D-Glucose Acts via Sodium/Glucose Cotransporter 1 to Increase NHE3 in Mouse Jejunal Brush Border by a Na+/H+ Exchange Regulatory Factor 2–Dependent Process

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BACKGROUND & AIMS: Oral rehydration solutions reduce diarrhea-associated mortality. Stimulated sodium absorption by these solutions is mediated by the Na^+/H^+ hydrogen exchanger NHE3 and is increased by Na^+-glucose co-transport in vitro, but the mechanisms of this up-regulated process are only partially understood. **METHODS:** Intracellular pH was measured in jejunal enterocytes of wild-type mice and mice with disrupted *Na+/H+ exchange regulatory co-factor 2* (*NHERF2−/−* mice) by multiphoton microscopy. Diarrhea was induced by cholera toxin. Caco-2BBe cells that express NHE3 and the sodium/glucose cotransporter 1 (SGLT1) were studied by fluorometry, before and after siRNA-mediated knockdown of NHERF1 or NHERF2. NHE3 distribution was assessed by cell-surface biotinylation and confocal microscopy. Brush-border mobility was determined by fluorescence recovery after photobleaching and confocal microscopy. **RESULTS:** The nonmetabolized SGLT1 substrate α-methyl-D-Glu (α-MD-G) activated jejunal NHE3; this process required Akt and NHERF2. α-MD-G normalized NHE3 activity after cholera toxin–induced diarrhea. α-MD-G–stimulated jejunal NHE3 activity was defective in *NHERF2−/−* mice and cells with *NHERF2* knockdown, but occurred normally with *NHERF1* knockdown; was associated with increased NHE3 surface expression in Caco-2 cells, which also was NHERF2-dependent; was associated with dissociation of NHE3 from NHERF2 and an increase in the NHE3 mobile fraction from the brush border; and was accompanied by a NHERF2 ezrin-radixin-moesin–binding domain-dependent increase in co-precipitation of ezrin with NHE3. **CONCLUSIONS: SGLT1-mediated Na-glucose cotransport stimulates NHE3 activity in vivo by an Akt- and NHERF2-dependent signaling pathway. It is associated with increased brush-border NHE3 and association between ezrin and NHE3. Activation of NHE3 corrects cholera toxin–induced defects in Na absorption and might contribute to the efficacy of oral rehydration solutions.**

Keywords: Intestinal Na Absorption; Cholera Toxin; Exocytosis; Ezrin; NHERF2.

BASIC–ALIMENTARY TRACT

The human small intestine absorbs approximately 7.5 L of water and 650 mEq of Na daily. Most of this is accomplished by cotransport of Na and nutrients, particularly D-glucose, and neutral NaCl absorption, which is the BB Na/H exchanger 3 (SLC9A3) linked to a Cl/HCO_3 exchanger, either SLC26A3 or A6.[1] These 2 processes were assumed to be separate until recent in vitro studies using polarized intestinal epithelial cell monolayers showed enhanced NHE3 activity and surface expression after sodium/glucose cotransporter (SGLT)1-mediated Na-glucose cotransport.[2–5] However, whether SGLT1-dependent NHE3 activation occurs in vivo under basal or pathologic conditions has not been explored. If present in vivo, this NHE3 activation may represent a mechanism by which oral rehydration solution (ORS) stimulates intestinal Na and water absorption.

In vitro studies suggested that SGLT1-dependent NHE3 activation occurs via sequential activation of p38 mitogen-activated protein kinase/mitogen-activated protein kinase MAPK2, Akt2, and ezrin. NHE3 regulation involves signaling complexes that form on the NHE3 C-terminus, many of which are scaffolded by Na+/H+ exchange regulatory co-factor (NHERF) proteins, a multi-PDZ domain–containing gene family.[6–9] It is unknown whether NHERF proteins are necessary for the SGLT1-NHE3 linkage. The studies presented here used mouse jejunum with NHERF2 knockout (KO) and Caco-2/SGLT1/HA-NHE3 cells with NHERF2 knockdown (KD)

Abbreviations used in this paper: α-MD-G, α-methyl-D-Glu; CT, cholera toxin; ERM, ezrinradixin-moesin; KD, knockdown; KO, knockout; NHERF, Na+/H+ exchange regulatory co-factor; ORS, oral rehydration solution; SGLT, sodium/glucose cotransporter; WGA, wheat-germ agglutinin; WT, wild-type.

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to explore whether α-methyl-D-Glu (α-MD-G) stimulates NHE3 in intact tissue and whether the effect requires NHERF2, as well as the mechanism involved in these effects. These results suggest that optimization of the physiologic NHE3 regulatory pathway identified may increase the utility of ORS solutions for severe diarrheal diseases.

Materials and Methods

Animals

Male NHERF2−/− mice bred into the C57BL/6 background (Charles River Laboratories, Inc, Wilmington, MA) for at least 6 generations were produced from heterozygotes and with wild-type male C57BL/6 mice were studied between 10 and 12 weeks of age. These mice initially were produced by a retroviral trapping method by Lexicon Pharmaceuticals (Woodlands, TX) using Omnibank clone OST2298.[10] Protocols used were approved by the Johns Hopkins University Animal Use Committee.

NHE3 Activity Determination by Two-Photon Microscopy

Na^+/H^+ exchange activity in intact mouse jejunum was determined as rates of Na^+- dependent alkalinization using a 2-photon microscope (MRC-1024MP; Bio-Rad, Hercules, CA) and the pH-sensitive dye, SNARF-4F acetoxymethyl ester (Invitrogen, Carlsbad, CA), as we have described,[11] with minor modifications. Details are provided in the Supplementary Materials and Methods section. Full-thickness jejunum was studied starting 1 cm distal to the ligament of Treitz.

Cholera Toxin–Induced Acute Watery Diarrhea Model

An acute watery diarrhea model using purified holo-cholera toxin (CT) (Sigma, St. Louis, MO) was standardized in vivo, as previously described.[12] Mice were gavaged with CT (10 μg in 7% $NaHCO_3$) through an orogastric feeding tube, with $NaHCO_3$ as control. Six hours later, mice were killed and fluid was collected by gravity from the entire small intestine.

Measurement of NHE3 Activity in Caco-2/bbe Cells

Caco-2 cells stably transfected with SGLT1[3] were infected with HA-NHE3 adenovirus (see the Supplementary Materials and Methods section), grown on pieces of polycarbonate membranes (0.4-μm pore size, called filterslips), and NHE3 activity was determined using the intracellular pH-sensitive dye 2′,7′-Bis(2-carboxyethyl)-5(6)-carboxy fluorescein acetoxymethyl ester (Invitrogen, Carlsbad, CA) and a computerized fluorometer, as described.[13] Na^+/H^+ exchange rates (H^+ efflux) were calculated as the initial rates of Na^+-dependent change in pHi over approximately 1 minute (ΔpH/Δmin).[13] Means ± standard error were determined from at least 3 independent experiments.

Apical Cell Surface Biotinylation of NHE3 in Caco-2/bbe Cells

The Caco-2/bbe cell surface biotinylation protocol was modified from the protocol for PS120 cell surface biotinylation published previously using NHS-SS biotin (Pierce Chemicals, Rockford, IL).[14] The details are provided in the Supplementary Materials and Methods section. The immunoblots produced were probed with a monoclonal anti-HA antibody (16B12; Covance, Princeton, NJ), with normalization to β-actin using monoclonal anti–β-actin antibody (A2228; Sigma).

Immunoprecipitation and co-precipitation and immunofluorescence techniques are described in the Supplementary Materials and Methods section. Fluorescence recovery after photobleaching/confocal microscopy was performed as described[10] and is detailed in the Supplementary Materials and Methods section.

Statistics

Results were expressed as the mean ± standard error of the mean. Statistical evaluation was performed by analysis of variance or the Student's *t* test.

Results

α-MD-G Stimulates NHE3 Activity in Mouse Jejunum via SGLT1

NHE3 activity in intact mouse jejunum was measured by 2-photon microscopy using the pH-sensitive dual-emission dye, SNARF-4F.[11,15] Because the fluorescence of SNARF-4F in different solutions and intracellular conditions often differs, the PKa of SNARF-4F in jejunum was measured by the K^+/nigericin (10 mmol/L) method.[11] Supplementary Figure 1 shows the jejunal calibration of the pH response. Jejunum was perfused sequentially with tetramethylammonium (TMA) (20 min) and then Na buffer containing either D-mannose or α-MD-G (25 mmol/L) (Figure 1*A*). α-MD-G is a nonmetabolized analogue of D-glucose and is specifically transported by SGLT1. Figure 1*B* shows the pH-dependent emission shifts from acidic to basic, comparing conditions before and after Na^+ treatment. Compared with the D-mannose group, α-MD-G significantly stimulated NHE3 activity (0.45 ± 0.03 ΔpH/min [n = 9] vs 0.31 ± 0.03 ΔpH/min [n = 5]; $P < .01$) (Figure 1*C*). The specific SGLT1 inhibitor phloridzin (1 mmol/L) abolished the α-MD-G–induced stimulation (0.45 ± 0.03 ΔpH/min [n = 9], α-MD-G/no phloridzin vs 0.21 ± 0.02 [n = 3], α-MD-G/phloridzin; $P < .01$). These results show that luminal α-MD-G stimulates NHE3 activity in intact mouse jejunum by a mechanism involving SGLT1.