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Regulation of Electroneutral NaCl Absorption by the Small Intestine

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Abstract

Na⁺ and Cl[−] movement across the intestinal epithelium occurs by several interconnected mechanisms: (1) nutrient coupled Na⁺ absorption; (2) electroneutral NaCl absorption; (3) electrogenic Cl[−] secretion by CFTR; and (4) electrogenic Na⁺ absorption by ENaC. All of these transport modes require a favorable electrochemical gradient maintained by the basolateral Na⁺-K⁺-ATPase, a Cl[−] channel and K⁺ channels. Electroneutral NaCl absorption is observed from the small intestine to distal colon. This transport is mediated by apical Na⁺/H⁺ (NHE2/3) and Cl[−]/HCO₃[−] (Slc26a3/a6, others) exchangers that provide the major route of NaCl absorption. Electroneutral NaCl absorption and Cl[−] secretion by CFTR are oppositely regulated by the autonomic nerve system, immune system, and endocrine system via PKAα, PKCα, cGKII, and/or SGK1. This integrated regulation requires the formation of macromolecular complexes, which mediated by NHERF family of scaffold proteins, and involve internalization of NHE3. Using knockout mice and human mutations, a more detailed understanding of the integrated as well as subtle regulation of electroneutral NaCl absorption by the mammalian intestine has emerged.

Keywords

NHE; SLC26; NHERF; CFTR; neuroendocrine system

Introduction

The mammalian intestine is responsible for digestion and absorption of ingested food. However, secretion and absorption of electrolytes and fluid are also essential functions of the intestine, in particular intestinal epithelial cells. In humans, the gastrointestinal (GI) tract secretes 8–10 L/day of fluid in total in the face of ingested food containing 1.5–2 L/day of fluid. Most fluid is (re)absorbed by the GI tract: small intestine (~95%) and large intestine (~4%). The small intestine secretes ~1 L/day and (re)absorbs ~6.5 L/day, making it the major net fluid-absorber. Because there is no “active” water movement in the human body, the fluid movement is driven by active movement of Na⁺ and Cl[−] (this review) or HCO₃[−] (beyond this review) by GI epithelial cells. In the intestine, there are four modes of Na⁺ and

Cl^- movement: (1) nutrient coupled Na^+ absorption; (2) electroneutral NaCl absorption; (3) electrogenic Cl^- secretion by CFTR; and (4) electrogenic Na^+ absorption by ENaC.

Nutrient coupled Na^+ absorption

In 1960s, transepithelial sugar and amino acid movement was measured using the short-circuit current technique (Ussing chamber). These studies found that absorption is dependent on extracellular (luminal) Na^+ . This nutrient coupled Na^+ absorption is now explained by the function of Na^+ /glucose cotransporters (SGLTs, aka, Slc5 proteins), several Na^+ /amino acid cotransporters (Slc6, 38, etc) and Na^+ coupled solute carriers (see <http://www.bioparadigms.org/slc/menu.asp>). The transepithelial Na^+ movement generates a lumen-negative (mucosal-negative) transepithelial voltage facilitating paracellular Cl^- and fluid absorption. This absorption can be regulated by enterotoxins, and thus is also used as route for therapeutically increase Na^+ absorption via oral rehydration.

Electroneutral NaCl absorption

During the 1960s–1980s, short-circuit current studies also demonstrated that the small intestinal and proximal colonic mucosa has basal NaCl and fluid absorption in the absence of nutrients and not associated with transepithelial currents (Figure 1A). This was in contrast to other modes of Na^+ and Cl^- transport, all of which caused transepithelial currents. Therefore, this absorption mode is called “electroneutral NaCl absorption.” These studies demonstrated that the absorption is mediated by the coupled function of apical Na^+/H^+ exchanger(s) and $\text{Cl}^-/\text{HCO}_3^-$ exchanger(s), the details of which are highlighted in this review.

Electrogenic Cl^- secretion

When the intestinal mucosa (apical or luminal surface) is stimulated by agents that increase intracellular cAMP, Ca^{2+} , or cGMP, electroneutral NaCl absorption is inhibited and Cl^- secretion is activated (1) (Figure 1B). The secretion is mediated by apical Cl^- channel CFTR (2). Inside negative electric potential of epithelial cells provides driving force to secrete Cl^- . This movement generates luminal negative electrical difference that results in paracellular Na^+ secretion, and CFTR mediated electrogenic Cl^- secretion is observed all over the regions of the small and large intestines.

Electrogenic Na^+ absorption (3, 4)

This rheogenic mode is specific to distal colon. Here the transepithelial electrical resistance is higher than that of other GI segments. Colonic transepithelial voltage (V_t) exceeds -20 mV. In the colon, luminal Na^+ is much lower than other segments and decreases proximal to distal. This lumen negative V_t provides the thermodynamic driving force allowing the mucosa to absorb Na^+ against a large Na^+ concentration gradient. The Na^+ absorption is mediated by epithelial Na^+ channel ENaC (5), which is predominantly regulated by aldosterone (6).

In early 1990s, two mammalian Na^+/H^+ exchangers, NHE2 (Slc9a2) and NHE3 (Slc9a3), were identified as molecules that are predominantly expressed in the apical membrane of intestinal and renal epithelium. At least two mammalian $\text{Cl}^-/\text{HCO}_3^-$ exchangers, Slc26a3

(DRA) and Slc26a6 (PAT-1), are highly expressed in the apical membrane of intestinal epithelium (Figure 1C). Recently Slc26a9, which functions as a $\text{nCl}^-/\text{HCO}_3^-$ exchanger and Cl^- channel (7), has also been located at the apical membrane of the small intestine and colon (8). In 2000s, knockout mice for *Nhe2*, *Nhe3*, *Slc26a3*, and *Slc26a6* were studied and their roles *in vivo* have been analyzed. Initial analysis of *Slc26a9*^{-/-} mice show defective stomach acid secretion (9), but have not been assessed for intestinal absorption phenotypes. In this review, we describe the function and the regulation of these transporters particularly as related to electroneutral NaCl absorption in the mammalian small intestine.

Molecules involved in Electroneutral NaCl Absorption by the Small Intestine

NHE2 and NHE3, Na^+/H^+ exchangers for apical Na^+ absorption

Luminal Na^+ absorption by the small intestine is mediated by Na^+/H^+ exchange. Two Na^+/H^+ exchangers are localized to the intestinal brush border membrane: NHE2 (Slc9a2) and NHE3 (Slc9a3). Intestinal expression and function of NHE2 and NHE3 significantly overlap. Analyses of *Nhe2*^{-/-} and *Nhe3*^{-/-} mice demonstrate that NHE3 is the dominant Na^+/H^+ exchanger in the small intestine.

Slc9 is a family of Na^+/H^+ exchangers that consists of 8 membrane proteins (10, 11). NHE2 (12, 13) and NHE3 (14, 15) were first identified as NHE1 homologs, and their transcripts are highly expressed in the GI tract (stomach, small intestine, large intestine) and the kidney. In contrast to NHE1 at the basolateral membrane, NHE2 and NHE3 are found at the brush border (apical) membrane of the intestinal epithelium (jejunum, ileum, and colon) and the renal tubule (proximal tubule and thick descending limb of Henle's loop). Activities have been studied by expressing the recombinant proteins in NHE-null cell lines (PS120 or SP-1). Both NHE2 and NHE3 mediate the exchange of Na^+/H^+ with a stoichiometry of $1\text{Na}^+:1\text{H}^+$ (16). The inward Na^+ gradient (low intracellular $[\text{Na}^+]$) maintained by the basolateral Na^+/K^+ -ATPase (NKA) provides the continued driving force.

NHE3 is important for normal GI physiology. *Nhe3*^{-/-} mice have slight diarrhea, mild acidosis, reduced blood pressure, and increased intestinal segment size and weight (17). The contents of the small intestine, cecum, and colon of *Nhe3*^{-/-} mice are somewhat alkaline (17). In the kidney, proximal tubule fluid and HCO_3^- absorption are significantly reduced in *Nhe3*^{-/-} mice (17). The *Nhe3*^{-/-} jejunum exhibits reduced Na^+ absorption (18). Apical membrane Na^+/H^+ exchange activity of jejunal, midvillous epithelium is decreased in *Nhe3*^{-/-} mice (19). In the colon of *Nhe3*^{-/-} mice, ENaC and H^+/K^+ ATPase expression are upregulated. These alterations increase amiloride-inhibitable short circuit current. Thus, electrogenic Na^+ absorption by ENaC compensates for NHE3 loss-of-function (17). The NHE2 intestinal function can be compensated by NHE3 (20–22). Yet, intestinal NHE3 function cannot be compensated by NHE2.

Apical $\text{Cl}^-/\text{HCO}_3^-$ exchangers – Slc26 proteins

Luminal Cl^- absorption by the small intestine is mediated by $\text{Cl}^-/\text{HCO}_3^-$ exchange. Thusfar, two $\text{Cl}^-/\text{HCO}_3^-$ exchanger have been localized to the intestinal brush border membrane.

These $\text{Cl}^-/\text{HCO}_3^-$ exchangers are not related to the Band 3 $\text{Cl}^-/\text{HCO}_3^-$ exchangers (AE1-3), but rather belong to Slc26 family, i.e., Slc26a3 and Slc26a6. Intestinal expression of Slc26a3 and Slc26a6 overlap (23). Melvin and Shull were the first to show any Slc26 protein functions as a $\text{Cl}^-/\text{HCO}_3^-$ exchanger (24), i.e., Slc26a3. Later Slc26a6 was shown to function as an electrogenic $\text{Cl}^-/\text{HCO}_3^-$ exchanger (25–28). Muallem's group found the Slc26a3 is also electrogenic having the opposite coupling of Slc26a6 (26, 28). Recent analyses of Slc26a3-null (*Slc26a3*^{-/-}) and Slc26a6-null (*Slc26a6*^{-/-}) mice demonstrated that both transporters have significant roles in Cl^- absorption and HCO_3^- secretion by the intestine (19, 23, 29). However, Slc26a3 and Slc26a6 have significant roles in unstimulated HCO_3^- secretion by the duodenum but Slc26a6 is more predominant. On the other hand, Slc26a3 plays a more significant role during cAMP-stimulated duodenal HCO_3^- secretion. In the jejunum, Slc26a3 and Slc26a6 both have significant roles in Cl^- absorption; however, here Slc26a3 is more abundant. Slc26a6 has also plays significant roles in duodenum SO_4^{2-} absorption and oxalate excretion (25, 30–34). Finally, Slc26a3 plays a major role in colonic Cl^- absorption (35, 36).

Slc26 is an anion transporter and channel family with 10 gene members (8, 27, 34). Slc26 proteins transport monovalent anions (Cl^- , iodide, formate, oxalate, hydroxyl ion (OH^-), and HCO_3^-) and divalent anions (SO_4^{2-} and oxalate (37). SLC26A3 was first identified in the colon, and its expression is down-regulated in colonic adenomas (DRA) (38). The SLC26A3 transcript is expressed in the intestine from the duodenum to the distal colon, but is most abundant in the duodenum and the colon. Immunohistochemical analyses demonstrated that Slc26a3 is localized to the apical membrane of enterocytes of surface and crypt in the colon (34, 35, 39) apical membrane of pancreatic duct (40). When exogenously expressed in *Xenopus* oocytes and mammalian culture cells, Slc26a3 mediates electrogenic $\text{Cl}^-/\text{HCO}_3^-$ and Cl^-/OH^- exchange with stoichiometries of $2\text{Cl}^-:1\text{HCO}_3^-$ (28).

SLC26A6 was first identified through database mining and named putative anion transporter-1 (PAT-1) (41). Its anion-transport activity was later established (25, 30, 42, 43). In contrast to SLC26A3 mRNA (mainly intestine), SLC26A6 transcripts are expressed in the small intestine as well as kidney, pancreas, heart, and placenta. In the intestine, Slc26a6 is most abundant in the duodenum, jejunum, and ileum, with lower expression in the colon. Immunohistochemical analyses demonstrated that Slc26a6 is localized the apical membrane of the gastric parietal cells (44) and duodenal enterocytes (43), as well as the apical membrane of renal proximal tubule (42, 45). When exogenously expressed in *Xenopus* oocytes and mammalian culture cells, Slc26a6 mediate electrogenic $\text{Cl}^-/\text{HCO}_3^-$, Cl^-/OH^- exchange $\text{Cl}^-/\text{oxalate}$, and $\text{Cl}^-/\text{SO}_4^{2-}$, as well as electroneutral $\text{Cl}^-/\text{formate}$ and $\text{SO}_4^{2-}/\text{oxalate}$ exchange. The stoichiometry of $\text{Cl}^-/\text{HCO}_3^-$ exchange by Slc26a6 is $1\text{Cl}^-:2\text{HCO}_3^-$ (28, 46), which is opposite to Slc26a3 (28). This coupling issue has been questioned as one group found electrogenic $\text{Cl}^-/\text{ox}^{2-}$ exchange for mouse Slc26a6 but electroneutral $\text{Cl}^-/\text{HCO}_3^-$ exchange for mouse as well as human SLC26A6 (47).

In humans, recessive loss-of-function mutations in the *SLC26A3* gene result in severe congenital chloride losing diarrhea, CLD (48). Since SLC26A3 mutations cause CLD, this Slc26a3 exchanger is crucial for the absorption of $\text{Na}^+\text{-Cl}^-$ in the colon. Accordingly, *Slc26a3*^{-/-} mice exhibited high chloride content diarrhea (35). Apical Cl^-/OH^- and

$\text{Cl}^-/\text{HCO}_3^-$ exchange activities were significantly decreased in the colons of *Slc26a3*^{-/-} mice, and the luminal content is more acidic in the *Slc26a3*^{-/-} colon. These observations also suggest that *Slc26a3* is the major colonic Cl^- /base exchanger (35). In addition to the colon, Cl^- absorption is essentially abolished in the jejunum of *Slc26a3*^{-/-} mice (19). Basal $\text{Cl}^-/\text{HCO}_3^-$ exchange activity is also reduced by 30–40% in the *Slc26a3*^{-/-} duodenum (49). Unstimulated and cAMP-stimulated HCO_3^- secretions in the *Slc26a3*^{-/-} duodenum are reduced ~55–60% and ~50%, respectively, in the duodenum of *Slc26a3*^{-/-} mice (50).

Slc26a6 knockout mice develop a high incidence of calcium oxalate urolithiasis (33). Duodenal oxalate efflux is significantly reduced in the *Slc26a6*-null mice (31), and that result in increased dietary-oxalate absorption and increased [oxalate] in plasma and urine (33). In the *Slc26a6*^{-/-} mouse duodenum, basal HCO_3^- secretion and Cl^- absorption are significantly decreased, yet cAMP-stimulated HCO_3^- secretion is not altered compared to the wild-type mice (31, 51). Basal $\text{Cl}^-/\text{HCO}_3^-$ exchange activity is reduced by 65%–80% in *Slc26a6*^{-/-} duodenum, which is more severe than that of *Slc26a3*^{-/-} mice. In addition, $\text{SO}_4^{2-}/\text{HCO}_3^-$ exchange activity is almost abolished in the *Slc26a6*^{-/-} duodenum. In the *Slc26a6*^{-/-} jejunum, Cl^- absorption seems decreased (29), but other work found that the reduction of jejunal Cl^- absorption in *Slc26a6*^{-/-} mice is much weaker than that of *Slc26a3*^{-/-} mice (19). In the isolated microperfused renal tubules of *Slc26a6*^{-/-} mice, the apical $\text{Cl}^-/\text{HCO}_3^-$ exchanger activity is reduced (31).

The intestinal Cl^- absorption deficiency in *Slc26a3*^{-/-} mice is more severe than that of *Slc26a6*^{-/-} mice, even though both transporters are highly expressed in the intestine and have high $\text{Cl}^-/\text{HCO}_3^-$ exchange activity *in vitro*. These results suggest that *Slc26a6* role in intestinal Cl^- absorption can be compensated by *Slc26a3*, while the opposite is not true for the small intestine or the colon electrogenic NaCl absorption. Conversely, only *Slc26a6*^{-/-} mice have defective duodenum SO_4^{2-} and oxalate transport, suggesting that these functions cannot be compensated by *Slc26a3*. These mouse physiological results are not surprising as neither SO_4^{2-} nor oxalate are substrates for *Slc26a3*-mediated exchange (27).

Slc26a9 is another anion transporter in the GI tract (52) with functional multiplicity (Figure 1): $\text{Cl}^-/\text{HCO}_3^-$ exchanger, Cl^- channel and $\text{Na}^+/\text{anion}^-$ cotransporter (7). As for *Slc26a3* and *Slc26a6*, *Slc26a9* is also localized to the apical pole of epithelial cells (7, 46, 52, 53). The activity of *Slc26a3/a6* are stimulated by interaction with the R-region of CFTR and the *Slc26*-STAS domain (26) (Figure 1C). Interestingly, this same STAS / R-region interaction leads to inhibition of *Slc26a9* activity (Figure 1C) (54). *Slc26a9*^{-/-} mice have poor stomach acid secretion and loss of tubulovesicles in parietal cells (9). While *Slc26a9* is present in the small intestine (52), the *Slc26a9*^{-/-} mice are not reported to have an obvious intestinal phenotype. This would seem to make sense as *Slc26a9* in the intestine seems secondary to both CFTR Cl^- channels as well as *Slc26a3/a6*. Nonetheless, since *Slc26a9* has opposite interaction-regulation, it is possible that *Slc26a9* activity might be unchecked in the absence or misdirection of CFTR. This of course, would be predicted to effect the severity of intestinal cystic fibrosis phenotypes.

Basolateral transport

Although apical transporters and channels in the intestine have been the subject of intense recent study, the basolateral (serosal, blood-side) transporters and channel are equally important. That said, details of basolateral transport in NaCl absorption have been limited. Figure 1A illustrates that once Na^+ and Cl^- are apically absorbed, a functional combination of a Cl^- channel (CIC-2, (55–58)), NKA and a K^+ channel (Kir 7.1, (59, 60)) are the major players in the basolateral step. Moreover, CIC-2 is necessary (in jejunum) to recover paracellular permeability (barrier function) after ischemia (61) and is associated at lateral membranes with villus and tight junction function (62). Clinically, lubiprostone (SPI-0211; Cl^- channel activator) is used to treat (reverse) constipation (63). Lubiprostone activates intestinal CIC-2 channels (64), but in some circumstances also requires CFTR function (65). The role of CFTR, however, is controversial (66). CIC-2 can be turned down by $\alpha 1$ -adrenoregic nerves (67). The K^+ channel (presumably Kir 7.1) is needed to recycle K^+ after the NKA exchanges 2K^+ for 3Na^+ to complete the NaCl blood exit step (Figure 1A).

REGULATION OF ION TRANSPORTERS IN EPITHELIAL CELLS

In the epithelial cells of the small intestine and proximal colon, electroneutral NaCl absorption is regulated by intracellular second messengers: cAMP, Ca^{2+} and cGMP. All of these inhibit NHE3 at the apical (brush border) membrane. Interestingly, the same signals also regulate electrogenic Cl^- and fluid secretion, i.e., increased cAMP, cGMP, or Ca^{2+} “activate” CFTR (Cl^- channel) on the apical membrane. Interestingly, those same signals have no effect on the glucose-coupled Na^+ absorption (cAMP, (68); Ca^{2+} , (69); cGMP, (70)). For example, glucocorticoids activate NHE3 via SGK1 (71, 72) as well as SGLT1 (Na^+ -coupled glucose cotransporter) (71). Mineralocorticoids have weaker effects on electroneutral NaCl absorption by the small intestine (73, 74), while effectively activating colonic Na^+ absorption by NHE3 (proximal colon, (73)) and ENaC (distal colon, (6)).

Regulation of NHE3 by cAMP, Ca^{2+} , and cGMP

When the intestinal mucosa is stimulated by enterotoxins, neurotransmitters, or drugs, all of which increase intracellular cAMP (68, 75), Ca^{2+} (75–77), or cGMP (70), electroneutral NaCl absorption is inhibited and electrogenic Cl^- secretion is activated. NHE3 is one of the main targets of these second messengers. These inhibitory mechanisms have been analyzed mainly in exogenous expression systems (mammalian culture, for review, see (10, 78, 79)) and knockout mice (80, 81). This inhibition requires both second messenger-activated protein kinases and scaffold proteins NHERF(s). Cultured cells and mice lacking NHERF(s) do not show second messenger mediated inhibition of NHE3. NHERF(s) mediate interaction among C-terminal PDZ-binding motif of NHE3, other membrane proteins, cytoskeleton, protein kinases, etc. cAMP-mediated inhibition of NHE3 requires NHERF1 (NHERF), NHERF2 (E3KARP) (82), and NHERF3 (PDZK1) (80). Similarly, Ca^{2+} -mediated inhibition of NHE3 requires NHERF2 (83, 84) and NHERF3 (80), but not NHERF1. NHE3 inhibition by cGMP requires NHERF2 but not NHERF1 and NHERF3 (80, 85). cAMP activate protein kinase A (PKA) II, which anchors to NHERF1/2 via cytoskeletal protein ezrin, directly phosphorylate multiple serine residues in the cytoplasmic domain of NHE3 (82, 86). Ca^{2+} induce membrane localization of PKC α that interact with NHERF2 and α -actinin 4 (83, 84,

87) and phosphorylate NHE3 (88). cGMP activate cGMP-dependent protein kinase (cGK or PKG) II that interact with NHERF2, yet it is not known if cGKII directly phosphorylates NHE3. In the renal proximal tubule (89) and cultured cells Caco-2 (90), NHE3 is present both in apical membrane and clathrin-associated subapical endosomes which is the major endocytic pathway. NHE3 internalization is stimulated by cAMP (91) and Ca^{2+} (90) in Caco-2 cells; and this endocytic pathway requires synaptotagmin 1 and adaptor protein 2 (AP2) (91). *In vitro*, NHE3 phosphorylated by PKA α is still active (92). Thus, phosphorylation-mediated internalization of NHE3 seems the dominant mechanism for the NHE3 inhibition by second messengers.

Conversely, activation of NHE3 is by decreased intracellular pH (pH_i) or increased cellular metabolism (e.g., glucose transport, also decreasing pH_i) (93). This NHE3 activation (by brush border translocation) is controlled by ezrin (aka, cytovillin, villin2) (94).

Regulation of NHE3 and NKA by glucocorticoid, SGK1, and PI3K

In small intestine epithelial cells, glucocorticoids activate electroneutral Na^+ absorption (74), NKA (74), and the Na^+ -coupled glucose cotransporter 1 (SGLT1) (71, 95). The promoter of rat *Nhe3* gene has binding sites for glucocorticoid receptor (GR) and is activated by glucocorticoids (96). NHE3 is also activated by glucocorticoids via SGK1 phosphorylation (72). Glucocorticoids activate SGK1 by inducing SGK1 gene expression (~20 min) and by stimulating PI 3-kinase (PI3K) which phosphorylates SGK1 to activate (6, 97, 98). The NHE3 activation by SGK1 is dependent on their combined interaction with NHERF2 and then phosphorylation at S663 of NHE3 by SGK1 (72, 99). Analyses of *Sgk1*^{-/-} mice demonstrated that glucocorticoids enhance intestinal NHE3 and SGLT1 protein abundance at the brush-border of wild-type but not of *Sgk1*^{-/-} mice (71).

In the proximal colon, mineralocorticoids also activate NHE3 (6, 73). There is a differential effect of mineralocorticoids in small vs. large intestine on NHE3 activation. This difference arises from the differential expression and activity of 11 β -hydroxysteroid dehydrogenase (11 β -HSD2) which is required for mineralocorticoids function mediated by mineralocorticoids receptors (100).

Regulation of Slc26a3/a6 by second messengers

Studies of isolated intestinal mucosa demonstrated that Cl^- influx is reduced and Cl^- efflux is increased after stimulation by enterotoxins or neurotransmitters (via $\uparrow$ [cAMP] (68, 75), $\uparrow$ [Ca^{2+}] (75–77), or $\uparrow$ [cGMP] (70)). Thus, these signals seem to inhibit Cl^- absorption perhaps by Slc26a3 and Slc26a6 activating Cl^- secretion by CFTR (101). In contrast to NHE3-regulation, the inhibitory mechanism of Slc26a3 and Slc26a6 are poorly understood.

SLC26A3 has C-terminal PDZ-binding motif, which can interact with PDZ-2 domain of NHERF2 (102) as well as PDZ-2/-3 of NHERF3 (103). In cultured cells, exogenously expressed Slc26a3 is inhibited by increased intracellular [Ca^{2+}] (104, 105) and increased [cAMP] (105). Interestingly, this Slc26a3 inhibition does not occur after PMA (PKC activator) suggesting that the $\text{Cl}^-/\text{HCO}_3^-$ exchange inhibition is not mediated by PKC (104). Cyclic-AMP and Ca^{2+} cause internalization of Slc26a3 (similar to NHE3) in both

cultured cells and intestinal mucosa (105), suggesting that internalization a major pathway to regulate Slc26a3 expression and thus its apical $\text{Cl}^-/\text{HCO}_3^-$ exchange. The interactions of Slc26a6 and NHERFs have not been directly demonstrated. Nevertheless, SLC26A6 has no transport activity after removal of its C-Terminal PDZ-binding motif (106, 107).

In contrast to Slc26a3, PKC does regulate Slc26a6 activity (108). In HEK293 cells or in *Xenopus* oocytes, PKC seems to dissociate intracellular carbonic anhydrase II (CAII) from its binding site on Slc26a6 (109) (108). CAII catalyzes the reversible conversion between CO_2 and HCO_3^- which supplies the HCO_3^- substrate to Slc26a6. Slc26a6 has a binding site for CAII, the deletion of which decreases $\text{Cl}^-/\text{HCO}_3^-$ exchange activity (109). Mechanistically, PKC activation reduces Slc26a6/CAII association resulting in the reduced Slc26a6 activity in HEK293 cells (109). In *Xenopus* oocytes, PKC- δ mediates PMA induced Slc26a6-inhibition and internalization (108). CAII is also seems required for full Slc26a3 activity in HEK293 cells, but Slc26a3 transport activity is not stimulated by direct interaction with CAII (110).

Cross-regulation of NHE3, SLC26s, and CFTR

In the small intestine, it is widely held that villus cells absorb mainly NaCl and nutrients, while crypt cells mainly secrete fluid containing Cl^- and HCO_3^- . These ideas are the synthesis of immunohistochemical analyses demonstrating variable distribution of the ion and nutrition transporters and channels (50, 111). At the same time, it is also believed that the electroneutral NaCl absorption and electrogenic Cl^- secretion occurs in the same cells where transporters form macromolecular complexes, which presumably allows the transporters to regulate and be regulated by each other. How can this occur?

At the brush border, NHERFs scaffold transporters/channels (NHEs, Slc26s, and CFTR), cytoskeletal molecules, and kinases as described above. Furthermore several Slc26s are directly associated with CFTR. For Slc26a3 and Slc26a6, this interaction is mediated by phosphorylation of the CFTR R-region by PKA apparently allowing the interaction with the Slc26-STAS domain (26, 101) When two proteins are exogenously co-expressed, the cAMP mediated interaction activates $\text{Cl}^-/\text{HCO}_3^-$ and Cl^-/OH^- exchange activities of SLC26s and overall open probability of CFTR (26, 101). This model is developed for the fluid secretion of pancreatic duct cells, but similar system may be present in the intestinal epithelium. Conversely, when Slc26a9-STAS and R-CFTR interact, Slc26a9 functions are inhibited and this process does not seem to require phosphorylation (54).

Studies using jejunal mucosa of *CFTR*^{-/-} mice indicated that the mice lack not only cAMP-mediated Cl^- secretory activity but also lack cAMP-mediated inhibition of electroneutral NaCl absorption (112). These data suggest an *in vivo* involvement of CFTR on regulation of NHEs and Slc26s. (4, 113) CFTR activation reduces the cell volume in villus epithelium and induces cell shrinkage. Hypertonic medium (causing cell shrinkage)_also inhibits electroneutral NaCl absorption (114). On the other hand, inhibition of duodenal NHEs by certain inhibitors can stimulate CFTR and HCO_3^- secretion (111, 115). Cultured cells co-expressing NHE3, CFTR and NHERF2, show inhibition of PKA-mediated CFTR activity, which is dependent on interaction of NHE3 and NHERF2 (116).

Coexpression of Slc2aA3 and NHE (NHE2 or NHE3) results in transport activation (105). In this system, anion inhibitors (DIDS and niflumic acid) block not only the Slc26a3 activity but also NHE activity. Likewise, dimethyl-amiloride (NHE inhibitor) of NHEs blocks NHE2/3 activity as well as Slc26a3 activity.

Increased cellular cAMP is restored to basal levels by (a) hydrolysis into 5'-AMP by phosphodiesterases (PDEs) or (b) cAMP efflux by MRP4 (ABCC4, ABC transporter). MRP4 physically associates with CFTR via NHERF3, and thereby inhibits CFTR (117). MRP4 may also control NHE3 activity by regulating local intracellular [cAMP].

REGULATION OF EPITHELIAL NaCl ABSORPTION

Epithelial absorption and secretion in the small intestine are regulated by endocrine system, autonomic nerve system, and immune system (Figure 2). The intestine has a tremendous number of enteric nerves which form interconnected networks within the intestinal wall and project directly to the epithelium. Thus, both direct and enteric nerve-mediated regulation of epithelial cell transport are operative.

The enteric nerves are composed of the myenteric plexus and the submucosal plexus, which can function even when disconnected from the central nerve system (118). The enteric nerves and the epithelial cells are both regulated by sympathetic nerves (pro-absorptive), parasympathetic nerves (secretory /anti-absorptive), endo- or paracrine system (pro- and anti-absorptive), and immune system (secretory) (119–122). Although the mechanisms of the enteric nerve-mediated regulation is not fully understood, the end result is predominantly mediated by norepinephrine (pro-absorptive), somatostatin (pro-absorptive), acetylcholine (secretory), and VIP (secretory).

Secretory regulation by neuroendocrine systems (acetylcholine, VIP, substance P, 5-HT)

The secretory (anti-absorptive) neural effect is mediated by parasympathetic neurons (cholinergic), cholinergic secretomotor neurons, and VIP secretomotor neurons. Acetylcholine and VIP inhibit electroneutral NaCl absorption and induce electrogenic Cl⁻ secretion in the small intestine epithelium. This secretory effect is mediated by M₃ muscarinic receptors (↑ cellular [Ca²⁺]) and VIP receptors (↑ [cAMP]) on the epithelial cells (123).

Substance P is an 11 amino-acid peptide and its receptor is neurokinin 1 receptor (NK1). Substance P is found in myenteric and submucosal neurons and has a secretory effect (124). The substance P secretory effect is largely inhibited by tetrodotoxin, suggesting the effect is mediated by secretomotor neurons. Both cholinergic and noncholinergic secretomotor neurons are involved, and the cholinergic effect is mediated by NK1 (124, 125).

Serotonin (5-hydroxytryptamine, 5-HT) is secreted both from the enteric nerves in myenteric plexus and enterochromaffin (EC) cells (126, 127) which sense luminal molecules (128). The anti-absorptive and pro-secretory effect of 5-HT is mediated predominantly by cholinergic and VIP secretomotor neurons. In Caco-2 cells, 5-HT₄ receptor mediate activation of PKCα, inhibition of Na⁺/H⁺ exchange activity, and reduction of transcription

of NHE3 (129). 5-HT also modifies the brush border architecture which in turn reduces NHE3 function (130).

Absorptive regulation by neuroendocrine systems (catecholamines, somatostatin, opioids)

Norepinephrine (secreted by adrenal gland or sympathetic nerves termini) and other catecholamines increase electroneutral NaCl absorption and decrease electrogenic Cl^- secretion by intestinal mucosa. Catecholamines act at the α_2 -adrenergic receptor (131) on the epithelial cells (132). The α_2 -receptor couples with inhibitory G-proteins Gi_2 and Gi_3 (133) which both antagonize cAMP production. Additionally, second messenger-independent inhibition of Cl^- secretion by G_i has been suggested for colonic epithelium (134). Catecholamines (α_2 -receptor) also elicit noradrenergic inhibitory postsynaptic potentials (IPSP) of VIP secretomotor neurons in the submucous plexus (119, 135).

Somatostatin is a 14 or 28 amino-acid peptide secreted by extrinsic and intrinsic neurons of intestinal myenteric and submucosal plexus, as well as endocrine D cells in the epithelium throughout gut (136, 137). Somatostatin analogs activate electroneutral NaCl absorption in the intestine. Secretory diarrhea caused by VIP-producing (the Verner-Morrison syndrome) or serotonin-producing (carcinoid syndrome) tumors demonstrate an inhibitory effect of somatostatin (138). Somatostatin receptors (SSTR1 and SSTR3) are expressed in the epithelium as well as enteric neurons of submucosal and myenteric plexuses (139). SSTR2 is expressed in the enteric neurons but not in epithelial cells (140). Somatostatin (via SSTR1/SSTR2) also elicits nonadrenergic IPSPs of the VIP secretomotor neurons (119, 135).

Opioids (morphine, enkephalin) cause small intestine absorption (141, 142). The δ -opioid receptor is found in submucosal and myenteric neurons and mediates the inhibition of VIP secretomotor neurons by enkephalin. Prolonged morphine use results in astriction by stimulating NaCl absorption. Accordingly, enkephalinase is a drug target for diarrhea.

Neuropeptide Y is a 36 amino-acid peptide and is present in both myenteric and submucous neurons (143, 144). In the submucous plexus, neuropeptide Y is found in cholinergic and noncholinergic secretomotor neurons. Neuropeptide Y inhibits VIP-induced cAMP synthesis and Cl^- secretion, as well as prostaglandin elicited Cl^- secretion. The absorptive effect of neuropeptide Y is mediated by norepinephrine (α_2 -adrenergic receptors) in the ileum (145), while using Y_1 receptors in the human colonic epithelium (143).

Regulation by para- and endocrine systems

Guanylin and uroguanylin are 15–16 amino-acid peptides and are present in serotonin-positive EC cells of the small intestine (146). Luminal peptide secretion is stimulated by salt ingestion, and elicit an increase of intracellular cGMP in epithelial cells via the apical receptor for guanylate cyclase C (GC-C) (147, 148).

Peptide YY (PYY) is a gut hormone of 36 amino acid residues released from endocrine L-cells of the ileal mucosa following a meal. PYY shares sequence homologies with NPY and

accordingly also shares a receptor. Like NPY, PYY is absorptive (anti-secretory) in the small intestine (144).

As mentioned above, glucocorticoids activate electroneutral NaCl absorption as well as nutrient coupled Na⁺ absorption in the small intestine. Mineralocorticoids play a significant role in stimulation of colonic Na⁺ absorption, but have little effect on the regulation of small intestinal NaCl absorption. Low doses of angiotensin II (AT₂ receptor) stimulate intestinal electroneutral NaCl absorption (149) indirectly via norepinephrine secretion by sympathetic nerves (150, 151). Angiotensin II also antagonizes the secretory effect of VIP (152).

Atrial natriuretic peptide (ANP) and its relating peptides (BNP and CNP) are a family of peptides which reduce blood pressure and induce natriuresis. Natriuretic peptides reduce intestinal NaCl and water absorption and increase intestinal fluid content (153–156). This natriuretic action effects the jejunum but not ileum in dog (154). Atrial natriuretic peptide (ANP) stimulates the cGMP synthesis in cultured rat ileal cells via their receptor guanylate cyclase A (156, 157). However, the intestinal role of natriuretic peptides is still controversial and detailed regulatory mechanisms have not been clarified.

Immune regulation of secretion

Mediators of enteric immune system also have secretory (anti-absorptive) effects on intestinal epithelium (158). Prostaglandin E₂ (PGE₂) increases electrogenic Cl[−] secretion and inhibits electroneutral NaCl absorption (159). Because the effect is not inhibited by atropine or tetrodotoxin (160), PGE₂ may directly induce intracellular cAMP activated by its receptors present in the plasma membrane of intestinal epithelium (161). PGE₂ is secreted by activated fibroblasts (162) and the secretion is inhibited by indomethacin (160).

Histamine is secreted by activated mast cells and elicits a short-circuit current in intestinal epithelium and this current is blocked by atropine or tetrodotoxin in rat jejunum (160), suggesting the mediation of cholinergic enteric nerves. In the colon, the histamine H₁ receptor (↑ intracellular [Ca²⁺]) directly mediates secretion by the epithelial cells (163). Activated mast cells secrete 5-HT (160, 163) which can also cause epithelial secretion.

T-cell activation inhibits intestinal Na⁺ absorption, increases Cl[−] secretion, increases intestinal permeability, and causes diarrhea. These T-cell elicited physiological changes are mediated by TNF-α and IFN-γ. In mouse jejunum, TNF-α inhibits epithelial NHE3 (apical) and Na⁺-K⁺-ATPase (basolateral) (164, 165) thereby decreasing transepithelial NaCl absorption. IFN-γ reduces the transcriptional activity of *nhe3* gene in rat ileum, colon and Caco-2/bbe cells (166) reducing the number of NHE3 transporters and thus reducing NaCl absorption. Finally, IFN-γ also reduces Slc26a3 and Slc26a6 expressions in Caco-2 cells (167, 168). likely reducing Cl[−] uptake. Again, the net result is decreased intestinal NaCl absorption.

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List of Acronyms

ANP	Atrial natriuretic peptide and its relating peptides (BNP and CNP) are peptides → ↓ blood pressure and ↑ natriuresis
CA	Carbonic anhydrase
CFTR	Cl ⁻ channel mutated in cystic fibrosis; ABC transporter
cGK	cGMP-dependent protein kinase
ENaC	epithelial Na ⁺ channel
IPSP	inhibitory postsynaptic potential
5-HT	5-hydroxytryptamine, serotonin
NHE	Na ⁺ /H ⁺ exchanger
NHERF	NHE related factor, PDZ-binding protein
NKA	Na ⁺ /K ⁺ ATPase, Na ⁺ pump
PKA	protein kinase A; cAMP-dependent protein kinase
PKC	protein kinase C
SGLT1	Na ⁺ /glucose cotransporter1
SGK1	serum- and glucocorticoid-regulated kinase 1
Slc# / SLC#	HUGO nomenclature for “Solute carrier”, # indicates gene family; all capital is specific for human genes
Slc26a3	Cl ⁻ /HCO ₃ ⁻ exchanger; DRA, down-regulated in adenoma; CLD, Cl ⁻ loosing diarrhea; activated by R-region of CFTR
Slc26a6	Electrogenic Cl ⁻ / <u>n</u> HCO ₃ ⁻ exchanger; PAT1; CFEX; exchanges Cl ⁻ for HCO ₃ ⁻ , sulfate, oxalate or formate; activated by R-region of CFTR
Slc26a9	Electrogenic <u>n</u> Cl ⁻ /HCO ₃ ⁻ exchanger; Cl ⁻ channel; Na ⁺ /anion cotransporter; inhibited by R-region of CFTR
STAS	Sulfate transporter anti-sigma domain
VIP	vasoactive intestinal peptide

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Summary Points

1. Electroneutral NaCl absorption by the small intestine is mediated by NHE2, NHE3, Slc26a3, Slc26a6, and Slc26a9 at the apical membrane of the small intestine, and the basolateral NKA. Physiological analyses of knockout mice demonstrated facilitating electroneutral NaCl absorption is the dominant functions of NHE3, Slc26a3, and Slc26a6 in the intestine.
2. Intracellular pH and intracellular $[\text{HCO}_3^-]$ control directly (H^+ and HCO_3^- as substrates) and indirectly (pH dependence of transporters and metabolic HCO_3^- production) intestinal NaCl absorption
3. Direct interaction or NHERF-mediated interaction among NHE3, Slc26s, CFTR, and protein kinases, regulate transport activities. Electroneutral NaCl absorption and electrogenic Cl^- secretion are oppositely controlled by intracellular cAMP, Ca^{2+} , and cGMP.
4. The endocrine system, autonomic nerve system, and immune system can regulate epithelial NaCl transport function directly or indirectly via the enteric nervous system and transporter gene expression.

Future Issues

1. Is control of the basolateral transporters and channels in the intestine more complicated or merely a few key proteins?
2. How does diet effect intestinal NaCl absorption, e.g., can abundance of apical transport substrates (sulfate, oxalate, etc) control efficacy of NaCl absorption?
3. What is the clinical effect of intestinal resection on NaCl absorption?
4. Does systemic acid-base status effect intestinal NaCl absorption?

Figure 1. Mechanism of electroneutral NaCl absorption by the intestinal epithelial cells
 (A) Measurement of electroneutral NaCl absorption and cAMP-stimulated electrogenic Cl⁻ secretion. The basal (*upper left*) and cAMP stimulated (*upper right*) transepithelial movement of Na⁺ and Cl⁻ by rabbit ileal mucosa (1.12 cm² exposed area) were measured by the short-circuit technique (using Ussing chambers). Mucosal→serosal (absorption: abs.) and serosal→mucosal (secretion: sec.) movement are both shown. Net ion flux is shown in *lower panel*. In *lower panel*, positive values indicate net absorption, and negative value indicates net secretion. [Modified from Field (68)]. (B) Molecules involved in electroneutral NaCl absorption. Apical absorption of NaCl is mediated by Na⁺/H⁺ exchanger (NHE3) and anion exchangers (Slc26a3/6/9). Basolateral movement of NaCl is mediated by Na⁺/K⁺-ATPase (NKA) and ClC-2 chloride channel. Metabolic CO₂ is the source of H⁺ and HCO₃⁻ ions via intracellular carbonic anhydrase II (CA) catalysis. Electrogenic Cl⁻ secretion is mediated by CFTR. (C) Direct or NHERF-mediated interaction between CFTR and transporters. Known interactive activation (+) and inhibition (-) are shown; “?” is unknown. R, R-region of CFTR; S, STAS domain of Slc26s.

Figure 2. Regulation of NHE3: endocrine, nervous, and immune

Absorptive (anti-secretory, red) and secretory (anti-absorptive, blue) signals are indicated. NE, norepinephrine or norepinephrinergic neuron; ACh, acetylcholine or cholinergic neuron; VIP, vasoactive intestinal peptide or VIPergic neuron; AII, angiotensin II; NPY, neuropeptide Y; 5-HT, 5-hydroxytryptamine or serotonin; SP, substance P; SST, somatostatin; EK, enkephalin; PYY, peptide YY; PGE₂, prostaglandin E₂; IFN-γ, interferon-γ; GC, glucocorticoid; GN, guanylin; UGN, uroguanylin; PKAα, cAMP-dependent protein kinase α; PKCα, protein kinase Cα; cGKII, cGMP-dependent protein kinase II; SGK1, serum and glucocorticoid-dependent protein kinase 1.