Lactobacillus acidophilus upregulates intestinal NHE3 expression and function

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Submitted 31 August 2012; accepted in final form 10 October 2012

Singh V, Raheja G, Borthakur A, Kumar A, Gill RK, Alakkam A, Malakooti J, Dudeja PK. Lactobacillus acidophilus upregulates intestinal NHE3 expression and function. Am J Physiol Gastrointest Liver Physiol 303: G1393–G1401, 2012. First published October 18, 2012; doi:10.1152/ajpgi.00345.2012—A major mechanism of electroneutral NaCl absorption in the human ileum and colon involves coupling of Na+/H+ and Cl-/HCO3- exchangers. Disturbances in these mechanisms have been implicated in diarrheal conditions. Probiotics such as Lactobacillus have been indicated to be beneficial in the management of gastrointestinal disorders, including diarrhea. However, the molecular mechanisms underlying diarrheal effects of probiotics have not been fully understood. We have previously demonstrated Lactobacillus acidophilus (LA) to stimulate Cl-/HCO3- exchange activity via an increase in the surface levels and expression of the Cl-/HCO3- exchanger DRA in vitro and in vivo. However, the effects of LA on NHE3, the Na+/H+ exchanger involved in the coupled electroneutral NaCl absorption, are not known. Current studies were, therefore, undertaken to investigate the effects of LA on the function and expression of NHE3 and to determine the mechanisms involved. Treatment of Caco2 cells with LA or its conditioned culture supernatant (CS) for 8–24 h resulted in a significant increase in Na+/H+ exchange activity, mRNA, and protein levels of NHE3. LA-CS upregulation of NHE3 function and expression was also observed in SK-CO15 cells, a human colonic adenocarcinoma cell line. Additionally, LA treatment increased NHE3 promoter activity, suggesting involvement of transcriptional mechanisms. In vivo, mice gavaged with live LA showed significant increase in NHE3 mRNA and protein expression in the ileum and colonic regions. In conclusion, LA-induced increase in NHE3 expression may contribute to the upregulation of intestinal electrolyte absorption and might underlie the potential anti diarrheal effects of probiotics; NHE3; diarrhea; intestinal sodium absorption.

DISTURBANCES IN ELECTROLYTE ABSORPTION IN CASES OF INFLAMMATION AND INFECTION MAY CAUSE DIARRHEA. DIARRHEA IS CONSIDERED TO BE A MULTIFACTORIAL EVENT THAT MAY OCCUR DUE TO EITHER INCREASED SECRETION OF FLUID AND ELECTROLYTES, DECREASED ABSORPTION, OR BOTH. THE ELECTRONEUTRAL COMPONENT OF ELECTROLYTE ABSORPTION IN THE MAMMALIAN INTESTINE OCCURS VIA COUPLED ACTIVITIES OF Na+/H+ AND Cl-/HCO3- EXCHANGERS PRESENT ON THE APICAL SURFACE OF INTESTINAL EPITHELIAL CELLS (28). NHE2 AND NHE3 ARE THE MAIN SODIUM-ABSORBING ISOFORMS OF NHEs, WHEREAS DRA AND PAT-1 ARE THE Cl-/HCO3- EXCHANGERS.

NHE3 (SLC9A3) IS A MEMBER OF THE SOLUTE CARRIER FAMILY 9, EXPRESSED ON APICAL MEMBRANES OF EPITHELIAL CELLS OF KIDNEY AND THE GASTROINTESTINAL TRACT. THE IMPORTANCE OF NHE3 IN ELECTROLYTE AND FLUID HOMEOSTASIS IS EVIDENT BY REDUCED Na+ ABSORPTION IN NHE3 KNOCKOUT MICE AND ITS INHIBITION IN DIARRHEAL DISEASES (29). PREVIOUS REPORTS FROM OUR LABORATORY HAVE SHOWN THAT THE ENTEROPATHOGENIC E. coli (EPEC) INFECTION OF HUMAN INTESTINAL Caco2 MONOLAYERS SIGNIFICANTLY INHIBITED THE ACTIVITIES OF NHE3 AND DRA, DEFINING THE PATHOPHYSIOLOGICAL BASIS OF EPEC-INDUCED EARLY DIARRHEA (15). IN THIS CONTEXT, ANY AGENT THAT CAN STIMULATE LUMINAL NaCl ABSORPTION MAY ACT AS ANTI DIARRHEAL AGENT AND COULD BE INVESTIGATED FOR ITS THERAPEUTIC POTENTIAL. IN THIS RESPECT, PROBIOTICS HAVE EMERGED AS A POTENTIAL CANDIDATE FOR THE TREATMENT OF VARIOUS GASTROINTESTINAL DISORDERS, INCLUDING DIARRHEA.

PROBIOTICS ARE NONPATHOGENIC LIVING MICROORGANISMS THAT HAVE BENEFICIAL EFFECTS ON HOSTS. A NUMBER OF MECHANISMS HAVE BEEN PROPOSED THAT UNDERLIE THEIR BENEFICIAL EFFECTS. THESE INCLUDE PRODUCTION OF ANTIBACTERIAL SUBSTANCES (33), MAINTAINING THE INTEGRITY OF THE INTESTINAL BARRIER (13), AND COMPETITION WITH PATHOGENIC MICROORGANISMS FOR ENTEROCYTE BINDING (4). HOWEVER, THE PRECISE MOLECULAR MECHANISMS OF MODULATION OF INTESTINAL EPITHELIAL HEALTH BY PROBIOTICS ARE NOT COMPLETELY UNDERSTOOD.

IN THIS REGARD, WE HAVE PREVIOUSLY SHOWN THAT SHORT-TERM TREATMENT OF Caco-2 MONOLAYERS WITH Lactobacillus acidophilus (LA) OR ITS CULTURE SUPERNATANT STIMULATED Cl-/HCO3- EXCHANGE ACTIVITY BY INCREASING THE SURFACE LEVELS OF DRA VIA A PHOSPHATIDYLINOSITOL-3 KINASE-MEDIATED MECHANISM (6). WE ALSO HAVE SHOWN THAT LONG-TERM TREATMENT OF Caco-2 CELLS WITH LA SUPERNATANT ENHANCES DRA EXPRESSION AND FUNCTION VIA TRANSCRIPTIONAL MECHANISMS (22). FURTHERMORE, LA HAS BEEN SHOWN TO ATTENUATE TUMOR-NECROSIS FACTOR-α-INDUCED Cl− SECRETION IN INTESTINAL EPITHELIAL CELLS INVOLVING P38 MAPK, ERK1, ERK2, AND PI3K PATHWAYS (23). HOWEVER, TO DATE, THE EFFECTS OF PROBIOTICS ON INTESTINAL Na+/H+ EXCHANGERS HAVE NOT BEEN Examined. SINCE DRA IS FUNCTIONALLY COUPLED TO NHE3, IT IS VERY IMPORTANT TO INVESTIGATE THE NET IMPACT OF LA ON INTESTINAL NaCl ABSORPTION. IN THIS REPORT, WE HAVE DEMONSTRATED THAT LONG-TERM TREATMENT WITH LA OR ITS CULTURE SUPERNATANT STIMULATED Na+/H+ EXCHANGE ACTIVITY BY UPREGULATING NHE3 EXPRESSION VIA TRANSCRIPTIONAL MECHANISMS. UPREGULATION OF NHE3 EXPRESSION HAS ALSO BEEN SHOWN IN VIVO IN THE ILEUM AND COLON OF MICE THAT WERE ORALLY ADMINISTERED WITH LIVE LA.

MATERIALS AND METHODS

Materials. Radionucleotide 22Na was obtained from PerkinElmer (Waltham, MA) and Caco2 cells from the American Type Culture Collection (Manassas, VA). HOE-694 and S3226 were generous gifts...
from Dr. Hans J. Lang (Aventis, Pharma Deutschland Chemical Research, Frankfurt/Main, Germany). RNeasy mini kit for RNA extraction was obtained from Qiagen (Frederick, MD), and the real-time qRT PCR kit was from Stratagene (La Jolla, CA). Antibodies for NHE3 were purchased from Alpha Diagnostic (rabbit polyclonal-NHE3-A) (San Antonio, TX) and Millipore (mouse monoclonal-MAB3138) (Billerica, MA). All other chemicals were of at least reagent grade and were obtained from Sigma (St. Louis, MO) or Fisher Scientific (San Jose, CA).

**Cell culture.** Caco2 cells were grown in T-75 cm² culture flasks at 37°C in a 5% CO₂–95% air incubator in Minimum Essential Medium (Invitrogen Life Technologies, Carlsbad, CA) containing 20% FBS, 20 mM HEPES, 100 IU/ml penicillin, and 100 mg/ml streptomycin. Cells (2 x 10⁶) were plated per well in a 24-well culture plate and were used between passages 25 and 45. Fully differentiated confluent monolayers were used for the experiments (12–14 days postplating). Human colon adenocarcinoma cell line SK-CO15 (32) was maintained in DMEM containing 2 mM pyruvate supplemented with 10% FBS, 1 mM HEPES, and x1 nonessential amino acids. Confluent monolayers (7–10 days postplating) were used for the experiments.

**Bacterial culture.** The Lactobacillus species Lactobacillus acidophilus (LA) (4357), Lactobacillus rhamnosus (LR) (53103), Lactobacillus plantarum (LP) (14917), and Lactobacillus casei (LC) (393) were procured from ATCC. Bacteria were grown overnight in MRS broth (Mann-Rogosa-Sharpie, Difco) for 24 h at 37°C without shaking. Next day, bacteria were spun down by centrifuging at 3,000 rpm for 10 min. For in vitro studies, culture supernatant was separated from spun-down bacterial pellet, filtered through a 0.22-μm filter, and diluted in the cell culture media for further use. For in vivo studies, 3 x 10⁶ CFU of live bacteria suspended in 100 μl of sterile 1× PBS or sterile PBS as vehicle was gavaged per animal that were killed after 24 h.

**Measurement of Na⁺/H⁺ exchange activity.** The function of NHEs, measured as Na⁺/H⁺ exchange activity, was determined in acid-loaded postconfluent Caco2 monolayers as ethylisopropylamiloride (EIPA)-sensitive ²²Na uptake, as previously described (2). Briefly, confluent cell monolayers were preincubated for 20 min at room temperature following incubation in a medium containing (in mM) 50 NH₄Cl, 70 choline chloride, 5 KCl, 1 MgCl₂, 2 CaCl₂, 5 glucose, and 15 MOPS (pH 7.0). The cells were then washed with a solution containing (in mM) 120 choline chloride and 15 Tris·HEPES (pH 7.5) and were incubated in uptake buffer containing (in mM) 3 NaCl, 110 choline chloride, 1 MgCl₂, 2 CaCl₂, and 20 HEPES (pH 7.4) and 1 μCi/ml of ²²Na with or without 50 μM EIPA or 50 μM HOE-694. After 5 min, the uptake of ²²Na was terminated by washing the monolayers twice with ice-cold PBS. The cells were then solubilized by incubation with 0.5 N NaOH, and incorporated radioactivity was determined. The protein content of the cell lysate was estimated by the method of Bradford, and incorporated radioactivity was determined using Packard Tri-Carb 1600TR Liquid Scintillation analyzer (Packard Instruments: Perkin Elmer). The values of ²²Na uptake were expressed as nanomoles ²²Na per milligram protein per 5 min.

**Transfections.** Caco2 cells were transfected with NHE3 promoter (−1,507/+131) fragment cloned upstream of the luciferase reporter gene in pGL-2 basic vector along with β-galactosidase expression vector using Lipofectamine 2000 (Invitrogen) as described previously (21). After 24 h, cells were treated with the culture supernatant from various Lactobacillus species for 16 h or with LA-CS for 8, 16, and 24 h. Activities of Firefly Luciferase and β-galactosidase were measured according to the manufacturer’s instructions (Promega).
moter activity was expressed as relative luciferase activity normalized to β-galactosidase activity to correct for transfection efficiency.

**In vivo studies.** In vivo studies performed in C57BL/6J mice were approved by the Animal Care Committee of the University of Illinois at Chicago and Jesse Brown VA Medical Center. Mice were gavaged with LA (3 × 10^9 CFUs) or sterile PBS as vehicle for 14–24 h. Intestine was removed, and mucosa was scraped for RNA extraction for real-time PCR and preparation of cell lysate for measurement of NHE protein levels by immunoblotting.

**Western blotting.** LA-CS-treated and untreated Caco2 cells or tissue lysates were prepared from the scraped mucosa of ileum and colon using cell lysis buffer (Cell Signaling, Danvers, MA). Lysates were run on an 8% gel and then transferred onto nitrocellulose membrane. Membranes were then incubated with anti-rabbit polyclonal antibodies against NHE3 (Alpha Diagnostic, San Antonio, TX) (1:100) (for Caco2 cells) or mouse monoclonal NHE3 antibody (catalog no. MAB3136, Chemicon, Temecula, CA) (1:75 dilution) (for tissue lysates) in the blocking buffer containing 1 TBS and EIPA-sensitive 22Na+ uptake

**Fig. 2.** Dose-dependent activation of apical Na+/H+ exchange activity by *L. acidophilus* (LA)-CS in Caco2 cells. Na+/H+ exchange activity in Caco2 cells following 16 h of treatment with increasing dilutions of LA-CS. Results are means ± SE of four independent experiments performed in triplicate. *Significant differences between groups (control vs. LA treated; *P* < 0.05).

**Fig. 3.** Contribution of NHE2 and NHE3 to total NHE activity. NHE activity was determined in the presence of 50 μM EIPA or 50 μM HOE-694. Values are means ± SE of four separate experiments performed in triplicate. White bars, control; gray bars, LA-CS. *Significant difference compared with control (*P* < 0.05).

**Fig. 4.** Effect of LA-CS on NHE3 expression in Caco2 cells. Changes in NHE3 mRNA (*A*) and NHE2 mRNA (*B*) levels in Caco2 cells in response to treatments with LA-CM for 8-, 16-, or 24-h time periods. Values of mRNA levels for NHE3 were normalized against histone mRNA levels. C: control and treated cell lysates were subjected to 8% SDS-PAGE followed by transfer to nitrocellulose membrane. The blot was probed with anti-NHE3 or anti-β-actin antibody. The data were quantified by densitometric analysis and expressed as % of control in arbitrary units. Results represent means ± SE of five different experiments. *Significant differences between control vs. LA-treated groups (*P* < 0.05).
0.5% (for Caco2 cells) or 5% (for tissue lysate) nonfat dry milk overnight at 4°C. Membranes were then incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit (for Caco2 cells) or goat anti-mouse (for tissue lysate) IgG antibody (1:2,000 dilution) for 1 h at room temperature and washed in 1× TBS with 0.1% Tween-20 for 30 min with agitation, during which the wash buffer was changed every 5 min. The antigen-antibody complexes on the membranes were visualized by chemiluminescence (ECL Western blotting detection reagents, Amersham Pharmacia Biotech), and the image was captured on light-sensitive imaging film (Hyperfilm ECL, Amersham Biosciences).

Statistical analysis. Data are presented as means ± SE of three to five independent experiments. Difference between control and treated was analyzed using one-way ANOVA with Tukey’s test. Differences were considered significant at P < 0.05.

RESULTS

Effect of different Lactobacilli species on Na⁺/H⁺ exchange activity. Previous studies from our laboratory have shown that live LA or its culture supernatant (CS) stimulated Cl⁻/HCO₃⁻ exchange activity (6). To examine the effects of Lactobacillus on NHE3 activity, Caco2 monolayers were treated with CS of LA (4357), L. rhamnosus (LR) (53103), L. plantarum (LP) (14917), and L. casei (LC) (393) for 16 h, and EIPA-sensitive ²²Na uptake was measured. As shown in Fig. 1A, LA caused a significant increase (approximately threefold) in Na⁺/H⁺ exchange activity (EIPA-sensitive ²²Na uptake) compared with control, whereas, LR, LP, and LC did not show any significant increase in Na⁺/H⁺ exchange activity. A time course experiment showed that LA did not affect Na⁺/H⁺ exchange activity at 3 h, but by 8 h Na⁺/H⁺ exchange activity increased significantly (~2.5-fold), with maximum increase at 16 h (approximately threefold), and remained elevated until 24 h (Fig. 1B). A 16-h time point was used to treat cells with LA in subsequent studies. Similar results were obtained in Caco2 cells grown on transwells (data not shown).

Stimulation of Na⁺/H⁺ exchange activity by LA-CS is dose dependent. We next investigated the dose response of LA-CS on Na⁺/H⁺ exchange activity. The Na⁺/H⁺ exchange activity increased (approximately twofold) in response to treatments with both 1:10 and 1:50 dilutions of LA-CS (Fig. 2). However, higher dilutions had no significant effect on Na⁺/H⁺ exchange activity. These studies indicate that the effects of LA are mediated by soluble factor(s) secreted by LA, which also appeared to be concentration dependent, since higher dilutions of the CS had no significant effects in stimulating Na⁺/H⁺ exchange activity. For subsequent experiments, we used either 1:10 or 1:50 dilution of the CS.

Effect of LA on NHE3 vs. NHE2 exchange activity. NHE2 and NHE3 are the two main luminal membrane sodium hydrogen exchangers and are expressed in intestinal epithelial cells. Among these, NHE3 is considered to be the predominant Na⁺-absorbing isoform (26). To dissect the effects of LA-CS on apical isoforms of NHE3, the amiloride analog EIPA and NHE2 specific inhibitor HOE-694 were used. NHE2 activity was calculated as NHE activity sensitive to 50 μM HOE-694. NHE3 activity was calculated by subtracting 50 μM HOE-694-
sensitive NHE activity from total NHE activity (50 μM EIPA-sensitive NHE activity). Treatment of Caco2 cells with LA-CS significantly increased NHE3 activity by approximately twofold (P < 0.05) compared with the control values. Under control conditions, 72% of total NHE activity was contributed by NHE2, and 28% was contributed by NHE3 (Fig. 3). However, NHE2 activity essentially remained almost the same for control as well as for LA-CS. These results indicate that the increases in Na+/H+ exchange activity in response to LA-CS are specifically due to increased NHE3 activity. These results were further confirmed by utilizing specific NHE3 inhibitor S3226 (data not shown).

LA-CS enhances NHE3 mRNA and protein expression in Caco2 cells. To examine whether the LA-mediated stimulation of Na+/H+ exchange activity occurs due to increased expression of NHE3, we used real-time quantitative RT-PCR to measure NHE2 and NHE3 mRNA levels in Caco2 cells in response to treatments with LA-CS (1:50 dilution) for 8, 16, and 24 h. Results revealed that NHE3 mRNA levels were enhanced significantly (approximately twofold) by LA-CS at all time points studied (Fig. 4A). Interestingly, similar to our functional studies, NHE2 mRNA levels remained unaltered in response to LA-CS treatments (Fig. 4B), suggesting that NHE3 is the major Na+ absorbing isoform involved in mediating the stimulatory effects of LA on Na+/H+ exchange activity. We also measured the effects of long-term treatments with LA-CS on NHE3 protein expression by immunoblotting. As shown in Fig. 4C, NHE3 protein level significantly increased (~1.5-fold) in cells treated with LA-CS for 24 h compared with control.

LA-CS increases NHE3 promoter activity. To examine whether LA-CS-induced increase in NHE3 expression involves transcriptional regulation, we investigated the effect of LA-CS on NHE3 promoter activity. Caco2 cells were transfected with NHE3 promoter luciferase reporter plasmid using Lipofectamine 2000 reagent and 24-h posttransfection, cells were treated with CS of Lactobacillus species (1:50) for 16 h or LA-CS (1:50 dilution) for 8, 16, and 24 h. NHE3 promoter activity was markedly increased in response to LA (Fig. 5A), whereas other Lactobacillus species did not show any significant increase in promoter activity. A time course experiment showed that LA-CS significantly enhanced NHE3 promoter activity (1.5-fold; P < 0.05) as early as 8 h and remained elevated until 24 h (Fig. 5B). These results suggest that long-term treatments of Caco2 cells with LA-CS increase NHE3 expression via transcriptional mechanisms.

LA-CS enhances NHE3 function and expression in SK-CO15 cells. Recent studies showed SK-CO15 to be a better in vitro model to study NHE3 regulation since this cell line predominantly expresses NHE3 on the apical membrane (32). Therefore, we examined NHE3 activity (S3236-sensitive 22Na uptake) and NHE3 mRNA and protein levels in SK-CO15 cells in response to long-term (24 h) treatments with LA-CS. Results are shown in Fig. 6. Similar to the results obtained in Caco2 cells, LA-CS significantly enhanced inhibitor (S3226)-sensitive NHE3 activity (Fig. 6A), NHE3 mRNA (Fig. 6B) and protein (Fig. 6C) levels. These results confirm that the effects of LA-CS on NHE3 function and expression are not cell-line specific.

Live LA enhances intestinal NHE3 expression in vivo. To validate our in vitro results, we also investigated the effects of
LA on the expression of NHE3 in native intestinal tissue. For this, C57BL/6J mice were gavaged with live LA as described in MATERIALS AND METHODS. Mice were euthanized after 24 h, and mucosa from different regions of the intestine were processed for measuring NHE3 mRNA and protein levels. To assess NHE3 mRNA expression in different regions of mice intestine, real-time quantitative PCR was done on RNA samples from mice gavaged with live LA for 24 h. Results showed that LA administration significantly increased NHE3 mRNA levels in the ileum (Fig. 7B) and the colon (Fig. 7C), whereas there was no significant change in the jejunum (Fig. 7A), after 24 h. Also, NHE2 mRNA levels did not show any change in colon of these mice (Fig. 7D). Changes in NHE3 protein levels in mice gavaged with LA were measured by immunoblotting. Results showed that, 24 h after gavage, NHE3 protein levels significantly increased in the ileum (approximately twofold) (Fig. 8A) and colon (approximately threefold) (Fig. 8B).

**DISCUSSION**

Diarrheal diseases continue to be the major cause of mortality and morbidity in young children throughout the world. In addition to the mainstream medicine, probiotics have emerged as promising candidates for the primary and secondary prevention of diarrhea. However, until recently, most of the studies have emphasized the overall gastro-protective roles of probiotics, although their mechanisms of action at the molecular level, with respect to their anti-diarrheal effects, are not fully understood.

We have recently demonstrated that live LA or the soluble factor(s) in the culture supernatant of LA stimulate Cl⁻/HCO₃⁻ exchange activity in vitro as well as in vivo. The acute effects were mediated via PI-3 kinase-dependent increase in cell surface DRA (6), whereas the long-term effects involved LA-induced increase in the expression of DRA via transcriptional mechanisms (22). The present report, for the first time, demonstrates that LA or the soluble effector molecule(s) secreted by LA in culture supernatant increased Na⁺/H⁺ exchange activity via modulation of NHE3 expression and function. The effect was specific to LA since the other species (LR, LP, and LC) showed no significant effect. These results indicate that the effects of probiotics on the upregulation of NHE3 are species specific and not a generalized effect of bacteria.

Intestinal epithelial cells express four different NHE isoforms: NHE1, 2, 3, and 8. NHE1 is localized to the basolateral
LA secreted soluble effector molecule(s) on apical Cl⁻/HCO₃⁻ exchange activity involved distinct mechanisms. However, in the present study, we demonstrate that LA stimulates Na⁺/H⁺ exchange activity only at a longer treatment time point with no effect on short-term treatments. These results suggest that, although DRA is functionally coupled to NHE3, the effects of LA on Cl⁻/HCO₃⁻ and Na⁺/H⁺ exchange activities are different at least at a shorter time period (1–3 h). Also, the stimulation of Na⁺/H⁺ exchange activity by LA-CS was dose dependent, since increasing the dilution of LA-CS decreases the extent of increase in Na⁺/H⁺ exchange rate.

The increase in NHE3 function was consistent with increased expression of NHE3 mRNA and protein in the Caco2 cells as well as in vivo in the mouse ileal and colonic mucosa. This increase was mediated partly via transcriptional mechanisms, since LA also increased NHE3 promoter activity in Caco2 cells. We have earlier reported that Cl⁻/HCO₃⁻ exchange activity was increased by LA-CM through the increased expression of major Cl⁻/HCO₃⁻ exchanger DRA via modulation at the transcriptional level (22). These results are supported by other recent studies showing that secreted bioactive factor(s) by probiotics can modulate the expression of various genes implicated in important physiological processes such as cell growth and proliferation, differentiation, angiogenesis, and apoptosis (12, 16, 17). Lactic acid bacteria are known to secrete metabolites that possess anti-inflammatory properties (14). Probiotics are well known to produce short-chain fatty acids like butyrate from complex carbohydrates by bacterial fermentation, which in turn has been reported to stimulate sodium absorption (18) via transcriptional activation of NHE3 promoter in intestinal epithelial cell lines, which require Sp1 and Sp3 binding to the NHE3 core promoter region (3). Sequence analysis of NHE3 promoter region revealed many regulatory cis-acting elements like SP1, SP3, cAMP response element, AP-2, Cdx-2, Mzf-1, Myo-D, Egr-1, glucocorticoid, and thyroid hormone receptor binding sites (8, 21). Some of these are the potential transcription factor binding sites that are involved in the tissue-specific differentiation, e.g., Cdx-2 modulates gene expression and differentiation in the intestine, suggesting the possible role of these factors in mediating the stimulatory effects of LA on NHE3 function and expression. However, detailed deletion and mutagenesis studies are needed to further elucidate the regulatory elements involved in transcriptional regulation of NHE3 promoter by LA and should be the subject of future investigations.

The microenvironment of the native intestine that houses >400 species of bacteria is complex. Several studies have reported altered composition of microbiota with decreased Lactobacilli and increased population of Clostridia and Enterobacteriacea in patients with Crohn's disease or ulcerative colitis (34). Therefore, complementing the gut microflora with health-promoting bacteria is a very stimulating facet of alternative therapy with probiotics. In our in vivo model, oral administration of LA to C57BL/6 mice showed increased NHE3 mRNA expression in both ileum and colon, whereas mRNA levels of NHE3 were unchanged in jejunum of these mice. This could be due to more persistence of Lactobacilli strains in ileum and colon (1). Site-specific colonization in the gastrointestinal tract could be considered as a contributory factor for differential upregulation of protein, which they can affect either directly or through their secreted factors (9).

**Fig. 8. NHE3 protein expression in mice samples in response to LA (3 × 10⁹ CFUs) treatment for 24-h time period. Total lysates extracted from the mucosal tissues of ileum (A) and colon (B) of control and treated mice were subjected to 8% SDS-PAGE followed by transfer to nitrocellulose membrane. The blot was probed with anti-NHE3 or anti-β-actin antibody. A representative blot is shown. The data were quantified by densitometric analysis and expressed as % of control in arbitrary units. Results are means ± SE of five different experiments. *Significant differences between control and LA-treated groups (P < 0.05).**
Increased NHE3 protein expression in ileum and colon as assessed by Western blot analysis in colon clearly demonstrated that increased mRNA levels did translate into higher protein levels. Consistent with our in vitro results, LA administration did not alter NHE2 mRNA levels in colon of these mice.

Our results suggest that probiotics, via their pro-absorptive effect, could act as anti-diarrheal agents by increasing NHE3 function and expression. Importantly, the pro-absorptive effects are mediated by LA-secreted soluble factors. However, the exact identity of the bioactive factors secreted by LA that modulate NHE3 is not known. An earlier study has shown that Lactobacillus GG produces small peptides that had cytoprotective effects similar to the live bacteria (27). Probiotics may produce certain growth inducers like spermine and spermidine during their transit through the gastrointestinal tract that increase the overall mass of mucosa and expression of the brush border enzymes such as sucrase and alkaline phosphatase (7).

Conjugated linoleic acids (CLAs) produced by Lactobacillus are shown to be effective in STIMULATES NHE3 EXPRESSION AND FUNCTION


