

TRANSLATIONAL PHYSIOLOGY |

Neutral Na-amino acid cotransport is differentially regulated by glucocorticoids in the normal and chronically inflamed rabbit small intestine

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Sundaram U, Wisel S, Coon S. Neutral Na-amino acid cotransport is differentially regulated by glucocorticoids in the normal and chronically inflamed rabbit small intestine. *Am J Physiol Gastrointest Liver Physiol* 292: G467–G474, 2007; doi:10.1152/ajpgi.00503.2005.— Neutral Na-amino acid cotransport by system ATB⁰ [e.g., Na-alanine cotransport (NAcT)] is an important means of assimilation of amino acids in the intestine. NAcT is inhibited during chronic intestinal inflammation by an alteration in the affinity for the amino acid. How glucocorticoids, a standard of treatment for diseases characterized by chronic intestinal inflammation, may affect NAcT during chronic enteritis is not known. Thus we first demonstrated that methylprednisolone (MP) stimulated NAcT in the normal intestine. The mechanism of stimulation was secondary to an increase in cotransporter numbers without an alteration in the affinity for the amino acid. Treatment with MP reversed the reduction in NAcT in villus cells from the chronically inflamed intestine. MP also alleviated the decrease in Na-K-ATPase activity in villus cells during chronic enteritis. However, MP treatment reversed the NAcT inhibition in villus cell brush border membrane vesicles from the inflamed intestine, which suggested an effect of MP at the level of the cotransporter itself. Kinetic studies demonstrated that the reversal of NAcT inhibition by MP was secondary to restoration in the affinity for the amino acid without a change in the V_{max} . Unaltered steady-state mRNA and immunoreactive protein levels of NAcT also indicated that the number of cotransporters was unchanged after MP treatment in the chronically inflamed intestine. These results indicated that MP reversed NAcT inhibition in the chronically inflamed intestine by restoring the affinity of the transporter for the amino acid while it stimulated NAcT in the normal intestine by increasing the cotransporter numbers. Therefore, MP differentially regulates NAcT in the normal and chronically inflamed intestine.

glucocorticoids; inflammation; amino acid transport; sodium transport; intestinal absorption

AMINO ACIDS ARE CRITICAL FUEL SOURCES for the small intestinal epithelium (31). Na-coupled amino acid uptake is the primary means of assimilation of amino acids in the small intestine (9, 17). It has been demonstrated in the mammalian small intestine both in animals as well as in humans (9, 10). Na-alanine cotransport (NAcT) is representative of Na-dependent neutral amino acid in the intestine. NAcT has been demonstrated in the rabbit intestine (9, 16). Candidate genes for NAcT are SLC1A4 (ASCT1, Ala/Ser/Cys/Thr transporter 1), SLC1A5 (ASCT2 or ATB⁰), SLC38A3 (SN1, System N amino acid transporter 1),

and SLC38A5 (SN2) (6, 9, 32). Our studies as well as other studies indicate that ATB⁰ may be the candidate for the rabbit small intestinal NAcT. We have demonstrated that NAcT is present on the brush border membrane (BBM) of villus, but not crypt cells in the normal rabbit intestine (29).

One of the most common intestinal disorders is inflammatory bowel disease (IBD, e.g., Crohn's disease). The most common morbidity of IBD is malabsorption of electrolytes, fluid, and nutrients, which leads to malabsorption, malnutrition, and weight loss in this condition (1, 4, 5). However, the mechanism of alteration of Na-dependent amino acid uptake in human inflammatory bowel diseases is unknown. In a mammalian model of chronic intestinal inflammation it was previously demonstrated that NAcT was inhibited (29). At the cellular level, NAcT was inhibited secondary to an effect at the level of the cotransporter in the BBM and as well as Na-K-ATPase on the basolateral membrane. However, inhibition of NAcT in villus cell BBM vesicles (BBMV) from the chronically inflamed intestine indicated that effect is indeed at the level of the cotransporter itself. Further kinetic and molecular studies demonstrated that the mechanism of inhibition was secondary to an alteration in the affinity for the amino acid without a change in the numbers of cotransporters (29). Given that Na-dependent amino acid uptake is present in the human small intestine and our demonstration that NAcT is reduced during chronic enteritis in rabbits (29) clearly raises the possibility that NAcT may be affected in IBD.

A wide variety of immune-inflammatory mediators are known to be endogenously produced in IBD, which may, at least in part, have an effect on electrolyte and nutrient transport pathways (5, 18, 20). However, it is not known whether a given immune-inflammatory mediator pathway is responsible for alterations seen with a specific transport pathway during chronic enteritis. Broad-spectrum immune suppression with glucocorticoids, a commonly used treatment of IBD, at least partially alleviates the malabsorption seen in these conditions (14). However, the cellular mechanism of action of glucocorticoids on NAcT in the chronically inflamed intestine, let alone in the normal intestine, is not known.

Given this background (9, 15, 17, 20, 23, 24), we looked at the effect of the glucocorticoid methylprednisolone (MP) on NAcT in the normal and chronically inflamed rabbit intestine. Glucocorticoids have been demonstrated to stimulate NAcT in

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neonatal pig intestine (13) while having no effect on this cotransport process in the rat small intestinal cell line IEC-6 (12). However, the mechanism of NAcT stimulation by glucocorticoids was not addressed in the former study. Thus, the effect of MP on NAcT is unclear in the normal mammalian small intestine. Furthermore, whether it may alleviate the inhibition of NAcT during chronic intestinal inflammation is unknown. Therefore, goals of this study were to 1) determine whether MP regulates NAcT in the normal mammalian small intestine and delineate this mechanism, 2) determine whether MP can reverse the inhibition of NAcT in the chronically inflamed intestine and delineate this mechanism, and 3) test the hypothesis that MP may regulate NAcT differentially in the normal and chronically inflamed intestine.

METHODS

Induction of chronic inflammation and drug treatment. New Zealand White male rabbits (2.0–2.2 kg) were utilized. Chronic intestinal inflammation was produced in rabbits as previously reported (25). 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 intestinal inflammation during days 13–15. Only enterocytes from those animals that had histologically confirmed chronic ileal inflammation were utilized for experiments. Control or inoculated rabbits were treated intramuscularly with saline (days 12 and 13 postinoculation) or with water-soluble 20 mg of MP sodium succinate per kilogram body weight per day (days 12 and 13 postinoculation) and killed on day 14 postinoculation. Animals were killed with an overdose of pentobarbital sodium through the ear vein according to protocols of the West Virginia University and the Ohio State University departments of Laboratory Animal Medicine.

Cell isolation. Villus cells were isolated from the intestine by a calcium chelation technique as previously described (25, 27). 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 imposed acid or alkaline gradient and return to baseline pH after perturbations. At least one, frequently two, or all three criteria are used to assess viability of all cell preps. The cells were maintained in short-term culture for up to 6–8 h (25, 27).

Na-K-ATPase measurement. Na-K-ATPase was measured as P_i liberated (8) in cellular homogenates from the same amount of cells from normal or inflamed intestine as previously described (28). Enzyme specific activity was expressed as nanomoles of P_i released per milligram protein per minute.

BBMV preparation. BBMV from rabbit ileal villus cells were prepared by CaCl₂ precipitation and differential centrifugation as previously reported (28). BBMV were resuspended in a medium appropriate to each experiment. BBMV purity was assured with marker enzyme enrichment (e.g., alkaline phosphatase).

Uptake studies in villus cells. Intact cell uptakes were done as previously described (28–30). In brief, villus cells (100 mg wet wt) were washed and resuspended in HEPES buffer containing (in mM) 0.1 alanine, 4.5 KCl, 1.2 KH₂PO₄, 1.0 MgSO₄, 1.25 CaCl₂, 20

HEPES, and either 130 mM sodium chloride or choline chloride and was gassed with 100% O₂ (pH 7.4 at 37°C). Ten μCi of ³H-alanine were added to 1 ml cell suspension in the HEPES buffer and 100 μl aliquots were removed at desired time intervals. The uptake was arrested by mixing with 10 ml ice-cold stop solution (choline-HEPES buffer). The mixture was filtered on 0.65 μm Millipore (HAWP) filters. Following two washes with ice cold-stop solution, the filter was dissolved in 5 ml Ecocint and the radioactivity was determined.

BBMV uptake studies. Uptake studies were performed by rapid filtration technique as previously described (28–30). In brief, 5 μl of BBMV resuspended in 100 mM choline chloride, 0.10 mM MgSO₄, 50 mM HEPES-Tris (pH 7.5), 50 mM mannitol, and 50 mM KCl were incubated in 95 μl of reaction medium that contained 50 mM HEPES-Tris buffer (pH 7.5), 0.1 mM alanine, 20 μCi ³H-alanine, 0.10 mM MgSO₄, 50 mM KCl, 50 mM mannitol, 100 mM of either NaCl or choline chloride. The vesicles were voltage clamped with 10 μM valinomycin and 100 μM carbonyl cyanide *p*-(tri-fluoromethoxy) phenyl-hydrozone (FCCP). 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, 75 mM KCl, and 100 mM choline chloride]. The mixture was filtered on 0.45 μm Millipore (HAWP) filter and washed with 10 ml ice-cold stop solution. Filters with BBMV were dissolved in Ecocint and radioactivity was determined.

Northern blot studies. Total RNA was extracted from rabbit ileal villus cells by the guanidinium isothiocyanate-cesium chloride method as previously reported (7, 29). Messenger RNA was isolated from total RNA by oligo(dT)-cellulose chromatography (3). After denaturation messenger RNA was electrophoresed on 1.8% agarose-formaldehyde gel, transferred to nylon membrane (Schleicher and Schuell, Keene, NH), and incubated with prehybridization solution. Membranes were hybridized with ³²P-labeled cDNA. Hybridized membrane was exposed to autoradiography film (NEN Research Products, Boston, MA). Human β-actin DNA was utilized to ensure equal loading of messenger RNA onto the electrophoresis gels. β-Actin DNA was random labeled with [³²P]CTP with Klenow polymerase.

cDNA probe synthesis for Northern blot studies. On the basis of rabbit Na-dependent neutral amino acid cotransporter sequence a 292-base pair fragment was selected for amplification (14, 29). Sense (5'-GTCTGCALLATCATCAACGT-3') and antisense (5'-CCG-GTCTGTCCGTTTACCCAT-3') primers were designed and synthesized (GIBCO-BRL). Using rabbit enterocyte cDNA as template the 292 base pair probe was amplified by polymerase chain reaction (PCR). 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 MgCl₂, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 0.5% cDNA synthesis product, 0.2 mM oligonucleotide primers, and 2–5 units of Ampli-Taq gold polymerase. PCR profile consisted of 35 cycles each at 94, 52, and 72°C. Hot PCR with α-dCTP-³²P was simultaneously performed, and the PCR product was used as probes for Northern hybridization (29).

Western blot studies. BBMV (4 μg) 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 ATB⁰ antiserum (Alpha Diagnostics) 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).

Data presentation. When data are averaged, means ± SE are shown in the figures except when error bars are inclusive within the symbol. All uptakes were done in triplicate. The *n* number 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.

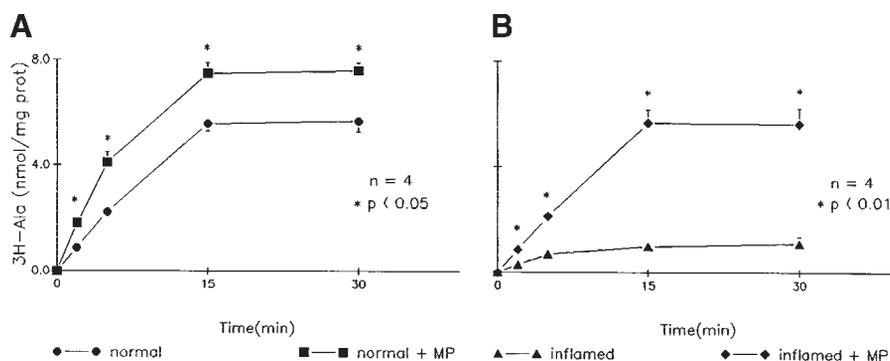


Fig. 1. Effect of methylprednisolone (MP) treatment on Na-alanine cotransport (NACT) in villus cells from the normal and chronically inflamed intestine. Na-dependent alanine uptake [nmol/mg of protein (prot)] is defined as alanine (Ala) uptake in the presence of extracellular Na minus that in the absence of Na. Statistical comparisons are made of uptakes of different conditions for each time point. A: Na-dependent alanine uptake as a function of time in villus cells from control rabbits. This uptake was significantly increased at all time points measured in villus cells from MP-treated normal rabbits. B: in villus cells from the chronically inflamed intestine Na-dependent alanine uptake was significantly reduced. Treatment of rabbits with chronic enteritis with MP almost completely reversed the inhibition of Na-dependent alanine uptake.

RESULTS

Na-Amino acid cotransport in intact cells. We had previously demonstrated that Na-dependent amino acid uptake was present in villus, but not crypt cells from the normal and the chronically inflamed intestine. Furthermore, Na-dependent amino acid uptake was significantly diminished in villus cells from the chronically inflamed intestine (29). In this study we looked at Na-dependent amino acid uptake in villus cells from sham inoculated rabbits (control) and rabbits with chronic enteritis treated with MP. Na-stimulated alanine uptake was significantly enhanced in villus cells from control rabbits treated with MP (Fig. 1A). Also, Na-stimulated alanine uptake was significantly diminished in villus cells from the chronically inflamed intestine (Fig. 1). Furthermore, the inhibition of Na-stimulated alanine uptake seen in villus cells from the chronically inflamed intestine was almost completely reversed by MP treatment (Fig. 1B). These data indicated that MP stimulates NACT in normal villus cells and reverses the inhibition of NACT in villus cells from the chronically inflamed intestine.

Na-K-ATPase activity. Alteration of Na-amino acid cotransport 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 that 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 significantly reduced in villus cells from the chronically inflamed intestine compared with the normal intestine (Fig. 2). MP treatment did not alter Na-K-ATPase activity in villus cells from the normal intestine. However, MP treatment completely reversed the reduction in Na-K-ATPase activity seen in villus cells from the chronically inflamed intestine (Fig. 2). These data suggested that the increase in Na-amino acid cotransport in villus cells from normal rabbits treated with MP was not secondary to a stimulation of Na-K-ATPase by MP. These data also suggested that the reversal of Na-amino acid cotransport inhibition by MP treatment in the chronically inflamed intestinal villus cells might, at least in part, be due to the restoration of Na-electrochemical gradients across the BBM.

Na-amino acid cotransport in BBM. To determine whether MP treatment had an effect at the level of the Na-amino acid

cotransporter itself, alanine uptake was determined in villus cell BBMV prepared from rabbits with normal and chronically inflamed intestine treated with MP or saline. In normal rabbits treated with MP, Na-dependent alanine uptake was significantly enhanced in villus cell BBMV (Fig. 3). As previously observed (25), Na-dependent alanine uptake was reduced in villus cell BBMV from the chronically inflamed intestine (Fig. 3). However, MP treatment resulted in a significant reversal of Na-dependent alanine uptake reduction in villus cell BBMV from the chronically inflamed intestine (Fig. 3). These data demonstrated that MP stimulates NACT at the level of the cotransporter in the normal intestine. These data also demonstrate that MP reverses the NACT inhibition at the level of the cotransporter in the chronically inflamed intestine.

Kinetic studies. To further decipher the mechanism of glucocorticoid-mediated stimulation of NACT in the normal intestine and reversal of inhibition of NACT in the chronically inflamed intestine, kinetic studies were performed. Uptake for all the concentrations were carried out at 6 s since initial uptake studies for Na-dependent amino acid uptake in BBMV was linear for at least 10 s (29). Figure 4 shows the uptake of Na-dependent alanine as a function of varying concentrations of extravascular alanine in villus cell BBMV from the normal

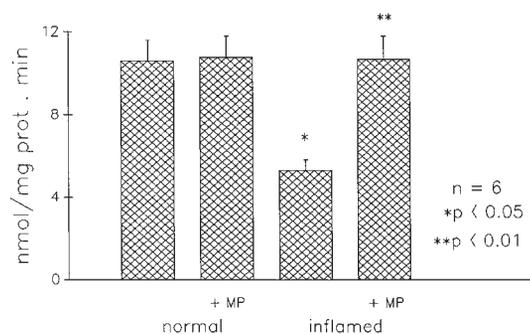


Fig. 2. Effect of MP treatment on Na-K-ATPase in villus cells from the normal and chronically inflamed intestine. Villus cell Na-K-ATPase activity is expressed as nanomoles of P_i released per milligram protein per minute. Villus cell Na-K-ATPase levels were not affected by treatment with MP in the normal intestine. In villus cells from the chronically inflamed intestine the activity of this enzyme is significantly reduced. Treatment of rabbits with chronic enteritis with MP almost completely reversed the inhibition of Na-K-ATPase in villus cells.

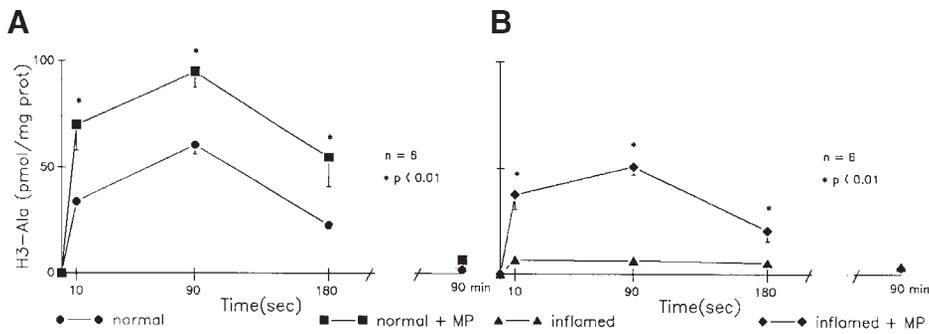


Fig. 3. Effect of MP treatment on NaCT in villus cell brush border membrane vesicle (BBMV) from the normal and chronically inflamed intestine. Na-dependent alanine uptake (pmol/mg of protein) is defined as alanine uptake in the presence of extracellular Na minus that in the absence of Na. Statistical comparisons are made of uptakes of different conditions for each time point. All uptakes were done in triplicate, and each n represents BBMV preparations from different animals. Statistical comparisons are made of uptakes of different conditions for each time point. A: Na-dependent alanine uptake as a function of time in villus cell BBMV from control rabbits. This uptake was significantly increased at all time points measured in villus cell BBMV from MP-treated normal rabbits. B: in villus cell BBMV from the chronically inflamed intestine Na-dependent alanine uptake was significantly reduced. Treatment of rabbits with chronic enteritis with MP almost completely reversed the inhibition of Na-dependent alanine uptake.

and MP-treated intestine. As the concentration of extravascular alanine was increased, the uptake of Na-dependent alanine was stimulated and subsequently became saturated in all conditions (Fig. 4A). By using Enzfiter, kinetic parameters derived from these data demonstrated that the affinity ($1/K_m$) for alanine uptake was not altered by MP treatment (K_m for alanine uptake in BBMV was 10.8 ± 1.1 mM in normal and 13.3 ± 1.2 in normal + MP, $n = 4$). However, the maximal rate of uptake (V_{max}) of alanine was significantly increased in rabbits treated with MP (V_{max} for alanine uptake in BBMV was 3.20 ± 0.10 nmol·mg protein⁻¹·6 s⁻¹ in normal and 7.50 ± 0.30 in normal + MP, $n = 4$, $P < 0.05$). These data suggest that the mechanism of NaCT stimulation by MP in the normal intestine was secondary to an increase in the number of cotransporters rather than altered affinity for alanine. In contrast, as shown on Fig. 5A, treatment of rabbits with chronically inflamed intestine with MP did not alter the maximal rate of uptake of alanine (V_{max} for alanine uptake in BBMV was 3.84 ± 0.15 nmol·mg protein⁻¹·6 s⁻¹ in inflamed and 3.66 ± 0.20 in inflamed + MP, $n = 4$). However, treatment of rabbits with chronic enteritis with MP restored the diminished affinity for alanine seen during chronic enteritis as shown on Fig. 5B (K_m was

31.6 ± 1.8 mM in inflamed and 11.0 ± 1.2 in inflamed + MP, $n = 4$, $P < 0.05$). These data suggest that the mechanism of reversal of inhibited NaCT by MP during chronic enteritis was secondary to a restoration in the affinity for the amino acid rather than an increase in cotransporter numbers.

Molecular studies. To confirm the findings of the kinetic studies the steady-state levels of mRNA for the Na-dependent neutral amino-acid cotransporter (ATB⁰) in villus cells for all of the conditions were determined. The probe recognized a band at the expected size of 2.9 kb in villus cells from the normal and the chronically inflamed intestine. Treatment with MP of the normal intestine results in an increase in the message for this cotransporter (Fig. 6). In contrast, the intensity of the ATB⁰ band appears similar in villus cells from the normal and chronically inflamed intestine (Fig. 7). Furthermore, treatment of the chronically inflamed intestine with MP also does not alter the steady-state mRNA levels of this cotransporter (Fig. 7). Because steady-state mRNA levels may not directly correlate with functional protein levels on the BBM, immunoreactive ATB⁰ levels on the BBM were also determined. Western blot analysis of villus cell BBM showed that the immunoreactive protein levels of ATB⁰ were also increased in animals

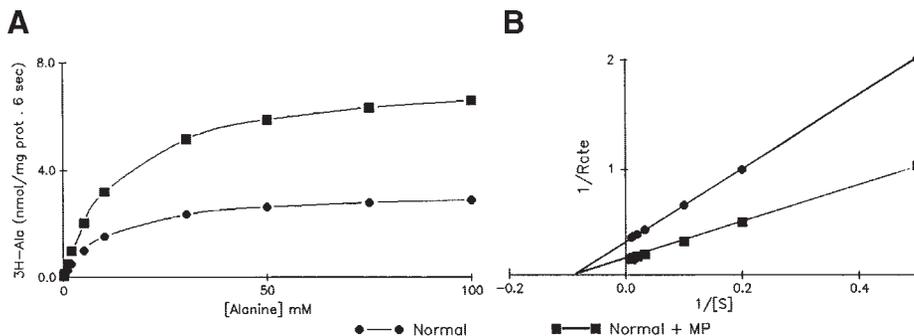


Fig. 4. Kinetics of alanine uptake in villus cell BBMV from the normal and MP-treated intestine. A: this figure, representative of 4 experiments, shows the Na-dependent uptake of alanine as a function of varying concentrations of extravascular alanine. Uptake for all concentrations was determined at 6 s. Isomolarity was maintained by adjusting the concentration of mannitol. As the concentration of extravascular alanine was increased, the uptake of alanine was stimulated and subsequently became saturated in villus cell BBMV from the normal and MP-treated intestine. B: conversion of the Michaelis-Menten equation to a straight line using Lineweaver-Burk technique with Enzfiter yielded kinetic parameters. The affinity ($1/K_m$) for alanine uptake was unchanged by MP treatment (K_m for alanine uptake in BBMV was 10.8 ± 1.1 mM in normal and 13.3 ± 1.2 in normal + MP, $n = 4$). However, the maximal rate of uptake (V_{max}) of alanine was significantly increased in rabbits treated with MP (V_{max} for alanine uptake in BBMV was 3.20 ± 0.10 nmol·mg protein⁻¹·6 s⁻¹ in normal and 7.50 ± 0.30 in normal + MP, $n = 4$, $P < 0.05$).

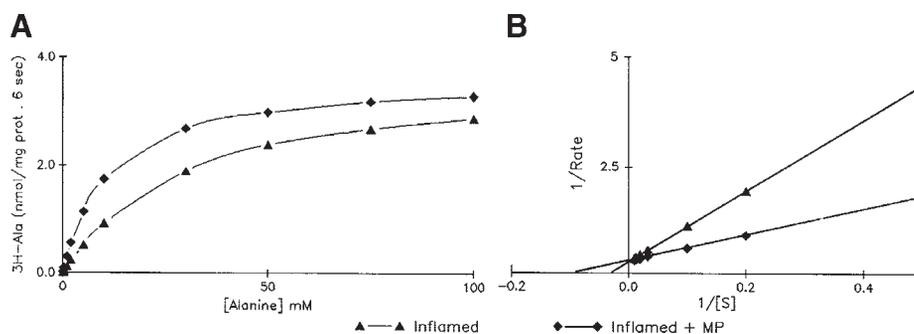


Fig. 5. Kinetics of alanine uptake in villus cell BBMVs from the chronically inflamed intestine and MP treatment. *A*: this figure is representative of 4 such experiments. Uptake of alanine as a function of varying concentrations of extravascular alanine is shown. As the concentration of extravascular alanine was increased, the uptake of alanine was stimulated and subsequently became saturated in all conditions. *B*: analysis of these data with the Enzfiter provided the kinetic parameters. The V_{max} of alanine was not affected by MP treatment of the chronically inflamed intestine (V_{max} for alanine uptake in BBMVs was 3.84 ± 0.15 nmol · mg protein⁻¹ · 6 s⁻¹ in inflamed and 3.66 ± 0.20 in inflamed + MP, $n = 4$). However, the affinity ($1/K_m$) for alanine uptake was significantly increased in the chronically inflamed intestine treated with MP (K_m was 31.6 ± 1.8 mM in inflamed and 11.0 ± 1.2 in inflamed + MP, $n = 4$, $P < 0.05$).

treated with MP (Fig. 8). Densitometric quantitation demonstrated that ATB⁰ levels increased threefold in villus cells from MP-treated rabbits. However, the intensity of the ATB⁰ immunoreactivity appears similar in villus cells from the normal and chronically inflamed intestine (Fig. 9). Finally, treatment of the chronically inflamed intestine with MP also does not alter the BBM protein of this cotransporter (Fig. 9). Densitometric quantitation demonstrated that ATB⁰ levels were not significantly different in villus cells from these rabbits. These molecular biology studies are consistent with the kinetic studies and demonstrate the following mechanisms: 1) MP stimulates NAcT in the normal intestine by increasing cotransporter numbers without altering the affinity for the amino acid; 2) during chronic enteritis NAcT is inhibited secondary to a decrease in the affinity for alanine without an alteration in the cotransporter numbers; and 3) MP-mediated reversal of inhibition of NAcT during chronic enteritis is via the restoration of the affinity for the amino acid and not via the upregulation of cotransporter numbers.

DISCUSSION

This study demonstrates that Na-amino acid cotransport is differentially regulated by glucocorticoids in the normal and

chronically inflamed intestine. Treatment with glucocorticoids stimulates NAcT in the normal intestine. The mechanism stimulation by MP in the normal intestine is not secondary to an alteration in the Na-extrusion capacity of the villus cells. At the level of the cotransporter, the MP-mediated stimulation in the normal intestine is secondary to an increase in cotransporter numbers without an alteration in the affinity. The increase in cotransport numbers may be secondary to increased synthesis of the protein and/or membrane trafficking from cytosol to BBM.

As previously noted, glucocorticoids' effect on Na-amino acid cotransport in the mammalian small intestine is poorly understood. It was shown to stimulate NAcT in neonatal pig intestine (13), but the mechanism of this stimulation was not deciphered. In another study it was suggested that glucocorticoids acutely accelerated intestinal nutrient transport possibly via diffusion (11). Yet in another study it was shown to have no effect on this cotransport process in the rat intestinal enterocytes (12). Thus the effect of an all too common medication, glucocorticoids, and the exact mechanism of regulation by this drug of an important pathway of amino acid assimilation in the

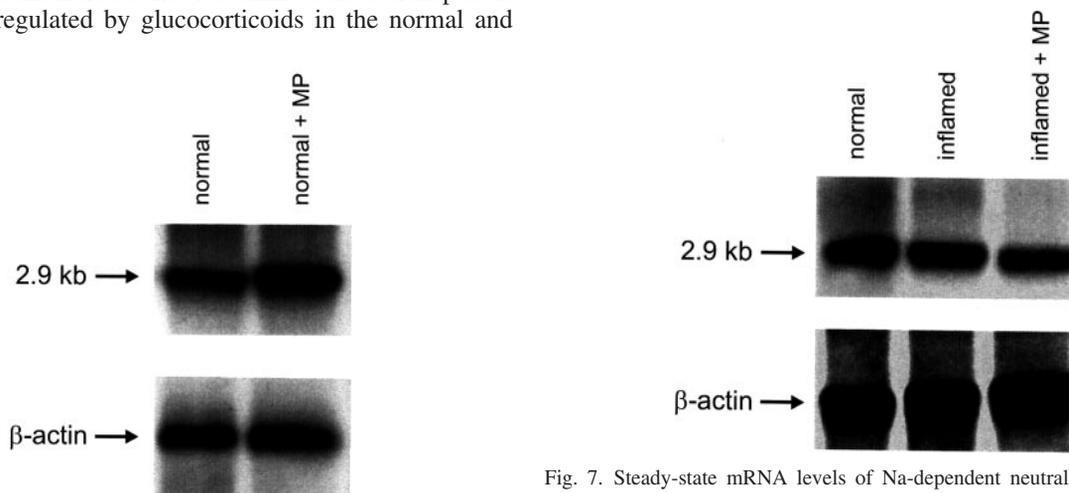


Fig. 6. Steady-state mRNA levels of Na-dependent neutral amino acid cotransporter in the normal rabbit intestine. This figure is representative of 4 such experiments. Northern blot analysis demonstrated that the steady-state levels of mRNA were increased in normal intestine treated with MP. β -Actin was utilized to ensure equal loading of mRNA in the gel.

Fig. 7. Steady-state mRNA levels of Na-dependent neutral amino acid cotransporter in the normal and chronically inflamed intestine. This figure is representative of 3 such experiments. Northern blot analysis demonstrated that steady-state mRNA levels were unchanged in villus cells from the normal and chronically inflamed intestine. Furthermore, mRNA levels were also unchanged in the chronically inflamed intestine and in the MP-treated inflamed intestine.

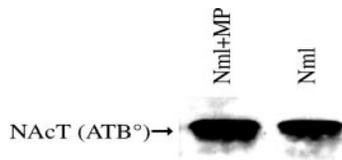


Fig. 8. Immunoreactive levels of Na-dependent neutral amino acid cotransporter in the normal (Nml) rabbit intestine. Representative data of 4 experiments each with different animals are shown. Western blot analysis demonstrated that the amounts of immunoreactive NAcT were increased in normal intestine treated with MP.

mammalian small intestine were not well understood before this study.

In the chronically inflamed intestine, treatment with MP reverses the inhibition of NAcT. The mechanism of reversal by MP during chronic enteritis is not entirely secondary to an alteration in Na-K-ATPase activity. The MP-mediated reversal of inhibition in the chronically inflamed intestine is secondary to a restoration of the affinity of the cotransporter for the amino acid without an alteration in the cotransporter numbers.

Whereas malabsorption of electrolytes and nutrients have been well documented in IBD (1, 4, 5, 18, 20, 22), until recently the alterations that occur in Na-nutrient cotransport processes during chronic intestinal inflammation had not been investigated. In a rabbit model of chronic intestinal inflammation, unique alteration in Na-nutrient cotransport processes were observed (28–30). At the cellular level, Na-glucose, Na-amino acid, and Na-bile acid cotransport were inhibited secondary to an effect at the level of the cotransporter as well as a reduction in Na-K-ATPase in the chronically inflamed intestine. However, at the cotransporter level each pathway was uniquely altered during chronic enteritis. Na-glucose cotransport (SGLT-1) was inhibited by a decrease in the number of cotransporters without a change in the affinity for glucose in the chronically inflamed intestine (28). In contrast, NAcT was inhibited by a reduction in the affinity for the amino acid without a change in the number of cotransporters during chronic enteritis (29). Unlike these two Na-solute cotransport processes, Na-bile acid cotransport was inhibited by both a decrease in the affinity for the bile acid as well as in cotransporter numbers in the chronically inflamed ileum (30). Therefore, these three types of Na-dependent solute cotransport processes are inhibited by different mechanisms during chronic enteritis. Since a variety of immune-inflammatory mediators are known to be released in the chronically inflamed intestine, it is hypothesized that different immune-inflammatory mediators may regulate these three cotransport pathways in the chronically inflamed intestine.

This hypothesis may be further supported if a broad-spectrum immune modulator such as glucocorticoid were to reverse the malabsorption during chronic enteritis by reversing the same unique mechanism that resulted in the inhibition of each of the cotransporters during chronic enteritis. Indeed, we have previously demonstrated that MP reversed SGLT-1, which was inhibited by a decrease in cotransporter numbers, by restoring the cotransporter numbers without affecting the affinity for glucose (22). However, as this study demonstrates, NAcT, which was inhibited during chronic enteritis by a decrease in the affinity for the amino acid, was reversed by glucocorticoids by restoring the affinity for the amino acid without altering the

cotransporter numbers. Thus it is likely that MP acts as an immune modulator to reverse the inhibition of SGLT-1 and NAcT in the chronically inflamed intestine rather than exerting a direct effect on the cotransporter itself.

Our studies to date demonstrate that, during chronic enteritis while both SGLT1 and NAcT are inhibited, their mechanisms of inhibition suggest that different molecular alterations are resulting in our observations. For example, inhibition of SGLT1 is secondary to a reduction in SGLT1 mRNA as well as a reduction in the protein on the BBM. Thus it is likely that SGLT1 is regulated at the transcriptional and posttranslational levels. The posttranslational alterations could be at the level of synthesis of the protein and/or secondary to altered trafficking of protein to the BBM. In contrast, NAcT is inhibited secondary to a reduction in the affinity of the cotransporter for the amino acid. Thus, unlike SGLT-1, NAcT is likely not regulated at the transcriptional level. Furthermore, at the posttranslational level it is also probably not regulated like SGLT-1. Most likely at this level it is regulated secondary to altered glycosylation and/or phosphorylation. Future studies should yield important insight into the regulation of these two Na-dependent cotransport processes during chronic intestinal inflammation.

It is also noteworthy that MP selectively regulates Na-solute cotransport processes in the normal intestine. For example, our previous studies demonstrated that MP did not affect SGLT-1 in the normal rabbit intestine. As previously noted, whether MP affected NAcT in prior studies was unclear. In any event, no mechanistic determinations were performed in these studies. This study demonstrated that, unlike SGLT-1, MP stimulates NAcT in villus cells from the normal rabbit intestine and the mechanism is by increasing BBM cotransporter numbers. The likely mechanisms of this increase in cotransporter numbers are at the level of transcription of mRNA and/or at the posttranslational level. With respect to the latter, the possible mechanisms include enhanced synthesis of the protein and/or altered trafficking to the BBM.

The hypothesis that MP acts as an immune modulator to reverse the inhibition of NAcT in the chronically inflamed intestine is also supported by the fact that MP had a different effect on NAcT in the normal intestine (MP increased cotransporter numbers) than it did in the chronically inflamed intestine. Similarly MP also had different effects on SGLT-1 (26) in the normal (no effect) and chronically inflamed intestine (MP reversed SGLT-1 inhibition by restoring cotransporter numbers). Therefore glucocorticoids most likely act as a broad-spectrum immune modulator to reverse specific alterations in cotransport processes seen

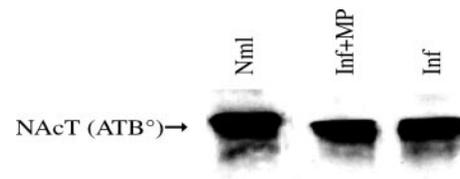


Fig. 9. Immunoreactive levels of Na-dependent neutral amino acid cotransporter in the normal and chronically inflamed intestine. This figure is representative of 3 such experiments. Western blot analysis demonstrated that the amounts of immunoreactive NAcT were unchanged in villus cells from the normal and chronically inflamed intestine. Furthermore, levels were also unchanged in the chronically inflamed intestine and in the MP-treated inflamed intestine.

during chronic enteritis. In contrast, glucocorticoids have different effects on at least some of these cotransporters in the normal intestine.

MP as an immune modulator may exert its effect at many levels. One possibility is that it normalizes the histological alterations seen in the chronically inflamed intestine (e.g., villus blunting, crypt hypertrophy). However, we have previously demonstrated that this brief duration of treatment with MP is not sufficient to restore the morphological alterations seen during chronic enteritis (26). Another more likely possibility is that formation of an immune-inflammatory mediator, which was responsible for the specific cotransport alteration during chronic enteritis, is inhibited by MP, allowing for the restoration of cotransporter activity. Specifically which immune-inflammatory mediators are responsible for the inhibition of Na-amino acid and Na-glucose cotransport in the chronically inflamed intestine will need to be delineated in future studies using specific pathway inhibitors, agonists, and antagonists.

This hypothesis of glucocorticoids having an effect on circulating immune-inflammatory mediators, which 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 electrolyte and fluid transport in nonhistologically involved areas of the intestine (4). In another study a single dose of methylprednisolone partially reversed the inhibition in Na and fluid absorption in patients with ulcerative colitis (21).

Methylprednisolone has also been demonstrated to partially reverse the inhibition of glucose stimulated Na flux in acute enteritis without altering the severe histological distortions seen in this condition. In that study, MP had no effect on glucose-stimulated Na flux in the normal piglet jejunum or on Na-K-ATPase in the acutely inflamed intestine. The mechanism of glucocorticoid-mediated reversal of Na-glucose cotransport inhibition was not further deciphered in that model of acute intestinal inflammation (19).

In conclusion, this study demonstrates that in the normal intestine glucocorticoids stimulate Na-amino acid cotransport by increasing cotransporter numbers without altering the affinity for the amino acid. It also demonstrates that the inhibition of Na-amino acid cotransport in the chronically inflamed intestine can be reversed by treatment with glucocorticoids. However, at the cotransporter level the mechanism of glucocorticoid-mediated reversal is secondary to restoration of affinity for the amino acid rather than altered cotransporter numbers. Therefore, it is postulated that glucocorticoids may act directly on the cotransporter in the normal intestine whereas they act as an immune modulator to reverse the inhibition of Na-amino acid cotransport in the chronically inflamed intestine. Thus the effects of MP on Na-amino acid cotransport are profoundly different at the cotransporter level in the normal and chronically inflamed intestine.

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