Hyposecretion, Not Hyperabsorption, Is the Basic Defect of Cystic Fibrosis Airway Glands**

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Human airways and glands express the anion channel cystic fibrosis transmembrane conductance regulator, CFTR, and the epithelial Na⁺ channel, ENaC. Cystic fibrosis (CF) airway glands fail to secrete mucus in response to vasoactive intestinal peptide or forskolin; the failure was attributed to loss of CFTR-mediated anion and fluid secretion. Alternatively, CF glands might secrete acinar fluid via CFTR-independent pathways, but the exit of mucus from the glands could be blocked by hyperabsorption of fluid in the gland ducts. This could occur because CFTR loss can disinhibit ENaC, and ENaC activity can drive absorption. To test these two hypotheses, we measured single gland mucus secretion optically and applied ENaC inhibitors to determine whether they augmented secretion. Human CF glands were pretreated with benzamil and then stimulated with forskolin in the continued presence of benzamil. Benzamil did not rescue the lack of secretion to forskolin (50 glands, 6 CF subjects) nor did it increase the rate of cholinergically mediated mucus secretion from CF glands. Finally, neither benzamil nor amiloride increased forskolin-stimulated mucus secretion from porcine submucosal glands (75 glands, 7 pigs). One possible explanation for these results is that ENaC within the gland ducts was not active in our experiments. Consistent with that possibility, we discovered that human airway glands express Kunitz-type and non-Kunitz serine protease inhibitors, which might prevent proteolytic activation of ENaC. Our results suggest that CF glands do not display excessive, ENaC-mediated fluid absorption, leaving defective, anion-mediated fluid secretion as the most likely mechanism for defective mucus secretion from CF glands.

Mucus clearance is an important component of airway innate defenses, and defects in clearance appear to be an important problem in cystic fibrosis (CF) airway disease (1). Submucosal glands are the major sites for mucus production in large airways. They secrete the gel-forming mucin MUC5B, many innate defense molecules, and the serine protease activator inhibitor; ENAc; epithelial Na⁺ channel; VIP, vasoactive intestinal peptide; 1-EBIO, 1-ethyl-benzimidazolone; KRB, Krebs-Ringer bicarbonate; HAL, hepatocyte growth factor activator inhibitor; NEI, inhibitor of human neutrophil elastase.

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‡ These results have been independently confirmed (6, 7). The direct explanation for these results is that CFTR-mediated fluid secretion is lost in CF glands. However, both CFTR and ENaC are expressed in human submucosal glands (8–11), and primary cultures of human large airway submucosal gland acini showed evidence of both CFTR-dependent Cl⁻ transport and amiloride-sensitive Na⁺ transport (12).

What role does ENaC play in submucosal glands, and how is it regulated? Nothing is known about the activity or function of ENaC within submucosal glands, but in the ciliated cells of the airway surface ENaC is complexly regulated. For example, CFTR expression appears to inhibit ENaC in the airways via an unknown mechanism, while ENaC is activated extracellularly by diverse membrane proteases (13–15) and is deactivated by protease inhibitors (13–16). The function of ENaC within glands is of increased interest because of a recent study which suggested that gland acini contain only scant CFTR (11), while the ducts contain abundant CFTR, raising the question of how the non-responsiveness of CF glands can be explained. One possibility is that the ducts actively secrete the glandular fluid. Another is that the co-localization of ENaC and CFTR in the ciliated ducts of glands may mean that ENaC is disinhibited in CF glands by the loss of CFTR (17), leading to increased volume absorption of fluid from CF glands. That might be sufficient to block secretion from CF glands.

To test this latter hypothesis, we used reverse transcriptase PCR to confirm the presence of ENaC subunits in human submucosal glands and then used optical methods to study secretion rates of single submucosal glands from CF subjects and from normal pigs in the presence or absence of ENaC inhibitors. We found no evidence that ENaC inhibitors altered secretion rates in either normal or CF glands. To try to understand why this might be, we used reverse transcriptase PCR to show that glands express multiple serine protease inhibitors. These results are discussed in terms of a hypothesis that innate anti-serine proteases modify ENaC activity both within glands and on the surface of the airways.

EXPERIMENTAL PROCEDURES

Airway Tissues and Cell Cultures—Human CF lungs and tracheal scraps from donors were obtained from lung transplants or, in one CF case, from an autopsy specimen harvested less than 2 h post mortem at Stanford Hospital. The average ages of the CF transplants were 29 ± 2 years, and three of them were male. Our study was approved by the Institutional Review Board of Stanford University. Post-mortem (<1 h)
Yorkshire pig tracheas were obtained from the animal facility at Stanford University after acute experiments unrelated to our studies. The human airway gland serous cell line, Calu-3, was maintained as described previously (2). The human airway surface epithelial cell line, H441, was kindly provided by Robin Tiouvanziam, Stanford University, and maintained in a T25 tissue culture flask containing a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12 nutrient mixture supplemented with 10% fetal bovine serum (Sigma), 100 U/ml penicillin, and 2 mM glutamine at 37 °C in a humidified atmosphere containing 5% CO2. Cells were passaged once at 7–10 days with a density of ~1.5 × 106/cm2.

**Tissue Preparations and Optical Measurements**—Harvested tissues were kept until use in ice-cold Krebs-Ringer bicarbonate (KRB) buffer gassed with 95% O2 and 5% CO2. The KRB buffer composition was 115 mM NaCl, 2.4 mM K2HPO4, 0.4 mM KH2PO4, 25 mM NaHCO3, 1.2 mM MgCl2, 1.2 mM CaCl2, and 10 mM glucose (pH 7.4) adjusted to ~290 mosM with a Wescor vapor pressure osmometer. To minimize endogenously generated prostaglandins during tissue preparation, 1.0 mM indomethacin was present in the KBS buffer. Tissue preparation for optical measurements and the experimental setup for single gland mucus secretion measurements were described previously (4, 18, 19).

Briefly, a piece of dissected human CF bronchial preparation was mounted as apical-side-up in a Sylgard-lined 35-mm plastic Petri dish and placed onto an optical chamber where temperature and pH are controlled. The surface of the tissue was cleaned, dried, and covered with water-saturated mineral oil. The rate at which spherical mucus bubbles were secreted from the gland ducts into the oil layer was optically recorded at intervals of 1–5 min using a computer-controlled Nikon digital camera. Stored images were analyzed by Scion Image software (Scion Corp.). To determine the effects of ENaC blockers, tissue preparations were pretreated with 10 μM amiloride or 10 μM benzamil for various durations (5–60 min) and exposed either apically only or apically + basolaterally. They were then stimulated with 10 μM forskolin in the continued presence of the inhibitor in the bath.

**Reverse Transcriptase PCR**—Total RNA was extracted from isolated human airway glands, Calu-3 cells, and H441 cells using the Qiagen total RNA isolation kit (Qiagen, CA). Primer pairs were designed to span an exonic region. PCR amplifications were done for 35 cycles of 45 s at 94 °C followed by 45 s at 53–56 °C and 60 s at 72 °C using the Qiagen HotStarTaq DNA polymerase kit (Qiagen, CA). PCR products were separated on a 1.5% agarose gel and then visualized by ethidium bromide staining.

**Electrophysiology**—Porcine tracheal tissue preparations were mounted in EasyMount Ussing chambers (Physiologic Instruments, San Diego, CA) with an exposed surface of 0.45 cm2 and bathed in KRB buffer. Transepithelial short circuit current (Isc) was measured using a VCC-600 voltage clamp (Physiologic Instruments), and Isc data were captured and displayed with PowerLab Chart4 software (AD Instruments, Mountain View, CA). Tissue preparations were pretreated with 10 μM benzamil or a vehicle before being mounted in the chambers and variably stimulated or inhibited with 0.1 mM carbachol, 1 mM 1-ethylbenzimidazolone-1 (1-BEI), 0.1 mM bumetanide, and 20 μM ouabain to an apical or basolateral chamber as indicated in the legend to Fig. 2.

**Reagents**—PCR kits were stored according to manufacturer’s protocols. Chemicals (Sigma) were made fresh or maintained at −20 °C as aliquots of stock concentrations. Stock solutions of carbachol, amiloride, ouabain, and forskolin were made in deionized water, while forskolin, benzamil, and 1-BEI were dissolved in dimethyl sulfoxide (Me2SO).

**Statistics**—Data are expressed as mean ± S.E. unless indicated otherwise. Student’s t test for unpaired data was used to compare means of different treatment groups. The difference between the two means was considered to be significant when p < 0.05.

**RESULTS AND DISCUSSION**

**Confirmation of Minimal Responses of CF Glands Stimulated with VIP/Forskolin**—Human airway glands from donors and from patients with lung diseases other than CF, as well as airway glands from pigs, secret mucus in response to VIP or forskolin (4, 18, 20). In marked contrast, glands from CF subjects show virtually no response to these agents, even though they continue to respond to carbachol (4). The lack of mucus secretion in response to VIP and forskolin shows in Fig. 1A, while carbachol produced a good response (sustained carbachol secretion rates: 5.38 ± 1.08 nl/min for 8 glands).

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**TABLE 1**

**PCR primer sequences of ENaC and serine protease inhibitors**

| Primer set    | Sequence          | Product size | RefSeq   |
|---------------|-------------------|--------------|----------|
| ENaC-α forward | 5’TGGACTCGTCCTCAATGC-3’ | 188          | NM 001038|
| ENaC-α reverse| 5’TGGACTCGTCCTCAATGC-3’ | 173          | NM 000336|
| ENaC-β forward| 5’TGGACTCGTCCTCAATGC-3’ | 155          | NM 000109|
| ENaC-β reverse| 5’TGGACTCGTCCTCAATGC-3’ | 221          | Ref. 29  |
| ENaC-γ forward| 5’TGGACTCGTCCTCAATGC-3’ | 269          | Ref. 29  |
| HAI-1 forward | 5’TGGACTCGTCCTCAATGC-3’ | 233          | NM 021102|
| HAI-1 reverse | 5’TGGACTCGTCCTCAATGC-3’ | 150          | NM 006287|
| HAI-1B forward| 5’TGGACTCGTCCTCAATGC-3’ | 441          | NM 006928|
| HAI-1B reverse| 5’TGGACTCGTCCTCAATGC-3’ | 244          | NM 002177|
| HPI2 forward  | 5’TGGACTCGTCCTCAATGC-3’ | 237          | NM 009934|
| HPI2 reverse  | 5’TGGACTCGTCCTCAATGC-3’ | 223          | NM 030666|
| NEI forward   | 5’TGGACTCGTCCTCAATGC-3’ | 173          | NM 000336|
| NEI reverse   | 5’TGGACTCGTCCTCAATGC-3’ | 155          | NM 000109|

Defective Cystic Fibrosis Gland Secretion

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In an attempt to detect any small responses to VIP or forskolin that might be present in CF glands, and to distinguish between responses and the unstimulated or "basal" secretion often observed in glands, we used our previously developed optical methods (4, 18, 19) to analyze single gland mucus secretion rates in response to forskolin/VIP from a subset of 151 glands among more than 1000 observed glands from 18 different CF subjects. These glands were selected based on the presence of mucus bubbles prior to carbachol stimulation, which ensured that the ducts were not blocked. The mean secretion rate in response to forskolin/VIP in this set of CF glands was 0.01 ± 0.01 nl/min. This contrasts...
with response rates to forskolin of 0.94 ± 0.19 nl/min for 139 glands from 16 normal donor control subjects and 0.99 ± 0.23 nl/min for 79 glands from 12 disease control subjects. This ~100-fold difference in response rates was previously interpreted to be the result of the loss of CFTR in CF gland serous cells and the consequent absence of the HCO₃⁻ and Cl⁻-mediated fluid secretion thought to occur via CFTR (4). This interpretation is based on the assumption that Calu-3 cells, which secrete copious fluid in response to VIP and forskolin (21), are a reasonable model for gland serous cells, as well as on the observation that gland serous cells express abundant CFTR (9).

However, a reinvestigation of human airways with a new set of monoclonal antibodies found only sparse and variable CFTR in the gland serous cells but abundant CFTR in the airway surface and in the ducts (11). The different, antibody-dependent distributions of CFTR remain to be resolved. One approach to resolving the differences is to use physiology to assess some possible functional consequences of each pattern. Because CFTR in the ducts is accompanied by ENaC (10, 11), and because of evidence that the loss of CFTR increases ENaC activity in CF airways (17), an alternative explanation for the lack of CF gland secretion is that VIP/forskolin still stimulates CF glands to secrete fluid via non-CFTR pathways, but the lack of observed fluid secretion from the openings of CF gland ducts results from enhanced, ENaC-driven fluid volume absorption in the CF gland ducts. The volume absorption need not completely offset secretion; it could be that hyperabsorption in the small diameter ducts could lead to blockage.

Confirmation of ENaC Expression in Human Airway Glands—Two prior studies, taken together, provided evidence for ENaC expression in glands, but neither study alone demonstrated expression of α-, β-, and γ-ENaC subunits (8, 10). Fig. 1B shows two representative isolated human submucosal glands after injections with Brilliant Blue (right one: note dark blue collecting ductal parts) followed by whole gland staining with Neutral Red dye to more clearly visualize the glands and permit certainty that the tissue was glandular. Reverse transcriptase PCR for with Neutral Red dye to more clearly visualize the glands and permit certainty that the tissue was glandular. Reverse transcriptase PCR for CF glands, Calu-3 cells, and H441 human airway surface epithelial cells as sources for RNA. We extracted total RNA from each sample and ran reverse transcriptase PCR as described. All three ENaC subunits were demonstrated in RNA from isolated human glands and from H441 surface epithelial cells (Fig. 1C). Calu-3 cells only expressed the α-ENaC subunit. Calu-3 cells lack amiloride-sensitive Na+ transport (22). These PCR results confirm previous reports that ENaC is expressed in human airway surface epithelia and submucosal glands (8, 10).

Continued Lack of Responses to Forskolin in CF Glands Exposed to ENaC Inhibitors—To test the hypothesis that defective gland secretion is caused by enhanced ENaC-driven fluid absorption within CF glands, CF airway glands were incubated with a 10 μM concentration of the potent ENaC inhibitor benzamil for 5–60 min and were then stimulated with forskolin in the continued presence of the inhibitor in the bath. If 1) forskolin-stimulated CF glands still secrete fluid via non-CFTR pathways, and if 2) ENaC is active or hyperactive in CF glands as it appears to be in surface epithelia of CF airways, and if 3) ENaC mediates the absorption of fluid volume at it appears to do for surface epithelia (17), then it might be expected that the inhibition of ENaC could rescue, at least partially, forskolin-stimulated fluid secretion of CF glands.

Results from two representative experiments with benzamil are shown in Fig. 2. In the first experiment, benzamil was added to a CF tissue containing glands that were secreting low levels of mucus basally. Benzamil treatment did not affect the basal mucus secretion nor was there any significant stimulation of secretion by subsequent treatment with 10 μM forskolin in the presence of benzamil (Fig. 2A). This result is significant because the low rate of basal secretion proves that the ducts were not blocked; hence even small increases in the net rate of fluid flow from the ducts should have been observed if secretion were the net result of opposing volume secretion and ENaC-based volume absorption. However, a potential criticism of this kind of experiment is that the benzamil might not gain access to ENaC in the lumen of the duct.

To partially address that concern, some CF gland preparations were pretreated with benzamil for up to 60 min both apically and basolaterally before the preparation was stimulated with forskolin. Despite this prolonged exposure, there was still no rescue of forskolin-stimulated secretion, as shown for a representative preparation treated for 60 min (Fig. 2B). Similar experiments were carried out with a total of 50 glands from 6 different CF subjects with the same results: there was no indication of any increase in either basal secretion, when present, and no rescue of the lack of forskolin-stimulated secretion.

To verify tissue viability, the tissue was stimulated with 10 μM carbachol in the presence of benzamil at the end of experiment. The sustained response to carbachol from 5 amiloride or benzamil pretreated subjects was 2.0 ± 0.35 nl/min (40 glands from 5 CF subjects) versus 2.96 ± 0.52 nl/min for carbachol responses from a larger series of CF tissues that were not treated with ENaC inhibitors (143 glands from 17 CF subjects, t test, p = 0.14, not significant).

No Effect of Benzamil- on Forskolin-stimulated Mucus Secretion from Healthy Pig Glands—To determine whether ENaC-driven volume absorption plays a role in normal airway glands, experiments like those above were also carried out with porcine tracheal preparations. These were pretreated with the ENaC inhibitors benzamil or amiloride with vehicle controls and were then stimulated with 10 μM forskolin in the continued presence or absence of the inhibitors (Fig. 2C). No significant differences in mucus secretion rates were observed in the presence of either ENaC inhibitor. Secretion rates from individual glands were 0.73 ± 0.14 nl/min in the benzamil/amiloride-pretreated group (n = 8, 75 glands from 7 pigs) and 0.67 ± 0.14 nl/min in the control group (n = 8, 75 glands from 7 pigs, p = 0.74, not significant).

Evidence That Benzamil Reaches and Persists in the Glands—Because we observed no effect of benzamil or amiloride on gland volume secretion, we cannot eliminate the possibility that benzamil failed to reach ENaC in the glands or did not persist after it was removed from the apical surface. Here, three observations make that less likely. First, in humans benzamil has an IC₅₀ of <40 nM (23), and we used 10 μM. Second, although benzamil is absorbed from mucosal surfaces via an unknown mechanism (23); it is not certain that this process entirely eliminates the effects of benzamil; indeed it may prolong those effects, because the Iₑₑ of human and sheep airway epithelia treated with benzamil showed persistent inhibition following brief, 30-s exposures to benzamil; only 60% recovery was observed after 13 washes, and recovery was slower with longer drug exposures (23). Third, basolateral application of benzamil slowly reduced the amiloride-sensitive Iₑₑ in Ussing experiments (data not shown).

As a positive control, and to determine the persistence of benzamil effects in tissues treated via our protocol, porcine tracheal preparations were pretreated either with 10 μM benzamil or vehicle (0.1% Me₂SO) exactly as we treated the CF tissues and were then mounted in Ussing chambers in the absence of apical inhibitors. The inhibitory effects of benzamil pretreatment on Iₑₑ continued for at least 2 h (compare bottom Iₑₑ trace in Fig. 2D with control trace above). The stimulatory Iₑₑ responses to forskolin, carbachol, and 1-EBIO (Fig. 2D) and the inhibition of Iₑₑ with bumetanide and ouabain were all comparable between the tissue preparations pretreated with benzamil or vehicle (Fig. 2D).
CF Glands Hyposecrete Rather than Hyperabsorb

The main result from these studies is that ENaC inhibitors failed to rescue forskolin-stimulated secretion from CF glands, arguing against the possibility that the loss of secretion is secondary to ENaC-mediated hyperabsorption.

A secondary, but potentially important, finding is the lack of effect of ENaC inhibitors on the volume of secretion from normal glands. This could come about for at least two reasons. First, it is conceivable that the ducts might absorb salt, but not fluid volume, by analogy with sweat ducts (24). That possibility has not been formally tested, and the levels of Na⁺, Cl⁻, and HCO₃⁻ in gland mucus are lower than expected for isotonic secretion (5), even though far higher than in sweat. Second, ENaC in the ducts might be inactive. We have no evidence for ENaC activity in the glands under any condition we tried. Because we saw the transcripts of all ENaC subunits, and because cultures of human airway submucosal glands express a basal Isc that is inhibited by amiloride (12), we think it is likely that ENaC in the glands is capable of being functional. Indeed, one study has seen evidence for a contribution of ENaC to volume secretion from glands (25). Phillips et al. (25) used the tantalum powder-hillocks technique to study the effects of amiloride pretreatment on methacholine-induced gland secretion in porcine tracheas; in contrast with our results, they found that volumes were enhanced by amiloride. The differences they observed occurred in the rapid secretory pulse that occurs in the first 3 min rather than in the longer term sustained responses we studied. Thus, if ENaC is transiently active only at the beginning of secretion, we would likely have missed its contribution. It is also possible the hillocks technique measures, in part, absorption by the airway surface epithelia, although attempts were made to minimize that effect. In either case, our results, which indicate that ENaC does not contribute to volume absorption during prolonged secretion, caused us to look for factors that might silence ENaC in the glands.

FIGURE 2. ENaC inhibitors fail to rescue the lack of mucus secretion to forskolin stimulation in CF airway glands. A and B, mucus bubble volume as a function of time/stimulation in CF gland preparations. A, after a 60-min basal secretion, glands were treated with 10 μM benzamil (Bz) for 60 min and then stimulated with 10 μM forskolin (Fsk) for 60 min in the presence of benzamil. B, the tissue preparation was submerged in the presence of 10 μM benzamil for 60 min to achieve full effects of benzamil and then started a 60-min basal period followed sequentially by forskolin stimulation for 90 min and 10 μM carbachol (Carb) stimulation in the presence of benzamil. In both conditions, forskolin stimulation did not increase mucus secretion in CF glands. Two representatives (16 glands from two CF different subjects) are shown here from 50 glands of six different CF subjects. C, porcine tracheal preparations were pretreated with 10 μM amiloride (A) or 10 μM benzamil (B, filled column) to determine ENaC activity in healthy normal glands. No significant difference in mucus secretion rates stimulated by 10 μM forskolin was observed (75 glands, n = 8 from 7 pigs). D, Isc responses in the absence (top Isc traces) and presence (bottom Isc traces) of 60-min pretreatment of porcine tracheal preparations with 10 μM benzamil. Note that stimulatory (10 μM carbachol and 1 mM 1-EBIO: a, apical; bl, basolateral treatment) and inhibitory (0.1 mM bumetanide (Bm) and 20 μM ouabain (Ouab)) effects are comparable except for lack of benzamil effect on the bottom Isc traces. Representative Ussing results are shown here from n = 5 similar experiments.
Presence of Serine Protease Inhibitors in Human Submucosal Glands—Serine proteases and Kunitz-type serine protease inhibitors appear to be natural regulators of ENaC in airway surface epithelia (13). In prior work, we proposed that submucosal glands are the major source of serine protease inhibitors in the airways and hence may play an important role in dampening ENaC activity (2). While nothing is known about the regulation of ENaC within the glands, it is clear that ENaC will be exposed to high concentrations of gland-produced proteins and so should be affected powerfully by the balance of proteases and anti-proteases in airway mucus. Because our results can be interpreted to indicate that ENaC in the glands is inactive, we used reverse transcriptase PCR to look for evidence that glands contain Kunitz-type anti-proteases in addition to α-1 antitrypsin and α-1-antichymotrypsin (2).

Of seven Kunitz-type serine protease inhibitors that we assayed in human submucosal glands and the serous cell line Calu-3, three were found in both glands (Fig. 3A) and Calu-3 cells (Fig. 3B), and four were absent from both glands and Calu-3 cells. The three expressed Kunitz-type inhibitors were the hepatocyte growth factor activator inhibitors type 1 (HAI-1), its splice variant (HAI-1B), and HAI-2 (also called plasminogen activator inhibitor type 1, its splice variant, type 1, and inhibitor of human neutrophil elastase, respectively). Expected sizes of amplicons are: 221 bp (HAI-1), 269 bp (HAI-1B), and 233 bp (HAI-2), and 233 bp (NEI). NEI is not a Kunitz-type serine protease inhibitor. From our conditions of reverse transcriptase PCR, we failed to detect any message of other Kunitz-type serine protease inhibitors tested in the present study: tissue factor pathway inhibitor, its type 2, inter-α-trypsin inhibitor, and serpinF2 using primer sets shown in Table 1.

Potential Role of Proteolytic Imbalance in Early CF Airway Disease—Why is ENaC expressed in glands, and do proteases and antiproteases play a role in controlling its activity within the glands? Regional localization of ENaC within the glands is not yet definitive, but ENaC appears to be expressed in both ducts and acini (8, 10). One possible glandular function for ENaC is suggested by the observation that glands secrete K⁺, and at least in kidney cortical collecting duct cells, K⁺ secretion is strongly influenced by Na⁺ absorption through ENaC and can be inhibited by amiloride (27). This suggests that proteolytic balance might influence K⁺ secretion via ENaC regulation.

Our present study evaluated the possibility that ENaC-mediated hyperabsorption of fluid in CF airway glands was the cause of the failed secretion observed when CF glands are stimulated with VIP or forskolin (4). The evidence did not support that hypothesis, leaving a defect in CCl⁻ and HCO₃⁻-mediated fluid secretion as the most likely cause for the failure of gland fluid secretion. Our evidence that ENaC is silent within normal and CF glands is in contrast with the situation in surface airway epithelia, where ENaC is variably active in normal airways, where it helps control the depth of the ASL, and where it appears to be hyperactive in CF airways (17). Regardless of the mechanism, the loss or reduction of gland mucus secretion in CF airways is expected to diminish the levels of effective anti-proteases in the CF airways. It is well known that a large imbalance favoring proteolysis exists in infected CF airways, stemming mainly from the huge populations of neutrophils in the airways. Excessive proteolytic activity has a host of effects, including the stimulation of gland and goblet cell secretion (28), tissue destruction leading to bronchiectasis, and cleavage of many innate defense proteins. However, while the destructive cascade triggered by inadequately treated infections in CF airways is a major topic of interests for CF researchers, less attention has been paid to the state of affairs in the uninfected or recently infected CF airways. Why do early infections start in the first place, and why can they not be properly resolved? We look to gland dysfunction as a critical component of any answer to that question, and we think gland dysfunction has multiple consequences. We hypothesize that one consequence is a shift in the proteolytic balance of CF airways toward proteolysis.

We hypothesize that excess proteolytic activity also exists in uninfected CF airways, because gland-derived anti-proteases are not properly secreted from CF glands (2, 4). That could promote more rapid fluid absorption, secondary to enhanced proteolytic activation of surface ENaC in uninfected airways by intrinsic proteases. Once infection is under way, abundant proteases from inflammatory cells will tip the balance even further toward proteolysis. Infected CF airways have high levels of neutrophil elastase, which has been shown to activate near-silent ENaC channels and to increase Na⁺ absorption in a human bronchial cell line (14). Neutrophil elastase is inhibited by NEI (16), which we have shown to be expressed by glands; NEI abolished the activation of ENaC by neutrophil elastase (14).

In summary, our results suggest that CF glands do not display excessive, ENaC-mediated fluid absorption, leaving defective, anion-mediated fluid secretion as the most likely mechanism for defective mucus secretion from CF glands. This conclusion, based on the prevalence of protease inhibitors in glands and evidence that ENaC in the gland is silent, leads us to suggest that decreased availability of gland-derived protease inhibitors on the airway surface might shift the proteolytic balance toward increased proteolysis, leading to increased ENaC activation, increased absorption, and decreased mucus clearance.

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