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

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Abstract

Diarrheal diseases remain a leading cause of morbidity and mortality for children in developing countries while representing an important cause of morbidity worldwide. The WHO recommended low osmolarity oral rehydration solutions plus zinc save lives in patients with acute diarrhea[1], but there are no approved, safe drugs which 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 which occur in diarrhea. Mechanisms of Cl^- secretion and approaches to anti-Cl^- secretory therapies of diarrhea are discussed in a companion review.

Introduction

Acute diarrheal diseases are a global public health problem. In developing countries, diarrhea is the second leading cause of mortality in children less than 5 years of age with an estimated 1.7 billion cases and 0.76 million deaths yearly[2]. Childhood mortality from diarrhea in the USA is much less frequent. Rather it is the aged who appear to be dying most from diarrheal diseases[3]. Recently, the Bill and Melinda Gates Foundation supported Global Enteric Multicenter Study (GEMS) documented the organisms producing acute diarrhea in children < 5yo in low income countries [3]. Although there was variability in the responsible organisms, the major causes included rotavirus, enterotoxigenic *E. coli* producing heat stable enterotoxin with or without heat labile enterotoxin, *Cryptosporidium*, *Shigella*, and *V. cholera*[4].

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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 [5]. Racecadotril reduces the secretion of water and electrolytes into the intestine, and has had generally positive although inconsistent success for acute diarrhea in children [6, 7]. Also Racecadotril is not approved by FDA.

In fact, it is oral rehydration therapy (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 argues 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 transports electrolytes normally must be understood to allow understanding of the changes which 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 while those in the lower crypt are primarily Cl^- and HCO_3^- secretory. The plasma membrane of each cell is divided into two defined regions, the apical (BB) and basolateral (serosal) membrane (**Figure**). 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 which contribute to intestinal Na^+ absorption is presented to provide a model on which to consider potential drug therapy which is aimed at either reversing the changes in transport which occur in diarrhea and/or stimulating other transport processes which can compensate for these changes. (**Figure)**

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-ATPase. The Na+ pump lowers intracellular [Na^+] and makes the inside of the cell electrically negative. Apical Na^+ absorptive proteins (**Figure**) create water filled pores in the plasma membrane which allows Na^+ to enter the cell from the lumen down this electrochemical gradient (inside of cell Na^+, ~10μM; electrically negative compared to intestinal lumen). These apical or brush boarder (BB) transporters differ in their distribution based on 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 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) and in jejunum and ileum it is SLC26A6 (PAT-1)[9, 10]. While these proteins are member 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**). Neutral NaCl absorption occurs throughout the

GI tract excluding the distal colon, but is less in the jejunum than ileum and proximal colon. In the jejunum there are also brush border symporters which absorb substrates such as D-glucose or L-amino acids generated from macromolecules via digestion. These are generally linked to Na^+ [12], 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 SGLT1 transports 2Na:1 D-glucose or D-galactose across the BB, while 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 which 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 both enterotoxigenic and inflammatory diarrheas, the two 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]. Based on these changes in intestinal electrolyte transport in diarrhea, a strategy to develop drug therapy for diarrheal diseases is to seek drugs which stimulate intestinal Na^+ (and Cl^-) absorption. While stimulation of SGLT1 and Na^+-L-amino acid transporters have been used in ORS treatment of diarrhea, drug stimulators of these transporters have not been developed.

Brush Border Na^+/H^+ Exchanger (NHE3) and Cl^-/HCO_3^- Exchanger SLC26A3 (DRA)

The BB Na^+/H^+ exchanger NHE3 accounts for the largest amount of intestinal Na^+ absorption, while BB Cl^-/HCO_3^- exchange is involved both in anion secretion and NaCl absorption. NHE3 is highly regulated as part of normal digestion. Both stimulation and inhibition can be mimicked by neurohumoral substances that are released as part of digestion, as well as by multiple other factors some of which affect bowel function. NHE3 is directly stimulated in the intestine by $alpha_2$ adrenergic agonists and PYY and indirectly stimulated by somatostatin and mu receptor opiate agonists. NHE3 is also inhibited by the second messengers cAMP, cGMP, and elevated Ca^{2+} [14]. Common second messenger changes are caused by agents which contribute to the mechanism of fluid secretion that occurs physiologically as a part of digestion as well as cause diarrheal diseases. Examples include secretin and cholera toxin (elevation of cAMP); guanylin and *E. coli* heat stable enterotoxin (elevation of cGMP); acetylcholine and *C. difficile* colitis (elevation of Ca^{2+}). Less is known about the acute regulation of DRA. However, DRA is stimulated by LPA (Lysophosphatidic acid), butyrate and probiotics and is inhibited by elevated Ca^{2+} [15]. Both NHE3 and DRA are targets of pathogens which cause diarrheal diseases[16, 17]. Nonetheless, NHE3 and DRA have the potential to be targeted for development of anti-diarrheal drugs. In fact, a peptide that has the sequence of a part of NHE3 works from the lumen of the intestine to stimulate baseline intestinal Na^+ absorption and to overcome cholera toxin-induced intestinal secretion[18].

SGLT1: Na^+ D-Glucose Linked Co-Transporter 1—In addition to taking up Na^+ and D-glucose across the small intestinal BB (brush border), SGLT1, when exposed to D-glucose, initiates a signaling pathway that stimulates NHE3 activity under basal conditions by increasing the amount of NHE3 in the BB, and importantly, reverses cholera toxin inhibition of NHE3[16]. While not yet a drug target by itself, this SGLT1/D-glucose effect can reverse the NHE3 inhibition that occurs in most diarrheal diseases and appears to allow NHE3 to respond to additional drug stimulation[18]. This effect may be an unrecognized benefit of ORS and thus consideration should be given to developing drugs to stimulate SGLT1 as potentially useful in treating diarrhea.

ENaC (Epithelial Na^+ Channel)—This is a heteromeric tetrameric channel that is the rate limiting factor for electrogenic Na^+ absorption in the BB of the descending colon. ENaC is activated by apical extracellular proteases; it is also stimulated by hormones, short chain fatty acids and cAMP [19]. However its role in normal GI physiology and in acute diarrhea has not been defined. Drug stimulation of ENaC would seem to be a high likelihood target for treating diarrhea given its distal location in the GI track in an intestinal segment in which highly efficient Na^+ absorption occurs.

Additional Stimulators of Intestinal Na^+ Absorption

Modified ORS including Zn—A recent modification in ORS (osmolarity reduced to 245 mOsm/L) is now the solution sanctioned by the WHO[20]. By reducing the ORS osmolarity, a transepithelial osmotic force drives water and electrolytes across the jejunum and appears to increase its effectiveness[20]. Another new concept for treatment of ORS has begun being tested. Current ORS stimulates Na^+ absorption primarily in jejunum which has SGLT1, Na^+ L-amino acid transporters, and a di-Tri peptide transporter, PEPT1, as well as NHE3 (jejunum). The new approach uses ORS to add colonic Na^+ absorption by replacing the D-glucose or protein/L-amino acids from conventional ORS with a relatively pancreatic amylase resistant “non-hydrolyzable” starch[21]. Corn starch or maize is relatively resistant to hydrolysis by pancreatic amylase. When taken orally, while some of the corn starch is broken down in the jejunum to stimulate Na^+ absorption like conventional ORS, most of the corn starch enters the colon where it is broken down by bacteria and metabolized to short chain fatty acids such as propionate, butyrate and acetate. In the colon there is a neutral linked Na^+ absorptive process that exchanges short chain fatty acids (SCFA) for OH^- ions plus BB Na^+/H^+ exchange (an alternative model of H^+/SCFA symport has also been suggested). Preliminary experiments have demonstrated that this ORS shortens the duration and volume loss of severe acute diarrhea, an effect not seen with conventional ORS[21].

Zn shortens the duration of diarrhea and is part of the WHO recommended ORS. Whether its effect is related to Zn deficiency is not established but seems unlikely, indicating its action probably occurs by effects on intestinal electrolyte transport proteins affected in diarrhea. In fact, it has already been shown that Zn in vitro inhibits stimulated Cl^- secretion via blocking basolateral membrane K^+ channels[22]. It also appears to stimulate NHE3 activity.

Calcium-sensing Receptor—There are additional potential drug targets to consider for treating diarrhea. The intestinal calcium-sensing receptor (CaSR) regulates intestinal secretion and absorption and is thus a potential target for the treatment of secretory diarrhea of various etiologies. It is a 7 membrane spanning domain G-protein coupled receptor expressed on both the BB and basolateral membranes of intestinal epithelial cells. It is involved in the regulation of calcium homeostasis in the body. Concerning its potential role in treating diarrhea, stimulating the CaSR with either Ca^{2+} or sensitizing (calcimimetic) compounds stopped changes in colonic Na^+ absorption and Cl^- secretion caused by cholera toxin and heat stable *E. coli* enterotoxin. While this effect involved reversal of changes in second messengers cause by these enterotoxins, the stimulation of the CaSR also appears to directly affect function of NHE3 and CFTR[23, 24].

Challenges, Pitfalls and Future Perspective

What are the roadblocks and possibilities of overcoming them to develop a drug to treat diarrhea via stimulation of Na^+ absorption? Stimulation of neutral NaCl absorption, either via effects on NHE3 and perhaps on DRA has been identified as appropriate drug targets for treatment of diarrheas, with the potential to be useful for most forms of diarrhea. Drugs that are currently approved which stimulate neutral NaCl absorption are restricted to analogues

of physiologic regulators including mu agonist opiates, somatostatin, and clonidine (an alpha$_2$ adrenergic agonist). Pharmacologic preparations of these agonists have all been used to treat diarrhea but are limited by side effects (clonidine is used to treat hypertension), high costs, or requirement for parenteral administration, or limited potency (loperamide). While modifications of these classes of drugs might be developed, alternate ways to stimulate Na^+ absorption are needed. Proof of principle has been provided based on studies with an NHE3 mimetic peptide in cell and animal models, including in intact intestine with stimulation of basal absorption and reversal of cholera toxin and carbachol stimulation of fluid secretion [18]. However, these findings have not yet been translated into a drug for consideration for testing in people. Recent scientific advances make us optimistic that development of such a drug is now possible. The advances include: 1) Availability of a human"mini-intestinal" model for study ex vivo, developed through the work of Clevers and associates starting with human intestinal stem cells[25]. Failure of drugs for human use have been attributed to lack of studies in humans with efficacy and drug toxicity being different in humans compared to animals or the cancer cell lines that are usually tested. 2) Development of high throughput approaches (a popular approach of screening and assaying a large number of potential biological modulators and effectors against a chosen set of defined targets often on a plate form that allows performance of multiple analyses at the same time) that allow rapid screening of drugs already FDA approved for repurposing, drug libraries and collections of natural products [26, 27]. There are several natural products used to treat diarrhea around the world, especially in countries in which acute diarrhea is very common, that should be tested for potential efficacy in stimulating neutral NaCl absorption. 3) Commitment by leaders of the NIH to develop drug treatment for diarrhea as a global health responsibility to further address the mortality posed by diarrheal diseases.

In spite of this optimistic view, drug development is notoriously slow and as of now no lead compounds which stimulate neutral NaCl absorption have been identified that over the short term are likely to emerge as useful for drug treatment of diarrhea.

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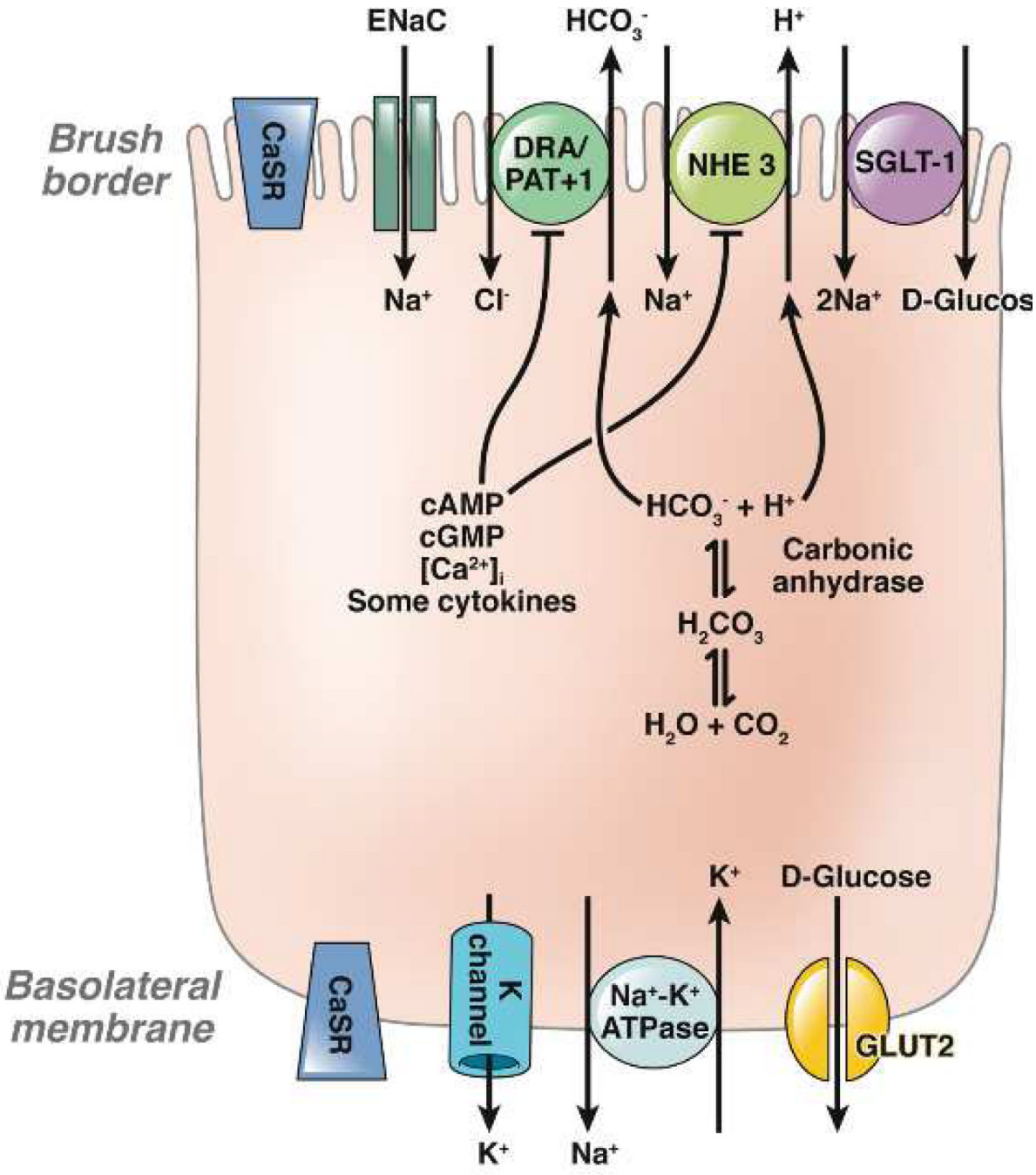

Figure.

Intestinal Sodium Absorptive Cells: Neutral NaCl absorption is made up of NHE3 linked to either DRA or PAT-1. Functional linkage is by changes in intracellular pH with carbonic anhydrase generating H^+ and HCO_3^- used to link these two transporters. In diarrhea, intestinal NaCl absorption is inhibited by elevated cAMP, cGMP, $[Ca^{2+}]$, and cytokines such as TNF-α with effects exerted on NHE3 and/or DRA