Ca\textsuperscript{2+}-Activated Cl\textsuperscript{−} Channels: A Newly Emerging Anion Transport Family

Catherine M. Fuller and Dale J. Benos

A new family of Cl\textsuperscript{−} channels widely expressed in epithelia has been identified. These proteins are associated with Ca\textsuperscript{2+}-sensitive conductive Cl\textsuperscript{−} transport when heterologously expressed. This family may underlie the Ca\textsuperscript{2+}-mediated Cl\textsuperscript{−} conductance responsible for rescue of the cystic fibrosis knockout mouse from significant airway disease.

Despite over 40 years of investigation, the exact mechanisms regulating the conductive movement of Cl\textsuperscript{−} across the apical membrane of secretory epithelia are still largely unknown. Although many of the components of the system (Na\textsuperscript{+} pump, cotransporter, K\textsuperscript{+} channel) have been described and their contribution to secretion is fairly well understood, a detailed picture of the Cl\textsuperscript{−} exit pathway at the apical membrane has, in many systems, proved refractory to identification. The problem lies in the sheer number of separate Cl\textsuperscript{−} conductance pathways that can contribute to secretion and the multiple opportunities that these conduits provide for different and sometimes contradictory modes of regulation. Moreover, the lack of high-affinity inhibitors of Cl\textsuperscript{−} channels has prohibited rapid molecular identification of specific channel proteins. Thus this class of ion transport proteins comprise a formidable system for the physiologist to dissect; determining which conductances contribute to physiological Cl\textsuperscript{−} secretion in any particular tissue has been hard to decipher, in large measure because of a lack of molecular information about the constituent channels that are present.

However, with the molecular cloning of several anion channels, including the cystic fibrosis transmembrane conductance regulator (CFTR), the Cl\textsuperscript{−} channel family of anion channels, and the ligand-gated anion channels (e.g., \&-aminobutyric acid and glycine receptor-operated channels), this void is gradually dissipating. It is our intent in this article to describe the novel characteristics of an additional new family of anion channels, the Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels (CaCCs), and to discuss why these particular ion channels are well suited as pharmacological targets for the treatment of cystic fibrosis (CF).

Mechanism of epithelial Cl\textsuperscript{−} secretion

The ability to secrete Cl\textsuperscript{−} is of fundamental importance to the maintenance of epithelial fluid and solute transport in a secreting epithelium. In secretory epithelial cells, Cl\textsuperscript{−} is accumulated to a concentration greater than that predicted by the Nernst equation for the equilibrium distribution of ions. This is dependent on both the concerted action of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, a K\textsuperscript{+} channel and a Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{−} cotransporter, and on the polarized nature of the epithelium itself. Following an increase in the permeability of the apical cell membrane to Cl\textsuperscript{−}, Cl\textsuperscript{−} exits the cell down its electrochemical gradient accompanied by Na\textsuperscript{+} and water. The resultant primary secretion is essentially isotonic NaCl. The predominant Cl\textsuperscript{−} exit pathway in the majority of secretory epithelia is the CFTR protein. The primary physiological pathway for the activation

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TABLE 1. Concentration of Ca\textsuperscript{2+} required to achieve maximum open probability of the bCaCC under different conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>[Ca\textsuperscript{2+}] at Maximum $P_o$</th>
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<tbody>
<tr>
<td>Ca\textsuperscript{2+}</td>
<td>&gt;10 µM</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+} + CaMK II</td>
<td>1 µM</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+} + IP\textsubscript{4} (20 nM)</td>
<td>0.25 µM</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+} + CaMK II + IP\textsubscript{4} (20 nM)</td>
<td>0.035 µM</td>
</tr>
</tbody>
</table>

$P_o$, open probability; bCaCC, bovine Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels; [Ca\textsuperscript{2+}], Ca\textsuperscript{2+} concentration; CaMK II, calmodulin-dependent kinase II; IP\textsubscript{4}, d-myo inositol (3,4,5,6)-tetrakisphosphate.

Cloning of a new Cl\textsuperscript{−} channel: the bCaCC

Although there was considerable, if circumstantial, evidence for a distinct CaCC at the apical membrane of several epithelial cells, evidence of a distinct polypeptide that subserved this function had, until recently, been lacking. The first distinct CaCC to be cloned was identified in the bovine airway. With the use of classic biochemical purification techniques, Ran and co-workers (12) isolated a protein from the bovine tracheal epithelium that migrated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 140 kDa under nonreduced conditions, with minor components at 90 kDa, 60–64 kDa, and 32–38 kDa. When incorporated into planar lipid bilayers, this protein had a single-channel conductance of ~25 pS under symmetrical ionic conditions, an ion selectivity of I > Cl\textsuperscript{−} (reversed from that observed for both CFTR and ClC-2), and was inhibited by DIDS. The protein could also be activated by Ca\textsuperscript{2+}, albeit at rather high (5–10 µM) levels that would be unlikely to be seen by the cell under normal physiological conditions. However, the channel could be activated and phosphorylated by multifunctional calmodulin-dependent kinase II (CaMK II) in the presence of calmodulin and ATP at much lower (0.5–1 µM) concentrations of Ca\textsuperscript{2+} that more closely fell into the physiological range (Table 1 and Fig. 1). These results suggest that this CaCC is primarily regulated via Ca\textsuperscript{2+}-dependent kinases. In addition, this channel was insensitive to regulation by PKA, unlike the PKA sensitivity exhibited by both CFTR and the outwardly rectifying Cl\textsuperscript{−} channel (ORCC). In the presence of the reducing agent dithiothreitol (DTT), only polypeptides migrating at a relative molecular mass (Mr) of 32,000–38,000 were observed by SDS-PAGE, and the protein could no longer form a channel when incorporated into a lipid bilayer. With the use of a polyclonal antibody raised against this 32- to 38-kDa protein to screen a tracheal cDNA expression library, a cDNA that coded for a 903 amino acid protein was isolated (2).

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block by niflumic acid, a drug that is an effective blocker of CaCC regulation. The cloned channel was also insensitive to PKC, these findings additionally implicated this kinase in any of these tissues, including gut, lung, and brain. Interestingly, some cDNAs have been identified, mostly by homology screening. Until very recently, the bCaCC was the only cloned protein shown to act as a CaCC. Because mRNA for this isoform was not detected in a variety of other bovine tissues as determined by reverse transcription-polymerase chain reaction (RT-PCR) analysis, it was thought that the channel might be unique to the tracheal epithelium. However, this view has had to be revised as several new members of what is now apparently a large and intriguing family of related proteins has recently emerged. The first new family member identified, lung endothelial cell adhesion molecule 1 (Lu-ECAM-1, bCLCA2), was cloned from bovine aortic endothelial cells (BAEC) raised on a pulmonary matrix (3). This polypeptide is 91% homologous to the bCaCC at the amino acid level and is indeed post-translationally processed to a smaller polypeptide of ~25 pS. Channel activity was significantly increased in the presence of CaMK II and protein kinase C (PKC), consistent with a protein subject to calmodulin (and in Xenopus oocytes and COS-7 cells in the case of the cloned protein), the in vitro translated product of the cDNA migrated with an M, of 100,000 (140,000 following core glycosylation in the presence of pancreatic microsomes) and was resistant to reduction by DTT. This conundrum led to the proposal of two alternate hypotheses: either the native and cloned proteins were unrelated or the primary translated product was subject to post-translational processing to a smaller polypeptide of ~32–38 kDa. It was proposed that this smaller fragment could reassemble to form a functional homomeric Cl– channel at the membrane. This interpretation would account for the ability of DTT to act as a channel blocker; indeed, in bilayer experiments, channel activity could be reestablished by addition of a cross-linking reagent, Cu2+-1,10-phenanthroline (12). Similarly, DTT effectively reduced the size of the 140-kDa protein to a predominant smaller form of 32–38 kDa (12). This latter hypothesis was partially substantiated by the presence of several consensus sites for monobasic proteolytic cleavage within the translated protein.

### A new family of Cl– channels

Expression and biochemical characteristics. Until very recently, the bCaCC was the only cloned protein shown to act as a CaCC. Because mRNA for this isoform was not detected in a variety of other bovine tissues as determined by reverse transcription-polymerase chain reaction (RT-PCR) analysis, it was thought that the channel might be unique to the tracheal epithelium. However, this view has had to be revised as several new members of what is now apparently a large and intriguing family of related proteins has recently emerged. The first new family member identified, lung endothelial cell adhesion molecule 1 (Lu-ECAM-1, bCLCA2), was cloned from bovine aortic endothelial cells (BAEC) raised on a pulmonary matrix (3). This polypeptide is 91% homologous to the bCaCC at the amino acid level and is indeed post-translationally processed to two major forms closely associated at the membrane (Tables 2 and 3). Lu-ECAM-1 exhibits a highly restricted pattern of expression, being found in BAEC, spleen, and lung. Immunohistochemical analysis with a polyclonal antibody raised against the 90-kDa fragment of Lu-ECAM-1 revealed staining at the apical membrane of the bovine trachea and in vesicles in the bronchus (3). Given the homology between bCaCC and Lu-ECAM, staining in the trachea probably represents cross-reactivity with the bCaCC, whereas staining in the bronchus may represent a contribution of both proteins.

Since the identification of Lu-ECAM-1, other related cDNAs have been identified, mostly by homology screening. Four human and two mouse cDNAs are now known (Tables 2 and 3; Refs. 1, 6, 8, and 9) and have been identified in a variety of tissues, including gut, lung, and brain. Interestingly, several of the CaCCs exhibit highly restricted patterns of expression, e.g., the human CaCC-1 (hCaCC1; GenBank...
nomenclature hCLCA1) seems to be expressed almost exclusively in the crypt and goblet cells of the small and large intestine and in the fetal spleen as determined by Northern blotting and in situ hybridization (6). One isoform, hCaCC-3 (hCLCA3), encodes a truncated 37-kDa version of the protein, corresponding to the NH2-terminal region of its homologs (8). When heterologously expressed in HEK 293 cells, this protein is secreted. The likely function of this isoform is currently unknown. Two mouse isoforms [mCaCC-1, Genbank nomenclature mCLCA1 (4) and mGob-5 (11)] have also been identified from mouse lung and intestinal goblet cell libraries, respectively (Tables 2 and 3). Although overall both mCaCC isoforms are very similar to the other CaCCs, mCaCC-1 was thought to be very widely distributed, being particularly highly expressed in the respiratory epithelia of the bronchi, trachea, and submucosal glands (7), but this is likely due to the expression of multiple, closely related mRNAs in several tissues (13).

There is good evidence that this new family of proteins undergoes post-translational processing. Lu-ECAM-1 is processed from a 120- to 130-kDa precursor to two proteins that migrate at 90 kDa and 32–38 kDa on SDS-PAGE. Both hCaCC-1 and -2 and mCaCC are thought to be subject to post-translational cleavage from larger precursors. Glycosylation site scanning of hCaCC-2 has shown that the larger 86-kDa fragment has three transmembrane regions, whereas the smaller cleaved 34-kDa portion contains two transmembrane domains (9). Because this detailed topology mapping has not yet been carried out for the other members of the family, it may be that the assignment of four transmembrane domains to the larger portion of the molecule, which was based on hydrophobicity plotting, was premature.

**Functional characteristics.** Despite uncertainties over the exact number of transmembrane domains, the high degree of structural similarity among all of these polypeptides strongly argues for a commonality of function. To date, hCaCC-1 and -2 and mCaCC-1 have been expressed either in *Xenopus* oocytes and/or transfected HEK 293 cells. Expression of each isoform in *Xenopus* oocytes was associated with an increase in Ca2+-sensitive Cl− current as assessed by dual-electrode voltage clamp. This current could be induced by ionomycin and was inhibited by DIDS and DTT, compounds that were previously demonstrated to be effective blockers of the bCaCC in oocytes. However, niflumic acid, which was used in earlier studies to inhibit the endogenous CaCC of the oocyte and that in our hands had no effect on the bCaCC, markedly inhibited both hCaCC1 and mCaCC. This observation prompted the use of the HEK 293 cell system. When expressed in the HEK cell system, both hCaCC-1 and -2 and mCaCC-1 were associated with increases in Ca2+-sensitive current as determined under whole cell patch-clamp conditions. Cells were patched with low Ca2+ (~25 nM) in the pipette and superfused with 2–4 μM ionomycin in the presence of a bath Ca2+ concentration of 1 mM. This treatment resulted in an immediate increase in an outwardly rectified current. In other experiments, whole cell recording was carried out in the presence of 2 mM Ca2+ in the pipette; on achieving the whole cell configuration, the current was immediately activated. The current was nearly completely

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**TABLE 3. Functional characteristics of CaCC family members**

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Major Fragments</th>
<th>Distribution (mRNA)</th>
<th>Regulation</th>
<th>I/V</th>
<th>Selectivity</th>
<th>Inhibited by</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCLCA1</td>
<td>130–140 32–38*</td>
<td>Trachea, upper airways</td>
<td>Ca2+, PKC</td>
<td>Linear*</td>
<td>I&gt;NO3&gt;Br&gt;Cl</td>
<td>DIDS, DTT</td>
<td>2</td>
</tr>
<tr>
<td>hCLCA1†</td>
<td>120 90 32–38</td>
<td>Lung, pulmonary arteries and venules, spleen</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>3</td>
</tr>
<tr>
<td>hCLCA1</td>
<td>125 90 37–41</td>
<td>Crypts, goblet cells of small and large intestine</td>
<td>Ca2+</td>
<td>OR*</td>
<td>?</td>
<td>DIDS, DTT</td>
<td>1,6</td>
</tr>
<tr>
<td>hCLCA2</td>
<td>120 86 34</td>
<td>Lung, trachea, mammary tissue, cornea</td>
<td>Ca2+</td>
<td>OR*</td>
<td>?</td>
<td>DIDS, DTT, NFA, TMX</td>
<td>1,9, Itoh et al.</td>
</tr>
<tr>
<td>hCLCA3</td>
<td>37</td>
<td>Secreted</td>
<td>?</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>8</td>
</tr>
<tr>
<td>mCLCA1</td>
<td>125/130 90 32–38</td>
<td>Skin, kidney, trachea</td>
<td>Ca2+</td>
<td>OR*</td>
<td>?</td>
<td>DIDS, DTT</td>
<td>4,7,13</td>
</tr>
</tbody>
</table>

Sizes (in kDa) of major fragments on SDS-PAGE (where known), distribution of mRNA, and electrophysiological characteristics (where known), are shown. * predicted; †, 4 related clones identified; *, following incorporation into planar lipid bilayers; ‡, recorded from *Xenopus* oocytes and/or transfected HEK 293 cells; ?, unknown; OR, outwardly rectified; N/A, not applicable; DIDS, 4,4'-disothiocyanostilbene-2, 2'-disulfonic acid; DTT, dithiothreitol; NFA, niflumic acid; TMX, tamoxifen.
inhibited by DIDS (300 µM), niflumic acid (100 µM), and DTT (2 mM). In one series of experiments with hCaCC-2, the current was also inhibited by tamoxifen (10 µM) (9), a compound also widely used as an inhibitor of anion channels. With the use of the cell-attached patch recording configuration, single channels from HEK cells expressing hCaCC-1 have also been recorded. The slope conductance of the channel was estimated at 13.4 pS, and the current-voltage curve exhibited a reversal potential of approximately −45 mV. The current across the patch was also increased in the presence of 2 µM ionomycin in the bath. These observations are consistent with these proteins being the initial members of a family of Ca2+-activated Cl− channels. However, it is also consistent with these proteins subserving a potential role as regulators of an endogenous, otherwise cryptic channel. In the absence of functional mutations, the role of these proteins in epithelial Cl− secretion still needs to be established.

**CaCCs: multifunctional proteins?**

One highly intriguing aspect of this new family of anion conductance proteins is exhibited by Lu-ECAM-1. This protein functions as a bona fide endothelial cell adhesion molecule; antibodies to the protein prevent the attachment of metastatic melanoma cells to BAEC cells grown on a lung matrix (3). Whether or not Lu-ECAM-1 functions simultaneously as a channel protein and an adhesion molecule or whether the two roles are mutually exclusive is not known at present. However, the ability of ion channels to subserve multiple roles is not unique; for example, both the β-subunit of the voltage-gated brain Na+ channel and one of the family of Na+ channels of *Caenorhabditis elegans* (unc-105) have been postulated to have roles in cell adhesion. Cl− channels have also been proposed to be multifunctional; the best example of this is undoubtedly CFTR, which regulates at least three other ion channels (the epithelial Na+ channel ENaC, the renal outer medulla K+ channel ROMK1, and an ORCC) and has been proposed to regulate several other transporters and channels. Furthermore, diphenylamine carboxylic acid (DPC), which is often used as a blocker of Cl− channels, including CFTR, was reported to block adherence of endothelial cells to an artificial substrate. CFTR has itself been reported to act as an attachment factor for bacteria, including *Pseudomonas aeruginosa*. Whether or not this new class of proteins represents sticky ion channels or leaky glues remains to be determined.

**Is the CaCC a candidate for the Cl− conductance upregulated in the CF knockout mouse?**

Given the experimental evidence that an ionomycin-sensitive, Ca2+-dependent Cl− secretion is upregulated in the nasal epithelia of CF mice, it is tempting to speculate that the protein underlying these observations is a member of this newly defined ion channel family. Other studies that used UTP (an agonist whose effect is mediated by Ca2+) to activate secretion have reported that both nasal and rectal potential differences in long-living subgroups of CF mice are reduced by DIDS. However, the specificity of DIDS to block a CaCC in these studies has been questioned, because this compound may also inhibit the metabotropic P2Y2 (UTP) receptor. Other candidates that have been proposed to fulfill the role of alternate Cl− channel in CF, including CIC-2, are less satisfactory alternatives either because 1) they are downregulated in the airways at birth (CIC-2), 2) rely on the presence of CFTR or voltage for activation (CIC-2, ORCC), or 3) are inhibited by Ca2+-mediated agonists such as phorbol ester (PKC) and even Ca2+ itself (CIC-3).

The cloned CaCC would therefore appear to be the best candidate for the upregulated Cl− conductance in CF mouse tissues. Since a Ca2+-mediated Cl− conductance pathway is also preserved in CF cells of human origin, why does the CaCC not effectively substitute for CFTR in humans? One possibility is that the human CaCC is not expressed either in appropriate cell types or in the appropriate cellular location. It has been suggested that if a CaCC does exist, its most likely location will be at the basolateral membrane. Preliminary evidence in the rat parietal cell suggests that a CaCC is indeed present at the basolateral membrane of this tissue.

**“If the CaCC is likely to present in the correct location, why then can it not substitute for CFTR?”**

However, protein(s) immunoreactive with antibodies raised against either the 90-kDa fragment of Lu-ECAM-1 or the 32- to 38-kDa fragment of bCaCC is found at the apical membrane of bovine tracheal epithelium and at the apical membranes of bovine tracheal submucosal glands, consistent with prevailing electrophysiological evidence garnered from the upper airways and CF cells of human origin.

If the CaCC is likely to present in the correct location, why then can it not substitute for CFTR? The answer to this riddle may lie in some findings concerning the effects of a by-product of the inositol trisphosphate (IP3)/phospholipase C (PLC)/Ca2+ signaling cascade, D-(3,4,5,6)-inositol tetrasphosphate (IP4). Observations from two independent laboratories have shown that this compound is an effective inhibitor of both Ca2+-mediated Cl− secretion and Ca2+-activated Cl− current in T84 cells (14, 15). Studies of the bCaCC incorporated into planar lipid bilayers further demonstrated that this family member is exquisitely sensitive to 20 nM IP4 in the presence of submicromolar levels of Ca2+ (10).

The interaction of IP4 with the bCaCC is complex and highly dependent on the prevailing concentration of Ca2+. In Ca2+-free solutions (<1 nM free Ca2+ concentration), the open probability (Po) of the bCaCC in the presence of 20 nM IP4 was on the order of 0.4. However, in the presence of CaMK II, channel Po began to increase in direct proportion to the Ca2+ concentration until near-maximal Po (0.9) was observed at a Ca2+ concentration of ~30 nM. Because Ca2+ concentration was increased further, however, the channel began to shut down until, at a Ca2+ concentration of ~300 nM, Po was on the order of 0.36. At 1 µM free Ca2+ concentration, channel Po was negligible (Fig. 1 and Table 1). A similar biphasic behavior was observed in the absence of CaMK II, with the
exception that the peak $P_o$ was in the range of 0.6–0.7 at 300 nM Ca$^{2+}$ concentration.

If extrapolated to the situation in the intact epithelial cell, these findings suggest that, in response to repeated cholinergic or $\alpha$-adrenergic stimulation, i.e., at a time when Cl$^-$ secretion is required, and under conditions when IP$_4$ has accumulated (14), the CaCC may have such a low $P_o$ as to be irrelevant for functional Cl$^-$ secretion, compared with CFTR and the ORCC. However, in the case of a CF epithelial cell, three potential Cl$^-$ exit pathways (CFTR, ORCC, and CaCC) would be inactive, contributing at a fundamental level to the deleterious consequences of the disease (Fig. 2). Why then in isolated human CF cells can a Ca$^{2+}$-regulated Cl$^-$ secretory pathway be demonstrated? The answer to this question probably rests in the way in which the experiments are done. Electrophysiological experiments to determine the presence of a Ca$^{2+}$-stimulated Cl$^-$ conductance in isolated CF cells are frequently done using an ionophore to circumvent the receptor. In other cases, only a single application of an agonist such as carbamylcholine is used. In each of these experimental maneuvers, little or insufficient IP$_4$ would be expected to accumulate to effectively block the CaCC.

**Is the CaCC a candidate for pharmacological intervention in CF?**

For the CaCC to be considered a viable target for therapy in CF, several questions need to be addressed. First, is the cloned CaCC protein synonymous with the Ca$^{2+}$-sensitive Cl$^-$ conductance recorded in several normal and CF epithelial cell types? Although many of the characteristics of the expressed and endogenous currents are the same (anion selectivity, sensitivity to DIDS, outward rectification) and, importantly, are not shared by other cloned Cl$^-$ channels (CFTR, ClC family), the lack of functional mutants, i.e., those affecting single-channel conductance or anion selectivity, precludes definitive assignment of channel function to all the CaCCs. Data obtained to date for CaCC family members are consistent with these proteins acting as functional anion channels in their own right. However, with the exception of the bCaCC, the data for the other CaCC family members could also be interpreted as the proteins acting as regulators of an endogenous, and possibly cryptic, channel protein, conferring on it properties similar to those of the Ca$^{2+}$-sensitive Cl$^-$ current recorded in native cells. Structure/function analysis to determine channel architecture in terms of identifying gates and pores is clearly required. Second, if IP$_4$ is such an effective brake serving to limit Cl$^-$ secretion, why is the CF mouse able to escape the airway disease characteristic of CF? One possible explanation could be that the mouse airway expresses a CaCC isoform that is markedly less sensitive to the effects of IP$_4$ than is its human homolog. Third, are these new channels expressed in CF tissues, and, importantly, what is their cellular location? Preliminary evidence suggests that they are likely to be at the luminal membrane of epithelia, but additional and more detailed studies are clearly required. Fourth, what is the mechanism of activation of the channel, i.e., how do these channels interact with regulators such as Ca$^{2+}$, CaMK II, PKC, and IP$_4$? All of these issues will have to be clarified before we approach the CaCC as a therapeutic target.

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**FIGURE 2.** Alternate pathways for Cl$^-$ secretion in epithelial cells. Secretory epithelia are subject to regulation by the autonomic nervous system, principally norepinephrine (NEpi) and acetylcholine (ACh) via adrenergic and muscarinic receptors, respectively. Activation of cystic fibrosis transmembrane conductance regulator (CFTR) chiefly involves both a cAMP-dependent phosphorylation step and ATP binding to the nucleotide-binding folds. In addition, CFTR plus protein kinase A (PKA) can regulate an additional outwardly rectifying Cl$^-$ channel (ORCC) that is only active in the presence of CFTR. An alternate pathway, involving Ca$^{2+}$ as a second messenger and activated by acetylcholine or norepinephrine, is also available. In this case, Ca$^{2+}$ released from the endoplasmic reticulum may be able to activate a channel directly, although it seems more likely that it would act to potentiate the actions of protein kinase C (PKC) or multifunctional CaMK II. Consistent with this dual pathway for regulating Cl$^-$ secretion, evidence exists for two populations of K$^+$ channels that are sensitive to either cAMP or Ca$^{2+}$-mediated signals, although the latter channel is by far the best characterized. Other modifications of the system could include Ca$^{2+}$-coupled purinergic and cGMP-coupled receptors located on the apical membrane and an inhibitory role for a side product of the inositol trisphosphate (IP$_3$) cascade, IP$_4$, as indicated by the dashed line.
In summary, a new family of proteins whose function is consistent with their proposed role as epithelial Cl\(^{-}\) channels has recently been identified. These proteins may underlie the Ca\(^{2+}\)-dependent Cl\(^{-}\) secretion observed in some epithelial tissues, but resolution of their function will require a greater understanding of these new potential channels, in particular in terms of their precise cellular location, tissue distribution, and regulatory characteristics. These channels may be appropriate targets for therapeutic intervention in CF. However, mechanisms designed to increase Ca\(^{2+}\) in the cell are unlikely to be successful; not only can Ca\(^{2+}\) have deleterious consequences on cell function, but the CaCC at least is in fact not very sensitive to Ca\(^{2+}\) in the physiological range in the absence of CaMK II. It will likely be more appropriate to focus on the IP\(_{4}\) inhibitory pathway or on drugs that could maintain channel P\(_{o}\) in the presence of IP\(_{4}\) and CaMK II.

We would like to acknowledge the work of all of those laboratories whose studies, for reasons of space limitations, were not cited directly.

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References