Unique mechanism of inhibition of Na$^+$-amino acid cotransport during chronic ileal inflammation

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Sundaram, U., S. Wisel, and J. J. Fromkes. Unique mechanism of inhibition of Na$^+$-amino acid cotransport during chronic ileal inflammation. *Am. J. Physiol.* 275 (*Gastrointest. Liver Physiol.* 38): G483–G489, 1998.—In the chronically inflamed ileum, unique mechanisms of alteration of transport processes suggest regulation by different immune-inflammatory mediator pathways. We previously demonstrated that Na$^+$-glucose cotransport in the chronically inflamed ileum was inhibited by a decrease in cotransporter number without a change in glucose affinity. The aim of this study was to determine the alterations in Na$^+$-amino acid cotransport in chronically inflamed ileum produced by coccidial infection in rabbits. [^{3}H]alanine uptake was performed in cells and vesicles by rapid filtration. In villus cells from chronically inflamed ileum, Na$^+$-K$^+$-ATPase was reduced 50% and Na$^+$-alanine cotransport was also reduced (5.8 ± 1.2 in normal and 1.4 ± 0.5 nmol/mg protein in inflamed; $n = 6$, $P < 0.05$). [^{3}H]alanine uptake in brush-border membrane vesicles was reduced in chronically inflamed ileum (73.2 ± 1.2 in normal and 21.5 ± 3.2 pmol/mg protein in inflamed; $n = 3$, $P < 0.05$), suggesting a direct effect on the cotransporter itself. Na$^+$-amino acid cotransport in chronically inflamed ileum was inhibited by a decrease in affinity without a change in the maximal rate of uptake, and unaltered steady-state mRNA levels also suggested that the number of cotransporters was unchanged. Thus the mechanisms of inhibition of Na$^+$-amino acid cotransport and Na$^+$-glucose cotransport in chronically inflamed ileum are different. These observations suggest that different immune-inflammatory mediators may regulate different transport pathways during chronic ileitis.

electrolyte transport; inflammatory bowel disease; intestinal nutrient absorption; immune regulation of nutrient transport; sodium-potassium-ATPase

IN DIARRHEAL DISEASES characterized by chronic inflammation of the intestine, inhibition of NaCl and nutrient absorption has been well described (1, 3, 4, 16). The wide variety of immune-inflammatory mediators known to be endogenously produced in chronically inflamed ileum may, at least in part, have an effect on electrolyte and nutrient transport pathways (4, 16, 21). At present it is not known whether a given immune-inflammatory mediator pathway is responsible for alterations seen with a specific transport pathway during chronic ileitis. Unique mechanisms of alteration of the various electrolyte and nutrient transport pathways in chronically inflamed ileum would suggest that different immune-inflammatory pathways may regulate different transport pathways.

In the normal ileum a group of similar cotransporters exist that are important not only for Na$^+$ absorption but also for nutrient assimilation (8, 9, 23). Whether the different Na$^+$-dependent cotransporters that transport different solutes are altered by different mechanisms in chronically inflamed ileum is not currently known. This is undoubtedly the result of a lack of animal models of chronic ileitis and the inability to isolate viable enterocytes suitable for the study of electrolyte transport from chronically inflamed intestine.

In a rabbit model of chronic ileal inflammation we have previously demonstrated that one of the Na$^+$-dependent nutrient cotransport pathways, Na$^+$-glucose cotransport, was inhibited. The mechanism of inhibition of Na$^+$-glucose cotransport during chronic ileal inflammation was due to a decrease in the number of cotransporters and was not secondary to an alteration in the affinity for glucose (27).

Another important Na$^+$-dependent nutrient cotransport process in the normal ileum is Na$^+$-amino acid cotransport. Some amino acids (e.g., glutamine) are thought to be important energy sources for the ileal mucosa (8, 28). Thus an alteration of Na$^+$-dependent amino acid cotransport in the chronically inflamed ileum will affect not only Na$^+$ absorption but also the assimilation of important nutrients. However, the effect of chronic inflammation on Na$^+$-amino acid cotransport is unknown.

Given this background, we studied Na$^+$-dependent alanine uptake as representative of Na$^+$-amino acid cotransport in this model of chronic ileitis. Na$^+$-alanine cotransport has previously been demonstrated in the rabbit ileum (11). The favorable Na$^+$ gradient for this cotransport is provided by Na$^+$-K$^+$-ATPase (8, 9, 13, 23). Thus during chronic ileal inflammation cellular alterations in Na$^+$-alanine cotransport may be at the level of the cotransporter and/or secondary to an alteration in Na$^+$-K$^+$-ATPase.

Therefore, the aims of this study were to test the hypothesis that chronic inflammation uniquely alters Na$^+$-alanine cotransport in the rabbit model of chronic ileitis and to determine the cellular mechanisms of this alteration.

METHODS

Induction of chronic inflammation. Chronic ileal inflammation was produced in rabbits as described previously (26). 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 ileum by a Ca^{2+} chelation technique as previously described (25–27). Briefly, a 3-ft section of ileum was filled with buffer containing (in mM) 0.1 EDTA, 115 NaCl, 25 NaHCO$_3$, 2.4 K$_2$HPO$_4$, 0.4 KH$_2$PO$_4$, 2.5 L-glutamine, 0.5 β-hydroxybutyrate, 0.5 dithiothreitol, 5,000 U/l penicillin, 5 mg/l streptomycin, and 10 mg/l gentamicin and gassed with 95% O$_2$-5% CO$_2$, pH 7.4, at 37°C. The intestine was incubated in this solution for 3 min and gently palpated for another 3 min to facilitate cell dispersion. The fluid was then drained from the ileal loop, 1 mM CaCl$_2$ and phenylmethylsulfonyl fluoride were added, and the suspension was centrifuged at 500 g for 5 min. The pellet was resuspended in Leibowitz-15 medium (L-15 medium; GIBCO) with 20 mM HEPES, 10% rabbit serum, 5,000 U/l penicillin, 5 mg/l streptomycin, and 10 mg/l gentamicin and gassed with 100% O$_2$, pH 7.4, at 37°C. Similarly, five more cell fractions were collected. These six fractions represented the sequential collection of cells from the villus tip to the crypt base. The fractions were incubated for 10 min with collagenase (100 mg/l, type IV; Sigma) and then washed free of collagenase with modified L-15 medium, filtered through 30-μm nylon mesh, pelleted, resuspended at a final concentration of 0.1 g of cells in 40 ml of modified L-15, and incubated at 37°C.

Cells used for brush-border membrane vesicle (BBMV) preparation were frozen immediately in liquid nitrogen and stored at −70°C until required. Previously established criteria were used to validate good separation of villus and crypt cells. These criteria included *1*) marker enzymes (e.g., thymidine kinase, alkaline phosphatase), *2*) transporter specificity [e.g., Na$^+$-glucose cotransport and Na$^+$/H$^+$ exchange are present on the brush-border membrane (BBM) of villus but not crypt cells], *3*) differences in intracellular pH (e.g., intracellular pH is higher in crypt cells compared with villus cells), and *4*) morphological differences (e.g., villus cells are larger, with better developed BBM compared with crypt cells) (25–27).

Good cell viability was ensured using the following set of criteria: *1*) trypan blue exclusion, *2*) the demonstration of Na$^+$/H$^+$ and Cl$^-$/HCO$_3^-$ exchange activities, and *3*) the ability of the cells to maintain a baseline pH or an imposed acid or alkaline gradient and to return to baseline pH after perturbations (25–27).

BBMV preparation. BBMV from rabbit ileal villus cells were prepared by CaCl$_2$ precipitation and differential centrifugation as previously described (27). Briefly, frozen villus cells were thawed and suspended in 2 mM Tris·HCl buffer (pH 7.5) containing 50 mM mannitol. The suspension was homogenized and 10 mM CaCl$_2$ was added. The suspension was then centrifuged at 8,000 g for 15 min, and the resulting supernatant was centrifuged at 20,000 g for 30 min. The pellet was then resuspended in 10 mM Tris·HCl buffer (pH 7.5) containing 100 mM mannitol and homogenized. Vesicles were formed by adding MgCl$_2$ (10 mM). The homogenate was centrifuged at 2,000 g for 15 min to remove debris, and the BBMV were precipitated by centrifugation at 27,000 g for 30 min. BBMV were resuspended in a medium appropriate to each experiment. BBMV purity was assured with marker enzyme (e.g., alkaline phosphatase) enrichment.

Uptake studies in villus and crypt cells. Villus or crypt cells (100 mg wet wt) were washed and resuspended in HEPES buffer containing (in mM) 0.1 alanine, 4.5 KCl, 1.2 KH$_2$PO$_4$, 1.0 MgSO$_4$, 1.25 CaCl$_2$, 20 HEPES, and either 130 mM NaCl or choline chloride and gassed with 100% O$_2$ (pH 7.4 at 37°C).

[^{3}H]alanine (10 μCi) was added to a 1-ml cell suspension in HEPES buffer, and 100-μl aliquots were removed at desired time intervals. The uptake was arrested by mixing with 3 ml of ice-cold stop solution (choline-HEPES buffer). The mixture was filtered on 0.65-μm Millipore (HAWP) filters. After two washes with ice-cold stop solution, the filter was dissolved in 4 ml Optifluor. The radioactivity retained on the filter was counted in a Beckman LS-5 liquid scintillation counter.

BBMV uptake studies. Uptake studies were performed by rapid-filtration technique as previously described (27). In brief, 10 μl of BBMV resuspended in 100 mM choline chloride, 0.10 mM MgSO$_4$, 50 mM HEPES-Tris (pH 7.5), 50 mM mannitol, and 50 mM KCl were incubated in 90 μl reaction medium containing 50 mM HEPES-Tris buffer (pH 7.5), 0.1 mM alanine, 20 μCi [^{3}H]alanine, 0.10 mM MgSO$_4$, 50 mM KCl, 50 mM mannitol, and 100 mM of either NaCl or choline chloride. The vesicles were voltage clamped with 10 μM valinomycin and 100 μM carbonyl cyanide p-trifluoromethoxyphenylhydrazone. At desired times, uptake was arrested by mixing with ice-cold stop solution (in mM: 50 HEPES-Tris buffer, pH 7.5, 0.10 MgSO$_4$, 75 KCl, and 100 choline chloride). The mixture was filtered on a 0.45-μm Millipore (HAWP) filter and washed with 3 ml ice-cold stop solution. Filters with BBMV were dissolved in Optifluor, and the radioactivity retained on the filters was counted in a Beckman LS-5 liquid scintillation counter.

Na$^+$-K$^+$-ATPase measurement. Na$^+$-K$^+$-ATPase was measured as P$_i$ liberated by the method of Forbush (7) in cellular homogenates from the same amount of cells from normal or inflamed ileum as previously described (23). Enzyme-specific activity was expressed as nanomoles of P$_i$ released per milligram protein per minute.

Northern blot studies. Total RNA was extracted from rabbit ileal villus cells by the guanidinium isothiocyanate-cesium chloride method as previously reported (5, 27). mRNA was isolated from total RNA, using oligo(dT)-cellulose chromatography (2). After denaturation, mRNA was electrophoresed on 1.8% agarose-formaldehyde gel, transferred to a nylon membrane (Schleicher & Schuell, Keene, NH), and incubated with prehybridization solution. Membranes were hybridized with ^{32}P-labeled cDNA. Hybridized membrane was exposed to autoradiography film (NEN Research Products, Boston, MA). Human β-actin was used to ensure equal loading of mRNA onto the electrophoresis gels. β-Actin DNA was randomly labeled with [^{32}P]CTP with Klenow polymerase (Boehringer Mannheim, Indianapolis, IN).

cDNA probe synthesis for Northern blot studies. On the basis of the rabbit Na$^+$-dependent neutral amino acid cotransporter sequence, a 292-bp fragment was selected for amplification (10). Sense (5′-GTCTGCALLATCATCAACGT-3′) and antisense (5′-CCGGTCTGTCCGTTTACCCAT-3′) primers were designed and synthesized (GIBCO BRL). The 292-bp probe was amplified by PCR, using rabbit enterocyte cDNA as a template. PCR was performed in a thermal cycler (Perkin-Elmer 2400) in a reaction volume of 100 μl consisting of 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl$_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 0.5% cDNA synthesis product, 0.2 mM oligonucleotide primers, and 2–5 U of AmpliTaq gold polymerase (Perkin-Elmer, Branchburg, NJ). The PCR profile consisted of 35 cycles each at 94°C, 52°C, and 72°C. Hot PCR with [α-^{32}P]dCTP was simultaneously performed, and the PCR products were used as probes for Northern hybridization.

Data presentation. When data are averaged, means ± SE are shown except when error bars are inclusive within the symbol. All uptake measurements were performed in triplicate. For any set of experiments, n refers to the number of
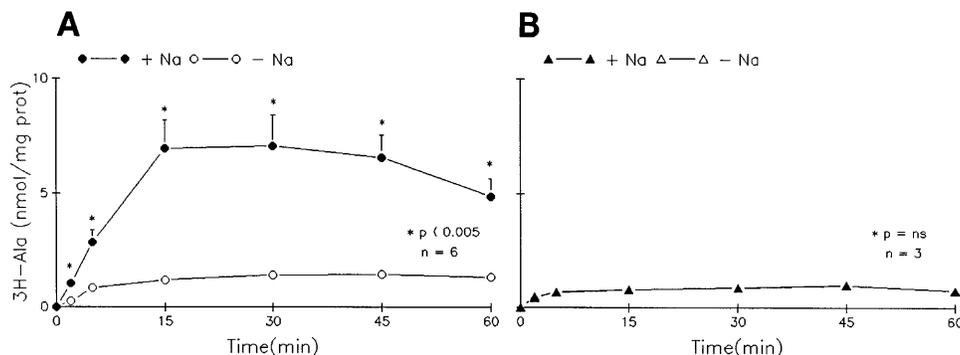
Fig. 1. Effect of extracellular Na$^+$ on [^{3}H]alanine uptake as a function of time in villus and crypt cells from normal rabbit ileum. In this and all subsequent figures only least-significant P values are shown. A: villus cells. Extracellular Na$^+$ significantly stimulated [^{3}H]alanine uptake at all time points. B: crypt cells. Extracellular Na$^+$ does not stimulate [^{3}H]alanine uptake. ns, Not significant.

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

The distribution of Na$^+$-alanine cotransport along the crypt-villus axis of normal rabbit ileum was initially determined. In villus cells from normal ileum (Fig. 1A), alanine uptake was significantly stimulated by extracellular Na$^+$ for up to 60 min. However, Na$^+$-stimulated alanine uptake was not observed in crypt cells from normal ileum (Fig. 1B).

To confirm these findings, steady-state mRNA levels for the Na$^+$-dependent amino acid cotransporter in villus and crypt cells were measured. The designed PCR primers amplified a single band of the expected size of 292 bp (Fig. 2A). This DNA probe then identified a band at the expected size of 2.9 kb primarily in villus cells (Fig. 2B). These results suggest that the message and the functional protein for Na$^+$-amino acid cotransport are present in villus but not crypt cells from normal rabbit ileum.

Na$^+$-stimulated alanine uptake was then determined in intact villus and crypt cells from chronically inflamed ileum. Extracellular Na$^+$ stimulated alanine uptake for up to 60 min in villus cells from chronically inflamed ileum (Fig. 3A). However, similar to normal ileum, Na$^+$-stimulated alanine uptake was not present in crypt cells from chronically inflamed ileum (Fig. 3B).

Figure 4 compares the Na$^+$-dependent uptake of alanine in villus cells from normal and chronically inflamed ileum. Na$^+$-dependent alanine uptake was significantly reduced in intact villus cells from chronically inflamed ileum. These data suggested that Na$^+$-alanine cotransport was inhibited in intact villus cells from chronically inflamed ileum.

Inhibition of Na$^+$-alanine cotransport at the cellular level may represent a direct effect on the cotransporter on the BBM and/or may be the result of inhibition of Na$^+$-K$^+$-ATPase on the basolateral membrane (BLM), which provides the favorable Na$^+$ electrochemical gradient for this cotransport process. Thus Na$^+$-K$^+$-ATPase activity was measured in homogenates of villus cells from normal and chronically inflamed ileum.
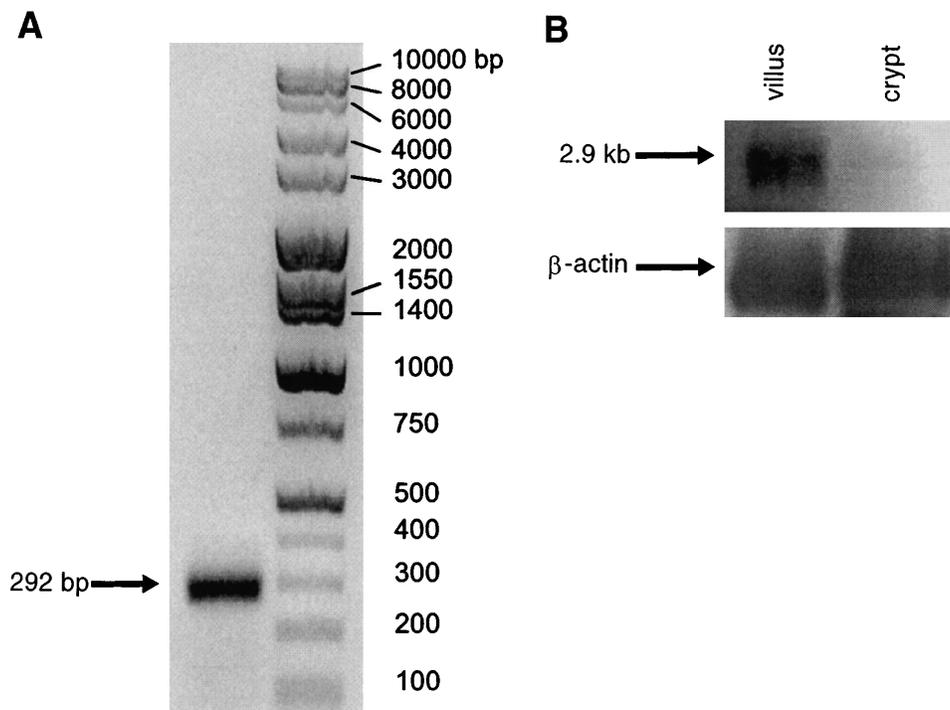

Fig. 2. Steady-state mRNA levels of Na$^+$-dependent neutral amino acid cotransporter crypt-villus distribution in normal ileum. Data are representative of 4 such experiments. A: PCR amplification identified an expected 292-bp fragment. B: Northern blot analysis demonstrated that steady-state levels of mRNA for the Na$^+$-dependent neutral amino acid cotransporter are present in villus but not crypt cells from normal ileum. Human β-actin was used to ensure equal loading of mRNA in the gel.

Fig. 3. Effect of chronic ileal inflammation on [³H]alanine uptake in villus and crypt cells. *A*: villus cells. Extracellular Na⁺ significantly stimulated [³H]alanine uptake at all time points. *B*: crypt cells. Extracellular Na⁺ does not stimulate [³H]alanine uptake.
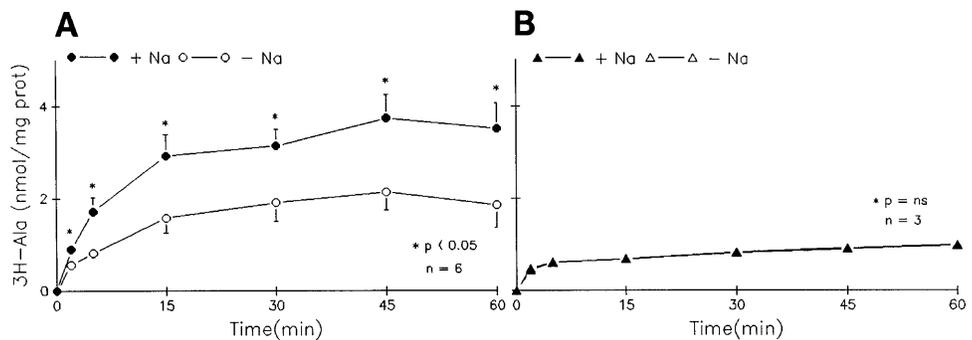
Na^+-K^+-ATPase activity was reduced ~50% in villus cells from inflamed ileum (11.2 ± 2.0 in normal and 6.0 ± 1.3 nmol·mg protein^{-1}·min^{-1} in inflamed; $n = 6$, $P < 0.05$). These data suggested that the inhibition of Na^+-alanine cotransport in inflamed ileal villus cells may, at least in part, be due to reduced electrochemical gradients of Na^+ across the BBM of these cells.

To determine whether chronic inflammation has a direct effect on the Na^+-alanine cotransporter itself, alanine uptake was determined in BBMV prepared from villus cells from normal and chronically inflamed ileum. Extravesicular Na^+ significantly stimulated alanine uptake in villus cell BBMV from normal (Fig. 5*A*) and chronically inflamed ileum (Fig. 5*B*). However, Na^+-dependent alanine uptake was significantly reduced in villus cell BBMV from chronically inflamed ileum (Fig. 5*C*). Thus these data indicate that the Na^+-alanine cotransporter itself was directly affected during chronic ileal inflammation.

Kinetic studies were performed to determine whether the inhibition of Na^+-alanine cotransport during chronic ileal inflammation was due to an alteration in the affinity for alanine and/or in the rate of uptake of alanine. Because Na^+-dependent alanine uptake in BBMV was linear for at least 10 s in normal as well as inflamed ileum, uptake measurements for all the concentrations were carried out at 5 s (data not shown). Figure 6 demonstrates the kinetics of alanine uptake in villus cell BBMV from normal and chronically inflamed ileum. Figure 6*A* shows the uptake of alanine as a function of varying concentrations of extravesicular
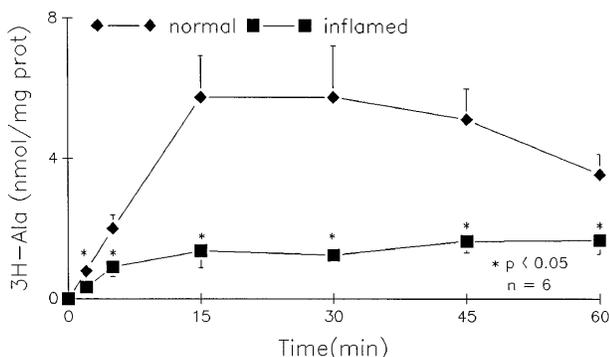
alanine. As the extravesicular concentration of alanine was increased, the uptake of alanine was stimulated and subsequently became saturated in normal as well as in chronically inflamed ileum. A Lineweaver-Burke plot of these data was generated with the use of Enzfitter software and is shown in Fig. 6*B*. Kinetic parameters derived from these data demonstrate that the maximal rate of uptake (V_{max}) of alanine did not differ between normal and chronically inflamed ileum [V_{max} for alanine uptake in BBMV was 5.02 ± 0.2 pmol/mg protein at 5 s in normal and 4.46 ± 0.3 pmol/mg protein in inflamed ileum; $n = 3$, not significant (NS)]. However, the apparent Michaelis constant (K_m) for alanine uptake was increased 2.5-fold in the inflamed ileum (8.5 ± 0.7 mM in normal and 21.0 ± 2.0 mM in inflamed ileum; $n = 3$, $P < 0.01$). These data suggested that Na^+-alanine cotransport was inhibited in chronically inflamed ileum as a result of a decrease in the affinity for alanine rather than an altered number of cotransporters.

To confirm these findings, the steady-state levels of mRNA for the Na^+-dependent neutral amino acid cotransporter in villus and crypt cells from normal and chronically inflamed ileum were determined (Fig. 7). The probe recognized a band at the expected size of 2.9 kb in villus cells from normal and chronically inflamed ileum. Qualitatively, at least, the intensity of the Na^+-dependent neutral amino acid cotransporter band appears similar in villus cells from normal and chronically inflamed ileum. Furthermore, similar to normal ileum, the message for this cotransporter was also not present in crypt cells from chronically inflamed ileum. These data also suggest that the number of Na^+-alanine cotransporters is unaltered in villus cells from chronically inflamed ileum.

DISCUSSION

This study demonstrates that Na^+-alanine cotransport is present in villus but not crypt cells in normal and chronically inflamed rabbit ileum. However, Na^+-alanine cotransport is significantly inhibited in intact villus cells from chronically inflamed ileum. This inhibition may be at the level of the cotransporter on the BBM or may be the result of a reduction in the activity of Na^+-K^+-ATPase on the BLM, which provides the favorable Na^+-gradient for this cotransporter. However, this inhibition of Na^+-alanine cotransport during

Fig. 4. Effect of chronic ileal inflammation on Na^+-dependent [³H]alanine uptake in villus cells. Na^+-dependent [³H]alanine uptake, defined as [³H]alanine uptake in the presence minus that in the absence of Na⁺, was significantly reduced in villus cells from chronically inflamed ileum at all times measured.
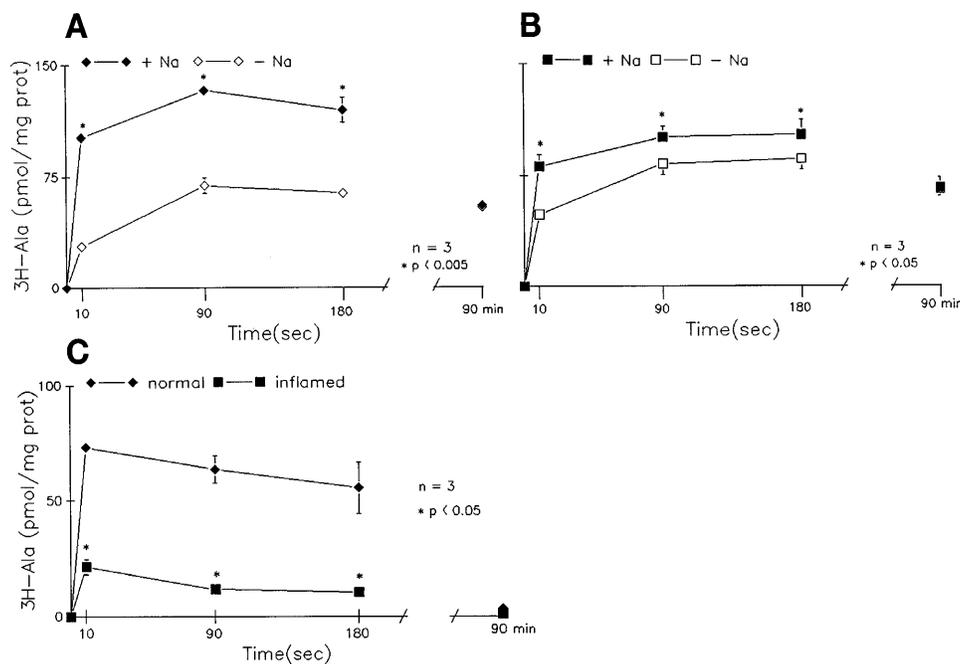
Fig. 5. Effect of extravesicular Na+ on [3H]alanine uptake as a function of time in villus cell brush-border membrane vesicles (BBMV) from normal and chronically inflamed rabbit ileum. Measurement of uptake in this and subsequent figures was carried out in triplicate. *A*: normal ileum. [3H]alanine uptake in villus cell BBMV from normal ileum was significantly stimulated by extravesicular Na+. *B*: chronically inflamed ileum. [3H]alanine uptake in villus cell BBMV from inflamed ileum was also significantly stimulated by extravesicular Na+. *C*: Na+-dependent alanine uptake, defined as [3H]alanine uptake in the presence minus that in the absence of Na+, was significantly reduced in villus cell BBMV from chronically inflamed ileum.

chronic ileal inflammation is not solely secondary to an alteration in Na+ extrusion from the cell facilitated by Na+-K+-ATPase. In fact, Na+-glucose cotransport is reduced at the level of the cotransporter in chronically inflamed ileum, as demonstrated by the BBMV studies. It should be noted that it may not be appropriate to directly correlate transport in intact cells with that in BBM vesicles, as measurement in whole cells may reflect the combined transport at the BBM and BLM levels.

Kinetic studies demonstrate that the mechanism of inhibition of Na+-alanine cotransport during chronic ileal inflammation is secondary to a decrease in the affinity for alanine rather than a decrease in the number of cotransporters. The K_m for alanine in normal rabbit ileum shown in this study agrees fairly well with previous observations in the rabbit (8.5 ± 0.7 mM in this study; 9 mM from Ref. 24). However, the K_m for alanine in the rabbit is higher than that observed in bovine kidney BBMV (2.47 mM, Ref. 12), guinea pig small intestine BBMV (0.6 mM, Ref. 22), bovine renal epithelial cell line NBL-1 (0.17 mM, Ref. 6), or Caco-2 cells (0.164 ± 0.026 mM, Ref. 15). The differences in K_m for alanine may be due to differences in species, organs, transformed cell lines, and/or experimental protocols.

The steady-state mRNA level of the Na+-dependent neutral amino acid transporter does not differ in villus cells from normal and chronically inflamed ileum. These findings are consistent with the kinetic studies, which demonstrate that the cotransporter numbers are unaffected in chronically inflamed ileum. Of course, it should be noted that although the cloned transporter
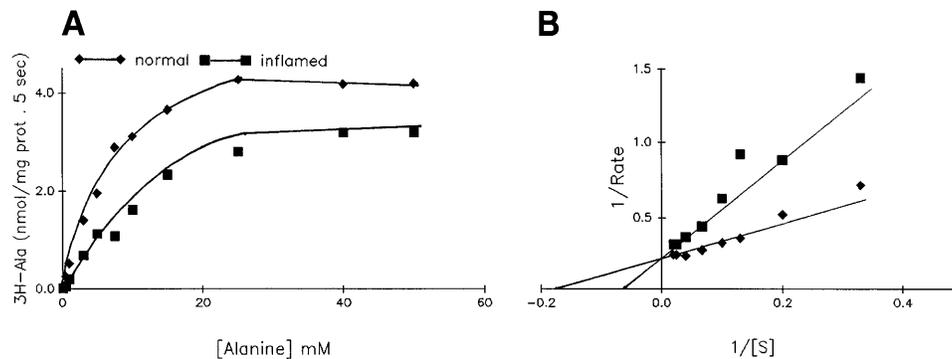

Fig. 6. Kinetics of [3H]alanine uptake in villus cell BBMV from normal and chronically inflamed ileum. Data are representative of 3 such experiments. *A*: uptake of [3H]alanine as a function of varying concentrations of extravesicular alanine. Uptake for all concentrations was measured at 5 s. Isosmolarity was maintained by adjusting the concentration of mannitol. As extravesicular alanine concentration was increased, uptake of [3H]alanine was stimulated and subsequently became saturated in normal and inflamed ileum. *B*: analysis of these data with a Lineweaver-Burke plot provided the kinetic parameters. The maximal rate of uptake (V_{max}) of alanine was not affected during chronic ileal inflammation. However, the affinity ($1/K_m$) for alanine uptake was significantly reduced in chronically inflamed ileum.
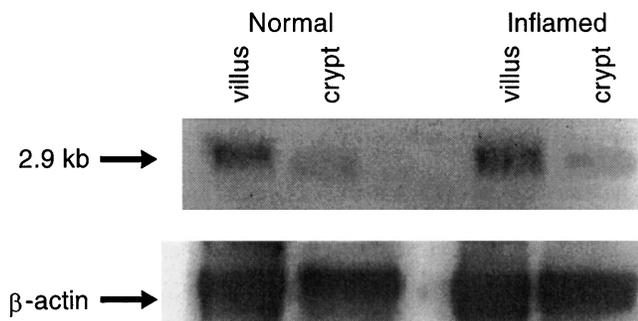
Fig. 7. Steady-state mRNA levels of Na⁺-dependent neutral amino acid cotransporters in chronically inflamed ileum. Data are representative of 4 such experiments. Northern blot analysis demonstrated that mRNA for Na⁺-dependent neutral amino acid cotransporter is present in villus but not crypt cells from chronically inflamed ileum. Furthermore, steady-state levels of mRNA are unchanged in villus cells from normal and chronically inflamed ileum. Human β-actin was used to ensure equal loading of mRNA in the gel.

most likely represents the BBM transporter, there is currently no direct evidence for this.

Although it is clear that inhibition of nutrient and NaCl absorption occur in diarrheal illnesses characterized by chronic inflammation of the intestine (e.g., Crohn's disease), the cellular mechanism of these alterations is poorly understood. Undoubtedly, this is the result of a lack of good animal models of chronic ileal inflammation. Two other models of chronic small intestinal inflammation, peptidoglycan polysaccharide-induced enterocolitis in rats (20) and alloimmunization-induced enterocolitis in guinea pigs (14), have not yet been used for transport studies.

Although the effect of chronic ileal inflammation on Na⁺-alanine cotransport was not previously known, the effect of acute inflammation on this process has been studied. In acute enteritis caused by rotavirins in pigs, Na⁺-alanine cotransport measured as alanine-dependent net Na⁺ flux was, if anything, enhanced. In acute enteritis produced by transmissible gastroenteritis virus, Na⁺-dependent alanine uptake in BBMV was initially reported to be enhanced and was subsequently diminished with a different BBM preparation method (17–19). The mechanism of inhibition at the cellular level was not addressed in these studies, possibly owing to the fact that in acute enteritis there is near-complete loss of mature villus cells containing this cotransporter.

Unlike acute intestinal inflammation, in which severe morphological damage probably accounts for most of the transport abnormalities, in this model of chronic ileitis the mucosal architecture is fairly well preserved (26). Thus this chronic ileitis model may be more suitable to study the effect of immune-inflammatory mediator pathways on epithelial transport function during chronic ileal inflammation.

In this and in a previous study (27), we have demonstrated that during chronic ileal inflammation Na⁺-dependent nutrient cotransport processes are inhibited by different mechanisms at the cotransporter level. In the first study (27), Na⁺-glucose cotransport was inhibited by a decrease in the number of cotransporters without a change in the affinity for glucose in chroni-

cally inflamed ileum. In contrast, this study demonstrates that Na⁺-alanine cotransport was inhibited by a change in the affinity for alanine without a change in the number of cotransporters during chronic ileitis. Thus, these two types of Na⁺-dependent nutrient uptake pathways are inhibited by different mechanisms in chronically inflamed ileum. Because a variety of immune-inflammatory mediators are known to be released in chronically inflamed ileum, it is hypothesized that different immune-inflammatory mediators may regulate these two transport pathways in chronically inflamed ileum.

This hypothesis is further supported by other specific transport pathway alterations in villus and crypt cells previously reported in this model of chronic ileal inflammation (26). Sundaram and West (26) demonstrated that coupled NaCl absorption, which occurs by the dual operation of Na^+/H^+ and Cl^-/HCO_3^- exchange on the BBM of villus cells, was inhibited due to a reduction in Cl^-/HCO_3^- but not Na^+/H^+ exchange. Unlike the villus cells, in the crypt cells, Na^+/H^+ exchange, known to be present only on the basolateral membrane (26), was stimulated in chronically inflamed ileum. This stimulation of basolateral Na^+/H^+ exchange alkalinizes the crypt cell, which may subsequently stimulate BBM Cl^-/HCO_3^- exchange, resulting in the secretion of HCO_3^- by these cells (26).

Together these studies demonstrate that specific transport pathways are altered in villus and crypt cells during chronic ileal inflammation. Furthermore, a related family of Na⁺-dependent nutrient cotransport processes is uniquely altered during chronic ileitis. Given the numerous immune-inflammatory mediators that are produced in chronically inflamed ileum and given that at least some of them are capable of altering transport pathways, it is reasonable to postulate that different immune-inflammatory mediators may regulate different transport pathways during chronic ileitis. Which of these agents is responsible for the transport abnormalities observed in this rabbit model of chronic ileal inflammation has yet to be delineated.

In conclusion, Na⁺-alanine cotransport is inhibited during chronic ileal inflammation. The inhibition is not entirely a consequence of a reduction of the Na⁺-extrusion capacity of the cell. At the level of the cotransporter, the mechanism of inhibition of Na⁺-alanine cotransport is secondary to a decrease in the affinity for alanine without a change in the number of transporters. This mechanism of inhibition Na⁺-amino acid cotransport differs from that of Na⁺-glucose cotransport during chronic ileal inflammation.

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