Mechanism of inhibition of Na⁺-glucose cotransport in the chronically inflamed rabbit ileum

U. Sundaram, S. Wisel, V. M. Rajendren, and A. B. West.

Inhibition of Na⁺-glucose cotransport in the chronically inflamed rabbit ileum. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G913–G919, 1997.—In a rabbit model of chronic ileal inflammation, we previously demonstrated that coupled NaCl absorption was reduced because of an inhibition of Cl⁻/HCO₃⁻ but not Na⁺/H⁺ exchange on the brush-border membrane (BBM) of villus cells. In this study we determined the alterations in Na⁺-stimulated glucose-[Na⁺-O-methyl-D-glucose (OMG)] absorption during chronic ileitis. Na⁺-OMG uptake was reduced in villus cells from the chronically inflamed ileum; Na⁺-K⁺-adenosinetriphosphatase (ATPase), which provides the favorable Na⁺ gradient for this cotransporter in intact cells, was found to be reduced also. However, in villus cell BBM vesicles isolated from the inflamed ileum Na⁺-OMG uptake was reduced as well, suggesting an effect at the level of the cotransporter itself. Kinetic studies demonstrated that Na⁺-OMG uptake in the inflamed ileum was inhibited by a decrease in the maximal rate of uptake for OMG without a change in the affinity. Analysis of steady-state mRNA and immunoreactive protein levels of this cotransporter demonstrates reduced expression. Thus Na⁺-glucose cotransport was inhibited in the chronically inflamed ileum, and the inhibition was secondary to a decrease in the number of cotransporters and not solely secondary to an inhibition of Na⁺-K⁺-ATPase or altered affinity for glucose.

Another important Na⁺-absorptive pathway in the normal ileum is Na⁺-nutrient cotransport (e.g., Na⁺-glucose cotransport). An alteration of this cotransport process in the chronically inflamed ileum will not only affect Na⁺ absorption but also the assimilation of important nutrients.

Na⁺-glucose cotransport is known to be present on the BBM of villus but not crypt cells in the normal ileum. In the intact cell, Na⁺-K⁺-adenosinetriphosphatase (ATPase) provides the favorable Na⁺ gradient for this cotransporter (7, 10, 15, 16, 25, 26, 28). Thus, during chronic ileal inflammation, cellular alterations in Na⁺-glucose cotransport may be at the level of the cotransporter and/or Na⁺-K⁺-ATPase. Therefore, the aims of this study were to test the hypothesis that chronic inflammation alters Na⁺-glucose cotransport and to determine the cellular mechanisms of this alteration.

Methods

Induction of chronic inflammation. Chronic ileal inflammation was produced in rabbits as previously reported (27). Pathogen-free rabbits were intragastrically inoculated with 10,000 oocytes of the coccidian protozoan Eimeria magna or sham inoculated with 0.9% NaCl (control animals). Oocytes were isolated from feces of infected rabbits by the method of Jackson (12). 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 utilized for experiments.

Measurement of epithelial dynamics. Histological sections were analyzed to determine alterations in epithelial morphology during chronic ileal inflammation. The villus-to-crypt ratio was defined as total villus height from the base of the crypt divided by the total crypt height. Ten different villus/crypt units were measured in three control or inflamed intestines from different animals.

Cell isolation. Villus and crypt cells were isolated from the normal and inflamed ileum by a Ca²⁺-chelation technique as previously described (26, 27). Established criteria were utilized to validate good separation of villus and crypt cells. These criteria included 1) marker enzymes (e.g., thymidine kinase), 2) transporter specificity, 3) differences in intracellular pH, 4) morphological differences, and 5) differing rates of protein synthesis.

The following set of criteria was utilized to exclude cells that showed evidence of poor viability: 1) trypan blue exclusion, 2) the demonstration of Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchange activities, and 3) the ability of the cells to maintain a baseline pH or imposed acid or alkaline gradient and return to baseline pH after perturbations. The cells were maintained in short-term culture for up to 6–8 h. For short-term culture, cells were resuspended at a final concentration of 0.1 g cells in

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40 ml of Lebowitz-15 medium (GIBCO) with 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 10% rabbit serum, 5,000 U/l penicillin, 5 mg/ml streptomycin, and 10 mg/ml gentamicin and gassed with 100% O2, pH 7.4 at 37°C and kept in sterile flasks until needed. 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 ileal villus cells were prepared by CaCl2 precipitation and differential centrifugation (17). Frozen villus cells were thawed and suspended in 2 mM tris(hydroxymethyl)aminomethane (Tris)-HCl buffer (pH 7.5) containing 50 mM mannitol. The suspension was homogenized, and 10 mM CaCl2 was added. The homogenate was centrifuged at 8,000 g for 15 min, and the supernatant was centrifuged at 20,000 g for 30 min. Then the pellet was resuspended in 10 mM Tris-HCl buffer (pH 7.5) containing 100 mM mannitol and homogenized. Vesicles were formed by adding MgCl2 (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) 1.25 3-O-methyl-d-glucose (3-OMG), 4.5 KCl, 1.2 KH₂PO₄, 1.0 MgSO₄, 1.25 CaCl₂, 20 HEPES, and either 130 mM NaCl or choline chloride and were gassed with 100% O2 (pH 7.4 at 37°C). Ten microcuries of [3H]DMDG (Amersham) were added to 1-ml cell suspension in the HEPES buffer, and 100-µl aliquots were removed at the desired time intervals. The uptake was arrested by mixing with 3 ml ice-cold stop solution (choline-HEPES buffer). After two washes with ice-cold stop solution, the filter was dissolved in 4 ml Optifluor and the radioactivity was determined.

BBMV uptake studies. Uptake studies were performed by the rapid filtration technique as previously described (17). In brief, 10 µl of BBMV resuspended in (in mM) 100 choline chloride, 0.10 MgSO₄, 50 HEPES-Tris (pH 7.5), 50 mannitol, and 50 KCl were incubated in 90 µl reaction medium that contained 50 mM HEPES-Tris buffer (pH 7.5), 1 mM OMDG, 20 µM 3-OMG, 0.10 mM MgSO₄, 50 mM KCl, 50 mM mannitol, 100 mM of either NaCl or choline chloride, 10 µM vanilomycin, 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, 0.10 MgSO₄, 75 KCl, and 100 choline chloride, pH 7.5). The mixture was filtered on 0.45-µm Millipore (HAWP) filters. After two washes with ice-cold stop solution, the filter was dissolved in 1 ml Optifluor, and the radioactivity was determined.

Data presentation. When data are averaged, 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

The histological observation of villus blunting in the inflamed ileum was confirmed by measurements of crypt and villus height. Villus-to-crypt ratio was noted to be diminished from 7.97 ± 0.7 to 3.98 ± 0.2 (P < 0.0001) from the normal to the chronically inflamed ileum. The morphological changes have been observed in detail previously (27).

Crypt-villus cell separation is an important concern in intestine in which the normal maturation process is altered. Thus the following criteria were used to ensure good cell separation (27): 1) marker enzymes (alkaline phosphatase for villus cells and thymidine kinase for crypt cells), 2) higher intracellular baseline pH in crypt compared with villus cells, 3) presence of Na+/H+ exchange in the BBM of villus but not crypt cells, 4) a better developed BBM on villus cells, 5) higher rate of protein synthesis in crypt cells compared with villus cells, and 6) the presence of Na+-nutrient cotransport on the BBM of villus but not crypt cells.

Cell viability by trypan blue exclusion was observed in 94 ± 4% of villus cells from the normal rabbit ileum and in 93 ± 5% of villus cells from the inflamed ileum (n = 11). We have previously demonstrated that Na+-dependent glucose uptake is present in villus but not crypt cells of the normal rabbit ileum (26). The initial study was performed to confirm this observation. In villus cells from the normal ileum 3-OMG uptake was signifi-
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stantly stimulated by extracellular Na⁺ (e.g., 8.56 ±
0.90 nmol/mg protein at 15 min in the presence of Na⁺
and 1.03 ± 0.13 nmol/mg protein in the absence of Na⁺,
n = 6, P < 0.0001). However, Na⁺-stimulated 3-OMG uptake
was not observed in crypt cells from the normal ileum (e.g., 1.10 ± 0.36 nmol/mg protein at 15 min in
the presence of Na⁺ and 1.20 ± 0.11 nmol/mg protein in
the absence of Na⁺, n = 6, P not significant).

Na⁺-stimulated 3-OMG uptake in villus and crypt
cells from the inflamed ileum was next determined
(Fig. 1). In villus cells from the inflamed ileum 3-OMG uptake
was also significantly stimulated by extracellu-
lar Na⁺ (Fig. 1A). Similar to the normal ileum, Na⁺-
stimulated 3-OMG uptake was also not present in crypt
cells from the inflamed ileum (Fig. 1B). Thus these data
demonstrate that Na⁺-stimulated glucose uptake is
present in villus but not crypt cells from the normal
and inflamed ileum.

Figure 2 compares the Na⁺-dependent uptake of
3-OMG in villus cells from the normal and inflamed ileum.
Na⁺-dependent 3-OMG uptake was significantly
diminished in villus cells from the inflamed ileum.
These data indicate that Na⁺-glucose cotransport was
reduced in intact villus cells from the inflamed ileum.

Inhibition of Na⁺-glucose cotransport at the cellular
level may represent a direct effect on the cotransporter
located on the BBM and/or may be secondary to an
inhibition of Na⁺-K⁺-ATPase on the basolateral mem-
brane (BLM), which provides the favorable Na⁺-
electrochemical gradient for this cotransport process.
Thus Na⁺-K⁺-ATPase activity was measured as previ-
ously described (14) in homogenates of villus cells from
normal and inflamed ileum. Na⁺-K⁺-ATPase activity
was reduced ∼50% in villus cells from the inflamed ileum
compared with the normal ileum (Fig. 3). These
data suggest that the reduction in Na⁺-glucose cotrans-
port in inflamed ileal villus cells may, at least in part,
be due to reduced electrochemical gradients of Na⁺
across the BBM resulting from an alteration in Na⁺
extrusion capacity by the cells.

To determine whether chronic inflammation has a
direct effect on the Na⁺-glucose cotransporter itself,
3-OMG uptake was determined in BBMV prepared
from villus cells from the normal and inflamed ileum.
Na⁺-dependent 3-OMG uptake was significantly re-
duced in villus cell BBMV from the inflamed ileum (Fig.
4). These data suggest that the cotransporter itself was
directly inhibited during chronic ileal inflammation.

Altered Na⁺ permeability would be expected to dimin-
ish the Na⁺ gradient across the membrane and may
explain the inhibition of 3-OMG uptake seen in BBMV
from the inflamed ileum. Therefore, 22Na⁺ uptake in
villus cell BBMV from the normal and inflamed ileum
was determined and was found not to be different (0.10
nmol·mg protein⁻¹·15 s⁻¹ in normal and 0.11 nmol·mg
protein⁻¹·15 s⁻¹ in inflamed, n = 3, P not significant).
Unaltered amiloride-sensitive 22Na⁺ uptake (i.e.,
Na⁺/H⁺ exchange) also indicates that the villus cell
BBM from the inflamed ileum is not significantly
contaminated by crypt cell BBM, because it is known
that Na⁺/H⁺ exchange is only present on the BBM
of villus but not crypt cells in the rabbit ileum (16).

BBM purity was also ensured by a similar degree of
enrichment of the villus cell BBM marker enzyme,
alkaline phosphatase (11.0 ± 1.0-fold enrichment in
normal villus cell BBM and 10.1 ± 1.1-fold enrichment
in inflamed villus cell BBM, n = 3).

To determine whether the inhibition of Na⁺-glucose
cotransport during chronic ileal inflammation was due
to an alteration in the affinity for glucose and/or in the
maximal rate of uptake (Vₘₐₓ) of glucose, kinetic stud-
ies were performed. Uptake for all the concentrations
was carried out at 6 s because in initial uptake studies
Na⁺-dependent glucose uptake in BBMV was linear for
at least 10 s (data not shown) in the normal and the
inflamed ileum. Figure 5 demonstrates the kinetics of
glucose uptake in villus cell BBMV from the normal
and inflamed ileum. Figure 5A shows the uptake of
Na⁺-dependent 3-OMG as a function of varying concen-
trations of extravesicular glucose. As the concentra-
tion of extravesicular glucose was increased, the uptake of
Na⁺-dependent 3-OMG was stimulated and subse-
dually became saturated in the normal as well as in
the inflamed ileum. With use of Enzfitter, a Lineweaver-
Burk plot of these data was generated and is shown in
Fig. 5B. Kinetic parameters derived from these data
demonstrate that the affinity (1/Michaelis constant
(Kₘ)) for 3-OMG uptake was not different between
the normal and inflamed ileum (Kₘ for 3-OMG uptake
in BBMV was 6.6 ± 1.5 mM in normal and 7.0 ± 2.0 in
inflamed ileum, n = 3, P not significant). However, the
Vₘₐₓ of 3-OMG was reduced sevenfold in the inflamed
ileum (Vₘₐₓ for 3-OMG uptake in BBMV was 5.0 ± 0.5
nmol·mg protein⁻¹·6 s⁻¹ in normal and 1.2 ± 0.4
nmol·mg protein⁻¹·6 s⁻¹ in inflamed ileum, n = 3, P <
0.05). These data suggested that Na⁺-glucose cotrans-

![Fig. 1. Effect of extracellular Na⁺ on 3-O-methyl-D-glucose (3-OMG) uptake as a function of time in intact villus and crypt cells from chronically inflamed rabbit ileum. A: villus cells, n = 6, *p <
0.005. Extracellular Na⁺ significantly stimulated 3-OMG uptake at all time
points in villus cells from inflamed ileum. B: crypt cells, n = 6. Similar to the
normal ileum, Na⁺-stimulated 3-OMG uptake was also not present in crypt
cells from inflamed ileum. ●, Na⁺ present; ○, Na⁺ absent.](http://ajpgi.physiology.org/ by 10.220.33.5 on June 25, 2017)
port was inhibited in the chronically inflamed ileum secondary to a decrease in the number of cotransporters rather than altered affinity for glucose. To confirm these findings we next looked at steady-state levels of mRNA for SGLT1 in villus cells.

Steady-state levels of mRNA transcripts for SGLT1 were markedly reduced in villus cells from the chronically inflamed ileum (Fig. 6). Because steady-state mRNA levels may not directly correlate with functional protein levels on the BBM, immunoreactive SGLT1 levels on the BBM were also determined. Western blot analysis of BBMV showed that the anti-SGLT1 antibody recognized one major immunoreactive protein band at the expected size of 70 kDa, which was reduced in intensity in the chronically inflamed ileum (Fig. 7).

**DISCUSSION**

This study demonstrates that Na\(^+\)-glucose cotransport is inhibited in the chronically inflamed ileum. This inhibition is not entirely a consequence of a reduction in the capacity of the villus cells to extrude Na\(^+\). In the chronically inflamed ileum there is an inhibition at the level of the Na\(^+\)-glucose cotransporter itself. Kinetic parameters indicate that while the affinity for glucose is not affected, the \(V_{\text{max}}\) of glucose is reduced. Diminished levels of steady-state mRNA for SGLT1 and SGLT1 immunoreactive protein are observed in the chronically inflamed ileum. These data indicate that the mechanism of Na\(^+\)-glucose cotransport inhibition is due to a decrease in the number of Na\(^+\)-glucose cotransporters in the chronically inflamed ileum.

Our laboratory had previously demonstrated the presence of Na\(^+\)-glucose cotransport in isolated villus but not crypt cells from the normal rabbit ileum (16, 26). This distribution was shown to be preserved in the chronically inflamed ileum as well (Fig. 1). However, previous studies have indicated the presence of Na\(^+\)-glucose cotransport in both villus and crypt cells from the normal rabbit ileum (19). Inadequate cell separation criteria resulting in the contamination of crypt cells with villus cells may account for this finding. Indeed, immunocytochemistry and in situ hybridization studies have demonstrated that the Na\(^+\)-glucose cotransporter (SGLT1) is present only in the mature villus cells in the normal rabbit ileum (11, 28). Thus most of the currently available evidence supports the observation that Na\(^+\)-glucose cotransport is limited to villus cells in the normal ileum.

Although the location of Na\(^+\)-glucose cotransport along the crypt-villus axis in the ileum is fairly clear, how it is altered during chronic ileal inflammation is not known. Previous studies have looked at the effect of acute inflammation on Na\(^+\)-glucose cotransport in several animal models. Differing observations about the effect of acute inflammation on Na\(^+\)-glucose cotransport have been reported; Na\(^+\)-glucose cotransport was found to be unaltered in acute enteritis produced by Giardia in gerbils (4), whereas inhibition of Na\(^+\)-glucose cotransport was observed in acute enteritis produced by Cryptosporidium in pigs (2) and Yersinia enterocolitica in rabbits (21). It was not possible to address the mechanism of alteration in Na\(^+\)-glucose cotransport at the cellular level based on these intact tissue studies because in acute enteritis there is near complete loss of mature villus cells that contain the
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Na⁺-glucose cotransporter. In acute enteritis produced by transmissible gastroenteritis virus in piglets (9, 13, 14) the inhibition of Na⁺-glucose cotransport has been postulated to be secondary to the loss of high affinity D-glucose carrier. However, multiple Na⁺-glucose co-transport systems have not been described in other animals, including rabbits (19). Thus the mechanism of alteration of Na⁺-glucose cotransport during acute intestinal inflammation is not completely understood.

The alterations that occur in Na⁺-glucose cotransport during chronic ileal inflammation have not previously been investigated. Undoubtedly, this is a 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 (23) and alloimmunization-induced enterocolitis in guinea pigs (20), have not yet been utilized for electrolyte transport studies. At present there are no perfect animal models of chronic ileitis comparable to the human disease. Although this rabbit model of chronic intestinal inflammation should not be considered as an example of the human disease, it does possess many of the same features: the ileum is thickened with a cobblestone appearance, the villi are blunted, the crypts are hypertrophied, and the immune response is characterized by chronic rather than acute inflammatory cells. Thus it may be a suitable animal model to study the effect of chronic ileal inflammation on electrolyte transport at the cellular level.

Inhibition of Na⁺-glucose cotransport in villus cells from the chronically inflamed ileum may occur at the level of the cotransporter and/or secondary to an alteration in Na⁺ extrusion from the cell facilitated by Na⁺-K⁺-ATPase. This study indicates that during chronic ileal inflammation the mechanism of inhibition of Na⁺-glucose cotransport is at the level of the cotransporter and is not exclusively secondary to an alteration in the Na⁺ extrusion capacity of the villus cell. Kinetic studies and Northern and Western blot studies indicate that the number of Na⁺-glucose cotransporter is reduced in villus cells from the chronically inflamed ileum.

One possible explanation for the reduction in the number of Na⁺-glucose cotransporters in the chronically inflamed ileum may be a significant contamination of the isolated villus cell population with crypt cells or immunocytes. Fortunately, in the chronically inflamed ileum many of the characteristics of mature villus cells are clearly preserved, which allowed us to separate villus and crypt cells consistently (27). Furthermore, the cell isolation process did not result in any

**Fig. 5.** Kinetics of glucose uptake in villus cell BBMV from normal (●) and chronically inflamed (○) ileum. A: representative of 3 experiments. Na⁺-dependent uptake of [3H]OMG is shown as a function of varying concentration of extravesicular D-glucose. Isosmolarity was maintained by adjusting the concentration of mannitol. Uptake for all concentrations was determined at 6 s. As concentration of extravesicular glucose was increased, uptake of glucose 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 3-OMG uptake is not affected during chronic ileal inflammation. However, maximal rate of uptake of 3-OMG is reduced severalfold in inflamed ileum.

**Fig. 6.** Northern blot analysis demonstrates that steady-state levels of SGLT1 mRNA are reduced in villus cells from chronically inflamed ileum. Representative of 4 experiments each with different animals.

**Fig. 7.** Western blot analysis demonstrates reduction in amount of immunoreactive SGLT1 in BBMV from chronically inflamed ileum.
significant contamination of epithelial cells with immunocytes (27). Thus it is unlikely that the inhibition of Na⁺-glucose cotransport during chronic ileal inflammation demonstrated in this study is a result of the contamination of villus with crypt cells or immuno-
cytes.

Alterations in absorption and secretion by ileal villus and crypt cells undoubtedly occur as a result of the numerous immune-inflammatory mediators endog-

enously produced in the chronically inflamed intestine (5, 6, 22, 24). In this model of chronic inflammation we have previously demonstrated that coupled NaCl ab-
sorption, which occurs by the dual operation of Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchange on the BBM of villus cells, is inhibited as a result of impairment of Cl⁻/HCO₃⁻ but not Na⁺/H⁺ exchange. Unlike the villus cells, in the crypt cells Na⁺/H⁺ exchange, known to be present only on the BLM of crypt cells, was stimulated in the chronically inflamed ileum. The BLM Na⁺/H⁺ ex-
change stimulation alkalinizes the crypt cells, which may subsequently stimulate the BBM Cl⁻/HCO₃⁻ ex-
change resulting in HCO₃⁻ secretion by these cells (27).

The previous findings (27) and this study demon-
strate that specific transport pathways are altered in villus and crypt cells during chronic ileal inflammation to inhibit coupled NaCl and glucose-stimulated Na⁺ absorption and promote HCO₃⁻ secretion. Given the numerous immune-inflammatory mediators known to be released in the chronically inflamed intestine and at least some of them are capable of affecting electrolyte transport pathways, it is hypothesized that different immune-inflammatory mediators released in the chronically inflamed ileum may have unique effects on transport pathways in villus and crypt cells. Which of these agents are responsible for the transport abnor-
malities observed in this model of chronic ileal inflamma-
tion has yet to be elucidated.

In conclusion, Na⁺-glucose cotransport is inhibited during chronic ileal inflammation. The mechanism of inhibition is not solely secondary to a diminution in the Na⁺ extrusion capacity of the villus cell. In fact, our studies suggest that the number of Na⁺-glucose cotrans-
porters is reduced in villus cells in the chronically inflamed ileum.

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Address for reprint requests: U. Sundaram, Div. of Gastroenterology, Ohio State Univ. School of Medicine, N-214 Doan Hall, 410 W. Tenth Ave., Columbus, OH 43210.

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