and rat colon have been shown to be insensitive to anion-exchange inhibitors of the stilbene-derivative class (e.g., DIDS). However, the BLM SCFA/HCO₃⁻ exchanger in the rat colon was shown to be sensitive to DIDS (10). The rabbit ileal BBM SCFA/HCO₃⁻ exchanger is sensitive to DIDS and in this regard resembles the rat BLM SCFA/HCO₃⁻ exchanger more than the BBM SCFA/HCO₃⁻ exchanger in human or rat colon.

It is reasonably clear that SCFAs are important nutrients for colonocytes and that they stimulate Na⁺ and fluid absorption in the colon (1). It is also fairly well accepted that local instillation of SCFA in diarrheal diseases characterized by chronic inflammation of the colon alleviates, at least partially, the malabsorption of fluid and electrolytes and improves the colonic inflammation (2, 7, 12). On the basis of these observations it has been hypothesized that SCFAs help to maintain the health and functional integrity of the colonic epithelium. However, how SCFA absorption or SCFA/HCO₃⁻ exchange may be altered during chronic intestinal inflammation is unknown.

In a rabbit model of chronic ileal inflammation this laboratory has demonstrated multiple unique alterations in electrolyte and nutrient transport processes. NaCl absorption was inhibited by an inhibition of Cl⁻/HCO₃⁻ but not Na⁺/H⁺ exchange on the BBM of villus cells during chronic ileitis (17). Na⁺-dependent nutrient cotransport processes were also inhibited in the chronically inflamed ileum by different mechanisms of inhibition. For example, Na⁺-glucose cotransport was inhibited by a decrease in cotransporter numbers without a change in the affinity for glucose (18). In contrast, Na⁺-amino acid cotransport was inhibited by a decrease in the affinity for amino acid without a change in the cotransporter numbers (19). Unlike these two Na⁺-nutrient cotransport processes, Na⁺-bile acid cotransport was inhibited by both a decrease in cotransporter numbers and a decrease in the affinity for the bile acid (20). In view of the unique nature of the transport alterations observed in the chronically inflamed rabbit ileum, it was hypothesized that different immune in-

Fig. 7. Effect of chronic ileal inflammation on SCFA/HCO₃⁻ exchange in villus cell BBMV. HCO₃⁻ gradient-stimulated [¹⁴C]butyrate uptake is significantly diminished in villus cell BBM from chronically inflamed ileum. pH and HCO₃⁻ gradient-stimulated [¹⁴C]butyrate uptake is also significantly reduced in villus cell BBMV from chronically inflamed ileum. Results of 2 of the 4 experiments illustrated are also included in Figs. 1A and 4A.

Fig. 8. Kinetics of SCFA/HCO₃⁻ exchange inhibition in chronically inflamed ileal villus cell BBMV. A: pH and HCO₃⁻ gradient-stimulated [¹⁴C]butyrate uptake is shown as a function of varying concentrations of extravesicular butyrate. Isomolarity was maintained by adjusting concentration of N-methyl-D-glucamine gluconate. Uptake for all concentrations was determined at 3 s. As concentration of extravesicular butyrate was increased, uptake of butyrate was stimulated and subsequently became saturated in villus cell BBMV from both normal and inflamed ileum. B: analysis of these data with Lineweaver-Burk plot yielded kinetic parameters. Affinity for butyrate uptake in villus cell BBMV was not affected during chronic ileal inflammation. However, maximum rate of uptake of butyrate was reduced severalfold in chronically inflamed ileum.
flammatorv mediators may have unique effects on different transport processes during chronic ileitis (19, 20).

The effect of chronic intestinal inflammation on SCFA transport has not previously been studied. In one study, using intact tissue, acute inflammation was noted to inhibit SCFA absorption in the rabbit colon (3). However, this study did not demonstrate the presence of SCFA/HCO₃⁻ exchange or delineate the mechanism of inhibition of SCFA absorption. Furthermore, in acute intestinal inflammation the severe loss of absorptive cells may be the primary reason for the alterations in transport processes seen in these conditions. In chronic intestinal inflammation the architectural changes are not as severe as those seen on acute inflammation, and thus the mucosa during chronic intestinal inflammation is a more suitable model to determine the effect of immune-inflammatory mediators on transport processes. Because local instillation of SCFA in chronically inflamed colon improves the inflammation and malabsorption, it may be hypothesized that the absorption of SCFA may not be completely affected in these tissues. However, alterations in SCFA absorption during chronic intestinal inflammation are at present unknown. Similarly, the effect of chronic intestinal inflammation on SCFA/HCO₃⁻ exchange is also at present unknown. This study demonstrates that SCFA/HCO₃⁻ exchange is inhibited during chronic ileal inflammation. The mechanism of inhibition is secondary to a reduction in transporter numbers rather than an altered affinity for SCFA.

In conclusion, this study presents several novel observations about SCFA/HCO₃⁻ exchange: 1) it is only present in the BBM of villus cells in the normal and chronically inflamed rabbit ileum; 2) it is sensitive to anion-exchange inhibitors such as DIDS in the rabbit ileum; 3) it is inhibited during chronic ileitis; and 4) its mechanism of inhibition is secondary to a reduction in transporter numbers in the chronically inflamed ileum.

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