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APICAL NA⁺/H⁺ EXCHANGERS IN THE MAMMALIAN GASTROINTESTINAL TRACT

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The Slc9a family of nine Na⁺/H⁺ exchangers (NHE) plays a critical role in neutral sodium absorption in the mammalian intestine as well as other absorptive and secretory epithelia of digestive organs. These transport proteins mediate the electroneutral exchange of Na⁺ and H⁺ and are crucial in a variety of physiological processes, including the fine tuning of intracellular pH, cell volume control and systemic electrolyte, acid-base and fluid volume homeostasis. Here, we review the role of the Na⁺/H⁺ exchange mechanism as it relates to the physiology of organs and cells involved in nutrient absorption, and we describe physiological and molecular aspects of individual isoforms, including their structure, tissue-, and subcellular distribution, as well as their regulation by physiological stimuli at the transcriptional and post-transcriptional levels. A particular emphasis is placed on Na⁺/H⁺ exchanger isoforms expressed on the apical (brush border) membrane of the epithelial cells, and the consequences of gene-targeted mutation of individual isoforms are discussed in the context of the physiology of digestive organs. Where available, we also provide a review of pathophysiological states related to aberrant expression and/or activity of Na⁺/H⁺ exchangers within the confines of the digestive system.

Key words: Na⁺/H⁺ exchangers, absorption, brush border, postnatal development

INTRODUCTION TO MAMMALIAN NA⁺/H⁺ EXCHANGER GENE FAMILY

The existence of a Na⁺/H⁺ exchange mechanism in mammalian membranes was suggested nearly forty years ago, first by Mitchell and Moyle (1) and Brierley et al. (2) in mammalian membranes, and later by Harold and Papineau (3) and by West and Mitchell (4) in prokaryotic plasma membrane. In 1987, Goldberg et al. (5) cloned the first Na⁺/H⁺ antiporter from E. Coli, later termed
nhaA. Its mammalian counterpart was cloned shortly after by Sardet et al. (6). Cloning of this basolateral Na⁺/H⁺ exchanger, initially described as growth factor-activatable Na⁺/H⁺ antiporter, later termed NHE1 and considered a prototypical mammalian Na⁺/H⁺ exchanger, initiated the explosion of knowledge about function, structure and regulation of what was soon found to be a family of nine proteins involved in membrane Na⁺/H⁺ antiport mechanism, termed NHE1 through NHE9. Families of Na⁺/H⁺ exchangers were identified in bacteria, yeast, plants and animals and implicated in numerous physiological processes such as transepithelial ion transport, intracellular and organellar pH and volume regulation, and regulation of cell proliferation and apoptosis.

This review focuses primarily on the structure, function and regulation of the Na⁺/H⁺ exchangers expressed on the apical membrane of the mammalian gastrointestinal tract epithelia and involved in vectorial transepithelial sodium transport. The reader is referred to the following publications (7-10) and (11, 12) for an overview or original articles on plants, Caenorhabditis elegans, and procaryotic Na⁺/H⁺ antiport, respectively. Comprehensive reviews on mammalian Na⁺/H⁺ exchange were recently published by Orlowski & Grinstein (13), by Zachos et al. (14) and by Kiela and Ghishan (15), the latter two with a particular emphasis on intestinal Na⁺/H⁺ exchange. Phylogenetic analysis of evolutionary relations among NHE sequences from all phyla, including cloned genes and sequences electronically annotated based on sequence similarity, has been recently published by Brett, Donowitz and Rao (16).

The nine proteins forming the family of mammalian Na⁺/H⁺ exchangers demonstrate considerable variation in their amino-acid sequence, ranging from under 12% (hNHE1 vs. hNHE9) to over 70% identity (hNHE6 vs. hNHE7) (15). With the exception of NHE5, all known NHE isoforms have been detected in the gastrointestinal tract with segmental differences, crypt-villus gradients of expression, and different cellular localizations, all of which determines their demonstrated or alleged functions. Na⁺/H⁺ exchangers expressed on the plasma membrane of the gastrointestinal epithelia include NHE1-4, NHE6 and NHE8. Of those, NHE6 was found to appear on the plasma membrane only transiently at a temperature non-permissive for endocytosis (4°C, (17)) while under physiological conditions it resides primarily in the recycling compartment of the endoplasmic reticulum (17). Ubiquitously expressed NHE1 is consistently expressed on the basolateral membrane where it is believed to play housekeeping roles to regulate intracellular pH and cell volume (18). NHE4 represents another basolateral NHE isoform, with weaker specificity as a cation/H⁺ exchanger (19), and is expressed primarily in the parietal, chief and to a lesser extent in the mucous cells of the gastric gland epithelium (20, 21). It has been postulated that NHE4 is coupled with the basolateral Cl⁻/HCO₃⁻ exchanger AE2, and that it plays a critical role in regulating gastric acid secretion and foveolar epithelial cell differentiation (22). In this review we describe the two Na⁺/H⁺ exchangers expressed primarily on the brush border membrane of the intestinal epithelial cells, NHE2 and NHE3, and
discuss the expression and an emerging role of another apical Na\(^{+}\)/H\(^{+}\) exchanger isoform, NHE8, in the intestinal electrolyte and water absorption.

**NHE2**

NHE2 was first cloned from rat and rabbit intestinal cDNA libraries by Collins *et al.* and Wang *et al.* (23, 24) and by Tse *et al.* (25), respectively. Human NHE2 was cloned by Ghishan *et al.* (26), and later corrected by Malakooti *et al.* (27). NHE2 protein shares the most similarity with NHE4, especially within the cytoplasmic C-terminus. Interestingly, in the human, rat, and mouse, the Slc9a2 and Slc9a4 genes co-segregate on chromosomes 2, 9, and 1, respectively (28, 29). The adjacent chromosomal location of the two NHEs in all three species strongly suggests that they arose by gene duplication early in the evolution. The predicted molecular weights of NHE2 protein in rat, rabbit, and human are ~91 kDa, although its mobility on SDS-PAGE gels does not confirm these calculations. Mature rabbit NHE2, when expressed in NHE-deficient PS120 fibroblasts, was shown to be an *O*-linked sialoglycoprotein (30). In these studies, neuraminidase shifted the mobility of NHE2 protein from 85 kDa to 81 kDa, and *O*-glycanase further shifted the mobility of the 81 kDa protein to 75 kDa. Incubation of PS120/NHE2 cells with an *O*-glycosylation inhibitor benzyl N-acetyl-alpha-D-galactosaminide reduced the size of the 85 kDa protein to 81 kDa, although this was without consequence for the Na\(^{+}\)/H\(^{+}\) exchange activity in these cells (30).

NHE2 is expressed in the epithelia of all digestive organs, with particularly high expression in the proximal colon (21, 31-34). With the exception of gastric epithelium, NHE2 was unambiguously demonstrated on the apical membrane of polarized epithelial cells. In the intestinal epithelium, expression of NHE2 along the crypt-villus axis shows some species-dependent differences. In rabbits, NHE2 is present in the brush-border of the entire villus of the small intestine, in colonic surface cells, and in the apical membrane of the upper half of the crypt (34). In the mouse colon, however, NHE2 is predominantly expressed in the crypt cells (35, 36), suggesting a role for this isoform in cryptal pH and volume homeostasis.

**NHE3**

Rabbit and rat NHE3 were first cloned by Tse *et al.* and Orlowski *et al.*, respectively (37, 38). The second report also included partial cloning of a human ortholog, later fully cloned by Brant *et al.* (39), and mapped to chromosome 5p15.3 (40). NHE3 transcript codes for an 831-834 amino acid protein with a calculated molecular weight of ~93 kDa, with highest homology to NHE5 (51.3\%) and NHE2 (33.4\%). Based on the presence of potential *N*-glycosylation sites in the NHE3 protein of all three species, it was at first believed to be a glycoprotein. Glycosylation of NHE3 may, however, be species-specific. Rabbit
and pig renal NHE3 were shown to be glycosylated and sensitive to glycopeptidase F and general N-linked glycosylation inhibitor, tunicamycin (41, 42), while glycosylation of rat or canine NHE3 was not detected (42, 43). The functional consequences of glycosylation are not clear. In vivo inhibition of N-glycosylation in tunicamycin-treated LLC-PK cells significantly decreased NHE3 activity, as measured by pH-dependent $^{22}$Na uptake and by Na-dependent $\text{pH}_i$ recovery from an acid load (41). This decrease in tunicamycin-treated cells was accompanied by an intracellular accumulation of seemingly unglycosylated forms of NHE3 protein, and a conceivably compensatory 3-fold increase in NHE3 mRNA. Based on these studies, it has been postulated that glycosylation of porcine NHE3 plays a role in membrane trafficking and ultimately in NHE3 activity. In rabbits, however, deglycosylation of renal brush border protein did not impact acid-stimulated, amiloride-sensitive $^{22}$Na influx into the vesicles (42). Therefore, the physiological significance of NHE3 glycosylation is still unclear.

The secondary structure of NHE3 follows the general model for all members of the \textit{Slc9a} family and is discussed in more detail in the section on Membrane Topology.

The range of NHE3 gene expression in various tissues has been found to differ among species. In rabbits and rats, NHE3 is consistently expressed at high levels in the absorptive epithelia of kidney cortex, colon, and small intestine, with lower levels detected in the stomach, brain, and heart (37, 38), whereas human NHE3 is also expressed in relatively high levels in testes, ovaries, prostate, and thymus (39). Rat NHE3 is expressed in both acinar and ductal cells of the salivary glands (31), although its role in salivary secretions appears to be negligible. Expression of NHE3 in cholangiocytes and gallbladder epithelium implies a role for this isoform in bile formation, and possibly in pathogenesis of gallstones (44-46). Expression, cellular localization, and functional relevance of NHE3 in the gastric epithelium are somewhat controversial and are described in more detail in the section on the \textit{Na}$^+$/\textit{H}$^+$ exchange and gastric physiology.

Expression of NHE3 appears to be higher in the ileum than in other intestinal segments in both rabbits and humans (34, 47). In humans, NHE3 mRNA levels are also higher in the colon, where NHE3 is present at approximately the same levels in both the ascending and descending segment (47). In the small intestinal and colonic epithelium, NHE3 may be considered a marker for the absorptive epithelial cells, since it is expressed only in the villus or surface epithelium, and not in the crypts (34, 48). Ectopic expression of NHE3 has been demonstrated in the colonic crypts of NHE2$^{-/-}$ mice, where it may play a compensatory role in regulation of crypt cell volume and $\text{pH}_i$ (35). In polarized intestinal epithelial cells, the majority of NHE3 protein is localized to the apical membrane, where it can be found both on the microvilli as well as in the intervillus clefts (34). In Caco-2 cells, about 20% of total NHE3 protein is localized to a diffuse subapical pool, and recycling between plasma membrane and this endosomal compartment represents a mode of regulation of NHE3 via endocytosis/exocytosis (49, 50).
When expressed in AP-1 fibroblasts, ~90% of NHE3 protein was found in the juxtanuclear endomembrane vesicles, a pool further increased by inhibition of phosphatidylinositol 3'-kinase (PI3-K) (51). It has been suggested that the constitutive activity of PI3-K is important in the maintenance of the steady state level of NHE3 on the plasma membrane. NHE3 regulation via endosomal recycling is discussed in more detail in the section on post-transcriptional regulation.

In the brush border membrane of rabbit ileal enterocytes, NHE3 is equally split between a detergent-soluble and detergent-insoluble fractions, and a part of the latter fraction is present in cholesterol-enriched lipid microdomains (lipid rafts) (52). Li et al. demonstrated that the lipid raft pool and its association with actin cytoskeleton play an important role in regulation of NHE3 activity through endocytosis.

NHE8

NHE8 isoform was recently cloned from a mouse kidney cDNA library by Goyal et al. (53) and was described as a sequence encoding a 576 amino acid protein, sharing 96% identity with its likely human ortholog (NM_015266) (54). Human NHE8 protein shares less than 24% homology with other known NHE isoforms (15). Hydropathy analysis predicts membrane topology similar to the general models for all NHEs with 10-12 transmembrane domains in the N-terminal portion of the protein, followed by a relatively short (~100 amino acids) hydrophilic C-terminal tail (53). The molecular mass of NHE8 detected by Western blotting is ~85 kDa, significantly higher than the 64 kDa predicted from the length of open reading frame. Consistent with the prediction of four N-glycosylation sites, inhibition of glycosylation with tunicamycin reduced the size of detected protein (53).

Initial expression analysis indicated ubiquitous expression of NHE8 in mouse tissues with predominant expression in the liver, skeletal muscle, kidney, and testes (53). In the kidney, NHE8 mRNA was localized by in-situ hybridization primarily to the proximal tubules of the outer stripe of the outer medulla and to a lesser extent to the renal cortex, while NHE8 protein co-purified with brush-border membranes. Further immunolocalization studies determined that renal NHE8 is expressed on both microvillar surface membranes and the coated pit regions in the epithelial cells of proximal tubules (55). The co-localization of NHE8 with megalin in the intermicrovillar coated pits and sub-apical tubules suggests that similar to NHE3, NHE8 may be regulated by endocytic retrieval and recycling. Plasma membrane localization of NHE8 has not been confirmed in the heterologous expression system of human NHE8 in COS-7 cells, where NHE8 was convincingly shown to be expressed in the mid- to trans-Golgi compartments (54) and not on the plasma membrane. Although human NHE8 is expressed in the
liver, small intestine, and colon (54), its role in the gastrointestinal tract is not known. Recently, our laboratory described cloning of rat NHE8 cDNA (56). Polyclonal antibodies raised against both N- and C-terminal peptides detected NHE8 in the apical membrane of jejunal enterocytes both by western blotting and immunohistochemistry (56). The apparent discrepancies between subcellular localization of NHE8 in vitro and in vivo remain unresolved. They may represent true variation in protein trafficking, or may stem from methodological differences or differences in protein distribution in polarized epithelial cells and non-polarized fibroblast cell lines.

MEMBRANE TOPOLOGY AND FUNCTIONAL DOMAINS

Of the nine NHEs cloned to date, two-dimensional structure of NHE1 and NHE3 has been most extensively studied utilizing a variety of experimental approaches including cysteine substitution and accessibility (57), C-terminal truncation (58, 59), identification of glycosylation sites (30, 43), proteolytic cleavage (60), and epitope immunolocalization (51, 61-63) and has been discussed in more detail elsewhere (15). Modeling algorithms predicting hydrophobic and hydrophilic regions of the NHE proteins suggest the same general arrangement, with approximately 60% of the amino-terminal of the protein being amphipathic and forming 10-12 membrane spanning \( \alpha \)-helices. This configuration is relatively conserved among different isoforms. Much more hydrophilic and less conserved carboxyl-terminus faces the cytoplasm, and has been predicted or experimentally demonstrated to contain multiple phosphorylation sites and sites responsible for interaction with accessory proteins, all of which are believed to serve regulatory functions.

Vast majority of knowledge about the functional domains within the amphipathic region come from studies on NHE1. Other NHE isoforms have not been studied in such detail, although general location and functional consequences of the described functional domains are likely to be consistent among the Scl9a family. Transmembrane domain 4 (TM4) is crucial for NHE1 function, with the residues Phe\(^{161} \), Phe\(^{162} \), Leu\(^{163} \), and Gly\(^{173} \) affecting affinity for Na\(^+ \) and/or its resistance to inhibitors (64-66). Slepkov et al. recently showed that within this transmembrane helix, Pro\(^{167} \) and Pro\(^{168} \) are critical in NHE1 activity, expression, and membrane targeting (67). Within the seventh transmembrane domain (TM7), Glu\(^{262} \) and Asp\(^{267} \) are indispensable for NHE1 activity, with their charge and acidity being the most critical (68). Transmembrane domain 9 (TM9) contains a sequence conferring sensitivity to antagonists. A hybrid NHE1, in which this transmembrane helix has been replaced with analogous segment of the amiloride-resistant NHE3, was resistant to amiloride, ethylisopropylamiloride, HOE694, and cimetidine (69). Within this transmembrane domain, His\(^{349} \) may be one of the critical moieties bestowing
sensitivity to amiloride compounds, as described by Wang et al. (70). Mutation of amino acids Tyr<sup>454</sup> and Arg<sup>458</sup> within the eleventh transmembrane domain (TM11) has shown that both amino acids are essential in targeting NHE1 to the cell surface (71). TM11 and its neighboring intracellular loop (IL5) have also been implicated in pH sensing. Mutation of Gly<sup>455</sup> or Gly<sup>456</sup>, although it did not affect the protein’s affinity for Na<sup>+</sup> or H<sup>+</sup> ions, resulted in an alkaline shift in NHE1 pH-dependence. In contrast, mutation of Arg<sup>440</sup> in IL5 had the opposite effect. Based on these data, it has been postulated that both Arg<sup>440</sup> in IL5 and glycine residues in the conserved segment of TM11 constitute the putative pH<sub>i</sub> sensor in NHE1.

The long, hydrophilic cytosolic domain of NHE1 (amino acids 500–815), which regulates the activity of the amphipathic N-terminal domain, is a target for phosphorylation by protein kinases and participates in binding with regulatory proteins. The reader is referred to review articles published recently by Putney et al. (72), Slepkov and Fliegel (73), Baumgartner et al. (74), and references therein for more detailed descriptions of modes of NHE1 activity regulation. The role of the cytoplasmic C-terminal domain of NHE3 is described in a later section on post-transcriptional regulation of NHE3 activity.

TRANSPORT CHARACTERISTICS AND PHARMACOLOGY OF APICAL Na<sup>+</sup>/H<sup>+</sup> EXCHANGERS

Substrate specificity: The steady-state velocities of most NHE isoforms show a saturating, first-order dependence on the outside Na<sup>+</sup><sub>e</sub> concentration (with K<sub>Na</sub> values 3-50 mM), consistent with simple, saturating, Michaelis-Menten kinetics, which is indicative of a single binding site (75-77). Both NHE2 and NHE3 isoforms specifically transport Na<sup>+</sup> in exchange for H<sup>+</sup>, with much lower efficiency towards Li<sup>+</sup> or NH<sub>4</sub><sup>+</sup> and essentially no affinity for K<sup>+</sup>. NHE8, however, which is described as a both intracellular (54) and plasmalemmal isoform (55, 56), is also capable of K<sup>+</sup>/H<sup>+</sup> exchange when reconstituted in artificial liposomes (54). Contrary to a typical first-order dependence of exchange kinetics for Na<sup>+</sup>, hydrogen concentration dependence does not follow a simple Michaelis-Menten equation which assumes no cooperativity, but rather displays characteristics typical of allosteric effect, with more than one binding site for H<sup>+</sup>. The kinetic analysis of Na<sup>+</sup>/H<sup>+</sup> exchange appears to be in agreement with structural data suggesting a presence of a H<sup>+</sup> sensor in the 11<sup>th</sup> transmembrane domain, and an element regulating pH set-point located in the intracellular loop 5.

ATP dependence: Na<sup>+</sup>/H<sup>+</sup> exchangers have been categorized into the monovalent cation proton antiporter family (CPA1; 2.A.36) and represent electrochemical-potential-driven transporters. Cation fluxes via NHE mechanism are driven exclusively by the transmembrane gradients of substrates and are only secondarily dependent on ATP. Although NHE proteins do not bind or consume ATP directly, despite a maintained transmembrane H<sup>+</sup> gradient, depletion of
cellular ATP results in marked inhibition of NHE1, NHE2, and NHE3 activities (78). In the case of NHE1 and NHE2, ATP depletion reduces their sensitivity to intracellular pH (H<sup>i</sup>), while NHE3 demonstrates both impaired H<sup>i</sup> sensing as well as reduced maximal velocity of transport (V<sub>max</sub>). The precise mechanism of this interaction is not clear. It is plausible, however, that reduced interaction of these Na<sup>+</sup>/H<sup>+</sup> exchangers with plasmalemmal phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), may participate in this phenomenon.

**Cl<sup>-</sup> dependence:** Chloride-dependent Na<sup>+</sup>/H<sup>+</sup> exchange has been functionally identified in the epithelium of the rat distal colon (79-82). This phenomenon was postulated to be the result of functional coupling of the chloride channel to a novel NHE isoform, later cloned as a putative Cl<sup>-</sup>-dependent Na<sup>+</sup>/H<sup>+</sup> exchanger (82). Mouse studies failed, however, to demonstrate Cl<sup>-</sup>-dependent NHE in the colonic crypts (35, 36). Although a number of factors can account for the described discrepancies (e.g. species differences, site of analysis, or simply the use of different methodological approaches) one has to consider that Cl<sup>-</sup> dependence of the colonic Na<sup>+</sup>/H<sup>+</sup> exchange may not be a ubiquitous function of colonic crypt epithelia.

**NHE Inhibitors:** Pharmacology of NHE inhibitors was recently reviewed by Masereel et al. (83). Amiloride, a potassium-sparing diuretic, was the first described NHE inhibitor (84), which could also inhibit a electrogenic Na<sup>+</sup> channels and the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger. NHE1 and NHE2 isoforms are the most sensitive to amiloride inhibition (IC<sub>50</sub> ~1µM), whereas NHE3 is relatively amiloride-resistant with IC<sub>50</sub> >100µM. Development of several pyrazine or phenyl derivatives of amiloride increased their potency towards NHEs, particularly NHE1, and more importantly increased their selectivity by eliminating inhibition of the Na<sup>+</sup> channel and Na<sup>+</sup>/Ca<sup>2+</sup> exchanger. Of these molecules, DMA, EIPA, HOE-694, and HOE-642 are the most frequently used in experimental settings. Several NHE inhibitors based on a bicyclic template have recently been introduced, such as zoniporide, SM-20550, BMS-284640, T-162559, or TY-12533. Other compounds not related to amiloride have also proven useful, especially S-3226, as the first NHE3-specific inhibitor with IC<sub>50</sub> of 20 nM (85). Cimetidine, clonidine, and harmaline, although not frequently used, have also been reported to act as weak and non-specific inhibitors of Na<sup>+</sup>/H<sup>+</sup> exchange (86). The sensitivity of more recently described isoform, NHE8 is currently being evaluated in our laboratory.

**PHYSIOLOGICAL ROLES OF THE APICAL GASTROINTESTINAL NA<sup>+</sup>/H<sup>+</sup> EXCHANGERS**

**NHE2 and NHE3 in Gastric Physiology**

Interestingly, while in the small and large intestine, NHE2 and NHE3 are exclusively localized at the apical membrane of the enterocytes, they are both believed to be expressed at the basolateral site in gastric epithelium. Unusually
high sensitivity of NHE2 to extracellular pH led investigators to speculate that basolateral alkalinization, during stimulated acid secretion by parietal cells, results in increased basolateral NHE activity – likely mediated by NHE2. Increased NHE2 activity at the basolateral membrane in response to interstitial alkalinization could permit this isoform to participate in acid secretion, viability of parietal cells, and mucosal protection. Indeed, NHE2 homozygous knockout mice exhibited marked alterations in gastric mucosal histology and function. NHE2" mice demonstrate a loss of net acid secretion coinciding with a reduced number of parietal and chief cells (87). The reduction of the number of zymogenic cells may be secondary to decreased viability of parietal cells, which although develop normally, undergo premature necrosis (87). This process is accompanied by progressive inflammation in the form of diffuse corporal gastritis that ranges from transmural neutrophilic infiltration to a profound atrophy consistent with chronic achlorhydria, dependent on age and stage of inflammation (88).

Expression of the NHE3 isoform in the stomach is somewhat controversial. Although documented in the rat (38), human and guinea pig (89), it has not been detected in rabbit gastric mucosa (21, 37). Kulaksiz et al. (89) demonstrated NHE3 protein expression at the basolateral membrane of surface mucous cells in both human and guinea pig specimens. These findings were later challenged by functional studies in perfused isolated rat gastric glands, which demonstrated NHE3-like activity in the parietal cell apical membrane (90). No descriptions of gastric abnormalities were reported in studies on NHE3-deficient mice (91) therefore, the expression and physiological role of NHE3 in the gastric epithelium still remain obscure. The expression and potential role of NHE8 in gastric physiology has not yet been described.

Small Intestinal Na+/H+ Exchange

Sodium enters the enterocyte primarily by means of co-transport with glucose and amino acids, through apically expressed Na+/H+ exchangers and through electrogenic Na+ channels. NHE2 and NHE3 are the two apically expressed NHE isoforms believed to participate in vectorial transport of Na+ across the intestinal epithelium. Apical expression of NHE8 has recently been described in rat jejunum, and participation of NHE8 in the small intestinal Na+/H+ exchange in suckling animals was postulated (56). The exact contribution of this isoform to intestinal electroneutral Na+ transport remains, however, to be determined. Although NHE2 appears to be the major functional isoform in the chicken ileum and colon (92), it is NHE3 and not NHE2 that mediates the majority of both basal and meal-stimulated Na+ absorption in the dog ileum (93-95). In rabbit ileum, NHE2 and NHE3 contribute equally to the Na+/H+ exchange, with the NHE3 contribution increasing to ~68% after glucocorticoid treatment (96). The relevance of NHE2 to intestinal Na+/H+ exchange in mice seems to be negligible, since NHE2-null mice have no apparent intestinal absorptive defect (87) and no
known compensatory mechanism, e.g. increased expression of NHE3 (97). Contrasting with these results and emphasizing the physiological role of NHE3 in Na\(^+\) absorption, NHE3\(^{-/-}\) mice display a number of phenotypical changes: moderate diarrhea, distention and accumulation of alkaline fluid in all intestinal segments, mild metabolic acidosis, low blood pressure, decreased body fat, and increased mortality when deprived of Na\(^+\) intake (91, 97). Hypertrophy of the small intestine and colon likely represents a compensatory mechanism. NHE2 expression was not altered in the jejunal epithelium of NHE3\(^{-/-}\) mice (97).

Although a residual, EIPA-sensitive component of Na\(^+\) absorption remained in NHE3\(^{-/-}\) mice, this probably did not represent NHE2 activity, since it could be inhibited by cAMP (97). Also, in double knockout mice (NHE2\(^{-/-}\)/NHE3\(^{-/-}\)), the additional loss of NHE2 in NHE3-deficient mice caused no reduction in viability, no further impairment of systemic acid-base balance, and no apparent worsening of the diarrhea (98). The observed residual activity in NHE3\(^{-/-}\) and in double knockout mice may be mediated by NHE8, although kinetics of this isoform and its sensitivity to inhibitors and cAMP have not yet been evaluated. Increased local intestinal expression of IFN\(\gamma\), as well as a five-fold increase in serum level of the cytokine, was found in NHE3\(^{-/-}\) mice (99). This was accompanied by increased expression of a number of IFN\(\gamma\)-inducible genes identified by microarray analysis (99). There was no evidence of inflammation in the intestine of NHE3\(^{-/-}\) mice, and it has been suggested that increased IFN\(\gamma\) might participate in compensation for the defective Na\(^+\) absorption mechanisms by its antisecretory effects, such as decreased expression of CFTR (99).

Colonic Na\(^+\)/H\(^+\) Exchange

The bulk of colonic electrolyte absorption occurs via electroneutral NaCl transport and takes place in both crypts and surface epithelium of proximal and distal colon. This electroneutral absorption is mediated by coupled luminal Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3^-\) exchange. The remaining electrolyte absorption is mediated by electrogenic sodium channels (ENaC) and by transcellular and/or paracellular absorption of Cl\(^-\). For a more comprehensive review of colonic electrolyte absorption, the reader is referred to recent articles by Kunzelmann and Mall (100) and by Geibel (101).

Electroneutral NaCl absorption is generally believed to represent coupled apical membrane Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3^-\) exchanges. This HCO\(_3^-\) -dependent component of electroneutral NaCl absorption is enhanced by adrenergic agonists and inhibited by cAMP, aldosterone, and increases in intracellular Ca\(^{++}\) (100). Colonic NaCl absorption is also enhanced by short-chain fatty acids (SCFA), especially by butyrate. Several components have been proposed in this interaction. SCFA absorption may stimulate electroneutral Na\(^+\) uptake by acidification of colonocytes and activation of apical Na\(^+\)/H\(^+\) exchangers (102). Cl\(^-\) absorption is mediated via the apical Cl\(^-\)/HCO\(_3^-\) exchanger, stimulated in turn by
increased HCO$_3^-$ production during SCFA metabolism. Another model has been proposed, in which butyrate is taken up via nonionic diffusion and/or SCFA/HCO$_3^-$ exchange mechanism mediated by MCT1 (monocarboxylate transporter 1) (103). Subsequently electroneutral NaCl absorption is activated by parallel Cl$^-$/butyrate and Na$^+$/H$^+$ exchange (104). SCFA absorption activates basolateral volume-sensitive Cl$^-$ channels, whereas basal and cAMP-activated, CFTR-mediated Cl$^-$ secretion is inhibited (105, 106). Activation of Na$^+$/H$^+$ exchange and inhibition of chloride secretion are most likely the basis of the anti-diarrheal effects of butyrate (100, 107, 108). In vitro functional studies with the rat distal colon indicated that butyrate-dependent Na$^+$ absorption was mediated by both NHE2 and NHE3 (109). In C2bbe clone of Caco-2 cells, however, only NHE3 and not NHE2 activity was stimulated by butyrate (110). The increase in NHE3 activity was likely due to transcriptional activation of NHE3, as both protein and mRNA were induced by SCFAs in vitro as well as in the colonic epithelium of rats fed 5% pectin-supplemented diet (110). This hypothesis is further supported by strong induction of rat NHE3 promoter by sodium butyrate in transient transfections (111).

There are segmental differences with respect to the predominant mode of Na$^+$ absorption present in the proximal and distal colon of various species. Overall, Na$^+$ transport in the proximal colon is thought to be mediated primarily by Na$^+$/H$^+$ exchange, while in the distal colon it is mixed (rat and mouse) or dominated by electrogenic absorption mediated by ENaC (rabbit, human, and guinea pig). Of the nine cloned NHEs, expression of NHE1-4, NHE7, and NHE8 has been described in the colon. The ubiquitous NHE1 is not regulated by dietary Na$^+$ depletion (112) and is expressed in the basolateral membrane of all epithelial cells of the colon with no detectable segmental differences (47). NHE2 and NHE3 are both expressed on the apical side of colonic epithelial cells (34). While NHE3 has been unambiguously described in the surface epithelium (47, 48), expression of NHE2 is somewhat unclear. Studies with human colonic biopsies showed uniform distribution of NHE2 mRNA along the vertical axis of the colonic crypts (47); however, later studies in mice demonstrated that in the distal colon NHE2 functions mainly in the crypts cells (35, 36), which added to the body of evidence about absorptive properties of colonic crypts, e.g. Singh et al. (113). Although the Cl$^-$-dependent Na$^+$/H$^+$ exchange mechanism has been described in the crypts of rat distal colon (81, 82, 114), others have failed to demonstrate such a mechanism in mouse colonic crypts (35, 36). The reasons for this discrepancy remain unknown, as indicated in the earlier discussion on Cl$^-$-dependency above. Despite the high expression of NHE2 in the colon and its established participation in pH$_i$ and cell volume regulation (35), NHE2$^{-/-}$ mice display no obvious intestinal absorptive defect (87, 97). It has been speculated that ectopic expression of NHE3 in colonic crypts of NHE2$^{-/-}$ mice may compensate for the lack of the latter isoform (35). In birds, NHE2 mediates most (~85%) of the colonic Na$^+$/H$^+$ activity both under basal conditions and under Na$^+$ depletion (92). In mice, on the other hand, based on
defective Na\(^+\) absorption in NHE3-deficient animals, NHE3 appears to be the major Na\(^+\) transporter in the mouse proximal colon (91), estimated to contribute ~70\% of basal net Na\(^+\) absorption (115). Consistent with this observation, and as described earlier, NHE3\(^{-/-}\) mice exhibit moderate diarrhea with colonic distention and fluid accumulation, mild metabolic acidosis, lower blood pressure, and a high mortality when deprived of dietary Na\(^+\) (91, 97). Colonic compensatory mechanisms counteracting the loss of NHE3 include hypertrophy and increased expression and activity of the apical ENaC in the distal colon and a dramatic induction of H\(^+\)/K\(^+\)-ATPase (cHKA) mRNA, with the latter presumably representing a K\(^+\) sparing mechanism in a state of increased electrogenic Na\(^+\) absorption (91). These compensatory increases in ENac and cHKA were likely mediated by a ~five-fold increase in circulating aldosterone (91).

**EXPRESSION AND ACTIVITY OF THE APICAL NA\(^+\)/H\(^+\) EXCHANGERS DURING POSTNATAL DEVELOPMENT**

Intestinal Na\(^+\)/H\(^+\) exchange activity increases during development to reach maximal values after weaning. Not only the expression of all three described apical NHE isoforms, NHE2, NHE3, and NHE8, but also their contributions to total NHE activity in the small intestine significantly change during the dietary transition from mother’s milk to solid foods. In developing rats, the contribution of NHE3 to jejunal Na\(^+\)/H\(^+\) exchange dramatically increases with age from 59\% in 2-to-3-week-old to ~92\% in 6-week-old animals (116). Therefore, it has been postulated that NHE2 plays a greater role in small intestinal Na\(^+\) absorption in the pre-weaning period than in adulthood. NHE2-mediated Na\(^+\) uptake in rat jejunum is lowest in suckling rats, higher in weanling and adult rats, and still higher in adolescent rats (117). A similar pattern is also seen in the NHE2 protein and mRNA expression; NHE2 protein levels are lowest in sucklings and higher in weanling and adult rats (4-6-fold increase), with a similar pattern of mRNA expression (lowest in suckling rats and 3-5-fold higher after weaning) (117). This ontogenic regulation of NHE2 function is thought to be mediated by changes in NHE2 transcription rate as evidenced by nuclear run-on assay (117). Interestingly NHE2 expression in the rat kidney follows a reciprocal pattern, with highest expression in the suckling period and a decline towards adulthood (118), implying tissue-specific mechanisms regulating postnatal changes in NHE2 expression.

Epidermal growth factor (EGF) has been postulated to participate in this postnatal increase in expression and activity of NHE2. In suckling rats, EGF treatment increases jejunal NHE2 activity by almost 2-fold in suckling rats. This increase correlates with the elevated steady state mRNA level and NHE2 gene promoter activity in transiently transfected rat intestinal epithelial (RIE) cells (119). In adult mice, however, neither exogenous EGF nor salivarectomy affected
NHE2 mRNA expression in the small intestine (120), suggesting that this regulation may be species and/or age dependent. Although EGF administration has no effect on NHE3 gene expression in young rats, small intestinal NHE3 function, and gene expression are also subject to developmental regulation with a similar pattern of activity as NHE2 (suckling = weanling < adult « adolescent rats) (116). Interestingly, while brush border NHE3 protein expression is lowest in suckling rats and increases up to 7-fold after weaning, NHE3 mRNA level increases during this period only approximately 2-fold after weaning (116). These observations suggest that ontogenic regulation of NHE3 expression and activity likely involves both transcriptional and posttranscriptional mechanisms. Glucocorticoid hormones, implicated in postnatal maturation of the gut are likely candidate factors in the described ontogenic NHE3 regulation. The increase in rat small intestinal NHE3 expression after weaning coincides with a surge in levels of endogenous glucocorticoid hormones (121). Glucocorticoids have been demonstrated to enhance NHE3 gene transcription in transient transfections (122, 123), as well as act to increase NHE3 activity via non-genomic mechanisms. These include serum- and glucocorticoid-induced kinase 1 (SGK1)-mediated increase in NHE3 activity (124, 125), likely involving phosphorylation of NHE3 at Ser\(^{336}\) by SGK1 and ensuing increased exocytosis of the protein into the plasma membrane (126, 127). Interestingly, the transcriptional component of glucocorticoid regulation of NHE3 activity is age- and region-specific. In suckling rats, the responsiveness to methylprednisolone treatment is observed only in the proximal, but not in the distal small intestine (128). Conversely, in adult rats, the stimulatory effect of methylprednisolone is observed only in the distal, but not in the proximal, small intestine. We have demonstrated by Western blot and \(^{3}\)H\)dexamethasone mesylate binding, that the responsiveness of NHE3 to glucocorticoids is directly related to the expression of glucocorticoid receptor (GR) in the small intestine (128).

In the proximal small intestine, expression of the novel NHE8 isoform is also regulated during development, but contrary to the expression pattern of NHE2 and NHE3, NHE8 mRNA and protein expression decreases ~2-fold after weaning (56). Since the sensitivity of NHE8 to NHE inhibitors used in earlier studies on NHE ontogeny is not yet known, the contribution of all three apical NHE isoforms (NHE2, NHE3, and NHE8) to intestinal Na\(^{+}\) absorption in the early postnatal period may need to be re-evaluated.

Transcriptional Regulation

Rat and human NHE2 promoters have been cloned and characterized (129, 130). Both proximal promoters lack canonical TATA and CAAT boxes, are highly GC rich, and share about 59% homology with a number of conserved, predicted, regulatory elements. Initial analysis of the human NHE2 promoter predicted a number of putative binding sites for the following trans-acting factors: Sp1, AP-
2, Egr-1, p300, NF-κB, Oct-1, zinc finger protein-1, MyoD, two caudal-related homeobox (Cdx) family members, CdxA and Cdx-2, glucocorticoid receptor (GRE), thyroid hormone receptor, a CACCC site, and several polyoma viral enhancer 3 sites (129). Of all these sites, only Sp1, AP-2, CACCC, NF-κB, and Oct-1 were conserved in human and rat NHE2 promoters. A minimal promoter region necessary for transcription of human NHE2 gene in colonic epithelial cells was identified at –85/+249 nt (131), and of the predicted regulatory factors, Egr-1 has been experimentally confirmed to transactivate hNHE2 gene promoter and mediate the effects of phorbol 12-myristate 13-acetate (PMA) on hNHE2 gene transcription (131). Rat NHE2 gene promoter, its regulation by Sp transcription factors, and its response to hyperosmotic stress was well characterized in the mouse renal inner medullary collecting duct cells, mIMCD-3 (132, 133). Interestingly, the minimal promoter sufficient to drive reporter gene expression in the renal epithelium was not active in the RIE cells (119) suggesting that other upstream elements are indispensable for active NHE2 gene transcription in the intestinal epithelium.

Rat NHE3 gene promoter was cloned at about the same time by Kandasamy and Orlowski (123) and by Cano (122). Both reports demonstrated the transcriptionally mediated effects of glucocorticoids on NHE3 gene expression. We have subsequently resolved a discrepancy in the transcriptional start site in those two reports, and showed that the atypical TATA box located at bp -26/-31 (numbers according to the major transcription start site mapped in (123) was not necessary and even detrimental for promoter activity, and that a -20/+8-bp fragment represents a functional, albeit atypical, initiator (134). Within the -81-bp upstream region, three Sp transcription factor binding sites were critical because their mutation drastically reduced promoter activity. The roles of Sp1 and Sp3 were further demonstrated by electromobility shift assay and by transactivation of the NHE3 promoter in SL2 cells by forced expression of Sp1 and Sp3. Both of these transcription factors were found to act synergistically with GATA-5 bound to a GATA box in exon 1 (+20/+23 bp). These studies demonstrated that rat NHE3 promoter is initiator-driven and controlled mainly by Sp1 and Sp3, which functionally interact with GATA-5. This interaction may represent a regulatory mechanism participating in a gradient of intestinal gene expression along the crypt-villus axis (134). Rat NHE3 promoter reporter construct, when transiently transfected into Caco-2 cells, was also significantly induced by SCFAs, especially butyrate (111). The mechanism of this induction involves Ser/Thr kinase activity with a likely permissive role for PKA, as the activation of the promoter by butyrate was abrogated by H-7, Rp-cAMPS and H-89 inhibitors, as well as by overexpression of a dominant-negative mutant form of the regulatory subunit of PKA (111). Cloning of the human NHE3 5′-regulatory region (135) defined a maximal promoter activity in the -95/+5 nt region, a sequence with very high homology with the proximal promoter of the rat NHE3, with overlapping and functional regulatory elements for Sp and AP-2
transcription factors (135). This minimal promoter was recently implicated in Egr-1-mediated but protein kinase C-independent induction of hNHE3 transcription by PMA (136).

Chronic metabolic perturbations in systemic acid-base balance can affect Na\(^+\) absorptive functions of the gut (137). Metabolic acidosis induced in rats by 5% NH\(_4\)Cl in drinking water induced ileal expression of NHE2 and NHE3 mRNA, protein, as well as their activities (138). Transcriptional regulation of NHE3 was confirmed in opossum kidney cells (OKP) transfected with NHE3 promoter construct and subjected to prolonged (24-hour) exposure to acidified media (122). The precise mechanism of this induction has not been described.

As an adaptive response to enhance the intestinal absorptive capacity, rat small intestinal Na\(^+\)/H\(^+\) activity was shown to increase, primarily in the segment distal from the resection (139). It was later shown that this increase was associated with \(~3\)-fold elevation of NHE3 mRNA and protein expression after a 50% massive proximal small bowel resection in rats (140). The increase was again observed only in the ileal segment distal from the anastomosis site, suggesting that dietary rather than humoral factors might be responsible. Similar results were also obtained in enterectomized mice (120).

**Post-transcriptional Regulation**

NHE2 protein has a relatively short half life (~3 hours) compared to other NHE isoforms (NHE1 – 24 hours, NHE3 – 14 hours) and is subject to lysosomal degradation, as determined in PS120 fibroblasts and Caco-2 cells (141). This suggests that changes at the level of gene transcription or translation may be more critical for NHE2 regulation than for other isoforms with longer half lives. NHE2 is a residual plasma membrane protein and unlike NHE3, it does not appear to undergo endosomal recycling (141). Glycosylation of NHE2 may affect its cellular localization, since unglycosylated 75 kDa rabbit NHE2 was found predominantly intracellularly (142), although it is not clear whether this represents a regulatory mechanism or is simply related to the maturational stage of NHE2 protein synthesis. Compared to NHE3, NHE2 activity is considered relatively stable and is not regulated by many factors. Extracellular alkalinization activates NHE2, which is believed to propel increased proton extrusion in gastric parietal cells during secretagogue-stimulated acid secretion. The maximal rate of exchange (\(V_{max}\)) mediated by NHE2 was shown to be stimulated by serum, fibroblast growth factor (FGF), and protein kinase C activator phorbol mirystyl acetate (PMA) in PS120 fibroblasts (143). Intracellular ATP depletion reduced the NHE2 activity by a dramatic decrease in H\(^+\) affinity as well as \(V_{max}\), with virtual elimination of the allosteric effect of H\(^+\) (143). ATP depletion also eliminated the stimulatory effect of serum, suggesting that growth factor-stimulated NHE2 activity is mediated via its pH-sensing mechanism. Thrombin increased NHE2 \(V_{max}\), without altering the Hill coefficient (143), although it is not
clear if this could be attributed to increased intracellular Ca\textsuperscript{++} ascribed to thrombin-treated fibroblasts. In the same study, thrombin also increased NHE3 activity, whereas it was shown later that elevation of intracellular Ca\textsuperscript{++} by thapsigargin in Caco-2/bbe cells inhibited NHE3 (144).

Vast majority of knowledge on acute regulation of NHE3 activity comes either from heterologous cell expression systems or from renal epithelial cells, although the described mechanisms are likely ubiquitous and will, perhaps with certain exceptions, apply to the epithelial cells of the digestive tract. These mechanisms were reviewed in more detail by Zachos et al. (14), Moe (145), Weinman et al. (146), and Yun (124). The C-terminal domain of NHE3 contains numerous putative phosphorylation sites for various kinases (38). Deletion of this domain renders NHE3 activity constitutive, with partially preserved transport activity. Domain swapping experiments have shown that regulatory characteristics of one NHE isoform can be transferred to another by the cytoplasmic domain of the first. For example, replacement of the C-terminal cytoplasmic tail of NHE1, an isoform that is largely cAMP insensitive, with an analogous domain of NHE3, transfers cAMP-mediated inhibition to the hybrid molecule. The experiments strongly suggested the existence of functionally relevant phosphorylation sites located at the cytoplasmic C-terminal tail of NHE3. In response to elevated intracellular cAMP, protein kinase A (PKA) phosphorylates NHE3 on multiple sites in the intact cell. Ser\textsuperscript{605} and Ser\textsuperscript{634} of rat NHE-3 are crucial for regulation of NHE3 by PKA. However, of the two serines, only Ser\textsuperscript{605} was found to be been phosphorylated in vivo (147). In this study, phosphorylation of Ser\textsuperscript{552} was also shown to participate in the NHE3 response to cAMP, although in another study by Kurashima et al. (148), Ser\textsuperscript{552} was not functionally important. The functional consequence of PKA-mediated NHE3 phosphorylation is its reduced $V_{\text{max}}$, decrease in the surface amount, presumably due to increased endocytosis, and decreased exocytosis. The recruitment of PKA to the C-terminus of NHE3 involves a multiprotein complex including NHE regulatory factors (NHERF1 and NHERF2) and a scaffolding protein ezrin. NHERF1 and NHERF2 proteins contain two 80-90 amino acid PDZ domains consisting of GLGF repeats mediating physical interaction with short peptide sequences located at the C-terminus of interacting proteins. Both NHERF1 (initially cloned and described as NHE3 Kinase A Regulatory Protein, E3KARP (149)) and NHERF2 reconstitute PKA-dependent NHE3 inhibition when expressed in NHERF-deficient cells (149). NHERF1 and NHERF2 interact through their C-terminal 29 amino acids with cytoskeleton-associated ezrin (150, 151), which functions as A Kinase anchoring protein (AKAP). Phosphorylation of NHE3 by PKA is therefore facilitated by bringing the catalytic subunit of PKA to the vicinity of the NHE3 cytoplasmic tail by a protein complex containing either of the two NHERF factors and cytoskeleton-associated AKAP protein, ezrin.

Somewhat similar scaffolding mechanism lays behind glucocorticoid-stimulated NHE3 activity. In this case, however, the mediating kinase (serum and glucocorticoid inducible kinase, SGK1) interacts directly and specifically with
NHERF2, acting as a bridge between the kinase and NHE3, to stimulate activity of the latter (124, 152). It has also been postulated that the mechanism of post-transcriptional regulation of NHE3 by glucocorticoids is biphasic, with an initial phase involving phosphorylation of the pre-existing membrane NHE3, and a later phase in which SGK1 and NHERF2 facilitate translocation of the newly synthesized NHE3 to the cytoplasmic membrane (124). By using in vitro SGK1 kinase assay and site-directed mutagenesis, Ser^{663} was identified in NHE3 protein as the major site of phosphorylation by SGK1. Mutation of Ser^{663} blocked the effect of dexamethasone, demonstrating the importance of this site (126).

The NHERF-mediated link with ezrin suggests the association of NHE3 with cytoskeleton as a likely mechanism controlling NHE3 activity. Consistent with this notion, NHE3 was found to co-sediment with F-actin, and pharmacological disruption of cytoskeleton induced a profound inhibition of NHE3 activity (153). Inhibition of two kinases controlling cytoskeletal assembly, RhoA and ROCK, also inhibited NHE3 activity in Chinese hamster ovary cells stably transfected with dominant-negative mutants of a respective kinase without altering NHE3 abundance in the cytoplasmic membrane (154). This mechanism may at least in part account for the inhibitory effect of cAMP on NHE3 activity. Elevated PKA activity inhibits RhoA, resulting in altered cell morphology with disruption of the microfilament actin network (155). By analogy, expression of constitutively-active forms of RhoA and ROCK kinases attenuates PKA-mediated NHE3 inhibition by stabilizing actin filaments (156). Similarly, disruption of actin cytoskeleton by hyperosmotic stress may be responsible for the associated decrease in NHE3 activity (157).

In addition to being present at the cell surface, NHE3 is detectable in intracellular vesicles of the juxtanuclear compartment; consistent with recycling endosomes (158-160). NHE3 remains in a state of dynamic equilibrium between the cell surface and the intracellular compartment: it undergoes internalization via clathrin-coated vesicles (158) and is exocytosed back to the cytoplasmic membrane in a phosphatidylinositol 3-kinase-dependent manner (161). PI3-kinase inhibition leads to decreased NHE3 activity correlating with depletion of the plasma membrane pool of NHE3 protein (51), while constitutively active PI3-kinase or AKT transfected into NHE3-expressing PS120 cells stimulates the exchanger and increases the percentage of NHE3 present on the plasma membrane (161). EGF and FGF growth factors have been shown to stimulate NHE3 activity by increasing the surface protein pool in a PI3-kinase-dependent manner (162, 163). Other factors increasing the apical pool of NHE3 include lysophosphatidic acid (LPA) (164) and endothelin-1 (165). Conversely, decreased NHE3 surface expression has been associated with inhibition of the transporter by PKC (50), by parathyroid hormone (166), and by dopamine (167). Collectively, these studies strongly suggest that redistribution of NHE3 between subcellular compartments is an effective means of transport regulation.
In the cell, NHE3 exists in large multiprotein complexes which range from 400 kDa in the intracellular pool to ~1,000 kDa at the plasma membrane. These complexes are dynamic and are influenced by physiological stimuli participating in acute NHE3 regulation. This dynamic assembly, association with cytoskeletal proteins, endosomal recycling, and protein phosphorylation events all act in concert to provide highly regulated turnover and activity of NHE3 protein (14).

**APICAL NA⁺/H⁺ EXCHANGE IN GUT PATHOPHYSIOLOGY**

In healthy individuals, daily ileocecal flow is approximately two liters of electrolyte-rich fluid. Of this amount, 1.5-1.9 liters is absorbed in the colon, although the maximal capacity of the human large intestine to absorb fluids may be as high as 5-6 liters per day (168). Therefore, there is a large margin within which a healthy colon can compensate for increased ileocecal flow ensuing from defective small intestinal absorption. Exceeding the maximal capacity will result in diarrhea. On the other hand, in colonic disease, relatively small changes in water and electrolyte absorption will produce a significant increase in stool water output, emphasizing the relevance of fine tuning of colonic transport processes.

Downregulation of NHE2 activity and gene expression has been documented in rats and in colonic epithelial Caco-2/bbe cells treated with interferon γ, implicating a role for NHE2 in inflammation-associated diarrhea (169). The lack of absorptive defect in the intestine of NHE2−/− mice, however, suggests that cytokine-mediated changes in NHE2 function may not be critical for electrolyte absorption in the inflamed intestinal mucosa. IFNγ also down-regulates NHE3 mRNA and protein expression both *in vivo* and *in vitro* (169). In interleukin-2 knockout mice, a mouse model of human ulcerative colitis (170), a drastic reduction in colonic transepithelial net Na⁺ flux was paralleled by a reduction in electroneutral NaCl absorption and decreased NHE3 mRNA and protein expression in the proximal colon, and abrogated aldosterone-stimulated electrogenic Na⁺ transport with decreased ENaC expression in the distal colon (115). This strongly suggests an involvement of NHE3 in the pathogenesis of diarrhea in ulcerative colitis. On the other hand, in a rat model of necrotizing enterocolitis, ileal expression of NHE3 was not affected (171), implying differential involvement of inflammatory mediators in regulation of NHE3 expression and activity.

Enteropathogenic *E. coli* invasion of intestinal epithelial cells increased NHE2 activity by ~300%, while it inhibited activities of NHE3 and Cl⁻/OH⁻ exchange (172). The authors speculated that NHE2 activity might represent a potential compensatory response to increased luminal fluid resulting from inhibition of NHE3 activity, disruption of tight junctions, inflammatory response, or alterations in anion exchanger activity.
Holmberg and Perheentupa (173) and Booth et al. (174) described a form of congenital secretory diarrhea (CSD) due to defective sodium/hydrogen exchange (OMIM %270420). Based on a close phenotypical resemblance between this rare disease and symptoms displayed by NHE3\(^{-/-}\) mice, NHE3 became a likely candidate for linkage. Recent homozygosity mapping and multipoint linkage analysis studies in four candidate regions known to contain NHE1, NHE2, NHE3, and NHE5 genes have shown that CSD is an autosomal recessive disorder but is not related to mutations in the NHE1, NHE2, NHE3, and NHE5 genes (175). Since location of the human NHE2 gene is most likely adjacent to NHE4, that latter gene can also be excluded as a candidate for CSD. It would seem, therefore, that another NHE isoform or a regulatory factor may be directly responsible for the loss of Na\(^+\)/H\(^+\) exchange in this disease.

Altered expression and activity of NHE3 in primary and diabetes-related hypertension have implied a potential role for this isoform in the pathogenesis of high blood pressure. Spontaneously hypertensive rats (SHR) have elevated NHE3 activity in the ileal brush-border membranes (176) and in renal proximal tubules (177), suggesting that increased intestinal sodium absorption and renal reabsorption may contribute to systemic sodium retention and the pathogenesis of hypertension. In both streptozotocin-induced diabetes and in BB/W autoimmune diabetic rats, renal cortex brush border Na\(^+\)/H\(^+\) exchange (presumably mediated by NHE3) was significantly induced, likely due to acidosis and not hypoinsulinemia (178). The effect of diabetes on intestinal Na\(^+\)/H\(^+\) exchange is not well described. In streptozotocin-induced diabetes in rats (used as a model for secondary vitamin D deficiency), ileal NHE3 mRNA was induced two-fold (179). It is likely, however, that the observed difference was due to vitamin D deficiency, as repletion of diabetic mice with 1,25(OH)\(_2\)D3 brought NHE3 mRNA expression down to control levels.

CONCLUSIONS

Since the initial description of electroneutral Na\(^+\)/H\(^+\) antiport nearly forty years ago, an explosion of physiological, biochemical and molecular studies have yielded a vast body of knowledge that brought a wide appreciation of this transport process in organellar, cellular, organ and systemic physiology and pathophysiology. In mice, gene targeting of the two major intestinal apically expressed NHE isoforms, NHE2 and NHE3, revealed and continues to reveal their important roles in the gastrointestinal physiology and pathology, particularly as it relates to regulation of gastric acid secretion and intestinal and colonic sodium and water absorption. The relevance of the more novel isoform, NHE8, in the intestinal absorptive processes remains to be elucidated in more detail.

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