Effects of secretagogues on net and unidirectional liquid fluxes across porcine bronchial airways

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Martens CJ, Ballard ST. Effects of secretagogues on net and unidirectional liquid fluxes across porcine bronchial airways. Am J Physiol Lung Cell Mol Physiol 298: L270–L276, 2010. First published November 13, 2009; doi:10.1152/ajplung.00253.2009.—Rates of liquid secretion and absorption across the bronchopulmonary airways are important for regulating airway surface liquid volume and maintaining mucociliary transport. The current study demonstrates the feasibility of measuring not just net liquid movements but unidirectional liquid movements across isolated intact bronchi from swine. Airways were liquid filled to assess both net liquid movements, and, in the presence of NPPB to selectively inhibit secretion, unidirectional absorption. Unidirectional liquid secretion rates were determined by subtraction. For comparison, net liquid movements were assessed in air-filled airways in parallel. In the absence of secretagogues, unidirectional absorption was observed (4.63 ± 0.53 μl·cm⁻²·h⁻¹) with little unidirectional secretion (1.42 ± 0.36 μl·cm⁻²·h⁻¹). ACh, substance P (SP), and vasoactive intestinal peptide (VIP) all induced unidirectional secretion (10.64 ± 1.52 μl·cm⁻²·h⁻¹, 14.16 ± 1.39 μl·cm⁻²·h⁻¹, and 4.25 ± 0.25 μl·cm⁻²·h⁻¹, respectively) without affecting unidirectional absorption. Net liquid secretion in air-filled airways was close to that in liquid-filled airways except with VIP. VIP luminal liquid is generally assumed to be coupled to the active epithelia have been extensively characterized. Absorption of epithelium imbedded in the submucosa possesses transepithelial and volume, the airway surface epithelium and the glandular becomes too thin or too deep (9). To regulate the ASL depth absorption was observed (4.63 ± 0.53 μl·cm⁻²·h⁻¹). One critical function of this continuous, thin layer of liquid. Proton extrusion across the transcellular transport of Na⁺ and Cl⁻ creates an osmotic gradient across the barrier, and water thus follows the ion movement since the airway epithelium is relatively permeable to water (12, 28, 42). Secretion of liquid is accomplished through the active transepithelial secretion of Cl⁻ and HCO₃⁻ (40). Chloride enters the epithelial cells across the basolateral membrane along with Na⁺ and K⁺ through NKCC cotransporters (44) and exits across the apical membrane through anion channels, which include the cystic fibrosis transmembrane conductance regulator (CFTR) channels and Ca²⁺-activated Cl⁻ channels (CaCC) (37). HCO₃⁻ secretion occurs by at least two mechanisms in airway epithelia (4). HCO₃⁻ can be synthesized along with H⁺ in the cytoplasm of airway epithelia through the actions of carbonic anhydrase.

The ion transport processes that exist in mammalian airway epithelia have been extensively characterized. Absorption of luminal liquid is generally assumed to be coupled to the active transepithelial absorption of Na⁺. This process involves absorption of Na⁺ across the apical membrane of the surface epithelial cells through epithelial Na⁺ channels (ENaC) and extrusion of this cation across the basolateral membrane through the Na⁺-K⁺-ATPases (9). This process is electrogenic in that the transepithelial transport of Na⁺ creates an interstitial-positive (lumen-negative) voltage that pulls anions (chiefly Cl⁻ as the most abundant anion in luminal solution) across the epithelial barrier through the paracellular pathway. This transepithelial absorptive transport of Na⁺ and Cl⁻ creates an osmotic gradient across the barrier, and water thus follows the ion movement since the airway epithelium is relatively permeable to water (12, 28, 42). Secretion of liquid is accomplished through the active transepithelial secretion of Cl⁻ and HCO₃⁻ (40). Chloride enters the epithelial cells across the basolateral membrane along with Na⁺ and K⁺ through NKCC cotransporters (44) and exits across the apical membrane through anion channels, which include the cystic fibrosis transmembrane conductance regulator (CFTR) channels and Ca²⁺-activated Cl⁻ channels (CaCC) (37). HCO₃⁻ secretion occurs by at least two mechanisms in airway epithelia (4). HCO₃⁻ can be synthesized along with H⁺ in the cytoplasm of airway epithelia through the actions of carbonic anhydrase.

Proton extrusion across the basolateral membrane via Na⁺/H⁺ exchangers (NHE) permits net synthesis of HCO₃⁻, which exits the cell across the apical membrane via anion channels. This general mechanism has been identified in both surface epithelium and in submucosal gland cells (5, 35). Alternatively, in gland secretory cells, depending on the stimulant, HCO₃⁻ can enter the epithelial cells directly across the basolateral cell membrane through Na⁺-HCO₃⁻ cotransporters (NBC) and then exit across the apical membrane through anion channels (6, 14). In both Cl⁻ and HCO₃⁻ transport models, it is assumed that the trancellular transport of anions is accompanied by cations (principally Na⁺ as the most abundant cation in extracellular solution) through the paracellular pathway with water following. Water flow across the airways is likely to occur both transeptally, where its flux is facilitated by the presence of aquaporins in both glandular and surface epithelial cells (42), and paracellularly, through the tight junctions. When secretion is induced, the tight junctions between the cells of glandular serous cells dilate, which likely facilitates liquid movement (34). Liquid secretion from glands can be voluminous and is well documented (5, 26, 30). On the other hand, liquid secretion across the native surface epithelium has proven very difficult to identify and quantitate. Typically, to reveal liquid secretion, Na⁺ and liquid absorption must be blocked with amiloride, as shown by Jiang and coworkers (24) in studies with cultured human airway epithelium. Unfortunately, this maneuver always raises the possibility that blockade of Na⁺ absorption per se induces anion and liquid secretion by the surface epithelium. To our knowledge, the only empirical evidence for anion and liquid secretion in “restiting” surface epithelia comes from studies of regulation of ASL in planar cultures of airway surface epithelial cells (38) where this process appears to play an important role in fine-tuning the

THE LUMINAL SURFACES of the airways of the lung are lined with a continuous, thin layer of liquid. One critical function of this airway surface liquid (ASL) is support of mucociliary clearance, which can be compromised if the depth of the ASL becomes too thin or too deep (9). To regulate the ASL depth and volume, the airway surface epithelium and the glandular epithelium imbedded in the submucosa possess transepithelial active ion transport mechanisms that are capable of driving the secretion or absorption of liquid across the barrier.

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ASL depth, particularly that of the periciliary liquid, which is critical for normal mucociliary transport.

For many years, studies of ion transport in airway epithelia have been performed predominantly with the short-circuit current technique of Ussing and Zerahn (41). This technique, where the transepithelial voltage is clamped at zero voltage by passing depolarizing current sufficient to null the voltage, provides a quantitative measure of the net active transepithelial ion (current) flow that gives rise to the transepithelial potential. Active ion transport across the surface epithelium, by the mechanisms described above, generates a voltage that can be quantitated by the Ussing technique. Unfortunately, active anion secretion by submucosal gland epithelium is largely electrically silent (10, 40); thus, the Ussing short-circuit current technique fails to adequately account for the contribution of glands to the net transepithelial ion transport. The reason for this anomaly is unclear, but it may be due to the very leaky nature of the glandular tight junctions, which may blunt development of the transepithelial potential when liquid secretion is induced. Consequently, the contribution of submucosal glands to the net transepithelial ion and liquid transport in tracheobronchial airways has not been fully appreciated until recent years.

Despite the great interest in the forces that drive fluid movements in these tissues, comparatively few studies have made measurements of water transport across intact airway epithelia, which must be considered to be more reliable estimates of in vivo behavior than measurements with cultured cells, whose physical properties and behavior could be unpredictably influenced by the conditions of culture. The capacitance probe technique, which measures changes in the volume of a sealed liquid-filled compartment that bathes one surface of native airway tissues mounted as a planar sheet in a flux chamber, has been used to approximate net transepithelial liquid volume movements in intact canine tracheas (43). A soluble volume marker has also been used to make similar measurements of liquid transport in intact porcine tracheas (12). Although informative, all of these techniques measure net liquid movements (i.e., the sum of liquid secretion and absorption). By desiccating the surface epithelium and layering it with oil, Quinton (30) and later Joo et al. (27) were able to isolate the unidirectional liquid secretion rates from individual submucosal glands, but this technique destroys the surface epithelium. Measurements of both unidirectional absorptive and secretive liquid fluxes in native airways could be very informative yet have not been made to our knowledge. The ability to quantitate these respective pathways is important considering the present controversies regarding the pathogenesis of cystic fibrosis (CF) airway disease. Loss of CFTR activity in CF airways reportedly causes both reduced unidirectional anion and liquid secretion from intact airway glands (25) and accelerated unidirectional Na+ and liquid absorption from cultured airway surface epithelial cells (24). Either of these two mechanisms could independently give rise to a net reduction of airway surface liquid volume and account for the reductions in mucociliary clearance and thickened luminal mucus that characterize this disease. Given the likely availability of non-rodent, mammalian animal models of CF in the near future (32, 36), a quantitative technique that would be capable of distinguishing between these two liquid-handling scenarios by native intact airways would be of potential benefit.

In the present study, we modified the cannulated porcine airway preparation to allow estimation of both unidirectional secretion and unidirectional absorption of liquid by the airway epithelium. We achieved this by filling the air space of cannulated bronchi with liquid and measuring changes in this volume over time under two conditions: 1) in the presence of 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), which selectively blocked anion and liquid secretion, thus revealing unidirectional liquid absorptive flux and 2) in the absence of this inhibitor, which represented the net liquid flux. From these two conditions, the unidirectional liquid secretive flux was calculated as the difference between the net and unidirectional absorptive fluxes. The effects of three major physiological secretagogues on the net and unidirectional liquid fluxes were assessed.

METHODS

All procedures involving animal use were reviewed and approved by the University of South Alabama Institutional Animal Care and Use Committee and complied with U.S. Department of Health and Human Services guidelines for the care and use of laboratory animals. Young pigs (10–15 kg) were obtained from a local vendor. The animals were sedated with intramuscular injections of xylazine (0.8 mg/kg) and ketamine (16 mg/kg) and euthanized with an intravenous overdose of pentobarbital sodium through an ear vein. The lungs were excised and placed in Krebs Ringer bicarbonate solution (KRB) at room temperature. Intrapulmonary bronchi (~30–40 mm in length and 3–4 mm in diameter) were dissected intact from lung lobes. Side branches of the airways were ligated with 5–0 silk suture. The isolated bronchi were placed in 80-ml KRB baths at room temperature and slowly warmed (~0.25°C/min) to 37°C.

Once the solutions reached physiological temperature, one of three bronchi was pretreated with 300 μM NPPB to block anion and liquid secretion. The other two airways were pretreated only with the DMSO vehicle (1:1000 dilution). After a 45-min pretreatment, the airways were removed from the bath solutions, and all accessible mucus and liquid were aspirated from the airway lumina. The airways were then tied onto polyethylene cannulas with suture and returned to their respective bath solutions where they were suspended vertically. The lower cannulas were plugged in advance with silicone glue. In this way, airway secretions and instilled liquid solutions would not leak out of the airway lumina during the experiments.

After cannulation, the lumina of the NPPB-pretreated airway and one of the other airways were filled with a measured volume of KRB (82.2 ± 0.8 μl) that was sufficient to completely fill the lumens. The third bronchus remained air-filled. At this point, all three bronchi were treated with one of three secretagogues at concentrations sufficient to induce near-maximal liquid secretion responses: 10 μM ACh, 1 μM substance P, or 100 nM vasoactive intestinal peptide (VIP). These concentrations of the secretion agonists are sufficient to provide near-maximal liquid secretion rates in porcine bronchi (Ballard, unpublished observations). One group of airways received no secretagogue treatment. Fifteen minutes before substance P addition, 1 μM phosphoramidon, a protease inhibitor that prevents breakdown of substance P, was added to the bath. Similarly, fifteen min before VIP addition, the protease inhibitors phosphoramidon (1 μM) and thiorphan (5 μM) were added to the bath to prevent VIP breakdown. A control group of airways was not exposed to the secretagogues to assess the resting behavior of the tissues.

After the 2-h incubation period with the secretagogues, the bronchi were removed from their cannulas and sectioned lengthwise. All accessible mucus liquid was collected from the lumina and transferred.
to tared microcentrifuge tubes. The tubes were then weighed to determine secretion volume. The length (L) and outer diameter (D) of each bronchus was measured and used to calculate luminal surface area (SA = 0.682DπL). This formula was previously derived to account for differences in surface area between the inner and outer airway surfaces (40). It was considered that a small yet measurable volume of liquid may have remained in the airways following the initial aspiration that preceded cannulation. To determine this volume, four bronchi were dissected and cannulated as described above. Then, the airways were immediately removed from their cannulae and sectioned lengthwise, and all residual luminal liquid was collected with pipettes. The average liquid volume collected from each of these four airways was 4.85 μL. Liquid volume flux rates (J_v) were calculated from the change in luminal liquid volume, airway surface area, and the period of exposure to the secretagogues. Positive flux values indicated secretion (i.e., fluid movement into the airway lumen), whereas negative flux values indicated absorption (i.e., fluid movement out the airway lumen). The 4.85-μL residual volume was mathematically added to the initial instillate volumes in the liquid-filled airways and the sum taken as the initial instillate volume in the air-filled airways for the purpose of the J_v calculations. It is important to emphasize that unidirectional liquid fluxes could not be assessed in air-filled airways because the liquid instillate, which was the substrate for absorption, was not added to the lumen, and the endogenously secreted liquid was not produced in volumes sufficient to assess absorptive capacity. Net liquid fluxes in air-filled bronchi, which more closely mimic the in vivo condition, were, however, performed in parallel to provide a physiological comparator to the liquid-filled airways.

J_v was measured under three conditions. First, in initially air-filled airways, it was assumed that the lumen contained only the small residual volume at the beginning of the experiment; thus, even minimal liquid absorption (negative J_v), if it occurred, could not be directly assessed with this preparation. A gain in luminal volume in the initially air-filled airways was likely to have been the consequence of a combination of unidirectional secretory J_v and some magnitude of unidirectional absorptive J_v. This initially air-filled condition was deemed to more closely represent the native state and behavior of airways in the lung. Second, airways were initially filled with a known volume of KRB, but the airways were treated with NPPB to block anion and liquid secretion, unidirectional absorptive J_v occurred (−4.63 ± 0.53 μL·cm⁻²·h⁻¹, n = 9) and significantly different (P < 0.05) from the net J_v in air-filled airways. When liquid-filled airways were pretreated with NPPB to block anion and liquid secretion, unidirectional absorptive J_v occurred (−5.09 ± 0.52 μL·cm⁻²·h⁻¹, n = 9) that approximated the net absorptive J_v. Unidirectional secretory J_v, determined by subtraction, was negligible (±0.46 ± 0.54 μL·cm⁻²·h⁻¹, n = 9). These measures indicate that under basal, unstimulated conditions, excised porcine bronchial airways absorb liquid with little, if any, secretion occurring.

When bronchi were treated with ACh, net secretory J_v was observed in both air-filled (+10.64 ± 1.52 μL·cm⁻²·h⁻¹, n = 7) and liquid-filled bronchi (+8.38 ± 1.51 μL·cm⁻²·h⁻¹, n = 7) (Fig. 2). Although net secretory J_v was higher in the air-filled bronchi, this difference was not significant. Unidirectional absorptive J_v (−6.38 ± 0.88 μL·cm⁻²·h⁻¹, n = 7) was similar to that seen in control tissues. ACh induced a substantial unidirectional secretory J_v (±14.76 ± 1.25 μL·cm⁻²·h⁻¹, n = 7). These results indicate that ACh selectively stimulates the unidirectional secretory pathway without affecting the unidirectional absorptive pathway in pig bronchi.

Substance P induced changes in J_v that were nearly identical to those seen with ACh (Fig. 3). Increased net secretory J_v occurred in both air-filled (+12.20 ± 1.36 μL·cm⁻²·h⁻¹, n = 9) and liquid-filled bronchi (+8.87 ± 1.18 μL·cm⁻²·h⁻¹, n =

RESULTS

In control air-filled tissues (no secretagogues present), a small secretory J_v was observed (+1.42 ± 0.36 μL·cm⁻²·h⁻¹, n = 9) (Fig. 1). However, when the airway lumina were filled with KRB, net J_v was absorptive (−4.63 ± 0.53 μL·cm⁻²·h⁻¹, n = 9) and significantly different (P < 0.05) from the net J_v in air-filled airways. When liquid-filled airways were pretreated with NPPB to block anion and liquid secretion, unidirectional absorptive J_v occurred (−5.09 ± 0.52 μL·cm⁻²·h⁻¹, n = 9) that approximated the net absorptive J_v. Unidirectional secretory J_v, determined by subtraction, was negligible (±0.46 ± 0.54 μL·cm⁻²·h⁻¹, n = 9). These measures indicate that under basal, unstimulated conditions, excised porcine bronchial airways absorb liquid with little, if any, secretion occurring.

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![Fig. 1. Net and unidirectional liquid fluxes across porcine bronchial airways under resting conditions](http://ajplung.physiology.org/)

Net liquid fluxes (J_v-net) were measured in air-filled and liquid-filled bronchi. Unidirectional liquid absorptive flux (J_v-ab) was measured in liquid-filled bronchi that had been pretreated with NPPB to block unidirectional anion and liquid secretion. Unidirectional liquid secretory flux (J_v-sc) was determined by subtracting J_v-net from J_v-net in air-filled bronchi was significantly different (*) from J_v-net in liquid-filled bronchi. Asterisk indicates that the secretory J_v-net in air-filled airways was significantly (P < 0.05) greater than J_v-net in liquid-filled airways.
Fig. 2. Effect of ACh on net and unidirectional liquid fluxes across porcine bronchi. The ordinate indicates the rate of Jv. ACh was added to all tissues. Jv-net were measured in air-filled and liquid-filled bronchi. Jv-ab was measured in liquid-filled bronchi that had been pretreated with NPPB to block unidirectional anion and liquid secretion. Jv-sc was determined by subtracting Jv-ab from Jv-net.

9). As with ACh, the net secretory Jv was higher in the air-filled bronchi than in the liquid-filled bronchi, but this difference was insignificant. Unidirectional secretory Jv was substantial (+14.16 ± 1.39 μl/cm²-h⁻¹, n = 9), whereas unidirectional absorptive Jv closely resembled the untreated controls (–5.28 ± 0.53 μl/cm²-h⁻¹, n = 9). Thus, substance P, similar to ACh, substantially increased unidirectional secretion without appreciably affecting the unidirectional absorptive rate.

VIP induced a secretory Jv in air-filled bronchi (+4.25 ± 0.25 μl/cm²-h⁻¹, n = 8) that was smaller in magnitude than observed with ACh or substance P treatment (Fig. 4). Surprisingly, net Jv in VIP-treated liquid-filled bronchi was close to zero (–1.07 ± 1.09 μl/cm²-h⁻¹, n = 8), which was significantly different (P < 0.05) than the air-filled Jv. Unidirectional secretory Jv (+4.44 ± 1.26 μl/cm²-h⁻¹, n = 8) was very similar in magnitude to the net Jv seen in air-filled airways. Unidirectional absorptive Jv in VIP-treated airways (–5.51 ± 0.81 μl/cm²-h⁻¹, n = 8), as with ACh- and substance P-treated airways, was very close to control values. As with ACh and substance P responses, VIP preferentially stimulated unidirectional secretory Jv and had essentially no effect on unidirectional absorptive Jv. There was, however, a noticeable disconnect between the net Jv in the air-filled airways, where liquid secretion occurred, and the net Jv in liquid-filled airways, where little change was observed.

One possible explanation for the discrepancy between the VIP-induced net Jv in the liquid-filled airways and the air-filled airways is that the liquid secreted in the air-filled airways had a high macromolecular content, which formed a thicker mucus gel that could have retarded liquid absorption. To test this notion, the liquid secreted by the cannulated bronchi in response to each of the three agonists was collected, and the nonvolatile solids content was determined. The nonvolatile solids were equivalent in the ACh-treated airways (2.06 ± 0.07%, n = 8) and substance P-treated airways (2.15 ± 0.07%, n = 8) (Fig. 5). However, nonvolatile solids content of the secreted liquid in the VIP-treated airways was significantly (P < 0.001) higher (3.98 ± 0.26%, n = 14) than liquid secreted in response to ACh or substance P. When the contribution of the nonvolatile solids content of the Krebs buffer alone (1.25%) was accounted for, the nonvolatile mucus solids in the VIP-induced liquid was approximately three times that of the ACh- and substance P-induced liquid.

**DISCUSSION**

This study demonstrates that it is feasible to measure unidirectional liquid fluxes across the epithelium of intact bronchial airways. Using three physiological secretagogues (ACh, substance P, and VIP), we document that these agents variably induced net and unidirectional liquid secretion in these tissues. We also show that unidirectional liquid absorption was relatively consistent in the liquid-filled airways and not appreciably affected by the presence of any of the three secretagogues.
In the air-filled airways, VIP induced the secretion of a thicker (i.e., higher solids content) mucous liquid than ACh or substance P administration, which corresponded to the lower net liquid absorption rates in the VIP-treated tissues. To achieve measures of unidirectional secretive \( J_v \) in these studies, it was necessary to block, as completely as possible, either unidirectional absorptive \( J_a \) or unidirectional secretive \( J_s \) in one set of airways. For this purpose, we used NPPB, which has been shown in previous studies to be an efficacious inhibitor of bronchial liquid secretion responses to ACh (5), substance P (39), and forskolin (6), agents that all appeared to induce liquid secretion that originates largely, if not entirely, from the submucosal glands. NPPB is well documented as an inhibitor of anion channels, including CFTR (19, 46) and CaCC (2), as well as some K\(^+\) channels (17, 23). Despite possible targetting of multiple channels, this agent does not appear to alter absorption of ions and liquid, since the basal rate of liquid absorption in the control bronchi (without secretagogues) was unaffected by NPPB (Fig. 1). Initially, we considered that ENaC inhibitors could be used to block active Na\(^+\) and liquid absorption. However, preliminary studies indicated that absorption of liquid from the air space of porcine-cannulated bronchi was insensitive to benzamil, a relatively selective ENaC inhibitor (7). Additional preliminary experiments similarly indicated that amiloride was an ineffective blocker of liquid absorption from porcine bronchi (Ballard, unpublished observations). This was surprising to us since ENaC inhibitors have long been recognized as blockers of active Na\(^+\) absorption across airway epithelium. Indeed, a previous study from our laboratory of porcine bronchi documented that amiloride partially inhibited the transepithelial potential in perfused bronchi of similar size and generation as those in the present study (8). Nonetheless, amiloride and its congeners appear to be relatively poor blockers of liquid absorption in intrapulmonary bronchi from pigs, and thus could not be used by us to block the unidirectional liquid absorption in these tissues. We speculate that the poor efficacy of these ENaC inhibitors in our studies is due to their rapid absorption across the apical membrane of the surface epithelial cells, a phenomenon that has been documented in airway epithelial cell cultures (21). It is of note that luminal amiloride only blocks 40% of the rate of liquid absorption from the air space of resected, fluid-filled human lungs, a process that is also presumed to be driven by an ENaC-dependent process (33).

The three secretagogues used in this study are all known inducers of anion and liquid secretion from airway submucosal glands (5, 26, 39). In the present study, ACh and substance P both induced high rates of unidirectional secretive \( J_s \). In contrast, VIP induced a comparatively modest unidirectional secretive \( J_s \). Indeed, in the liquid-filled condition, the VIP unidirectional secretion and unidirectional absorption rates were nearly equal in magnitude, resulting in negligible net \( J_s \). Attributing physiological relevance to these quantitative rates is difficult, but clearly ACh and substance P are capable at these maximal rates of flooding the air space with mucous liquid. One must assume that VIP-induced secretion, which in humans is entirely CFTR dependent (25), must play a lesser role in the regulation of ASL fluid dynamics. This concept has been recognized and discussed by Wine (45), who suggested that CFTR-dependent ion and liquid secretion perhaps served to fine-tune the balance of ASL and mucociliary transport rather than to radically accelerate clearance. Interestingly, substance P, whose effects on secretion are CFTR dependent (11, 22), is a less effective agonist for liquid secretion in humans (11).

Although the magnitude of the differences was variable, a consistent observation in these studies was that the rate of net secretive \( J_s \) in the air-filled bronchi exceeded the net secretory flux in the liquid-filled bronchi. Reduced liquid absorption was particularly notable in the VIP-treated airways where virtually no reabsorption of secreted liquid occurred when the airways were air-filled (Fig. 5). Two possible explanations could account for this observation. First, not all of the secreted liquid in the air-filled airways may have been capable of being reabsorbed. This notion is plausible since secreted liquid contains some level of gel-forming mucous macromolecules, and not all of the water can be absorbed from such gels due to the forces of molecular crowding and the Donnan effects on electrolyte distributions that also influence hydration in negatively charged, interstitial matrix gels (3, 18). Unfortunately, little information is available about the forces of hydration in airway mucous gels and whether this property is sufficient to account for this observation. Second, the lower volumes of ASL in the air-filled airways may have inhibited reabsorption by concentrating secreted protease inhibitors that reduced ENaC activity by blocking protease activation of this channel by endogenous mechanisms (29). Unfortunately, it would be difficult to assess the importance of this possible mechanism in our preparation since preliminary experiments indicated that ENaC inhibitors were largely ineffective at blocking liquid absorption.

Studying transepithelial ion transport in intact tracheas from sheep, Acevedo (1) reported that ACh both stimulated active \( \text{Cl}^- \) secretion and inhibited active Na\(^+\) absorption. While our studies of unidirectional liquid transport in porcine bronchi corroborate the increase in anion secretion, we did not observe changes in liquid absorption. We cannot currently account for this discrepancy, although it could have resulted from either regional (trachea vs. intrapulmonary bronchi) or species (sheep vs. pig) differences between the two studies. Acevedo did not measure liquid transport in her study but made conclusions...
from the bioelectric properties and radioisotopic fluxes across these excised airways.

We are aware of only one study where the capacitance probe technique was used to measure net liquid fluxes across intact native airways. Working with isolated canine trachea, Welsh and coworkers (43) measured the net liquid transport rates under both basal conditions and following stimulation with aminophylline, a phosphodiesterase inhibitor that induces Cl− secretion. In contrast to our results, these investigators observed a small level of liquid secretion (2.6 µl·cm⁻²·h⁻¹) under basal conditions; however, the aminophylline-induced secretion rates (8.2 µl·cm⁻²·h⁻¹) were comparable to secretion rates measured in the present study. It is assumed that aminophylline activated CFTR in canine trachea due to its cAMP-elevating activity. Using cultures of human tracheal epithelial cells, Jiang et al. (24) also used the capacitance probe technique to study liquid movements and reported resting absorptive Jv rates of 4.3 ± 0.5 µl·cm⁻²·h⁻¹, which are close to the resting values observed in the present study. Crews et al. (12) used blue dextran as a volume probe to measure liquid transport properties of porcine trachea under resting, unstimulated conditions. In this study, absorptive Jv was ~37 µl·cm⁻²·h⁻¹, and liquid absorption across the pig trachea was abolished by amiloride and substantially reduced by isomolar replacement of Na⁺ with choline in the luminal solution. This magnitude of liquid absorption is roughly eightfold greater than observed in the porcine intrapulmonary bronchi. We speculate that these high secretion rates reflect differences in transport activity between the trachea and intrapulmonary bronchi in swine.

Grubb and coworkers (20) cultured airway epithelial cells on the inside walls of hollow permeable biofibers and measured rates of liquid flux. They reported resting absorption rates of 0.65 µl·cm⁻²·h⁻¹ and 2.21 µl·cm⁻²·h⁻¹ in canine tracheal and bronchial epithelial cells, respectively.

The driving force for transepithelial liquid secretion and absorption in the airways is assumed to be transepithelial active ion transport. Several studies of the transepithelial ion transport properties of intact airways were performed in the 1980s before the current pervasive use of cultured epithelial cells. Assuming that the airway epithelial barriers were permeable to water and that the net volume of transported fluid was essentially isotonic saline, transepithelial liquid transport can be estimated from the unidirectional and net transepithelial fluxes of radioisotopes of Na⁺ and Cl⁻ in these studies. Boucher and Gatzy (10) reported that under unstimulated, open-circuit conditions, canine intraparenchymal bronchi actively absorbed 1.78 µeq Na⁺·cm⁻²·h⁻¹ and 1.65 µeq Cl⁻·cm⁻²·h⁻¹. Assuming that absorbed fluid was composed of 145 meq Na⁺/l and 145 meq Cl⁻/l, these values indicate that the tissues absorbed ~12 µl·cm⁻²·h⁻¹, which is about two to three times greater than the unidirectional absorptive rates measured in the present study. In their study, liquid absorption was driven by active absorption of Na⁺. Application of ACh to the bronchi under open-circuit conditions reduced the net absorption of these ions, presumably by inducing secretion of ions and liquid that opposed their absorption. The authors noted that the effects of ACh were electrically silent in canine bronchi in that they were not reflected in the transepithelial PD or short-circuit current of the tissues (10). Trout and coworkers (40) similarly concluded that ACh-induced liquid secretion was poorly reflected in the bioelectric properties of porcine bronchi.

We believe that the technique described here could be useful in future studies of the pathogenesis of CF airway disease. Numerous hypotheses have been forwarded over recent decades to explain the unusual pulmonary pathology of this disease. CF patients typically develop progressive obstruction of the airways with unusually thick mucus, increased susceptibility to bacterial colonization, and reductions in mucociliary clearance (13). CF disease is caused by loss of function mutations in the gene that codes for the CFTR (31), an anion channel that is found in the apical membrane in both the surface epithelial cells and serous cells of the submucosal glands (16). Unquestionably, the normally functioning CFTR is capable of supporting transepithelial secretion of Cl⁻, HCO₃⁻, and liquid. Indeed, liquid secretion from submucosal glands, induced by either VIP or substance P, has been shown to be CFTR dependent, whereas cholinergically induced liquid secretion appears to be largely CFTR independent (11, 25).

Loss of the capability to secrete significant quantities of liquid in CF airways could presumably account for the airway disease in CF. On the other hand, considerable evidence indicates that loss of CFTR activity in CF airways results in hyperabsorption of Na⁺, and complications related to reduced ASL volume (reduced mucociliary clearance and thickened mucus) could alternatively be due to this mechanism (15). The technique used in the present study should prove useful for distinguishing between increased unidirectional absorptive Jv and decreased unidirectional secretive Jv in the recently generated porcine (32) and ferret (36) models of CF airway disease that should be available in the near future.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


