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Ion transport in the intestine

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Abstract

Purpose of review—In recent years, the field of intestinal physiology has witnessed significant progress in our understanding of the expression and function of ion transport proteins and their genes under physiological and pathophysiological conditions. This review will present some of these most recent advances in the small intestinal ion transport mechanisms.

Recent findings—One of the new and exciting aspects of this field has been the integration of function and structure of several intestinal transport processes. This is well exemplified by the discussed intricacies of intestinal bicarbonate secretion as well as the role of scaffolding PDZ proteins interacting with several transporters. We also discuss some of the most recent data pointing to the role of ion transporters in the pathogenesis of inflammation-associated diarrhea and their potential role in the maintenance of epithelial integrity.

Summary—Mouse models deficient in some of the key genes encoding ion transporters and their adapter proteins continue to provide important clues into intestinal transport processes. Several of the new in-vivo findings revise or complement past paradigms, many of which were derived from in-vitro approaches. New data on the interdependent functions of multiple transporters, as exemplified here by intestinal bicarbonate secretion, increase the complexity of the intestinal ion transport mechanisms and continue to contribute to a more integrated view of the transport phenomena in the gut. Data from patients and mouse models of intestinal inflammation also increase our understanding of the pathophysiology of inflammation-associated diarrhea.

Keywords

anion exchange; bicarbonate secretion; inflammation; Na^+/H^+ exchange; PDZ proteins

Introduction

Research on intestinal and epithelial transport in general can be viewed historically as proceeding in phases. The first phase was represented by physiological studies on solute transport without the knowledge of the molecular identity of the transport proteins and their

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genes. In the next stage, with the advent of molecular biology, recombinant DNA techniques, and later successful genome sequencing projects, most of the critical transport genes have been identified, cloned, and characterized for their tissue and cellular distribution, substrate specificity, transport kinetics, gene organization, transcriptional and post-transcriptional control under basal conditions and as regulated by physiological stimuli. Later, with the advent of gene targeting technologies and evolving animal models of human disorders, intestinal transport physiologists focused their attention on evaluating the physiological roles of intestinal transporters in mice carrying targeted inactivating mutations, and on regulation of intestinal transport processes under pathophysiological conditions related to an array of disorders affecting electrolyte homeostasis in the gut. Another new and exciting aspect of recent progress in this field has been the integration of function and structure of several intestinal transport processes. Although the realization that many of the membrane transporters do not function independently is not a new concept, many recent studies explain it with significantly more biochemical detail, highlighted by the role of common scaffolding proteins participating in the formation of membrane-associated signaling complexes effectively regulating the activity, cellular localization, and degradation rate of the intestinal transport proteins.

Integration of transporter function in duodenal bicarbonate secretion

One prime example of the complexity and integration of function in intestinal ion transport is bicarbonate secretion. Duodenal HCO_3^- secretion is a critical nonstructural protection mechanism against the acidic gastric output, against changes in luminal pH, which could adversely affect mucin viscosity and bacterial binding, and against mucosal ulcerations in the otherwise ‘leaky’ duodenal epithelium. Several key transport pathways participate in this defense mechanism, resulting not only in neutralization of the luminal content, but also providing high intracellular buffering capacity to counteract the effects of highly acidic millieu. These include basolateral uptake and enzymatic generation of HCO_3^-, and its luminal secretion, functions secondarily affected by abundantly expressed apical and basolateral Na^+/H^+ exchangers. HCO_3^- entry into the epithelial cells is provided by the activity of the basolateral Na^+/HCO_3^- cotransporter NBC1 along with NHE1 [1]. Intracellularly, bicarbonate is generated from the ambient CO_2 by the activity of several soluble or membrane-bound isoforms of carbonic anhydrase, although the relative contribution of each of these enzymes is still not defined. Duodenal bicarbonate secretion has been believed to be mediated by transport mechanisms provided by CFTR, and three anion/HCO_3 exchangers, SLC26A3 (DRA: downregulated in adenoma), SLC26A6 (PAT1), and SLC4A9 (AE4). The contribution of each of these four pathways to basal and acid stimulated HCO_3^- secretion as well as their mutual relationship has been a subject of debate in recent years.

CFTR is expressed primarily in the crypt cells whereas the three anion exchangers are expressed at different levels in the brush border membrane of the villus epithelial cells. In a recent report Simpson *et al.* [2••], using in-vitro techniques, measured anion exchange in the upper villus epithelium of wild type, SLC26A3, SLC26A6, and SLC4A9 knockout mice. AE4 had relatively low expression and did not significantly contribute to basal Cl^-/HCO_3^- or SO_4^{2-}/HCO_3^- exchange or to changes in intracellular pH (pH_i). DRA-deficient mouse

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epithelium displayed a 30–40% reduction of Cl^- – but not SO_4^{2-}-dependent HCO_3^- flux. Interestingly, the increase in pH_i in $DRA^{-/-}$ epithelia suggested that this cytoplasmic alkalinization may be due to disrupted coupling between DRA and Na^+/H^+ exchange activities. Indeed, a very recent study by Walker *et al*. [3•] elegantly confirmed that DRA couples with NHE3 for electroneutral NaCl absorption across the mammalian small intestine. It remains unclear whether other apical NHE isoforms are in similar relationship with DRA. We have reported that NHE2 (SLC9A2) contributes to small intestinal Na^+/H^+ exchange, albeit with a rapid decline with age [4]. Similarly, the role of NHE8, also expressed on the apical membrane of the small intestinal epithelial cells [5•] has not yet been elucidated in this context. The third anion/HCO_3^- exchanger, PAT-1, is the most versatile in terms of substrate specificity, demonstrating Cl^-, SO_4^{2-}, oxalate, formate, HCO_3^-, and OH^- transport [6]. In the quoted study by Simpson *et al*. [2••], PAT-1 was responsible for 65–80% of the Cl^- -dependent HCO_3^- flux, and for essentially all of the SO_4^{2-}-dependent HCO_3^- flux in the villus epithelial cells. Interestingly, contrary to the observed cytoplasmic alkalinization in DRA-deficient mice, baseline pH_i was reduced in $PAT\text{-}1^{-/-}$ mice, a phenomenon that could not be attributed to changes in Na^+/H^+ activity. The authors speculated that PAT-1 might function as a bidirectional exchanger, whereby membrane depolarization, e.g., resulting from electroneutral Na^+/H^+ exchange would favor PAT-1-mediated HCO_3^- influx. This would imply that PAT-1 stoichiometry for anion/HCO_3^- exchange may be electrogenic under certain conditions. This indeed has been described in other systems, although it may not be universal across species, cell type, or the exchange substrates [7-9].

Tightly controlled conditions of an in-vitro study such as Simpson's [2••] are both an asset and a disadvantage. They do not account for the neural tone, regional (crypt-villus) distribution and activity of transporters, hormone and neurotransmitter receptors or the levels of their ligands under basal and acid-stimulated conditions, etc. Importantly, such focused designs may miss cooperative aspects of transporter function, such as that reported for CFTR and anion/HCO_3^- exchangers. There is a number of reports emphasizing the role of CFTR in the duodenal bicarbonate transport, which includes not only the passive facilitating coupling effect of CFTR-mediated Cl^- secretion on Cl^-/HCO_3^- exchange, but also active and direct HCO_3^- conductance. CFTR's anion selectivity is not intrinsically fixed, but can undergo a dynamic shift to conduct bicarbonate by a process involving ATP hydrolysis, especially when activated by glutamate. A recent article by Singh *et al*. [10••] attempted to address some of the issues related to the roles of PAT-1 and CFTR in a more physiologically relevant in-vivo settings using luminal perfusion in anesthetized mice. This study utilized CFTR and SLC26A6 knockout mice to investigate the resting, luminal acid stimulated, and high plasma $HCO3^-$-stimulated duodenal $HCO3^-$ secretion. Contrary to the in-vitro study by Simpson *et al*. [2••], PAT-1 deficiency resulted in only a slight reduction in basal duodenal HCO_3^- secretion (by approximately 18% under normal plasma HCO_3^- concentration), but it almost completely eliminated the increase in HCO_3^- secretion in response to intraarterial perfusion with bicarbonate. CFTR deficiency similarly blunted the response to high arterial bicarbonate, but it also reduced basal HCO_3^- secretion by as much as 70%. PAT-1 status had little effect on luminal acidification-induced bicarbonate secretion, suggesting that *in vivo*, the role of PAT-1 is limited to basal rather than acid-

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stimulated HCO_3^- secretion, and that its contribution may be highly dependent on the systemic acid –base status. CFTR deficiency, on the contrary, completely eliminated the effects of luminal acidification on bicarbonate secretion, an effect that was not influenced by arterial HCO_3^- infusion.

This, like several other studies, points to a critical role of CFTR and its coupling with anion/HCO_3^- exchange in basal and particularly in acid-stimulated bicarbonate secretion. This finding, however, also takes us back to the 'CFTR paradox' formulated by J.D. Kaunitz, that is the lack of mucosal ulcerations in cystic fibrosis patients despite a defect in duodenal HCO_3^- secretion. It is not inconceivable, that the role of CFTR in murine duodenal mucosa is much greater than that of human, particularly that there are significant species differences in the distribution of CFTR-expressing cells in mice vs. humans or rats. Unlike in humans, mice lack the scattered highly expressing epithelial cell, with more prominent expression in Brunner glands, crypts, and on the villi. CFTR expression in the villus cells is significantly greater than in humans or rats [11]. This could partially explain the phenotypic differences in cystic fibrosis symptoms in human patients and CFTR knockout mice, with the latter more affected by intestinal dysfunction. It has also been postulated, that in duodenal epithelium, CFTR deficiency may be partially protective against an acidic insult due to intracellular accumulation of HCO_3^- and increased cytosolic buffering capacity. A recent article by Mizumori *et al*. [12] adds another dimension to the explanation of this 'CFTR paradox' by demonstrating that in rats, CFTR inhibition results in increased NHE3 activity. The authors postulate that NHE3-mediated proton extrusion could neutralize luminal HCO_3^- and facilitate CO_2 absorption, which could then serve as a substrate for carbonic anhydrases, or for basolateral NBC1 transporter. An alternative, though not mutually exclusive theory is that HCO_3^- is taken up by the reverse activity of PAT-1, as we described earlier in this review. One complicating factor stems from a recent demonstration of coupling of NHE3 and DRA activities in the murine small intestine [3•]. One could extrapolate the results of this study and hypothesize that an increase in NHE3 activity in response to CFTR inhibition or ablation would be accompanied by DRA-mediated HCO_3^- secretion, which could, at least to a degree, counteract PAT-1 mediated HCO_3^- uptake.

In conclusion, the emerging picture of small intestinal ion transport is increasingly complex, with multiple players acting in a synchronized and interdependent manner. New models of targeted gene ablation continue to unravel novel aspects of this integration, as exemplified by the described mechanism of duodenal bicarbonate secretion and absorption.

PDZ protein family and their role in intestinal ion transport

Another plane of structural and functional integration of intestinal ion transporters is provided by PDZ domain-containing proteins. These domains constitute one of the largest families of interaction domains and function by binding the C termini of their target proteins [13,14]. They serve as adapter proteins between integral membrane and cytoskeletal proteins, and have been shown to bind directly to actin *in vitro*. A group of these proteins, highly concentrated in the apical aspect of polarized epithelial cells, has attracted considerable attention in the field of intestinal and renal ion transport; this includes

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NHERF-1 (also known as NHERF, EBP50), NHERF-2 (E3KARP), NHERF-3 (CAP70, PDZ-dc1), NHERF-4 (IKEPP), CAL (CFTR-associated ligand), and Shank2/ CortBP1. A comprehensive (change to lower case) overview of this topic is well beyond the limits of this brief article and the reader is referred to two recent reviews published by Lamprecht and Seidler [15•], and Donowitz and Li [16•]. Here, we will discuss some of the most recent developments describing the structural and functional relationship between PDZ proteins and intestinal ion transporters.

NHERF-1 has been demonstrated to associate with multiple ion transporters in a heterologous expression systems, including the discussed NHE3, DRA, PAT-1, and CFTR. Although NHERF-1 and NHERF-2 were initially described as mediating the inhibitory effects of cAMP on NHE3 activity and Na^+ uptake *in vitro*, and as potentially viable targets of antidiarrheal therapies, a recently published report utilizing NHERF-1 deficient mice demonstrates reduced small intestinal fluid absorption in vivo, as well as reduced in-vitro Na^+ absorption in isolated jejunal and colonic, though not ileal, mucosa [17••]. Moreover, contrary to renal proximal tubules [18••] and jejunum [17••], cAMP-mediated inhibition of both, ileal Na^+/H^+ exchange and fluid absorption remained unaffected by NHERF-1 ablation [17••,18••]. These new findings point to a tissue-specific and segment-specific involvement of NHERF-1 in the regulation of water and electrolyte transport, as well as to a potentially profound impact of this scaffolding protein in diarrheal disorders. Indeed, a very recent report by Sullivan *et al*. [19••] suggests that inflammation (IBD)–associated diarrhea may be related to reduced expression of NHERF-1 and NHERF-2 in the ileal and colonic mucosa.

NHERF-1 and NHERF-2 are known to enhance CFTR-mediated Cl^- secretion due to the anchoring of protein kinase A (PKA) to the CFTR complex. NHERF-1 and NHERF-2 have also been described to reduce CFTR endosomal recycling and lysosomal degradation by interfering with the negative effects of CAL, a phenomenon facilitated by the recently illustrated low affinity of the CAL–CFTR interaction compared with NHERF–CFTR binding [20]. This mechanism of CFTR regulation may explain the observed decrease in forskolin-stimulated Cl^- secretion in NHERF-1-deficient mice [17••]. Somewhat unexpectedly, however, NHERF-2 deficiency had no effect on cyclic nucleotide-induced CFTR activation in mouse jejunum, a phenomenon that may be related to the described very low expression of this adapter protein in the mouse small intestinal epithelium [21•].

In summary, some of the new in-vivo findings revisit and revise the roles of the PDZ proteins in epithelial ion transport, bringing new complexity to the involvement of these crucial adapter proteins in intestinal electrolyte and water homeostasis. The unraveling of the intricate network of functional and structural relationships continues to provide clues into their tissue-specific, segment-specific, and transporter-specific roles.

Regulation of intestinal ion transport in inflammatory states

In conjunction with the kidney, the gastrointestinal tract is uniquely positioned, both structurally and functionally, to maintain the homeostatic control of electrolyte balance and the ensuing fluid homeostasis and passive mucosal defense. Under normal circumstances in an adult human, the gastrointestinal tract handles an average luminal daily load of 9 L of

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water and 800 mEq of Na^+ originating from ingestion as well as secretory fluids. Small intestinal absorption of water is largely secondary and controlled by ion flux across the epithelial barrier. Small intestine has a substantial capacity to absorb osmolytes and water, with nearly 80% of the daily load absorbed and approximately 2 L/day of resulting ileocecal flow. Large intestine has the capacity to absorb 1.5–1.9 L/day, although in pathological states with defective small intestinal absorption, the initiated adaptive mechanisms allow the colon to compensate with absorption rate reaching 5–6 L/day. Exceeding this maximal capacity will result in diarrhea, commonly observed in response to intestinal inflammation such as in chronic inflammatory bowel diseases or secondary to microbial infections. Pathogenic mechanisms involved in infectious diarrhea have been recently reviewed by Navaneethan and Giannella [22•] and will not be discussed in detail. The reader is also referred to a recent review on the altered ion transport in IBD by Martinez –Augustin *et al*. [23•]. Several key findings have been recently described that contribute to our understanding of the pathogenesis of inflammation-associated diarrhea. Intestinal electroneutral Na^+/H^+ exchange, mediated primarily by NHE3, is known to be negatively affected by colonic inflammation and inflammatory mediators (nitric oxide, TNFa, IFN-g) as well as enteropathogenic bacteria [24]. In a recent report, Sullivan *et al*. [19••] describes that in ileal or sigmoid biopsies of a considerable number of patients with active Crohn's disease or ulcerative colitis, there is reduced expression of NHE3, NHERF-1, NHE1, epithelial sodium channel (EnaC) (Na^+ channel), and CLC-5 protein, with the latter suggested to participate in endosomal NHE3 trafficking rather than epithelial Cl^- conductance. These findings were recapitulated in mouse models of chemically induced colitis [19••], thus further highlighting and explaining the role of altered transepithelial Na^+ and fluid transport in IBD-associated diarrhea. Colitis or systemic inflammation may also affect small intestinal ion transport as recently demonstrated for ileal bicarbonate transport [25•] and NHE8 expression in the jejunum (unpublished data and [26]). Our group has also recently described spontaneous development of distal colitis in NHE3-deficient mice, and suggested the role of NHE3 as a modifier gene in intestinal inflammation [27•]. We have also presented data demonstrating severe susceptibility of NHE3 knockout mice to DSS-induced mucosal injury, which unprecedently extends to the small intestine (unpublished data and [28••]). These findings, combined, strongly suggest a vital role of NHE3 not only in the pathogenesis of inflammation-associated diarrhea, but also in maintenance of epithelial integrity and perhaps in modulating mucosal inflammation and injury when down regulated.

In summary, intestinal inflammation is associated with defects in epithelial barrier function and ion flux, both contributing to impaired fluid homeostasis and diarrhea. Disturbances in ion and fluid absorption are less well characterized but the emerging data points to both the electroneutral and electrogenic Na^+ absorption. Infammation and inflammatory mediators are associated with changes in the expression and function of key epithelial transporters, including the Na^+/K^+ ATPase, sodium/potassium/chloride cotransporter 1 (NKCC1), sodium/hydrogen exchangers, and the ENaC. There is also emerging data suggesting that those changes may negatively feed back to the ability to maintain epithelial integrity in the face of microbial or noxious challenge. Further studies will be needed to provide a more comprehensive picture of the role of ion transport in intestinal inflammation.

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Conclusion

Although recent months have not yielded major break-throughs in the field of physiology and pathophysiology of intestinal ion transport, gene targeting and generated mouse models deficient in some of the key genes encoding transporters and their adapter proteins continue to provide important clues into intestinal transport processes. Several of the new in-vivo findings revise or complement past paradigms, many of which were derived mostly from in-vitro approaches. Novel information on the interdependent functions of multiple transporters, as exemplified here by intestinal bicarbonate secretion, increases the complexity of the intestinal ion transport mechanisms and continues to contribute to a more integrated view of the transport phenomena in the gut. Data from patients and mouse models of intestinal inflammation also increase our understanding of the pathophysiology and etiology of inflammation-associated diarrhea.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 160 –161).

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