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Topical Review

Regulation of Chloride Channels in Secretory Epithelia

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Abstract. Fluid and electrolyte secretion from secretory epithelia is a highly regulated process. Chloride channel activity at the apical membrane determines the rate and direction of salt and water secretion. Multiple classes of Cl^- channels with distinct gating mechanisms are involved in moving ions and water. Secretory agonists that induce intracellular increases in two second messenger systems, cAMP and $[Ca^{2+}]_i$, are generally associated with secretion. However, changes in cell volume and the membrane potential may also play a role in regulating fluid and electrolyte secretion in some tissues. In this review we discuss the regulation of the different types of Cl^- channels found in secretory epithelia.

Key words: Secretion — Regulation — cAMP-activated Cl^- channel — Volume-sensitive Cl^- channel — Voltage-gated Cl^- channel — Ca^{2+}-dependent Cl^- channel

Introduction

The primary function of Cl^- channels in secretory cells is transepithelial ion transport. Figure 1 shows a fluid secretion model first described by Silva et al. [78] that generally applies to Cl^--secreting epithelia. According to this model transepithelial Cl^- movement is dependent upon secondary active transport mechanisms. The energy for Cl^- uptake is provided by the inward-directed Na^+ chemical gradient created by the Na^+ pump. Intracellular accumulation of Cl^- is predominantly mediated by $Na^+/K^+/2Cl^-$ cotransporters located in the basolateral membrane. In most nonepithelial cells, the intracellular Cl^- concentration is near its electrochemical equilibrium. However, the $Na^+/K^+/2Cl^-$ cotransporter concentrates intracellular Cl^- above its electrochemical gradient as much as 5-fold in secretory epithelia [24].

Thus, Cl^- is poised to exit across the lumenal membrane of the cell once an apical Cl^- channel is activated. The apical Cl^- conductance pathway consequently plays a key role in determining the rate at which fluid and electrolyte secretion occurs. An electrically uncompensated Cl^- efflux would depolarize the cell membrane potential and so inhibit further Cl^- efflux. This does not occur in epithelial fluid secretion since there is a simultaneous opening of K^+ channels and an associated flux of K^+ ions into the interstitial fluid. The transepithelial electrical potential difference created by the oppositely directed movements of Cl and K ions tends to be neutralized by paracellular transport of cations (mostly Na^+) across tight junctions. The net result, then, is the creation of a transepithelial osmotic gradient which drives the movement of water creating a plasmalike primary secretion. Transcellular Cl^- movement is a very efficient mechanism for driving fluid and electrolyte secretion, requiring the hydrolysis of only one ATP for every six Cl ions transported across the cell [86].

Classifications of Cl^- Channels in Secretory Epithelial

The molecular identity is known for two types of Cl^- channels present in secretory epithelia, i.e., CFTR and ClC-2 (*see below*). However, because several other important structural classes of Cl^- channels have not been

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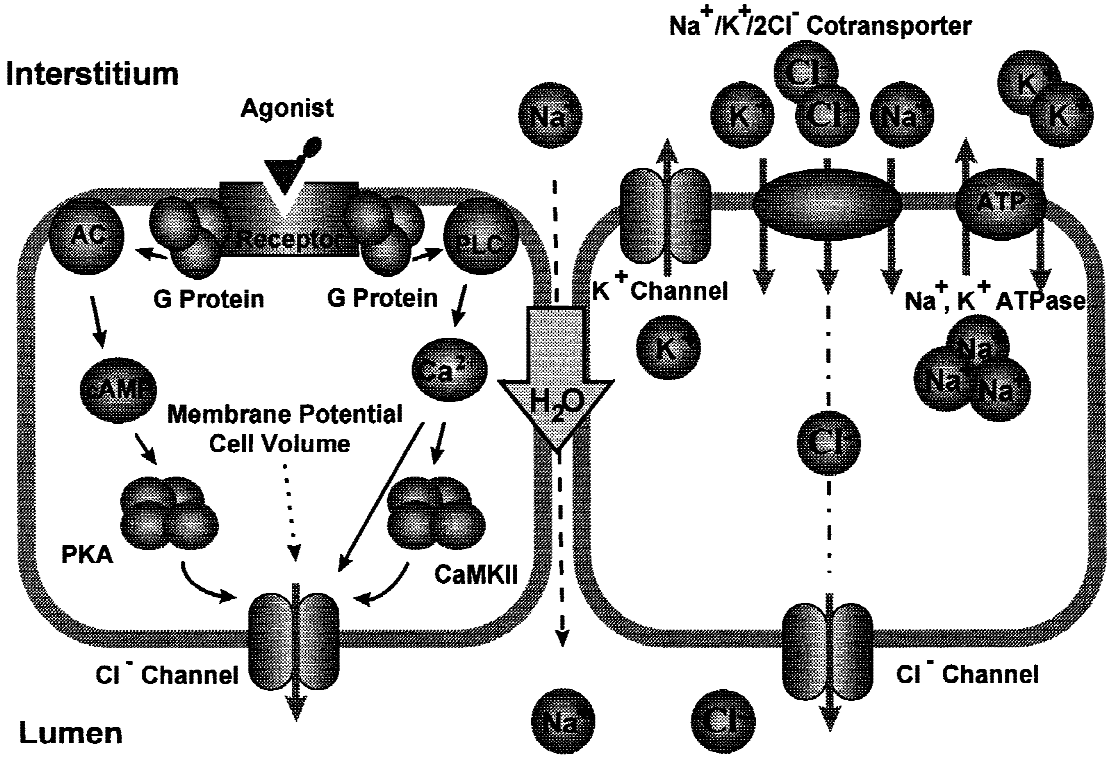

Fig. 1. Fluid secretion model—*Right cell:* Ion transport pathways in secretory epithelia. This cell shows the four essential ion transport mechanisms involved in fluid and electrolyte movement in Cl^--secreting epithelia: basolateral Na^+,K^+ ATPase with a stoichiometry of 3 Na^+:2 K^+, the electroneutral $Na^+/K^+/2Cl^-$ cotransporter located in the basolateral membrane, and basolateral K^+ and apical Cl^- channels. Transepithelial Cl^- movement drives the fluid and electrolyte secretion process. *See text for details. Left cell:* Activation mechanisms for Cl^- channels in secretory epithelial cells. The cell on the left demonstrates the various activation mechanisms for the different classes of Cl^- channels found in secretory epithelia. Binding of an agonist to its receptor activates G proteins which in turn stimulate either adenylate cyclase (AC) or phospholipase C (PLC) to increase the intracellular concentrations of cAMP and free Ca^{2+}, respectively. Phosphorylation of channel proteins by protein kinase A (PKA) or Ca^{2+}/calmodulin–dependent protein kinase II (CaMKII) results in the opening of cAMP- or Ca^{2+}-activated Cl^- channels, respectively. In some types of epithelial cells Ca^{2+} may directly activate Cl^- channels. Agonist induced changes in cell volume and membrane potential have been shown to activate additional classes of Cl^- channels (volume-sensitive and voltage-gated channels) independent of the intracellular concentrations of cAMP and free Ca^{2+}.

cloned, the most logical way to classify Cl^- channels in secretory epithelia is according to their mechanism of activation. At least 4 general classes of Cl^- channels are expressed in secretory epithelia including those activated by: (i) intracellular cAMP, (ii) cell swelling, (iii) hyperpolarization, and (iv) intracellular Ca^{2+}. It must be kept in mind, however, that this classification is an oversimplification as many Cl^- channels are regulated by more than one mechanism.

Figure 2 shows examples of currents from these 4 general classes of Cl^- as expressed in secretory epithelia. Currents activated by cAMP (upper left) exhibit no time- or voltage-dependent properties as seen by the time-independent currents (inset) and linear steady-state current-voltage relation. Volume-sensitive Cl^- channel currents display a weakly rectifying steady-state current-voltage relation (Fig. 2, upper right) and, at least on the time scale of this example, little or no time-dependence (inset). Recorded with longer pulses, the currents at rather positive potentials are seen to decline over a several second time course [3]. Cl^- channels gated by voltage (lower left) exhibit a strongly inward rectifying steady-state current-voltage relation. The kinetics of activation at negative potentials is in the range of a few hundred msec (inset). Ca^{2+}-dependent Cl^- channels have a strongly outward rectifying steady-state current-voltage relation (lower right). The activation kinetics are both Ca^{2+}- and voltage-dependent and, for the example shown, occur over a 1–2-sec time course at rather positive potentials (inset). In addition to differences in the gating mechanisms and kinetics, the anion and inhibitor sensitivity of the different classes of channels are typically unique as well. However, each class of Cl^- channel almost certainly includes several different channels which may differ as to anion and inhibitor specificity.

It is not entirely clear whether the apical Cl^- chan-

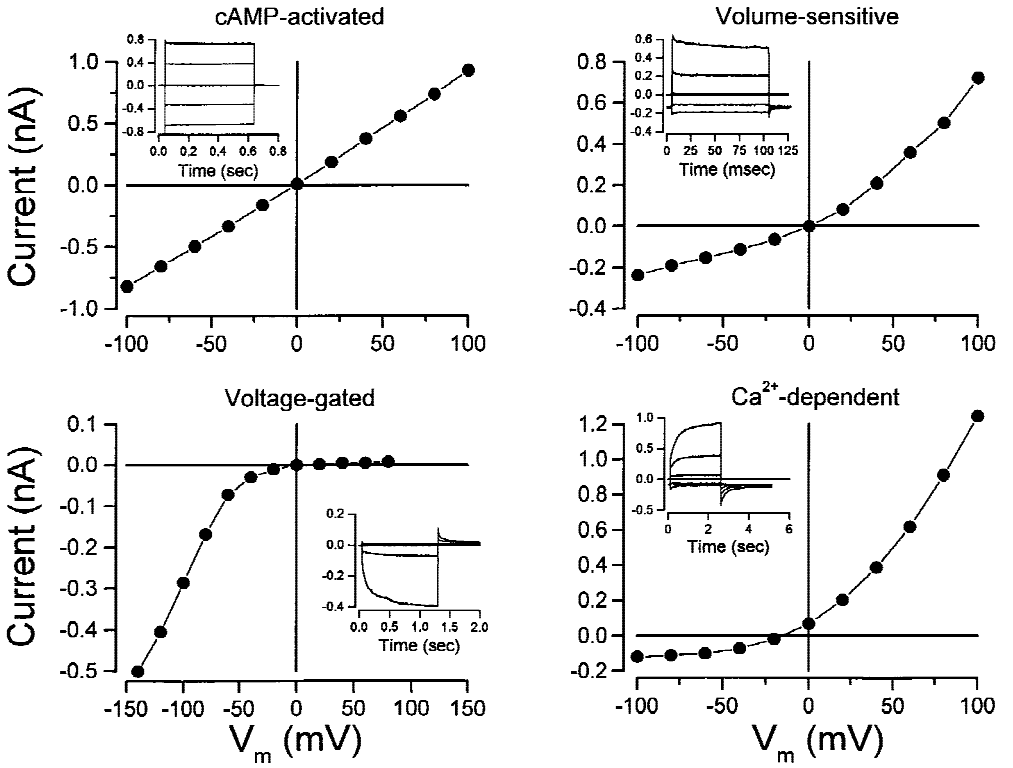

Fig. 2. Properties of Cl^- currents in secretory epithelia recorded with whole-cell patch clamp techniques. Examples of Cl^- currents (insets) and the corresponding steady-state current-voltage relations are given for the different types of Cl^- channels in secretory epithelial cells. *Upper-left panel:* Channels activated by cAMP (CFTR) have no voltage- or time-dependent activation and exhibit linear steady-state current-voltage relations. The example given is a rat submandibular gland acinar cell in symmetrical internal and external Cl^- solutions (140 mM) activated by including a nonhydrolyzable ATP analogue (AMP-PMP) in the patch pipette. Inset: Currents (in nA) recorded during 0.6 sec pulses to voltages of −80, −40, 0, +40, and +80 mV (bottom to top) from a 0 mV holding potential. Figure: Currents measured at the end of the pulses to the voltages indicated. Data from W. Zeng, *see* [95] for more examples and detailed methods. *Upper-right panel:* Volume-sensitive channels in a rat parotid gland acinar cell activated by a 36 mosm hypotonic gradient recorded with symmetrical internal and external Cl^- solutions (approximately 140 mM). Inset: Currents (in nA) recorded during 100 msec pulses to −80, −40, 0, +40, and +80 mV from a −50 mV holding potential. Figure: Currents recorded at the end of the pulse at the voltages indicated. Data from J. Arreolo, *see* [3] for additional examples and detailed methods. *Lower-left panel:* Voltage-gated channels in a mouse parotid gland acinar cell recorded in a bath solution of 135 mM Cl^- with an internal Cl^- of 70 mM. Inset: Currents (in nA) recorded during 1.25 sec pulses to −120, −60, 0, and +60 mV from a 0 mV holding potential. The voltage following the pulses was +50 mV. Figure: Currents measured at the end of the pulses to the voltages indicated. *Unpublished data from A.* Rich. *Lower-right panel:* Ca^{2+}-dependent Cl^- channels recorded from a rat parotid acinar cell activated by a buffered 100 nM Ca^{2+} concentration in the patch pipette. Currents recorded with 135 mM external and 47 mM internal Cl^-. Inset: Currents (in nA) recorded during 2.5 sec pulses to −80, −40, 0, +40, and +80 mV from a −50 mV holding potential. Figure: Currents measured at the end of the pulses to the indicated voltages. Data from J. Arreola, *see* [4] for additional examples and details.

nels involved in fluid and electrolyte secretion are the same as those that regulate intracellular pH, cell volume and membrane potential. It seems likely that the different classes of Cl^- channels expressed in secretory cells have unique functions not only because gating is regulated by different types of stimuli but also because the biophysical properties are distinct. There may be some overlap in function, however, as apical Cl^- channels involved in fluid and electrolyte secretion are primarily activated by cAMP in some epithelia [9,16,29], whereas activation is Ca^{2+} dependent in others [4, 17, 23, 56, 58]. Indeed, in several types of secretory epithelia such as colonic T84 cells [16, 26, 91] and salivary acinar cells [5, 94], all four types of channels are evidently expressed concomitantly.

cAMP-activated Cl^- Channels

A cAMP-activated channel is encoded by the CFTR gene (Cystic Fibrosis Transmembrane Conductance Regulator), a member of the ABC (ATP-binding cassette) transporter superfamily. CFTR was the first of the epithelial Cl^- channels to be cloned [72]. Mutational inactivation of the CFTR gene causes the lethal, autosomal recessive disease cystic fibrosis. Immunohistochemical analysis

has localized CFTR to the apical membrane of several Cl^--secreting epithelia [20, 54, 94]. The apical location of this channel as well as the functional consequences of cystic fibrosis, e.g., airway and pancreatic insufficiency due to obstruction, and reduced electrolyte absorption by sweat glands, clearly demonstrate that this channel is involved in transepithelial ion transport.

Of the putative Cl^- channel genes described to date in secretory epithelia, CFTR is the only channel protein to have been purified and functionally analyzed in lipid bilayers, thus proving that CFTR is a cAMP-activated Cl^- channel [7]. It appears however that CFTR may also modulate the function of other transport proteins. For example, CFTR expression has been shown to inhibit the activity of expressed epithelial Na^+ channels (ENaC) in a cAMP-dependent fashion [83]. In bronchial epithelial cells, an outward rectifying Cl^- current distinct from CFTR is activated by cAMP when CFTR is present. Regulation of this channel by cAMP disappears in cells lacking functional CFTR indicating that CFTR regulates more than one conductance pathway in airway cells [22].

Some of the basic properties of the CFTR channel include linear current-voltage relationship, anion selectivity sequence of $Br^- > Cl^- > I^-$ and insensitivity to DIDS (4,4-diisothiocyanatostilbene-2,2′-disulfonic acid). Many of these properties apply to cAMP-activated Cl^- currents in secretory epithelia some of which are apparent in the example in Fig. 2 (upper left). Single channel data indicate that the channel conductance of CFTR is about 8 pS [77]. Channel opening requires phosphorylation by protein kinase A as well as hydrolyzable ATP. The roles of phosphorylation and ATP have been extensively reviewed elsewhere [31, 90].

Many Cl^--secreting epithelia rely on cAMP to activate fluid secretion via CFTR, although other Cl^- channels distinct from CFTR are also gated by cAMP. For example, the functional properties of the cAMP-activated channel in rat choroid plexus include strong inward rectification and a selectivity sequence of $I^- > Cl^- > Br^-$, clearly different from CFTR [45]. Consistent with the functional properties of this channel, *in situ* hybridization studies failed to detect CFTR transcripts in rat choroid plexus [46]. Furthermore, there was no significant change in the magnitude of cAMP-activated currents in choroid plexus cells isolated from transgenic mice with a disrupted CFTR gene [47].

Volume-sensitive Cl^- Channels

Volume-sensitive Cl^- channels, the so-called volume sensitive organic osmolyte and anion channel (VSOAC), are expressed in virtually all animal cells (for a recent review of volume-sensitive anion channels see Strange et al. [82]). As the name implies, these anion channels have a broad specificity for anions as well as organic solutes such as sorbitol, taurine and betaine. The functional properties of VSOAC are very similar in all cell types suggesting that these channels may belong to a single gene family; however, because no members have been cloned to date this may be an over-simplification. Volume-sensitive channels are outwardly rectifying, as the example in Fig. 2 (upper right) illustrates, and at positive membrane potentials the swelling-activated whole-cell current inactivates. Other properties include an anion selectivity of $I^- > NO_3^- > Br^- > Cl^-$ and sensitivity to inhibitors such as NPPB (5-nitro-2-(3-phenylpropyl-amino)-benzoic acid), DDF (1,9-dideoxyforskolin) and stilbene derivatives as well as extracellular nucleotides like cAMP and ATP. Single channel current measurements predict that the volume-sensitive anion channel has a conductance of 15–50 pS depending on the membrane potential [37].

Several putative volume-activated Cl^- channel clones have been reported in the literature, including pl_{Cln} [69], MDR1 [32, 88], phospholemmen [59], and ClC-2 [33, 84]. It appears upon further scrutiny, however, that many of these proteins may act as Cl^- channel regulatory proteins and not as volume-sensitive Cl^- channels [50,71,85]. When the voltage-gated ClC-2 Cl^- channel is expressed in oocytes, it also acts as a cell swelling-activated Cl^- channel [33,42,84]. However, the functional properties of ClC-2 currents are distinctly different from VSOAC (*see below*).

Voltage-activated ClChannels

Since Jentsch and coworkers [41] first cloned a voltage-gated Cl^- channel from electroplax, ClC-0, nine distinct mammalian members of the ClC gene family have been described (*see* [38, 39, 40]). Schmidt-Rose and Jentsch [75] suggest that the topology of ClC chloride channels includes 10 (possibly 12) transmembrane spans with the N- and C-terminals facing the cytoplasm.

It has proved difficult to demonstrate that all of ClC family gene products are Cl^- channel proteins. Of the mammalian isoforms, ClC-1, ClC-2 and ClC-5 are the only members of the ClC family unequivocally expressed as functional Cl^- channels. Multiple members of this family are expressed in epithelial cells including several isoforms primarily found in the kidney (ClC-K1, ClC-K2, and ClC-5).

Of the known *functional* ClC channels, only ClC-2 is expressed in secretory epithelia. This ubiquitously expressed Cl^- channel is inward rectifying with an anion selectivity of $Cl^- > Br^- > I^-$ and is sensitive to inhibitors like Zn^{2+} [81], 9-AC (anthracene-9-carboxylate) and DPC (diphenylamine carboxylate), but is evidently not blocked by DIDS [84]. The voltage-gated Cl^- currents in secretory epithelia are similar to expressed ClC-2 currents, showing inward rectification and time-dependent activation at voltages more negative than −20 mV (Fig. 2, lower left panel). The single channel conductance of

ClC-2 is predicted from noise analysis to be 3–5 pS [40]. Expression of the ClC-2 Cl^- channel in oocytes suggests that cell swelling can activate this channel [33, 42], although this has not been definitively established for native mammalian cells (*see* discussion below). The apical location of this channel in rat small intestine, renal and airway epithelia suggests that, at least in these tissues, ClC-2 may play a role in regulating fluid and electrolyte movement [61].

Ca^{2+}-activated Cl^- Channels

Ca^{2+}-dependent Cl currents exhibit a characteristic voltage- and time-dependent kinetic behavior. The steady-state current-voltage relation shows strong outward rectification (Fig. 2, lower right panel); however, the open channel current-voltage relation is near linear [4]. Several factors influence the activation kinetics of the Ca^{2+}-activated Cl^- channel including the type of anion being transported as well as the internal Cl^- and Ca^{2+} concentrations [4, 23, 35, 66]. High internal Ca^{2+} concentrations and anions with greater permeability than Cl^- increase the rate of activation at positive potentials [4, 23, 66]. Ca^{2+}-dependent currents in secretory epithelia are inhibited by blockers such as DIDS, niflumic acid, NPPB and have an anion selectivity sequence of $I^- > NO_3^- > Br^- > Cl^-$. The single-channel conductance typically measured for Ca^{2+}-dependent Cl^- channels in secretory epithelial cells is 1–15 pS [3, 55, 56, 60, 74].

A Cl^- channel activated by Ca^{2+}/calmodulin protein kinase II (CaMKII), Ca-CC, was recently cloned from a bovine tracheal cDNA expression library using an antibody to a 38 kDa protein [18]. This antibody precipitates a protein that acts as a CaM kinase II-modulated Cl^- channel when reconstituted in a lipid bilayer [30, 70]. It is not presently clear whether this gene product is structurally related to all Ca^{2+}-dependent Cl^- channels or is a unique protein only expressed in bovine trachea. The latter seems likely because RT-PCR failed to detect transcript for Ca-CC in any bovine tissue tested besides trachea [18]. Moreover, the protein against which the antibody was generated is considerably smaller than the product translated from Ca-CC (38 kDa *vs.* 100 kDa). The functional relationship between these two different molecular weight proteins is uncertain at this time.

Regulation of Cl^- Channels in Secretory Epithelia

Fluid and electrolyte movement by secretory epithelia is highly regulated [28]. Recent reviews have described the regulation of cAMP-dependent CFTR [31, 90] and volume-sensitive [82] Cl^- channels and these channels will not be further discussed here. The remainder of this article will focus on the regulation of Ca^{2+}-dependent and voltage-activated Cl^- channels in secretory epithelia.

Regulation of Ca^{2+}-dependent Cl^- Channels

Although CFTR is clearly involved in fluid and electrolyte secretion in many secretory epithelia [28], in some tissues such as salivary and lacrimal glands, Ca^{2+}-dependent Cl^- channels are apparently the primary Cl^- channel activated in the apical membrane during stimulated secretion [17, 23, 58]. Indeed, even in tissues that are thought to rely on CFTR, Ca^{2+}-activated secretion can be elicited from cells isolated from patients with CF [2, 29] and in mice lacking CFTR gene expression [15].

Activation of Ca^{2+}-dependent Cl^- channels in secretory epithelia is both voltage and Ca^{2+} dependent; however, membrane voltage alone is unable to activate these channels [4]. This is clearly different from Ca-CC which is open even in the absence of Ca^{2+} [18]. The kinetics of the time-dependent activation is sensitive to the intracellular Ca^{2+} concentration, the time constant decreasing as the Ca^{2+} concentration increases [4].

Ca^{2+}-dependent channels in many secretory epithelia [11, 60, 74, 89, 92] and other cell types [63, 76] are activated by CaMKII. Peptides that block binding of calmodulin and peptides that occlude the catalytic site of CaMKII prevent channel activation in these tissues. However, activation by CaMKII is apparently not a universal property of all Ca^{2+}-dependent Cl^- channels. We found that selective inhibitors of CaMKII failed to block activation of this channel in rat parotid acinar cells [6]. This result is consistent with earlier results demonstrating that Ca^{2+} activation of the acinar cell Cl^- channel is voltage-dependent and suggests the possibility that intracellular Ca^{2+} may bind directly to the channel to open the pore [4]. Consistent with this interpretation, Cl^- channels can be activated by Ca^{2+} applied to the intracellular surface in cell free patches from salivary [55] and airway epithelia [29]. In contrast, Cl^- channels from canine airway cells appear to be indirectly activated by Ca^{2+} because changes in $[Ca^{2+}]_i$ are temporally dissociated from changes in channel gating [13]. However, when these cells are depleted of ATP, Ca^{2+}-induced anion efflux is not perturbed suggesting that Ca^{2+}-dependent activation of the channel does not require phosphorylation [14]. Therefore it appears that Ca^{2+} may act depending on the cell type, either directly, indirectly, or through phosphorylation-dependent mechanisms to activate Ca^{2+}-dependent Cl^- channels.

There is also growing evidence that some types of Ca^{2+}-dependent Cl^- channels are regulated by inositol tetrakisphosphate and annexin IV. The agonist-induced elevation in 3,4,5,6-IP_4 correlates with the inhibition of Ca^{2+}-dependent Cl^- channels in secretory cells [43,87]. The concentrations of 3,4,5,6-IP_4 required to inhibit these channels are comparable to the increases seen in vivo. Other inositol tetrakisphosphates such as 1,4,5,6-IP_4 and 1,3,4,6-IP_4 fail to inhibit these channels [36,93].

The Ca^{2+}-binding protein, Annexin IV, is concentrated along the apical membrane of many secretory epithelia and also appears to inhibit Ca^{2+}-activated Cl^- channels [10,44]. Annexin IV blocked Ca^{2+}-dependent Cl^- channels when introduced into T84 cells through the patch clamp pipette. In agreement with annexin IV acting as an inhibitor of these channels, both a neutralizing antibody and antisense oligonucleotide to annexin IV enhanced activity of these channels [44].

The regulation of Ca^{2+}-dependent Cl^- channels is further complicated by complex interactions between CaMKII, 3,4,5,6-IP_4 and annexin IV. For example, annexin IV acts synergistically with 3,4,5,6-IP_4 to inhibit Ca^{2+}-activated Cl^- channels. At concentrations of annexin IV that produce no inhibition of this channel, the potency of 3,4,5,6-IP_4 is approximately doubled [94]. Ismailov et al. [36] showed that 3,4,5,6-IP_4 can both activate and inhibit this Ca^{2+}-activated Cl^- channel. The biphasic nature of this phenomenon is dependent on the intracellular Ca^{2+} concentration as well as on the CaMKII-induced phosphorylation state of the channel [36].

Physiological changes in intracellular pH modulate stimulus-secretion coupling by regulating the activation of Ca^{2+}-dependent Cl^- channels in secretory epithelia. The pH sensitivity is such that as the pH decreases, inhibition of Ca^{2+}-dependent Cl^- channels occurs [3,51,67]. Intracellular pH modulation may be a unique property of Ca^{2+}-dependent Cl^- channels. Indeed, low intracellular pH appears to increase, not inhibit, the activity of CFTR due to an increase in channel conductance [34]. The mechanism whereby low intracellular pH inhibits Ca^{2+}-activated Cl^- channels may differ depending on whether or not the channel requires CaMKII for activation. In T84 cells, low intracellular pH inhibits CaMKII-dependent Cl^- currents due to changes in open probability of the channels, as the single channel current amplitude is insensitive to intracellular pH [3]. In the case of these CaMKII-dependent Cl^- channels, acidic conditions may inhibit channel gating by decreasing CaMKII activity or by protons competing with calcium ions for binding sites on calmodulin. For parotid and lacrimal acinar cells, where Ca^{2+} may directly activate the channel, competition is predicted to occur between H^+ and Ca^{2+} at binding sites on the channel protein itself. In addition protons may block single channels.

Modulation of Ca^{2+}-dependent Cl^- channel activity by intracellular pH may play an important role in regulating the rate of fluid and electrolyte secretion. Typically, following the addition of a fluid secretion-inducing agonist, the intracellular pH initially drops due to HCO_3^- efflux, apparently via these same Ca^{2+}-activated Cl^- channels [57,73,95]. The magnitude of this pH drop is partially buffered by upregulation of Na^+/H^+ exchangers [57,73,95]; however, reduction of Ca^{2+}-dependent Cl^- channel current may provide an additional mechanism to prevent an excessive acidification of the cytoplasm. When Na^+/H^+ exchangers are blocked by amiloride derivatives, the intracellular pH decreases to approximately 6.9 [57,95]. If protons were to equilibrate according to the electrochemical gradient the pH would be expected to drop nearly another 0.5 pH unit. Therefore, HCO_3^- efflux and the resulting intracellular pH drop is somehow prevented, most likely by inhibition of the Ca^{2+}-activated Cl^- channel.

On the other hand, the most important property of the pH-sensitivity of Ca^{2+}-activated Cl^- channels may be in sustaining fluid secretion during prolonged stimulation. Upregulation of Na^+/H^+ exchangers not only reduces the magnitude of the initial acidification, but in the continued presence of agonist, raises the intracellular pH at least 0.15 units higher than the original resting pH [57,73,80]. Thus, as the pH rises the Ca^{2+}-sensitivity of the Cl^- channel increases, evoking continued fluid and electrolyte movement even as the cytosolic Ca^{2+} concentration decreases to near resting levels.

Regulation of Voltage-activated Cl^- Channels

Evidence is accumulating that the inward rectifying, voltage-gated Cl^- currents present in secretory epithelia correlate with ClC-2 expression. In native cells the activity of the ClC-2-like currents is in the physiological voltage range, activation generally occurring at membrane potentials more negative than −20 to −40 mV [5, 8, 12, 48, 64, 68, 79]. This voltage-sensitivity is similar to rat ClC-2 expressed in HEK 293 cells [68] and DRG neurons [81], but is less negative than rat ClC-2 expressed in oocytes [84].

The ability of secretory cells to concentrate Cl^- well above the Cl^- electrochemical gradient suggests that these voltage-gated channels are not very active relative to Cl^- influx mechanisms in ''resting'' cells. However, it is possible that the Cl^- channels are ''active'' but overwhelmed by Cl^- uptake pathways. In any case, hyperpolarization-activated Cl^- currents grow with time after obtaining the whole-cell patch clamp mode in T84 cells [26], rat parotid acinar cells and in HEK 293 cells expressing rat ClC-2 [68]. This suggests that the resting activity of this channel may be minimal but certainly increases as regulatory factor(s) are removed by intracellular perfusion using the whole-cell patch clamp method. Activation of this channel may therefore require an additional signal such as a change in cell volume, internal Cl^- concentration, pH, or the state of phosphorylation.

One potential mechanism to modulate gating is the ability of ClC-2 to sense cell volume. Cell volume regulation is a critical response for epithelia that move large quantities of fluid and electrolytes. Over the course of stimulated secretion, secretory cells initially shrink and

then routinely swell once stimulation ceases [25,62]. Using the oocyte expression system Jentsch and coworkers have observed that ClC-2 is activated by cell swelling [33, 42, 84]. Swelling not only increased the magnitude of the currents but also produced a shift of the voltage dependence from voltages more negative than −80 to about −60 mV [84]. The ClC-2-like currents in a human colon carcinoma T84 cell line [27], rat Leydig cells [64] and pig pancreatic acinar cells [8] increase following cell swelling. Inspection of the currents in T84 cells before and after swelling show that not only does the magnitude increase with swelling but the time constant becomes more than an order of magnitude faster [27]. It is not clear if this change in the time constant reflects two distinct channels with different time constants, or whether swelling modulates this activation property of ClC-2 in T84 cells. In contrast, cell swelling does not appear to alter the time constant for ClC-2 current expressed in occytes [42].

Mutagenesis studies have located the domain responsible for volume-sensitive gating in the N-terminus region of ClC-2. This region is also involved in activation by the membrane potential [33]. More recently, Jordt and Jentsch [42] have shown that mutations in the cytoplasmic loop between transmembrane domains D7 and D8 prevent activation by cell swelling and remove the voltage sensitivity of ClC-2. Taken together, it appears that the gating of ClC-2 can be described by a model where the N-terminal domain occludes the channel by binding to a ''receptor'' formed by the cytoplasmic loop between transmembrane domains D7 and D8. Two observations support this model: first, the functional behavior of the occluding domain was position-independent [33]; and second, mutations in the putative receptor domain produced a channel phenotype identical to deletion of the N-terminal occluding domain [42].

It must be stressed that the endogenous ClC-2-like Cl^- currents in mammalian cells do not entirely resemble the expressed ClC-2 currents activated by cell swelling in oocytes. Indeed, in rat osteoblasts [12] and rat submandibular duct cells [49], cell swelling appears to inhibit ClC-2-like currents.

One problem with studying volume regulation of ClC-2 is the large outward rectifying Cl^- current stimulated by swelling that is present in many cell expression systems [1, 68]. It may be possible to isolate the ClC-2 current by using an appropriate form of stimulation. Cells can apparently differentiate the mechanism by which the cell volume increases, that is, swelling produced by a hypotonic shock verses swelling induced by the accumulation of electrolytes may selectively activate distinct signaling mechanisms and therefore different channels [82]. In the case of an increase in cytosolic electrolytes, an increase in internal Cl^- concentration can apparently activate ClC-2-like channels. For example, the ClC-2-like currents in rat submandibular duct cells [21] and T84 cells [26,27] are very sensitive to the intracellular Cl^- concentration, activity increasing as the Cl^- content increases. Thus, cell swelling induced by salt accumulation may activate ClC-2 channels due to an increase in Cl^- content and not via the change in cell volume per se.

Extracellular pH also modulates ClC-2 gating. The activity of rat ClC-2 expressed in oocytes increases at low external pH [42]. This was also found to be true for a Cl^- channel isolated from rabbit gastric mucosa and studied in a lipid bilayer [19]. Studies in lipid bilayers of expressed rabbit ClC-2, which is 93% similar to rat ClC-2, suggest that the ClC-2 channel protein is responsible for the Cl^- currents expressed in rabbit gastric mucosa [53]. Interestingly, the channel activity of a rat ClC-2 mutation that lacks the N-terminal occluding domain is no longer modulated by external pH [42]. This suggests an interaction between external H ions and the cytoplasmic occluding domain or its receptor.

An additional mechanism that may also regulate ClC-2 is the phosphorylation state of the protein. The number and position of consensus sites for PKC-, cAMP- and Ca^{2+}/calmodulin kinase II-dependent phosphorylation of ClC-2 varies according to the species. Rabbit gastric ClC-2 [53] and the ClC-2-like currents in rat Leydig cells [64,65] are activated by cAMP. In contrast, the ClC-2-like currents expressed in human T84 cells appear to be inhibited by a cAMP-dependent process [26]. Moreover, there was no effect of raising cAMP on human ClC-2 when expressed in oocytes [42]. PKC activation blocked channel activity in DRG neurons expressing rat ClC-2 [81] as well as the ClC-2-like currents in rat pyramidal cells [52] and T84 colonic cells [26]. The complicated nature of the phosphorylation-dependent regulation suggests that functional channels may contain an as yet unidentified regulatory subunit along with the ClC-2 gene product.

Conclusion

The specific contributions of the various classes of Cl^- channels to the fluid secretion process in secretory epithelia is unclear at this time. In addition the details of the regulation of the contributions of these various channels are lacking. The sensitivity of Ca^{2+}-dependent Cl^- channels to modulators such as intracellular pH, CaMKII, 3,4,5,6-IP_4, and annexin IV, and of voltage-activated Cl^- channels to cell volume and its phosphorylation state, may provide tissue specific mechanisms for regulating the activity of the different classes of Cl^- channels.

A molecular physiology approach will be required to gain insight into these types of questions. In particular, the structural identities of Ca^{2+}-dependent and volume-sensitive channels are yet to be determined. Functional

expression of these channels will aid in defining the regulatory properties of the different classes of Cl^- channels. Determination of their structure-function relationship by site-directed mutation analysis will play a large role in advancing our knowledge of the regulatory and kinetic properties of these important channels. Molecular physiology in genetically engineered animals will provide models to dissect the functions of the various classes of Cl^- channels expressed in Cl^--secreting epithelia. Knock out of the CFTR gene has already provided in vivo verification for the function of this cAMP-activated channel in numerous tissues [15,90,94].

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