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FLUID SECRETION BY SUBMUCOSAL GLANDS OF THE TRACHEOBRONCHIAL AIRWAYS

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Abstract

Submucosal glands of the tracheobronchial airways provide the important functions of secreting mucins, antimicrobial substances, and fluid. This review focuses on the ionic mechanism and regulation of gland fluid secretion and examines the possible role of gland dysfunction in the lethal disease cystic fibrosis (CF). The fluid component of gland secretion is driven by the active transepithelial secretion of both Cl^- and HCO_3^- by serous cells. Gland fluid secretion is neurally regulated with acetylcholine, substance P, and vasoactive intestinal peptide (VIP) playing prominent roles. The cystic fibrosis transmembrane conductance regulator (CFTR) is present in the apical membrane of gland serous cells and mediates the VIP-induced component of liquid secretion whereas the muscarinic component of liquid secretion appears to be at least partially CFTR-independent. Loss of CFTR function, which occurs in CF disease, reduces the capacity of glands to secrete fluid but not mucins. The possible links between the loss of fluid secretion capability and the complex airway pathology of CF are discussed.

Introduction

Submucosal glands populate the tracheobronchial airways of most higher mammals (Goco *et al.*, 1963). They are responsible for secretion of a wide variety of macromolecules including numerous mucins (prominently MUC5B) and antibacterial substances (including lysozyme, lactoferrin, collectins, and β -defensins). In addition, glands secrete liquid which serves both as the vehicle for carrying the macromolecules from the gland secretory tubules to the airway surface and for providing critical volumes of fluid to the airway surface that are necessary for the support of mucociliary transport. Together, the mucins (which bind pathogens), the antimicrobial substances (which kill or disable pathogens), and mucociliary transport (which physically removes pathogens from the airways) form the cornerstone of airway innate host defense. This review focuses specifically on the liquid secretion component of gland secretion and what is known about its mechanism, its regulation, and the consequences of its disruption in lethal genetic disease cystic fibrosis (CF). Please note that we do not wish to imply by omission that other non-glandular theories of CF pathogenesis are without merit. We focus primarily on studies that report direct measurements of fluid secretion from intact airway tissues, where important structure-function relationships are maintained. For additional

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information on gland fluid secretion, the reader is referred to other reviews on the subject (Ballard & Inglis, 2004; Inglis & Wilson, 2005; Verkman *et al.*, 2003; Wine & Joo, 2004).

Submucosal Gland Structure

A tracheobronchial submucosal gland consists of 1) secretory tubules, 2) a collecting duct, and 3) a ciliated duct that opens to the airway surface (Meyrick *et al.*, 1969). A schema detailing this arrangement is shown in Fig. 1. The secretory tubules are populated by two principal cell types: serous and mucous cells. The serous cells contain numerous apical electron-dense secretory vesicles and are known to secrete a number of macromolecules including lysozyme, lactoferrin, secretory IgA, peroxidases, and albumin (Basbaum *et al.*, 1990). Because serous cells undergo morphological changes when treated with fluid-inducing secretagogues (Quinton, 1981), these cells are generally assumed to be the primary site of fluid secretion in airway glands. Serous cells are the dominant cell type in the most distal, acinar portion of the secretory tubules (Meyrick *et al.*, 1969). Mucous cells, which as the name implies, secrete primarily gel-forming mucins, are typically located more proximal within the tubules and ducts (Fig. 1). This arrangement is witness to the proposed functions of these cell types: the serous cells elaborate the watery, fluid component of gland secretion that flushes the mucin gel component, which is secreted in the more proximal tubules, through the larger ducts, and out of the gland.

The secretions produced in the secretory tubules drain into the collecting duct (Fig. 1). The collecting ducts may form a distended cavity or “antrum” in the submucosal space (Inglis *et al.*, 1997a; Meyrick *et al.*, 1969) although this structure is not seen in all glands (Inglis *et al.*, 1997a). The collecting duct is drained by a single duct that passes through the smooth muscle and lamina propria of the mucosa to the airway surface (Fig. 1). The duct that passes from the collecting duct to the airway surface contains many ciliated cells that morphologically resemble the ciliated cells of the surface epithelium; therefore, this portion of the ductal system is sometimes referred to as the “ciliated duct” (Meyrick *et al.*, 1969). Ciliated cells often penetrate well into the collecting ducts. In excised porcine airways, ciliary activity can be observed within the collecting ducts as secretory vesicles are whisked about within the gland antrum (Inglis *et al.*, 1997a). It is tempting to speculate that the beating cilia within these ducts facilitate expulsion of gland secretions; however, no evidence to date indicates that secretory products are cleared from the ducts by any process other than bulk flow of fluid.

Regulation of Secretion

Liquid secretion from submucosal glands is largely under neural control. Stimulation of the vagus nerve causes vigorous gland secretion that is mediated largely by local release of ACh (Ueki *et al.*, 1980). Exposure of glands to cholinergic agonists mimics this response (Quinton, 1979), which is mediated primarily through stimulation of M₃ muscarinic receptors (Ishihara, 1992). Adrenergic agonists also induce gland secretion although the response is species-specific. Cat and ferret airway glands secrete fluid when stimulated with α -agonists (Quinton, 1979; Borson *et al.*, 1980), whereas pig and sheep glands respond poorly if at all to these agents (Joo *et al.*, 2001). β_2 -adrenergic agonists are generally weak stimulators of airway gland secretion in several species including cats, pigs, humans, and sheep (Quinton, 1979; Joo *et al.*, 2001; Joo *et al.*, 2002b). Two neuropeptides, substance P and vasoactive intestinal peptide (VIP), are thought to play important regulatory roles in submucosal gland secretion. Substance P is a potent and efficacious glandular fluid secretagogue (Haxhiu *et al.* 1990; Trout *et al.* 2001; Phillips *et al.* 2003), and its secretory effects are primarily mediated through stimulation of NK₁ receptors (Phillips *et al.* 2003). VIP induces liquid secretion in submucosal glands through stimulation of VPAC₂ receptors (Groneberg *et al.* 2001). Nitric oxide synthase co-localizes in airway neurons with substance P and VIP (Dey *et al.*, 1996) where it probably

mediates local vasodilation through generation of nitric oxide (NO), a response that should facilitate fluid secretion by increasing blood flow to gland tissue.

Much remains to be learned about the second messenger pathways involved in gland secretion, but some generalities can be made based on studies with airway submucosal glands and other similarly-functioning tissues. Muscarinic stimulation of feline gland cells causes mobilization of Ca^{+2} from IP_3 -sensitive intracellular stores and activation of protein kinase C (Sasaki *et al.*, 1994). Nagaki and coworkers (1994) report that substance P elevates intracellular Ca^{+2} in gland cells as well. Thus, it is likely that both of these neurotransmitters transduce their responses in glands through stimulation of phospholipase C. In contrast, the VPAC_2 receptors couple with adenylyl cyclase and elevate intracellular cAMP concentrations when stimulated though more complex effects on intracellular Ca^{+2} homeostasis may occur as well (Groneberg *et al.*, 2006).

While the identity of these major neural controllers of gland secretion have long been known, much less is known about the subtleties of how each of these neural elements respond and interact to physiologic and pathologic stimuli. For a detailed discussion of the finer issues of neural control of gland secretion, the reader is referred to a recent review by Wine (2007). In addition to these well-studied neurotransmitters, we expect that a host of autocrine, neurocrine, and paracrine substances such as histamine, ATP, prostanoids, platelet activating factor, and human neutrophil elastase are also capable of stimulating or modulating submucosal gland secretion.

Ionic mechanism of secretion

Liquid secretion by the submucosal glands is driven by the active secretion of both Cl^- and HCO_3^- . *In vitro*, porcine bronchi secrete approximately $15 \mu\text{L} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ when treated with ACh and virtually all of this fluid originates from submucosal glands (Trout *et al.*, 1998a; Ballard *et al.*, 1999). Seventy percent of this secreted fluid is driven by $\text{Na}^+ \text{-} \text{K}^+ \text{-} 2\text{Cl}^-$ cotransport (NKCC)-dependent Cl^- secretion whereas about 20% is supported by Na^+/H^+ exchange (NHE)-dependent HCO_3^- secretion (Trout *et al.*, 1998a)(Fig. 2). The magnitude of HCO_3^- secretion that occurs following muscarinic stimulation is sufficient to induce measurable alkalization of the luminal solution in isolated liquid-perfused bronchi (Inglis *et al.*, 2003). Individual gland secretion rates from carbachol-treated sheep airways varies from transient peak rates of 15.7 nL/min to sustained rates of 4.5 nL/min (Joo *et al.*, 2001). The partial HCO_3^- dependence of gland fluid secretion has also been observed in individual submucosal glands where carbachol-induced secretion is reduced by one-half to two-thirds by removal of HCO_3^- and CO_2 from the bath solution (Joo *et al.*, 2001; Joo *et al.*, 2002b). Substance P induces nearly equal rates of gland secretion in porcine bronchi with similar fractional contributions from Cl^- and HCO_3^- secretion to that seen with ACh (Trout *et al.*, 2001). Similar to muscarinic agonists, substance P-induced Cl^- secretion is dependent on NKCC and HCO_3^- secretion requires NHE (Trout *et al.*, 2001).

VIP stimulates liquid secretion from submucosal glands, but the rates are much lower than that seen with muscarinic stimulation. In pig glands, peak and sustained rates of secretion with VIP are about 9% and 40%, respectively, of carbachol rates (Joo *et al.*, 2002b). VIP-induced secretion is also mediated by a combination of Cl^- and HCO_3^- secretion with each contributing about one-half to the liquid secretion response (Joo *et al.*, 2002b). Forskolin, which directly activates adenylyl cyclase to produce cAMP, mimics the secretion response to VIP (Joo *et al.*, 2002b). When intact porcine bronchi are treated with forskolin, fluid is secreted that originates almost entirely from glands and is driven by a combination of Cl^- and HCO_3^- secretion (Ballard *et al.*, 2006). Unlike the response to ACh and substance P, forskolin induces HCO_3^- secretion by two mechanisms. In addition to the dimethylamiloride-sensitive pathway

that is induced following muscarinic or substance P stimulation, forskolin also induces a Na^+ -dependent, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS)-sensitive pathway that most likely depends upon a basolateral membrane Na^+ - nHCO_3^- cotransporter (NBC) (Ballard *et al.*, 2006)(Fig. 2). Devor and colleagues (1999) demonstrated a functional role for an NBC transporter in transepithelial HCO_3^- secretion by Calu-3 cells, a human lung adenocarcinoma cell line that expresses many characteristics of serous cells (Shen *et al.* 1994). Evidence has been reported for the existence of an anion exchanger (AE2) in the basolateral membrane of gland cells that could potentially support Cl^- or HCO_3^- dependent secretion (Loffing *et al.*, 2000). However, it is yet unclear whether this transporter plays a significant role in fluid secretion.

Anion channels most likely conduct Cl^- and HCO_3^- across the apical membrane of the secretory cell (Fig. 2). As discussed in the following section, there is evidence that the cystic fibrosis transmembrane conductance regulator (CFTR) mediates VIP-dependent secretion whereas an alternate anion channel, perhaps a Ca^{+2} -activated Cl^- channel (CaCC), appears to mediate the response to muscarinic agonists.

Active secretion of anions across glandular epithelia generates a lumen negative potential difference which pulls cations across the barrier, most likely through the paracellular tight junctions (Fig. 2). The aggregate movement of anions and cations generates an osmotic gradient across the epithelial barrier, and water moves to preserve isosmotic conditions. Aquaporin 5 water channels (AQP5), which are present in the apical membrane of serous submucosal gland cells, facilitate this water movement (Song & Verkman, 2001) although some water probably moves through the tight junctional pathway as well.

The ducts of the submucosal glands express CFTR (Engelhardt *et al.*, 1992) and subunits of ENaC (Burch *et al.*, 1995). These findings have led to speculation that more proximal regions of the gland ducts might have the capability to secrete or absorb fluid to respectively augment or attenuate the acinar secretion volumes. To address this issue, Wu and colleagues (2007) dissected individual gland ducts from the surrounding airway tissue, ligated the ducts at various points, and stimulated the airways with forskolin. No evidence of volume changes were observed in the ligated segments suggesting that no significant secretion or absorption was occurring in these gland duct regions. However, the authors did not rule out the possibility that these transporters might be capable of altering ion composition of fluid as it passes through the ducts.

Role in CF lung disease

Some of the earliest literature on CF suggest that submucosal glands could play an important role in the etiology of the airway disease. A prominent feature of CF is the presence of a thick, poorly-cleared, inspissated mucus that obstructs the intrapulmonary airways, contributes to chronic bacterial colonization, and, ultimately, leads to the development of bronchiectasis and death in most patients. Zuelzer *et al.* (1949) recognized as early as the 1940's that the gland ducts from CF airways were prominently dilated with dense mucous secretions and proposed that a defect in submucosal gland secretion could be at the heart of the disease process. The increased quantity of mucin in the gland ducts led some to surmise that there was an increase in mucin secretion in CF glands (Lamb & Reid, 1972). In the ensuing years, many investigators searched for the endogenous factor(s) that were responsible for the hypersecretion of mucus in CF; however, it was Quinton's discovery in 1983 that sweat ducts in CF patients lacked a prominent Cl^- conductance that first indicated that this disease was instead caused by a fundamental defect in anion transport (Quinton, 1983). This finding was eventually confirmed in 1989 by the landmark studies that identified the CF gene (Kerem *et al.* 1989; Riordan *et al.*, 1989; Rommens *et al.*, 1989.), which was found to code for the cystic fibrosis

transmembrane conductance regulator (CFTR), a cAMP-activated anion channel (Anderson *et al.*, 1991). Using CFTR antibodies and mRNA probes, Engelhardt and coworkers (1992) showed that this protein was present not just in the surface epithelium of airways but was highly expressed in the serous cells of the submucosal glands as well. This study provided evidence for the first time that a defect in anion and liquid transport might exist in the submucosal glands of CF airways. Using CFTR antibodies, other researchers subsequently corroborated this glandular distribution pattern in human, bovine, and porcine airways (Jacquot *et al.*, 1993; Ballard *et al.*, 1999). Though generally accepted, the notion that serous cells highly express CFTR has not been without its detractors. Kreda *et al.* (2005), using CFTR antibodies and mRNA probes, reported weak and inconsistent labeling of human bronchial gland cells compared to the airway surface epithelial cells. Contemporaneous to the Engelhardt study, Snouwaert and colleagues (1992) reported the creation of the first CFTR gene “knockout” mouse which, surprisingly, did not develop the overt lung disease that typifies human CF. Though mouse airways were found to express Ca^{+2} -activated anion channels that could possibly substitute for the lost CFTR in murine airway epithelia (Grubb & Boucher, 1999), the absence of significant lung disease in CF mice was provocative in that this species does not express submucosal glands in their intrapulmonary airways (Borthwick *et al.*, 1999). These findings raised an intriguing question: “Could an anion transport defect in submucosal glands significantly contribute to human CF lung disease?”

To answer this question, it was necessary to document that CFTR specifically mediates liquid secretion from airway glands. This proved problematic in the years immediately following the Engelhardt study because 1) available pharmacological inhibitors of the CFTR were notoriously non-specific, 2) CF airway tissues were difficult to obtain for study because lung transplantation had not yet become commonplace as treatment for advanced disease, and 3) no other genetic model of CF besides mice was available. Despite these limitations, our laboratory undertook the task of demonstrating that anion and liquid secretion by airway glands (whether by CFTR-dependent or independent means) was critical to lung function and that its inhibition led to pathological correlates with CF airway disease. As described in the previous section, we found that selective inhibition of Cl^- secretion with the NKCC inhibitor bumetanide and HCO_3^- secretion inhibition with the NHE inhibitor dimethylamiloride, blocked about 85–90% of the ACh-induced liquid volume secretion from airway glands (Trout *et al.*, 1998a). This strategy appeared to inhibit the liquid secretion but not the mucin glycoprotein secretion response to ACh, thus uncoupling the secretion of these products from glands as we expected might occur in CF (Inglis *et al.*, 1998; Trout *et al.*, 1998b). In the presence of Cl^- and HCO_3^- secretion inhibitors, the secreted mucus was thick and poorly hydrated, and the rheological properties of this material were altered in a manner that predicted poor cough clearance (Trout *et al.*, 1998b). When both Cl^- and HCO_3^- secretion were inhibited, gland ducts became clogged with dense mucous material (Inglis *et al.*, 1997b; Inglis *et al.*, 1998) similar to that observed in CF airway glands (Easterly & Oppenheimer, 1968). Interestingly, gland duct occlusion required approximately 85–90% inhibition of gland liquid secretion that could only be obtained by inhibiting both Cl^- and HCO_3^- secretion. It did not occur when only Cl^- secretion was inhibited (Inglis *et al.*, 1998) suggesting that the critical level of liquid secretion inhibition required to occlude gland ducts was greater than the 70% inhibition that could be obtained with bumetanide alone (Trout *et al.*, 1998a). We also found that the combination of Cl^- and HCO_3^- secretion inhibitors, or 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), an anion channel blocker that is known to inhibit CFTR, substantially reduced mucociliary transport in porcine tracheas (Ballard *et al.*, 2002), a finding that reproduces another important correlate to CF airway pathology. This effect of the inhibitors was presumably caused by the reduction in periciliary liquid volume at the airway surface since the mucociliary transport impairment could be ameliorated with inhibitors of Na^+ -dependent liquid absorption by the surface epithelium. A subsequent study with isolated perfused porcine lungs confirmed that Cl^- and HCO_3^- secretion inhibitors indeed depleted periciliary fluid in

the intrapulmonary bronchi causing cilia to become trapped between the apical cell membrane and the thin, dense layer of surface mucus (Trout *et al.*, 2003). Unfortunately, because intact airways were used in these studies, it was not possible to attribute the inhibitory effects of these anion secretion inhibitors on mucociliary transport solely to submucosal glands (i.e., these inhibitors would have also blocked anion and liquid secretion by surface epithelium as well). Indeed, similar depletion of periciliary fluid also occurs in monolayer cultures of surface epithelial cells from CF airways which do not express submucosal glands (Matsui *et al.*, 1998). Nonetheless, these studies, while not specifically targeting CFTR, clearly demonstrate that liquid secretion is essential to airway function and that its inhibition mimics critical aspects of CF airway disease.

In recent years, lung transplantation has been increasingly utilized as a treatment option for end-stage CF lung disease. This development has benefitted not just the patients but also researchers in that airways from the explanted CF lungs can be routinely obtained for study by laboratories that are convenient to transplant centers. Using an optical technique for estimating liquid secretion rates from individual submucosal glands, Joo and coworkers (2002a) observed that CF airway glands did not secrete when treated with either VIP or forskolin but the secretion response to carbachol, a muscarinic agonist, remained intact. These authors noted that the gland mass in the CF airways was substantially larger than in normal airways and implied that the carbachol secretion rates, if normalized to gland mass, might actually be reduced in CF. This important study documented for the first time that there is indeed an anion transport defect in CF airway submucosal glands, but it indicated that gland secretory function may be only partially disabled in this disease. A more recent study adds additional support for this notion. Ianowski and colleagues (2007), using the same technique, examined the mechanism of liquid secretion of the perilaryngeal airway glands of CFTR knockout mice. Mice only express glands in the most cranial portions of the trachea (Borthwick *et al.*, 1999), which could explain the paucity of pulmonary pathology in the absence of functional CFTR. Their findings, which mirror those of the earlier Joo study, showed that the perilaryngeal glands responded to carbachol but did not secrete to either VIP or forskolin (Ianowski *et al.*, 2007). This study also documented that the perilaryngeal glands from the CF mice failed to secrete when they were exposed to chili oil, a treatment that likely induces secretion through stimulation of sensory nerves and release of tachykinins such as substance P.

Salinas and coworkers (2005) demonstrated that the diffusional viscosity of CF gland fluid, assessed by fluorescence recovery after photobleaching (FRAP), was 2.2-fold greater than that of non-CF gland fluid, a property that is consistent with a thicker, poorly-hydrated mucus. They also observed that the gland fluid secretion response to pilocarpine, a muscarinic agonist, was 2.7-fold lower in CF glands than in non-CF glands. This finding suggests that muscarinic fluid and macromolecule secretion is at least partially uncoupled in CF and supports the notion that the abnormally thick mucus that is found in the airway lumen of CF patients, which contains 10–20% nonvolatile solids (Chernick & Barbero, 1959), is the result of an abnormality in gland secretion.

These observations raise several important and intriguing questions. First, is it possible that the secretion response of CF glands to muscarinic stimulation is related to contraction of myoepithelial cells and not stimulation of anion and liquid secretion? When stimulated, myoepithelial cells, which surround the secretory tubules, contract and compress the glands thus facilitating expulsion of gland secretions from the ducts. Cholinergic stimulation induces myoepithelial cell contraction, but VIP does not (Shimura *et al.*, 1986); therefore, carbachol could appear to stimulate liquid secretion in CF glands by causing myoepithelial cells to contract and squeeze resident thick mucus from the tubules and out through the duct openings rather than directly stimulating secretion. Myoepithelial cell contraction is unlikely to account for these results because it would be expected to induce a transient effect, whereas Joo and

colleagues (2002a) show that the carbachol effect on CF gland secretion is sustained. Second, could loss of only the VIP component of fluid secretion, which is substantially less than the response to muscarinic agonists, cause the degree of gland duct occlusion and obstruction seen in histological sections of CF airways? One would expect that the muscarinic component of fluid secretion, if it indeed remains at least partially intact and normal in CF glands, would periodically flush thick secretions from the gland ducts and deliver sufficient quantities of normally hydrated mucus liquid to airway surfaces. However, recent evidence suggests that substance P-induced liquid secretion might be disabled as well (Ianowski *et al.*, 2007). Because substance P is the equal of ACh as a glandular secretagogue in normal airways, loss of this fluid component would likely be more damaging to gland function. Third, if the muscarinic component of fluid secretion from CF glands remains intact, could this pathway be selectively manipulated to increase mucus fluidity and hydration of airway surfaces in CF patients? If so, the muscarinic pathway could potentially be the savior of the CF airway. Unfortunately, much remains to be learned about the conditions under which VIP and ACh, as well as the raft of other neurotransmitters and paracrine substances, are secreted and how these agents act in concert to control gland secretion. The recent review by Wine (2007) of neural regulation of gland secretion addresses some of these critical issues.

Because normal submucosal glands are capable of secreting HCO_3^- (Inglis *et al.*, 1998; Trout *et al.*, 1998a) and the CFTR is conductive to this anion (Poulsen *et al.*, 1994), there has been speculation that CFTR might be required for normal HCO_3^- secretion by airway glands. Jayaraman *et al.* (2001) used fluorescent pH-sensitive probes injected directly into liquid droplets as they emerged from the gland ducts to measure gland fluid pH. They saw no significant difference in the pH of gland fluid from normal human donor airways and explanted CF airways. However, when this same laboratory looked at gland fluid pH in freshly harvested nasal glands from CF and normal subjects, they found that the pH of this fluid was significantly lower in CF gland fluid (6.57 ± 0.09) than in non-CF gland fluid (7.18 ± 0.06) (Song *et al.*, 2006). These authors attributed the lack of significance of the earlier study to small sample numbers, variability in specimen quality, and the secondary effects of advanced disease. Song and coworkers (2006) additionally found that the selective CFTR inhibitors GlyH-101 and CFTR_{inh}-172, as well as NPPB, significantly reduced gland fluid pH in pig tracheobronchial airways by approximately 0.5 pH unit. This more acidic gland liquid could be important in CF pathogenesis since the rheological properties of mucus are sensitive to changes in pH (Holma, 1998) and significant changes in the physical properties of this material could contribute to its reduced cough and mucociliary clearance in CF disease.

In summary, CFTR appears to play a significant role in the secretion of Cl^- , HCO_3^- , and fluid from submucosal glands. In CF disease, defects in CFTR function are sufficient to produce a significantly thicker mucus that is acidic compared to normal gland fluid. However, this defect in liquid secretion is agonist-dependent with the VIP- mediated secretion being completely disabled while the muscarinic component remains at least partially intact. Thus, the possibility remains that the residual functional component of CF glands could be manipulated to salvage airway function in these affected airways.

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FIGURE 1.

Schema of a tracheobronchial submucosal gland. The gland consists of a ciliated duct, a collecting duct, and numerous secretory tubules. The secretory tubules are populated by serous and mucous cells. The serous cells are most plentiful in the distal and acinar regions of the tubules whereas the mucous cells populate the more proximal regions. This arrangement allows the secreted mucins to be easily flushed from the ducts of the tubules by the fluid secreted from the serous cells. See text for details.

FIGURE 2.

Cell model for anion and liquid secretion by submucosal gland cells. Anion secretion is generally attributed to serous cells. Cl^- is the dominant anion that is secreted and it enters the cell across the basolateral membrane through the $\text{Na}^+/\text{K}^+-2\text{Cl}^-$ cotransporter (NKCC) and exits across the apical membrane through anion channels. CFTR mediates secretion induced by VIP and forskolin whereas an alternate anion channel, perhaps a Ca^{+2} -activated anion channel, likely participates in the secretion induced by muscarinic agonists. HCO_3^- secretion is mediated through two potential pathways. HCO_3^- generated intracellularly results from the actions of carbonic anhydrase, which catalyzes the synthesis of HCO_3^- and H^+ from CO_2 and H_2O . Removal of H^+ from the cytoplasm by Na^+/H^+ exchange (NHE) results in the net synthesis of intracellular HCO_3^- that exits the cell through apical membrane anions channels. This pathway is induced by both forskolin and muscarinic agonists. It is likely that forskolin also induces transepithelial HCO_3^- secretion where HCO_3^- enters cells across the basolateral membrane via a $\text{Na}^+/\text{nHCO}_3^-$ cotransporter (NBC) and exits across the apical membrane through anion channels. The mechanisms for forskolin- and VIP-induced secretion are probably very similar and require CFTR. Transepithelial secretion of anions creates an electrical gradient for cations (mostly Na^+) to follow through the paracellular pathway and the resultant osmotic gradient pulls water across the barrier. For convenience, water is shown moving through the paracellular pathway, but, due to the presence of AQP5 in the apical membrane, a significant fraction of water movement is transcellular. See text for details.