Regulatory Mechanisms of Secretory Diarrhea and Electrolyte Imbalance in Acute and Chronic Diarrhea in Infancy

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Until relatively recently the small intestine was considered to be largely an organ for the absorption of water and solutes. Over the past decade, however, evidence has accumulated that it is capable of marked secretion of water and electrolytes, and this chapter summarizes some of the important advances that have occurred in our understanding of the mechanisms of the small intestine that regulate this secretion. Most of the experimental work has employed purified enterotoxins and/or pharmacological agents to produce intestinal secretion in vivo and in vitro, and as a result the available data are more directly relevant to acute than to chronic diarrhea. The pathophysiological mechanisms of acute diarrhea must therefore at present serve as a model for chronic diarrhea, which is much more difficult to reproduce in the laboratory. Other chapters in this volume discuss a variety of luminal, neurohumoral, and pharmacological agents that influence intestinal transport, and this chapter therefore concentrates on those intracellular regulators and mediators that may act as a final common pathway for a variety of extracellular secretagogues already noted.

THE NATURE OF SECRETORY DIARRHEA

Intestinal secretion may be defined as the net accumulation of water and electrolytes in the intestinal lumen. In leaky epithelia such as the small intestine there are large mainly paracellular fluxes of water and electrolyte from both lumen to serosa and from serosa to lumen; net transepithelial absorption occurs when the flux from the lumen to serosa exceeds that from serosa to lumen. Net secretion occurs as a result of a decrease in the flux from lumen to serosa and/or as a result of an increase in flux from serosa to mucosa (1). Clinically, net small intestinal secretion results in diarrhea only when the considerable reabsorptive capacity of the colon is exceeded (2). Net secretion may occur either as the result of the presence of large amounts of osmotically active solute within the lumen (osmotic diarrhea) or as the result of an alteration of the intracellular metabolism of the enterocyte (secretory diarrhea). In osmotic diarrhea, luminal
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Hyperosmolality results in a low luminal concentration of water, and water enters the lumen passively down a concentration gradient, whereas in secretory diarrhea, electrolyte and water secretion occurs in the absence of a favorable electrochemical or concentration gradient and results from active electrolyte secretion and/or inhibition of active absorption.

These differences produce a marked difference in stool composition in osmotic as opposed to secretory diarrhea (Table 1). In secretory diarrhea, stool volumes are usually large, diarrhea persists during fasting, and the anion gap in the stool is low, in contrast to osmotic diarrhea, which remits on fasting and is characterized by a large solute gap in the stool. However, in clinical practice, for example, protracted diarrheal states in infancy, the cause of intestinal secretion is often multifactorial and these differences are frequently blurred.

Intracellular Regulation of Intestinal Secretion

A large number of intestinal secretagogues have been identified, the classic example of prototype of which is cholera toxin (3), and there is now considerable evidence that cholera toxin (and *E. coli* heat-labile toxin)-induced secretion is mediated by raised intracellular concentrations of cyclic AMP (4). Two other intracellular mediators of secretion, cyclic GMP and calcium, have also been identified, and the relationships among these three intracellular messengers and a variety of secretagogues have been summarized by Field (5) (Table 2).

Microtubules have also been shown to play a part in the intracellular regulation of secretion (6); defective Na\(^+\)-K\(^+\) ATPase activity (“sodium pump”) within the enterocyte may also be important (7). The roles of these various intracellular factors will now be considered in more detail.

Cyclic AMP

When small intestinal mucosa is mounted in Ussing chambers *in vitro* (8), exposure of the tissue to an agent that raises cyclic AMP (e.g., cholera toxin or theophylline) results in sustained increases in potential difference and short-

<table>
<thead>
<tr>
<th>TABLE 1. Stool composition in osmotic and secretory diarrhea</th>
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<tbody>
<tr>
<td><strong>Osmotic</strong></td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
</tr>
<tr>
<td>Na(^+) (mM)</td>
</tr>
<tr>
<td>K(^+) (mM)</td>
</tr>
<tr>
<td>Na(^+) + K(^+) (mM)</td>
</tr>
<tr>
<td>(Na(^+) + K(^+)) × 2 (mM)*</td>
</tr>
<tr>
<td>Solute gap**</td>
</tr>
</tbody>
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* Multiplied by 2 to account for anions.
** Osmolality minus (Na\(^+\) + K\(^+\)) × 2.

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TABLE 2. Stimulus-secretion coupling in small intestine

<table>
<thead>
<tr>
<th>Intracellular mediator</th>
<th>Extrinsic stimulus</th>
<th>Contraluminal</th>
</tr>
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<tbody>
<tr>
<td><strong>Cyclic AMP</strong></td>
<td>Luminal</td>
<td>Contraluminal</td>
</tr>
<tr>
<td>Bacterial enterotoxins: cholera and heat-labile E. coli</td>
<td>Vasoactive intestinal peptide</td>
<td></td>
</tr>
<tr>
<td>Dihydroxy bile acids</td>
<td></td>
<td>Prostaglandins</td>
</tr>
<tr>
<td>Hydroxy fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cyclic GMP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial enterotoxins: heat-stable E. coli</td>
<td></td>
<td>?</td>
</tr>
<tr>
<td><strong>Ca^{2+}</strong></td>
<td></td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>?Detergents: bile salts, fatty acids</td>
<td>Serotonin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Substance P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neurotensin</td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td>Other bacterial enterotoxins</td>
<td>Calcitonin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bombesin</td>
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<tr>
<td></td>
<td></td>
<td>Vasopressin</td>
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</tbody>
</table>

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circuit current. As a result of a decrease in the lumen-to-serosa flux and an increase in the serosa-to-lumen flux, net Cl⁻ absorption is reversed to secretion. Net Na⁺ absorption is abolished as a result of a reduction in the flux from lumen to serosa. In vitro, HCO₃⁻ secretion, as assessed by the residual ion flux, does not occur but has been observed in vivo (9,10). A model has been produced to explain these effects (11,12) (Fig. 1) that suggests that cyclic AMP inhibits coupled NaCl transport across the brush border while at the same time stimulating active Cl⁻ secretion.

The exact nature of the NaCl cotransport that is inhibited by cyclic AMP remains undecided. Studies employing brush border vesicles (13,14) have not corroborated suggestions made on the basis of observations on intact epithelium (15) that electroneutral Na⁺ and Cl⁻ coupling occurs. In vitro (13,14) and in vivo (16) studies suggest that the coupling is indirect and that Na⁺/H⁺ and Cl⁻/HCO₃⁻ (OH⁻) exchange mechanisms exist. Hyun and Kimmich (17) have recently provided further evidence that tight Na⁺ and Cl⁻ coupling does not occur. They were able to demonstrate, using intact chicken enterocytes (mainly villus cells), that under optimal conditions for cyclic AMP production (cholera toxin plus theophylline) up to 60% of Na⁺ influx could be inhibited, whereas Cl⁻ influx was not altered, and that inhibition of Na⁺ influx was not Cl⁻ dependent.

Active Cl⁻ secretion in vitro (12) is dependent on the presence of serosal Na⁺ and Na⁺–K⁺ ATPase and is thought to occur by a mechanism that may be summarized as follows (12). A low concentration of intracellular Na⁺ is maintained by Na⁺–K⁺ ATPase situated in the basolateral membrane; as a result, NaCl cotransport across the basolateral membrane occurs, Na⁺ passing down a concentration gradient and thereby enabling Cl⁻ to accumulate intracellularly
above its electrochemical equilibrium. In the absence of secretory stimuli, Cl\(^-\) permeability of the apical membrane is low, and accumulated Cl\(^-\) recirculates to the serosa. In the presence of cyclic AMP (and/or Ca\(^{2+}\)), apical Cl\(^-\) permeability increases, thereby inducing active Cl\(^-\) secretion, Na\(^+\) entering the lumen by a paracellular route.

It has been suggested that absorption is a property of mature villus enterocytes whereas the ability to secrete electrolytes and water resides in the immature enterocytes that line the crypts. Field (5) has recently reviewed the evidence that supports this notion:

1. Micropipette studies have shown that secretion arises from the intervillus region in small intestinal and colonic epithelium (18,19).

2. In intestinal epithelia that are devoid of crypts (e.g., winter flounder), exposure to cyclic AMP inhibits absorption but does not stimulate Cl\(^-\) secretion (20,21).
3. Exposure of rat small intestine to cholera toxin for 5 min results in activation of villus cell but not crypt cell adenylate cyclase, and this is associated with inhibition of NaCl absorption; exposure for 30 min results in activation of villus and crypt cell enzyme and the production of Cl⁻ secretion in addition to inhibition of NaCl absorption (21).

4. Destruction of villus but not crypt cells by hyperosmotic sodium sulfate does not impair cholera toxin-induced secretion (22).

More recently, two further pieces of evidence derived from novel techniques (selective Ca²⁺ deprivation to damage villus or crypt cells (23); existence of a tissue osmolality gradient along the villus (24) has been produced, which adds weight to this hypothesis.

Cyclic GMP

Cyclic GMP has also been identified as an intracellular mediator of intestinal secretion (25,26), largely as a result of investigations on the mode of action of *E. coli* heat-stable toxin (ST). The same enterotoxin has recently been isolated and purified from enteropathogenic strains of *Yersinia enterocolitica* (27) and shown to produce similar effects on intestinal transport to those of *E. coli* ST.

Exposure of short-circuited rabbit terminal ileum in vitro to ST (26) results in a rapid rise on potential difference and short-circuit current, inhibition of NaCl cotransport, and stimulation of active Cl⁻ secretion, although this latter effect is not as marked as when produced by cyclic-AMP-related agonists (26). The ST brings about an increase in cyclic GMP concentration in intestinal epithelial cells as a result of activation of guanylate cyclase (25,28). Further evidence that supports the notion that cyclic GMP is functionally as important follows. Cyclic GMP analogs produce intestinal secretion (28,29); ST-induced rises in short-circuit current and cyclic GMP concentration are both maximal within 5 min of toxin addition and persist for over 30 min, whereas cyclic AMP concentrations are unchanged by *E. coli* ST (25). Epinephrine, an antisecretory agent, causes an apparently paradoxical rise in mucosal cyclic GMP concentration (29); Guandalini et al. (26) have recently shown, however, that the concentration achieved by epinephrine is insufficient to produce changes in Cl⁻ transport.

Calcium

Calcium ions play an important role in the control of cellular function in a wide variety of cell types (30), including secretion by several different epithelia. In 1977, Bolton and Field (31) and Frizzel (32) provided evidence that Ca²⁺ was important in controlling intestinal secretion. Addition of the divalent cation ionophore A23187 to the serosal bathing solution of rabbit terminal ileum mounted in Ussing chambers (31) resulted in electrical and ion flux changes similar to those produced by cyclic AMP, but these changes occurred in the
absence of raised cyclic AMP concentrations. The response was dependent on the presence of Ca\(^{2+}\) in the serosal bathing solution. Similarly, the responses to carbamylcholine and serotonin, secretagogues that do not produce raised cyclic AMP, were Ca\(^{2+}\) dependent. In contrast, secretion associated with raised cyclic AMP concentrations (vasoactive intestinal peptide, prostaglandin E) was not Ca\(^{2+}\) dependent. Addition of A23187 to the luminal bathing solution of colonic mucosa \textit{in vitro} (32) resulted in active Cl\(^{-}\) secretion, which was once again Ca\(^{2+}\) dependent but not cyclic AMP mediated. These data suggest that not only does intracellular Ca\(^{2+}\) play an important role in regulating intestinal secretion but that certain secretagogues act as Ca\(^{2+}\) ionophores.

Further evidence in support of an important physiological role for Ca\(^{2+}\) has been published by Donowitz and Asarkof (33). Sodium and Cl\(^{-}\) absorption by rabbit ileal mucosa \textit{in vitro} was enhanced by bathing in calcium-free solutions, and this was associated with a concurrent fall in ileal Ca\(^{2+}\) content. Addition of the Ca\(^{2+}\) channel blocker verapamil to the serosal bathing solution of non-Ca\(^{2+}\)-deprived mucosa resulted in a similar enhancement of absorption, providing further evidence that Ca\(^{2+}\) is an important physiological regulator.

There is some evidence to suggest that some of the effects of Ca\(^{2+}\) in intestinal epithelial cells is exerted through a Ca-dependent regulator protein (CDR; calmodulin). Calmodulin is a highly conserved protein of 16,500 daltons which has a broad if not ubiquitous distribution in the tissues of eukaryotes and is responsible for Ca\(^{2+}\)-dependent activation of a wide variety of enzyme systems (34–37). Calmodulin possesses four Ca\(^{2+}\) binding sites, and binding of Ca\(^{2+}\) results in a conformational change (50% of the molecule adopts an \(\alpha\)-helical configuration), which is necessary for CDR to regulate enzyme systems.

The role of CDR in regulating intestinal secretion has been investigated by the use of pharmacological agents that bind to CDR. The phenothiazine trifluoperazine binds to the Ca\(^{2+}\)•CDR complex with high affinity (38) and has been shown to inhibit secretion induced by choleragen, theophylline, or A23187 in the absence of any change in the raised concentrations of mucosal cyclic AMP induced by the choleragen or theophylline (39). Trifluoperazine also inhibited the increase in passive Cl\(^{-}\) permeability induced by theophylline, and it was possible to demonstrate indirectly, using the Ca\(^{2+}\)-dependent uptake of \(^{3}\)H-labeled trifluoperazine by mucosal CDR as an assay for intracellular Ca\(^{2+}\), that free intracellular Ca\(^{2+}\) is raised during secretion induced by choleragen, theophylline, or A23187. Furthermore, the antidiarrheal potency of several pharmacological agents, including chlorpromazine and loperamide, may correlate positively with their ability to bind to CDR (40), suggesting not only that CDR is important in regulating secretion but that the basis of the therapeutic effect of certain agents is an inhibition of a conformational change in CDR.

Additional roles for Ca\(^{2+}\) within the enterocyte have also been suggested. Calcium may indirectly increase intracellular concentrations of the arachidonic acid metabolites 5-hydroxyperoxyeicosatetraenoic acid (5-HPETE) and 5-hydroxyeicosatetraenoic acid (5-HETE), which are themselves secretagogues (41).
Bradykinin-induced secretion (6) appears to be mediated by arachidonic acid and may involve bradykinin-related opening of membrane Ca\textsuperscript{2+} channels.

Intracellular Ca\textsuperscript{2+} may also be the messenger by which the apical membrane of epithelial cells communicates with the basolateral membrane (42); inhibition of basolateral Na\textsuperscript{+}-K\textsuperscript{+} ATPase reduces the Na\textsuperscript{+} permeability of the apical membrane, and stimulation with CO\textsubscript{2} results in an increase (42). A hypothetical model has been proposed to explain these observations (42) (Fig. 2); evidence exists that there is a Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange system located at the basolateral membrane (43,64), which maintains intracellular Ca\textsuperscript{2+} at low concentrations; entry of Na\textsuperscript{+} down a chemical gradient drives Ca\textsuperscript{2+} uphill, out of the cell, such that a rise in the intracellular concentration of Na\textsuperscript{+} would result in an increase in Ca\textsuperscript{2+} concentration. It is speculated that such a rise in intracellular Ca\textsuperscript{2+} brings about a reduction in apical membrane Na\textsuperscript{+} permeability, thereby protecting the cell against osmotic lysis. Kimmich and Randies (44) have recently made the intriguing observation that labeled Na\textsuperscript{+} accumulated in isolated enterocytes via an ATP-regulated Na\textsuperscript{+} channel is extruded by a mechanism that is highly Ca\textsuperscript{2+} dependent. Clearly, Ca\textsuperscript{2+} plays an important part in the regulation of intestinal ion transport.

**Microtubules**

Microtubules are fibrous cytoplasmic organelles that have an architectural and contractile role in nearly all eukaryotic cells (45,46). They are hollow, tubular, and rigid and built from a helical array of tubulin subunits, each of which is a dimer comprising two 55,000-dalton subunits (α and β tubulin). Assembly and disassembly of microtubules occur at opposite ends of the organelle, and the tubulin–microtubule system is in a state of dynamic equilibrium. Assembly is accompanied by hydrolysis of GTP (47) and is blocked by colchicine and vinblastine (7), although the specificity of these agents is not proven beyond
doubt. Notis et al. (48) have recently shown that an intact microtubule system is necessary for cyclic-AMP- and Ca\textsuperscript{2+}-dependent intestinal secretion.

In the rat small intestine in vivo, intraperitoneal colchicine inhibited cholera toxin- and prostaglandin-induced secretion but did not alter adenylate cyclase activation or cyclic AMP concentration. In the rat terminal ileum in vitro, colchicine and vinblastine reduced the short-circuit response to dibutyl cyclic AMP and theophylline; vinblastine reduced the response to carbamylcholine, a Ca\textsuperscript{2+}-dependent secretagogue. The structural isomer of colchicine, lumicolchicine, which does not block microtubule assembly, did not, however, inhibit the secretory response to dibutyl cyclic AMP or to theophylline. The part that microtubules may play in concert with cyclic nucleotides and Ca\textsuperscript{2+} in regulating secretion is considered next; it represents a promising area for the development of more specific blockers as experimental tools.

INTEGRATION OF CYCLIC NUCLEOTIDES, CALCIUM, AND MICROTBULBLES

At present there is no generally accepted hypothesis that explains how the intracellular factors known to regulate secretion are functionally integrated, but phosphorylation of a brush border protein is probably important at some point in regulating ionic channels.

Schlatz et al. (49) have demonstrated the presence of endogenous protein kinases in brush border and basolateral membranes that phosphorylate endogenous membrane proteins; cyclic AMP and cyclic GMP were both found to be effective enzyme activators. In addition, endogenous phosphatase activity was observed that was not, however, regulated by cyclic nucleotides. A unique polypeptide was identified in both types of membrane (50), which acted as a specific phosphate-accepting substrate for endogenous kinase activity and which, in brush border membranes, had a molecular weight of 103,000 and in basolateral membranes, 113,000. De Jonge and Van Dommelen (51) have recently demonstrated the presence of a cyclic-GMP-dependent protein kinase in the microvillus membrane that is capable of autophosphorylation. These findings raise the attractive possibility that a conformational change in a membrane protein occurring as a result of phosphorylation is an integral part of the process whereby the permeability of ionic channels is regulated.

Calcium ions, by an action mediated by the Ca\textsuperscript{2+}·CDR complex, may also regulate membrane protein kinases. A Ca\textsuperscript{2+}·CDR-dependent protein kinase activity has been identified in smooth and skeletal muscles and in a cerebral membrane preparation (52), and the possibility exists, as suggested by de Jonge and van Dommelen (51), that cyclic nucleotides and Ca\textsuperscript{2+} act in concert to regulate membrane protein kinase activity. The exact nature of any interaction is at present uncertain, but there is evidence to suggest that cyclic nucleotides mobilize Ca\textsuperscript{2+} from intracellular storage sites and that Ca\textsuperscript{2+}·CDR may act at a site on a pathway distal to that at which cyclic and nucleotides act. (1) Cyclic
AMP, when added to strips of colonic mucosa preloaded with $^{45}$Ca, results in 
$Ca^{2+}$ efflux from the tissue (32). (2) Cyclic AMP appears to raise the free in-
tracellular $Ca^{2+}$ concentration (39). (3) The secretory effects of cyclic-nucleotide-
dependent secretagogues can be blocked by antagonists of $Ca^{2+}$·CDR or of 
microtubule assembly (38,48) and by loperamide (53) in the absence of a fall 
in cyclic nucleotide concentration. The observation that cyclic AMP is capable 
of eliciting secretory responses in rabbit ileum (31) and colon (32) in vitro 
even in the absence of external $Ca^{2+}$ would be possible if sufficient amounts of $Ca^{2+}$ 
are already stored within the secretory cell to initiate secretion following mo-
bilization by cyclic AMP.

As previously outlined, Notis et al. (48) have provided evidence that intact 
microtubules play an important part in regulating secretion and have suggested 
that microtubules may do this by producing changes in apical membrane com-
position and/or structure, thereby altering membrane permeability. Of relevance 
to their proposal are the observations that bovine brain microtubules possess 
intrinsic protein kinase activity and act as a substrate for a cyclic-AMP-dependent 
protein kinase (54). Similarly, the observations that dibutyryl cyclic AMP, cholera 
toxin, and theophylline promote microtubule elongation from existing tubulin 
pools in a variety of cell types (55) add weight to their hypothesis. An apparent 
paradox with regard to the relationship among microtubules, intestinal ion 
secretion, and $Ca^{2+}$·CDR arises. Although CDR has been found to be associated 
with microtubules by immunofluorescence and electron microscopy (56–58) 
$Ca^{2+}$ even at micromolar concentrations inhibits rather than promotes micro-
tubule assembly, a function probably mediated by CDR (55). This inhibition 
is only expressed, however, at high, possibly unphysiological concentrations of 
CDR (34). Thus, a single unifying hypothesis incorporating microtubules, cyclic 
AMP, and $Ca^{2+}$ is difficult to formulate at present.

THE ROLE OF $Na^{+}$–$K^{+}$ ATPase IN PROTRACTED DIARRHEA

Disturbances in the regulatory factors mediating intestinal secretion previously 
outlined have been extensively investigated in the laboratory using experimental 
models of acute diarrhea. Direct clinical observations have been made in patients 
with cholera (9,59), but little information exists regarding the intracellular 
pathogenesis of protracted diarrhea.

Severe protracted diarrhea in infancy (SPDI) (60,61) carries a high mortality, 
and knowledge of the pathophysiology is fragmentary. Histology of the jejunal 
mucosa varies from normal to hyper- or hypoplastic villus atrophy, and patho-
genesis is probably multifactorial. Milla et al. (62) have shown, using a steady-
state jejunal perfusion technique, that in a group of patients with SPDI, net 
secretion of water occurred; glucose absorption was impaired, but fructose was 
less affected; the glucose-evoked potential difference response was also decreased. 
In contrast to cholera, adenylate cyclase activity in the jejunal mucosa of these 
patients was normal, and $Na^{+}$–$K^{+}$ ATPase activity was depressed. These findings
suggest that sodium-coupled hexose transport is defective in SPDI as a result of a previously unrecognized defect in the "sodium pump" within the basolateral membrane of the enterocyte. Under these circumstances, Na\(^+\) absorption is diminished as a result of a decrease in the favorable Na\(^+\) gradient across the brush border membrane. Net small intestinal secretion occurs, sufficient to overwhelm what appears to be normal reabsorptive capacity in the colon, with resulting secretory diarrhea.

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