HHS Public Access

Author manuscript

Cell Mol Gastroenterol Hepatol. Author manuscript; available in PMC 2017 January 13.

Published in final edited form as:

Cell Mol Gastroenterol Hepatol. 2017 January ; 3(1): 27–40. doi:10.1016/j.jcmgh.2016.09.010.

Pathophysiology of Intestinal Na^+/H^+ exchange

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Abstract

Several members of the *SLC9A* family of Na^+/H^+ exchangers are expressed in the gut, with varying expression patterns and cellular localization. Not only do they participate in the regulation of basic epithelial cell functions, including control of transepithelial Na^+ absorption, intracellular pH (pH_i), cell volume, and nutrient absorption, but also in cellular proliferation, migration, and apoptosis. Additionally, they modulate the extracellular milieu in order to facilitate other nutrient absorption and to regulate the intestinal microbial microenvironment.

Na^+/H^+ exchangers are frequent targets of inhibition in gastrointestinal pathologies, either by intrinsic factors (e.g. bile acids, inflammatory mediators) or infectious agents and associated microbial toxins. Based on emerging evidence, disruption of NHE activity via impaired expression or function of respective isoforms may contribute not only to local and systemic electrolyte imbalance, but also to the disease severity via multiple mechanisms. Here, we review the current state of knowledge about the roles Na^+/H^+ exchangers play in the pathogenesis of disorders of diverse origin and affecting a range of GI tissues.

Keywords

Barrett's esophagus; esophageal adenocarcinoma; epithelial injury; epithelial restitution; diarrhea; infection; inflammation; microbiota; Inflammatory Bowel Disease; hypertension

Introduction

Na^+/H^+ exchange (NHE) is an evolutionarily conserved membrane transport mechanism attributed to members of the CPA (cation/proton antiporters) superfamily. [1,2] Among the four inclusive families: CPA1, CPA2, PSE, and NaT-DC, the CPA1 (*SLC9A*) family contains the best-characterized plasmalemmal and intracellular isoforms (*SLC9A1–9*). Additionally, the *SLC9B* subgroup consists of two Na^+/H^+ antiporter (NHA) isoforms, NHA1 and NHA2 (*SLC9B1* and *SLC9B2*). In the gut, expression of all NHE isoforms but

Competing interests: The authors have declared that no competing interests exist.

Disclosures: All authors declare that there is no conflict of interest to disclose

NHE5 has been described. NHE1 (*SLC9A1*), NHE2 (*SLC9A2*), NHE3 (*SLC9A3*), and NHE8 (*SLC9A8*) have not only been implicated in regulating the basic functions of the epithelial cells, including control of the intracellular pH (pH_i), acidic mucosal microclimate, cell volume, and nutrient absorption, but also in cell proliferation[3], cell migration[3, 4], and apoptosis.[5] Intestinal expression of NHE4 (*SLC9A4*) in the small and large intestine remains controversial. While gastric expression and function has been confirmed,[6, 7] its presence in the intestine, at least in rodents, was contested.[7] In light of these observations, the interpretation of the more recent studies on the functional contribution of NHE4 to pH regulation in the human colonic crypts[8, 9] has to be done with care, although species differences may account for the reported discrepancies.

As a principle, Na^+/H^+ exchangers utilize energy stored in the form of the electrochemical Na^+ concentration gradient developed across the plasma membrane (secondary active transport) by the basolateral Na^+/K^+-ATPase (NKA). NHE is a mechanism critical for transepithelial movement of Na^+ and HCO_3^- (and thus for luminal and systemic salt, volume, and acid-base homeostasis) (Figure 1). It also serves supporting roles for other nutrient transporters by providing the proton gradient for the proton-coupled absorption of amino-acids, peptides, organic anions, short-chain fatty acids, folate, and iron[10, 11]. Only mutations in the *NHE6* or *NHE9* genes have been linked to human disease (X-linked mental retardation and familial autism, respectively).[12, 13] Although long assumed, only recently Janecke et al.[14] demonstrated novel recessive missense, splicing, and truncation mutations in the NHE3-encoding *SLC9A3* gene as being linked to the pathogenesis of congenital sodium diarrhea. Non-genetically based functional alterations of intestinal NHE's have also been linked with epithelial dysfunction. Details on molecular mechanisms of regulation of expression and function of NHEs are beyond the scope and limit of this article, but have been comprehensively covered in other reviews.[15–18] In this review, we summarize the current state of knowledge regarding the physiological roles of NHE isoforms in the gut, and roles that aberrant epithelial Na^+/H^+ exchange plays in the context of gastrointestinal disorder pathogenesis.

1. Na^+/H^+ exchange in esophageal and gastric pathology

The role of NHE1 in the esophageal epithelium in Barrett's esophagus and in the progression to esophageal adenocarcinoma

Barrett's esophagus is a precancerous condition characterized by the metaplasia of columnar epithelium with goblet cells that replace the normal squamous stratified epithelium in response to chronic reflux of gastric acid and bile acids. Though it is thought to represent a protective adaptive response to noxious components of the gastric chyme, metaplasia is also associated with increased risk of esophageal adenocarcinoma. When esophageal luminal acidity reaches pH 2.0, the microenvironment adjacent to the surface cells of the stratified squamous epithelium is reduced to a pH of 2.0–3.0[19]. Upon exposure to acid, esophageal epithelial cells regulate their pH primarily via the combined actions of Na^+/H^+ antiport and Na^+-dependent Cl^-/HCO_3^- exchange[20]. NHE1 is the sole plasmalemmal Na^+/H^+ exchanger isoform expressed in the rabbit and rat esophagus[21] and is allosterically regulated (activated) by reduced pH_i in a PKC-dependent mechanism.[22] Additionally, epidermal growth factor

(EGF), abundant in the saliva, exerts a cytoprotective effect in acid-exposed cells via Ca^{2+}/calmodulin- and PKC-dependent activation of NHE1[23]. Consistent with that, patients with low salivary EGF levels were found to be susceptible to severe oesophageal damage as a potential consequence of gastroesophageal reflux and to form a high-risk group for development of Barrett's oesophagus[24, 25]. NHE1 expression is increased in patients with gastroesophageal reflux disease[26] and in Barrett's esophagus[27], where it likely represents a cellular defensive mechanism to manage the acute and chronic acid overload. Bile acids present in reflux chyme reduce the ability of the cells to control their pH_i by nitric oxide-mediated NHE1 inhibition, thus leading to increased DNA damage and potentially to mutations and cancer progression.[27]

However, NHE1 has diverse physiological roles extending well beyond pH_i and cell volume control, including cell proliferation, growth, migration, and apoptosis, and contributes to pathological processes such as cancer cell invasion and heart failure[28, 29]. In a Barrett's adenocarcinoma cell line acid pulse-induced NHE1 activity correlated with increased proliferation, which could be reduced by inhibition of NHE1 or PKC[30]. This finding led to a somewhat paradoxical proposal that NHE1 inhibition may be of therapeutic value in Barrett's esophagus and prevention of its progression to cancer.

NHE's in gastric epithelial injury and repair

The integrity of the gastric epithelium is essential for protection from noxious luminal contents, which include gastric acid, proteases, and food-borne pathogens. As a consequence of damage to the surface epithelium, adjacent healthy cells rapidly migrate into the injured area to restore the mucosal barrier, and failure of this restitution process may lead to ulceration. Expression and activity of NHE1, and to a lesser extent NHE2 and NHE3, has been described in gastric myofibroblasts, cells that play a central role in wound healing, deposition of the extracellular matrix, and epithelial function.[31] These functions are tightly correlated with the myofibroblasts' ability to migrate and proliferate within the subepithelial compartment. NHE1 and its stimulation by insulin-like growth factor-II (IGF-II) and carbachol has been postulated to contribute to human gastric myofibroblast (HGF) migration and to be indispensable for IGF-II-induced HGF proliferation.[31] Gastric epithelium expresses five NHE isoforms: apical NHE2 (in surface/neck mucous cells), apical NHE3 (in rat but not rabbit parietal cells), apical NHE8 (mouse fundic and pyloric glands), basolateral NHE1 (all cells), and basolateral NHE4 (in parietal and chief cells). [32–34] Initially, NHE1 has been implicated in gastric repair in guinea pig mucosa *in vitro*.[35, 36] Later studies with more definitive mouse knockout models, and the use of intravital two-photon laser microscopy following injury, showed that it is NHE2 and not NHE1 that is required for the cytoprotective effects of trefoil factor 3 in the gastric epitheliaum.[37] These findings were also in agreement with the *in vitro* studies by Furukawa et al.[38], and in the description of progressive gastritis and gastric atrophy with loss of parietal cells in $NHE2^{-/-}$ mice.[39, 40] Consistent with its expression pattern, loss of NHE4 in knockout mice resulted in reduced numbers of parietal cells, a loss of mature chief cells, increased numbers of mucous and undifferentiated cells, and an increase in the number of necrotic and apoptotic cells.[6] More recent studies from our laboratory also showed that gastric expression of NHE8 was critical for the maintenance of bicarbonate secretion by the Cl^-/HCO_3^- exchanger downregulated-

in-adenoma (DRA; *SLC26A3*) and the associated mucosal protection. Compared to their wild-type littermates, mice lacking NHE8 had a higher incidence of gastric ulcer formation. [32] Although the mechanism responsible for DRA downregulation is not yet clear, it appears to be a phenomenon consistent across organs, as it was described also in the colon,[41] conjunctiva, the cornea, and the lacrimal glands.[42]

2. Intestinal Na^+/H^+ exchange in diarrheal diseases

Congenital sodium diarrhea

Congenital sodium diarrhea (CSD) is a rare autosomal recessive diarrheal disorder originally described in two patients in 1984 and 1985.[43, 44] To date, there have been fewer than 50 CSD cases reported in the literature.[45] Prior to birth, polyhydroamnios (an excess of amniotic fluid in the amniotic sac) is observed, which is suggestive of an increase in colonic output by the fetus. After birth the disease is characterized by the secretion of a large volume of 'nonstopping' high Na^+ diarrhea, often mistaken for urine, which leads to irritability, dehydration, and metabolic acidosis.[45] Although profuse diarrhea continues, electrolyte replacement therapy promotes normal growth and development.[46]

Two of the early reports of CSD linked diarrhea to defective NHE in the jejunum[44, 46], although the identity of the transporter remained unknown. In an attempt to identify the specific isoform, Muller et al studied a small CSD cohort of five infants from two inbred Austrian families and found no contribution from any of the six then known NHE's[47]. Subsequent analyses demonstrated additional intestinal and corneal pathophysiology (intestinal epithelia dysplasia and corneal epithelial erosions) among other symptoms accompanying high Na^+ diarrhea. These affect about a third of CSD patients, are referred to as the syndromic CSD form, and have been linked to a mutation in *SPINT2*.[48] The classical or non-syndromic form of CSD is strongly linked to defective NHE activity, and recently, using a cohort of 18 patients from 16 families, Janecke et al. determined that a variety of mutations (point, missense, and truncation) in the NHE3-encoding *SLC9A3* gene occurred in a subset (9/18) of the studied CSD cases.[45] The identified *SLC9A3* mutations included one whole-gene deletion, one splicing and two frame-shift mutations, all of which are expected to abolish protein production. Four missense mutations/variants, p.Arg382Gln, p.Ala311Val, p.Ala269Thr, p.Ala127Thr were tested in vitro, and all but p.Ala127Thr (benign variant) conferred decreased basal Na^+/H^+ exchange activity. [45]

Additionally, an activating mutation in the catalytic domain of the guanylate cyclase c gene (*GUCY2C*) was also identified and may account for 20% of sporadic CSD cases.[49, 50] This mutation is likely mechanistically linked to hyperactivation of the cystic fibrosis transmembrane regulator (CFTR)[49] and reduced NHE3 function via elevated intracellular cGMP and NHE3 inhibition via a cGKII kinase-dependent mechanism.[51, 52] Consequently, NHE3 inhibition may contribute to at least 70% of CSD cases. Additional research is required to identify the remaining contributing factors, especially in the fetal and neonatal period when the contribution of NHE3 to total epithelial NHE is thought to be minimal. Although NHE8 has a reciprocal ontogenic pattern of pattern of expression, with higher levels in young than older animals[53, 54], no mutations were found in the exonic regions of *SLC9A8* gene in a small cohort of five CSD patients.[54]

Inhibition of Na^+/H^+ exchange during infectious diarrhea

Diarrhea caused by enteric infections is a major factor in morbidity and mortality worldwide. The mechanisms are multifactorial and include alterations in motility, changes in paracellular permeability, loss of absorptive surface, and changes in electrolyte fluxes. A significant component of altered electrolyte flux in infectious diarrhea is increased chloride secretion via activation of apical chloride channels, including the cystic fibrosis transmembrane conductance regulator (CFTR) and Ca^{2+}-activated Cl^- channels.[55] However, there is a significant component or subset of infectious diarrheal cases related to inhibition of Na^+ and fluid absorption. The underlying mechanism for the latter is a reduction in Na^+ absorption via impaired NHE activity, with the affected NHE isoforms and the mechanisms involved varying among pathogens. During infection with *Vibrio cholerae*, a prime example of secretory diarrhea, cholera toxin is capable of reducing epithelial Na^+ absorption via cAMP-dependent inhibition of NHE2 and NHE3.[56] Extrapolating from the known mechanisms governing NHE regulation[57, 58], one could generalize that bacterial pathogens which increase intracellular Ca^{2+}, cAMP, and/or cGMP concentration inhibit electroneutral NaCl absorption. Indeed, this was shown in mouse models of infection with *Salmonella typhimurium, Shigella dysenteriae* type 1 toxin, and *Campylobacter jejuni*.[59–61] However, other mechanisms leading to NHE inhibition have also been described.

Clostridium difficile, the leading cause of nosocomial diarrhea and pseudomembranous colitis, also exerts inhibitory effects on epithelial NHE. *C. difficile* Toxin B (TxB) inhibits Rho-family GTPases and alters the interaction between NHE3 and the microvillar actin cytoskeleton to facilitate its internalization and loss of function.[62] TxB was also shown to disrupt the stimulating effects of β-PIX (Rho Guanine Nucleotide Exchange Factor 7), which along with Shank2 maintains apical membrane NHE3 expression.[63] More recently, Engevik et al.[64] postulated that NHE3 inhibition by *C. difficile* toxin resulted in alteration of the intestinal environment and gut microbiota, which could facilitate colonization and expansion of the pathogen. They showed that when compared to healthy controls, decreased expression of NHE3 in infected patients correlated with elevated Na^+ and pH of the stools, increased *Bacteroidetes* and *Proteobacteria*, and decreased *Firmicutes* phyla. Interestingly, *in vitro* growth of *C. difficile* was promoted by elevated Na^+ and an alkaline media pH; consequently, the authors concluded that inhibition of NHE3 creates an altered environment favored by *C. difficile*.[64]

Several classes of pathogenic *E. coli* strains are responsible for diarrheal outbreaks. These include enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic E. coli (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli*, and diffusely adherent *E. coli* (DAEC). A variety of NHE-independent mechanisms that contribute to diarrheagenic effects of EPEC are known (outside the scope of this review).[65] Surprisingly, the effects of EPEC *in vitro* (Caco-2 BBE, HT-29 cells and T84 cells) were isoform-specific, with significant increase in NHE1 (basolateral) and NHE2 (apical) activity and a concurrent 50% inhibition of NHE3.[66] In a follow-up study, Hodges et al.[67] showed that the inhibitory effects of EPEC on NHE3 were dependent on EspF, a component of EPEC type III secretion system. On the host side, Chen et al.[68] showed that *E. coli* heat-stable enterotoxin (STp), which binds to guanylate cyclase-C in the luminal enterocyte

membrane and increases intracellular cGMP levels,[69] requires the presence of the regulatory PDZ adaptor protein NHERF2.[68] The downstream effector of elevated cGMP, type II isoform of cGMP-dependent protein kinase cGKII is a part of a NHE3 signaling complex,[70] and inhibition of NHE3 activity by cGMP/cGKII requires NHE3 protein phosphorylation at Ser^{554}, and Ser^{607},[52] although the relationship of NHERF2/ cGMP/cGKII in the context of exposure to *E. coli* enterotoxin is not yet clear. Increased apical NHE2 activity may be compensatory to the loss of NHE3, although it remains unknown whether it outweighs NHE3 inhibition *in vivo*. Contrary to $NHE3^{-/-}$ mice[71], targeting of *SLC9A2* gene did not result in diarrhea[39, 72], suggesting that NHE2 is not a major absorptive Na^+/H^+ exchanger. Inhibition of NHE4 in T-84 cells by the heat-stable enterotoxin of enterotoxigenic E. coli has also been reported, although the relevance of this finding is unclear considering inconsistent the data on intestinal expression of this isoform. [7, 9]

Less is known about the role of NHE in diarrhea during viral gastroenteritis. Astroviral infections have been associated with Na^+ malabsorption, and one study suggested that infection leads to decreased levels of NHE3 in the insoluble protein fraction in the enterocytes, presumed to represent the apical membrane.[73] Recently communicated data from rotavirus-infected patients showed that both NHE2 and NHE3 proteins are downregulated, and the remaining NHE3 is mislocalized.[74]

Contribution of Na^+/H^+ exchange to the efficacy of oral rehydration therapy

The glucose transporter SGLT1 uses a Na^+ gradient to transport Na^+ and glucose at a 2:1 stoichiometric ratio against a glucose gradient.[75] In each cycle, a sugar molecule is co-transported with Na^+ across the cell, which is accompanied by 260 water molecules.[76] This mechanism was calculated to account for 5 liters of water absorbed per day in the human intestine and formed the molecular basis of oral rehydration therapy aimed to control mortality associated with cholera and other infectious diarrheal diseases.[77] Lin et al.[78] demonstrated a key NHE3 contribution to the efficacy of oral rehydration solution. They showed that SGLT1-mediated Na-glucose co-transport stimulates NHE3 activity *in vivo* by an Akt/PKB- and NHERF2-dependent pathway. This increase was associated with increased brush-border NHE3 recruitment through its release from the endosomal storage pool. Activation of NHE3 by glucose reversed cholera toxin-induced NHE3 inhibition, a phenomenon that may at least partially explain the efficacy of oral rehydration solutions (ORS).[78] In 2004, both the World Health Organization and the United Nations Children's Fund suggested the supplementation of ORS with zinc. This recommendation was based on observations linking a reduction in tissue and an increase in fecal zinc levels in infants and children with diarrhea[79] and that zinc deficiency is responsible for decreased net water and sodium transport from the small and large intestine.[80] Clinical studies showed a decline in diarrheic stool volume and episode duration when zinc was administered in conjunction with ORS.[81] In addition to the anti-secretory effects of zinc[82], Hoque et al. showed that zinc not only stimulated basal NHE3 activity by 50%, but it also counteracted forskolin-induced cAMP-mediated NHE3 inhibition.[83] While conventional ORS targets primarily small intestinal Na^+ and water absorption, an improved formulation has been proposed to also target the colon.[84] In this approach, D-glucose is replaced with a relatively amylase resistant cornstarch. While some of the starch is enzymatically broken down in the jejunum to

stimulate Na^+ absorption, most of it would enter the colon where it could be fermented to short chain fatty acids (SCFA) such as propionate, butyrate and acetate by the resident bacteria. Butyrate increases expression and/or activity of apical NHE's[85–88] and aids in transepithelial Na^+ absorption via a neutral linked Na^+ absorptive process that exchanges SCFA for OH^- ions along with the apical Na^+/H^+ exchange. In a small, randomized clinical trial, this ORS formulation performed better than standard ORS by reducing both the duration and volume loss of severe acute diarrhea.[84] Two other randomized controlled trials with the addition of high-amylose maize starch (HAMS) to ORS carried out in South India have demonstrated a substantial decrease in diarrhea duration in both adults and children hospitalized for acute diarrhea.[89, 90] One drawback of such modified ORS is the precipitation of the starch in the ORS formulation. New formulations containing an anti-settling agent have been proposed and clinical trials have been reported in 2014 by Binder et al.[91] as under way.

Modulation of intestinal Na^+/H^+ exchange in noninfectious diarrhea associated with immunosuppression (rapamycin)

Rapamycin or Sirolimus is a drug isolated from the actinomycete *Streptomyces hygroscopius* that exhibits immunosuppressive, antiproliferative, and antifungal properties. Rapamycin and its analogs (Rapalogs) have been shown to inhibit the activity of mTOR (mammalian or mechanistic Target of Rapamycin).[92] mTOR is a serine-threonine kinase that is known to form two different catalytic complexes, the mTOR Complex 1 or 2 (mTORC1 or mTORC2) and to induce autophagy.[92] Rapamycin administration inhibits mTORC1, while long-term use partially inhibits mTORC2. Its use to prevent organ transplant rejection and to treat lymphangioleiomyomatosis is associated with several side effects, which include diarrhea. Among patients receiving post-renal transplant rapamycin treatment, in those hospitalized for noninfectious diarrhea, ileal NHE3 expression was reduced at the apical membrane.[93] Of 367 records of renal transplant cases at the Albany Medical College, 20 patients had 39 events of acute, severe diarrhea requiring hospitalization. In all events, rapamycin levels at the time of diarrhea were significantly elevated (2–7 times higher than the baseline levels), and in all cases, diarrhea resolved within 3–5 days after the serum rapamycin levels returned to baseline.[93] This finding was reproduced in rapamycin-treated wild-type, but not in autophagy-resistant $Atg7^{-/-}$ mice. Deletion of mTOR in mice also led to reduced NHE activity, luminal accumulation of fluid, and reduced levels of NHE3 and NHERF1.[93]

3. Intestinal Na^+/H^+ exchange in clinical and experimental Inflammatory Bowel Diseases

Inflammatory Bowel Disease (IBD) is a group of chronic inflammatory diseases that include Crohn's disease (CD) and ulcerative colitis (UC). Both conditions are characterized by immune system activation, microbial dysbiosis, and disruption of the epithelial barrier, which may lead to bacterial translocation, the latter being a more prominent feature of CD.[94] Alterations in the function of epithelial cells in IBD extend into transcellular nutrient transport.[95] In some instances, those alterations of transport protein complexes at the apical or basolateral membranes have consequences transcending their basal functions as nutrient

or electrolyte transporters to modulate epithelial barrier function and gut microbiota, potentially shaping the immune response in the course of IBD.

Inhibition of intestinal Na^+/H^+ exchange as a contributor to inflammation-associated diarrhea

Diarrhea is one of the common symptoms in patients with IBD, occurring in about 50% of acute flare-ups in CD and in nearly all UC patients.[96] The pathophysiology of diarrhea in IBD is complex and multifactorial. It involves altered intestinal motility as well as abnormal epithelial ion transport and defective Na^+ absorption in the colonic mucosa in particular.[97, 98] Contrary to infectious diarrhea where active and excessive Cl^- secretion is predominant, in IBD, both electrogenic Na^+ transport mediated by sodium channels[99–101] as well as electroneutral NHE and coupled Na-Cl absorption are reduced.[102] . At least for UC, a model was proposed whereby the associated diarrhea is the result of a combination of factors: increased paracellular permeability to monovalent ions, reduction of electrogenic Na^+ absorption (secondary to decreased apical Na^+ channel and basolateral NKA expression), electroneutral Na^+ absorption and coupled Na^+-H^+/ Cl^--HCO_3^- exchange, and as a result compromised active Cl^- absorption.[102]

The mechanisms involved in the inhibition of epithelial NHE's in IBD are not well defined and conflicting results have been published. IFN-γ inhibits the expression and function of NHE3 and NHE2 both *in vitro* and *in vivo*.[103] Inhibition of NHE3 expression and activity has also been described in several experimental models of colitis, including IL-$2^{-/-}$ mice[104], and in DSS- and TNBS-induced colitis.[105] The latter study described decreased NHE3 protein expression in sigmoid mucosal biopsies from most of the cases of active UC and/or CD, in ileal mucosal biopsies of active CD, as well as in ~50% of sigmoid biopsies from inactive UC or CD. Siddique et al. showed that in both the untreated and treated patients with CD and UC, NHE3 protein and activity were also reduced.[106] Furthermore, NHE3 mRNA was reduced only in CD, but not in patients with UC. However, in IL-$10^{-/-}$ mice NHE3 activity, measured in the apical enterocytes within isolated colonic crypts, was significantly decreased without altered NHE3 expression and localization.[107] The authors speculated that decreased expression of two key NHE3-regulatory PDZ adaptors NHERF2 and PDZK1 may be responsible for decreased NHE3 activity.[107] Additional studies with UC patients by Yeruva et al.[108] and Farkas et al.[109] showed a significant reduction of NHE3 activity despite the preserved protein and mRNA expression, thus arguing for post-translational regulation of the symporter activity. In an *in vivo* model of diarrhea mediated by anti-CD3 monoclonal antibody-induced T cell activation, Clayburgh et al.[110] showed that in the jejunum, TNF induces NHE3 internalization. PKCα activation triggered by TNF was responsible for NHE3 internalization and the resulting Na^+ malabsorption.[110] While it is clear that the NHE3-mediated apical Na^+/H^+ exchange and epithelial Na^+ absorption are inhibited in IBD, the exact mechanism remains unclear and may depend on the specific disease, segment involved, and/or the severity of inflammation.

NHE8 has a broader expression pattern than other apical NHE's.[53, 111, 112] Moreover, its expression is not limited to the apical membrane; *in vitro* studies with ectopically expressed NHE8 show it's both present and functional in the trans-Golgi network and multivesicular

bodies.[113] It is broadly expressed in the colon, including the goblet cells, where it participates in the regulation of Muc2 expression and mucous secretion.[41, 114, 115] Although we observed a dramatic decrease in the expression of NHE8 in UC patients (Li et al., unpublished data), loss of NHE8 expression and activity may not contribute to inflammation-associated diarrhea directly as NHE8$^{-/-}$ mice do not exhibit symptoms of impaired Na^+ absorption or diarrhea.[41] The consequences of NHE8 deficiency in IBD may be more related to microbial-host interactions as discussed in the next section.

Both the role of the basolateral NHE1 and the potential changes in its expression and/or activity in the colonic epithelial cells in clinical and experimental IBD remains unclear, and the available data are inconsistent. Expression of NHE1 mRNA and protein was increased in both acetic acid and TNBS-induced colitis in rats,[116] and non-specific pharmacological NHE inhibition with amiloride was deemed beneficial in rodent models of IBD.[117, 118] However, in human patients, opposing data have been published. Contrary to the same group's earlier rat data, Khan et al.[119] and Siddique and Khan[120] showed decreased NHE1 mRNA in the biopsies of UC and CD patients, whereas Farkas et al.[109] showed consistently elevated NHE1 activity and expression in the inflamed mucosa of UC patients. The importance of these controversial findings remains unclear. While it may be consequential to some aspects of epithelial cell function, the notion that "NHE1 suppression may reduce an uptake of sodium chloride and water from the inflamed colonic lumen and thus contribute to diarrhea"[119] currently lacks experimental support.

Apical Na^+/H^+ exchange as a modulator of the gut microbiome and inflammatory response during colitis

The lumen of the gastrointestinal tract is a habitat for 100 trillion bacterial cells, which form a virtual organ.[121] This gastrointestinal bacterial consortium, collectively referred to as the gut microbiome, is a highly functional, complex, and dynamic entity. The composition of this virtual organ, known as the gut microbiota or microbiome, is generally conserved at the phylum level and is unique for each individual at lower taxonomic (genus and species) levels. The adult colon is predominantly colonized by two phyla *Bacteroidetes* and *Firmicutes*, followed by *Proteobacteria* and *Actinobacteria*.[122] Cells of both the innate immune system (including epithelial cells) as well as of the adaptive immune system can sense microorganisms or their metabolic products and translate the signals into host physiological responses, which in turn can regulate the gut microbial ecology.[123, 124] This cross-talk is essential for normal gut development, maturation of the immune system, optimal nutrient bioavailability, participation in xenobiotic and hormone metabolism, and the maintenance of the epithelial and immune cell microenvironment. Any perturbations in microbial-host homeostasis results in dysbiosis, a detrimental shift in the composition or microbial abundance, with consequences ranging from enteric infections[125, 126], colorectal cancer[127], and autoimmune diseases[128], through metabolic syndrome (type 2 diabetes and obesity)[129], to neuropsychiatric diseases and autism spectrum disorders.[130–133]

Dysbiosis in both IBD patients and animal models is characterized by reduced general biodiversity,[134] expanded relative abundance of the *Bacteroidetes, Proteobacteria and Actinobacteria* phyla, decreased abundance of *Firmicutes*,[135] and importantly the presence

of *Clostridia spp*. belonging to the clostridia IV and XIVa clusters, the major butyrate-producing bacteria and inducers of colonic $CD4^+FoxP3^+$ T regulatory cells.[136] Similar changes are observed in mouse models of IBD.[137–139] The mechanisms by which the gut microbial community is affected by the inflammatory process, or whether dysbiosis is sufficient to induce and/or modulate mucosal immune responses, remain a subject of debate. While some studies indicate that dysbiotic communities can be colitogenic upon transmission to wild-type mice, [140] others have shown that mucosal bacteria from UC patients only confer susceptibility and are not sufficient to induce disease in untreated or otherwise non-genetically manipulated hosts.[141]

We hypothesized that inhibition of epithelial NHE, common in IBD patients, may lead to altered epithelial cell function and mucosal milieu that could lead or contribute to the observed shifts in microbial gut ecology and, as a consequence, influence mucosal inflammation. Indeed, mice lacking the dominant epithelial apical NHE isoform NHE3, when raised in a conventional facility, spontaneously develop distal colitis with mild diarrhea.[142] Colitis symptoms were alleviated by broad-spectrum antibiotics and reduced by rederivation into a barrier facility.[142, 143] $NHE3^{-/-}$ mice develop microbial dysbiosis similar to IBD patients.[143] Although they appear to have normal mucous thickness, structure, and Muc2 mRNA expression, higher bacterial penetrance with increased bacterial adhesion to the mucosa and bacterial translocation was described, similar to $IL\text{-}10^{-/-}$ mice.[142, 144] These findings suggested that mucus thickness alone is not a major determinant of bacterial penetration, and that other factors may contribute to mucosal bacterial penetration in IBD.[144] It is plausible, however, that the molecular structure of MUC2 polymers within the inner layer may change with impaired apical Na^+/H^+ exchange. As MUC2 is packed into secretory granules in the goblet cells, the organellar pH changes from 7.2 in the ER to 6.0 in the trans-Golgi network and to 5.2 in the secretory granulae, along with increasing Ca^{2+} concentration.[145] Upon secretion, and before the 1,000-fold expansion, it remains densely packed to form the impenetrable for bacteria inner stratified layer, which is best preserved at pH 6.2 in form of sheets of N-terminal trimers.[145] Disruption of the regulatory mechanisms responsible for maintenance of the acidic microclimate could make the MUC2 network structure more penetrable for bacteria. This would, however, require experimental confirmation.

Importantly, $NHE3^{-/-}$ mice are highly susceptible to experimental colitis, [146, 147] although the relative contributions of epithelial dysfunction and microbial dysbiosis are still unclear. Most recently, our group showed that in adoptive T cell transfer colitis NHE3 deficiency and the associated dysbiosis dramatically accelerated and exacerbated the disease, increased mucosal $CD4^+$ T cell and neutrophil homing, and increased intestinal permeability, all of which was attenuated with broad-spectrum antibiotics.[148] Strikingly, a dysbiotic microbiome was more strongly associated with NHE3 deficiency than with T cell mediated colitis *per se*.[148] We concluded that Na^+/H^+ exchange is, therefore, a crucial process for maintaining microbial homeostasis within the gut and that its disruption in IBD is likely a strong contributor to dysbiosis, which in turn affects the progression and severity of disease. Another group also showed that higher ileal luminal Na^+ concentration, and not changes in pH, is responsible for the relative expansion of *Bacteroides thetaiotaomicron* and expression and activity of fucosylase FUT2.[149] *B. thetaiotaomicron* has been shown to be

colitigenic,[150] and an aberrantly high IL-8 response to *B. thetaiotaomicron* has been demonstrated in CD patients, [149] findings that may provide a link between inhibition of NHE3 and mucosal neutrophil influx in IBD.

As suggested earlier in this review, NHE8 may also contribute to mucosal homeostasis through mechanisms distinct from that of NHE3, possibly through its yet undefined roles in the goblet cell function. Indeed, it is downregulated and confers higher susceptibility during experimental colitis with a Th2-like response.[151, 152] Reduced expression or absence of NHE8 is also related to decreased Muc2 mRNA expression in goblets cells, and decreased mucosal expression of antimicrobial peptides.[114] NHE8$^{-/-}$ mice have reduced Muc2 expression, which could also be reproduced in colonic organoids from this strain (Xu et al. unpublished data), and show a reduced inner mucous layer and closer proximity of luminal bacteria to the brush border membrane.[152] Consequently, loss of NHE8 *in vivo* led to higher adhesion of *Salmonella typhimurium*.[152] *In vitro*, NHE8 siRNA knockdown showed similar increase in *S. typhimurium* adhesion, which appeared to be selective and did not affect adhesion of a probiotic strain of *Lactobacillus plantarum* JDM1.[152] In summary, data from NHE3 and NHE8 knockout mice suggests that their inhibition during inflammation or infection, albeit acting via different mechanisms, may be critical in shaping the host-microbial homeostasis in the gut and affect mucosal immune responses during IBD. Development of new pharmacological approaches to either prevention of inhibition of the two apical NHEs, or restoration of their activity, may be of significant benefit to IBD patients not only as means of reducing episodes of diarrhea, but possibly as means of modulating the gut microbiome and host's inflammatory response.

4. Therapeutic targeting of intestinal Na^+/H^+ exchange

Potential for modulation of intestinal fluid and Na^+ absorption in diarrheal and inflammatory conditions by targeting NHE3 activity

As described earlier in this review, part of the success of oral rehydration therapy and its modifications relies on the modulation of electroneutral epithelial Na^+/H^+ exchange. Considering the role of the C-terminus of the NHE3 protein in forming regulatory complexes responsible for NHE3 endocytic retrieval,[18] Zachos et al.[153] postulated that a peptide designed to mimic the region of the NHE3 protein involved in the formation of the Inhibitor Regulatory Complex (IRCX; NHERFs 1–4, PLC-γ, CK2, and CaM kinase II) may increase the apical pool of NHE3 and improve its activity. This 38-amino acid decoy peptide dubbed IRCX-CP stimulated basal NHE3 activity by 40% and prevented inhibition of NHE3 activity by both forskolin and carbachol, without altering the stimulatory effect of EGF on NHE3 activity.[153] Additionally, *in vivo*, IRCX-CP prevented the cholera toxin-induced increase in luminal fluid accumulation. It remains to be seen whether these promising data translate into a viable treatment. However, it clearly shows that the formation of the regulatory complexes at the C-terminus of NHE3 represents an attractive target for compound screening and development of drugs to stimulate NHE3 as potentially useful in the treatment of infectious and non-infectious diarrhea, and perhaps in modulating microbial and inflammatory responses in IBD.

Inhibition of intestinal NHE3 activity as a treatment modality for hypertension, constipation-predominant irritable bowel syndrome (IBS-C), and hyperphosphatemia for end-stage renal disease (ESRD) patients on dialysis

On the opposite spectrum, excessive sodium intake and/or reduced excretion in the intestine leads to sodium-fluid imbalances that result in hypertension, which can then lead to vision impairment in addition to a variety of cardiovascular and renal diseases such as heart failure, chronic kidney disease, stroke, and coronary heart disease. Typically, hypertension is considered to be one of the most common modifiable risk factor for cardiovascular disease and death, [154] as it can be controlled by reducing dietary sodium intake.[155] Unfortunately, compliance with a low-salt diet is low.[156] Drugs that limit intestinal sodium absorption, given alone or in conjunction with other antihypertensive medication, have been considered. To this end, two oral non-absorbable NHE3 inhibitors have been developed, SAR218034 (SAR) and Tenapanor. Pharmacokinetic analyses in rats, dogs, and humans demonstrated that neither of these compounds crossed the intestinal barrier in biologically active doses,[157, 158] and Tenapanor was well tolerated in phase I clinical study.[159] Both drugs were found to increase fecal and reduce urinary Na^+ concentrations in rats (SAR and Tenapanor) and humans (Tenapanor). Not surprisingly, both drugs caused an increase in luminal fluid resulting from elevated Na^+, leading to loose stools (a laxative effect). [157, 158] In a spontaneously hypertensive rat model, SAR administration in conjunction with sodium chloride laden drinking water markedly reduced systolic blood pressure.[158] Furthermore, in a salt-fed 5/6th nephrectomized rat model (a Chronic Kidney Disease model associated with hypertension, hypervolemia, cardiac hypertrophy, and arterial stiffening), Tenapanor reduced extracellular volume expansion, albuminuria, and blood pressure, in addition to promoting protective cardiorenal effects such as reducing left ventricular hypertrophy.[157] Both drugs exhibit enhanced effects if administered in conjunction with an ACE-inhibitor, which was deemed important in cases where hypertension cannot be controlled by the administration of a single medication. [157, 158] However, more recently, the development of Tenapanor and SAR, and the general concept of inhibition of intestinal Na^+/H^+ exchange as an antihypertensive strategy appears to have been abandoned by Ardelyx and Sanofi, respectively. Ardelyx continues the investigation into the use of Tenapanor and its close analogs in patients with constipation-predominant irritable bowel syndrome (IBS-C) and for the treatment of hyperphosphatemia in ESRD patients on dialysis.[160, 161] The potential of NHE3 inhibitors as stool softeners in IBS-C is obvious, though it remains to be seen if they can be applied without significant adverse effects. A somewhat similar strategy to modulate ion transport in constipation has been recently applied to develop CFTR activator to increase the net chloride output in the gut.[162] The concept behind the use of NHE3 inhibition in hyperphosphatemia is based on the described increased fecal P_i excretion and reduced urinary P excretion in NHE3 inhibitor-treated rats with chronic kidney disease with vascular calcification, where Tenapanor markedly reduced ectopic calcification and protected renal function.[161] The mechanism of this phenomenon remains unclear. It is also not yet evident whether targeting NHE3 would be more efficacious and with less adverse effects that the P_i binders currently used clinically.

CONCLUSIONS

NHEs are important regulators of a wide array of processes on a cellular, tissue, and systemic levels. The majority of current knowledge is limited to the function of plasmalemmal isoforms, which play protective roles during disease pathogenesis in a segment- and isoform-specific fashions and are frequent targets of inhibition by microbial or inflammatory stimuli. As such, future studies may bring new approaches that selectively target NHEs in the gut to enhance or inhibit their function for the benefit of the patients. Importantly, a large body of knowledge relies on studies focused on individual isoforms, frequently with knockout mice, while their true function always occurs in the larger context of frequently inter-dependent transporters, also subject to regulation during physiological and pathophysiological conditions. Thus, more integrative approaches to function, regulation, and targeting of NHEs in gut-associated pathologies may be needed. While the usefulness of orally available inhibitors remains to be established, the emerging focus on development of agonists of intestinal apical Na^+/H^+ exchange may provide significant benefits to the future treatment of infectious and inflammatory disorders of the gut.

Acknowledgments

Funding: This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases Grant 2R01 DK-041274 (to FK Ghishan and PR Kiela). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abbreviations used in this paper

β-PIX	Rho Guanine Nucleotide Exchange Factor 7
CaM kinase II	Ca^{2+}/Calmodulin-dependent Protein Kinase II
cAMP	Cyclic Adenosine Monophosphate
CD	Crohn’s Disease
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
cGMP	Cyclic Guanosine Monophosphate
cGMPKII	Cyclic Guanosine Monophosphate Kinase II
CK2	casein kinase 2
CPA	Cation/Proton Antiporter
CSD	Congenital Sodium Diarrhea
DAEC	diffusely adherent *E. coli*
DRA	Downregulated-in-Adenoma
DSS	Dextran Sulfate Sodium
EGF	Epidermal Growth Factor

EAEC	enteroaggregative *E. coli*
EHEC	enterohemorrhagic *E. coli*
EPEC	Enteropathogenic *E. coli*
ERSD	End-Stage Renal Disease
ETEC	enterotoxigenic *E. coli*
GTP	Guanosine Triphosphate
GUCY2C	guanylate cyclase c gene
HGF	Human Gastric Myofibroblast
IBD	Inflammatory Bowel Disease
IBS-C	Irritable Bowel Syndrome with Constipation
IFG-II	Insulin-like Growth Factor
IFN-γ	Interferon gamma
IL-2	Interleukin 2
IL-8	Interleukin 8
IL-10	Interleukin 10
IRCX	Inhibitor Regulatory Complex
IRCX-CP	IRCX Complex Formation
mTOR	Mechanistic or Mammalian Target of Rapamycin
mTORC1	mTOR Complex 1
mTORC2	mTOR Complex 2
Muc2	Mucin 2
NaT-DC	Na^+-transporting carboxylic acid decarboxylase
NHERF	Sodium Hydrogen Exchange Regulatory Factor
NHA	Na^+/H^+ Antiporter
NHE	Na^+/H^+ Exchange
NKA	Na^+/K^+-ATPase
ORS	Oral Rehydration Solution
PDZ	structural domain [combines the first letters of the following proteins - Post Synaptic Density Protein

	(PSD95), Drosophila Disc Large Tumor Suppressor (DLG1), and Zona Occludens-1 Protein (ZO-1)]
PDZK1	Sodium Hydrogen Exchange Regulatory Factor 3
pH_i	intracellular pH
PKC	Protein Kinase C
PLC-γ	Phospholipase C-γ
PSE	Pho1 phosphate permease
SAR	SAR218034
SCFA	Short-Chain Fatty Acids
Shank2	SH3 and Multiple Ankyrin Repeat Domains 2
Th2	T helper 2
TNBS	Trinitrobenzene sulfonic acid
TxB	*C. difficile* Toxin B
UC	Ulcerative Colitis

REFERENCES

1. Chen JS, Reddy V, Chen JH, et al. Phylogenetic characterization of transport protein superfamilies: superiority of SuperfamilyTree programs over those based on multiple alignments. J Mol Microbiol Biotechnol. 2011; 21:83–96. [PubMed: 22286036]
2. Brett CL, Donowitz M, Rao R. Evolutionary origins of eukaryotic sodium/proton exchangers. Am J Physiol Cell Physiol. 2005; 288:C223–C239. [PubMed: 15643048]
3. Walker J, Undem C, Yun X, et al. Role of Rho kinase and Na+/H+ exchange in hypoxia-induced pulmonary arterial smooth muscle cell proliferation and migration. Physiol Rep. 2016:4.
4. Rigiracciolo DC, Scarpelli A, Lappano R, et al. GPER is involved in the stimulatory effects of aldosterone in breast cancer cells and breast tumor-derived endothelial cells. Oncotarget. 2016; 7:94–111. [PubMed: 26646587]
5. Zhao Y, Cui G, Zhang N, et al. Lipopolysaccharide induces endothelial cell apoptosis via activation of Na(+)/H(+) exchanger 1 and calpain-dependent degradation of Bcl-2. Biochem Biophys Res Commun. 2012; 427:125–132. [PubMed: 22995319]
6. Gawenis LR, Greeb JM, Prasad V, et al. Impaired gastric acid secretion in mice with a targeted disruption of the NHE4 Na+/H+ exchanger. J Biol Chem. 2005; 280:12781–12789. [PubMed: 15684419]
7. Bookstein C, Xie Y, Rabenau K, et al. Tissue distribution of Na+/H+ exchanger isoforms NHE2 and NHE4 in rat intestine and kidney. Am J Physiol. 1997; 273:C1496–C1505. [PubMed: 9374634]
8. Beltran AR, Ramirez MA, Carraro-Lacroix LR, et al. NHE1, NHE2, and NHE4 contribute to regulation of cell pH in T84 colon cancer cells. Pflugers Arch. 2008; 455:799–810. [PubMed: 17943310]
9. Arena EA, Longo WE, Roberts KE, et al. Functional role of NHE4 as a pH regulator in rat and human colonic crypts. Am J Physiol Cell Physiol. 2012; 302:C412–C418. [PubMed: 22049213]
10. Thwaites DT, Anderson CM. H+-coupled nutrient, micronutrient and drug transporters in the mammalian small intestine. Exp Physiol. 2007; 92:603–619. [PubMed: 17468205]

11. Shawki A, Engevik MA, Kim RS, et al. Intestinal brush-border Na+/H+ exchanger-3 drives H+-coupled iron absorption in the mouse. Am J Physiol Gastrointest Liver Physiol. 2016 ajpgi 00167.
12. Takahashi Y, Hosoki K, Matsushita M, et al. A loss-of-function mutation in the SLC9A6 gene causes X-linked mental retardation resembling Angelman syndrome. Am J Med Genet B Neuropsychiatr Genet. 2011; 156B:799–807. [PubMed: 21812100]
13. Kondapalli KC, Hack A, Schushan M, et al. Functional evaluation of autism-associated mutations in NHE9. Nat Commun. 2013; 4:2510. [PubMed: 24065030]
14. Janecke AR, Heinz-Erian P, Yin J, et al. Reduced sodium/proton exchanger NHE3 activity causes congenital sodium diarrhea. Hum Mol Genet. 2015; 24:6614–6623. [PubMed: 26358773]
15. Kiela, PR.; Ghishan, FK. Na^+-H^+ Exchange in Mammalian Digestive Tract. In: Johnson, LR., editor. Physiology of the Gastrointestinal Tract. 4th. Vol. 2. Academic Press; 2006. p. 1847-1879.
16. Orlowski J, Grinstein S. Na+/H+ exchangers. Compr Physiol. 2011; 1:2083–2100. [PubMed: 23733698]
17. Donowitz M, Ming Tse C, Fuster D. SLC9/NHE gene family, a plasma membrane and organellar family of Na(+)/H(+) exchangers. Mol Aspects Med. 2013; 34:236–251. [PubMed: 23506868]
18. Donowitz M, Mohan S, Zhu CX, et al. NHE3 regulatory complexes. J Exp Biol. 2009; 212:1638–1646. [PubMed: 19448074]
19. Orlando RC. Esophageal epithelial defense against acid injury. J Clin Gastroenterol. 1991; (13 Suppl 2):S1–S5.
20. Layden TJ, Schmidt L, Agnone L, et al. Rabbit esophageal cell cytoplasmic pH regulation: role of Na(+)-H+ antiport and Na(+)-dependent HCO3- transport systems. Am J Physiol. 1992; 263:G407–G413. [PubMed: 1329529]
21. Shallat S, Schmidt L, Reaka A, et al. NHE-1 isoform of the Na+/H+ antiport is expressed in the rat and rabbit esophagus. Gastroenterology. 1995; 109:1421–1428. [PubMed: 7557121]
22. Malo ME, Fliegel L. Physiological role and regulation of the Na+/H+ exchanger. Can J Physiol Pharmacol. 2006; 84:1081–1095. [PubMed: 17218973]
23. Fujiwara Y, Higuchi K, Takashima T, et al. Roles of epidermal growth factor and Na+/H+ exchanger-1 in esophageal epithelial defense against acid-induced injury. Am J Physiol Gastrointest Liver Physiol. 2006; 290:G665–G673. [PubMed: 16306134]
24. Gray MR, Donnelly RJ, Kingsnorth AN. Role of salivary epidermal growth factor in the pathogenesis of Barrett's columnar lined oesophagus. Br J Surg. 1991; 78:1461–1466. [PubMed: 1773326]
25. Marcinkiewicz M, Grabowska SZ, Czyzewska E. Role of epidermal growth factor (EGF) in oesophageal mucosal integrity. Curr Med Res Opin. 1998; 14:145–153. [PubMed: 9787980]
26. Siddique I, Khan I. Regulation of Na/H exchanger-1 in gastroesophageal reflux disease: possible interaction of histamine receptor. Dig Dis Sci. 2003; 48:1832–1838. [PubMed: 14561010]
27. Goldman A, Shahidullah M, Goldman D, et al. A novel mechanism of acid and bile acid-induced DNA damage involving Na+/H+ exchanger: implication for Barrett's oesophagus. Gut. 2010; 59:1606–1616. [PubMed: 20876775]
28. Harguindey S, Orive G, Luis Pedraz J, et al. The role of pH dynamics and the Na+/H+ antiporter in the etiopathogenesis and treatment of cancer. Two faces of the same coin--one single nature. Biochim Biophys Acta. 2005; 1756:1–24. [PubMed: 16099110]
29. Karmazyn M, Sawyer M, Fliegel L. The Na(+)/H(+) exchanger: a target for cardiac therapeutic intervention. Curr Drug Targets Cardiovasc Haematol Disord. 2005; 5:323–335. [PubMed: 16101565]
30. Fitzgerald RC, Omary MB, Triadafilopoulos G. Altered sodium-hydrogen exchange activity is a mechanism for acid-induced hyperproliferation in Barrett's esophagus. Am J Physiol. 1998; 275:G47–G55. [PubMed: 9655683]
31. Czepan M, Rakonczay Z Jr, Varro A, et al. NHE1 activity contributes to migration and is necessary for proliferation of human gastric myofibroblasts. Pflugers Arch. 2012; 463:459–475. [PubMed: 22138972]
32. Xu H, Li J, Chen H, et al. NHE8 plays important roles in gastric mucosal protection. Am J Physiol Gastrointest Liver Physiol. 2013; 304:G257–G261. [PubMed: 23220221]

33. Pizzonia JH, Biemesderfer D, Abu-Alfa AK, et al. Immunochemical characterization of Na+/H+ exchanger isoform NHE4. Am J Physiol. 1998; 275:F510–F517. [PubMed: 9755122]
34. Rossmann H, Sonnentag T, Heinzmann A, et al. Differential expression and regulation of Na(+)/H(+) exchanger isoforms in rabbit parietal and mucous cells. Am J Physiol Gastrointest Liver Physiol. 2001; 281:G447–G458. [PubMed: 11447025]
35. Yanaka A, Suzuki H, Shibahara T, et al. EGF promotes gastric mucosal restitution by activating Na(+)/H(+) exchange of epithelial cells. Am J Physiol Gastrointest Liver Physiol. 2002; 282:G866–G876. [PubMed: 11960783]
36. Joutsi T, Paimela H, Bhowmik A, et al. Role of Na(+)-H(+)-antiport in restitution of isolated guinea pig gastric epithelium after superficial injury. Dig Dis Sci. 1996; 41:2187–2194. [PubMed: 8943971]
37. Xue L, Aihara E, Wang TC, et al. Trefoil factor 2 requires Na/H exchanger 2 activity to enhance mouse gastric epithelial repair. J Biol Chem. 2011; 286:38375–38382. [PubMed: 21900251]
38. Furukawa O, Matsui H, Suzuki N, et al. Epidermal growth factor protects rat epithelial cells against acid-induced damage through the activation of Na+/H+ exchangers. J Pharmacol Exp Ther. 1999; 288:620–626. [PubMed: 9918567]
39. Schultheis PJ, Clarke LL, Meneton P, et al. Targeted disruption of the murine Na+/H+ exchanger isoform 2 gene causes reduced viability of gastric parietal cells and loss of net acid secretion. J Clin Invest. 1998; 101:1243–1253. [PubMed: 9502765]
40. Boivin GP, Schultheis PJ, Shull GE, et al. Variant form of diffuse corporal gastritis in NHE2 knockout mice. Comp Med. 2000; 50:511–515. [PubMed: 11099134]
41. Xu H, Zhang B, Li J, et al. Impaired mucin synthesis and bicarbonate secretion in the colon of NHE8 knockout mice. Am J Physiol Gastrointest Liver Physiol. 2012; 303:G335–G343. [PubMed: 22575219]
42. Xu H, Zhao Y, Li J, et al. Loss of NHE8 expression impairs ocular surface function in mice. Am J Physiol Cell Physiol. 2015; 308:C79–C87. [PubMed: 25377091]
43. Holmberg C, Perheentupa J. Congenital Na+ diarrhea: a new type of secretory diarrhea. J Pediatr. 1985; 106:56–61. [PubMed: 3880821]
44. Booth IW, Stange G, Murer H, et al. Defective jejunal brush-border Na+/H+ exchange: a cause of congenital secretory diarrhoea. Lancet. 1985; 1:1066–1069. [PubMed: 2860286]
45. Janecke AR, Heinz-Erian P, Muller T. Congenital Sodium Diarrhea: A Form of Intractable Diarrhea, With a Link to Inflammatory Bowel Disease. J Pediatr Gastroenterol Nutr. 2016; 63:170–176. [PubMed: 26835907]
46. Fell JM, Miller MP, Finkel Y, et al. Congenital sodium diarrhea with a partial defect in jejunal brush border membrane sodium transport, normal rectal transport, and resolving diarrhea. J Pediatr Gastroenterol Nutr. 1992; 15:112–116. [PubMed: 1403458]
47. Muller T, Wijmenga C, Phillips AD, et al. Congenital sodium diarrhea is an autosomal recessive disorder of sodium/proton exchange but unrelated to known candidate genes. Gastroenterology. 2000; 119:1506–1513. [PubMed: 11113072]
48. Heinz-Erian P, Muller T, Krabichler B, et al. Mutations in SPINT2 cause a syndromic form of congenital sodium diarrhea. Am J Hum Genet. 2009; 84:188–196. [PubMed: 19185281]
49. Fiskerstrand T, Arshad N, Haukanes BI, et al. Familial diarrhea syndrome caused by an activating GUCY2C mutation. N Engl J Med. 2012; 366:1586–1595. [PubMed: 22436048]
50. Muller T, Rasool I, Heinz-Erian P, et al. Congenital secretory diarrhoea caused by activating germline mutations in GUCY2C. Gut. 2016; 65:1306–1313. [PubMed: 25994218]
51. Arshad N, Visweswariah SS. The multiple and enigmatic roles of guanylyl cyclase C in intestinal homeostasis. FEBS Lett. 2012; 586:2835–2840. [PubMed: 22819815]
52. Chen T, Kocinsky HS, Cha B, et al. Cyclic GMP kinase II (cGKII) inhibits NHE3 by altering its trafficking and phosphorylating NHE3 at three required sites: identification of a multifunctional phosphorylation site. J Biol Chem. 2015; 290:1952–1965. [PubMed: 25480791]
53. Xu H, Chen R, Ghishan FK. Subcloning, localization, and expression of the rat intestinal sodium-hydrogen exchanger isoform 8. Am J Physiol Gastrointest Liver Physiol. 2005; 289:G36–G41. [PubMed: 15731506]

54. Baum M, Martin MG, Booth IW, et al. Nucleotide sequence of the Na+/H+ exchanger-8 in patients with congenital sodium diarrhea. J Pediatr Gastroenterol Nutr. 2011; 53:474–477. [PubMed: 21666503]
55. Thiagarajah JR, Donowitz M, Verkman AS. Secretory diarrhoea: mechanisms and emerging therapies. Nat Rev Gastroenterol Hepatol. 2015; 12:446–457. [PubMed: 26122478]
56. Subramanya SB, Rajendran VM, Srinivasan P, et al. Differential regulation of cholera toxin-inhibited Na-H exchange isoforms by butyrate in rat ileum. Am J Physiol Gastrointest Liver Physiol. 2007; 293:G857–G863. [PubMed: 17690171]
57. Zachos NC, Tse M, Donowitz M. Molecular physiology of intestinal Na+/H+ exchange. Annu Rev Physiol. 2005; 67:411–443. [PubMed: 15709964]
58. Cha B, Donowitz M. The epithelial brush border Na+/H+ exchanger NHE3 associates with the actin cytoskeleton by binding to ezrin directly and via PDZ domain-containing Na+/H+ exchanger regulatory factor (NHERF) proteins. Clin Exp Pharmacol Physiol. 2008; 35:863–871. [PubMed: 18430067]
59. Khurana S, Ganguly NK, Khullar M, et al. Studies on the mechanism of Salmonella typhimurium enterotoxin-induced diarrhoea. Biochim Biophys Acta. 1991; 1097:171–176. [PubMed: 1932140]
60. Kaur T, Singh S, Gorowara S, et al. Role of enteric nervous system in Shigella dysenteriae type 1 toxin-induced fluid secretion in rabbit ileum. J Diarrhoeal Dis Res. 1995; 13:159–165. [PubMed: 8568191]
61. Kanwar RK, Ganguly NK, Kanwar JR, et al. Impairment of Na+,K(+)-ATPase activity following enterotoxigenic Campylobacter jejuni infection: changes in Na+, Cl- and 3-O-methyl-D-glucose transport in vitro, in rat ileum. FEMS Microbiol Lett. 1994; 124:381–385. [PubMed: 7851745]
62. Hayashi H, Szaszi K, Coady-Osberg N, et al. Inhibition and redistribution of NHE3, the apical Na +/H+ exchanger, by Clostridium difficile toxin B. J Gen Physiol. 2004; 123:491–504. [PubMed: 15078917]
63. Lee JS, Lee YM, Kim JY, et al. BetaPix up-regulates Na+/H+ exchanger 3 through a Shank2-mediated protein-protein interaction. J Biol Chem. 2010; 285:8104–8113. [PubMed: 20080968]
64. Engevik MA, Engevik KA, Yacyshyn MB, et al. Human Clostridium difficile infection: inhibition of NHE3 and microbiota profile. Am J Physiol Gastrointest Liver Physiol. 2015; 308:G497–G509. [PubMed: 25552580]
65. Lapointe TK, O'Connor PM, Buret AG. The role of epithelial malfunction in the pathogenesis of enteropathogenic E. coli-induced diarrhea. Lab Invest. 2009; 89:964–970. [PubMed: 19620958]
66. Hecht G, Hodges K, Gill RK, et al. Differential Regulation of Na+/H+ Exchange Isoform Activities by Enteropathogenic E. coli in Human Intestinal Epithelial Cells. Am J Physiol Gastrointest Liver Physiol. 2004
67. Hodges K, Alto NM, Ramaswamy K, et al. The enteropathogenic Escherichia coli effector protein EspF decreases sodium hydrogen exchanger 3 activity. Cell Microbiol. 2008; 10:1735–1745. [PubMed: 18433466]
68. Chen M, Sultan A, Cinar A, et al. Loss of PDZ-adaptor protein NHERF2 affects membrane localization and cGMP- and [Ca2+]- but not cAMP-dependent regulation of Na+/H+ exchanger 3 in murine intestine. J Physiol. 2010; 588:5049–5063. [PubMed: 20962002]
69. Vaandrager AB, Schulz S, De Jonge HR, et al. Guanylyl cyclase C is an N-linked glycoprotein receptor that accounts for multiple heat-stable enterotoxin-binding proteins in the intestine. J Biol Chem. 1993; 268:2174–2179. [PubMed: 8093618]
70. Cha B, Kim JH, Hut H, et al. cGMP inhibition of Na+/H+ antiporter 3 (NHE3) requires PDZ domain adapter NHERF2, a broad specificity protein kinase G-anchoring protein. J Biol Chem. 2005; 280:16642–16650. [PubMed: 15722341]
71. Schultheis PJ, Clarke LL, Meneton P, et al. Renal and intestinal absorptive defects in mice lacking the NHE3 Na+/H+ exchanger. Nat Genet. 1998; 19:282–285. [PubMed: 9662405]
72. Ledoussal C, Woo AL, Miller ML, et al. Loss of the NHE2 Na(+)/H(+) exchanger has no apparent effect on diarrheal state of NHE3-deficient mice. Am J Physiol Gastrointest Liver Physiol. 2001; 281:G1385–G1396. [PubMed: 11705743]

73. Nighot PK, Moeser A, Ali RA, et al. Astrovirus infection induces sodium malabsorption and redistributes sodium hydrogen exchanger expression. Virology. 2010; 401:146–154. [PubMed: 20219227]
74. Baetz NW, Gupta A, Kapoor A, et al. Rotavirus Infection in Patients Is Associated With Altered Trafficking of Apical Membrane Transport Proteins. Gastroenterology. 2016; 150:S113–S114.
75. Chen XZ, Coady MJ, Jackson F, et al. Thermodynamic determination of the Na+: glucose coupling ratio for the human SGLT1 cotransporter. Biophys J. 1995; 69:2405–2414. [PubMed: 8599647]
76. Loo DD, Zeuthen T, Chandy G, et al. Cotransport of water by the Na+/glucose cotransporter. Proc Natl Acad Sci U S A. 1996; 93:13367–13370. [PubMed: 8917597]
77. Hirschhorn N, Kinzie JL, Sachar DB, et al. Decrease in net stool output in cholera during intestinal perfusion with glucose-containing solutions. N Engl J Med. 1968; 279:176–181. [PubMed: 4968807]
78. Lin R, Murtazina R, Cha B, et al. 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. Gastroenterology. 2011; 140:560–571. [PubMed: 20977906]
79. Naveh Y, Lightman A, Zinder O. Effect of diarrhea on serum zinc concentrations in infants and children. J Pediatr. 1982; 101:730–732. [PubMed: 7131147]
80. Ghishan FK. Transport of electrolytes, water, and glucose in zinc deficiency. J Pediatr Gastroenterol Nutr. 1984; 3:608–612. [PubMed: 6090631]
81. Bhatnagar S, Bahl R, Sharma PK, et al. Zinc with oral rehydration therapy reduces stool output and duration of diarrhea in hospitalized children: a randomized controlled trial. J Pediatr Gastroenterol Nutr. 2004; 38:34–40. [PubMed: 14676592]
82. Hoque KM, Rajendran VM, Binder HJ. Zinc inhibits cAMP-stimulated Cl secretion via basolateral K-channel blockade in rat ileum. Am J Physiol Gastrointest Liver Physiol. 2005; 288:G956–G963. [PubMed: 15618279]
83. Hoque KM, Sarker R, Guggino SE, et al. A new insight into pathophysiological mechanisms of zinc in diarrhea. Ann N Y Acad Sci. 2009; 1165:279–284. [PubMed: 19538317]
84. Ramakrishna BS, Subramanian V, Mohan V, et al. A randomized controlled trial of glucose versus amylase resistant starch hypo-osmolar oral rehydration solution for adult acute dehydrating diarrhea. PLoS One. 2008; 3:e1587. [PubMed: 18270575]
85. Krishnan S, Rajendran VM, Binder HJ. Apical NHE isoforms differentially regulate butyrate-stimulated Na absorption in rat distal colon. Am J Physiol Cell Physiol. 2003; 285:C1246–C1254. [PubMed: 12878493]
86. Kiela PR, Kuscuoglu N, Midura AJ, et al. Molecular mechanism of rat NHE3 gene promoter regulation by sodium butyrate. Am J Physiol Cell Physiol. 2007; 293:C64–C74. [PubMed: 17344314]
87. Amin MR, Dudeja PK, Ramaswamy K, et al. Involvement of Sp1 and Sp3 in differential regulation of human NHE3 promoter activity by sodium butyrate and IFN-gamma/TNF-alpha. Am J Physiol Gastrointest Liver Physiol. 2007; 293:G374–G382. [PubMed: 17540780]
88. Xu H, McCoy A, Li J, et al. Sodium butyrate stimulates NHE8 expression via its role on activating NHE8 basal promoter activity. Am J Physiol Gastrointest Liver Physiol. 2015; 309:G500–G505. [PubMed: 26159698]
89. Ramakrishna BS, Venkataraman S, Srinivasan P, et al. Amylase-resistant starch plus oral rehydration solution for cholera. N Engl J Med. 2000; 342:308–313. [PubMed: 10655529]
90. Raghupathy P, Ramakrishna BS, Oommen SP, et al. Amylase-resistant starch as adjunct to oral rehydration therapy in children with diarrhea. J Pediatr Gastroenterol Nutr. 2006; 42:362–368. [PubMed: 16641573]
91. Binder HJ, Brown I, Ramakrishna BS, et al. Oral rehydration therapy in the second decade of the twenty-first century. Curr Gastroenterol Rep. 2014; 16:376. [PubMed: 24562469]
92. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012; 149:274–293. [PubMed: 22500797]
93. Yang J, Zhao X, Patel A, et al. Rapamycin Inhibition of mTOR Reduces Levels of the Na+/H+ Exchanger 3 in Intestines of Mice and Humans, Leading to Diarrhea. Gastroenterology. 2015; 149:151–162. [PubMed: 25836987]

94. Gutierrez A, Scharl M, Sempere L, et al. Genetic susceptibility to increased bacterial translocation influences the response to biological therapy in patients with Crohn's disease. Gut. 2014; 63:272–280. [PubMed: 23376290]
95. Ghishan FK, Kiela PR. Epithelial transport in inflammatory bowel diseases. Inflamm Bowel Dis. 2014; 20:1099–1109. [PubMed: 24691115]
96. Seidler U, Lenzen H, Cinar A, et al. Molecular mechanisms of disturbed electrolyte transport in intestinal inflammation. Ann N Y Acad Sci. 2006; 1072:262–275. [PubMed: 17057206]
97. Harris J, Shields R. Absorption and secretion of water and electrolytes by the intact human colon in diffuse untreated proctocolitis. Gut. 1970; 11:27–33. [PubMed: 5435265]
98. Rask-Madsen J, Hammersgaard EA, Knudsen E. Rectal electrolyte transport and mucosal permeability in ulcerative colitis and Crohn's disease. J Lab Clin Med. 1973; 81:342–353. [PubMed: 4631401]
99. Sandle GI, Higgs N, Crowe P, et al. Cellular basis for defective electrolyte transport in inflamed human colon. Gastroenterology. 1990; 99:97–105. [PubMed: 2344946]
100. Greig ER, Boot-Handford RP, Mani V, et al. Decreased expression of apical Na+ channels and basolateral Na+, K+-ATPase in ulcerative colitis. J Pathol. 2004; 204:84–92. [PubMed: 15307141]
101. Amasheh S, Barmeyer C, Koch CS, et al. Cytokine-dependent transcriptional down-regulation of epithelial sodium channel in ulcerative colitis. Gastroenterology. 2004; 126:1711–1720. [PubMed: 15188166]
102. Sandle GI. Pathogenesis of diarrhea in ulcerative colitis: new views on an old problem. J Clin Gastroenterol. 2005; 39:S49–S52. [PubMed: 15758659]
103. Rocha F, Musch MW, Lishanskiy L, et al. IFN-gamma downregulates expression of Na(+)/H(+) exchangers NHE2 and NHE3 in rat intestine and human Caco-2/bbe cells. Am J Physiol Cell Physiol. 2001; 280:C1224–C1232. [PubMed: 11287336]
104. Barmeyer C, Harren M, Schmitz H, et al. Mechanisms of diarrhea in the interleukin-2-deficient mouse model of colonic inflammation. Am J Physiol Gastrointest Liver Physiol. 2004; 286:G244–G252. [PubMed: 14715519]
105. Sullivan S, Alex P, Dassopoulos T, et al. Downregulation of sodium transporters and NHERF proteins in IBD patients and mouse colitis models: potential contributors to IBD-associated diarrhea. Inflamm Bowel Dis. 2009; 15:261–274. [PubMed: 18942765]
106. Siddique I, Hasan F, Khan I. Suppression of Na+/H+ exchanger isoform-3 in human inflammatory bowel disease: lack of reversal by 5'-aminosalicylate treatment. Scand J Gastroenterol. 2009; 44:56–64. [PubMed: 18785066]
107. Lenzen H, Lunnemann M, Bleich A, et al. Downregulation of the NHE3-binding PDZ-adaptor protein PDZK1 expression during cytokine-induced inflammation in interleukin-10-deficient mice. PLoS One. 2012; 7:e40657. [PubMed: 22848392]
108. Yeruva S, Farkas K, Hubricht J, et al. Preserved Na(+)/H(+) exchanger isoform 3 expression and localization, but decreased NHE3 function indicate regulatory sodium transport defect in ulcerative colitis. Inflamm Bowel Dis. 2010; 16:1149–1161. [PubMed: 20027604]
109. Farkas K, Yeruva S, Rakonczay Z Jr, et al. New therapeutic targets in ulcerative colitis: The importance of ion transporters in the human colon. Inflamm Bowel Dis. 2010
110. Clayburgh DR, Musch MW, Leitges M, et al. Coordinated epithelial NHE3 inhibition and barrier dysfunction are required for TNF-mediated diarrhea in vivo. J Clin Invest. 2006; 116:2682–2694. [PubMed: 17016558]
111. Xu H, Chen H, Dong J, et al. Gastrointestinal distribution and kinetic characterization of the sodium-hydrogen exchanger isoform 8 (NHE8). Cell Physiol Biochem. 2008; 21:109–116. [PubMed: 18209477]
112. Goyal S, Mentone S, Aronson PS. Immunolocalization of NHE8 in rat kidney. Am J Physiol Renal Physiol. 2005; 288:F530–F538. [PubMed: 15522984]
113. Lawrence SP, Bright NA, Luzio JP, et al. The sodium/proton exchanger NHE8 regulates late endosomal morphology and function. Mol Biol Cell. 2010; 21:3540–3551. [PubMed: 20719963]
114. Wang A, Li J, Zhao Y, et al. Loss of NHE8 expression impairs intestinal mucosal integrity. Am J Physiol Gastrointest Liver Physiol. 2015; 309:G855–G864. [PubMed: 26505975]

115. Xu H, Li Q, Zhao Y, et al. Intestinal NHE8 is highly expressed in goblet cells and its expression is subject to TNF-alpha regulation. Am J Physiol Gastrointest Liver Physiol. 2016; 310:G64–G69. [PubMed: 26564720]
116. Khan I, al-Awadi FM, Abul H. Colitis-induced changes in the expression of the Na+/H+ exchanger isoform NHE-1. J Pharmacol Exp Ther. 1998; 285:869–875. [PubMed: 9580638]
117. Nemeth ZH, Deitch EA, Szabo C, et al. Na+/H+ exchanger blockade inhibits enterocyte inflammatory response and protects against colitis. Am J Physiol Gastrointest Liver Physiol. 2002; 283:G122–G132. [PubMed: 12065299]
118. Khan I, Oriowo MA, Anim JT. Amelioration of experimental colitis by Na-H exchanger-1 inhibitor amiloride is associated with reversal of IL-1ss and ERK mitogen-activated protein kinase. Scand J Gastroenterol. 2005; 40:578–585. [PubMed: 16036511]
119. Khan I, Siddique I, Al-Awadi FM, et al. Role of Na+/H+ exchanger isoform-1 in human inflammatory bowel disease. Can J Gastroenterol. 2003; 17:31–36. [PubMed: 12560852]
120. Siddique I, Khan I. Mechanism of regulation of Na-H exchanger in inflammatory bowel disease: role of TLR-4 signaling mechanism. Dig Dis Sci. 2011; 56:1656–1662. [PubMed: 21221801]
121. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO Rep. 2006; 7:688–693. [PubMed: 16819463]
122. Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. Nature. 2011; 473:174–180. [PubMed: 21508958]
123. Thaiss CA, Zmora N, Levy M, et al. The microbiome and innate immunity. Nature. 2016; 535:65–74. [PubMed: 27383981]
124. Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. Nature. 2016; 535:75–84. [PubMed: 27383982]
125. Engevik MA, Yacyshyn MB, Engevik KA, et al. Human Clostridium difficile infection: altered mucus production and composition. Am J Physiol Gastrointest Liver Physiol. 2015; 308:G510–G524. [PubMed: 25552581]
126. Milani C, Ticinesi A, Gerritsen J, et al. Gut microbiota composition and Clostridium difficile infection in hospitalized elderly individuals: a metagenomic study. Sci Rep. 2016; 6:25945. [PubMed: 27166072]
127. Yamamoto M, Matsumoto S. Gut microbiota and colorectal cancer. Genes Environ. 2016; 38:11. [PubMed: 27350830]
128. Forbes JD, Van Domselaar G, Bernstein CN. The Gut Microbiota in Immune-Mediated Inflammatory Diseases. Front Microbiol. 2016; 7:1081. [PubMed: 27462309]
129. Sonnenburg JL, Backhed F. Diet-microbiota interactions as moderators of human metabolism. Nature. 2016; 535:56–64. [PubMed: 27383980]
130. Li Q, Zhou JM. The microbiota-gut-brain axis and its potential therapeutic role in autism spectrum disorder. Neuroscience. 2016; 324:131–139. [PubMed: 26964681]
131. Gacias M, Gaspari S, Santos PM, et al. Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. Elife. 2016:5.
132. Luna RA, Savidge TC, Williams KC. The Brain-Gut-Microbiome Axis: What Role Does It Play in Autism Spectrum Disorder? Curr Dev Disord Rep. 2016; 3:75–81. [PubMed: 27398286]
133. Yarandi SS, Peterson DA, Treisman GJ, et al. Modulatory Effects of Gut Microbiota on the Central Nervous System: How Gut Could Play a Role in Neuropsychiatric Health and Diseases. J Neurogastroenterol Motil. 2016; 22:201–212. [PubMed: 27032544]
134. Ott SJ, Musfeldt M, Wenderoth DF, et al. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut. 2004; 53:685–693. [PubMed: 15082587]
135. Miyoshi J, Chang EB. The gut microbiota and inflammatory bowel diseases. Transl Res. 2016
136. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013; 504:446–450. [PubMed: 24226770]
137. Garrett WS, Gallini CA, Yatsunenko T, et al. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host Microbe. 2010; 8:292–300. [PubMed: 20833380]

138. Morampudi V, Dalwadi U, Bhinder G, et al. The goblet cell-derived mediator RELM-beta drives spontaneous colitis in Muc2-deficient mice by promoting commensal microbial dysbiosis. Mucosal Immunol. 2016
139. Devkota S, Wang Y, Musch MW, et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10−/− mice. Nature. 2012; 487:104–108. [PubMed: 22722865]
140. Garrett WS, Lord GM, Punit S, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. Cell. 2007; 131:33–45. [PubMed: 17923086]
141. Du Z, Hudcovic T, Mrazek J, et al. Development of gut inflammation in mice colonized with mucosa-associated bacteria from patients with ulcerative colitis. Gut Pathog. 2015; 7:32. [PubMed: 26697117]
142. Laubitz D, Larmonier CB, Bai A, et al. Colonic gene expression profile in NHE3-deficient mice: evidence for spontaneous distal colitis. Am J Physiol Gastrointest Liver Physiol. 2008; 295:G63–G77. [PubMed: 18467500]
143. Larmonier CB, Laubitz D, Hill FM, et al. Reduced colonic microbial diversity is associated with colitis in NHE3-deficient mice. Am J Physiol Gastrointest Liver Physiol. 2013; 305:G667–G677. [PubMed: 24029465]
144. Johansson ME, Gustafsson JK, Holmen-Larsson J, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. Gut. 2014; 63:281–291. [PubMed: 23426893]
145. Ambort D, Johansson ME, Gustafsson JK, et al. Calcium and pH-dependent packing and release of the gel-forming MUC2 mucin. Proc Natl Acad Sci U S A. 2012; 109:5645–5650. [PubMed: 22451922]
146. Kiela PR, Laubitz D, Larmonier CB, et al. Changes in mucosal homeostasis predispose NHE3 knockout mice to increased susceptibility to DSS-induced epithelial injury. Gastroenterology. 2009; 137:965–975. 975 e1–10. [PubMed: 19450596]
147. Larmonier CB, Laubitz D, Thurston RD, et al. NHE3 modulates the severity of colitis in IL-10-deficient mice. Am J Physiol Gastrointest Liver Physiol. 2011; 300:G998–G1009. [PubMed: 21415416]
148. Laubitz D, Harrison CA, Midura-Kiela MT, et al. Reduced Epithelial Na+/H+ Exchange Drives Gut Microbial Dysbiosis and Promotes Inflammatory Response in T Cell-Mediated Murine Colitis. PLoS One. 2016; 11:e0152044. [PubMed: 27050757]
149. Engevik MA, Aihara E, Montrose MH, et al. Loss of NHE3 alters gut microbiota composition and influences Bacteroides thetaiotaomicron growth. Am J Physiol Gastrointest Liver Physiol. 2013; 305:G697–G711. [PubMed: 24072680]
150. Hickey CA, Kuhn KA, Donermeyer DL, et al. Colitogenic Bacteroides thetaiotaomicron Antigens Access Host Immune Cells in a Sulfatase-Dependent Manner via Outer Membrane Vesicles. Cell Host Microbe. 2015; 17:672–680. [PubMed: 25974305]
151. Xu H, Chen H, Dong J, et al. Tumor necrosis factor-{alpha} downregulates intestinal NHE8 expression by reducing basal promoter activity. Am J Physiol Cell Physiol. 2009; 296:C489–C497. [PubMed: 19109523]
152. Liu C, Xu H, Zhang B, et al. NHE8 plays an important role in mucosal protection via its effect on bacterial adhesion. Am J Physiol Cell Physiol. 2013; 305:C121–C128. [PubMed: 23657568]
153. Zachos NC, van Rossum DB, Murtazina R, et al. A Peptide Mimicking the NHE3 C-Terminus Stimulates Basal and Blocks CA2+ and cAMP Inhibition of NHE3 Activity, and Prevents Cholera Toxin-Induced Mouse Jejunal Fluid Accumulation: Potential Role as Drug Therapy for Diarrhea. Gastroenterology. 2012; 142:S–1.
154. Oparil S, Schmieder RE. New approaches in the treatment of hypertension. Circ Res. 2015; 116:1074–1095. [PubMed: 25767291]
155. Feyh A, Bracero L, Lakhani HV, et al. Role of Dietary Components in Modulating Hypertension. J Clin Exp Cardiolog. 2016:7.
156. Kwan MW, Wong MC, Wang HH, et al. Compliance with the Dietary Approaches to Stop Hypertension (DASH) diet: a systematic review. PLoS One. 2013; 8:e78412. [PubMed: 24205227]

157. Spencer AG, Labonte ED, Rosenbaum DP, et al. Intestinal inhibition of the Na+/H+ exchanger 3 prevents cardiorenal damage in rats and inhibits Na+ uptake in humans. Sci Transl Med. 2014; 6:227ra36.

158. Linz D, Wirth K, Linz W, et al. Antihypertensive and laxative effects by pharmacological inhibition of sodium-proton-exchanger subtype 3-mediated sodium absorption in the gut. Hypertension. 2012; 60:1560–1567. [PubMed: 23108657]

159. Johansson S, Rosenbaum DP, Knutsson M, et al. A phase 1 study of the safety, tolerability, pharmacodynamics, and pharmacokinetics of tenapanor in healthy Japanese volunteers. Clin Exp Nephrol. 2016

160. Zielinska M, Wasilewski A, Fichna J. Tenapanor hydrochloride for the treatment of constipation-predominant irritable bowel syndrome. Expert Opin Investig Drugs. 2015; 24:1093–1099.

161. Labonte ED, Carreras CW, Leadbetter MR, et al. Gastrointestinal Inhibition of Sodium-Hydrogen Exchanger 3 Reduces Phosphorus Absorption and Protects against Vascular Calcification in CKD. J Am Soc Nephrol. 2015; 26:1138–1149. [PubMed: 25404658]

162. Cil O, Phuan PW, Lee S, et al. CFTR activator increases intestinal fluid secretion and normalizes stool output in a mouse model of constipation. Cell Mol Gastroenterol Hepatol. 2016; 2:317–327. [PubMed: 27127798]

SUMMARY

Epithelial Na^+/H^+ exchange (NHE) is a pleiotropic membrane transport mechanism which participates in the intestinal NaCl transport, in the regulation of basic cellular functions and the extracellular milieu to facilitate other nutrient absorption and to regulate gut microbial microenvironment. In this review, we summarize the findings describing the basic roles of NHE and the consequences of its dysregulation in the pathogenesis of gastrointestinal disorders.

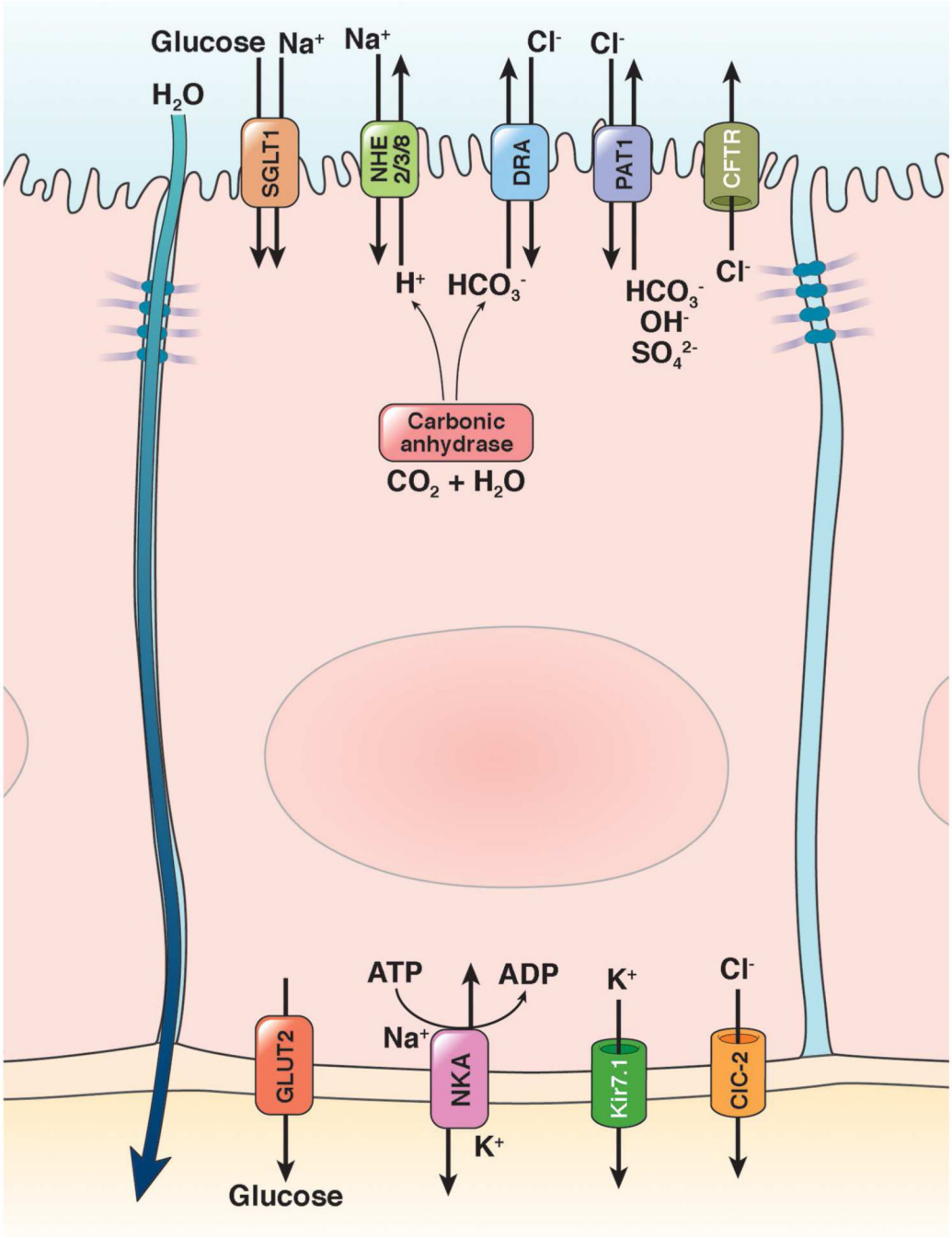

Figure 1. Simplified depiction of the apical Na^+/H^+ exchange in transepithelial sodium, glucose, and water absorption

The inward Na^+ gradient (low intracellular [Na^+]) is maintained by the basolateral Na^+/K^+-ATPase (NKA), which provides the driving force for several secondary active transporters membrane transport proteins that import glucose, amino acids (not shown), and other nutrients into the cell by use of the sodium gradient. The intracellular concentration of potassium is coordinated by basolateral efflux via Kir7.1 (Potassium inwardly-rectifying channel, subfamily J, member 13, KCNJ13). Electroneutral NaCl absorption is driven by parallel Na^+/H^+ and Cl^-/HCO_3^- exchange, the latter mediated by DRA (Down-regulated in

adenoma, SLC26A3), and PAT1 (putative anion transporter 1, SLC26A6). This coupled activity is made possible by carbonic anhydrase, which provides intracellular HCO_3^-. NHEs are cross-regulated with SLC26 family members and the Cl^- and HCO_3 transporting cystic fibrosis transmembrane conductance regulator (CFTR). In addition to Na^+/D-glucose apical transport, SGLT1 activity stimulates NHE3 via an increased amount of apical NHE3 (see section on ORS). GLUT2 (Glucose transporter 2, SLC2A2) provide a basolateral exit route for glucose. ClC-2 (Chloride channel protein 2, CLCN2) is capable of basolateral Cl^- secretion, though its key role appears to be the regulation of intestinal barrier function by altering tight junction composition and recovery from injury. Additional route of electrogenic Na^+ transport is provided by apical Na^+ channels (ENaC; not shown)

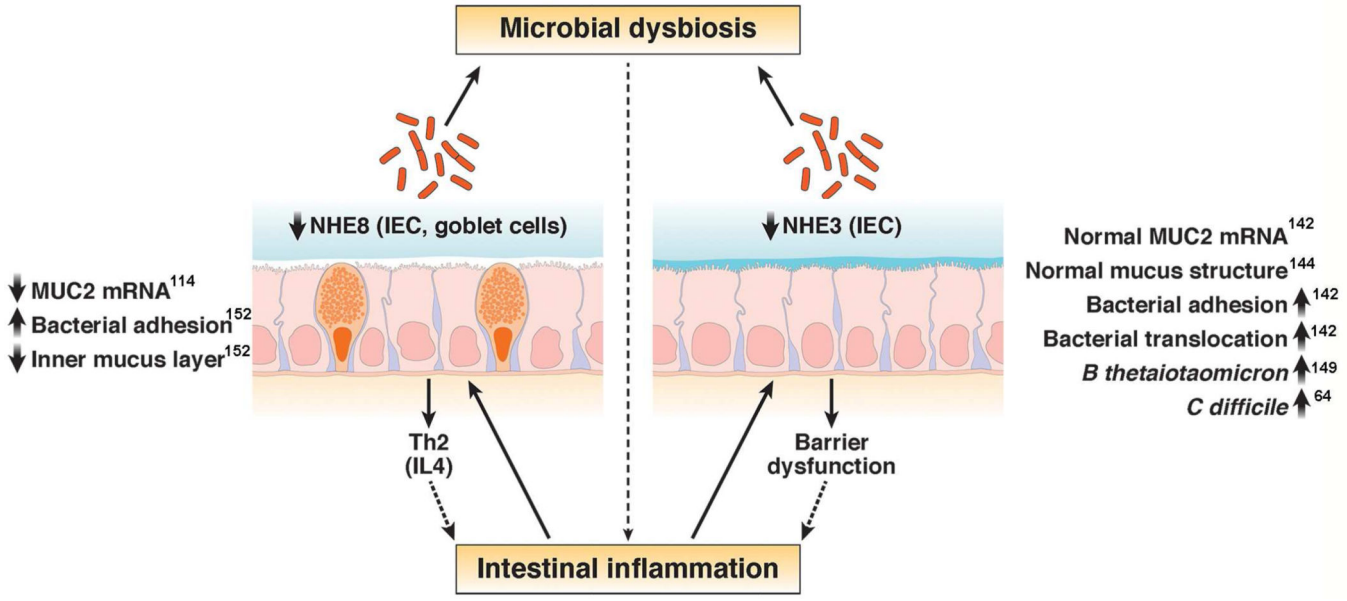

Figure 2.

Summary graph illustrating the roles of NHE3 and NHE8 and the consequences of their inhibition during mucosal inflammation.