Corticosteroids reverse the inhibition of Na-glucose cotransport in the chronically inflamed rabbit ileum

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Sundaram, Uma, Steve Coon, Sheik Wisel, and A. Brian West. Corticosteroids reverse the inhibition of Na-glucose cotransport in the chronically inflamed rabbit ileum. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G211–G218, 1999.—In a rabbit model of chronic ileal inflammation, we previously demonstrated inhibition of Na-glucose cotransport (SGLT-1). The mechanism of 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. In this study, we determined the effect of methylprednisolone (MP) on SGLT-1 inhibition during chronic ileitis. Treatment with MP almost completely reversed the reduction in SGLT-1 in villus cells from the chronically inflamed ileum. MP also reversed the decrease in Na-K-ATPase activity in villus cells during chronic ileitis. However, MP treatment reversed the SGLT-1 inhibition in villus cell brush-border membrane vesicles from the inflamed ileum, which suggested an effect of MP at the level of the cotransporter itself. Kinetic studies demonstrated that the reversal of SGLT-1 inhibition by MP was secondary to an increase in the maximal velocity for glucose without a change in the affinity. Analysis of immunoreactive protein levels of the cotransporter demonstrated a restoration of the cotransporter numbers after MP treatment in the chronically inflamed ileum. Thus MP treatment alleviates SGLT-1 inhibition in the chronically inflamed ileum by increasing the number of cotransporters and not solely secondary to enhancing the activity of Na-K-ATPase or by altering the affinity for glucose.

INHIBITION OF NaCl and nutrient absorption have been well described in human inflammatory bowel disease (1–3, 8). Immune modulation with corticosteroids is a mainstay of treatment for inflammatory bowel disease. This treatment, at least partially, alleviates the malabsorption seen in these conditions (11). However, how glucocorticoids affect alterations in coupled NaCl and nutrient-dependent Na absorption at the cellular level during chronic ileal inflammation is not known. This is primarily because of the lack of adequate animal models of chronic small intestinal inflammation. We recently reported a rabbit model of chronic ileal inflammation from which viable and relatively pure populations of villus and crypt cells can be isolated. In this model of chronic ileitis, specific alterations in electrolyte transport pathways were demonstrated. For example, it was shown that the mechanism of inhibition of coupled NaCl absorption was due to an inhibition of Cl/HCO3 but not Na/H exchange on the brush-border membrane (BBM) of villus cells (15). Na-nutrient cotransport processes, which are important for the assimilation of Na and nutrients, were also uniquely altered in the chronically inflamed ileum. Na-glucose cotransport (SGLT-1) was inhibited as a result of a decrease in the number of cotransporters without a change in the affinity for glucose (17). However, Na-amino acid cotransport was inhibited by a decrease in the affinity for amino acid without a change in the number of cotransporters (16). Unlike these two cotransport processes, Na-bile acid cotransport was inhibited by both a decrease in the affinity for the bile acid and a decrease in the number of cotransporters (18). These unique alterations in different transport processes suggest regulation by different immune inflammatory mediators, which may be released during chronic intestinal inflammation.

Given this background, we looked at the effect of a glucocorticoid, methylprednisolone (MP), on SGLT-1 inhibition during chronic ileitis. MP has previously been demonstrated to not have a direct effect on the Na-glucose cotransporter in the normal rabbit ileum (20). The MP-mediated increase in SGLT-1 observed in intact tissue studies was suggested to be secondary to an increase in mucosal Na-K-ATPase, which provides the favorable Na gradient for this cotransporter (5, 6, 10, 11, 13, 19).

In the chronically inflamed rabbit ileum, we previously demonstrated that, although the activity of Na-K-ATPase is diminished, it was not the sole explanation for the decrease in SGLT-1 (17). Thus MP may have an affect on SGLT-1 inhibition in the chronically inflamed ileum at the level of the cotransporter and/or Na-K-ATPase. Therefore, the aims of this study were to test the hypothesis that MP may alleviate the inhibition of SGLT-1 during chronic ileal inflammation and to delineate the cellular mechanisms of this reversal.

METHODS
Induction of chronic inflammation and drug treatment. Chronic ileal inflammation was produced in rabbits as previously reported (15, 17). Pathogen-free rabbits were intragastrically inoculated with 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 utilized for experi-
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Measurement of mucosal alterations. For histological evaluation, tissue samples were fixed in Formalin, embedded in paraffin, sectioned and, after hematoxylin and eosin staining, examined and graded in a blinded fashion by light microscopy. Histological sections were analyzed to determine alterations in epithelial morphology during chronic ileal inflammation and with drug treatment. Ten different fields were measured in every animal from each group. For villus blunting and fusion, an 0–2 scale (0 = normal, 1 = partial blunting/fusion, and 2 = marked blunting/fusion) was used. For intraepithelial (IEL) and lamina propria lymphocyte (LPL) count, a 0–3 scale (0 = normal, 1 = mild increase, 2 = moderate increase, and 3 = marked increase) was used.

Cell isolation. Villus and crypt cells were isolated from the ileum by a calcium chelation technique as previously described (14, 15, 17). Previously established criteria were utilized to validate good separation of villus and crypt cells. Some of these criteria included 1) marker enzymes (e.g., thymidine kinase, alkaline phosphatase, 2) transporter specificity (e.g., Na/H on the BBM of villus, but not crypt cells), 3) differences in intracellular pH (e.g., intracellular pH is higher in crypt cells compared with villus), 4) morphological differences (e.g., villus cells are larger and with better developed BBM compared with crypt cells) and, 5) differing rates of protein synthesis (e.g., higher synthesis rate in crypt cells compared with villus).

Previously established criteria were also utilized to study cells with good viability and 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 measured 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 (14, 15, 17).

BBM vesicle preparation. BBM vesicles (BBMV) from rabbit ileum villus cells were prepared by CaCl2 precipitation and differential centrifugation as previously reported (17). BBMV were resuspended in a medium appropriate to each preparation. BBMV resuspended in 100 mM choline chloride, 0.10 mM MgSO4, 50 mM HEPES-Tris (pH 7.5), 50 mM mannitol, and 75 mM KCl were incubated in 95 µl of reaction medium that contained mannitol, 100 mM of either NaCl or choline chloride, 10 µM valinomycin, and 100 µM carbonyl cyanide p-(trifluoromethoxy) phenyl-hydrazone (FCCP). At desired times, uptake was arrested by mixing with ice-cold stop solution (50 mM HEPES-Tris buffer, 0.10 mM MgSO4, 75 mM KCl, and 100 mM choline chloride, pH 7.5). The mixture was filtered onto a 0.45-µm Millipore (HAWP) filter and washed two times with 5 ml ice-cold stop solution. Filters with BBMV were dissolved in Optifluor, and radioactivity was determined.

Enzyme measurement. Na-K-ATPase was measured as P1 liberated in cellular homogenates from the same amount of cells as previously described (4, 17). Enzyme specific activity was expressed as nanomoles of P1 released per milligram protein per minute. Alkaline phosphatase activity was also measured in same amounts of cells as previously described (14).

Western blot studies. BBMV (4 mg) were diluted in SDS buffer, boiled, and electrophoresed on a 12% SDS-PAGE gel. The gel was electroblotted onto a polyvinylidene difluoride membrane and blocked for 2 h in 5% BSA at room temperature. The membrane was incubated at room temperature with 1:3,000 anti-rabbit SGLT-1 antiserum followed by goat anti-rabbit IgG coupled to horseradish peroxidase (1:10,000, Pierce, Rockford, IL). After each incubation, the membrane was washed extensively with PBS-0.2% Tween 20. The signal was developed with the chemiluminescence Western blot kit (NEN Research Products, Boston, MA). The SGLT-1 antibody was a generous gift from Dr. E. M. Wright. Densitometric analysis of the Western blots was done using Pharmacia LKB Ultrascan XL with Alpha Imager 2000.

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.

For statistical analysis of the morphological data, a Kruskal-Wallis nonparametric ANOVA with a Dunn multiple comparison test was utilized. All other data were analyzed with an ANOVA with a posttest when ANOVA revealed a significant difference among the groups.

RESULTS

Morphology. The ileum of sham-inoculated animals appeared normal throughout the study (Fig. 1A). However, in inoculated rabbits, chronic inflammation of the ileum resulted by day 12 postinoculation (Fig. 1B). No parasites were shed in the stool or observed microscopically in the mucosa. There was a marked increase in lymphocytes and plasma cells in the lamina propria. IEL were also present in increased numbers. Furthermore, villus blunting and crypt hyperplasia was noted in the chronically inflamed ileum. Treatment with MP (Fig. 1C) or AG (Fig. 1D) did not significantly alter the histology of the chronically inflamed ileum.

Histological sections were then analyzed to measure changes in epithelial morphology during chronic ileal inflammation and with drug treatment. Normal and MP- or AG-treated normal ileum did not demonstrate villus blunting and fusion (Fig. 2A). There was significant blunting and fusion in the chronically inflamed ileum that was not significantly altered by AG treatment. Although MP treatment demonstrated a trend toward improvement of the blunting and fusion of the...
inflamed ileum, the data were not statistically significant (Fig. 2A).

Treatment of the normal ileum with MP or AG did not significantly alter the IEL number (Fig. 2B). In the chronically inflamed ileum, IEL count was markedly increased, and treatment with AG did not alter this. Treatment with MP demonstrated a trend toward reduction of the IEL count, albeit this was not statistically significant (Fig. 2B).

Treatment of the normal ileum with MP or AG also did not significantly alter the LPL count (Fig. 2C). In the chronically inflamed ileum, LPL count was markedly increased, and treatment with AG did not significantly alter this. Treatment with MP demonstrated a trend toward reduction in the LPL count in the inflamed ileum. However, this was also not statistically significant (Fig. 2C).

Cell viability by trypan blue exclusion was observed in 95 ± 5% of villus cells from the normal rabbit ileum and 94 ± 5% from the chronically inflamed ileum (n = 6). Treatment with MP or AG did not alter this degree of viability (data not shown).

Marker enzyme activity. To determine whether treatment with MP also had an effect on a BBM marker enzyme, we measured alkaline phosphatase. The activity of this enzyme is markedly reduced in villus cells from the inflamed ileum (Fig. 3). Treatment with MP or AG had no effect on alkaline phosphatase activity in villus cells from the normal ileum. AG treatment also had no effect on the reduction in alkaline phosphatase activity in villus cells from the chronically inflamed ileum. However, treatment with MP almost completely reversed the inhibition in alkaline phosphatase activity in villus cells from the chronically inflamed ileum.

SGLT-1 in intact cells. We had previously demonstrated that Na-dependent glucose uptake was present in villus but not crypt cells from the normal and the chronically inflamed ileum. Furthermore, Na-dependent glucose uptake was significantly diminished in villus cells from the chronically inflamed ileum (17). AG treatment did not significantly affect the Na-dependent glucose uptake in villus cells from the normal or chronically inflamed ileum (Fig. 4). MP also did not significantly increase SGLT-1 in villus cells from the normal ileum (Fig. 4A). However, MP treatment almost completely reversed the inhibition of SGLT-1 seen in villus cells from the chronically inflamed ileum (Fig. 4B).
Na-K-ATPase activity. Alteration of SGLT-1 at the cellular level may represent a direct effect on the cotransporter located on the BBM and/or may be secondary to an effect on Na-K-ATPase on the basolateral membrane, which provides the favorable Na-electrochemical gradient for this cotransport process. Thus Na-K-ATPase activity was measured in homogenates of villus cells. Na-K-ATPase activity was reduced ~50% in villus cells from the chronically inflamed ileum compared with the normal ileum (Fig. 5). Neither AG nor MP treatment altered Na-K-ATPase activity in villus cells from the normal ileum. MP but not AG treatment completely reversed the reduction in Na-K-ATPase activity seen in villus cells from the chronically inflamed ileum (Fig. 5). These data suggested that the reversal of SGLT-1 inhibition by MP treatment in the chronically inflamed ileal villus cells may, at least in part, be due to the restoration of Na-electrochemical gradients across the BBM.

SGLT-1 in BBMV. To determine whether MP and AG treatment had an effect at the level of the Na-glucose cotransporter itself, 3-OMG uptake was determined in BBMV prepared from villus cells from treated and untreated normal and chronically inflamed ileum. AG did not affect Na-dependent glucose uptake in villus cell BBMV from the normal or chronically inflamed ileum (data not shown). MP also had no effect on the SGLT-1 in the normal ileum (Fig. 6). However, MP treatment resulted in a significant reversal of SGLT-1 reduction in BBMV from the chronically inflamed ileum (Fig. 6). These data demonstrated that, although MP does not have an effect at the Na-glucose cotransporter level in the normal ileum, it does reverse the reduction seen in the chronically inflamed ileum.

Kinetic studies. To decipher the mechanism of glucocorticoid-mediated reversal of inhibition of SGLT-1 in the chronically inflamed ileum, kinetic studies were performed. Uptake for all of the concentrations was carried out at 6 s, since initial uptake studies for Na-dependent glucose uptake in BBMV were linear for at least 10 s (17). Figure 7 demonstrates the kinetics of glucose uptake in villus cell BBMV from the normal, chronically inflamed ileum and MP-treated inflamed ileum. Treatment with AG did not alter the uptake in the normal or inflamed ileum (data not shown). Figure 7 shows the uptake of Na-dependent 3-OMG as a function of varying concentrations of extravesicular glucose. As the concentration of extravesicular glucose was increased, the uptake of Na-dependent 3-OMG was stimulated and subsequently became saturated in all conditions (Fig. 7A). Using Enzfitter, kinetic param-
eters derived from these data demonstrate that the affinity \([1/Michaelis constant (K_m)]\) for 3-OMG uptake was not altered by MP treatment \((K_m \text{ for 3-OMG uptake in BBMV was 5.9 mm in normal, 5.1 in inflamed, and 5.2 in inflamed + MP})\). However, the maximal rate of uptake \((V_{\text{max}})\) of 3-OMG, which was reduced in the chronically inflamed ileum, was almost completely reversed by treatment with MP \((V_{\text{max}} \text{ for 3-OMG uptake in BBMV was 4.5 nmol·mg protein}^{-1} \cdot \text{s}^{-1} \text{ in normal, 1.4 in inflamed, and 3.7 in inflamed + MP})\). These data confirmed the observation that SGLT-1 was inhibited in the chronically inflamed ileum secondary to a decrease in the number of cotransporters rather than altered affinity for glucose. Furthermore, treatment of the chronically inflamed ileum with MP appeared to restore the number of Na-glucose cotransporters without affecting the affinity of these cotransporters.

Western blot studies. To confirm the findings of the kinetic studies, immunoreactive SGLT-1 levels of the villus cell BBM were also determined. Western blot analysis of BBMV showed that the anti-SGLT-1 antibody recognized one major immunoreactive protein band at the expected size of 70 kDa, which was reduced in intensity in the chronically inflamed ileum \((\text{Fig. 8, A and B})\). AG treatment of the normal or the chronically inflamed ileum did not alter the immunoreactive levels of SGLT-1. Treatment of the normal ileum with MP also did not alter the levels of SGLT-1. However, treatment of the chronically inflamed ileum with MP almost completely restored the immunoreactive levels of SGLT-1. These data demonstrated that the mechanism of glucocorticoid-mediated reversal of inhibition of SGLT-1 in the chronically inflamed ileum is via the upregulation of cotransporter numbers \((\text{Fig. 8, A and B})\).
This study demonstrates that SGLT-1 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. At the level of the Na-glucose cotransporter, the mechanism of inhibition is due to a decrease in the number of cotransporters in the chronically inflamed ileum. Treatment with glucocorticoids does not affect SGLT-1 in the normal ileum. However, in the chronically inflamed ileum, treatment with MP almost completely reverses the inhibition of SGLT-1. The mechanism of reversal is not entirely secondary to a correction of reduced Na extrusion capacity of the villus cells seen in the chronically inflamed ileum. At the level of the cotransporter, the MP-mediated reversal of inhibition is secondary to a restoration of cotransporter numbers in the chronically inflamed ileum.

Although malabsorption of electrolytes and nutrients has been well documented in inflammatory bowel diseases, until recently the alterations that occur in SGLT-1 during chronic ileal inflammation had not 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 (12) and alloimmunization-induced enterocolitis in guinea pigs (7), have not yet been utilized for electrolyte transport studies. This rabbit model of chronic ileal inflammation possesses many of the same features as the human disease. Thus it was previously utilized to describe alterations in electrolyte and nutrient transport properties and now to study the effect of glucocorticoids on Na-dependent glucose uptake during chronic ileitis.

Glucocorticoids were chosen for this study because they are a mainstay of therapy of inflammatory bowel disease. Furthermore, although the mechanism of the glucocorticoid action on electrolyte and nutrient transport in inflammatory bowel disease is not known, this class of drugs has been demonstrated to at least partially alleviate the impairment of electrolyte and nutrient malabsorption in inflammatory bowel disease.
cally inflamed ileum, glucocorticoids do not increase Na-glucose cotransporter numbers (20) and are thought to stimulate Na-dependent glucose uptake by increasing Na-K-ATPase levels, which provide the favorable Na gradient for this cotransport (10, 11, 13). In the chronically inflamed rabbit ileum, SGLT-1 is inhibited secondary to a reduction in Na-K-ATPase level as well as a decrease in the number of cotransporters (17). Therefore, glucocorticoid action in the chronically inflamed ileum may be 1) an effect at the level of the transporter by altering transporter numbers or affinity for the substrate and/or 2) at the level of the Na-K-ATPase. These studies demonstrate that, in the chronically inflamed ileum, at the cellular level, MP reverses the impairment in Na-dependent glucose uptake. This is accomplished by both increasing Na-K-ATPase activity as well as an effect at the level of the cotransporter. At the level of the cotransporter, the reversal is due to a restoration of cotransporter numbers rather than an alteration of the affinity for glucose.

It is likely that MP acts as an immune modulator to reverse the inhibition of SGLT-1 in the chronically inflamed ileum rather than exerting a direct effect on the cotransporter itself. This hypothesis is supported by the fact that MP has no effect on SGLT-1 in the normal ileum at the cellular or cotransporter level. MP as an immune modulator may exert its effect at many levels. Thus one possibility is that the formation of an immune-inflammatory mediator is inhibited by MP, allowing for the restoration of Na-glucose cotransporter numbers. Specifically, which immune-inflammatory mediator is responsible for the inhibition of SGLT-1 in the chronically inflamed ileum should be delineated in future studies using specific pathway inhibitors and agonists and antagonists. Despite the broad spectrum immunoregulatory action of MP, the fact that it can reverse an altered transport process in the chronically inflamed ileum suggests that determining immune regulation of the transport pathway during chronic ileitis is possible in this animal model. This is an important observation, since, as previously stated, the study of immune regulation of electrolyte and nutrient transport in the chronically inflamed intestine is hampered by the lack of good animal models of chronic small intestinal inflammation.

This hypothesis of glucocorticoids having an affect on circulating immune-inflammatory mediators that can inhibit electrolyte transport in the intestine is supported by two previous studies. In one study, the findings were consistent with circulating immune-inflammatory mediators altering fluid and electrolyte transport in nonhistologically involved areas of the intestine (2). In another study, a single dose of MP partially reversed the inhibition in Na and fluid absorption in patients with ulcerative colitis (11).

MP has also been demonstrated to partially reverse the inhibition of glucose-stimulated Na flux in acute enteritis caused by transmissible gastroenteritis virus. In that study, MP had no effect on glucose-stimulated Na flux in the normal piglet jejunum. Furthermore, the severe histological distortion that is observed in acute enteritis was not affected by treatment with MP in that study. The mechanism of glucocorticoid-mediated reversal of SGLT-1 inhibition was not deciphered in that model of acute intestinal inflammation (9).

In conclusion, this study demonstrates that the inhibition of SGLT-1 in the chronically inflamed ileum can be reversed by treatment with glucocorticoids. The mechanism of this reversal at the cellular level is both at the cotransporter and is secondary to enhanced Na-K-ATPase activity. At the cotransporter level, the mechanism of glucocorticoid-mediated reversal is secondary to enhanced cotransporter numbers rather than an altered affinity for glucose. It is postulated that glucocorticoid may act as an immune modulator to reverse the inhibition of SGLT-1 in the chronically inflamed ileum.

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