TOPICAL REVIEW

Liquid secretion properties of airway submucosal glands

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The tracheobronchial submucosal glands secrete liquid that is important for hydrating airway surfaces, supporting mucociliary transport, and serving as a fluid matrix for numerous secreted macromolecules including the gel-forming mucins. This review details the essential structural elements of airway glands and summarizes what is currently known regarding the ion transport processes responsible for producing the liquid component of gland secretion. Liquid secretion most likely arises from serous cells and is principally under neural control with muscarinic agonists, substance P, and vasoactive intestinal peptide (VIP) functioning as effective secretogogues. Liquid secretion is driven by the active transepithelial secretion of both Cl^- and HCO_3^- and at least a portion of this process is mediated by the cystic fibrosis transmembrane conductance regulator (CFTR), which is highly expressed in glands. The potential role of submucosal glands in cystic fibrosis lung disease is discussed.

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Introduction

The submucosal glands of the tracheobronchial airways secrete liquid that is essential for flushing the macromolecular component of gland secretion from the gland ducts and for augmenting airway surface liquid (ASL) volume for the support of mucociliary transport. In this review, we provide an analysis of the current literature regarding the mechanisms of ion and liquid secretion by the tracheobronchial glands. Because the arrangement of glandular structural elements is important to their secretory function, when possible we emphasize studies performed with intact airways, where the complex architecture of glandular and surface epithelium is maintained. Because the cystic fibrosis transmembrane conductance regulator (CFTR) is known to mediate at least a portion of gland liquid secretion, we include a discussion of the potential role of submucosal glands in cystic fibrosis (CF) lung disease. Due to space constraints, however, we will not review the macromolecular component of gland secretion, about which a considerable literature exists owing to its importance in the aetiology of obstructive airway diseases. The reader is referred to several excellent reviews that provide more in-depth discussions of gland structure as well as fluid and macromolecular secretion (Tos 1966; Rogers, 1993; Shimura *et al.* 1994; Rogers 2000).

Gland morphology

Submucosal glands populate the trachea and bronchial airways of higher mammals including humans, monkeys, sheep, pigs, goats, oxen, opossums, cats and dogs (Goco *et al.* 1963; Sorkin, 1965; Choi *et al.* 2000). In adult humans, sheep, oxen, dogs and pigs, gland density is approximately $1\ mm^{-2}$ (Tos, 1976; Choi *et al.* 2000). In man, glands are well-expressed throughout the cartilaginous airways (Bloom & Fawcett, 1975), a pattern that is likely to hold for most higher mammals as well. Bronchioles, the compliant thin-walled distal airways that contain little cartilage, are aglandular; consequently, there is an abrupt transition in gland expression at the bronchial–bronchiolar junction, which occurs at about 1 mm airway diameter (Ballard *et al.* 1995). Rats, mice, guinea-pigs and hamsters express submucosal glands only in the most cranial portion of the trachea (Borthwick *et al.* 1999; Widdicombe *et al.* 2001). Rabbit airways are devoid of submucosal glands, but they do exhibit numerous shallow pits or depressions in the airway surface in which goblet cells are thought to cluster (Widdicombe *et al.* 2001).

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An individual airway gland typically consists of a primary (collecting) gland duct, lateral ducts and numerous secretory tubules (Tos, 1966). The primary gland duct passes from the surface epithelium through the lamina propria and smooth muscle layers into the submucosal space. The proximal segment of the primary duct (i.e. portion closer to the duct opening) is lined by ciliated cells whose morphology resembles that of the surface epithelium (Meyrick *et al.* 1969). The submucosal portions of the primary duct may form 'antra', i.e. distended duct regions whose diameters are 3- to 4-fold greater than the primary ducts (Meyrick *et al.* 1969; Inglis *et al.* 1997*a*) (Fig. 1). The functional significance of these distended duct regions, beyond simple conduction of glandular secretion products, is unclear. Many primary ducts do not form these antra, though their morphology may vary greatly from long straight segments to convoluted structures (Tos, 1966; Inglis *et al.* 1997*a*). The primary ducts serve as collectors for a pair of lateral ducts from which numerous secretory tubules arise (Tos, 1966; Meyrick *et al.* 1969). These secretory tubules are categorized as either mucous or serous depending on the relative predominance of these respective cell types (Meyrick *et al.* 1969). The mucous tubules may bifurcate once or more into other mucous tubules, but they always terminate in serous tubules.

The principal exocrine cells of the airway glands are the mucous and serous cells. Mucous cells closely resemble the goblet cells, which are found in the surface epithelium, in that their apices are packed with large mucin-containing granules that compress the nucleus and cytoplasm into the basal portions of the cells. The serous cells are pyramidal in shape and the nucleus is also basally located (Basbaum *et al.* 1990). The apices of the serous cells are filled with numerous electron-dense secretory granules that are 100–1800 nm in diameter. When stimulated with glandular secretogogues, serous cells undergo morphological changes that parallel the magnitude of fluid secretion (Quinton, 1981); consequently, serous cells are thought to be the principal mediators of fluid secretion in submucosal glands. Thus, because the serous tubules always lie distal to the mucous tubules, they are logically orientated to flush the mucin glycoprotein secretions of the mucous cells out of the ducts. Indeed, when fluid secretion is inhibited pharmacologically, the gland ducts become impacted with mucin glycoproteins (Inglis *et al.* 1997*b*) demonstrating the importance of this structural arrangement. In adult human airway glands, serous cells outnumber mucous cells by about 50% (Takizawa & Thurlbeck, 1971). Much of the basal surfaces of the glandular epithelial cells, including the mucous and serous tubules and portions of the gland ducts, are surrounded by myoepithelial cells. These cells are contractile and, when activated, facilitate emptying of the luminal contents (Shimura *et al.* 1986).

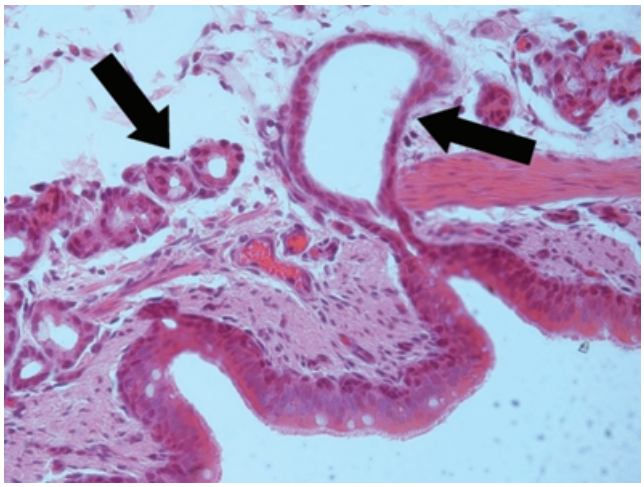

Figure 1. Slide section of submucosal gland from porcine bronchus
The right arrow identifies dilated segment, or antrum, of the primary (collecting) duct in the submucosa. The left arrow shows numerous secretory tubules.

Techniques for measuring liquid secretion from glands

Because glands are small and most of their mass is embedded in the submucosal space, study of the their exocrine function is problematic. Several experimental approaches have been employed. One approach is to cover the mucosal surface of the airways with a thin coating of tantalum power (Nadel & Davis, 1978). When glands are stimulated to secrete, the fluid that emerges from the ducts pushes the powder aside forming 'hillocks' at the duct openings. By modelling the hillock dimensions as liquid droplets, this technique permits useful estimates of short-term volume secretion rates (Hejal *et al.* 1995; Phillips *et al.* 2002*a*). Another technique, pioneered by Quinton (1979), involves drying the airway surface with a stream of gas and then layering the surface with water-equilibrated oil. As gland fluid emerges from the duct openings, it forms visible pools of liquid underneath the oil layer. This liquid can be collected with micropipettes for determination of volume and composition (Quinton, 1979; Ueki *et al.* 1980) or the volumes can be determined optically *in situ* from droplet dimensions (Joo *et al.* 2001). Others have isolated the glandular contribution to fluid secretion in intact airways by abrasively removing the surface epithelium whilst leaving the submucosal structures intact (Ballard

et al. 1999; Trout *et al.* 2001). Fluorescence imaging techniques have been employed to measure acid/base transport in the acinar cells of isolated glands (Hug & Bridges, 2001) or to measure pH and ion compositions of the gland fluid as it emanates from the gland duct openings (Jayaraman *et al.* 2001). Though technically challenging, it is feasible to dissect individual submucosal glands from the tracheobronchial airways. We are unaware of studies to date that achieve quantitative collection of secreted fluid from isolated glands, but the rate of ^{22}Na efflux from isolated glands (apparently across the basolateral membrane of gland cells via the Na^+,K^+-ATPase) has proven to be a useful correlate of secretory ion transport activity (Sasaki *et al.* 1990). Individual submucosal glands have even been attached to myographs for measurement of the contractile responses of myoepithelial cells (Shimura *et al.* 1986).

Useful information, particularly about electrolyte secretion, has also been obtained from studies of cultured gland cells. However, caution must be employed when extrapolating the results of these studies to overall glandular function since important structure–function relationships are lost. In addition, cells removed from their native environments and grown in complex media containing growth factors for prolonged periods may not accurately reflect *in vivo* behaviour. Indeed, isolated serous and mucous cells tend to dedifferentiate in primary culture expressing both serous and mucous cell proteins (Sommerhoff & Finkbeiner, 1990). The Calu-3 cell line, derived from a human lung adenocarcinoma, expresses many characteristics of submucosal gland serous cells including expression of CFTR (Shen *et al.* 1994). This cell line has proven to be a convenient model for evaluating serous cell function; but, since these cells are aneuploid, lacking chromosomes 1, 13, 15, and 17 (ATCC, Manassas VA, USA), studies of their function should be interpreted with caution.

Regulation of ion and liquid secretion

In the absence of secretogogues or neural stimulation, glands produce small quantities of liquid (Quinton, 1979; Joo *et al.* 2001; Ueki *et al.* 1980). However, vagal stimulation, either direct or through activation of sensory nerves, induces copious secretion of fluid from tracheal glands (Davis *et al.* 1982; Haxhiu *et al.* 1990). Direct application of acetylcholine (ACh) or other muscarinic agonists to excised airways mirrors this response demonstrating that fluid secretion from glands is under cholinergic control (Quinton, 1979; Ballard *et al.* 1999; Joo *et al.* 2002*b*). The response to these agonists is due to activation of M_3 muscarinic receptors (Ishihara *et al.* 1992) and perhaps M_1 receptors as well (Yang *et al.* 1988). Substance P, which is normally released from the terminals of sensory nerves, also induces vigorous fluid secretion from glands both *in vivo* (Haxhiu *et al.* 1990) and *in vitro* (Trout *et al.* 2001; Phillips *et al.* 2003). The secretion response to substance P is mediated predominantly through NK1 receptors on gland cells though prejunctional NK3 receptors are thought to reinforce secretion by inducing secondary release of ACh (Khawaja *et al.* 1999; Phillips *et al.* 2003). Calcitonin gene-related peptide (CGRP) and neurokinin A (NK-A) also stimulate gland secretion (Webber, 1989; Webber *et al.* 1991). Adrenergic control of fluid secretion appears to be less straightforward. α-Adrenergic receptor agonists are efficacious gland secretogogues in feline and ferret tracheas (Quinton, 1979; Borson *et al.* 1980; Ueki *et al.* 1980; Joo *et al.* 2001) but have little or no effect on volume secretion in pig, sheep, or human glands (Joo *et al.* 2001; Trout *et al.* 2001). β-Adrenergic agonists are ineffective fluid secretogogues for human, feline and pig glands (Quinton, 1979; Trout *et al.* 2001; Joo *et al.* 2001). Vasoactive intestinal peptide (VIP) induces liquid secretion from human and porcine glands though the rate of secretion is comparatively less than that seen with the muscarinic agonists (Joo *et al.* 2002*a*,*b*). The response to VIP is likely to be mediated through $VPAC_2$ receptors (formally VIP_2 receptors), which have been localized to acinar and ductal gland cells (Groneberg *et al.* 2001). In human airways, VIP-containing neurones are often coincident with cholinergic neurones, but this is not the case in all species (Fischer *et al.* 1996).

Recent studies suggest that autocrine and/or paracrine mechanisms play a role in the regulation of gland secretion. Stimuli such as hyposmotic and flow-induced stress induce the release of ATP from Calu-3 cells (Guyot & Hanrahan, 2002). Extracellular ATP, as well as UTP, is capable of directly stimulating a number of P_{2Y} (formally P_{2U}) receptor subtypes which in turn evoke a rise in intracellular Ca^{2+} and induce anion secretion in cultured human gland cells (Yamaya *et al.* 1996). In cultures of Calu-3 cells, ATP can also be broken down by ectonucleotidases to adenosine, which stimulates anion secretion through A_{2B} receptors via both protein kinase A and phospholipase A_2-dependent pathways (Huang *et al.* 2001; Cobb *et al.* 2002). The relative importance of these pathways in the moment-to-moment regulation of gland fluid secretion is not fully understood at this time. Platelet-activating factor (PAF) is also a potent stimulant of gland fluid secretion (Steiger *et al.* 1987). Human neutrophil elastase, a powerful inducer of mucus macromolecule secretion, is a likely

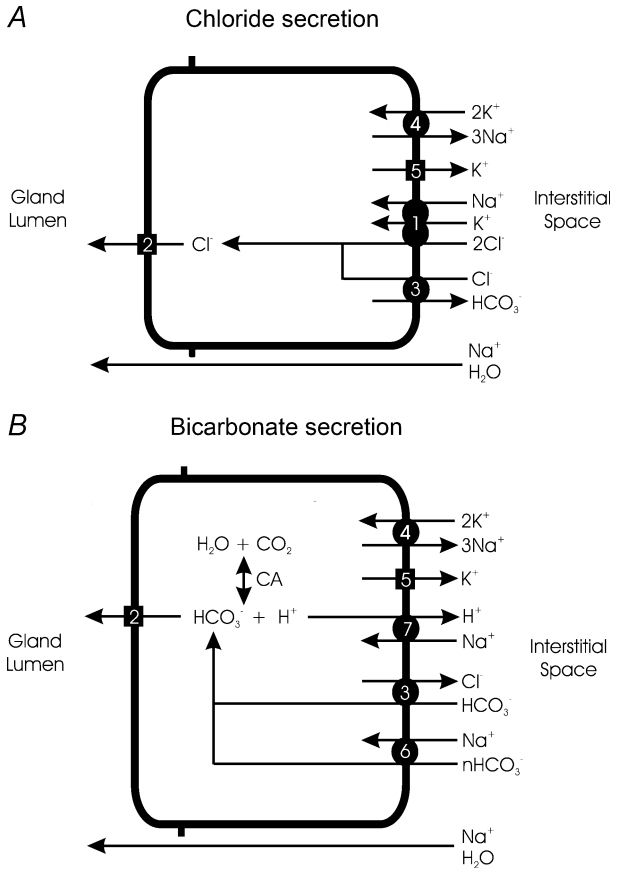

Figure 2. Summary of proposed cellular mechanisms for Cl^- and HCO_3^- secretion by serous submucosal gland cells
Though represented separately for clarity, it is likely that both anion secretion mechanisms exist in the same cell. See text for details. *A*, Cl^- secretion. Cl^- enters across the basolateral membrane by NKCC (1) and exits across the apical membrane through CFTR and possibly alternative anion channels (2). While NKCC is the major entry route for Cl^-, AE2 (3) could theoretically support Cl^- entry across the basolateral membrane if the electrochemical driving forces are appropriate. Na^+,K^+-ATPase (4) is localized to the basolateral membrane. Agonist stimulation is likely to result in activation of apical membrane anion channel(s) (2) and a population of K^+ channels on the basolateral membrane (5). *B*, HCO_3^- secretion. HCO_3^- is either transported across the basolateral membrane through electrogenic NBC (6) or AE2 (3), or it is generated intracellularly through the actions of carbonic anhydrase (CA). Intracellular generation of HCO_3^- also produces H^+, which is removed from the cell interior by NHE (7). HCO_3^- then exits across the apical membrane through the anion channels (2). Agonist stimulation similarly controls secretion by activating apical membrane anion channels (2) and basolateral membrane K^+ channels (5). Transepithelial secretion of either anion establishes a voltage gradient for cations, principally Na^+, to follow through the paracellular pathway. H_2O flows across the barrier, either paracellularly (as shown) or transcellularly, in response to the resultant osmotic gradient.

stimulant of gland fluid secretion as well (Schuster *et al.* 1992).

Mechanism of gland liquid secretion

Substantial information has been gathered on the cellular mechanism of ion and fluid secretion using a variety of preparations and species. A summary of the transport processes that are likely to be involved in this process, as discussed below, is shown in Fig. 2.

The secretion response to muscarinic agonists or substance P is vigorous and driven in large part by bumetanide-sensitive Cl^- secretion (Trout *et al.* 1998*a*, 2001; Joo *et al.* 2002*b*) indicating that this process is dependent upon Cl^- entry across the basolateral membrane by Na^+–K^+–$2Cl^-$ cotransport (NKCC) (Fig. 2*A*). Chloride exits the cell across the apical membrane through anion channels whose identity remains controversial. CFTR is a likely candidate for this role because antibodies to this protein localize to the apical membrane of gland serous cells *in situ* (Engelhardt *et al.* 1992; Jacquot *et al.* 1993) and bioelectric evidence for this channel has been found both in Calu-3 cells (Haws *et al.* 1994) and primary cultures of gland cells (Becq *et al.* 1993). However, the actions of ACh and substance P are likely to be transduced through inositol 1,4,5-triphosphate (IP_3), protein kinase C, and Ca^{2+} (Shimura *et al.* 1993; Nagaki *et al.* 1994; Sasaki *et al.* 1994) rather than the cAMP–protein kinase A pathway which is known to predominantly regulate CFTR (Tabcharani *et al.* 1991). This quandary has three possible explanations. First, CFTR in gland cells may be constitutively active even in the absence of cAMP-elevating agonists. Thus, anion efflux through the CFTR could be controlled chiefly by changes in cell membrane potential induced by alteration of basolateral K^+ conductance, a notion which has been proposed to explain the anion secretion responses to elevation of intracellular Ca^{2+} in both Calu-3 cells (Moon *et al.* 1997) and primary cultures of gland cells (Yamaya *et al.* 1993). Second, it is possible that CFTR-activating pathways lie downstream of Ca^{2+} and protein kinase C in the muscarinic and substance P signal transduction cascade. Third, it is possible that alternative Cl^- channels such as Ca^{2+}-activated Cl^- channels (CaCC) coexist with CFTR in the apical membrane and are activated by ACh and substance P through elevation of intracellular Ca^{2+} and/or activation of protein kinase C. Although functional studies have suggested that CFTR is the principal anion conductance pathway in the apical membrane of Calu-3 cells (Haws *et al.* 1994), muscarinic agonists do stimulate fluid secretion from glands in freshly excised CF airways,

which should not express functional CFTR (Joo *et al.* 2002*a*). While myoepithelial cell contraction, which occurs following cholinergic stimulation (Shimura *et al.* 1987), could mimic at least the initial secretion response of CF glands by compressing the glands and forcing the extrusion of resident mucus liquid from the lumen of the ducts, this effect should last only a few seconds and therefore minimally contribute to the observed volume secretion.

The electrical driving force for sustaining Cl^- efflux across the apical membrane of secretory epithelia is likely to be derived from increases in basolateral membrane K^+ conductance, which should hyperpolarize the cells (Smith & Frizzell, 1984). The identity of the specific population(s) of K^+ channels involved in the secretion responses to endogenous gland secretogogues, however, remains poorly defined. In Calu-3 cells, elevation of intracellular Ca^{2+} with thapsigargan activates both clofilium- and clotrimazole-sensitive conductances, which are likely to represent K_VLQT and intermediate-conductance Ca^{2+}-activated K^+ channels, respectively (Cowley & Linsdell, 2002). 1-EBIO activates intermediate-conductance Ca^{2+}-activated K^+ channels in Calu-3 cells that are clotrimazole- and charybdotoxin-sensitive (Devor *et al.* 1999), but this agonist has no measurable effect on fluid secretion by porcine airway glands (S. T. Ballard, unpublished observations).

In addition to Cl^- secretion, muscarinic agonists and substance P also stimulate HCO_3^- secretion from airway glands (Fig. 2*B*). In the presence of either ACh or substance P, HCO_3^- secretion appears to be primarily dependent upon intracellular generation of this anion since inhibitors of Na^+/H^+ exchange (NHE), such as dimethylamiloride, block this process (Trout *et al.* 1998*a*, 2001). The presence of carbonic anhydrase in gland cells suggests that these cells are capable of intracellular generation of HCO_3^- (Spicer *et al.* 1982). When intracellular HCO_3^- exceeds its electrochemical equilibrium, it can exit across the apical membrane through the CFTR (Poulsen *et al.* 1994) and perhaps through alternative anion channels as well. Trout *et al.* (1998*a*) report that the magnitude of HCO_3^- secretion relative to Cl^- secretion is low following application of these agonists as evidenced by the weak inhibitory effect of dimethylamiloride alone on ACh-induced liquid secretion (Trout *et al.* 1998*a*) and the relatively neutral pH of gland fluid (Jayaraman *et al.* 2001). Joo *et al.* (2002*b*) find that HCO_3^- may play a larger role in gland secretion since removal of HCO_3^- and CO_2 from the submucosal bath inhibits about half of the carbachol-induced gland liquid secretion in pig airways. In any event, ACh-induced secretion of HCO_3^- is sufficient to induce measurable alkalinization of an airway perfusate (Inglis *et al.* 2003). When Cl^- secretion is inhibited with bumetanide in the presence of ACh or substance P, HCO_3^- secretion is probably increased, evidenced by the nearly threefold increase in HCO_3^- concentration in secreted fluid, approximate doubling of the inhibitory effect of dimethylamiloride on the volume of secreted fluid (Trout *et al.* 1998*a*, 2001), and significantly increased alkalinization of luminal fluid (Inglis *et al.* 2003).

VIP is also an effective gland liquid secretogogue though sustained secretion rates induced with this agonist appear to be about 40% of that produced with cholinergic stimulation (Joo *et al.* 2002*b*). This peptide most likely induces secretion through cAMP and protein kinase A pathways because its effects are reproduced by forskolin, a direct activator of adenylyl cyclase. The anion channel which mediates the VIP response is certainly CFTR based on studies by Joo *et al.* (2002*a*) who showed that the VIP-sensitive fraction of human gland fluid secretion is absent in CF airways expressing severe CFTR mutations. Comparatively less is known about the mechanism of VIP-induced anion and liquid secretion in glands. The liquid secretion response of porcine glands to forskolin, as with ACh and substance P, is sensitive to bumetanide and removal of HCO_3^- from the bath, indicating that these airways secrete both Cl^- and HCO_3^- when stimulated with this agonist (Joo *et al.* 2002*b*). Several studies have investigated the actions of forskolin on anion secretion in the Calu-3 cell line. Devor *et al.* (1999) report that Calu-3 cells secrete HCO_3^-, rather than Cl^-, when exposed to forskolin and provide evidence that HCO_3^- transport is principally transcellular and dependent on Na^+ entry across the basolateral membrane via an electrogenic Na^+–HCO_3^- cotransporter (NBC). They reason that HCO_3^- is principally secreted when cell membrane potentials are near resting values. But, at hyperpolarizing membrane potentials that result from activating populations of basolateral membrane K^+ channels, HCO_3^- entry via NBC is inhibited, and a switch to Cl^- secretion is induced. In support of this notion, these investigators found that forskolin alone, which they expected to elevate cAMP and activate the CFTR, did not increase basolateral membrane K^+ conductance. However, this model may not fully represent the mechanisms by which these cells secrete anions since others report evidence that K_VLQT channels are activated by forskolin in Calu-3 cells (Cowley & Linsdell, 2002). The anion exchanger AE2 is also present in Calu-3 cells and reportedly capable of supporting HCO_3^--dependent Cl^- secretion (Loffing *et al.* 2000; Cuthbert

et al. 2003) and regulation of intracellular pH (Inglis *et al.* 2002) in this cell line. Given the appropriate driving forces, AE2 could potentially support Cl^--dependent HCO_3^- secretion as well.

Active secretion of Cl^- (and to a lesser extent HCO_3^-) across the glandular epithelial cells creates an electrical gradient for cations to passively follow through the paracellular pathway. Because of its abundance in extracellular liquid, Na^+ is the principal cation to move by this route and therefore is the major cation represented in gland secretions (Quinton, 1979; Jayaraman *et al.* 2001). The K^+ concentrations in gland liquid also resemble those of extracellular fluid suggesting that little, if any, active secretion of this cation occurs in submucosal glands (Quinton, 1979).

The osmotic gradient generated by ion secretion creates the driving force for water movement across the glandular epithelia. To support high rates of liquid secretion, the serous cells of glands must have a high permeability to water. Freeze-fracture studies show that the tight junctions which form between serous cells contain significantly fewer strands than junctions between mucous cells or the cuboidal epithelial cells that line the gland ducts (Schneeberger & McCormack, 1984). The tight junctions between serous cells are permeable to colloidal lanthanum whereas junctions between these other cell types are not (Schneeberger & McCormack, 1984). Because this marker solute penetrates spaces larger than 20 Å (Revel & Karnovsky, 1967), it is likely that the tubules lined by serous cells have a relatively high paracellular permeability to water and small solutes. Indeed, even though the model depicted in Fig. 2 is consistent with electrogenic anion secretion, induction of gland secretion is associated with little or no change in the transepithelial PD or short-circuit current of intact airways (Boucher & Gatzy, 1982; Trout *et al.* 1998*a*), consistent with the presence of 'leaky' paracellular junctions that typify secretory epithelia. Transcellular water movement across glandular epithelium is probably facilitated by expression of aquaporin 5 (AQP5), a mercury-sensitive water channel, in the apical membrane of submucosal gland cells (Kreda *et al.* 2001; Song & Verkman, 2001).

Currently, it is unclear whether the secreted acinar fluid is modified as it passes through the gland ducts to the airway surface. mRNA for both α and β subunits of epithelial Na^+ channels (ENaC) are expressed in human gland ducts, and it has been suggested that these channels might be involved in the absorption of salt and water (Burch *et al.* 1995). This notion is supported by observations that amiloride, an ENaC inhibitor, increases the rate of ACh-induced fluid secretion in porcine tracheal glands by 60% (Phillips *et al.* 2002*b*).

Role of submucosal glands in cystic fibrosis lung disease

The earliest histological indication of CF lung disease is seen in the third trimester of fetal life when the submucosal gland ducts become impacted with mucin (Ornoy *et al.* 1987). At birth, the lungs of CF neonates exhibit no overt signs of disease. However, infants afflicted with CF soon begin to express the clinical signs of the disease which include cough, production of a thick, inspissated mucus, impaired mucociliary transport, and unusually high susceptibility to microbial colonization. Hyperplasia of the submucosal glands and mucin occlusion of gland ducts are histological hallmarks of this disease (Zuelzer & Newton, 1949; Oppenheimer & Esterly, 1975). Because of the changes in gland morphology and the prominence of abnormal mucus in CF airways, a defect in submucosal gland secretion has been suspected as the root cause of this disease as far back as the 1940s (Zuelzer & Newton, 1949). For the decades that followed, the search for the primary defect in this disease focused largely on the physical and chemical properties of CF mucus which led to much confusion and little consensus about the mechanism of pathogenesis (Quinton, 1999). The discovery that CF was associated with a defect in epithelial Cl^- permeability (Quinton, 1983) dramatically shifted the emphasis of CF research away from mucus secretion and toward epithelial anion and fluid transport because of the lack of an obvious relationship between these processes. In 1989, the defective gene which causes CF was identified and found to normally code for the CFTR (Riordan *et al.* 1989), which was subsequently demonstrated to be a cAMP-activated anion channel (Anderson *et al.* 1991). Once antibodies and mRNA probes for this protein were available, CFTR was found to be highly expressed in the serous cells of the submucosal glands where it was speculated to mediate anion and liquid secretion (Engelhardt *et al.* 1992). Consequently, research into CF pulmonary pathogenesis has been gradually returning to the submucosal glands.

In the tracheobronchial airways of domestic pigs, pharmacological inhibition of ACh-induced Cl^- and HCO_3^- secretion from submucosal glands produces important correlates to CF pathology including occlusion of gland ducts with mucin (Inglis *et al.* 1997*b*), secretion of thick dehydrated mucus (Trout *et al.* 1998*b*), impairment of mucociliary transport (Ballard *et al.* 2002), and depletion of periciliary fluid with consequent flattening

of cilia at the airway surface (Trout *et al.* 2003). However, because of the unavailability of highly selective CFTR inhibitors, abolition of anion secretion inhibition in these studies required inhibition of basolateral membrane transporters or use of channel blockers that potentially target non-CFTR anion channels as well. Consequently, these studies could not conclude with certainty that CFTR is the sole channel that mediates anion and liquid secretion from glands. Indeed, as noted earlier, Joo *et al.* (2002*a*) report that the VIP/forskolin component of gland fluid secretion is absent in CF airways but that the muscarinic component remains at least partially intact. Because maximum liquid secretion rate with VIP is only about 40% of that produced with muscarinic agonists (Joo *et al.* 2002*a*), one must conclude that submucosal glands in CF airways retain the capacity, albeit reduced, to secrete liquid.

These observations establish important groundwork for future study into the role that submucosal glands play in the development of CF airway disease. For instance, how do the neuronal pathways that release VIP, ACh and substance P interact to control gland liquid and glycoprotein secretion? Under what conditions are each of these neurotransmitters released? Can loss of only the VIP component lead to the airway complications associated with CF? Do anion channels other than CFTR contribute significantly to gland secretion? If CF airway glands maintain the capability to secrete liquid to VIP-independent agonists, could these pathways be selectively manipulated to increase the delivery of low-viscosity fluid to the airway surface to rescue mucociliary transport in these patients? We are hopeful that the answers to these questions will lead to a better understanding of the mechanisms of submucosal gland secretion and CF pathogenesis.

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