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Enteroendocrine and Neuronal Mechanisms in Pathophysiology of Acute Infectious Diarrhea

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Abstract

Background—While enterocyte secretion is the predominant mechanism considered responsible for secretory diarrhea in response to acute enteric infections, there are several lines of evidence that support alternative mechanisms controlling fluid and electrolyte secretion in diarrhea.

Aim—To review enteroendocrine and neuronal mechanisms that participate in the development of acute infectious diarrhea.

Recent Advances—Acute infectious diarrheas due to bacterial toxins (e.g., cholera, *E. coli* heat-stable enterotoxin, *C. difficile*) and rotavirus are all associated with secretion of transmitters from enteroendocrine cells (e.g., 5-HT) and activation of afferent neurons that stimulate submucosal secretomotor neurons. The latter secrete acetylcholine (which binds to muscarinic receptors on epithelial cells) and VIP. Involvement of nerves was demonstrated by inhibition of bacterial toxin-induced secretion by hexamethonium (nicotinic), tetrodotoxin (Na⁺ channel blocker), and lidocaine (visceral/mucosal afferents). Nicotinic receptors are present on secretomotor neurons and these are activated by release of acetylcholine from enteric interneurons or extrinsic efferent fibers. Specific organisms also modify other mechanisms that may contribute to development of acute diarrhea. Thus, mucin secretion, activation of motor mechanisms, increased mucosal permeability and inhibition of bile acid absorption have been reported in specific types of acute infectious diarrhea.

Conclusion—New therapies targeting neural and transmitter mediation including 5-HT, VIP, NPY, as well as toxin receptors and channels activated during acute infectious diarrhea could usher in a novel approach to enhancing glucose–electrolyte solutions used in the treatment of acute diarrhea.

Keywords

Channel; Chloride; Cholera; CFTR; Goblet cell; Guanylin

Introduction

Acute infectious diarrhea is generally considered to be a manifestation of enterocyte secretion. The best example is diarrhea due to *Vibrio cholerae*. Thus, cholera toxin (CT) consists of a toxic active A subunit (CT_A), and a B subunit pentamer (CT_B), which binds the toxin to the enterocyte brush border membrane via a ganglioside (GM₁) receptor. The bound toxin is internalized into the intestinal epithelial cell, where the A subunit is cleaved into the A1 peptide which ADP-ribosylates G_s, the stimulatory subunit of heterotrimeric G protein, leading to activation of adenylate cyclase. The resultant increase in intracytoplasmic cAMP concentration leads to increased chloride secretion by crypt cells and reduced absorption of sodium and chloride ions by villous cells; these actions result in diarrhea [1].

However, the “doctrine” of diarrheal disease through enterocyte secretion has been challenged. Thus, Lucas [2] noted that, for almost 40 years, one of the principal causes of diarrheal disease has been considered fluid secretion from the enterocytes and colonocytes. This enterocyte secretion hypothesis rapidly displaced all other alternatives, such as vasodilatation coupled with enhanced paracellular permeability. Indeed, Ussing chamber measurements of short circuit current (I_{sc}), potential difference, flux measurements, or measurements of elevated second messenger concentrations that are linked to chloride ion secretion in vitro were supportive of the enterocyte secretion doctrine. However, there were several observations that were not supportive of enterocyte secretion as the sole mechanism of secretion in acute infectious diarrhea. For example, in contrast to its effects in vitro in the Ussing chamber, the heat-stable enterotoxin of *E. coli* (STa) was not secretory when fluid flux was measured by volume recovery [3]. In addition, knock-out studies fail to confirm the enterocyte secretion hypothesis, since deletion of the essential Na–K–Cl (NKCC1) co-transporter does not prevent the action of STa [4].

These observations suggest that alternative mechanisms must be considered to explain the mechanism of acute diarrhea induced by infectious organisms.

Intestinal Secretion as Part of an Enteroendocrine-Neural Reflex in Cholera

A hypothetical involvement of enteroendocrine–neural reflexes as the diarrheagenic “messenger” in cholera was proposed by Osaka et al. [5]. In their experiments, CT was introduced into the duodenum of young rabbits; this caused severe degranulation of the enteroendocrine cells as revealed by electron microscopy: basal granules were swollen up and opened to the basal and lateral cell surface. Osaka et al. [5] proposed the hypothesis that CT stimulates the apical receptor of the enteroendocrine cells, and in response to the stimulus, amine (such as serotonin or 5-HT) and peptide (possibly motilin) products of the cells were released and mediated the diarrheagenic action of cholera.

There was no controversy regarding the first step: the localization of CT in the mucosa was shown by immunohistochemistry in guinea pig jejunum [6]. Subsequent studies in animals and humans demonstrated release of 5-HT from the enteroendocrine cells and intestinal secretion in cats and humans.

Nilsson et al. demonstrated a relationship of relative 5-HT fluorescence in enteroendocrine cells and net fluid transport across the intestinal epithelium induced by CT in cats [7]; these effects were not replicated with heat-inactivated CT. Subsequently, the relationship of CT, 5-HT and intestinal secretion in human jejunum was also demonstrated in vivo [8]. The role of enteroendocrine cells could conceivably be paracrine or neurocrine.

Subsequent studies, so simple in design but so convincing and important, explored the role of neural elements through in vivo studies conducted in animal denervated jejunum. Lundgren et al. studied the effects of lidocaine (exploring nerve activity as it blocks sodium currents that are essential for generating action potentials) or the sodium channel blocker tetrodotoxin (TTX) and showed that CT-induced secretion in cat or rat denervated jejunum involved a neural mechanism [9, 10]. In addition to the known release of 5-HT from the cat small intestine exposed to CT, these authors also demonstrated release of vasoactive intestinal peptide (VIP) [11] in the cat small intestine.

The next major step was the proof that the VIP and other transmitters were originating from submucosal secretomotor neurons. Thus, for example, CT was shown to act in the mucosa and to induce specific and sustained hyperexcitability of secretomotor neuron in guinea pig jejunum [6]. In the same species, CT increases the number and the duration of action potentials fired during prolonged depolarizations in VIP and neuropeptide Y (NPY) neurons [6]. Overall, it became clear that effects of CT are neurally-mediated: TTX and hexamethonium (nicotinic acetylcholine receptor antagonist) blocked effects of CT in VIP and NPY neurons [6], and the neurally-mediated actions depend on 5-HT₃ and the neurokinin 1 (NK₁) receptors [6] in addition to the nicotinic ACh receptor.

Hubel [12] summarized in a review the secretory reflex circuit, acknowledging that much of the model was based on the body of work of Cassuto and colleagues, which was predominantly on cat small intestine [13]. In this model (Fig. 1), epithelial sensory cells (the “antecedent” to the tasting role of the enteroendocrine cells) liberate 5-HT or other chemical transmitters in response to intraluminal stimuli and stimulate afferent neurons. Through nicotinically-mediated reflexes, a submucosal neuron secretes a neuro-transmitter (e.g., acetylcholine) that influences ion transport in the enterocyte in the villus or crypt. In this model, extrinsic nerves, such as adrenergic nerves can also affect the enterocyte and enteroendocrine cells though the precise site of action was not clearly understood.

Enteroendocrine-Neural Reflex with Other Bacterial Toxins

E. coli STa activates guanylate cyclase C (GCC) receptors to induce chloride secretion. The receptors are confined to the luminal surface (microvillus border) of the intestinal epithelium throughout the length of both the small intestine and colon of several mammalian species examined [14]. In GCC receptor-deficient mice, baseline net bicarbonate absorption is comparable with that in normal mice. However, whereas in normal mouse proximal colon, mucosal 50 nM *E. coli* STa increased *I*_{sc}, in receptor-deficient mice, neither mucosal nor serosal 500 nM STa affected electrolyte transport in proximal or distal colon of mice [15]. There is little secretion of 5-HT from the pig intestinal mucosa in response to STa [16].

C. difficile toxin B activates human VIP submucosal neurons [17], and this increased expression is partly mediated by the cytokine, IL-1 .

Lundgren and colleagues summarized the literature demonstrating the effects of neuropharmacological agents tetrodotoxin, lidocaine and hexamethonium on net fluid secretion induced by various secretagogues including rotavirus in newborn mouse intestine [18], and parasites such as *Nippostrongylus brasiliensis* in inflamed rat jejunum in vivo [19, 20].

The Role of Goblet Cells

The “rice water” stool of cholera is an expression of the induction of mucin secretion. The release of mucin from goblet cells may be regulated by innate and adaptive immune responses; thus, the most potent effects are induced by T cell cytokines: interleukin-4 (IL-4)

and IL-13 produced by T helper 2 (T_H2) cells in response to parasitic infections. Interferon- (IFN) and IL-17, which are classically produced by T_H1 cells and T_H17 cells in response to intracellular and extracellular pathogens, also induce mucin production by goblet cells [21].

CT induces mucin secretion from goblet cells. As in the enterocyte, where CT induces cAMP production, Epple et al. [22] showed that CT caused a 116-fold increase of intracellular cAMP and stimulated the secretion of both preformed and newly synthesized mucin in a human goblet cell line HT-29/B6. These actions involved de novo synthesis of mucin molecules and microtubule-mediated secretion. This contrasts with carbachol, a cholinergic agonist, which only triggered the release of preformed mucin immediately after addition of this drug.

When CT was added to cloned human goblet cell monolayers (without submucosal neurons), it did not induce mucin secretion in contrast to effects of the calcium ionophore, A23187. This suggests that mucin secretion by CT involves submucosal nerves [23].

Guanylin is produced in the rat small intestinal and colonic goblet cells [24] and is structurally related to the STa. Given the production by goblet cells of the endogenous ligands of GCC receptor (guanylin and uroguanylin) on enterocytes that result in secretion of chloride ions, an intriguing question is: does goblet cell mucin secretion result in secretion of guanylin or uroguanylin? Guanylin-like immunoreactivity is present in the intracellular compartment occupied by the secretory granules in goblet cells. Li et al. [25] suggested that guanylin is packaged into these granules along with mucin in the rat intestine, and is released into the lumen when goblet cells are stimulated to secrete. The guanylin then has access to the GCC receptor on the luminal domain of the enterocyte. This results in activation of intracellular mediators including guanylate cyclase and cGMP, which ultimately influence chloride ion fluxes through the cystic fibrosis transmembrane regulator-chloride channel (CFTR, Fig. 2).

Effects on Bile Acid Absorption During Acute Infectious Diarrhea

Acute infectious diarrhea due to shigellosis in humans is associated with increased fecal bile acid excretion. The increased total bile acid excretion in shigellosis is associated with a greater proportion of cholic acid in stool. The fact that stool content of this primary bile acid is increased relative to the proportion of secondary bile acids suggests that bacterial dehydroxylation in the colon is deficient during the acute diarrheal stage [26].

Fecal bile acid and neutral sterol patterns were also studied by the same group in eight healthy adults challenged with a *Vibrio cholerae* strain. In contrast to the results with shigellosis, bile acid handling appears not to be affected in cholera: first, bacterial 7 - dehydroxylation of primary bile acids, cholic and chenodeoxycholic acid, was not altered despite the greatly increased fecal wet weight; and second, total concentrations of both bile acids and cholesterol in mg/g of fecal wet weight were decreased as a result of dilution due to diarrhea. However, total bile acid and neutral steroid excretions in mg/kg/day in subjects do not appear to be different with or without diarrhea [27]. Results obtained with cholera are similar to those observed in experimentally-induced travelers' diarrhea associated with toxigenic *E. coli* [27].

When bile acids are malabsorbed or cause intestinal secretion, there is evidence of the involvement of serotonin. Thus, it has been shown that sodium deoxycholate (4 mM) caused a release of 5-HT into the rat intestinal lumen, and this release was inhibited by calcium channel blockade; in addition, granisetron, a 5-HT₃ receptor blocker, partly inhibited the fluid secretion caused by bile salts [28].

The Role of Myenteric Neurons in Acute Infectious Diarrhea

There is evidence that the myenteric plexus is also involved during CT-induced net fluid secretion in rat small intestine. The role of the myenteric plexus was considered critical since treatment of the serosal surface of the intestinal loop with benzalkonium chloride (BAC), which selectively ablated the myenteric plexus, eliminated the ability of CT to induce rat intestinal secretion [29]. While the authors suggested that all afferent fibers in the intramural secretory reflex activated by CT are probably conveyed via the myenteric plexus, which functions as the integrating center in the enteric nervous system, it is conceivable that the topically active BAC may have penetrated beyond the myenteric plexus and also affected submucosal neurons. If the penetration of the BAC reached submucosal neurons, the secretory reflex at the submucosal level could be abolished. In fact, these careful semi-quantitative studies of Jodal et al. [29] showed that the calcitonin gene-related peptide (CGRP) neurons in the submucosa were depleted by BAC treatment relative to controls, even though the VIP, galanin and substance P submucosal neurons were not apparently altered. CGRP is an important transmitter in the control of gut submucosal arterioles [30], and it is conceivable that depletion of CGRP in these preparations may have influenced the vasodilatation that is ultimately involved in the secretion in response to CT.

Mathias et al. [31] used a different approach to assess the role of motility in CT-induced intestinal secretion. They studied repetitive action potentials or migrating action potential complexes (MAPCs) that may constitute a specific motor repertoire in response to these toxins or the non-specific effects of intestinal secretion or diarrhea. Mathias et al. compared the number of MAPCs of the intestinal musculature per hour for 10 loops of rabbit intestine infected with the live *Vibrio* culture, the whole cell lysate, or the purified enterotoxin. They showed that CT elicits MAPCs recorded by electrodes placed on the serosal surface distal to the segment exposed to CT. In addition, Nocerino et al. [32] showed that, in the rat, MAPCs can migrate past the ileocecal valve and into the colon. The MAPCs reflect contractile waves of the circular layer moving in an anal direction. They are eliminated by the same types of drugs as those that influence the local secretory reflex elicited by CT, i.e., by local anesthetics, nicotinic receptor agonists and atropine.

Stimulation of repetitive bursts of action potentials in the rabbit small intestine was also observed with *E. coli* STa application [33].

Figure 3 shows a schematic figure of the model proposed for the secretory nervous reflex activated by cholera toxin and incorporating the influence of the myenteric cholinergic neurons [28].

Increased Intestinal Permeability in Diarrhea Due to Invasive Organisms

The enteric nervous system, more specifically the enteric glial cells, has recently been demonstrated to significantly reduce the mucosal lesions and inflammatory response caused by *Shigella flexneri* in an ex vivo human colonic mucosa model [34]. These protective effects were mediated by S-nitrosoglutathione, the nitrosylated form of reduced glutathione, which is secreted by the glial cells. The involvement of the enteric glia in protection against intestinal secretion could be potentially relevant to other infectious diarrheas.

Infectious diarrhea caused by invasive pathogens results in increased intestinal permeability as well as inflammation. This was originally demonstrated by comparing the permeability to ⁵¹Cr-EDTA in controls and patients with infectious diarrhea [35]. Thus, acute gastroenteritis caused by infection with *Yersinia enterocolitica* is associated with altered tight junction proteins and induction of cell necrosis, both resulting in a decrease in transepithelial resistance in colonic HT-29/B6 cell monolayers [36]. This fall in trans-

epithelial resistance was manifested by an increased permeability to mannitol and fluorescein in a human colonic cancer cell line. In amebic dysentery caused by *Entamoeba histolytica*, the constitutive production and secretion of prostaglandin E₂ by the parasite alters ion permeability of the paracellular tight junctions of T84 cells grown to 100% confluence on polyethylene membrane inserts, resulting in increased sodium ion permeability towards the lumen in addition to the luminal chloride secretion by activation of CFTR [37]. Thus, the authors were able to demonstrate that *E. histolytica* is also capable of initiating disease in a contact-independent manner, in contrast to its direct effects on colon epithelium causing hemorrhage and inflammation that also contributes to diarrhea.

Noninvasive infectious diarrhea due to cholera is also associated with increased mucosal permeability in the small intestine. Fasano et al. showed that *V. cholerae* secretes an additional toxin (different from the CT) that affects the structure of the zonula occludens in the intercellular tight junctions, causing secretion in rabbit intestine in vitro [38].

Novel Treatment Strategies Targeting Enteroendocrine and Neural Mechanisms

Given the involvement of enteroendocrine and at least submucosal neurons in the development of acute infectious diarrhea, a number of experimental approaches suggest that new treatment strategies may be developed to enhance the traditional sugar-electrolyte-water approaches to treat acute dehydration.

Somatostatin exerts antisecretory effects by suppressing the firing of VIP secretomotor neurons rather than via a direct action on mucosal enterocytes in guinea-pig jejunum [39]. Somatostatin also decreases chloride secretion in response to 5-HT and a nicotinic agonist, dimethylphenylpiperazinium, a response that is sensitive to tetrodotoxin and reflects actions on both cholinergic and non-cholinergic secretomotor neurons in guinea-pig jejunum and rat colon [39, 40].

In rat distal colonic mucosa/submucosa preparations, pre-treatment with 5-HT₃ receptor antagonists *enhanced* 5-HT-induced increases in Isc; in mucosa-only preparations without retained neural elements, pre-treatment with 5-HT₃ receptor antagonist *inhibited* 5-HT-induced increase in Isc [41]. The overall effect of a 5-HT₃ receptor antagonist in the intact organ would be predicted to be deleterious in acute infectious diarrhea. Activation of 5-HT₃ receptors does not lead to secretion in human intestine, but it does in many animal models. For example, Michel et al. [42] showed that 2-methyl-5-HT (a moderately selective full agonist at the 5-HT₃ receptor) did not evoke chloride secretion in the human intestine, even though specific 5-HT_{3A} and 5-HT_{3B} receptor subunit immunoreactivity, as well as 5-HT_{3A} and 5-HT_{3B} receptor-specific messenger RNA were detected in the human intestinal tissue samples. On the other hand, 2-methyl-5-HT induced secretion in the guinea-pig intestine [42]. Others showed that mucosal stroking of muscle-stripped human jejunum mounted in modified Ussing chambers resulted in 5-HT release and chloride secretion mediated through a neural reflex that involved 5-HT₄ receptors [43]. In human jejunal perfusion studies, Bearcroft et al. showed a benefit of alosetron (a 5-HT₃ receptor antagonist) on basal fluid and sodium secretion, but did not reverse secretion in response to CT in human jejunum [44]. Overall, therefore, 5-HT₃ receptors activate human submucous neurons, but these neurons do not appear to play a significant role in reflexes controlling secretion. The lack of functional evidence for 5-HT₃ receptors in human intestine argues against a potential therapeutic role of 5-HT₃ antagonists in intestinal secretory states.

Acetorphan, an orally administered inhibitor of enkephalinase in the wall of the digestive tract (Fig. 4), prevents inactivation of endogenous opioid peptides released by submucosal

and myenteric neurons. Acetorphan completely prevented water and electrolyte secretion induced by CT in human jejunum [45].

Another experimental approach involves the inhibition of release of 5-HT from enteroendocrine cells. Kojima et al. [46] demonstrated that a lipophilic nitric oxide (NO)-releasing compound 5-amino-3-(3, 4-dichlorophenyl) 1,2,3,4-oxatriazolium (GEA3162) can suppress 5-HT release from colonic mucosa through a mechanism that does not involve cyclic GMP system or peroxynitrite generation. The effects of this agent on 5-HT secretion in response to infection have not been reported to date.

Other neurotransmitters that may be the target for future therapy are VIP and NPY. In perfused rat jejunum, PG 97-269 abolished the effects of VIP on fluid and electrolyte transport and attenuated cholera toxin and *Escherichia coli* heat labile toxin-induced net fluid and electrolyte secretion [47].

Y2 receptors are involved in mediating rat intestinal secretion in response to VIP [48]. Conversely, NPY Y2 receptor activation (e.g., with PYY₃₋₃₆) inhibited colonic motor function in mice, thus reducing intestinal fluid secretion and slowing colonic transit [49]; however, the efficacy in intestinal infections is unclear.

A novel approach to inhibition of secretion involves ligands that inhibit binding of the CT to the enterocytes' GM₁ glycoside receptor [50] (Fig. 4). Such an inhibition of toxin binding is a very attractive approach to the prevention of diarrhea, because it can be applied either prophylactically or after the infection has occurred [51]. However, these agents are at a preliminary stage of development.

In view of the pivotal role of CFTR in chloride secretion from the enterocyte, another approach in the treatment of cholera targets the CFTR chloride channel. The thiazolidinone CFTR inhibitor (CFTR_{inh}-172) reduced CT-induced fluid secretion in the mouse small intestine when injected intraperitoneally [52]. Despite being homologous to the sulfonylureas, such as glibenclamide that are used in the treatment of type II diabetes mellitus, CFTR_{inh}-172 did not inhibit other ion channels (including the ATP-sensitive K⁺ channels that are blocked by glibenclamide). Another class of compounds, called glycine hydrazide compounds (GlyH), blocks the CFTR: after intraluminal administration, GlyH-101 reduced intestinal fluid secretion caused by CT by approximately 80% [53]. Moreover, GlyH-101 is highly water-soluble, with rapid onset of action, and it binds close to the external pore entrance of the CFTR ion channel, in contrast to CFTR_{inh}-172, which is less water-soluble and binds to the nucleotide binding domain at the cytoplasmic surface of the CFTR ion channel.

Studies of pharmacological inhibition of chenodeoxycholate-induced fluid and mucus secretion and mucosal injury in the rabbit colon suggested that atropine markedly reduced and carbachol potentiated the fluid secretion, mucus output, and mucosal damage observed during bile acid perfusion. In contrast, pre-treatment of the colonic mucosa with lignocaine and parenteral administration of methysergide (5-HT antagonist) and somatostatin produced a modest reduction in the fluid secretory response without apparent effects on mucus output or mucosal damage. These data suggest that these effects were independent of the actions on mucosal protective factors, specifically mucus [54].

Binding of bile acids with resins such as cholestyramine has been tested in pediatric infectious diarrhea [55, 56]. Poor patient compliance precludes its widespread use, but tablet alternatives such as colesevelam (which is effective in diarrhea-predominant irritable bowel syndrome [57]) may be tested in the future.

Approaches to reduce intestinal permeability include the experimental drug 2,4,6-triaminopyrimidine [58]. The potential for agents to reduce intestinal permeability in infectious secretory diarrheas requires further study.

Summary and a Look to the Future

Enteroendocrine and neuronal mechanisms participate in the development of acute infectious diarrhea due to bacterial toxins (e.g., cholera, *E. coli* heat-stable enterotoxin, *C. difficile*) and rotavirus. These agents are all associated with secretion of transmitters from enteroendocrine cells (e.g., 5-HT) and activation of afferent neurons stimulating submucosal secretomotor neurons that secrete ACh (nicotinic) and VIP. Specific organisms also modify other mechanisms that may contribute to development of acute diarrhea, including mucin secretion, increased permeability and inhibition of bile acid absorption. New therapies targeting neural and transmitter mediation of acute infectious diarrhea could usher in novel approaches in the treatment of acute diarrhea.

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Abbreviations

5-HT	Serotonin
ACh	Acetyl choline
BAC	Benzalkonium chloride
cAMP	Cyclic adenosine monophosphate
CFTR	Cystic fibrosis transmembrane conductance regulator
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin gene-related peptide
CT	Cholera toxin
GCC	Guanylate cyclase C
GM₁	Ganglioside membrane receptor
IFN	Interferon
Isc	Short-circuit current
MAPC	Migrating action potential complex
NK₁	Neurokinin 1
NPY	Neuropeptide Y
STa	<i>E. coli</i> heat-stable enterotoxin
TTX	Tetrodotoxin
VIP	Vasoactive intestinal peptide

References

1. Navaneethan U, Giannella RA. Mechanisms of infectious diarrhea. Nat Clin Pract Gastroenterol Hepatol. 2008; 5:637–647. [PubMed: 18813221]

2. Lucas ML. Diarrhoeal disease through enterocyte secretion: a doctrine untroubled by proof. *Exp Physiol*. 2010; 95:479–484. [PubMed: 20233893]
3. Lucas ML, Thom MM, Bradley JM, O'Reilly NF, McIlvenny TJ, Nelson YB. *Escherichia coli* heat stable (STa) enterotoxin and the upper small intestine: lack of evidence in vivo for net fluid secretion. *J Membr Biol*. 2005; 206:29–42. [PubMed: 16440179]
4. Flagella M, Clarke LL, Miller ML, et al. Mice lacking the basolateral Na-K-2Cl cotransporter have impaired epithelial chloride secretion and are profoundly deaf. *J Biol Chem*. 1999; 274:26946–26955. [PubMed: 10480906]
5. Osaka M, Fujita T, Yanatori Y. On the possible role of intestinal hormones as the diarrhoeagenic messenger in cholera. *Virchows Arch B Cell Pathol*. 1975; 18:287–296. [PubMed: 809916]
6. Gwynne RM, Ellis M, Sjoval H, Bornstein JC. Cholera toxin induces sustained hyperexcitability in submucosal secretomotor neurons in guinea pig jejunum. *Gastroenterology*. 2009; 136:299–308. [PubMed: 19026646]
7. Nilsson O, Cassuto J, Larsson PA, et al. 5-Hydroxytryptamine and cholera secretion: a histochemical and physiological study in cats. *Gut*. 1983; 24:542–548. [PubMed: 6852634]
8. Bearcroft CP, Perrett D, Farthing MJ. 5-Hydroxytryptamine release into human jejunum by cholera toxin. *Gut*. 1996; 39:528–531. [PubMed: 8944560]
9. Cassuto J, Jodal M, Tuttle R, Lundgren O. The effect of lidocaine on the secretion induced by cholera toxin in the cat small intestine. *Experientia*. 1979; 35:1467–1468. [PubMed: 510483]
10. Cassuto J, Jodal M, Tuttle R, Lundgren O. On the role of intramural nerves in the pathogenesis of cholera toxin-induced intestinal secretion. *Scand J Gastroenterol*. 1981; 16:377–384. [PubMed: 16435479]
11. Cassuto J, Fahrenkrug J, Jodal M, Tuttle R, Lundgren O. Release of vasoactive intestinal polypeptide from the cat small intestine exposed to cholera toxin. *Gut*. 1981; 22:958–963. [PubMed: 7308850]
12. Hubel KA. Intestinal nerves and ion transport: stimuli, reflexes, and responses. *Am J Physiol*. 1985; 248:G261–G271. [PubMed: 2579577]
13. Cassuto J, Siewert A, Jodal M, Lundgren O. The involvement of intramural nerves in cholera toxin induced intestinal secretion. *Acta Phys Scand*. 1983; 117:195–202.
14. Krause WJ, Currie MG, Forte LR, et al. Distribution of heat-stable enterotoxin/guanylin receptors in the intestinal tract of man and other mammals. *J Anatomy*. 1994; 184:407–417.
15. Charney AN, Eqnor RW, Alexander-Chacko JT, Zaharia V, Mann EA, Giannella RA. Effect of *E. coli* heat-stable enterotoxin on colonic transport in guanylyl cyclase C receptor-deficient mice. *Am J Physiol*. 2001; 280:G216–G221.
16. Peterson JW, Whipp SC. Comparison of the mechanisms of action of cholera toxin and the heat-stable enterotoxins of *Escherichia coli*. *Infect Immun*. 1995; 63:1452–1461. [PubMed: 7890409]
17. Neunlist M, Barouk J, Michel K, et al. Toxin B of *Clostridium difficile* activates human VIP submucosal neurons, in part via an IL-1 β -dependent pathway. *Am J Physiol*. 2003; 285:G1049–G1055.
18. Lundgren O, Peregrin AT, Persson K, Kordasti S, Uhnöo I, Svensson L. Role of the enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. *Science*. 2000; 287:491–495. [PubMed: 10642552]
19. Lundgren O. Enteric nerves and diarrhoea. *Pharmacol Toxicol*. 2002; 90:109–120. [PubMed: 12071331]
20. Jodal M, Wingren U, Jansson M, Heidemann M, Lundgren O. Nerve involvement in fluid transport in the inflamed rat jejunum. *Gut*. 1993; 34:1526–1530. [PubMed: 8244137]
21. McGuckin MA, Lindén SK, Sutton P, Florin TH. Mucin dynamics and enteric pathogens. *Nat Rev Microbiol*. 2011; 9:265–278. [PubMed: 21407243]
22. Epple HJ, Kreusel KM, Hanski C, Schulzke JD, Riecken EO, Fromm M. Differential stimulation of intestinal mucin secretion by cholera toxin and carbachol. *Pflügers Arch*. 1997; 433:638–647. [PubMed: 9049150]
23. Lencer WI, Reinhart FD, Neutra MR. Interaction of cholera toxin with cloned human goblet cells in monolayer culture. *Am J Physiol*. 1990; 258:G96–G102. [PubMed: 2154122]

24. Cohen MB, Witte DP, Hawkins JA, Currie MG. Immunohistochemical localization of guanylin in the rat small intestine and colon. *Biochem Biophys Res Commun*. 1995; 209:803–808. [PubMed: 7733972]
25. Li Z, Taylor-Blake B, Light AR, Goy MF. Guanylin, an endogenous ligand for C-type guanylate cyclase, is produced by goblet cells in the rat intestine. *Gastroenterology*. 1995; 109:1863–1875. [PubMed: 7498651]
26. Huang CT, Woodward WE, Hornick RB, Rodriguez JT, Nichols BL. Fecal steroids in diarrhea. I. Acute shigellosis. *Am J Clin Nutr*. 1976; 29:949–955. [PubMed: 786002]
27. Huang CT, Levine MM, Daoud GS, Nalin DR, Nichols BL. Fecal steroids in diarrhea. IV. Cholera. *Lipids*. 1982; 17:612–616. [PubMed: 7144450]
28. Peregrin AT, Ahlman H, Jodal M, Lundgren O. Involvement of serotonin and calcium channels in the intestinal fluid secretion evoked by bile salt and cholera toxin. *Br J Pharmacol*. 1999; 127:887–894. [PubMed: 10433495]
29. Jodal M, Holmgren S, Lundgren O, Sjöqvist A. Involvement of the myenteric plexus in the cholera toxin-induced net fluid secretion in the rat small intestine. *Gastroenterology*. 1993; 105:1286–1293. [PubMed: 7693539]
30. Chen RY, Li DS, Guth PH. Role of calcitonin gene-related peptide in capsaicin-induced gastric submucosal arteriolar dilation. *Am J Physiol*. 1992; 262:H1350–H1355. [PubMed: 1590437]
31. Mathias JR, Carlson GM, DiMarino AJ, Bertiger G, Morton HE, Cohen S. Intestinal myoelectric activity in response to live *Vibrio cholerae* and cholera enterotoxin. *J Clin Invest*. 1976; 58:91–96. [PubMed: 932212]
32. Nocerino A, Iafusco M, Guandalini S. Cholera toxin-induced small intestinal secretion has a secretory effect on the colon of the rat. *Gastroenterology*. 1995; 108:34–39. [PubMed: 7806061]
33. Mathias JR, Nogueira J, Martin JL, Carlson GM, Giannella RA. *Escherichia coli* heat-stable toxin: its effect on motility of the small intestine. *Am J Physiol*. 1982; 242:G360–G363. [PubMed: 6278961]
34. Flamant M, Aubert PH, Rolli-Derkinderen M. Enteric glia protect against *Shigella flexneri* invasion in intestinal epithelial cells: a role for S-nitrosoglutathione. *Gut*. 2011; 60:476–484.
35. Zuckerman M, Watts M, Bhat B, Ho H. Intestinal permeability to [51Cr]EDTA in infectious diarrhea. *Dig Dis Sci*. 1993; 38:1651–1657. [PubMed: 8359077]
36. Hering N, Richter J, Krug S. *Yersinia enterocolitica* induces epithelial barrier dysfunction through regional tight junction changes in colonic HT-29/B6 cell monolayers. *Lab Invest*. 2011; 91:310–324. [PubMed: 20956974]
37. Lejeune M, Moreau F, Chadee K. Prostaglandin E2 produced by *Entamoeba histolytica* signals via EP4 receptor and alters claudin-4 to increase ion permeability of tight junctions. *Am J Pathol*. 2011; 179:807–818. [PubMed: 21683675]
38. Fasano A, Baudry B, Pumphlin D. *Vibrio cholerae* produces a second enterotoxin, which affects intestinal tight junctions. *Proc Natl Acad Sci USA*. 1991; 88:5242–5246. [PubMed: 2052603]
39. Foong JP, Parry LJ, Bornstein JC. Activation of neuronal SST₁ and SST₂ receptors decreases neurogenic secretion in the guinea-pig jejunum. *Neurogastroenterol Motil*. 2010; 22:1209–1216. [PubMed: 20626789]
40. Keast JR, Furness JB, Costa M. Investigations of nerve populations influencing ion transport that can be stimulated electrically, by serotonin and by a nicotinic agonist. *Naunyn Schmiedeberg Arch Pharmacol*. 1985; 331:260–266. [PubMed: 2418370]
41. Yang N, Liu SM, Zheng LF, et al. Activation of submucosal 5-HT(3) receptors elicits a somatostatin-dependent inhibition of ion secretion in rat colon. *Br J Pharmacol*. 2010; 159:1623–1625. [PubMed: 20233224]
42. Michel K, Zeller F, Langer R, et al. Serotonin excites neurons in the human submucous plexus via 5-HT₃ receptors. *Gastroenterology*. 2005; 128:1317–1326. [PubMed: 15887114]
43. Kellum JM, Albuquerque FC, Stoner MC, Harris RP. Stroking human jejunal mucosa induces 5-HT release and Cl⁻ secretion via afferent neurons and 5-HT₄ receptors. *Am J Physiol*. 1999; 277:G515–G520. [PubMed: 10484375]

44. Bearcroft CP, Andre EA, Farthing MJ. In vivo effects of the 5-HT₃ antagonist alosetron on basal and cholera toxin-induced secretion in the human jejunum: a segmental perfusion study. *Aliment Pharmacol Ther.* 1997; 11:1109–1114. [PubMed: 9663837]
45. Hinterleitner TA, Petritsch W, Dimsity G, Berard H, Lecomte JM, Krejs GJ. Acetorphan prevents cholera-toxin-induced water and electrolyte secretion in the human jejunum. *Eur J Gastroenterol Hepatol.* 1997; 9:887–891. [PubMed: 9355788]
46. Kojima S, Uchida K, Sasaki K, Sunagawa M, Ohno Y, Kamikawa Y. The suppressant effect of GEA3162 on spontaneous serotonin release from human colonic mucosa in vitro. *Eur J Pharmacol.* 2006; 550:162–165. [PubMed: 17022966]
47. Banks MR, Farthing MJ, Robberecht P, Burleigh DE. Antisecretory actions of a novel vasoactive intestinal polypeptide (VIP) antagonist in human and rat small intestine. *Br J Pharmacol.* 2005; 144:994–1001. [PubMed: 15711593]
48. Souli A, Chariot J, Voisin T, et al. Several receptors mediate the antisecretory effect of peptide YY, neuro peptide Y, and pancreatic polypeptide on VIP-induced fluid secretion in the rat jejunum in vivo. *Peptides.* 1997; 18:551–557. [PubMed: 9210175]
49. Moriya R, Shirakura T, Hirose H, Kanno T, Suzuki J, Kanatani A. NPY Y₂ receptor agonist PYY(3–36) inhibits diarrhea by reducing intestinal fluid secretion and slowing colonic transit in mice. *Peptides.* 2010; 31:671–675. [PubMed: 19925840]
50. Pickes J, Mitchell D, Liu J, et al. Nonspanning bivalent ligands as improved surface receptor binding inhibitors of the cholera toxin B pentamer. *Chem Biol.* 2004; 11:1205–1215. [PubMed: 15380181]
51. Thiagarajah J, Verkman AS. New drug targets for cholera therapy. *Trends Pharmacol Sci.* 2005; 26:172–175. [PubMed: 15808339]
52. Ma T, Thiagarajah J, Yang H, et al. Thiazolidinone CFTR inhibitor identified by high-throughput screening blocks cholera toxin-induced intestinal fluid secretion. *J Clin Invest.* 2002; 110:1651–1658. [PubMed: 12464670]
53. Munprasat C, Sonawane N, Salinas D, Taddei A, Galletta L, Verkman A. Discovery of glycine hydrazide pore-occluding CFTR inhibitors: mechanism, structure-activity analysis, and in vivo efficacy. *J Gen Physiol.* 2004; 124:125–137. [PubMed: 15277574]
54. Camilleri M, Murphy R, Chadwick VS. Pharmacological inhibition of chenodeoxycholic acid-induced secretion of fluid and mucus in the rabbit colon. *Dig Dis Sci.* 1982; 27:865–869. [PubMed: 6126321]
55. Isolauri E, Vähäsarja V, Vesikari T. Effect of cholestyramine on acute diarrhoea in children receiving rapid oral rehydration and full feedings. *Ann Clin Res.* 1986; 18:99–102. [PubMed: 3521444]
56. Vesikari T, Isolauri E. A comparative trial of cholestyramine and loperamide for acute diarrhoea in infants treated as outpatients. *Acta Paediatr Scand.* 1985; 74:650–654. [PubMed: 3901661]
57. Odunsi-Shiyanbade ST, Camilleri M, McKinzie S, et al. Effects of chenodeoxycholate and a bile acid sequestrant, colesteslam, on intestinal transit and bowel function. *Clin Gastroenterol Hepatol.* 2010; 8:159–165. [PubMed: 19879973]
58. Naftalin R, Simmons N. The effects of theophylline and cholera toxin on sodium and chloride ion movements within isolated rabbit ileum. *J Physiol.* 1979; 290:331–350. [PubMed: 469769]

Fig. 1. Secretory reflex circuit between enteroendocrine cell and enterocyte; other neural synapses from intrinsic to extrinsic nerves modulate this reflex (adapted from Hubel [12])

Fig. 2.

Three important cell types are involved in the response to acute enteric infections that result in diarrhea. The enteroendocrine cells serve a sensory function, secreting different transmitters into the lamina propria, and stimulating afferent neurons which may activate VIP- and acetylcholine-induced secretion either by direct effects on the enterocyte or indirectly through effects on submucosal secretomotor neurons. Note that both VIPergic and cholinergic secretomotor neurons are activated by cholinergic interneurons. Acetylcholine released from interneurons activates both types of secretomotor neurons via nicotinic receptors. The goblet cells discharge mucus in response to infection, and with it, the endogenous peptides guanylin and uroguanylin, which bind to the guanylate cyclase C-receptor on the luminal domain of enterocytes to cause guanylate cyclase production in the enterocyte, and ultimately chloride secretion through chloride channels or the cystic fibrosis transmembrane regulator. The GC-C protein is also the receptor for STa, the heat-stable enterotoxin of *E. coli*. Some infections are associated with invasion and inflammation and result in changes in tight junction permeability

Fig. 3.
Schematic figure of the model proposed for the secretory nervous reflex activated by cholera toxin (from Peregrin et al. [28])

Fig. 4. Schematic figure of working mechanism of CT induced secretion, and possible strategies for drug therapy of cholera including enhancement of endogenous enkephalin through enkephalinase inhibitor, and agents directed at the CFTR channel (reproduced from Thiagarajah et al. [51])