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ADVANCES IN TRANSLATIONAL SCIENCE

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Translating Molecular Physiology of Intestinal Transport Into Pharmacologic Treatment of Diarrhea: Stimulation of Na^+ Absorption

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Diarrheal diseases remain a leading cause of morbidity and mortality for children in developing countries, while representing an important cause of morbidity worldwide. The World Health Organization recommended that low osmolarity oral rehydration solutions plus zinc save lives in patients with acute diarrhea, but there are no approved, safe drugs that have been shown to be effective against most causes of acute diarrhea. Identification of abnormalities in electrolyte handling by the intestine in diarrhea, including increased intestinal anion secretion and reduced Na^+ absorption, suggest a number of potential drug targets. This is based on the view that successful drug therapy for diarrhea will result from correcting the abnormalities in electrolyte transport that are pathophysiologic for diarrhea. We review the molecular mechanisms of physiologic regulation of intestinal ion transport and changes that occur in diarrhea and the status of drugs being developed to correct the transport abnormalities in Na^+ absorption that occur in diarrhea. Mechanisms of Cl^- secretion and approaches to anti-Cl^- secretory therapies of diarrhea are discussed in a companion review.

Keywords: Diarrhea; Na Absorption; Intestine.

Acute diarrheal diseases are a global public health problem. In developing countries, diarrhea is the second leading cause of mortality in children younger than 5 years of age, with an estimated 1.7 billion cases and 0.76 million deaths yearly.[1] Childhood mortality from diarrhea in the United States is much less frequent. Rather, it is the aged who appear to be dying most from diarrheal diseases.[2] Recently, the Bill and Melinda Gates Foundation–supported Global Enteric Multicenter Study documented the organisms producing acute diarrhea in children <5 years old in low income countries.[2] Although there was variability in the responsible organisms, the major causes included rotavirus, enterotoxigenic *Escherichia coli* producing heat stable enterotoxin with or without heat labile enterotoxin, *Cryptosporidium*, *Shigella*, and *Vibrio cholerae*.[3]

Opiates have been used to treat diarrhea since the time of Hippocrates. These compounds only moderately decrease stool output, although they are widely used for treating otherwise refractory chronic diarrheas. Another antidiarrheal compound called racecadotril is a peripherally acting enkephalinase inhibitor.[4] Racecadotril reduces the secretion of water and electrolytes into the intestine and has had generally positive, although inconsistent, success for acute diarrhea in children.[5,6] Also, racecadotril is not approved by the Food and Drug Administration. Also, crofelemer is a Cl^- channel inhibitor that is FDA approved for treating HIV-related diarrhea.[7] It will be discussed in the accompanying article.

In fact, it is oral rehydration solution (ORS) that has accounted for the marked reduction of children dying from diarrhea in developing countries. Twenty-five years ago, childhood mortality from acute diarrhea primarily in developing countries was ~12 million/year. The reduction in mortality has correlated with ORS use. Importantly, ORS rehydrates the patients and reverses the killing dehydration but minimally reduces stool output or length of the diarrheal illness. These limitations plus that ORS is currently used in ~33% of cases of acute diarrhea argue for the need of an effective drug to treat diarrhea.

The purpose of this article is to review strategies for early drug development to treat diarrhea by stimulating intestinal Na^+ absorption. This topic will be described within a framework of reviewing the molecular mechanisms by which intestinal water and Na^+ are absorbed in healthy people and how those processes change in diarrhea.

Diarrhea Is Caused by Abnormalities in Intestinal Electrolyte Transport

Diarrheal diseases occur because of altered intestinal transport of electrolytes and water.[8] How the intestine

Abbreviations used in this paper: **BB, brush border; CaSR, calcium-sensing receptor; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; DRA, down-regulated in adenoma; ENaC, epithelial Na^+ channel; GI, gastrointestinal; NHE3, sodium/hydrogen exchanger 3; ORS, oral rehydration solution; SCFA, short-chain fatty acid; SGLT1, Na^+ D-glucose linked co-transporter 1; WHO, World Health Organization.**

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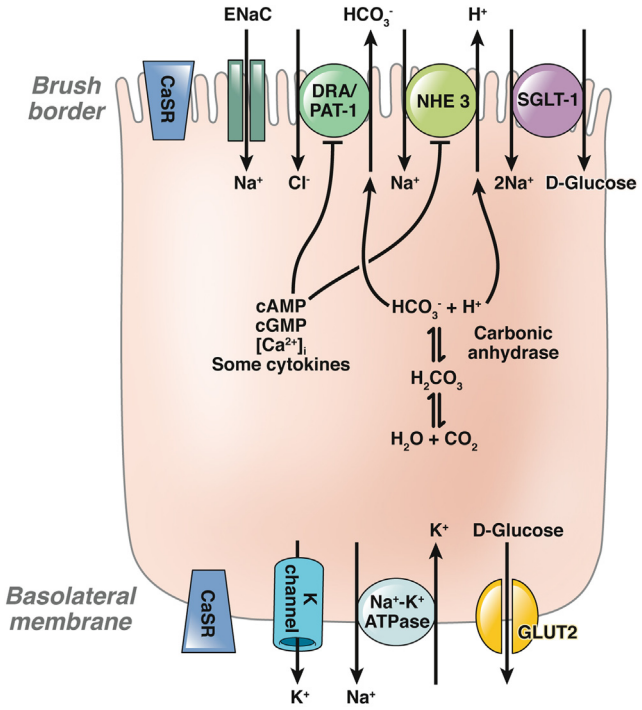

Figure 1. Intestinal sodium absorptive cells. Neutral NaCl absorption is made up of NHE3 linked to either Cl^-/HCO_3^- exchanger SLC26A3 (DRA) or A6 (PAT-1). Functional linkage is by changes in intracellular pH, with carbonic anhydrase generating H^+ and HCO_3^- used to link these 2 transporters. In diarrhea, intestinal NaCl absorption is inhibited by elevated cAMP, cGMP, $[Ca^{2+}]$, and cytokines such as tumor necrosis factor-α, with effects exerted on NHE3 and/or DRA.

transports electrolytes normally must be understood to allow understanding of the changes that occur in diarrhea. Epithelial cells in the villus of the small intestine or surface and upper crypt of the colon are primarily Na^+ absorptive cells, whereas those in the lower crypt are primarily Cl^- and HCO_3^- secretory. The plasma membrane of each cell is divided into 2 defined regions, the apical (brush border [BB]) and basolateral (serosal) membrane (Figure 1). Specific membrane transport proteins are segregated to either the apical or basolateral side of these cells. Concerted actions of these membrane proteins are required for transepithelial electrolyte transport (absorption and secretion). A cartoon version of the transport processes that contribute to intestinal Na^+ absorption is presented to provide a model on which to consider potential drug therapy that is aimed at either reversing the changes in transport that occur in diarrhea and/or stimulating other transport processes that can compensate for these changes (Figure 1).

Na^+ Absorptive Cells

Both the similar-appearing Na^+ absorptive villus and anion secretory crypt cells carry out active electrolyte transport energized by the basolateral membrane Na–K–adenosine triphosphatase. The Na^+ pump lowers intracellular $[Na^+]$ and makes the inside of the cell electrically negative. Apical Na^+ absorptive proteins (Figure 1) create water-filled pores in the plasma membrane that allows Na^+ to enter the cell from the lumen down this electrochemical gradient (inside of cell Na^+, $\sim$10 μmol/L; electrically negative compared with intestinal lumen). These apical or BB transporters differ in their distribution on the basis of the segment of the intestine. The Na^+ absorptive process most relevant for the pathophysiology of diarrheal diseases is neutral NaCl absorption. This is not a single transport protein but is made up of sodium/hydrogen exchanger 3 (NHE3), a BB Na^+/H^+ antiporter involved in intestinal Na^+ absorption, which is functionally linked to a Cl^-/HCO_3^- exchanger member of the SLC26A family. In duodenum and colon, the Cl^-/HCO_3^- exchanger is SLC26A3 (down-regulated in adenoma [DRA]), and in jejunum and ileum it is SLC26A6 (PAT-1).[9,10] Although these proteins are members of the same gene family, they differ in some properties, such as Cl^-/HCO_3^- stoichiometry and electrogenic properties.[11] Linkage is by small changes in intracellular pH, and the H^+ and HCO_3^- involved is generated by carbonic anhydrase (Figure 1). Neutral NaCl absorption occurs throughout the gastrointestinal (GI) tract excluding the distal colon, but it is less in the jejunum than in the ileum and proximal colon. In the jejunum there are also BB symporters that absorb substrates such as D-glucose or L-amino acids generated from macromolecules via digestion. These are generally linked to Na^+[12] by using the Na^+ pump generated electrochemical gradient as well as the concentration gradient across the BB for the substrate. Basolateral membrane transporters subsequently allow movement of the substrate from the cells to the blood. As an example, in the case of D-glucose, the BB symporter Na^+ D-glucose linked co-transporter 1 (SGLT1) transports 2 Na:1 D-glucose or D-galactose across the BB, whereas D-glucose is moved across the basolateral membrane by GLUT2. In the human distal colon, the epithelial Na^+ channel (ENaC) is located on the BB.

Potential Drug Targets for Treating Diarrhea by Stimulation of Intestinal Na^+ Absorption

Infectious agents that produce diarrheal diseases alter electrolyte transport and intestinal permeability by host-pathogen interactions. These include inhibition of Na^+ absorption and stimulation of anion and K^+ secretion. Neutral NaCl absorption is functionally inhibited in most diarrheal diseases including enterotoxigenic and inflammatory diarrheas, the 2 major categories of diarrhea. In some diarrheas in which there is damage to the epithelial cells, such as celiac disease, the amount of NHE3 is reduced.[13] On the basis of these changes in intestinal electrolyte transport in diarrhea, a strategy to develop drug therapy for diarrheal diseases is to seek drugs that stimulate intestinal Na^+ (and Cl^-) absorption. Although stimulation of SGLT1 and Na^+–L-amino acid transporters