Cholinergic ion secretion in human colon requires coactivation by cAMP


Cholinergic ion secretion in human colon requires coactivation by cAMP. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1274–G1281, 1998.—Cl− secretion in the colon can be activated by an increase of either intracellular Ca2+ or cAMP. In this study we examined a possible interdependence of the two second messenger pathways in human colonic epithelium. When measured in a modified Ussing chamber, carbachol (CCH; 100 µmol/L, basolateral) via an increase in cytosolic Ca2+ concentration ([Ca2+]i) activated a transient lumen-negative equivalent short-circuit current (Isc) [change (Δ) in Isc = −79.4 ± 7.5 µA/cm2]. Previous studies indicated that intracellular Ca2+ directly acts on basolateral K+-channels, thus enhancing driving force for luminal Cl− exit. Increased intracellular cAMP (by basolateral addition of 100 µmol/L IBMX and 1 µmol/L forskolin) activated a sustained lumen-negative current (ΔIsc = −42.4 ± 7.2 µA/cm2) that was inhibited by basolateral trans-6-cyano-4-(N-ethylsulfonyl-N-methylamino)-3-hydroxy-2,2-dimethyl-6-chromane (10 µmol/L), a blocker of KvLQT1 channels. In the presence of elevated cAMP, the CCH-activated currents were augmented (ΔIsc = 167.7 ± 32.7 µA/cm2), suggesting cooperativity of the Ca2+-and cAMP-mediated responses. Inhibition of endogenous cAMP production by indomethacin (10 µmol/L) significantly reduced CCH-activated currents and even reversed the polarity in 70% of the experiments. The transient lumen-positive Isc was probably due to activation of apical K+-channels because it was blocked by luminal Ba2+ (5 mmol/L) and tetraethylammonium (10 mmol/L). In the presence of indomethacin (10 µmol/L, basolateral), an increase of CAMP activated a sustained negative Isc. Under these conditions, CCH induced a large further increase in lumen-negative Isc (ΔIsc = −100.0 ± 21.0 µA/cm2). We conclude that CCH acting via [Ca2+]i can induce Cl− secretion only in the presence of cAMP, i.e., when luminal Cl− channels are already activated. The activation of a luminal and basolateral K+-conductance by CCH may be essential for transepithelial KCl secretion in human colon.

SODIUM CHLORIDE AND WATER secretion across the human colon is generated mainly by epithelial cells lining the crypts but also and to a lesser degree by the surface epithelium (9, 19). Secretion is under the control of a variety of hormones and neurotransmitters and is affected in common diseases like secretory diarrhea and cystic fibrosis (CF). Therefore, detailed knowledge about the ion conductances involved is essential for understanding the electrolyte secretion in human colon. Activators of electrolyte transport can be subdivided into those that act via the intracellular cAMP-, CGMP-dependent pathway and others that require an increase in intracellular Ca2+. During stimulation of electrolyte transport by either pathway, ion channels are activated in apical or basolateral membranes of colonic epithelial cells. Previous reports demonstrated that an apical Cl−-conductance is activated when intracellular CAMP is enhanced to upregulate Cl− secretion (13). This apical Cl−-conductance is formed by the CF transmembrane conductance regulator (CFTR), the protein that was demonstrated to be defective in CF (26). In addition to the opening of apical Cl−-conductances, basolateral K+-channels are activated (34) in rat colonic epithelium. The basolateral K+-conductance is formed by very small-conductance K+-channels, corresponding to the recently cloned KvLQT1 channel. This novel type of K+-conductance can be inhibited specifically by a new class of chromanol compounds (4).

Whereas CFTR and KvLQT1 are regulated by intracellular cAMP, other classes of ion channels are activated by intracellular Ca2+. Accordingly, basolateral K+-channels with a larger single-channel conductance (~16 pS) are activated by agonists that increase intracellular Ca2+- such as carbachol (CCH) (5, 28). These channels are inhibited by the common K+-channel blockers Ba2+ and tetraethylammonium (TEA+), but not by chromanol. It has been shown for the rat colonic epithelium that Ca2+-dependent K+-channels are shut off when the small-conductance K+-channels are turned on during an increase of intracellular CAMP (34). It is, however, not clear whether or not an increase of intracellular Ca2+ also leads to the activation of apically localized Ca2+-regulated Cl−-channels in colonic epithelial cells. Furthermore, CCH might further upregulate cAMP-dependent Cl−-channels. Alternatively, CCH-induced Cl−-secretion could be solely due to an activation of basolateral K+-channels, which hyperpolarizes these cells and thus enhances the driving force for luminal Cl− exit (6). These hypotheses have thus far not been examined in human colonic epithelium.

The aim of the present study was to gain a more detailed knowledge about the process of electrolyte secretion in the human colonic epithelium. To this end, we compared the use of freshly isolated human colonic epithelium rather than cultured cells. Using a novel type of miniature Ussing chamber allowing for the continuous exchange of luminal and basolateral...
bath solutions, we demonstrate the presence of a recently cloned new type of K\(^+\) channel in the basolateral membrane of human colonic epithelial cells. This channel could be an important pharmacological target for the treatment of secretory diarrhea (21). Moreover, the results uncover the relationship between Ca\(^{2+}\)- and cAMP-activated electrolyte secretion in the human colon. Former studies demonstrated a relationship between cholinergic and cAMP-dependent colonic ion secretion and described defective function for both in CF (11, 15, 31–33). The conclusions from this study are essential for the understanding of previous results showing altered Ca\(^{2+}\) (i.e., CCH)-induced electrolyte secretion in CF (32, 33).

EXPERIMENTAL PROCEDURES

Patients. Colonic tissue preparations were obtained from 29 patients with a mean age of 55.9 ± 4.5 yr (ranging from 1 mo to 88 yr) who underwent routine surgical procedures at the University Hospital Freiburg. Two- to three-millimeter forceps biopsies were taken either from surgical resections or directly from the patients. There was no muscle layer left after superficial forceps biopsies. Intestinal segments examined in the present study comprised distal descendent colon, sigmoidal colon, and rectum. The responses in the presence of indomethacin were similar in the preparations derived from the various segments. The tissues used for Ussing chamber experiments were not affected by the primary disease that was the cause for surgical intervention. The study was approved by the ethics committee and the patients had given their written informed consent.

Ussing chamber experiments. Small pieces of the removed colon that were not affected by the primary disease were immediately put into an ice-cold buffer solution of the following composition (mmol/l): 127 NaCl, 5 KCl, 5 d-glucose, 1 MgCl\(_2\), 5 sodium pyruvate, 10 HEPES, 1.25 CaCl\(_2\), and 10 g/l albumin. Small samples (2–4 mm in diameter) were taken from the tissue and mounted into a modified Ussing chamber. To obtain stable measurements even with small pieces of tissue, we constructed a sandwich chamber with a circular aperture of 0.95 mm\(^2\). The luminal and basolateral sides of the epithelium were perfused continuously at a rate of 10–20 ml/min (chamber volume 1 ml), allowing for the paired examination of the effects of CCH in the presence or absence of cAMP. The bath solution, which was replaced continuously, had the following composition (mmol/l): 145 NaCl, 0.4 K\(_2\)H\(_2\)PO\(_4\), 1.6 K\(_2\)HPO\(_4\), 5 d-glucose, 1 MgCl\(_2\), and 1.3 calcium gluconate. pH was adjusted to 7.4. Bath solutions were heated by a water jacket to 37°C. Experiments were carried out under open-circuit conditions with values for transepithelial voltage (V\(_t\)) referring to the serosal side of the epithelium. We found open-circuit measurements more adequate because 1) they more accurately reflect the in vivo situation, 2) we were able to keep the tissue preparations functional and responding for a longer time period (up to 7 h), and 3) the resulting calculated short-circuit current (I\(_sc\)) was larger and tissues responded better to stimulation with the agonists used in this study. Transepithelial resistance (R\(_t\)) was determined by applying short (1 s) current pulses [change (Δ) in I = 0.5 µA]. Voltage deflections obtained under conditions without the mucosa present in the chamber were subtracted from those obtained in the presence of the tissues. R\(_t\) was calculated according to Ohm’s law (R\(_t\) = ΔV/ΔI). Tissue preparations were only accepted if R\(_t\) exceeded that obtained for an empty chamber at least by a factor of 2. From each of patients 1–6, in most cases three biopsies were examined and recordings were usually stable for 3–4 h. Typically, after stabilization of basal V\(_t\) and R\(_t\), amiloride (10 µmol/l) was added to the luminal side of the colonic mucosa. Under these conditions the effect of basolaterally added CCH (100 µmol/l) was examined. Subsequently, the effect of CCH was examined in the presence of activators of the intracellular CAMP pathway (IBMX, 100 µmol/l, and forskolin, 1 µmol/l, basolateral solution). In another series of experiments, recordings were performed in the presence of indomethacin (10 µmol/l, basolateral solution) to suppress synthesis of endogenous prostaglandins and intracellular CAMP.

Compounds and analysis. Amiloride, indomethacin, TEA\(^+\), Ba\(^{2+}\), and IBMX were all obtained from Sigma and Merck (Deisenhofen and Darmstadt, Germany). Forskolin and trans-6-cyano-4-(N-ethylsulfonyl-N-methylamino)-3-hydroxy-2,2-dimethyl&2-chromane (293B) were obtained from Hoechst (Frankfurt, Germany). All used chemicals were of highest grade of purity available. Data are shown as individual recordings or as mean ± SE (n = number of observations). Paired Student’s t-test was used for analysis of paired data (P < 0.05).

RESULTS

Basal properties of human rectal mucosa. Under control conditions, I\(_sc\) of the tissue biopsies was −52.3 ± 5.3 µA/cm\(^2\) (n = 41). Basal V\(_t\) was −1.6 ± 0.3 mV and R\(_t\) was 27.8 ± 3.1 Ω·cm\(^2\). Addition of amiloride (10 µmol/l) to the mucosal side of the epithelium reduced V\(_t\) and I\(_sc\) slightly but significantly to −1.5 ± 0.2 mV and −48.1 ± 5.1 µA/cm\(^2\), respectively (n = 41). R\(_t\) under these conditions was 29.1 ± 3.0 Ω·cm\(^2\).

Cooperativity of Ca\(^{2+}\)- and cAMP-dependent Cl\(^−\) secretion. In the presence of amiloride, Ca\(^{2+}\)-dependent Cl\(^−\) secretion was stimulated by adding CCH (100 µmol/l) to the basolateral side of the epithelium. CCH invariably increased the lumen-negative I\(_sc\) from −41.3 ± 5.7 to −122.4 ± 22.7 µA/cm\(^2\) (n = 10). V\(_t\) was increased from −1.0 ± 0.4 to −2.4 ± 0.5 mV and R\(_t\) was slightly reduced from 24.6 ± 5.8 to 23.2 ± 5.2 Ω·cm\(^2\) (n = 10) (Fig. 1, A and B). The effect of CCH was only transient, and I\(_sc\) returned to control values within 2–4 min and was due to increase of intracellular Ca\(^{2+}\) without any change of intracellular CAMP (unpublished data from our laboratory).

Intracellular CAMP was enhanced by the inhibitor of phosphodiesterase IBMX (100 µmol/l) and the stimulator of the adenylate cyclase forskolin (1 µmol/l), both applied to the basolateral side of the epithelium. Intracellular Ca\(^{2+}\) was not affected by these agonists (unpublished data from our laboratory). This enhanced lumen-negative I\(_sc\) from −40.5 ± 7.1 to −71.8 ± 9.8 µA/cm\(^2\) (V\(_t\) increased from −1.0 ± 0.4 to −2.0 ± 0.5 mV; R\(_t\) decreased from 34.2 ± 3.1 to 33.6 ± 2.8 Ω·cm\(^2\), n = 28). After activation of the cAMP-dependent pathway, the effects of CCH on lumen-negative I\(_sc\) were significantly enhanced: I\(_sc\) was enhanced from −71.8 ± 9.8 to −248.3 ± 38.2 µA/cm\(^2\) (V\(_t\) was increased from −1.0 ± 0.4 to −5.2 ± 0.8 mV; R\(_t\) was decreased from 25.3 ± 3.8 to 22.7 ± 3.8 Ω·cm\(^2\), n = 10) (Fig. 1, A and B). These paired experiments indicate that Ca\(^{2+}\) and cAMP increase Cl\(^−\) secretion cooperatively.
Role of basolateral K⁺ channels for Cl⁻ secretion. We further examined the impact of basolateral K⁺ channels, activated by either cAMP or Ca²⁺, on Cl⁻ secretion in human colon epithelium. After stimulation of the tissues by IBMX and forskolin, the effects of BaCl₂ (5 mmol/l) and a specific blocker of the cAMP-activated KvLQT1 K⁺ channel [chromanol 293B (21)] were added to the basolateral side. In this series, IBMX and forskolin enhanced Iₛ_c from -43.4 ± 3.5 to -72.9 ± 5.0 µA/cm² (Vₛ was increased from -1.5 ± 0.2 to -2.4 ± 0.3 mV and Rₛ was decreased from 33.4 ± 3 to 32.1 ± 2.4 cm², n = 36). Addition of BaCl₂ (5 mmol/l) to the basolateral side completely inhibited Iₛ_c activated by increase of intracellular cAMP and reduced total Iₛ to -17.3 ± 3.1 µA/cm² (n = 6) (Fig. 2, A and C). This effect was mimicked by the chromanol 293B (10 µmol/l), which also led to complete inhibition of Iₛ_c activated by the increase of intracellular cAMP and reduced total Iₛ to -29.8 ± 3.0 µA/cm² (n = 36) (Fig. 2, B and C). Figure 2D depicts the concentration-response curve for 293B. The approximate IC₅₀ value was 5 µmol/l. Therefore, the activation of a basolateral K⁺ conductance is essential for cAMP-dependent stimulation of electrolyte secretion in the human colon, and the K⁺ channel involved is most likely the KvLQT1 channel (4).

To examine the impact of the above-described K⁺ channel blockers on Ca²⁺-dependent Cl⁻ secretion, the colonic tissue was first stimulated by IBMX and forskolin, and subsequently the effects of CCH were examined in the presence or absence of Ba²⁺ or 293B, respectively. CCH (100 µmol/l) enhanced Iₛ_c from -47.1 ± 6.6 to -151.4 ± 27.5 µA/cm² (n = 27). The effect of CCH was completely abolished in the presence of Ba²⁺ (∆Iₛ_c = 1.0 ± 3.3 µA/cm², ∆Rₛ = 1.4 ± 0.2 Ω·cm², n = 6) (Fig. 3, B and C). In contrast, 293B inhibited sustained lumen-negative Iₛ_c activated by cAMP from -72.3 ± 5.2 µA/cm² to -32.5 ± 3.5 µA/cm² (∆Rₛ = 0.7 ± 0.1 Ω·cm²), but the CCH-induced transient changes of Iₛ_c were not attenuated by 293B (∆Iₛ_c = -102.2 ± 14.4 µA/cm², Rₛ = 4.1 ± 0.31, n = 26) (Fig. 3, A and C). We conclude from these data that different types of K⁺ channels are activated during stimulation of electrolyte secretion in the human colon by the two second messengers cAMP and Ca²⁺. One type of K⁺ conductance must be activated to maintain electrolyte transport. In this respect, the properties of the human colon as found in the present study are very similar to those found in rat colon (34).

Ca²⁺-dependent Cl⁻ secretion requires activation of the cAMP-dependent pathway. To further investigate a possible cooperativity of Ca²⁺- and cAMP-activated Cl⁻ secretion, we examined in paired experiments the effects of CCH under three different conditions: 1) under control conditions, 2) in the presence of indo-
methacin (10 µmol/l) to suppress endogenous production of prostaglandins and thus intracellular cAMP, and 3) after maximal activation of the cAMP-dependent pathway by IBMX and forskolin. Before treatment with indomethacin, basal $I_{sc}$ was $232.1 \pm 6.3 \mu A/cm^2$ ($V_t = 52.1 \pm 6.0 mV, R_t = 33.2 \pm 4.5 V \cdot cm^2$) and was further increased by CCH to $299.6 \pm 13.7 \mu A/cm^2$ ($n = 26$). Subsequently, indomethacin was added to the basolateral side of the mucosa and the effect of CCH was examined repetitively in intervals of 10–20 min. After only 1 h of perfusion with indomethacin, the basal $I_{sc}$ was inhibited almost completely to $8.8 \pm 1.8 \mu A/cm^2$ ($V_t = -0.4 \pm 0.1 mV, R_t = 38.3 \pm 3.6 V \cdot cm^2, n = 26$). In 18 of 26 experiments (70%), we observed positive deflections of $V_t$ after the application of CCH (Fig. 4A) resulting in a transient increase of $I_{sc}$ to $14.3 \pm 4.7 \mu A/cm^2$ ($\Delta R_t = 3.6 \pm 0.5 V \cdot cm^2$). In 6 of these 18 experiments the response was monophasic and consisted only of a lumen-positive $I_{sc}$. In 20 experiments, residual negative deflections of $V_t$ were observed with $I_{sc}$ increasing to $-14.3 \pm 2.1 \mu A/cm^2$ ($\Delta R_t = 0.1 \pm 0.2 V \cdot cm^2$). In eight experiments the response was monophasic negative. This variability was observed for all colonic segments and rectal tissues, respectively, and is most likely due to variable inhibition of endogenous cAMP synthesis in different tissue preparation because of variable incubation with indomethacin.

After complete inhibition of the prostaglandin synthesis, cAMP production was again increased by basolateral addition of IBMX (100 µmol/l) and forskolin (1 µmol/l). This procedure enhanced lumen-negative $V_t$, which was completely blocked by basolateral $Ba^{2+}$ (5 mmol/l; A). Effect of $Ba^{2+}$ could be mimicked by the $K_1$ channel blocker trans-6-cyano-4-(N-ethylsulfonyl-N-methylamino)-3-hydroxy-2,2-dimethyl&2-chromane (293B; B), which was applied to the basolateral side. Time gaps between both records in A and B were 3 min. C: summary of $I_{sc}$ data calculated from experiments shown in A and B. IBMX- and forskolin-induced $I_{sc}$ were completely blocked by either $Ba^{2+}$ or 293B. D: concentration-response curve for effects of 293B on CAMP-activated $I_{sc}$. max. Maximum. All experiments were performed in the presence of 10 µmol/l amiloride. *Significantly different from control, §significantly different vs. IBMX ($P < 0.05$).
CCH response was significantly enhanced under these conditions. These results suggest that Ca\textsubscript{2+}-dependent Cl\textsuperscript{-} secretion in human colonic epithelium requires coactivation of the cAMP-dependent pathway and is only demonstrable when the endogenous cAMP pathway is activated, e.g., due to stimulation by the major autacoid prostaglandin. These results also suggest that the only relevant apical Cl\textsuperscript{-} conductance in human colon epithelial cells is that by cAMP-dependent Cl\textsuperscript{-} channels, corresponding to CFTR. Because, in CF, CFTR is mutated and cannot function as a Cl\textsuperscript{-} channel, colonic Cl\textsuperscript{-} secretion is defective, thus leading to the well-described intestinal manifestations of CF (12).

CCH activates luminal K\textsuperscript{+} secretion. The reversed lumen-positive response induced by CCH after inhibition of the cAMP pathway could be either due to activation of a basolateral Cl\textsuperscript{-} conductance or, more likely, due to an unmasked parallel activation of a luminal K\textsuperscript{+} conductance. We addressed this question by comparing the effects of CCH on lumen-positive \(V_t\) in the presence or absence of luminal BaCl\textsubscript{2} and TEA\textsuperscript{+}. In nine paired experiments with indomethacin in the bath, CCH (100 µmol/l) induced an increase of lumen-positive \(I_{sc}\) from \(-6.3 \pm 3.1\) to \(20.9 \pm 6.7\) µA/cm\textsuperscript{2} (\(\Delta R_t = 4.8 \pm 0.5\) Ω·cm\textsuperscript{2}) (n = 9). After addition of BaCl\textsubscript{2} (5 mmol/l) and TEA\textsuperscript{+} (10 mmol/l) to the luminal side, the lumen-positive CCH-induced \(I_{sc}\) was completely abolished and only a very small lumen-negative \(I_{sc}\) of \(-4.6 \pm 4.2\) µA/cm\textsuperscript{2} remained. Furthermore, inhibition of the lumen-positive \(I_{sc}\) by BaCl\textsubscript{2} and TEA\textsuperscript{+} was completely reversible on removal (\(\Delta I_{sc} = 29.5 \pm 5.7\) µA/cm\textsuperscript{2}, n = 9; Fig. 5, A and B). These experiments clearly indicate activation of a K\textsuperscript{+} conductance in the luminal membrane of human colon epithelial cells by CCH, which is unmasked when cAMP-dependent apical Cl\textsuperscript{-} channels are blocked by indomethacin.

**DISCUSSION**

cAMP and Ca\textsuperscript{2+} activate different types of K\textsuperscript{+} channels. The results of the present study indicate that at least two different types of K\textsuperscript{+} channels exist in the basolateral membrane of human colon epithelial cells: one activated by Ca\textsuperscript{2+} and the other by cAMP. Intracellular Ca\textsuperscript{2+} was increased by CCH, which binds to M3-type receptors on the basolateral side of colonic epithelial cells (25). Although cholinergic stimulation may increase intracellular inositol trisphosphate and Ca\textsuperscript{2+} as well as diacylglycerol and thus may activate protein kinase C (PKC), Ca\textsuperscript{2+} probably is the primary mediator because basolateral K\textsuperscript{+} channels in the colon are directly activated by an increase in intracellular Ca\textsuperscript{2+} (5).
Both types of K\textsuperscript{+} channels can be distinguished on the basis of their sensitivity toward the recently designed K\textsuperscript{+} channel blocker 293B (21); the cAMP-activated K\textsuperscript{+} channel is inhibited by 293B, whereas the Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel is not (5, 34). Thus electrophysiological properties of the human colon resemble those of the rat. Although the molecular nature of the Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel is not definitively clarified at this stage (5, 28), the present data strongly suggest that the cAMP-activated K\textsuperscript{+} channels in the basolateral membranes of human colonic epithelial cells are most likely identical to the KvLQT1 channels that were recently cloned from human heart (2, 29). In addition, overexpression of KvLQT1 in COS-7 cells and Xenopus oocytes identified KvLQT1 as the target for 293B (4, 7, 23). Activation of KvLQT1 channels by cAMP in the basolateral membrane of human colon epithelial cells is most likely essential for cAMP-dependent electrolyte secretion. In this respect, the compound 293B may add a new therapeutic tool for the treatment of secretory diarrhea (21).

Activation of luminal K\textsuperscript{+} conductance by CCH. Inhibition of the endogenous production of prostaglandins and hence a fall in cytosolic cAMP unmasked activation of apical K\textsuperscript{+} conductance by an increase in intracellular Ca\textsuperscript{2+}. This occurs in parallel to the activation of basolateral K\textsuperscript{+} channels. Apical K\textsuperscript{+} channels were identified in the rat colon in previous reports (8, 30). Normally, the positive I\textsubscript{sc} due to activation of apical K\textsuperscript{+} conductance is masked by the parallel activation of luminal Cl\textsuperscript{-} channels. As a net result, CCH enhances lumen-negative V\textsubscript{t} and depends on dietary K\textsuperscript{+} uptake and aldosterone (10, 22, 27). Thus colonic KCl secretion apparently is activated by an increase in intracellular Ca\textsuperscript{2+} and depends on dietary K\textsuperscript{+} uptake and aldosterone.

Cooperativity of Ca\textsuperscript{2+}- and cAMP-activated membrane conductances. Previous patch-clamp studies identified Ca\textsuperscript{2+}-activated Cl\textsuperscript{-} channels in nonpolarized cultured colonic epithelial cells, whereas other studies failed to demonstrate Ca\textsuperscript{2+}-activated Cl\textsuperscript{-} channels in colonic epithelial cells (6, 20). The data of the present study on native human colon tissue demonstrate that Ca\textsuperscript{2+}-induced Cl\textsuperscript{-} secretion requires activation of apical CFTR Cl\textsuperscript{-} channels and therefore confirm results of previous studies (3, 11, 14, 15, 24, 31). Moreover, inhibitors of Ca\textsuperscript{2+}-activated Cl\textsuperscript{-} channels such as DIDS failed to show inhibitory effects on CCH-induced Cl\textsuperscript{-} secretion when applied to the luminal side of the

![Fig. 4. Effect of indomethacin (10 µmol/l) on CCH (100 µmol/l)-induced changes in V\textsubscript{t}. A: effect of basolaterally applied CCH in the absence of indomethacin. Application of indomethacin inhibited lumen-negative V\textsubscript{t}. After 45 min of incubation with indomethacin, stimulation with CCH induced a lumen-positive V\textsubscript{t}. After recovery from CCH, stimulation with IBMX (100 µmol/l) and forskolin (1 µmol/l) induced a nontransient lumen-negative V\textsubscript{t} in the presence of indomethacin. The effect of CCH in the presence of both indomethacin and IBMX and forskolin was augmented. Time gaps between records were 40 min (first gap) and 20 min (second gap). B: summary of the equivalent I\textsubscript{sc} (I\textsubscript{sc} = V\textsubscript{t}/R\textsubscript{t}; R\textsubscript{t} was determined from the V\textsubscript{t} downward deflections obtained by pulsed current injection) under control conditions (solid bars), after inhibition with indomethacin, and after subsequent stimulation with forskolin and IBMX in the presence of indomethacin (n = 26; open bars). All experiments were performed in the presence of 10 µmol/l amiloride. All experimental I\textsubscript{sc} values were significantly different from the respective pre- and postexperimental controls (*Significantly different from control (P < 0.05; paired t-test)). Of 26 experiments, 18 showed positive deflections and 20 showed residual negative deflections of V\textsubscript{t} after the application of CCH. Twelve experiments showed a biphasic response.](http://ajpgi.physiology.org/10.1152/ajpgi.00978.2016)
epithelium (data not shown). Hence, a separate Ca\textsuperscript{2+}-regulated Cl\textsuperscript{−} conductance could not be demonstrated in the luminal membrane of human colonic crypts. The absence of a Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} conductance in the present experiments could also be caused if this specific conductance would require the coactivation of CFTR by cAMP. In fact, in previous experiments with HT-29 colonic carcinoma cells, the amplitude and time course of the Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} currents depended on the expression of CFTR and its prestimulation with cAMP (1). At any rate, although contribution of other cAMP-dependent Cl\textsuperscript{−} conductances cannot be ruled out completely by this and previous studies (17, 18), CFTR seems to be the predominant Cl\textsuperscript{−} conductance in the luminal membrane of colonic crypt cells.

CCH and thus an increase in intracellular Ca\textsuperscript{2+} seem to activate basolateral and apical K\textsuperscript{+} conductances in human colon epithelial cells. In addition, the increase of intracellular Ca\textsuperscript{2+} during stimulation by CCH apparently enhances the activity of CFTR Cl\textsuperscript{−} channels through the activated PKC pathway (16). When CFTR is mutated and thus the apical Cl\textsuperscript{−} conductance is impaired as in CF and in CFTR (−/−) knockout mice, colonic Cl\textsuperscript{−} secretion is abolished (3, 11, 14, 15, 31, 33). From these reports and the present study it becomes obvious that CFTR is essential not only for cAMP-dependent but also for Ca\textsuperscript{2+}-dependent Cl\textsuperscript{−} secretion in the colon.

CCH responses in CF colon and implications for the measurement of CFTR activity. As demonstrated in the present report, characteristic changes as they occur in CF can be mimicked by treatment of the tissue with inhibitors of the prostaglandin synthesis. In this respect the results of another report (12) have to be reconsidered. For that report, residual activation of I\textsubscript{sc} on CCH-dependent stimulation of colonic mucosa biopsies derived from CF patients were taken as a measure for the severity of the disease. To that end, the tissues were treated with indomethacin for only 10 min, which is, according to our data, too short for complete inhibition of prostaglandin synthesis. Therefore, data obtained under these conditions are difficult to interpret. We suggest that quantification of residual CFTR function by measuring CCH-induced I\textsubscript{sc} in colonic biopsies of CF patients with different CF phenotype should be obtained only in paired experiments. To that end, endogenous prostaglandin synthesis should be completely inhibited by indomethacin and the effect of CCH should be examined in both the absence or presence of cAMP in paired fashion.
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