Effect of anion secretion inhibitors on mucin content of airway submucosal gland ducts

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Inglis, Sarah K., Michel R. Corboz, and Stephen T. Ballard. Effect of anion secretion inhibitors on mucin content of airway submucosal gland ducts. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L762–L766, 1998.—In porcine bronchi, inhibition of both Cl− and HCO3− transport is required to block the anion secretion response to ACh and to cause mucin accumulation within ACh-treated submucosal gland ducts [S. K. Inglis, M. R. Corboz, A. E. Taylor, and S. T. Ballard. Am. J. Physiol. 272 (Lung Cell. Mol. Physiol. 16): L372–L377, 1997]. In this previous study, a combination of three potential HCO3− transport inhibitors [1 mM acetazolamide, 1 mM DIDS, and 0.1 mM dimethylamiloride (DMA)] was used to block carbonic anhydrase, Cl−/HCO3− exchange, and Na+/H+ exchange, respectively. The aim of the present study was to obtain a better understanding of the mechanism of ACh-induced HCO3− secretion in airway glands by determining which of the three inhibitors, in combination with bumetanide, was required to block anion secretion and so cause ductal mucin accumulation. Gland duct mucin content was measured in distal bronchi isolated from domestic pigs. Addition of either bumetanide alone, bumetanide plus acetazolamide, or DIDS plus DMA had no significant effect on ACh-induced mean gland duct mucin content. In contrast, glands treated with bumetanide plus DMA as well as glands treated with all four anion transport blockers were almost completely occluded with mucin after the addition of ACh. These data suggest that mucin is cleared from the ducts of bronchial submucosal glands by liquid generated from Cl− and DMA-sensitive HCO3− transport.

Acetyicholine; airway epithelium; bicarbonate secretion; bronchi; cystic fibrosis; mucus

METHODS

Airway excision. Young domestic pigs (8–18 kg) were obtained from a local vendor. The animals were sedated with intramuscular injections of ketamine (8 mg/kg) and xylazine (0.4 mg/kg) and killed with an intravenous overdose of pentobarbital sodium. Portions of the left and right lungs were rapidly excised and placed in Krebs-Ringer bicarbonate (KRB) solution at room temperature (25°C). Distal bronchi (external diameter 3–4 mm) were carefully dissected from the surrounding parenchyma, transferred to a tissue bath containing KRB solution, and slowly warmed (0.1–0.2°C/min) from room temperature to 37°C.

Histological analysis of glandular mucin content. Isolated, warmed distal bronchi were cut into 1-cm lengths, and side branches were severed flush with the main airway trunk. The bronchi were then incubated for 30 min in KRB solution containing blockers of Cl− and HCO3− secretion. Bumetanide (10 µM), a blocker of Na+–K+–2Cl− cotransport, was used to inhibit Cl− secretion. Blockers of HCO3− secretion were chosen based on the model for airway epithelial HCO3− secretion proposed by Smith and Welsh (18). According to this model, HCO3− and H+ are produced from CO2 and H2O by intracellular carbonic anhydrase. Protons are extruded from the cell by the CFTR. They can be proposed by Smith and Welsh (18). According to this model, HCO3− and H+ are produced from CO2 and H2O by intracellular carbonic anhydrase. Protons are extruded from the cell by the CFTR.
ANION SECRETION AND AIRWAY GLAND MUCIN

Histological analysis of glandular mucin content. To determine which anion transporters are required to keep submucosal gland ducts clear of mucin, bronchi were pretreated with various combinations of the anion transport blockers before the addition of ACh. The gland duct mucin contents for each of the five different treatments are shown in Figs. 2 and 3. Ductal mucin content was scored on a linear scale from zero (duct contains no mucin) to five (duct occluded with mucin; see METHODS). Figure 2 shows the frequency distribution of mucin scores, and Fig. 3 shows the average mucin content of the gland ducts. In bronchi treated only with ACh, >50% of the ducts contained no measurable mucin (Fig. 2) and mean ductal mucin content was 0.66 ± 0.07 (Fig. 3). In contrast, gland ducts from bronchi that were pretreated with all four anion transport blockers before ACh treatment contained significantly greater quantities of mucin (3.60 ± 0.09; Fig. 3). Forty percent of the ducts in these bronchi were completely occluded with mucin (Fig. 2). Mucin content of gland ducts pretreated with either bumetanide alone (1.33 ± 0.46), bumetanide and acetazolamide (1.95 ± 0.45), or bumetanide and DIDS (1.32 ± 0.45) was not significantly different from gland ducts treated only with ACh (Fig. 3). In contrast, the mucin content of gland ducts that were pretreated with bumetanide and DMA (3.61 ± 0.21) was comparable to the mucin content of gland ducts pretreated with all four anion transport blockers (Fig. 3) and was significantly greater than tissues that were pretreated with either no inhibitor, bumetanide alone, bumetanide and acetazolamide, or bumetanide and DIDS. Forty-one percent of the ducts that were pretreated with bumetanide and DMA were completely occluded with mucin (Fig. 2). These results confirm previous findings that glands pretreated with Cl⁻ and HCO₃⁻ secretion blockers become

\[ \text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{H}^+ + \text{OH}^- \]

\[ \text{HCO}_3^- + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{CO}_2 \]

\[ \text{Na}^+ + \text{HCO}_3^- \rightarrow \text{Na}^+ \text{H}_2\text{O} + \text{CO}_2 \]

\[ \text{Cl}^- + \text{HCO}_3^- \rightarrow \text{Cl}^- \text{H}_2\text{O} + \text{CO}_2 \]

\[ \text{K}^+ + \text{HCO}_3^- \rightarrow \text{K}^+ \text{H}_2\text{O} + \text{CO}_2 \]

Fig. 1. Model of potential anion secretion pathways in airway epithelial cells. Model of anion secretion is modified from Smith and Welsh (18) to account for possible existence of basolateral Cl⁻/HCO₃⁻ exchange. Cl⁻ secretion: Cl⁻ enters cell by basolateral Na⁺-K⁺-2Cl⁻ cotransport and exits through an apical anion channel. HCO₃⁻ secretion: HCO₃⁻ and H⁺ are formed inside cell by action of carbonic anhydrase (ca). H⁺ passes across basolateral membrane by Na⁺-H⁺ exchange and HCO₃⁻ exits cell through an apical anion channel. Alternatively, HCO₃⁻ could enter cell by basolateral Cl⁻/HCO₃⁻ exchange and exit through an apical anion channel. Dotted lines, intended site of inhibitor action.
occluded with mucin when ACh is added (9). Photomicrographs of tissues pretreated with either bumetanide and DMA or the DMSO vehicle before the addition of ACh illustrate the extent of gland duct occlusion induced by these agents (Fig. 4).

**DISCUSSION**

A previous study (9) of distal bronchi provided indirect evidence that 1) Cl⁻ and HCO₃⁻ secretion drives the glandular liquid secretion response to ACh and 2) ACh-induced glandular liquid secretion can be uncoupled from mucus secretion by pretreatment with blockers of both Cl⁻ (bumetanide) and HCO₃⁻ (acetazolamide, DMA, and DIDS) transport. Unfortunately, this previous study provided little insight into the mechanism of HCO₃⁻ secretion. In the present study, measurements of gland duct mucin content were used to determine which of the three HCO₃⁻ secretion blockers is specifically required to inhibit ACh-induced, HCO₃⁻-dependent liquid secretion. Pretreatment with either acetazolamide or DIDS, in combination with bumetanide, did not result in mucin accumulation after the addition of ACh. In contrast, DMA plus bumetanide was as effective as the cocktail of all three HCO₃⁻ blockers plus bumetanide at causing gland duct mucin accumulation after addition of ACh. These results suggest that DMA-sensitive Na⁺/H⁺ exchange is critical for glandular HCO₃⁻ and liquid secretion.

The finding that glands pretreated with DMA become occluded with mucin after addition of ACh is consistent with a major role for Na⁺/H⁺ exchange in airway gland
HCO₃⁻ secretion. Similar models of HCO₃⁻ secretion that require Na⁺/H⁺ exchange have been described in a variety of tissues including pancreatic ducts, ileum, and salivary acini (3, 13, 14). Muscarinic agonists are known to stimulate Na⁺/H⁺ exchange in acinar cells of the pancreas and salivary, lacrimal, and nasal glands (4, 6, 16, 22). The mechanism for ACh-induced stimulation of the Na⁺/H⁺ exchanger has not been fully determined, but elevation of intracellular calcium concentration is likely to play a role (22). An alternative explanation for the DMA-induced mucin occlusion of submucosal gland ducts could be that DMA stimulates gland mucin secretion. However, in a previous study, Inglis et al. (9) demonstrated that the combination of DMA, acetazolamide, and DIDS had no effect on gland duct mucin content in the absence of bumetanide. If DMA indeed stimulated mucin secretion, we would have expected an increase in ductal mucin content under those conditions. This previous study also demonstrated that the effect of the HCO₃⁻ blockers on gland mucin secretion is reversible, indicating that the blockers are unlikely to be toxic to the tissues.
The inability of DIDS to affect gland duct mucin content suggests that Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchange activity is low or absent from airway glandular epithelium. Taken together, the effects of DMA and DIDS on ductal mucin accumulation suggest that the majority of secreted HCO\(_3\)\(^{-}\) is generated within the cell cytoplasm and that little or no HCO\(_3\)\(^{-}\) enters the cells across the basolateral membrane (Fig. 1). It is surprising, therefore, that acetazolamide did not induce glandular mucin accumulation. It is possible that carbonic anhydrase was incompletely blocked despite the presence of acetazolamide or that the uncatalyzed generation of intracellular HCO\(_3\)\(^{-}\) supported sufficient liquid secretion to flush mucin from the gland ducts. Ductal mucin content is unlikely to be a truly quantitative measure of gland mucin from the gland ducts. Ductal mucin content is generated within the cell cytoplasm and that uncatalyzed generation of intracellular HCO\(_3\)\(^{-}\) supported sufficient liquid secretion to flush mucin from the gland ducts. Ductal mucin content is unlikely to be a truly quantitative measure of gland mucin from the gland ducts. Ductal mucin content is probably required to flush mucin from the gland ducts. If gland liquid secretion is only partially inhibited, mucin may still be adequately cleared.

Evidence for HCO\(_3\)\(^{-}\) secretion has been found in nasal glands (6) as well as in tracheal (18), bronchial (7), and bronchiolar epithelia (20). Although its physiological role has not yet been determined, airway epithelial HCO\(_3\)\(^{-}\) secretion may play an important role in the regulation of airway surface liquid pH. Because both the physical properties of mucus (19) and the activity of cilia (12) are sensitive to changes in H\(^{+}\) concentration, mucociliary clearance could be strongly influenced by the regulation of airway surface liquid pH.

In summary, this study suggests that ACh-induced HCO\(_3\)\(^{-}\) secretion in airway glandular epithelium is critically dependent on basolateral Na\(^{+}/H^{+}\) exchange. These findings reinforce the previous suggestion by Inglis et al. (9) that ACh-induced liquid secretion from airway submucosal glands is driven by both Cl\(^{-}\) and HCO\(_3\)\(^{-}\) secretion.

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