Electrolyte Transport in the Mammalian Colon: Mechanisms and Implications for Disease

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Kunzelmann, Karl, and Marcus Mall. Electrolyte Transport in the Mammalian Colon: Mechanisms and Implications for Disease. Physiol Rev 82: 245–289, 2002; 10.1152/physrev.00026.2001.—The colonic epithelium has both absorptive and secretory functions. The transport is characterized by a net absorption of NaCl, short-chain fatty acids (SCFA), and water, allowing extrusion of a feces with very little water and salt content. In addition, the epithelium does secret mucus, bicarbonate, and KCl. Polarized distribution of transport proteins in both luminal and basolateral membranes enables efficient salt transport in both directions, probably even within an individual cell. Meanwhile, most of the participating transport proteins have been identified, and their function has been studied in detail. Absorption of NaCl is a rather steady process that is controlled by steroid hormones regulating the expression of epithelial Na⁺ channels (ENaC), the Na⁺–K⁺-ATPase, and additional modulating factors such as the serum-
The purpose of this review is to give a summary of molecular properties of the Na⁺-2Cl⁻ cotransporter or the Na⁺-K⁺-ATPase, which have been reviewed extensively elsewhere (203, 240, 509). Instead, we focus on other aspects of electrolyte transport, under pathophysiological conditions during secretory diarrhea and CF. Other membrane transport processes occurring in the colonic epithelium, such as absorption of short-chain fatty acids (SCFA) and secretion of mucus are only discussed in the context of ion transport. They have been reviewed elsewhere (146, 184, 247, 428).

II. ANATOMY AND TASKS OF THE COLONIC EPITHELIUM

A. General Transport Properties

The primary nonmotor function of the human colon is absorption of ~1.3–1.8 liters electrolyte-rich fluid per day, which accounts for ~90% of the salt and water entering the proximal colon (125). Most data on colonic transport were obtained in studies on colonic tissues from rat, rabbit, mouse, and human. Despite considerable quantitative differences, the mechanisms are qualitatively very similar in the different species (123, 665). Epithelial cells coating the inside of the mammalian colon form a low-resistance epithelium in the proximal colon of only ~100 Ω·cm². The resistance is about two- to fourfold higher in rat distal colon, which can therefore be described as a “moderately” or “medium” resistance epithelium (100, 101, 667). The values are about twice as high in the mouse distal colon. Here the paracellular resistance is high compared with transcellular resistance (212). Colonic epithelial cells are highly conductive, with a greater density of ion channels in the luminal membrane of surface compared with crypt cells. The result of ion transport and describe new aspects of regulation of the participating transport proteins. Our focus is on the native tissue rather than cultured colonic epithelial cells. We do not aim to present a detailed review on molecular properties of the Na⁺-2Cl⁻-K⁺ cotransporter or the Na⁺-K⁺-ATPase, which have been reviewed extensively elsewhere (203, 240, 509). Instead, we focus on other aspects of electrogenic and electroneutral ion transport. This includes the contributing epithelial ion channels and transporters as well as their regulation in proximal and distal colon. We also describe specific aspects of dysfunction of epithelial transport, under pathophysiological conditions during secretory diarrhea and CF. Other membrane transport processes occurring in the colonic epithelium, such as absorption of short-chain fatty acids (SCFA) and secretion of mucus are only discussed in the context of ion transport. They have been reviewed elsewhere (146, 184, 247, 428).

The purpose of this review is to give a summary of the molecular aspects of electrolyte transport in the colon and describe new aspects of regulation of the participating transport proteins. Our focus is on the native tissue rather than cultured colonic epithelial cells. We do not aim to present a detailed review on molecular properties of the Na⁺-2Cl⁻-K⁺ cotransporter or the Na⁺-K⁺-ATPase, which have been reviewed extensively elsewhere (203, 240, 509). Instead, we focus on other aspects of electrogenic and electroneutral ion transport. This includes the contributing epithelial ion channels and transporters as well as their regulation in proximal and distal colon. We also describe specific aspects of dysfunction of epithelial transport, under pathophysiological conditions during secretory diarrhea and CF. Other membrane transport processes occurring in the colonic epithelium, such as absorption of short-chain fatty acids (SCFA) and secretion of mucus are only discussed in the context of ion transport. They have been reviewed elsewhere (146, 184, 247, 428).
is excretion of a stool containing <5 mM Na⁺, 2 mM Cl⁻, and 9 mM K⁺ (645). In addition to electrogenic and electroneutral absorption of NaCl, active absorption of K⁺ by luminal K⁺ pumps and absorption of SCFA produced by the intestinal flora are primary tasks of the colonic epithelium. Although net absorption of NaCl, KCl, and water are dominant housekeeping functions of the colon, secretion of NaCl and KCl also takes place and largely exceeds absorption in secretory diarrhea. In addition to the transport of NaCl and KCl, the colonic epithelium secretes HCO₃⁻ and mucus. Both have been demonstrated to have important protective and milieu functions.

B. Cell Types

According to morphological studies, at least three different cell types form the mammalian colonic epithelium. They comprise columnar epithelial cells, mucous, and argentaffin cells (84, 610). Columnar epithelial cells and goblet cells contribute to ~95% of all cells. In addition, enterochromaffin (enteroendocrine) cells make up another 5%. A surface epithelial layer is differentiated from colonic crypts. The columnar epithelial cells can be subdivided according to their degree of differentiation, which is based on their proliferative activity, expression of differentiation markers, and functional properties (266, 268, 274, 498, 506). Thus base crypt epithelial cells show the highest proliferative activity, demonstrate limited expression of differentiation markers, and have a high Cl⁻ secretory activity. In contrast, surface epithelial cells have a lower tendency to proliferate, show expression of differentiation markers and certain lectins, and have primarily absorptive function (266, 268, 337). The cells become increasingly differentiated the further they are located away from the crypt base and the closer they are to the surface. Thus highly proliferative and fairly undifferentiated epithelial cells in the crypt base form a constant source for replacement of the surface cells. These replacing cells differentiate while traveling along the crypts toward the surface (46, 157, 158) (Fig. 12).

C. Ion Transport in Surface Epithelium and Crypts

It is still a matter of debate whether crypts and surface epithelium represent two specialized compartments with distinct functions in either absorption (surface epithelium) or secretion (crypts) of electrolytes (175, 659). It has been suggested that secretion occurs to clear the crypts from mucus, which is secreted from goblet as well as columnar epithelial cells (247). However, mucus secretion also takes place in the surface epithelium. Therefore, this and further studies do not support the idea of exclusive secretion in the crypts. There is now clear evidence that electrolyte secretion is located in both surface epithelium and crypts (336). This comes from studies using vibrating electrodes (294, 336) as well as patch-clamp studies in which Cl⁻ channels could be demonstrated in both crypts and the surface (144). Moreover, CFTR Cl⁻ channels show a gradient of expression along the crypt/villus axis (606). Functional analysis of cAMP-activated Cl⁻ conductance and in situ hybridization suggest highest expression of CFTR in crypt cells, and studies in cultured colon epithelial cells indicate a higher mRNA expression in undifferentiated cells (220, 577, 585). Interestingly, it has been shown by in situ hybridization that expression switches from cystic fibrosis transmembrane conductance regulator (CFTR) to MDRI as the cells migrate across the crypt/villus boundary. Thus coordinated regulation of expression of these two genes has been assumed (607). However, expression of mRNA and CFTR Cl⁻ currents may not necessarily be tightly correlated (29). Nevertheless, CFTR Cl⁻ channels expressed in surface epithelial cells may be required for absorption rather than secretion of NaCl, since they are colocalized together with epithelial Na⁺ channels (ENaC). This and the fact that CFTR is likely to serve as a regulator of ENaC are outlined later in this review (341) (Figs. 3 and 4).

The localization of absorption within the colonic epithelium is even more controversial. As outlined below, absorption can be electrogenic via the ENaC or is electroneutral via parallel Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchange. According to the work of some groups, the high osmotic pressure gradient that is necessary to absorb water from the intestinal lumen and to generate the mammalian feces can only be created by a trapping mechanism for ions located in the crypts rather than the surface epithelium (434, 435, 457, 571). These interesting results are based on fluorescence shifts using fluorescent dextrans. At any rate, electroneutral NaCl absorption has been shown to take place in both crypts and surface epithelium (571) (Fig. 1).

In contrast, electrogenic absorption via ENaC is confined to the surface epithelium and upper part of the crypt, as determined by voltage scanning and whole cell patch-clamp experiments (220, 337). Expression of α,β,γ-ENaC subunits is limited to the surface and upper crypt, as verified by immunolabeling of ENaC (202). Accordingly, steroid-dependent regulation of ENaC expression was observed in the surface epithelium but not the crypts (202). The conclusion from the various studies is that bulk absorption occurs via electroneutral NaCl transport and takes place in both crypts and surface epithelium of proximal and distal colon. Evidence for electroneutral absorption in the crypts is more indirect, due to a lack of electrogenic Na⁺ absorption (39). The transport in rat and mouse colon is dominated by electroneutral absorption. Electrogenic absorption via luminal ENaC is confined to the surface epithelium of the distal colon and shows a larger contribution in rabbit, human, and guinea pig than
in rat and mouse (337, 397, 465). In general, less detailed information is currently available for human and mouse colon than that of rat (50, 230, 513) (Fig. 1).

III. ABSORPTIVE FUNCTION OF THE COLONIC EPITHELIUM

A. Electroneutral Absorption of NaCl

Bulk transport of NaCl in the colonic epithelium is due to electroneutral absorption by luminal Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchange. The remaining absorption is electrogenic and is due to absorption via luminal ENaC and transcellular/paracellular absorption of Cl⁻. The contribution of paracellular Cl⁻ absorption might be limited by the paracellular shunt resistance, which is roughly 20 times larger than the transepithelial resistance (212). However, the presence of a large lumen-negative transepithelial voltage, particularly in glucocorticoid-treated animals, and the fact that the paracellular shunt is not strictly ion selective, would allow for paracellular movement of Cl⁻ (5, 666, 667). Moreover, tight junctions consist of a complex array of proteins such as occludin, claudin, and paracellin and are probably actively regulated (5, 168, 569, 678). Thus it has been demonstrated that the tight junction permeability of the rat ileum is increased by cAMP (38).

There is a clear segmental heterogeneity with respect to Na⁺ absorption present in ascending (proximal) and descending (distal) colon in human and other species. In the ascending colon, Na⁺ transport is primarily mediated by an electroneutral process, while Na⁺ transport in the descending colon is dominated by electrogenic absorption via amiloride-sensitive Na⁺ channels under the influence of aldosterone (101, 367, 518, 679) (Fig. 1). In the absence of steroids, electroneutral absorption is the predominant transport process in both rat proximal and distal colon (39, 186, 187, 192). It should be mentioned, however, that significant species differences exist regarding the contribution of electroneutral and electrogenic Na⁺ absorption. For example, while electrogenic absorption dominates the rabbit distal colon, the rat colon is dominated by electroneutral absorption (39, 465). Limited

FIG. 1. Models for electrolyte transport in proximal and distal colonic epithelium and expression of different ion transporters along the crypt axis. Electroneutral NaCl absorption (parallel Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchange) dominates in the surface epithelium and is also present in the crypts. Electrogenic Na⁺ absorption via the epithelial Na⁺ channel (ENaC) takes place in the surface epithelium and upper crypts of the distal colon. The cystic fibrosis transmembrane conductance regulator (CFTR) is expressed throughout the colonic epithelium and dominates in the crypts.
information is available for human and mouse colon. For electroneutral absorption of NaCl, the presence of parallel Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3\)\(^-\) exchangers in luminal brush-border membranes of colonic epithelial cells is required (Fig. 2). It is driven by the action of the basolateral Na\(^+\)-K\(^+\)-ATPase and probably requires 1 mol ATP being hydrolyzed per 3 mol NaCl absorbed (307). The transport of Na\(^+\) and Cl\(^-\) is coupled via changes in intracellular pH and Cl\(^-\) (204, 480, 485). It is regulated by Na\(^+\) depletion or steroids (27, 186, 282, 671). The properties of these transport proteins are discussed in more detail below.

1. Role of Na\(^+\)/H\(^+\) exchange

Expression of three types of the Na\(^+\)/H\(^+\) exchanger (NHE) has been detected so far in the colonic epithelium. The abundant type 1 NHE is expressed in the basolateral membrane and does not seem to be affected by Na\(^+\) depletion. NHE2 and NHE3 are both expressed on the luminal side of colonic epithelial cells, with a larger contribution of NHE3 to Na\(^+\) absorption under control conditions (282). Regulation of NHE2 and NHE3 differ in proximal and distal colon, in as much as expression of both types is upregulated by Na\(^+\) depletion in the proximal colon, but is attenuated in the distal colon (282, 480). In addition to NHE2 and NHE3, a third and novel type of Cl\(^-\)-dependent NHE has been identified in apical membranes of rat crypt cells (485). This transporter is upregulated by Na\(^+\) depletion and increases in plasma aldosterone (487). According to \(^{22}\)Na\(^+\) uptake studies, the transporter is functionally coupled to a Cl\(^-\) channel rather than a Cl\(^-\)/anion exchange mechanism, and some data suggest that this Cl\(^-\) channel is identical to CFTR (486). Moreover, Na\(^+\)/H\(^+\) exchange is also detected on basolateral membranes and is due to the housekeeping function of the type 1 NHE (257, 282, 463).

Na\(^+\)/H\(^+\) exchange occurs in both surface and crypt epithelium, is tightly coupled to Cl\(^-\)/HCO\(_3\)\(^-\) exchange, and might be controlled by CFTR Cl\(^-\) channels (480, 485, 486). Although little is known about the impact of CFTR on cAMP-dependent regulation of the Na\(^+\)/H\(^+\) exchange in the colon, clear evidence has been found in the small intestine. In the normal (non-CF) human intestinal epithelium, an increase in intracellular cAMP inhibits electroneutral reabsorption of NaCl (43). In contrast, cAMP-dependent activation is not observed in the small intestine of CF patients carrying a defective CFTR. In the CF jejunum, an increase in intracellular cAMP even further activates absorption of Na\(^+\) (35, 446).

2. Role of luminal Cl\(^-\)/HCO\(_3\)\(^-\) exchange

Functional studies have shown the presence of at least two different types of Cl\(^-\) exchange mechanisms in apical membranes of colonic epithelial cells. In addition, a third type of Cl\(^-\)/HCO\(_3\)\(^-\) exchange is expressed in the basolateral membrane (484, 541, 594). The identified luminal Cl\(^-\)/HCO\(_3\)\(^-\) and Cl\(^-\)/OH\(^-\) exchange are represented by the anion exchanger type 1 (AE1) and a protein called DRA, which stands for downregulated in colonic adenomas (484, 541). DRA has been demonstrated to function...
as a transporter for $\text{SO}_4^{2-}$, as well as an anion exchanger (3, 546). Interestingly, DRA is upregulated in mice lacking the NHE3 but is otherwise not regulated by Na$^+$ depletion, whereas expression of AE1 is inhibited by aldosterone (269, 417, 484, 541). Mutations in human DRA are responsible for congenital chloride diarrhea and the most common form of syndromic deafness, described as the Pendred syndrome (269, 429, 546). Interestingly, in the airways, expression of DRA depends largely on the expression of the CFTR, a finding which could also apply to the colon (661). The Cl$^-$/HCO$_3^-$ exchange that is present in basolateral membranes of colonic epithelial cells is due to expression of the isoforms AE1 and AE2 (Fig. 2) (39, 482, 594). Like NHE3, Cl$^-$/HCO$_3^-$ exchange is also likely to be controlled by CFTR in the colonic epithelium. Regulation of Cl$^-$/HCO$_3^-$ exchange by CFTR has been demonstrated for other parts of the gastrointestinal tract, such as submandibular and pancreatic ducts. It is also observed after heterologous expression in NIH3T3 or HEK 293 cells (361, 362, 388). Taken together, the current studies indicate the presence of several types of Na$^+/H^+$ exchanger, along with three different anion exchangers. The results further suggest a regulation of both Na$^+/H^+$ as well as Cl$^-$/HCO$_3^-$ exchangers by CFTR, which therefore has an impact on electroneutral absorption of NaCl and regulation of cellular and mucosal pH in the colonic epithelium and other sections of the gastrointestinal tract (214, 466, 653).

**B. Electrogenic Absorption of Na$^+$**

In addition to electroneutral absorption by parallel exchange of Na$^+/H^+$ and Cl$^-$/HCO$_3^-$, epithelial cells of the distal colon possess a mechanism for electrogenic uptake of Na$^+$. The ENaC is responsible for electrogenic absorption and is located in the luminal membrane of colonic epithelial cells. ENaC is potently inhibited by amiloride and related diuretic compounds (18, 31, 74, 75, 202, 497). In addition to ENaC, other types of cation channels have been detected in the cecum (552, 553). However, the quantitative impact of these channels on electrolyte absorption remains to be demonstrated. The basic concept of electrogenic Na$^+$ absorption has been elaborated many years ago (123, 330). Due to the electrochemical gradient for Na$^+$ and the negative cell membrane voltage, there is a large driving force for luminal Na$^+$ uptake via ENaC. Absorption of Na$^+$ is accompanied by the counterion Cl$^-$, which is taken up by Cl$^-$ channels, localized in the apical membrane of absorptive epithelial cells, and is also likely to occur via the paracellular shunt, as outlined in section IIIA (220, 347, 397). CFTR is the predominant luminal Cl$^-$ channel in the colonic epithelium (396). Whole cell patch-clamp experiments and in situ hybridization suggested coexpression of CFTR and ENaC in surface and midcrypt epithelial cells of the rat colon (157, 220, 606). Due to the large driving force for Na$^+$ uptake in these cells and the depolarization of the luminal membrane voltage, we speculate that CFTR Cl$^-$ channels may also serve as an absorptive pathway for Cl$^-$ in these cells (Figs. 1 and 3). Thus the situation could be somewhat similar to that of the sweat duct (493). However, due to the inhomogeneous architecture of the native colonic epithelium and the lack of Na$^+$ transport in cultured colonic cells, it has not been possible to demonstrate Cl$^-$ absorption by CFTR in the colon. In contrast, crypt base cells express large amounts of CFTR but no ENaC, and therefore, CFTR has clearly a secretory function in this part of the colonic epithelium (220, 606). Na$^+$ that have been taken up into the cell are pumped out again on the basolateral side of the epithelium by the Na$^+-K^+$-ATPase. Cl$^-$ that have entered the cytosol via apical Cl$^-$ channels leave the cell via basolateral Cl$^-$ channels or Cl$^-$/HCO$_3^-$ exchangers (220, 473, 657). Water can move via various pathways, including the paracellular shunt and the transcellular flux through aquaporin water channels located in both luminal and basolateral membranes. However, a major role for aquaporins in the colon has not yet been established (640). In addition, water transport may potentially occur via substrate transporters (384, 415, 416, 682). The different membrane proteins participating in absorption of NaCl have to be regulated in parallel. Basolateral outward transport of Na$^+$ by Na$^+-K^+$-ATPases must keep up with the apical Na$^+$ entry via Na$^+$ channels (222, 225). The putative Na$^+$...
feedback mechanism and other regulatory functions are discussed in the following sections.

C. Amiloride-Sensitive ENaC

ENaC are expressed on apical membranes of absorptive colonic epithelial cells. These channels are highly selective for Na⁺ over K⁺ and have a rather small single-channel conductance of ~4 pS and a linear current-voltage relationship (249). So far, most studies have been done on other tissues, and single-channel analysis of ENaC on the colonic epithelium is not yet available. Na⁺ uptake by ENaC into colonocytes is the rate-limiting step during electrogenic absorption of Na⁺ (201). The amiloride-sensitive ENaC has been cloned initially from rat colon. It consists of three different subunits (α-, β-, and γ-ENaC) (74, 75). Probably four subunits (2α-, 1β-, 1γ) coassemble to form a functional Na⁺ channel (73, 179, 202). However, other studies claim that ENaC is composed of 9 subunits or even 17 transmembrane α-helices (164, 575). ENaC belongs to a large family of related proteins, which is discussed in several excellent review articles (4, 18, 73, 202, 450). The large extracellular domains of the ENaC subunits contain cysteine-rich boxes, implying a receptor-like structure (255, 623). Indeed, a serine protease (mouse channel activating protein; mCAP) was recently found to be coexpressed with ENaC in absorptive epithelial cells. It may enhance channel activity via interaction with the extracellular loop of the channel (93, 623). ENaC subunits, which traverse the lipid membrane only twice, contain proline-rich segments in the intracellular COOH terminus of each subunit (581). These segments are essential for the interaction with the ubiquitin ligase Nedd4, which leads to ubiquitination and endocytosis of the channel protein (581). Mutations in these proline-rich segments of different subunits lead to a salt-sensitive form of hypertension due to excessive absorption of Na⁺ in the kidney collecting duct in Liddle’s disease (505).

D. Regulation of Na⁺ Absorption

1. Feedback regulation

Recent studies demonstrate the importance of the COOH-terminal PY motifs present in all three ENaC subunits, for the so-called “feedback inhibition” of ENaC. Feedback inhibition describes the phenomenon that Na⁺ has a negative impact on the activity of Na⁺ channels (202, 614). Changes in the intracellular Na⁺ concentration during NaCl absorption have been suggested to down-regulate ENaC conductance. This mechanism causes a negative feedback loop, which controls luminal entry of Na⁺ and thus absorption of NaCl (148, 202, 331). Na⁺ feedback was demonstrated in turtle and rabbit colon some years ago (326), but until recently, the mechanisms of the inhibition of electrogenic Na⁺ absorption remained unclear (614–616). Interestingly, mutations causing Liddle’s disease reduce Na⁺-dependent downregulation of ENaCs in Xenopus oocytes (317). Recent studies examined the feedback regulation in more detail in mouse mandibular duct cells and demonstrated suppression of ENaC whole cell currents by enhanced intracellular Na⁺ concentration (149). This inhibitory pathway requires sensing of the intracellular Na⁺ concentration via an intracellular Na⁺-sensitive structure and also includes activation of certain subtypes of GTP binding proteins (68, 147, 148). Activation of these G proteins leads to binding of Nedd4 to ENaC with subsequent ubiquitination and a possible endocytotic retrieval of Na⁺ channels (147).

Interestingly, a very similar mechanism for Na⁺ feedback regulation was identified recently for Na⁺-dependent regulation of the NHE in mouse mandibular duct cells. However, this process does not seem to require the ubiquitin protein ligase Nedd4 (288). Along the same lines, an increase in intracellular Cl⁻ concentration was found to inhibit ENaC via activation of a different subtype of G protein (331). Similar to the Na⁺ feedback, Cl⁻ entry into colonocytes may trigger downregulation of Na⁺ channel activity and eventually endocytosis of ENaC. How luminal CFTR Cl⁻ channels and eventually other Cl⁻ channels may contribute to this process is outlined in section mD4.

2. Acute hormonal regulation of Na⁺ absorption via Ca²⁺, protein kinase C, and protein kinase A

Electroneutral absorption is acutely up- and down-regulated in response to some G protein-linked receptors, tyrosine kinase-coupled receptors, and protein kinases, which are summarized in a recent review (151). Activation of protein kinase C (PKC), Ca²⁺/calmodulin-dependent kinase, and increases in intracellular cAMP inhibit NHE3, whereas stimulation of α₁ or β₂-receptors activates NHE3 (246, 271, 377, 504, 609). Increases of intracellular cAMP inhibit the activity of NHE3, by a mechanism involving additional scaffolding proteins such as NHERF and the cytoskeleton binding protein ezrin (655, 681). Electrogenic reabsorption of Na⁺ and water occurs at a rather steady rate and does not seem to be affected by intestinal peptide hormones or autonomic nerve activity. In fact, early studies indicated that the rat colonic mucosa is set to almost maximal absorption and that the impact of regulatory mechanisms is typically to reduce absorption and to induce electrolyte secretion (7). Thus activation of electrolyte absorption in the intestinal epithelium by endogenous hormones is of limited importance (66, 252). Hormones such as norepinephrine (acting on α-receptors), somatostatin, and peptide neurotransmitters such as peptide YY and neuropeptide Y (NPY) can increase
electrolyte absorption by direct action on enterocytes (66, 140, 376, 604). However, an increase in net absorption is typically through inhibition of secretion (66, 140, 604).

Other forms of immediate regulation of ENaC have been described for some epithelial tissues. They include inhibitory effects of Ca\(^{2+}\) and PKC, activation of ENaC by the cAMP-dependent pathway, and the impact of actin filaments on channel activity (13, 18, 32, 76, 202, 291, 334, 562). Moreover, inhibition of epithelial Na\(^+\) absorption by intracellular cGMP has been recently reported (620). However, rat ENaC expressed in heterologous expression systems such as *Xenopus* oocytes is not activated by protein kinase A (PKA) (13, 64, 399, 561, 562). There is some evidence for phosphorylation of β- and γ-subunits of ENaC via PKA and acute activation of ENaC in the kidney by increases in intracellular cAMP (562). In contrast, these effects were not detected in the rat colon (62, 63). In a patch-clamp study with isolated rat colonic crypt cells, ENaC was inhibited in parallel with activation of CFTR by an increase in cytosolic cAMP (157). Similar observations were made in Ussing chamber experiments on human colonic and rectal mucosa, where activation of Cl\(^-\) secretion by increases in intracellular cAMP was paralleled by inhibition of amiloride-sensitive Na\(^+\) transport (397). Moreover, when rat ENaC was expressed in Madin-Darby canine kidney cells or NIH3T3 fibroblasts, amiloride-sensitive Na\(^+\) currents were enhanced by PKA in the absence of CFTR but were inhibited when CFTR was present (586, 588). Surprisingly, ENaC isolated from *Xenopus* kidney and guinea pig colon were shown to be activated by cAMP (372, 528). According to some studies, the effects of PKA are largely controlled by the presence of actin filaments (292), but so far it is not clear how important actin is for the regulation of Na\(^+\) channels. Taken together, the present results and the lack of conserved PKA phosphorylation sites in the α,β,γ-ENaC subunits in different species suggest additional, as yet unknown, proteins that are phosphorylated by PKA and that may activate ENaC in the absence of CFTR (528). Regulation of ENaC by PKA could be tissue specific, as has reported for PKC (81).

3. Nucleotide-mediated inhibition of Na\(^+\) absorption

Stimulation of purinergic receptors by extracellular nucleotides such as ATP or UTP is emerging as another mechanism for the acute regulation of colonic Na\(^+\) transport. Purinergic receptors are located on both luminal and basolateral membranes of rat colonic epithelial cells (323, 363). It has been shown that stimulation of these P2Y2 receptors induces an increase in intracellular Ca\(^{2+}\) and a transient activation of KCl secretion (323). Stimulation of purinergic receptors has also been shown to inhibit electrogenic absorption of Na\(^+\) in airway, kidney, and thyroid epithelial cells (54, 112, 133, 215, 287, 334, 402, 490). The mechanism of nucleotide-mediated inhibition of Na\(^+\) absorption is not yet defined, but different models have been suggested, such as an increase in intracellular Ca\(^{2+}\) (133, 287, 402), activation of PKC (334), and a possible contribution of GTP binding proteins (270, 440). Whether purinergic inhibition of Na\(^+\) absorption also takes place in the colonic epithelium of rat and human remains to be determined. In unpublished experiments performed in our laboratory, we found no evidence for functional expression of purinergic receptors on either luminal or basolateral sides of the native human and rabbit colonic epithelium. In contrast, in the mouse colon, both ATP and UTP elicited large Cl\(^-\) currents and inhibit ENaC. The results from these Ussing chamber experiments remain to be confirmed by patch-clamp analysis of isolated human colonic epithelial cells.

4. Regulation of Na\(^+\) absorption by CFTR

ENaC, NHE3 and CFTR are coexpressed in colonic epithelial cells (157, 220, 606). This has been outlined in section III.B. Evidence has grown over the past few years that CFTR regulates both electroneutral as well as electrogenic absorption of electrolytes in the intestinal epithelium (98, 229, 397). The impact of CFTR on Na\(^+\) absorption is probably twofold: in human airways and colon expressing wild-type CFTR, amiloride-sensitive Na\(^+\) absorption is significantly reduced compared with that of CF patients, even in the absence of cAMP, i.e., without activation of CFTR. After stimulation of wild-type CFTR by cAMP, amiloride-sensitive Na\(^+\) absorption is further inhibited. Thus the presence of wild-type CFTR seems to inhibit ENaC even in the absence of secretagogues. Similar has been observed in *Xenopus* oocytes coexpressing CFTR and ENaC (301, 348, 395, 397, 404). Enhanced amiloride-sensitive short-circuit currents have been detected in CF airways and intestine (51, 52, 229, 328, 366, 397, 449, 586). In addition to these Ussing chamber studies on the native human epithelium, inhibition of amiloride-sensitive Na\(^+\) conductance has been observed in patch-clamp studies on freshly isolated rat colonic epithelial cells (157). Furthermore, numerous in vitro studies and experiments in cultured cells have demonstrated interaction of CFTR and ENaC (366, 399, 586). Further insight into the regulation of Na\(^+\) transport was gained by the development of CFTR (−/−) knockout mice. CFTR has been shown to inhibit electroneutral absorption of NaCl in the mouse small intestine and pancreatic duct (2, 43, 98). CFTR knockout mice also show enhanced amiloride-sensitive short-circuit currents in the colonic epithelium compared with normal mice. These results correspond to measurements on the human CF colon, where enhanced Na\(^+\) absorption and lack of CFTR-dependent inhibition of ENaC was found (397). Thus CFTR may have a dual function in the mammalian colon. In the lower...
crypts, it is a cAMP-regulated Cl\textsuperscript{−} channel, essential for Cl\textsuperscript{−} secretion. In the upper crypt and particularly the surface epithelium, it may regulate other transport proteins such as ENaC and NHE3 (Fig. 4). In the mouse pancreatic duct, NHE3 is inhibited by an increase in intracellular cAMP even in the absence of CFTR. However, inhibition by cAMP is augmented when CFTR is present (2). Inhibition of ENaC by CFTR and current efforts in identifying the mechanism for this interaction are summarized in recent reviews (341, 352, 355, 542).

Interestingly, CFTR does not inhibit ENaC in the sweat duct epithelium. Here, CFTR is actually required for upregulation of ENaC and cAMP-dependent activation of Na\textsuperscript{+} absorption (473, 494). Correspondingly, not enhanced but rather a decreased Na\textsuperscript{+} conductance was detected in CF sweat ducts. The reason for the different regulation is currently unexplained but could be due to expression of additional, as yet unidentified proteins participating in the functional interaction (352). It may also reflect different morphological and functional properties of airways/colon and sweat duct. Colonic and respiratory epithelia consist of different types of epithelial cells, which show rather poor electrical coupling via gap junctions, at least in the colon (293). Therefore, individual cells seem to operate as single functional units. This is in contrast to the sweat duct epithelium, which operates as a syncytium of a single type of epithelial cell. These cells are intimately coupled via gap junctions and are devoted exclusively to absorption of electrolytes (220, 494). Moreover, the sweat duct is formed by a rather tight epithelium with a high paracellular resistance. Therefore, and in contrast to the colonic mucosa, electrolyte transport occurs only transepithelially (494). In the intestinal epithelium and probably in the airways, the situation is different since 1) epithelial cells work as individual units, and 2) they transport electrolytes in both secretory and absorptive directions. A change of vectorial transport could be achieved by inverse regulation of luminal CFTR and ENaC. In absorptive cells, coexpressing both CFTR and ENaC, activation of CFTR Cl\textsuperscript{−} channels would allow entry of Cl\textsuperscript{−} into the cell, which might shut off ENaC channels (64, 149). Thus inhibition of ENaC and NHE3 by CFTR provides a mechanism by which colonic epithelial cells limit absorption of NaCl, avoid cell swelling, and eventually switch from absorption under resting conditions to secretion when exposed to secretagogues. In summary, CFTR inhibits both electroneutral absorption of NaCl as well as electrogenic absorption of Na\textsuperscript{+}. It therefore contributes to redirection of epithelial ion transport in the colonic mucosa by switching epithelial cells from absorption toward secretion in the presence of secretagogues.

5. Regulation of Na\textsuperscript{+} absorption by proteases

Recently, another regulatory protein, the epithelial channel activating protease 1 (CAP1), has been identified that upregulates epithelial Na\textsuperscript{+} currents (623, 634). CAP1 is homologous to human prostasin and is coexpressed with ENaC in epithelial tissues, such as the cortical collecting duct of the kidney and in the colon. This protein is a serine protease that is secreted to the luminal side of the epithelium, where it interacts with the large extracellular domain of ENaC. Although clear evidence exists for CAP1 acting as a serine protease, no evidence was found for cleavage of the extracellular loops of either α-, β-, or γ-ENaC (623). CAP1 largely augments the activity of amiloride-sensitive Na\textsuperscript{+} channels, without altering the number of channels in the plasma membrane (623). CAP1 does regulate ENaC independent of CFTR as demonstrated recently in *Xenopus* oocytes (272). So far, this autocrine regulatory mechanism has been examined in detail only in the kidney. It will be interesting to learn in future studies about the role of CAP1 in regulating Na\textsuperscript{+} absorption in the colonic epithelium.
E. Steroid-Dependent Regulation of Ion Transport

In many tissues, expression of ENaC and further proteins participating in epithelial ion transport such as the Na⁺-K⁺-ATPase are upregulated by glucocorticoid and mineralocorticoid hormones. This leads to enhanced Na⁺ absorption and K⁺ secretion (40, 43, 505, 613). Glucocorticoid and mineralocorticoid receptors are present in proximal and distal colon in both surface epithelium and crypts. These steroids exert differential effects on Na⁺ absorption in the colon (25, 535). Low-dose glucocorticoids induce electroneutral Na⁺ absorption in both proximal and distal colon, whereas high concentrations activate both electroneutral and electrogenic absorption, particularly in the distal colon of human and rat (25–27, 91, 158, 512, 613). In contrast, aldosterone induces only electrogenic absorption and inhibits basal electroneutral absorption in the distal colon of rats (27). According to recent studies, increased transcription of β- and γ-ENaC subunits may form part of the early response. Early aldosterone action has been shown to enhance both number and activity of ENaC channels in the cell membrane (202, 411, 579, 631). In renal cells, aldosterone increases the open probability of Na⁺ channels (319).

Other proteins, such as the channel inducing factor (CHIF), have been demonstrated to be transcriptionally controlled by Na⁺ depletion and steroids. CHIF might participate in the regulation of luminal K⁺ channels in the colonic epithelium and the control of K⁺ homeostasis (12, 78, 637). It should be mentioned that upregulation of the activity of other transport proteins like the basolateral Na⁺-K⁺-ATPase by aldosterone is another mechanism by which aldosterone regulates Na⁺ absorption. Moreover, a fast and nongenomic action of aldosterone on Na⁺/H⁺ exchange was detected in the rat distal colon. Nongenomic activation of the Na⁺/H⁺ exchange may occur via an increase in intracellular Ca²⁺ and stimulation of PKC (671). This and other regulatory properties of aldosterone are the subject of a recent review (631).

F. Active Absorption of K⁺

Apart from the kidneys, the mammalian colon also contributes to the regulation of K⁺ homeostasis by secreting and absorbing K⁺ (515) (Fig. 5). Active K⁺ absorption is restricted to the mammalian distal colon of rat, rabbit, and guinea pig (153, 314, 460). It is mediated by at least two different types of H⁺-K⁺-ATPases, expressed in columnar surface epithelial cells and in the crypts (153). These ATPases are distinguished on the basis of their sensitivity toward ouabain and omeprazole. Although the ouabain-insensitive H⁺-K⁺-ATPase has been cloned, the ouabain-sensitive H⁺-K⁺-ATPase has not yet been identified at the molecular level (1, 128). Both types of H⁺-K⁺-ATPases have been detected in the surface epithelium, whereas the ouabain-sensitive isoenzyme has been identified only in apical membranes of colonic crypt cells. Colonic H⁺-K⁺-ATPases are members of the family of P-type ATPases, similar to the Na⁺-K⁺-ATPase and the gastric H⁺- K⁺-ATPase, consisting of α- (HKαα) and β-subunits (HKcβ, NaKβ). Expression of HKαα and NaKβ is upreg-
has also a significant impact on NaCl absorption (9, 10, 41, additional energy supply for colonic epithelial cells, but yet been identified at the molecular level (87, 407, 551, explain why Na$	extsuperscript{+}$ from the DIDS-sensitive Cl$	extsuperscript{-}$ absorption in rat colonic epithelial cells. This exchanger is distinct mechanism seems to be present in the luminal membrane (387). However, an additional SCFA butyrate, and acetate occurs primarily by nonionic diffusion and paracellular absorption in the proximal colon (387). However, an additional SCFA$	extsuperscript{-}$/HCO$	extsubscript{3}$$^{-}$ exchange mechanism seems to be present in the luminal membrane of rat colonic epithelial cells. This exchanger is distinct from the DIDS-sensitive Cl$	extsuperscript{-}$/HCO$	extsubscript{3}$$^{-}$ antiporter and has not yet been identified at the molecular level (87, 407, 551, 618, 633). Absorption of SCFA not only serves as an additional energy supply for colonic epithelial cells, but has also a significant impact on NaCl absorption (9, 10, 41, 119). SCFA stimulate electroneutral uptake of Na$	extsuperscript{+}$, presumably by acidification of colonocytes and activation of apical Na$	extsuperscript{+}$/H$	extsuperscript{+}$ exchangers (550). Cl$	extsuperscript{-}$ absorption is stimulated by increased HCO$	extsubscript{3}$$^{-}$ production during SCFA metabolism and stimulation of the apical Cl$	extsuperscript{-}$/HCO$	extsubscript{3}$$^{-}$ exchanger. Another model has been proposed, in which butyrate is taken up via nonionic diffusion or SCFA/HCO$	extsubscript{3}$$^{-}$ exchanger. Subsequently electroneutral NaCl absorption is activated by parallel Cl$	extsuperscript{-}$/butyrate and Na$	extsuperscript{+}$/H$	extsuperscript{+}$ exchange (481). During absorption of SCFA, basolateral volume-sensitive Cl$	extsuperscript{-}$ channels are activated, whereas basal and cAMP-activated Cl$	extsuperscript{-}$ secretion by CFTR is inhibited (41, 119, 146, 628). It was found that absorption of SCFA depolarizes colonic crypt cells due to cellular acidification and inhibition of K$	extsuperscript{+}$ channels (145). Because HCO$	extsubscript{3}$$^{-}$ secretion to the luminal side of the epithelium is activated, absorption of SCFA has a large impact on the regulation of luminal intestinal pH (95, 618). Thus SCFA transport is an important factor that regulates colonic fluid balance, absorption of NaCl, and luminal as well as cytosolic pH.

IV. SECRETORY FUNCTION OF THE COLONIC EPITHELIUM

A. Electrolyte Secretion

Another major function of the colon is secretion of electrolytes, which is balanced by absorption. It may facilitate the transport of mucus out of the crypts and maintain hydration of mucus, which is secreted by goblet and columnar epithelial cells in crypts and surface epithelium. Accordingly, mucus secretion is activated by an
increase in intracellular cAMP in parallel with electrolyte secretion (248). A limited KCl secretion under resting conditions does become a pronounced KCl secretion upon stimulation by secretagogues or when exposed to bacterial toxins. In the absorbing colon and in the absence of secretagogues, release of K+ to the luminal side is potential driven and largely maintained by the ENaC. This leads to a luminal K+ concentration which is above that of serum. As for the absorption of NaCl, polarized distribution of transport proteins is required for secretory salt transport. Thus secretory epithelial cells contain Cl− and K+ channels in their luminal membranes, allowing for secretion of KCl. In addition, after secretory stimulation and upon inhibition of absorption, paracellular transport of NaCl facilitates secretion of NaCl (225, 323, 404, 536). The apical Cl− conductance is formed predominantly by CFTR, which has a central role in colonic ion transport (219). On their basolateral membranes, secretory cells contain Na+−2Cl−−K+ cotransporters that take up Cl− from the serosal side of the epithelium together with Na+ and K+. Basolateral K+ channels allow for the recycling of K+ via the basolateral membrane, thus hyperpolarizing epithelial cells and maintaining the electrical driving force for Cl− secretion. This general scheme (Fig. 7) of electrolyte secretion in the colon has been established many years ago (123) and was originally described in rectal glands of Squamus acanthias (225–227). Water is driven osmotically through the paracellular shunt pathway and is transported via specialized aquaporin water channels (324, 663).

Secretion of KCl and NaCl is activated by a whole list of different secretagogues, which have been summarized elsewhere (22, 645). These secretagogues act via different intracellular messengers that are outlined below (141, 221, 228, 323, 404, 536) (Fig. 8). Coordinated action of apical and basolateral ion channels, together with basolateral cotransporters and the Na+−K+−ATPase, is essential. Thus, during secretion, Cl− uptake from the basolateral side has to keep up with luminal Cl− exit. Also, depolarization of cells by opening of luminal Cl− channels has to be compensated by activation of basolateral K+ channels to maintain the electrical driving force for luminal Cl− exit (136, 219, 221).

B. Participation of Na+−K+−ATPase and Na+−2Cl−−K+ Cotransporter

Both the Na+−2Cl−−K+ cotransporter and the Na+−K+−ATPase are essential for Cl− secretion. Both proteins have been cloned, and their expression in the colonic mucosa has been demonstrated (123). The properties of the Na+−K+−ATPase have been studied extensively and are summarized in detail elsewhere (203). In the colon as in other secretory tissues, the Na+−2Cl−−K+ cotransporter type 1 is expressed. The colonic Na+−2Cl−−K+ cotransporter is relatively insensitive to the loop diuretic furosemide but is blocked more potently by azosomide (219). Under control conditions, this cotransporter has a relatively low activity, and loop diuretics are rather ineffective. Only after stimulation of Cl− secretion by secretagogues do loop diuretics have an hyperpolarizing effect on the basolateral membrane. This is caused by inhibition of Cl− uptake, such that the intracellular Cl− concentration drops and reaches a new electrochemical Cl− equilibrium potential close to that of K+ (380, 381). The basolateral or secretory isoform of the Na+−2Cl−−K+ cotransporter (NKCC1) has been cloned recently (131). NKCC1 belongs to the family of cation-coupled Cl− transporters and is important for secretion of electrolytes as well as regulation of the cell volume (131, 240, 455). Impaired Cl− secretion has been described in NKCC1 knockout mice, confirming the participation of NKCC1 in epithelial Cl− secretion (183). However, although Cl− secretion was reduced, it could still be activated in the jejunum and cecum of these mice by an increase in intracellular cAMP and by heat-stable Escherichia coli toxin. This points to the existence of additional basolateral uptake mechanisms, such as coupled Cl−/HCO3− and Na+/H+ exchange, that might contribute to Cl− secretion. In addition, part of the residual cAMP activated short-circuit current is probably caused by secretion of HCO3−, whose uptake does not require transport by NKCC1 (231).

Regulation of the NKCC1 has been studied extensively and is reviewed elsewhere (240, 509). In brief, regulation of NKCC1 comprises increased cell surface
expression upon cAMP-dependent stimulation, direct phosphorylation by PKA, and regulation by other protein kinases and phosphatases (120, 129, 227, 240, 241, 265). The importance of phosphorylation by PKA is supported by the presence of respective PKA phosphorylation motifs in NKCC1 (241). Parallel activation of NKCC1 and ion channels makes perfect sense in adjusting basolateral uptake of $\text{Cl}^{-}$ to secretion via luminal CFTR $\text{Cl}^{-}$ channels.

According to the present data, a model is proposed in which activation of luminal CFTR turns on $\text{Cl}^{-}$ secretion and the amount of transport is determined by the activity of NKCC1. Thus NKCC1 is controlled by intracellular $\text{Cl}^{-}$, phosphorylation, and the actin cytoskeleton (59, 240, 409). In fact, adjusting the activity of NKCC1 to $\text{Cl}^{-}$ secretion may be more direct than assumed previously, mainly by changes in intracellular $\text{Cl}^{-}$ concentration and the cell volume. An incomplete list of hormones that antagonize secretion is shown, including neuropeptide Y (NPY), somatostatin, opiates, norepinephrine (NOR), and autocrine survival factor (ASF). Hormones and neurotransmitters are released from capillaries, by enteric nerves, or immune cells such as mast cells and lymphocytes. In addition, other factors are secreted in paracrine or autocrine fashion. Food components, bile acids, and bacterial or viral toxins may also exert secretory effects on the colonic mucosa.

C. CFTR $\text{Cl}^{-}$ Channels

CFTR was cloned about 11 years ago and was identified as the $\text{Cl}^{-}$ channel that is defective in CF (322, 500, 503, 636). It is the gene product that is affected by any of the more than 1,300 mutations causing CF. Structural analysis confirmed that CFTR is a member of the family of ATP-binding cassette (ABC) proteins (500). Numerous expression studies revealed CFTR’s function as that of a PKA-regulated $\text{Cl}^{-}$ channel with a single-channel conductance of $7–10 \text{pS}$ and a linear current-voltage relationship (559). Its structural and functional properties have been summarized recently in several comprehensive reviews (124, 341, 540, 542, 559). It has become clear in the meantime that CFTR is the predominant $\text{Cl}^{-}$ channel in airways, sweat duct, and native adult colon, where it is in charge of both cAMP- and $\text{Ca}^{2+}$-activated $\text{Cl}^{-}$ secretion (23, 396, 583).

CFTR is activated predominantly by PKA, but also by other second messenger pathways, including PKC, $\text{Ca}^{2+}$/calmodulin-dependent kinase, and cGMP-dependent kinase (85, 90, 303, 312, 318, 597, 621, 635). In addition, some of the phosphorylation sites located within the R
domain may even be inhibitory rather than activatory (664). Moreover, actin filaments seem to have a large impact on CFTR activity (180, 277, 470). In some but not all cell types, CFTR Cl\(^-\) conductance is activated during stimulation by cAMP through exocytosis of CFTR and insertion into the cell membrane (58, 275, 276, 350, 355, 599). It seems noteworthy that in sweat ducts, airways, and the colonic epithelium CFTR Cl\(^-\) conductance is already active under baseline conditions. This is probably due to the influence of endogenous secretagogues such as prostaglandins and adenosine (396, 476, 556). As outlined above, CFTR does act as a luminal Cl\(^-\) channel and also as a regulator of other ion conductances participating in electrolyte transport.

D. Other Cl\(^-\) Channels

1. Intermediate-conductance outwardly rectifying Cl\(^-\) channels

After the introduction of the patch-clamp technique, it became possible to examine single Cl\(^-\) channels in various epithelial cells (511). For technical reasons, ion channels expressed in the luminal membrane of native colonic cells were hard to study; therefore, cultures of colonic carcinoma cells such as HT29 or T84 were used rather than intact tissue preparations. A particular type of Cl\(^-\) channel was observed frequently in excised membrane patches. This channel had a single-channel conductance of \(-50\) pS and showed a characteristic outwardly rectifying current-voltage (I-V) relationship. On the basis of its single-channel properties, it was named the outwardly rectifying Cl\(^-\) channel (ORCC) or intermediate-conductance outwardly rectifying Cl\(^-\) channel (ICOR) (222, 259, 643, 656, 657). Because of its abundance, ORCC was assumed to be the apical Cl\(^-\) channel responsible for ion secretion. It was also shown to be activated by PKA (88, 193, 279, 370, 529). A PKA-dependent regulation of ICOR, however, was not found by our group (251). Subsequently, it was shown that ORCC is activated by extracellular ATP and stimulation of purinergic receptors (587). Recent studies have suggested that ORCC is activated by CFTR through an autocrine release of ATP (308, 544, 545). Because this is lost in nasal epithelial cells from CFTR (-/-) knockout mice, it was concluded that ORCC is regulated by CFTR (197). However, subsequent studies demonstrated limited contribution of ORCC to electrolyte transport in intact tissues: 1) although ORCC is present in excised membrane patches, the incidence in cell-attached patches is very low (144, 181, 351, 643, 673); 2) potent blockers of ORCC are rather ineffective in the intact epithelium even after stimulation of the intracellular cAMP pathway (224, 560, 601); 3) ATP or UTP applied to the luminal side may induce Cl\(^-\) conductance in cultured colonic epithelial cells, but they have no effect on ion transport in the native human colonic mucosa (354); and 4) the concept of CFTR-mediated ATP permeability is controversial and requires further evaluation (236, 237, 360, 369, 495, 589, 650). Thus a significant contribution of ICOR to colonic Cl\(^-\) secretion remains to be proven.

2. Ca\(^{2+}\)-activated Cl\(^-\) channels

Ca\(^{2+}\)-activated Cl\(^-\) channels (CaCC) are stimulated in luminal membranes of both non-CF and CF airway epithelia by Ca\(^{2+}\) ionophores, histamine, bradykinin, and extracellular nucleotides ATP and UTP (96, 198, 232, 408, 451, 668). In fact, in the airways of CFTR (-/-) knockout mice, the Ca\(^{2+}\)-activated Cl\(^-\) conductance is even upregulated and compensates for the lack of CFTR Cl\(^-\) channels, thus preventing the development of a lung disease in CF mice (233, 320). In contrast, CF mice demonstrate severe gastrointestinal manifestations, confirming the important role of CFTR in the gastrointestinal tract (230). However, Ca\(^{2+}\)-dependent Cl\(^-\) channels could be demonstrated in the intestine of an inbred strain of CFTR (-/-) mice. These mice do not exhibit CFTR-dependent Cl\(^-\) secretion, yet do not show any intestinal pathology and demonstrate a normal survival rate (507). Expression of Cl\(^-\) channels other than CFTR is likely to be age dependent. In 2- to 3-wk-old CFTR (-/-) knockout mice, Cl\(^-\) secretion was induced by carbachol in the small intestine, which suggests the presence of a separate non-CFTR Cl\(^-\) conductance (624). Furthermore, it was found that the rotavirus toxin NSP4, which induces severe gastroenteritis and diarrhea in infants and young animals, does induce a Ca\(^{2+}\)-mediated Cl\(^-\) secretion in parallel with inhibition of absorption in non-CF and CF mouse pup crypts (16, 427). NSP4-induced Cl\(^-\) transport was largely reduced in adult CF and non-CF mice. These results suggest age-dependent expression of CaCC, with only little contribution of these channels to Cl\(^-\) secretion in adult mice. It has been suggested that upregulation of CaCC or related regulatory proteins would determine the severity of CF. This would explain the poor correlation of genotype and phenotype in CF patients (507, 611, 626, 669). In fact, genetic linkage analysis indicates the presence of modifying loci on mouse chromosomes 7 and 19. Proteins encoded by these loci seem to be in charge of the reduced risk of developing a meconium ileus in class III CF mice (507, 683).

Alternative Ca\(^{2+}\)-dependent Cl\(^-\) channels have also been reported in several previous studies on cultured human colonic carcinoma cell lines (6, 102, 340, 419). This conductance has been characterized intensively in T84 colonic carcinoma cells (21, 24, 310). According to these and many other studies, cultured colonic carcinoma cells carry CaCC that are responsible for transient Ca\(^{2+}\)-mediated Cl\(^-\) secretion in these cells. Moreover, \(\alpha\)-myo-inositol 3,4,5,6-tetrakisphosphate (IP\(_4\)) has been identified as an inhibitory signal in T84 cells, which is the cause for the
only transient activation of CaCC and the long-term uncoupling from the Ca^{2+} signal (316, 622). However, both cAMP- and Ca^{2+}-activated Cl\(^{-}\) secretion are characterized abolished in the native colon of CF patients and CFTR knockout mice. This supports the idea that CFTR is the predominant if not only luminal Cl\(^{-}\) channel in the intact tissue (35, 253). It was shown that a residual cholinergic Cl\(^{-}\) secretion is preserved in a subset of CF patients with a mild phenotype, suggesting an alternative CaCC Cl\(^{-}\) channel (626). A recently cloned CaCC, hCLCA1, has been shown to be expressed in human intestinal crypt cells (234). Heterologous expression of hCLCA1 resulted in whole cell Cl\(^{-}\) currents, which were Ca^{2+} sensitive, outwardly rectifying, and inhibited by DIDS, dithiothreitol, and niflumic acid (234). According to this, hCLCA1 is a likely candidate for the CaCC in the human colon. However, hCLCA1 has not yet been characterized at the single-channel level. Furthermore, functional studies on isolated rat colonic crypts (55) and on human rectal and colonic tissues from non-CF and CF individuals failed to demonstrate a Ca^{2+}-dependent Cl\(^{-}\) conductance in native tissues. These studies show that Ca^{2+}-dependent activation of Cl\(^{-}\) secretion requires functional CFTR (396, 404): 1) cholinergic (Ca^{2+}-dependent) Cl\(^{-}\) secretion can be completely inhibited by deactivation of CFTR, 2) cholinergic activation fails to induce Cl\(^{-}\) secretion in rectal biopsies from CF patients, and 3) DIDS does not block cholinergic Cl\(^{-}\) secretion in concentrations lower than 1 mM. These findings suggest that residual cholinergic Cl\(^{-}\) secretion in CF tissues (626) depends on the residual function of mutant CFTR rather than an upregulation of alternative CaCC. Although there is some evidence for an alternative Ca^{2+}-activated Cl\(^{-}\) conductance in the mouse colon and in human colonic carcinoma cell lines, the contribution of this conductance is probably limited. The presence of mRNA coding for various types of Cl\(^{-}\) channels has been demonstrated in HT-29 cells (243). However, whether these results resemble the in vivo situation is questionable.

In summary, there is currently limited evidence that luminal Cl\(^{-}\) channels, other than CFTR, participate in secretion of electrolytes in the mammalian colon (55). In the adult human colonic mucosa, CFTR appears to be the Cl\(^{-}\) channel that is in charge for both cAMP- and Ca^{2+}-activated Cl\(^{-}\) secretion (55, 396, 404). This holds true also for rat and adult mouse colon. The situation is probably similar for the colon of other species such guinea pig and rabbit. It remains to be shown how the results obtained in mouse models for CF compare with the situation in humans and whether alternative CaCC modify disease severity in CF patients. Additional studies are also required to determine the role of CaCC in the developing human fetal or infant colon and their possible role in the pathogenesis of diarrhea caused by toxins of enteric viruses (16, 427).

3. **CIC Cl\(^{-}\) channels**

In recent years, Cl\(^{-}\) channels have been identified in the intestinal mucosa that belong to the large family of CIC Cl\(^{-}\) channels. These channels have been reviewed recently (299, 639). Several diseases are caused by defects in CIC Cl\(^{-}\) channels such as osteoporosis, Dent’s disease, Bartter’s syndrome, and myotonia (300, 333, 462, 568). These channels gained additional attention as a putative alternative non-CFTR Cl\(^{-}\) secretory pathway in CF (510). The CIC-2 channel is expressed in intestinal and respiratory epithelia and has been described as a time- and voltage-dependent inwardly rectifying Cl\(^{-}\) channel, which is regulated by changes in intracellular pH and cell volume (305, 543). In the choroid plexus, CIC-2 has been shown to be regulated by increases in intracellular cAMP (311). CIC-2 possibly contributes also to intestinal Cl\(^{-}\) secretion in the normal and CF intestine (422). An unusual and novel localization has been reported for CIC-2, at the tight junction complex, predominantly in the crypts (239, 325). These results support the concept of tight junctions as being dynamic structures that are regulated by cell signaling molecules, cytoskeleton, and PDZ domain-mediated interactions of proteins (79, 612). In the airways, CIC-2 is highly expressed during the fetal period but is then almost completely downregulated after birth, suggesting a role of CIC-2 in lung development (44, 94). The CIC-5 channel is predominantly expressed in colon and kidney showing properties such as a Cl\(^{-}\) > I\(^{-}\) selectivity and outward rectification. It is probably an intracellular Cl\(^{-}\) channel and is involved in calcium homeostasis and formation of kidney stones (510, 567). Moreover, the results of a recent study indicate a role of CIC-5 in endothycytotic pathways of intestinal cells (625). Based on these results, it is likely that CIC-2 rather than CIC-5 participates in Cl\(^{-}\) secretion in the colon. The extent of its contribution remains to be determined in future studies.

4. **Basolateral Cl\(^{-}\) channels**

Cl\(^{-}\) channels have also been detected in basolateral membranes of rat colonic epithelial cells (144). Some years ago, a Cl\(^{-}\) conductance was found in basolateral membranes of turtle colonic epithelial cells, which was regulated by cholinergic stimulation and increases in intracellular cAMP (83, 123, 629). Apart from these early studies and a few subsequent reports, not much is known about basolateral Cl\(^{-}\) channels and their function in the colon. They may take part in transcellular Cl\(^{-}\) absorption that is triggered by the uptake and release of SCFA (143, 145). More studies are required to examine whether these channels play a role in epithelial transport and whether they make a significant contribution to the regulation of cell volume during regulatory volume decrease (142).
5. Channel-forming peptides

Other studies have described the presence of small intestinal peptides that are able to form Cl\(^{-}\) channels; however, the role of these endogenous antimicrobial peptides in formation of a Cl\(^{-}\) conductive pathway remains to be confirmed (365, 420). These intestinal defensins or cryptdins are secreted by Paneth cells in vivo. They are able to permeabilize apical membranes of colonic epithelial cells and to form Cl\(^{-}\) conductive channels (365). It was speculated that the cryptdins 2 and 3 may function as novel intestinal secretagogues, due to reversible formation of ion-conductive channels when released into the crypt microenvironment (365). Another recent study detected formation of a probably mixed Cl\(^{-}/Na\(^{+}\) conductance by cryptdin-3 in T84 and CF epithelial cells (420).

E. Luminal K\(^{+}\) Channels

Luminal K\(^{+}\) secretion and a corresponding luminal K\(^{+}\) conductance have been suggested for many years (123). There have been some studies on luminal K\(^{+}\) channels of a rather large, ~150- to 200-pS single-channel conductance (71, 144). Other studies suggest the presence of luminal ROMK-type K\(^{+}\) channels (645). Apart from these preliminary reports, not much is known about the properties or the molecular identity of the luminal K\(^{+}\) conductance. It has been shown that chronic dietary K\(^{+}\) load increases the abundance of an apical K\(^{+}\) channel that is sensitive to changes in intracellular pH, Ca\(^{2+}\), and voltage (71, 188, 514, 515). K\(^{+}\) secretion was blocked by the chromanol 293B, a K\(^{+}\) channel blocker which potently blocks cAMP-activated K\(^{+}\) channels in the basolateral membrane of colonic epithelial cells (141). However, other properties of luminal K\(^{+}\) channels are clearly different from those of basolateral Ca\(^{2+}\)- and cAMP-activated K\(^{+}\) channels in the human colonic mucosa (404). Increases in intracellular cAMP affect colonic K\(^{+}\) transport by causing stimulation of K\(^{+}\) secretion and inhibition of K\(^{+}\) absorption (141). Activation of cAMP-dependent luminal K\(^{+}\) secretion was demonstrated in human CF rectal mucosa. This is masked in the non-CF rectum by the large CFTR-mediated Cl\(^{-}\) secretion (404). In rat and mouse distal colon, extracellular nucleotides have been shown to activate K\(^{+}\) secretion by binding to luminal purinergic P\(_{2X}\) receptors (323). In contrast, luminal ATP was ineffective when applied to either the luminal or basolateral side of the human colonic epithelium (unpublished results). Thus currently no evidence exists for regulation of K\(^{+}\) secretion by purinergic stimulation in the intact human colon.

Expression of luminal K\(^{+}\) channels is upregulated by aldosterone and glucocorticoids in parallel to Na\(^{+}\) channels (40). The effects of aldosterone seem to be restricted to the surface epithelium, although cAMP-mediated K\(^{+}\) secretion is located equally in crypts and surface epithelium (159, 228, 680). In addition, carbachol-induced K\(^{+}\) secretion has been described (262). A cholinergically controlled luminal K\(^{+}\) conductance was also detected in Ussing chamber recordings on human rectal mucosa. It is even enhanced in the colon of CF patients, suggesting regulation of apical K\(^{+}\) channels by CFTR (396, 404). It is currently not clear whether luminal K\(^{+}\) channels are activated directly by increases in intracellular Ca\(^{2+}\). Thus further patch-clamp studies are required to characterize luminal K\(^{+}\) channels and their regulatory properties. Unfortunately, this is hampered by the fact that patch-clamp analysis of the luminal membrane of colonic epithelial cells is rather difficult due to the brush-border membrane (645).

F. Basolateral K\(^{+}\) Channels

Basolateral K\(^{+}\) channels are essential to maintain a hyperpolarized membrane voltage and the electrical driving force that is required for Cl\(^{-}\) secretion and Na\(^{+}\) absorption (Figs. 2–4). In the mammalian colon, the basolateral K\(^{+}\) conductance is formed by at least two different types of K\(^{+}\) channels, which are either activated by increases in intracellular Ca\(^{2+}\) or cAMP (69, 383, 396, 414, 516, 537). These K\(^{+}\) conductances may work in concert when epithelial ion transport is costimulated by agonists increasing intracellular Ca\(^{2+}\) and cAMP. For rat colonic epithelial cells it has been shown that these K\(^{+}\) channels may function separately and independently. Ca\(^{2+}\)-activated K\(^{+}\) channels maintain the negative membrane voltage in resting epithelial cells and supply the driving force during Ca\(^{2+}\)-mediated stimulation of secretion (396). Under these conditions, cAMP-activated K\(^{+}\) channels have relatively little input. When intracellular cAMP is enhanced and luminal Cl\(^{-}\) channels are activated, the cells depolarize. Under these conditions, Ca\(^{2+}\) influx into the cell is limited and Ca\(^{2+}\)-dependent K\(^{+}\) channels become less active (47, 156, 645). Loss of activity of Ca\(^{2+}\)-activated K\(^{+}\) channels is compensated by parallel activation of cAMP-dependent K\(^{+}\) channels, repolarizing the membrane voltage (648). Apart from these two types of K\(^{+}\) channels, other K\(^{+}\) conductances could participate in maintaining the negative membrane voltage. In the reptilian colon, swelling-activated K\(^{+}\) channels have been examined extensively, and their molecular nature has been identified.

I. Basolateral cAMP-activated K\(^{+}\) channels

A basolateral K\(^{+}\) conductance is activated by increase in intracellular cAMP (379, 396, 414, 436, 648). As initially demonstrated for rat and rabbit colon, this type of
K⁺ channel is blocked specifically by the chromanol compound 293B (379, 648). The channel has been well characterized in patch-clamp and noise analysis on isolated rat colonic crypt cells (648). With a small single-channel conductance of ~3 pS, it can only be resolved by noise analysis. In the human colon, a similar 293B-sensitive K⁺ conductance has been detected (396, 414). The molecular nature of the cAMP-activated K⁺ channel has been identified recently (19, 45, 379, 396, 400, 524, 648, 676). The channel was initially cloned from heart muscle and was named KᵥLQT1 (KCNQ1), indicating its pathophysiological role in a congenital form of cardiac arrhythmia, the long Q-T syndrome. The channel is activated by intracellular cAMP and is also stimulated by Ca²⁺ increases in cardiac myocytes (645). Meanwhile, KᵥLQT1 channels have been cloned from different tissues and species, such as mouse, human, *Xenopus*, and shark (19, 45, 400, 524, 533, 676). Expression has been demonstrated in the colon and other epithelial tissues such as airways (344, 403). Interestingly, in other parts of the gastrointestinal tract, KᵥLQT1 is localized on the luminal side of epithelial cells. In parietal cells, it forms the luminal K⁺ channel together with the β-subunits KCNE2 and/or KCNE3 and has therefore a crucial role in acid secretion (216).

KᵥLQT1 expressed in basolateral membranes of native epithelial cells has a much higher affinity for the chromanol 293B, compared with KᵥLQT1 expressed in *Xenopus* oocytes. KᵥLQT1 K⁺ currents are voltage dependent in *Xenopus* oocytes but not in the native epithelium (344, 346). These and other differences in K⁺ channel properties are caused by the fact that KᵥLQT1 interacts with another regulatory protein in the native mucosa. KᵥLQT1 is the α-subunit in a K⁺ channel complex together with the small regulatory β-subunit KCNE3 (533). KCNE3 has a large impact on K⁺ channel properties and pharmacology, similar to those of minK (KCNE1, IsK) (70, 385, 502). When KᵥLQT1 is coexpressed in *Xenopus* oocytes together with KCNE3, K⁺ current properties are changed toward that of the native channel (533). Interestingly, coexpression of muscarinic M1 receptors and KᵥLQT1 in *Xenopus* oocytes leads to inhibition of KᵥLQT1 K⁺ currents upon cholinergic stimulation with oxotremorine-M (554). It will be interesting to see whether KᵥLQT1 K⁺ currents are also inhibited by activation of M3 receptors, which are expressed in the colonic epithelium. Apart from some preliminary results, no further data exist on the Ca²⁺- and PKC-dependent regulation of KᵥLQT1 in the colonic mucosa (48, 645).

Some evidence was presented for regulation of cAMP-dependent K⁺ conductance by CFTR in epithelial cells (386, 646). Furthermore, in experiments with *Xenopus* oocytes, the endogenous xKᵥLQT1 K⁺ conductance was activated by cAMP and blocked by 293B (400). These results suggest that KᵥLQT1 expressed endogenously in *Xenopus* oocytes is activated through stimulation of CFTR. However, subsequent experiments with oocytes coexpressing both KᵥLQT1 and CFTR did not indicate an interaction of KᵥLQT1 and CFTR (53). Moreover, cAMP-activated K⁺ channels were found in both normal and CF colon, indicating that CFTR is not required for activation of these K⁺ currents (402, 404). The presence of KᵥLQT1 channels explains why electrogenic Na⁺ absorption is further enhanced upon cAMP-dependent stimulation in the CF colon.

2. Basolateral Ca²⁺-activated K⁺ channels

The other type of K⁺ channel that has been demonstrated in the basolateral membrane of colonic epithelial cells is activated by an increase in intracellular Ca²⁺. Therefore, its basic characteristic is the pronounced sensitivity toward changes in intracellular Ca²⁺, which affects the open probability of the channel (47, 132, 136, 441). Moreover, evidence exists that the channel activity is modulated by phosphorylation (645). Evidence has been presented for cAMP-dependent modulation of Ca²⁺-activated SK4 and maxi K⁺ currents (209, 217). According to this, Cl⁻ secretion induced by either intracellular Ca²⁺ or cAMP does not necessarily require the support of different basolateral K⁺ channels. Both signaling pathways may converge on the same K⁺ conductance. Nevertheless, both types of K⁺ conductances coexist on basolateral membranes of colonic epithelial cells and have been demonstrated to function independently (645). Apart from regulation by Ca²⁺ and cAMP, SK4 channels are also regulated by changes in intracellular pH and are completely inhibited at pH 6.0 (456).

The channel has been isolated initially from human brain cells (hSK4), pancreas (hIK1), and T cells (hKCa4; KCNN4) (211, 290, 304, 378). Subsequently, the rat homolog was cloned, and expression in colonic crypt cells has been demonstrated (647). This K⁺ channel is activated by 1-ethyl-2-benzimidazolone (1-EBIO) (117, 136, 137, 508). Patch-clamp studies showed that the channel is blocked by low concentrations of the antifungal antibiotic clotrimazole and the imidazole compounds NS004 (137). In fact, clotrimazole was even suggested as an antidiarrheal compound. In T84 cells and colonic mucosa from mouse and rabbit, Cl⁻ secretion induced by increases in either intracellular Ca²⁺, cAMP, or cGMP was largely inhibited by clotrimazole (508). Therefore, it is possible that SK4 is activated by either of these second messenger pathways. Alternatively, the inhibitory effects of clotrimazole could be nonspecific at higher concentrations, thereby affecting various basolateral K⁺ channels. Because of that, clotrimazole may be useful for the treatment of secretory diarrhea, which is elicited in many instances by an inappropriate increase in intracellular cAMP or cGMP. Under these circumstances, CFTR and
cAMP-dependent K\(_{\text{rLQT1}}\) are maximally activated, while hSK4 might be turned off (645).

3. Other basolateral K\(^+\) channels

Other basolateral K\(^+\) channels have been detected in the colon, such as large-conductance K\(^+\) channels (132, 382). The physiological significance of these channels is currently not clear, since they were mostly found in excised membrane patches and probably have a low activity in the intact cell (132, 382). However, several studies have been performed on vesicles isolated from the basolateral membrane of colonic epithelial cells. K\(^+\) channels present in these vesicles have been studied after reconstitution into lipid bilayers and corresponding K\(^+\) transport was measured in uptake experiments (228, 327). The K\(^+\) channels that were detected in bilayer studies had a very large single-channel conductance varying from 160 to 270 pS. They were activated by Ca\(^{2+}\) and regulated by phosphorylation (228, 327). The channel was apparently much more frequent in vesicles obtained from surface epithelial cells compared with crypt cells and was therefore implicated in controlling Na\(^+\) absorption (235). The molecular identity of this Ca\(^{2+}\)-activated maxi K\(^+\) channel in the colon remains obscure, although some evidence exists that it belongs to a family of so-called slo channels (425, 645). More details about these and other putative basolateral K\(^+\) channels in the colonic epithelium are given in a recent review (645).

G. Nonselective Channels and Their Contribution to Electrolyte Transport

Nonselective cation channels have been detected in various types of epithelial cells including colonic epithelia (82). Data obtained from cultured colonic epithelial cells suggested a function in volume regulation (329). Nonselective cation channels have also been found in freshly isolated crypt cells from the rat colon (566). However, a physiological role for these channels has been questioned because it was only rarely detected in cell-attached patches and more frequently observed in cell excised membrane patches. As in most other cell types that express this type of channel, it requires unphysiologically high intracellular Ca\(^{2+}\) concentrations of larger than 10 \(\mu\)M to be activated (47). Moreover, nonselective channels probably do not contribute to cell volume regulation in colonic epithelial cells since activation of these channels could not be observed during hypertonic cell shrinkage and in patch-clamp analysis of crypt base cells (469, 660). Regulatory volume increase is due to activation of basolateral Na\(^+\)-2Cl\(^-\)-K\(^+\) cotransport and parallel inhibition of basolateral K\(^+\) channels (660).

Another separate role in epithelial electrolyte transport had been attributed to basolateral nonselective cation channels in colonic epithelial cells. According to two previous reports, nonselective cation channels, rather than Cl\(^-\) channels, are activated in epithelial cells in the mid-crypt and particularly the crypt base during cAMP-dependent stimulation (565, 566). These results were obtained in cation replacement experiments and were later questioned because Na\(^+\) replacement inhibits the basolateral Na\(^+\)-2Cl\(^-\)-K\(^+\) cotransporter (156). It was clearly shown that increases in intracellular cAMP activate a Cl\(^-\) conductance, but not a nonselective channel in both mid-crypt and crypt base cells (158). Taken together, there is currently little evidence that nonselective cation channels play a significant role in epithelial ion transport in the colon. It should be mentioned that some evidence exists for the presence of nonselective cation channels in the apical membrane of large intestinal epithelial cells (472, 552). cGMP-activated Na\(^+\) and Ca\(^{2+}\) absorption in the rat colon may occur via these nonselective cation channels. They are blocked by diltiazem but not by amiloride (472). These results have been confirmed by others, demonstrating electrogenic Ca\(^{2+}\) entry into the rat colonic epithelium through nonselective cation channels that are activated by stimulation of muscarinic receptors (191). Further studies will be needed to quantify the amount of Na\(^+\) and Ca\(^{2+}\) that is absorbed by these cyclic nucleotide-gated cation channels.

H. Regulation of Ion Secretion

1. General aspects

Secretion and absorption are controlled by endocrine, paracrine, autocrine, immunologic, and neuronal stimuli (Fig. 8). The major neuronal impact is due to the myenteric Auerbach plexus and the submucosal Meissner plexus. These plexuses innervate both epithelial and vascular smooth muscle cells, thereby controlling intestinal blood flow, secretion, and absorption in the colonic mucosa (315, 593). Compounds that are present in the intestinal lumen, such as food-derived components, bile acids, and bacterial toxins, may modulate absorption or induce release of secretagogues and cause hypersecretion (23, 138, 410, 477). Some of the effects mediated by these agonists are segment specific. For example, epinephrine induces different effects on ion transport in the proximal or distal part of the colon (273, 442). Secretion of electrolytes is evoked by a variety of secretagogues, such as acetylcholine, vasoactive intestinal polypeptide (VIP), PGE\(_2\), leukotrienes, bradykinin, and several other hormones. Discussion of the various secretagogues is beyond the scope of this review, since they are summarized in great detail elsewhere (23, 252, 458, 645). The secretory action of hormones and neurotransmitters is balanced by the inhibitory effects of neuropeptides, endogenous opiates, norepinephrine, growth hormones, and others,
which reduce intracellular cAMP levels or act via phosphatidylinositol (23, 252, 478, 573, 644).

There is an extensive cross-talk between various regulators of secretion (Fig. 8). Thus immune mediators or neurotransmitters may act directly on epithelial cells or may induce the release of mediators by other cell types. The interaction between different secretory pathways at the intact tissue level has been summarized in a recent review (160) and in Figure 8. Secretion is elicited via the second messengers Ca\(^{2+}\), cGMP, or cAMP and also other mediators such as diacylglycerol and PKC (139, 252, 381, 517, 645). The two most prominent transmitters, acetylcholine and VIP, act via increases in intracellular Ca\(^{2+}\) and cAMP, respectively (23). The most important immune mediator histamine is released from mast cells, binds to H\(_2\) receptors, and activates electrolyte secretion via an increase of intracellular Ca\(^{2+}\) (649). Another major secretagogue in the mammalian colon is PGE\(_2\), which is a metabolite of the arachidonic acid pathway and an important inflammatory mediator along with other prostanoids (23). Stimulation of PGE\(_2\) receptors triggers generation of

2. cAMP-dependent ion secretion

The primary target for intracellular cAMP is PKA, apart from possible direct effects of cAMP on CFTR (651). CFTR Cl\(^-\) channels are activated by PKA-dependent phosphorylation and binding of ATP, as outlined above (199, 341). In addition, exocytosis of CFTR from an intracellular pool may contribute to cAMP-dependent activation of Cl\(^-\) secretion in the human colon (396). The role of ion channels in regulation of ion transport is well established. However, it remains unclear to what degree regulation of the tight junction permeability and ion selectivity does contribute to the control of electrolyte transport. cAMP-dependent regulation of intestinal tight junction permeability was suggested in a previous report and has been examined recently in more detail for the ileum (38, 468).

3. Membrane compartmentalization and organization of transport proteins

CFTR is colocalized in the luminal membrane of epithelial cells, together with other proteins containing PDZ domains (342, 563). PDZ domains are known to link different proteins to a functional complex (167). Thus CFTR probably interacts with scaffolding proteins that are required for translocation of protein kinases to the luminal membrane (591, 592). It has also been shown that CFTR is localized to the apical membrane with the help of PDZ domain proteins and that mutations in the COOH-terminal region of CFTR could cause a defect in vectorial ion transport by mislocalization and alteration of cellular distribution of CFTR (430, 563). One of the proteins interacting with CFTR via PDZ domains in the subapical compartment is the NHERF, also termed EBP50 (431, 563, 641). There is evidence that NHERF acts as a scaffolding protein that anchors CFTR in the apical membrane. CFTR also inhibits NHE3 with the help of NHERF (2). Furthermore, NHERF has been shown to bind to other proteins, such as the Yes-associated protein 65 (YAP 65) via a second PDZ domain. YAP 65 itself is associated with a tyrosine kinase (c-Yes), which may play a role in subapical signal transduction (423). A similar association has been identified for CFTR and a PKA anchoring protein (AKAP), which mediates compartmentalization of CFTR with PKA, required for activation by agonists in the intact cell (591, 592). Thus CFTR interacts with other membrane proteins using PDZ domain proteins. These are likely to be colocalized in membrane microdomains together with other ion channels and membrane transporters. However, so far no evidence has been found for participation of PDZ domains and NHERF in the interaction of CFTR with ENaC (342).

4. cGMP-dependent ion secretion

cGMP induces Cl\(^-\) secretion by stimulation of CFTR through cGMP-regulated protein kinase G type II. It may also exert additional inhibitory effects on the phosphodiesterase, which leads to an increase in intracellular cAMP (116, 296, 532, 620). Expression of the cGMP-specific phosphodiesterase type PDE5 was demonstrated in human colonic epithelial cells and seems to play an essential role in regulation of intracellular cGMP levels (15). cGMP has gained attention through recent reports showing that the very common mediator nitric oxide (NO) is able to increase intracellular cGMP and trigger the release of...
The gastrointestinal peptide guanylin is the natural ligand of the guanylin receptor, which is a membrane-bound guanylate cyclase, located exclusively on the apical side of colonic epithelia (113).

5. Ca$^{2+}$- and PKC-dependent ion secretion

The effects of Ca$^{2+}$ on the colonic epithelium are quite diverse. Increases in intracellular Ca$^{2+}$ and stimulation of Cl$^-$ conductance by stimulation of cholinergic M3 receptors have been examined in great detail (447, 538). The secretory response elicited by increases in intracellular Ca$^{2+}$ is due to activation of basolateral K$^+$ channels, which enhance the driving force for luminal Cl$^-$ exit (396, 441, 647). As outlined above, there is no clear evidence that Ca$^{2+}$ directly activates CFTR or other Cl$^-$ channels in the adult colon (396, 583). Not much is known about the function of PKC as a second messenger for Cl$^-$ secretion. A contribution of both Ca$^{2+}$/calmodulin and PKC to carbachol-mediated regulation of both luminal and basolateral K$^+$ channels has been previously suggested (263). Several previous reports show that CFTR is activated through PKC (199, 303, 341). It is, however, not clear how relevant this PKC-dependent regulation of ion transport is for the intact colonic epithelium. In cultured cells expressing CFTR, it was shown that prestimulation of CFTR by PKC is even a prerequisite for further activation of CFTR by cAMP (303). Although we also found a PKC-mediated stimulation of CFTR in Xenopus oocytes, we were unable to detect any significant impact of PKC on Cl$^-$ transport in the human or mouse colonic epithelium (354). Taken together, the impact of Ca$^{2+}$ and PKC on electrolyte secretion in the colonic epithelium is probably limited to basolateral K$^+$ channels and increasing the driving force for Cl$^-$ luminal secretion.

6. Nucleotide-mediated ion secretion

The potential impact of the nucleotides ATP and UTP on Ca$^{2+}$-mediated Cl$^-$ secretion and regulation of electrogenic Na$^+$ absorption in the colon have been discussed already in sections νD and μD, respectively, of this review. The significance of nucleotide-controlled ion transport in the mammalian colon is currently not clear and seems to depend largely on the species examined. Moreover, dietary factors and the microflora in the intestine may modify the expression of purinergic receptors. In the mouse colon, activation of P2X2 receptors by ATP or UTP showed a strong activation of Cl$^-$ secretion. However, in mice lacking expression of P2X2 receptors, ion secretion was not impaired in the small intestine (109). Along these lines, the relevance of adenosine-mediated ion secretion in the colon remains to be demonstrated. Adenosine A$_2b$ receptors have been identified on both poles of T84 colonic carcinoma cells. Stimulation of these receptors by adenosine induces an increase in intracellular cAMP, which could contribute to the diarrhea observed in inflammatory bowel disease (584).

7. Proteinase activated receptor type 2-mediated activation of ion secretion

Proteinase activated receptors type 2 (PAR2) have been identified in the gastrointestinal tract on both sides of the intestinal epithelium (332, 439, 630). Activation of these receptors by trypsin, mast cell tryptase, or an activating peptide induces production and secretion of PGE$_2$ and PGF$_{1\alpha}$ in the small intestine. Basolateral PAR2 mediate an increase in intracellular Ca$^{2+}$ and activate Cl$^-$ secretion in rat small intestine, pancreatic duct epithelia, and kidney cortical collecting duct cells (36, 332, 439, 630). Similar findings have been reported for mouse and human colon, which indicates that proteinases are able to regulate intestinal transport (111, 354, 398). The presence of PAR2 receptors in the colonic epithelium and mesenteric afferent nerves implies an important pathophysiological role in inflammation. During inflammatory processes, tryptase is released from mast cells, which are resident in the mucosa-associated lymphatic tissue (MALT). Stimulation of PAR2 by tryptase activates secretion, which may promote clearance of the intestinal lumen from toxins and potentially harmful enzymes (439). This mechanism may also contribute to the diarrhea observed in chronic inflammatory bowel disease and Crohn’s disease, as outlined in section vi.
Electrolyte transport in the mammalian colon

**J. Secretion of Mucus**

Secretion of electrolytes is often paralleled by that of macromolecules. The largest and probably most important macromolecule is mucus, which creates a particular microclimate near the epithelial surface. Thus a barrier is formed that protects epithelial cells from abrasion and bacterial invasion (247). Mucus of different composition is released from goblet and crypt columnar epithelial cells when various agonists such as cAMP or Ca$^{2+}$ (162, 248, 339, 437). Cholinergic stimulation triggers the release of preformed mucus only, while increases in intracellular cAMP induce de novo synthesis of mucin and release of both newly synthesized and preformed mucin. Goblet cells secrete mucus upon stimulation with Ca$^{2+}$-enhancing agonists such as carbachol or histamine, while mucus release from columnar epithelial cells occurs through stimulation with prostaglandins. Some studies performed in cultured colonic carcinoma cells suggested a cAMP-induced mucin secretion, independent of Cl$^{-}$ movement. Other reports, however, indicate a clear link between both PGE$_2$- and adenosine-induced Cl$^{-}$ transport and mucus secretion in differentiated columnar epithelial cells (297). More details regarding colonic mucous secretion can be found in previous reports and reviews (185, 247, 437, 578).

In CF, mucus accumulation in the intestine is abnormally high and leads to severe intestinal obstruction. It was found that expression of several types of mucins is disturbed (453). The subtype Muc1 is overproduced in the intestine of CF mice. The role of Muc1 in intestinal obstruction is further emphasized by observations in CF mice lacking Muc1. These animals had less mucus obstruction and longer survival compared with Muc1-expressing littermates (453). In addition, CFTR has been demonstrated to play an important role in regulation of mucus secretion (357, 418, 424, 459). CFTR is expressed in mucous cells of tracheal glands, pancreas, gallbladder, and intestinal tract (177, 461, 674). The quantitative contribution of CFTR to mucus transport in the colonic epithelium, however, remains to be demonstrated.

**V. WATER TRANSPORT IN THE COLON**

**A. Paracellular or Transcellular Water Transport**

Fluid transport is one of the major functions of the human colonic epithelium, with ~1.5 liters of water being reabsorbed every day (42). The value refers to the net

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**FIG. 9. Cellular model for HCO$_3^-$ secretion in colonic epithelial cells.**

HCO$_3^-$ is secreted by different mechanisms, including electrogenic secretion via luminal CFTR Cl$^-$ channels, a luminal SCFA/HCO$_3^-$ exchanger, and the luminal Cl$^-$/HCO$_3^-$ exchanger DRA. CFTR may also serve as recycling pathway for Cl$^-$, which has been taken up by the luminal Cl$^-$/HCO$_3^-$ exchanger DRA or basolateral Na$^+$-coupled HCO$_3^-$ transporter. Electroneutral secretion of HCO$_3^-$ is paralleled by the activity of the Na$^+$/H$^+$ exchanger NHE3 or NHE2. K$^+$ recycles via basolateral K$^+$ channels, and Cl$^-$ is transported to the blood side via basolateral KCC1 cotransporter and probably Cl$^-$ channels.

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**TABLE 7**

<table>
<thead>
<tr>
<th>Electrotransport Mechanisms</th>
<th>CFTR role</th>
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<td>Cl$^-$ secretion</td>
<td>Essential</td>
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<tr>
<td>HCO$_3^-$ secretion</td>
<td>Essential</td>
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<tr>
<td>Water transport</td>
<td>CFTR-dependent</td>
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**FIG. 10. Cell model for HCO$_3^-$ secretion in colonic epithelial cells.**

HCO$_3^-$ is secreted via different mechanisms, including electrogenic secretion via luminal CFTR Cl$^-$ channels, a luminal SCFA/HCO$_3^-$ exchanger, and the luminal Cl$^-$/HCO$_3^-$ exchanger DRA. CFTR may also serve as recycling pathway for Cl$^-$, which has been taken up by the luminal Cl$^-$/HCO$_3^-$ exchanger DRA or basolateral Na$^+$-coupled HCO$_3^-$ transporter. Electroneutral secretion of HCO$_3^-$ is paralleled by the activity of the Na$^+$/H$^+$ exchanger NHE3 or NHE2. K$^+$ recycles via basolateral K$^+$ channels, and Cl$^-$ is transported to the blood side via basolateral KCC1 cotransporter and probably Cl$^-$ channels.
transport, but unidirectional transport rates might be substantially higher. This becomes immediately apparent when electrolyte transport is disturbed as in secretory diarrhea, where up to several liters per day are secreted. It is well accepted that the net movement of fluid is driven osmotically by active absorption and secretion. It is currently not clear to what degree water transport occurs via paracellular shunts and how much water is transported via epithelial cells (580). Moreover, it is still unclear how water is absorbed against the high osmotic gradient caused by the high effective osmolality of the feces. Models for absorption have been proposed, suggesting the presence of a standing gradient, a countercurrent concentration mechanism similar to that present in the kidney, and a hypertonic osmotic gradient. Movement of water through CFTR was suggested, because both anion replacement and the Cl− channel blocker 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB) inhibited CFTR-induced water permeability (256, 642). Subsequent studies arrived at different conclusions by showing that CFTR-induced osmotic water permeability is caused by activation of a separate conductance (530). CFTR activates AQP3 in non-CF but not in CF airway epithelial cells (531). Interaction of AQP3 with CFTR has been confirmed in Xenopus oocytes, Chinese hamster ovary cells, and airway epithelial cells (531, 532). However, no data are currently available demonstrating an interaction of CFTR and AQP3 in the native colon epithelium. In epithelial cells of small airways, AQP3 and CFTR are colocalized in apical membranes (338). However, in colonic epithelial cells, CFTR and AQP3 are expressed in opposite membranes. Thus interaction of AQP3 with CFTR would require a soluble factor or signal protein. Taken together, the pathway for water uptake has not been identified after all, and the impact of CFTR on water transport in the colonic epithelium remains to be demonstrated.

VI. DEFECTIVE ION TRANSPORT UNDER PATHOLOGICAL CONDITIONS

A. Secretory Diarrhea

Disturbances in colonic electrolyte transport may be either congenital or acquired. Congenital defects include chloride diarrhea caused by a defective luminal Cl−/HCO3− exchanger DRA and sodium diarrhea which is due to defective Na+/H+ exchange (176, 269, 321, 432). Intestinal colonization by pathogenic microorganisms is a major cause for acquired secretory and inflammatory diarrhea (280, 454). Secretory diarrhea in inflammatory bowel disease and hyperproliferative colonic mucosa is discussed in section viB. Here we focus on more recent molecular aspects of the transport defect in infectious diarrhea. Detailed information on the ion transport during secretory diarrhea is given in previous reviews (174, 175). Several species of bacteria induce secretory and inflammatory diarrhea, including E. coli, Shigella flexneri, Salmonella typhimurium, and Vibrio cholerae. These pathogens due so by alteration of ion transport, disruption of tight junctions, and activation of an inflammatory response (260, 364). Due to the exposure to enterotoxins,
intracellular second messengers are generated that lead to overstimulation of the secretory pathway by activating luminal CFTR. In parallel, electroneutral absorption by NHE3 and electrogenic absorption by ENaC are inhibited (352, 681). In addition to cholera toxin, *V. cholerae* produces several other toxins like zonula occludens toxin (ZOT), accessory cholera enterotoxin (Ace), and a hemagglutinin/protease, which increase the tight junction permeability and stimulate Ca\(^{2+}\)-dependent secretion (169, 608, 672). Cholera toxin and heat-labile *E. coli* toxin induce intestinal secretion by excessive increase in intracellular cAMP, due to irreversible activation of the adenylate cyclase (173, 313). Cholera toxin affects both small and large intestine. Moreover, cholera toxin increases intracellular cAMP in both crypts and villus epithelial cells, and thus both compartments are likely to contribute to generation of secretory diarrhea (126). Excessive secretion caused by cholera toxin is partially due to serotonin release from enterochromaffin cells (252). This activates secretion directly or stimulates epithelial cells indirectly by release of neurotransmitters from myenteric and submucosal plexus (252, 389). In parallel to the secretory effects of cholera and *E. coli* toxin, blood flow in the gut wall is enhanced due to vasodilation by VIP and NO (252). These mechanisms are necessary to maintain high secretory rates during diarrhea. Other toxins such as heat-stable *E. coli* toxin or *Y. enterocolitica* toxin enhance intracellular cGMP, leading to stimulation of protein kinase GII and activation of Cl\(^{-}\) secretion in crypts and apical membranes of the intestine (595, 620). The natural ligand for heat-stable toxin receptors is the regulatory peptide guanylin, which is secreted as proguanylin to the luminal side of the colonic epithelium. This process is probably triggered by choleric stimulation (267, 371, 406). Other microorganisms responsible for diarrhea, such as *Clostridium difficile*, produce clostridial toxin and increase intracellular Ca\(^{2+}\). The effects are further mediated by two large clostridial toxins that also modulate small GTP binding proteins, which maintain the cytoskeletal architecture and the tight junction integrity (261, 464). The significant increase in knowledge of the tight junction architecture made clear that many pathogens induced diarrhea by assaulting the tight junction complex (444, 547). Similar to cholera toxin, *C. difficile* toxins evoke electrolyte secretion also by indirect mechanisms via enteric nerves (23). Other invasive bacteria trigger secretion in a more complex way, by means of chemoattractants and transcriptional upregulation of proteins involved in secretion (23, 106, 309).

Independent of the intracellular second messenger, exposure to various bacterial toxins often results in activation of luminal CFTR. It therefore plays the central role in secretory diarrhea and the excessive loss of HCO\(_3\) with development of acidosis (85, 238, 404). Heat-stable *E. coli* toxin and guanylin, as well as cholera toxin, induce bicarbonate secretion due to activation of CFTR (238). Blocking luminal CFTR Cl\(^{-}\) channels would be the appropriate treatment for secretory diarrhea. This, however, is hampered by the fact that no potent or specific blockers of CFTR are available at the present time. New approaches to identifying blockers of CFTR by high throughput screening may deliver better blockers in future (200, 540). Chromanols such as 293B block basolateral cAMP-dependent KvLQT1 K\(^{+}\) channels, which are essential to maintaining the electrical driving force for luminal Cl\(^{-}\) secretion. These blockers could also be useful for the treatment of secretory diarrhea, because they act from both sides of the epithelium and show fairly low IC\(_{50}\) values (379).

The important role of CFTR in secretory diarrhea is demonstrated by the fact that bacterial toxins fail to induce secretory diarrhea in CF mice (114, 196). Therefore, it has been suggested that patients heterozygous for the CF defect have a genetic advantage due to a limited secretory response to bacterial or viral infections (28, 114, 116, 196, 230, 474, 600). Secretory diarrhea is caused by an imbalance between secretion and absorption of electrolytes. We have outlined above that CFTR also inhibits ENaC and NHE3 (2, 341). The pronounced activation of CFTR during secretory diarrhea should therefore lead to inhibition of both electrogenic absorption via ENaC and electroneutral absorption via NHE3. This may further contribute to the excessive secretion. It may be speculated whether activators of ENaC, such as proteases, could be useful in countering the secretory effects of these toxins (623). Apart from bacterial toxins, viral infections may also cause secretory diarrhea. Infection of the airways with influenza virus has recently been shown to inhibit epithelial Na\(^{+}\) absorption by a PKC-dependent mechanism (343). Preliminary results suggest that influenza viruses exert similar effects on the colonic mucosa (343). Further studies will be needed to demonstrate whether toxins of other enterotropic viruses, which typically lead to secretory diarrhea, also affect the epithelial Na\(^{+}\) conductance. Currently, exciting progress is made regarding the acute effects of pathogens on epithelial transport (106).

Rotavirus infections are a major cause for diarrhea in young children (16, 426, 427). NSP4 protein of rotavirus has been described as the first viral enterotoxin. Its potential ability to activate a Ca\(^{2+}\)-dependent Cl\(^{-}\) conductance in young children and mouse pups was outlined in section bB. In addition, rotaviruses also alter the permeability of the plasma membranes and tight junctions and even directly inhibit the intestinal glucose transporter SGLT (245, 438, 598). In addition to immunological host defense mechanisms, an increased Cl\(^{-}\) secretory response in the juvenile intestine could be another reason why infants and children are more susceptible to viral and
bacterial intestinal infection and present with a stronger diarrheal response than adults.

B. Inflammatory Bowel Diseases

Inflammatory bowel diseases (IBD), including the common Crohn’s disease (CD) and ulcerative colitis (UC), have been known for more than half a century and have been reviewed recently (20, 178). Despite intensive research, the reason for the chronic inflammatory process is not yet understood. Global gene expression profiles were obtained recently and identified UC and CD as distinct molecular entities (359). In that respect, CD was shown to be linked to a mutation in the NOD2 gene, but not UC (250). Interestingly, NOD2 activates the nuclear factor NFκB in response to bacterial lipopolysaccharides (250). These findings emphasize the importance of abnormalities in the immune function in CD. Because protruded diarrhea is observed in both UC and CD, we briefly summarize in this section the recent findings on defective electrolyte transport in IBD (see Fig. 10). The diarrhea might be caused by 1) malabsorption caused by a damage of absorptive area or defective absorption, 2) enhanced secretion, and 3) a leak flux due to an impaired epithelial barrier. Evidence has been accumulated over the years for both increased secretory transport and reduced absorption of electrolytes in IBD (150, 602). Inflammatory mediators are well known for their stimulatory effects on electrolyte secretion and their inhibitory effects on NaCl absorption (603). Arachidonic acid metabolites play a predominant role, which explains the beneficial effects of glucocorticoids, sulfasalazine, and aminosalicylic acid in the treatment of these diseases (150, 258, 281). Apart from increased levels of PGE₂ and proinflammatory cytokines, evidence for dysfunction of the Na⁺:K⁺-ATPase and the colonic Cl⁻/HCO₃⁻ exchanger has been collected (77, 254). In that respect, it is important to note that recent studies indicate a reduced expression of the transporter DRA during intestinal inflammation (244, 675). Thus a loss of transport function in the surface epithelium of the colon by attenuation of the expression of DRA or other membrane transporters may play a role in the pathogenesis of diarrhea in colitis.

An altered tight junction structure and permeability is likely to contribute to the impaired epithelial barrier and the diarrhea observed in UC (526). A study on inflamed human sigmoid colon identified a reduced trans-epithelial resistance that was paralleled by a change in the epithelial tight junction structure (526). This result parallels previous findings, which demonstrated control of the tight junction permeability by intracellular second messenger molecules such as cAMP and proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interferon-γ, and others, which are elevated in UC and CD (8, 155, 182). The contribution of TNF-α to the inflammatory process and the excessive electrolyte secretion (527) is supported by the finding that in recent clinical trials, treatment with TNF antibodies was very successful in downregulating the inflammatory process (14). The predominant role of TNF-α points to PAR2 as another class of inflammatory mediators that may control epithelial transport during IBD (111, 353). PAR2 is activated by tryptase, which is released during mast cell degranulation and stimulates electrolyte transport. In the human gut, mast cells are resident in the MALT, where they secrete proinflammatory cytokines, such as TNF-α (22). TNF-α and interleukin-1 as well as bacterial lipopolysaccharides have been shown to induce a sustained 10-fold increase in PAR2 expression in endothelial cells (445). IBD is characterized by mast cell infiltration, which forms an essential component of intestinal granuloma. In fact, mast cells have been implicated in affecting ion transport in the human intestine during IBD (110, 368, 491). In addition, mast cell tryptase activates colonic myocytes and enhances colonic motility, typical for CD (107). Therefore, epithelial PAR2 receptors are likely to contribute to IBD.

In summary, the profuse diarrhea observed in IBD is likely to be caused by inflammatory mediators enhancing secretion and inhibiting salt and water absorption and impaired epithelial barrier function.

C. Stress, Age-Related Changes in Ion Secretion, and Constipation

The influence of different types of stress and their role in development of the irritable bowel syndrome is being increasingly recognized (412). The physiological effects of psychological or physical stress on gut function are mediated primarily by autonomic, neuroendocrine, and pain modulatory responses. As a result, enhanced baseline Cl⁻ secretion is observed in jejunum and colon of stressed animals (576). It has also been demonstrated that stress alters the mucosal barrier, which is due to a change in release or response to neuroendocrine factors. Mucosal mast cells seem to play an essential role since they are activated via neurons releasing acetylcholine and other hormones (576).

The effects of aging on active ion transport and epithelial cell morphology were studied in detail in the rabbit colon (56). In this study, it was shown that cAMP-dependent Cl⁻ secretion, but not Na⁺ absorption, was significantly decreased in mature compared with young animals. This observation was paralleled by a reduction in nongoblet cells in colonic crypts and a decrease in stool water content in mature animals. These findings fit well with observations in human rectal and colonic biopsies, where both cAMP and cholinergic Cl⁻ secretion are highest in infants and decrease gradually during adulthood to...
old age (626). These age-dependent alterations in ion transport, in addition to changes in colonic water permeability (405), colonic motor function (413), and changes in colonic endocrine cell population (519), could contribute to age-related changes in colonic function. This may explain a delayed colonic transit and constipation in adults. 

FIG. 10. Activation of electrolyte secretion and mechanisms for diarrhea in infectious secretory diarrhea and inflammatory bowel disease. Bacterial toxins produced by Yersinia enterocolitica and heat-stable Escherichia coli toxin increase intracellular cGMP by activating the membrane-localized guanylate cyclase. Cholera toxin (CTX) and heat-labile E. coli toxin increase intracellular cAMP by acting on the adenylate cyclase (AC). Both lead to a large increase in luminal CFTR activity. The effects of CTX are also indirect, by stimulation of enteric nerves, which then secrete serotonin (5-HT), acetylcholine (ACh), vasoactive intestinal polypeptide (VIP), and nitric oxide (NO). Other toxins produced by Vibrio cholerae (ZOT, Ace, HA/P) enhance the tight junction permeability. A similar mechanism is used by the rotavirus toxin NSP4, which in addition may activate luminal Ca<sup>2+</sup>-dependent Cl<sup>-</sup> channels (CaCC). Stimulation of CFTR by CTX and other bacterial toxins and increase in intracellular cAMP leads to inhibition of electroneutral absorption by NHE3 and electrogenic absorption by epithelial Na<sup>+</sup> channels ENaC. Other bacterial toxins, such as Clostridium difficile toxins act via small intracellular G proteins (Rho, Rac). They cause an increase in intracellular Ca<sup>2+</sup> and depolymerize actin. In addition, some effects are indirect, via secretagogues released by immune cells. Some bacterial toxins, like those produced by Yersinia, Listeria, and some E. coli strains induce secretion in a more complex way, by enhanced transcription of inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-8 (IL-8), or granulocyte macrophage-colony stimulating factor (GM-CSF). In inflammatory bowel disease, secretion and diarrhea are caused by loss of surface area for absorption, enhanced tight junction permeability, and an activation of luminal CFTR Cl<sup>-</sup> channels. Secretion is activated by metabolites of the arachidonic acid pathway and by inflammatory mediators such as TNF-α, interferon-γ and IL-1. Some enteropathogenic bacteria induce diarrhea via structural changes in parallel with an increase in inflammatory mediators. Tryptase is secreted by the nearby mucosa-associated lymphatic tissue (MALT) and binds to PAR2 receptors. This leads to an increase in intracellular Ca<sup>2+</sup> and activation of Cl<sup>-</sup> secretion. In parallel to the activation of Cl<sup>-</sup> secretion, reduced electrogenic and electroneutral Na<sup>+</sup> absorption is observed, due to inhibition of NHE3 and ENaC. Attenuated absorption is also due to reduced expression of the Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchanger DRA.
In contrast, an increased secretory capacity in the juvenile colon is explained by an increased cell number and density of CFTR Cl\(^-\) channels (404, 626, 627). Although most studies indicate that CFTR is the only luminal Cl\(^-\) channel in the adult colon, some indirect evidence exists for expression of Ca\(^2+\)-activated non-CFTR Cl\(^-\) channels in the juvenile intestine (16, 624). A decrease in total Cl\(^-\) secretory capacity of the aged colon could explain the increased frequency of constipation in the elderly. Therefore, drugs that modulate the function of secretory ion channels such as CFTR or basolateral K\(^+\) channels could be beneficial for the treatment of constipation (134, 136, 647). This class of compounds has been realized already in the form of stimulant laxatives such as bisacodyl (479, 525).

D. CF

Many aspects of the ion transport defect in CF have been discussed already in previous sections of this review. Depending on the mutation, different properties of CFTR are affected, including 1) expression, 2) maturation, 3) channel regulation, 4) single-channel conductance, and 5) insertion into the plasma membrane (558, 611, 658, 670). The result is an impaired cAMP-regulated whole cell Cl\(^-\) conductance along with enhanced Na\(^+\) absorption in the large intestine (51). In previous studies, a clear correlation was found between expression of wild-type CFTR and stimulation of colonic Cl\(^-\) secretion by increase of intracellular cAMP through IBMX and forskolin (229, 230, 233, 395, 397) and an increase in intracellular Ca\(^2+\) by carbachol (396, 404). In these experiments, indomethacin and amiloride have been included to block generation of the main endogenous secretagogue PGE\(_2\) and to abolish the influence of Na\(^+\) absorption. These Ussing chamber studies showed activation of a Cl\(^-\) secretion in the non-CF colon, demonstrated by a negative voltage deflection, while tissues homozygous for the frequent CFTR mutation ΔF508 demonstrated only a K\(^+\) secretory response as indicated by a positive voltage deflection (Fig. 11). Patients, however, carrying a nonsevere mutation showed both K\(^+\) secretion and a residual Cl\(^-\) secretion. (Fig. 11, right trace). The large variability of phenotype in CF could be due to secondary genetic factors and expression of Ca\(^2+\)-activated or ClC-2 Cl\(^-\) channels (97, 166, 320, 507, 669, 683). CFTR mutations have been studied in great detail in transgenic CF animals (105, 127, 230, 492, 574). These mice lack pulmonary disease but demonstrate an intestinal phenotype with defects similar to those in CF patients. Defective Cl\(^-\) transport and abnormal intestinal electrolyte absorption probably contribute to meconium impaction and the distal intestinal obstruction syndrome observed in older CF patients (35, 49, 375, 397, 421). Interestingly, no intestinal symptoms are observed in mice carrying a defect in the secretory Na\(^+\)-2Cl\(^-\)-K\(^+\) cotransporter NKCC1, although cAMP-dependent Cl\(^-\) secretion is defective in the intestine of these animals (183). This reinforces the contribution of transport defects other than that of the defective Cl\(^-\) channel to the CF phenotype. Thus a defective regulation of ENaC and NHE3, with the result of an enhanced Na\(^+\) absorp-

![FIG. 11. Assessment of CFTR function and diagnosis of CF in Ussing chamber recordings of the transepithelial voltage (V(t)) and resistance (R(t)) in human rectal biopsies. In these experiments, indomethacin and amiloride have been included to block generation of the main endogenous secretagogue PGE\(_2\) and to abolish the influence of Na\(^+\) absorption. In non-cystic fibrosis (CF) subjects, increases in intracellular cAMP by IBMX and forskolin induce a sustained lumen-negative response caused by CFTR-mediated Cl\(^-\) secretion. Stimulation with carbachol (CCh) acts cooperatively and increases Cl\(^-\) secretion even further. In CF rectal biopsies homozygous for ΔF508-CFTR, cAMP-dependent Cl\(^-\) secretion is defective and CCh induces an inverse lumen-positive response, corresponding to K\(^+\) secretion. In rectal biopsies from a CF patient compound heterozygous for the CFTR mutations ΔF508 and S108F, cAMP-dependent stimulation induces an attenuated Cl\(^-\) secretory response, which is further increased during the plateau phase of the biphasic CCh response. This indicates residual CFTR function of the mutant S108F in native tissue.](http://physrev.physiology.org/10.220.32.247 on November 6, 2017)
tion, may have a larger impact in the development of CF than previously thought (118, 229, 397). Alternatively, the defective HCO_3^- secretion via CFTR may be a crucial factor that determines the symptoms and disease severity (92, 475).

Altered ion transport properties in jejunal and rectal biopsies of CF patients have been proposed to assist in the diagnosis of CF (35, 600, 626). An initial study found an apparently preserved cholinerigic Cl^- secretion in rectal biopsies of some CF patients (626). This Cl^- secretory response was ascribed to an alternative Ca^{2+}-dependent non-CFTR Cl^- channel and has been correlated to a milder phenotype in these patients (65, 626). However, other studies demonstrated that the apparent Ca^{2+}-activated Cl^- secretion observed in some CF rectal biopsies is due to a residual CFTR Cl^- channel function (396, 397, 404). Cholinergic secretion was completely abolished in non-CF and CF tissues by inhibition of CFTR. Thus, depending on the level of endogenous mediators such as PGE_2 which activate luminal CFTR Cl^- channels in rectal biopsy, the response toward cholinerigic stimulation is variable. It may range from a lumen-negative to a biphasic positive/negative response, or may generate only lumen-positive signals (Fig. 11) (396, 397, 404). Therefore, when measurements on rectal biopsies are used for the assessment of the CFTR function, it is mandatory to examine cholinerigic stimulation only when intracellular cAMP levels are controlled (65, 404). The appropriate use of this technique will supply valuable information regarding residual CFTR function and the CF phenotype and will help to find the diagnosis when the disease-causing mutation in CFTR cannot be identified. Abnormal ion transport assessed in the Ussing chamber is based on 1) missing activation of CFTR Cl^- conductance by cAMP or Ca^{2+}, 2) enhanced amiloride-sensitive Na^+ conductance, 3) lack of inhibition of ENaC by cAMP-dependent stimulation, and 4) activation of K^+ secretion by both Ca^{2+} and cAMP (396, 397, 404) (Fig. 11). According to this protocol, residual CFTR function in class IV and V mutations can be detected. Studies show that a residual CFTR function of 10–15% has a protective impact on pancreatic and intestinal function in these CF patients (154, 404). The present data also suggest a good correlation between CF-gastrointestinal phenotype and CFTR genotype, in contrast to the airway disease. Here, additional environmental factors, such as colonization by Pseudomonas aeruginosa and recurrent infections, have a major impact on progression of the lung disease (611).

Large efforts have been invested into the identification of compounds that are able to activate CFTR or basolateral K^+ channels, so as to drive Cl^- secretion. They are discussed at length in recent reviews (349, 540). Several compounds have been identified that are able to active CFTR such as NS004, phenylimidazothiazoles, genistein, psoralens, and 8-cyclopentyl-1,3-dipropylxan-thine (CPX) (11, 30, 80, 104, 134, 135, 283, 284). Flavonoids, such as genistein, are nutritional components and have been shown to induce Cl^- secretion in the rat colon. Genistein was particularly active on recombinantly expressed CFTR but was much less effective on native human airways and the colonic mucosa (213, 283, 284, 401). A similar variability was found for the compound CPX and phenylimidazothiazoles, such as levamisole. Bromotetramisole, psoralens, and NS004 demonstrated rather limited effects when applied at reasonable concentrations in experiments with Xenopus oocytes (11, 345, 349). In summary, depending on the tissue preparation, currently available CFTR activators show variable results. Moreover, beneficial effects in the treatment of CF patients remain to be demonstrated. Apart from directly activating CFTR, activation of basolateral K^+ channels may increase the electrical driving force for Cl^- secretion. 1-EBIO is a potent activator of Ca^{2+}-dependent hSK4 K^+ channels and could be useful for activation of Cl^- secretion in CF tissues (136). A combined application of both 1-EBIO together with NS004 could be even more effective in activation of Cl^- secretion in CF (134). Another compound, chlorzoxazone, seems to act on both luminal CFTR and basolateral K^+ channels and could therefore serve as a potential drug for the treatment of the CF defect (570, 596). However, it should be noted that successful pharmacological modulation of the CFTR Cl^- channel activity largely depends on the type of CFTR mutation and requires functional expression of CFTR in the luminal membrane. Therefore, additional strategies are being developed to increase protein maturation in cases where CFTR carries mutations that lead to decreased expression (349, 350, 499).

E. Change in Ion Transport During Dedifferentiation and Cancer

In this brief section we summarize recent data on the change in membrane transport during cellular differentiation and development of colonic cancer. Proliferative and undifferentiated epithelial stem cells are located in the crypt base and continuously replace surface cells. At 4–8 days, the life span of a colonocyte in the human colon is relatively short. Proliferation, migration, and parallel differentiation are precisely regulated and are adjusted to the process of exfoliation (266). During their journey from the crypt base toward the surface epithelium, crypt cells become increasingly differentiated (46). In parallel to the increase in differentiation and the loss of proliferative potential, the cells change their functional properties. Accordingly, epithelial cells located in the crypt base express large amounts of CFTR but no ENaC, while surface epithelial cells express less CFTR and show a pronounced amiloride-sensitive Na^+ conductance (156, 158,
Thus secretory epithelial cells in the crypts become absorptive as they migrate up the crypt axis and differentiate. Expression of another important transport protein, the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter NKCC1, is also regulated by cellular differentiation (302). The highest expression of NKCC1 is found in the crypt base. The differentiating agent butyrate selectively downregulates the expression of NKCC1 and therefore leads to profound decrease in transepithelial Cl\(^-\) secretion (302).

One may conclude from these and other studies that salt secretion is associated with a lower degree of cellular differentiation. Thus undifferentiated cells located in the crypt base are primarily secretory and do not show expression of ENaC (220). Other transport proteins such as the glucose transporters GLUT1 or GLUT5 are more abundant in undifferentiated crypt cells (242, 394). Expression of the sulfate transporter and Cl\(^-\)/HCO\(_3\)^\(^-\) antiporter DRA is largely dependent on cellular differentiation (541). As described in section III A2, DRA is expressed in apical membranes of normal colonic epithelial cells and is downregulated in adenomas and adenocarcinomas of the colon (541). Expression of DRA is also strongly reduced in the inflamed colon (675). Reduced expression of DRA along with a decrease of differentiation is likely to be one of the reasons for the chronic diarrhea frequently observed in patients with colonic carcinoma (86). Along this line, recent studies demonstrate an enhanced cAMP-activated Cl\(^-\) secretion in the hyperproliferative colonic mucosa, which is caused by elevated CFTR expression (617).

The carcinogen dimethylhydrazine (DMH) selectively induces colonic carcinoma when injected into rodents (46). Treatment of rats with DMH leads to a change in the transepithelial voltage and an altered carbachol response (46, 122). In the normal colonic mucosa, carbachol hyperpolarizes the cell membrane potential by activation of basolateral Ca\(^{2+}\)-dependent SK4 K\(^+\) channels. After treatment with DMH, carbachol depolarizes the membrane voltage, probably by activating Ca\(^{2+}\)-dependent Cl\(^-\) channels (46). In parallel, amiloride-sensitive Na\(^+\) conductance is reduced (46, 189). These changes resemble precisely the properties of colonic carcinoma cells in culture. T84 or HT-29 cells are dominated by a large Ca\(^{2+}\)-activated Cl\(^-\) conductance that is not detected in native colonic epithelial cells. These cells also lack amiloride-sensitive Na\(^+\) channels (223, 310). Moreover, DMH treatment uncouples Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3\)^\(^-\) exchange in the distal colon and thereby electroneutral Na\(^+\) absorption is no longer stimulated by the addition of Cl\(^-\) or HCO\(_3\)^\(^-\). These changes reflect probably an early event in the process of large bowel carcinogenesis (121). Taken together, proliferating and dedifferentiating colonocytes show a dramatic change in their ion transport properties, even before morphological changes occur and the carcinoma is detected. Thus the loss of sodium absorption and the

![FIG. 12. Cellular models of ion transport in the normal colon and in colonic tumors.](http://physrev.physiology.org/)}
increase in chloride secretion mimic a reverse ontogeny of the cells. The shift from the absorptive to the secretory cell type resembles a move backwards along the cells’ ontogenic path in the colonic crypts (Fig. 12). It is speculated that the epithelium changes from an absorptive into a more secretory epithelium, thereby causing tumor diarrhea.

F. Use of Cultured Colonic Carcinoma Cell Lines for Studying Ion Transport in the Colon

Colonic carcinoma epithelial cell lines such as HT-29, T84, and Caco-2 have been widely used as cell culture models for studying ion transport in native colonic epithelial cells. They were useful in identifying mechanisms of epithelial transport and the responsible ion channels (21, 130, 356). Many of the fundamental mechanisms of ion channel activation, stimulation of ion transport by secretagogues and intracellular signal transduction pathways as well as regulation of membrane transporters, have been discovered in studies on cultured colonic carcinoma cells (195, 222, 356). These cell models have also been widely used to study the process of intestinal differentiation and dedifferentiation (506). However, cultured carcinoma cells are different from native colonic epithelial cells regarding 1) missing ENaC expression, 2) presence of CaCC, and 3) presence of ORCC. Therefore, cell cultures probably come closest to the secretory crypt base cell type. However, different levels of CFTR expression are found in colonic cell lines, depending on their degree of differentiation (577). As mentioned above, expression of CFTR changes into expression of MDR1 along the crypt/villus axis (607) (Fig. 12). It will be interesting to further examine changes in ion channel expression during carcinogenesis. In that respect, voltage-gated K⁺ channels such as ether-a-gogo (EAG) have been shown to be crucial for cell proliferation and development of carcinoma (452, 677). More studies are needed to gain insight into the mechanisms of crypt cell proliferation and the oncogenic potential of ion channels. The results may deliver new tools for early diagnosis of precarcinogenic changes in the colon (189, 452, 677).

VII. SUMMARY AND CONCLUSION

The past 10 years have generated detailed insight into molecular mechanisms of ion transport in the colonic epithelium. There has been a large increase in knowledge, reflected by a tremendous number of publications. Although we have included a large number of citations, the list of references is necessarily incomplete. We therefore apologize for not including many other important reports in the field. It is now obvious 1) that ENaC is in charge of electrogentic Na⁺ absorption, 2) that CFTR is the most important Cl⁻ channel in the luminal membrane, 3) that at least two types of basolateral K⁺ channels facilitate Cl⁻ secretion, and 4) that transporters in charge of electroneutral absorption of NaCl and K⁺ and secretion of HCO₃⁻ have been identified. Regulation of electrolyte transport and the action of the intracellular second messengers cAMP, cGMP, and Ca²⁺ are well understood. Less knowledge exists regarding the cross-talk of the various transport proteins. A central role has been suggested for CFTR in coordinating electrolyte transport, by changing absorption into secretion. Gradually, a growing list of cofactors and additional protein subunits are being identified, including SGK, CAP1, kinase anchoring proteins, NHERF, and other PDZ domain proteins, as well as KCNE K⁺ channel subunits. These proteins could be organized in functional microdomains, allowing for coordinated ion transport. Unraveling this network of protein interactions and the contribution of cytoskeleton and membrane lipid metabolism will be the task for the future. Cloning of the ion transporters participating in absorption and secretion has improved our knowledge of intestinal diseases, such as secretory diarrhea, IBD, and CF. Studies on the changes of ion conductances during the development of colonic carcinoma have just begun to unmask the shift in ion channel expression and the impact on tumor growth. Ultimately, this knowledge will lead to the development of new pharmacological strategies for therapeutic intervention in colonic transport defects.

We gratefully thank Dr. D. Markovich for reading and correcting the manuscript. This review is in honor of Prof. Dr. Rainer Greger.

The work in the authors’ laboratories has been continuously and generously supported by the Deutsche Forschungsgemeinschaft.

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