Airway submucosal glands are important sites of cystic fibrosis transmembrane conductance regulator (CFTR) chloride (Cl\(^-\)) channel expression and fluid secretion in the airway. Whereas both mouse and human submucosal glands and their serous acinar cells express CFTR, human glands and serous cells secrete much more robustly than mouse cells/glands in response to cAMP-generating agonists such as forskolin and vasoactive intestinal peptide. In this study, we examined mouse and human serous acinar cells to explain this difference and reveal further insights into the mechanisms of serous cell secretion. We found that mouse serous cells possess a robust cAMP-activated CFTR-dependent Cl\(^-\) permeability, but they lack cAMP-activated calcium (Ca\(^{2+}\)) signaling observed in human cells. Similar to human cells, basal K\(^+\) conductance is extremely small in mouse acinar cells. Lack of cAMP-activated Ca\(^{2+}\) signaling in mouse cells results in the absence of K\(^+\) conductances required for secretion. However, cAMP activates CFTR-dependent fluid secretion during low-level cholinergic stimulation that fails to activate secretion on its own. Robust CFTR-dependent fluid secretion was also observed when cAMP stimulation was combined with direct pharmacological activation of epithelial K\(^+\) channels with 1-ethyl-2-benzimidazolinone (EBIO). Our data suggest that mouse serous cells lack cAMP-mediated Ca\(^{2+}\) signaling to activate basolateral membrane K\(^+\) conductance, resulting in weak cAMP-driven serous cell fluid secretion, providing the likely explanation for reduced cAMP-driven secretion observed in mouse compared with human glands.

Airway submucosal exocrine glands are major sites of fluid secretion in the lung and are likely important in the pathology of cystic fibrosis (CF)\(^\text{2}\) (1–3). CF is caused by defects in the function of the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel (4), which is expressed both in the surface airway epithelium and in the serous acinar cells at the distal ends of submucosal glands. Fluid secretion in response to intracellular cAMP ([cAMP])-elevating agonists is severely reduced in glands from CF patients (5–7), CFTR-knock-out pigs (8), and cftr\(^{tm1Unc}^{-/-}\) knock-out (cftr\(^{-/-}\)) mice (9, 10). We previously observed that agonist-evoked elevations of cAMP in porcine and human serous acinar cells evoke protein kinase A (PKA)-mediated fluid secretion that is dependent upon CFTR, which functions as an apical membrane secretory Cl\(^-\) channel (11). Defective cAMP-activated CFTR-dependent serous cell fluid secretion likely contributes to airway dehydration and/or altered mucus rheology seen in CF lung disease (1–3). Restoration of this fluid secretion may be an important therapeutic strategy for CF, and thus the elucidation of the molecular mechanisms of serous cell secretion is critically important.

Fluid secretion by exocrine acinar cells is driven osmotically by the primary secretion of NaCl (reviewed in Ref. 12). Cl\(^-\) is secreted through the cell by uptake mechanisms in the basolateral membrane operating in series with a Cl\(^-\) channel in the apical membrane that mediates its efflux. The resulting trans-epithelial voltage drives Na\(^+\) from the serosal side into the gland lumen via paracellular pathways. Regulation of apical membrane secretory Cl\(^-\) channels is a key step in the activation of fluid secretion. However, secretory cells also require sufficient counter-ion permeabilities to preserve electroneutrality during the robust efflux of cellular anion content that drives fluid secretion. The counter-ion permeability is typically provided by K\(^+\) channels localized on the basolateral membrane. A sufficiently high K\(^+\) conductance is necessary to maintain membrane potential (V_m) sufficiently hyperpolarized to provide an electrical driving force for Cl\(^-\) secretion (11–15). Although the apical membrane Cl\(^-\) conductance is usually rate-limiting for activation of fluid secretion in most exocrine cells, we previously discovered that activation of CFTR was insufficient to stimulate fluid secretion in human and porcine submucosal gland serous acinar cells because the basal K\(^+\) conductance was small and insufficient (11). Nevertheless, cAMP activated CFTR-dependent fluid secretion in porcine bronchial and human nasal serous acinar cells because it also elicited...
cytoplasmic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) signals that activated K\(^+\) channels to provide the necessary counter-ion permeability for fluid secretion (11). In contrast with human and pig serous acinar cells, cAMP failed to activate fluid secretion in mouse serous acinar cells (15). These observations were in accord with data demonstrating that intact mouse submucosal glands exhibit much lower rates of cAMP-activated, CFTR-dependent fluid secretion (normalized to the maximum rate of cholinergic-activated fluid secretion) compared with either human or porcine submucosal glands (3, 5–7, 9, 10, 16–19). Because we have shown that mouse, porcine, and human serous cells have similar apical membrane CFTR staining (11, 14, 15, 20), we hypothesized that, like human and pig serous acinar cells, mouse acinar cells lack a resting K\(^+\) conductance, but mouse cells differ in their ability to secrete fluid in response to [cAMP]\(_i\)-elevating agonists because of a lack of cAMP-activated robust K\(^+\) permeability, potentially due to the absence of cAMP-activated Ca\(^{2+}\) signaling.

Here, we have tested this hypothesis as well more thoroughly examined the role of CFTR in fluid secretion by mouse serous acinar cells. We utilized optical methods developed in rat salivary gland acinar cells (13, 21, 22) and adapted for mouse, human, and porcine airway submucosal gland serous acinar cells (11, 14, 15, 20) to measure fluid secretion in freshly isolated intact living cells. Fluorescence imaging of Ca\(^{2+}\) and Cl\(^-\) was combined with simultaneous differential interference contrast (DIC) imaging of cell volume that reflect changes in the secretory state of the cells (11, 14, 15, 20). Our results suggest that while human and mouse serous acinar cells share many of the mechanisms that drive fluid secretion, fundamental differences exist in the secretagogue-induced second messenger pathways that activate secretion. These data have important implications for explaining differences in the rates of cAMP-induced fluid secretion observed between mouse and human submucosal glands. In addition, they provide insights into the utility of mouse serous cells as a model for CFTR-dependent fluid secretion.

**EXPERIMENTAL PROCEDURES**

**Experimental Reagents and Solutions—**VIP was obtained from Advanced ChemTech/Creosalus (Louisville, KY). Fura-2-AM, SPQ, and BAPTA-AM were purchased from Invitrogen (Eugene, OR). Collagenase and DNase I were purchased from Worthington Biochemical (Lakewood, NJ). Vectashield (Eugene, OR). Collagenase and DNase I were purchased from Advanced ChemTech/Creosalus (Louisville, KY). Fura-2-AM, SPQ, and BAPTA-AM were purchased from Invitrogen (Eugene, OR). Collagenase and DNase I were purchased from Worthington Biochemical (Lakewood, NJ). Vectashield mounting medium was purchased from Cell-Tak (Grand Island, NY).

All solutions used were exactly as described in Refs. 11, 14, 15, 20. The primary physiological extracellular solution (Solution A) contained (in mM) 125 NaCl, 5 KCl, 1.2 MgCl\(_2\), 1.2 CaCl\(_2\), 1.2 NaH\(_2\)PO\(_4\), 11 glucose, and 25 NaHCO\(_3\) buffered to pH 7.4 by 95% O\(_2/5%\) CO\(_2\) gassing. Experiments performed in the absence of extracellular Ca\(^{2+}\) employed Solution A lacking CaCl\(_2\) with 1 mM EGTA added to chelate trace Ca\(^{2+}\). Tissue isolation was performed in Solution B, which was identical to Solution A except that it lacked NaHCO\(_3\) and contained 20 mM HEPES, 2 mM l-glutamate, 1 mM penicillin/streptomycin, 50 μg ml\(^{-1}\) gentamycin, and 1% MEM vitamins, amino acids, and nonessential amino acids. Collagenase digestion was performed in Solution B lacking added CaCl\(_2\) but with no EGTA added. The control solution for NO\(_3\) substitution experiments (Solution C (11, 23)) contained (in mM): 136.2 NaCl, 3.8 KCl, 1.2 KH\(_2\)PO\(_4\), 1.2 CaCl\(_2\), 1.2 MgCl\(_2\), 11 glucose, and 10 HEPES, pH 7.4 ([Cl\(^-\)]\(_i\) = 144.8 mM), gassed with 100% O\(_2\). The low [Cl\(^-\)]\(_i\) solution (Solution D) was identical, except NaCl was replaced isosmotically with NaNO\(_3\) ([Cl\(^-\)]\(_i\) = 8.6 mM).

**Isolation and Imaging of Primary Serous Acinar Cells—**All procedures were carried out exactly as described (11, 14, 15, 20). Mouse tissues were obtained from animals that were euthanized by CO\(_2\) inhalation for unrelated experiments. No animals were killed specifically for these studies. Human tissue was obtained with written patient consent from surgical specimens collected after turbinectomy procedures performed at the Rhinology Clinic of the Hospital of the University of Pennsylvania. Animal and human tissues were obtained with full approval of the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC) and Institutional Review Board (IRB), respectively. Minced mouse nasal turbinate and septum tissue or dissected human submucosal glands were incubated in Ca\(^{2+}\)-free Solution B containing 1.5 mg ml\(^{-1}\) Type IV collagenase and 10 μg ml\(^{-1}\) DNase I for 30–60 min at room temperature with gentle shaking. Following three washes (1000 RPM for 1 min in a clinical centrifuge) with collagenase-free-Solution B (containing Ca\(^{2+}\)), cells were resuspended in Solution A with 95% O\(_2/5%\) CO\(_2\) gassing and plated on washed, uncoated glass coverslips.

Cells were loaded with fura-2 (2 μM fura-2-AM for 10 min) or SPQ (20 mM SPQ for ~1.5 h) as described (15, 20) and imaged using simultaneous DIC and wide-field fluorescence microscopy as described (11, 13–15, 20, 21). Conversions of fura-2 340 nm/380 nm ratios to [Ca\(^{2+}\)]\(_i\) and normalization of SPQ fluorescence changes to fluorescence at time 0 (F/F\(_o\) = 0) were performed using methods previously described (11, 13–15). Relative cell/acini volume changes were determined by raising the DIC-imaged cross-sectional area to the 3/2 power (11, 14, 15, 20, 22), a method that yields results identical to those obtained by confocal three-dimensional reconstructions of mouse serous acinar cells (15). All experiments were performed at 37 °C under continuous perfusion as described (20).

CFTR and NKCC1 immunofluorescence staining was performed as previously described (11, 14, 15, 20). Isolated acini and cells were plated on Cell-Tak coated coverslips and fixed for 20 min in 4% formaldehyde at room temperature. Blocking and permeabilization was performed for 1 h in PBS containing 1% BSA, 2% normal goat serum, and 0.15% saponin. Primary antibody (anti-NKCC1 at 1:400 and/or anti-CFTR at 1:100) incubation was performed overnight at 4 °C. Secondary antibody incubation (both anti-rabbit and/or anti-mouse at 1:1000)
was performed for 2 h at 4 °C. Coverslips containing cells were mounted on slides using Vectashield Hard-Set mounting medium and viewed using a Perkin-Elmer Ultraview spinning disk confocal system and 488 nm and 568 nm laser lines as previously described (11, 14, 15, 20).

Statistics and Data Analysis—Images were acquired using Ultraview Software (PerkinElmer Waltham, MA). Data were analyzed using Excel, Igor Pro (Wavemetrics, Inc., Lake Oswego, OR) and/or ImageJ (W. Rasband, NIH, Bethesda, MD). All values are reported as means ± S.E. of the mean (S.E.). Student’s t test and one-way analysis of variance (ANOVA) with Bonferroni or Dunnett’s multiple comparison post-tests were used to determine statistical significance (p values) as appropriate; a p value of <0.05 was considered statistically significant. For all figures, one asterisk (*) indicates p < 0.05, two asterisks (**) indicate p < 0.01, and n.s. indicates no statistical significance.

RESULTS

Stimulation With [cAMP]i-elevating Forskolin Does Not Activate Robust Secretion by Mouse Serous Acinar Cells—Human serous acinar cells exhibit robust forskolin- and vasoactive intestinal peptide (VIP)-activated shrinkage (Fig. 1, A and B; n = 4 each) and (11)). As shown previously, shrinkage is caused by agonist-induced loss of cell KCl, through CFTR and K+ channels, and osmotically obliged water and reflects activation of fluid secretion (11, 14, 15, 20). In contrast, we observed no detectible shrinkage during forskolin or VIP stimulation of wild-type (Wt) mouse serous cells (Fig. 1C; n = 4; and previously in Ref. 15). However, the mouse cells were viable and capable of secreting Cl and fluid because subsequent exposure to 10 μM carbachol (CCh; a cholinergic agonist) triggered rapid and substantial cell shrinkage (Fig. 1C). Strong cholinergic stimulation activates fluid secretion by triggering a substantial [Ca2+]i rise that activates Ca2+-activated Cl channels (CaCCs; likely including Ano1 (14)), bypassing a requirement for CFTR in mouse cells as well as in human and pig serous acinar cells (11, 14, 15). Cl accumulation in mouse serous cells is overwhelmingly dependent on the activity of the Na-K-2Cl co-transporter isoform 1 (NKCC1) (15). To determine whether the failure to observe cAMP-induced shrinkage of mouse acinar cells was due to compensatory solute uptake, forskolin stimulation was performed in the presence of the NKCC1 inhibitor bumetanide (100 μM). Nevertheless, no forskolin-activated shrinkage was observed under these conditions (Fig. 1D; n = 4), despite intact secretory responses to CCh. Thus, cAMP fails to activate KCl loss from mouse serous acinar cells, in contrast to its effects in human cells.

Stimulation of Mouse Serous Cells With [cAMP]i-elevating Agonists Activates a PKA- and CFTR-dependent Plasma Membrane Anion Permeability—The forskolin- and VIP-induced secretory responses of human serous cells were inhibited by the
CFTR inhibitor CFTRinh172 (Fig. 1, E and F; n = 4 each), demonstrating that CFTR functions as a secretory Cl⁻ channel during human acinar cell fluid secretion. The lack of forskolin-induced secretory responses in mouse cells could be caused by lack of a CFTR-dependent Cl⁻ conductance. Although this seemed unlikely based on strong CFTR immunofluorescence signals observed in mouse submucosal gland serous cells (Fig. 2 and Ref. 15, 20), it is possible that CFTR, while highly expressed in these cells, may nevertheless be nonfunctional. To determine if CFTR is functional in the plasma membranes of mouse serous acinar cells, we employed a direct and sensitive method to measure plasma membrane Cl⁻ permeability. Mouse nasal serous acinar cells were loaded with the Cl⁻-sensitive fluorescent probe SPQ, previously used to track Cl⁻ secretion in these cells (15), and studied using a NO₃⁻ substitution protocol (23) that was previously employed in pig and human serous cells (11). SPQ is quenched by Cl⁻ but not by NO₃⁻. Because Cl⁻ channels have a high intrinsic permeability to NO₃⁻, replacement of bath Cl⁻ with NO₃⁻ results in electroneutral exchange of intracellular Cl⁻ for extracellular NO₃⁻, resulting in an increase in SPQ fluorescence that can be used as a measure of relative cellular anion permeability. All experiments were performed in the presence of 50 μM bumetanide and in the absence of extracellular CO₂/HCO₃⁻ (0-CO₂/HCO₃⁻) to minimize non-conductive Cl⁻ efflux through NKCC1 and Cl⁻/HCO₃⁻ (anion) exchanger (AE) activity, respectively.

In unstimulated serous acinar cells from Wt and cftr⁻/⁻ mice, replacement of bath Cl⁻ with NO₃⁻ resulted in a slow increase in normalized SPQ fluorescence (Fig. 3A; ΔF/F(0) = 0.0011 ± 0.0004 units·s⁻¹ in Wt and 0.0012 ± 0.0003 units·s⁻¹ in cftr⁻/⁻; n.s.; n = 4 each) reflecting a small basal Cl⁻ permeability. However, following stimulation of the same cells with 5 μM forskolin, introduction of NO₃⁻ caused a ~10-fold more rapid increase in SPQ fluorescence in Wt cells (ΔF/F(0) = 0.013 ± 0.02 units·s⁻¹) but not in cftr⁻/⁻ cells (0.0013 ± 0.0004 units·s⁻¹; p < 0.01 versus Wt). Thus, forskolin activates a CFTR-dependent plasma membrane anion permeability in mouse acinar cells, likely CFTR itself. To confirm this and to ensure that the responses in the Wt cells were not due to activation of CaCC, similar experiments were performed in Wt (average traces shown in Fig. 3B), cftr⁻/⁻, and cftr⁻/⁻ heterozygote (het) serous cells (average traces shown in Fig. 3, B–D, respectively) under similar 0-CO₂/HCO₃⁻/bumetanide conditions with the cells loaded with the Ca²⁺ chelator BATPA-AM (11, 14, 15, 20) and stimulated in the absence of extracellular Ca²⁺ (+1 mM EGTA; 0-[Ca²⁺])ₐ. SPQ fluorescence changes were recorded upon introduction of NO₃⁻ in unstimulated cells or cells stimulated for 180–200 s with either 5 μM forskolin, 3 μM VIP, or the β-adrenergic agonist isoproterenol (10 μM). Basal Cl⁻ permeabilities were identical in unstimulated Wt, cftr⁻/⁻, and het cells (Fig. 3E). In the Wt cells, anion permeability was increased ~10-fold after stimulation with forskolin, VIP, or isoproterenol (Fig. 3E). The forskolin-activated increase in anion permeability was abolished by 10 μM H89 (PKA inhibitor) or 12 μM CFTRinh172 (Fig. 3E). Again, no significant increase in anion permeability was observed after stimulation of cftr⁻/⁻ cells with forskolin, VIP, or isoproterenol (Fig. 3E). Het cells also exhibited a robust forskolin-induced increase in anion permeability (Fig. 3E). While the forskolin-induced permeability in het cells initially appeared smaller than the permeability in Wt cells, the two responses were not significantly different. These results demonstrate that Wt and het mouse cells respond to elevation of [cAMP], with robust CFTR activation, and suggest that the lack of significant cAMP-activated fluid secretion is not caused by insufficient Cl⁻ permeability.

Mouse Serous Acinar Cells Lack cAMP-activated [Ca²⁺], Signals That Are Correlated With and Required for Optimal Secretion—Acinar cell shrinkage during secretagogue stimulation requires both Cl⁻ efflux through secretory Cl⁻ channels as well as counter-ion K⁺ efflux (typically across the basolateral membrane) to preserve cellular electroneutrality. Since the mouse cells possess cAMP-activated CFTR Cl⁻ permeability, we hypothesized that the lack of CAMP-activated fluid secretion by mouse serous cells is due to a lack of sufficient K⁺ conductance. In human serous acinar cells, CAMP-activated cell shrinkage requires not only PKA-mediated activation of CFTR-de-
pendent anion permeability, but also PKA-activated elevation of [Ca$^{2+}$], (shown in Fig. 4, A and B) required to activate K$^+$ conductance (11), likely mediated by Ca$^{2+}$-activated K$^+$ channels. Because forskolin failed to cause shrinkage of mouse serous cell despite activation of a robust anion permeability, we speculated that basal K$^+$ conductance was low in mouse acinar cells, and that lack of cAMP-activated [Ca$^{2+}$], signaling might account for the failure of K$^+$ conductance activation during stimulation. We therefore examined whether mouse cells exhibit forskolin-induced [Ca$^{2+}$], signals as observed in pig and human acinar cells (11). Ratiometric imaging of [Ca$^{2+}$], with fura-2 revealed that neither forskolin nor VIP altered [Ca$^{2+}$], (Fig. 4, C and D; $n = 6$). In contrast, 10 $\mu$m CCh activated a robust elevation of [Ca$^{2+}$], that caused marked secretion (Fig. 4, C and D) likely mediated by CaCC (15). These data suggest that the low rate of cAMP-activated fluid secretion from mouse serous cells may be caused by lack of cAMP-dependent [Ca$^{2+}$], signals to activate counter-ion K$^+$ conductance.

**Forskolin Potentiates Secretory Responses of Mouse Serous Cells to a Sub-secretory [CCh] Through a PKA- and CFTR-dependent Mechanism**—Whereas cAMP stimulation results in minimal fluid secretion from mouse serous cells, we asked whether their CFTR anion permeability nevertheless plays a role in fluid secretion under particular conditions. Specifically,
we examined the fluid secretion responses to elevated cAMP during weak cholinergic stimulation that moderately raises \([\text{Ca}^{2+}]_i\). In Wt cells, stimulation with 100 nM CCh elicited a small \([\text{Ca}^{2+}]_i\) signal \((147 \pm 6 \text{ nm}; n = 6)\) that was associated with a minor shrinkage response \((<5\% \text{ volume change; Fig. 5A})\). However, after stimulation of the same cells with 5 \(\mu\text{M}\) forskolin, which alone elicited no \([\text{Ca}^{2+}]_i\), or cell volume responses (Figs. 1 and 4), subsequent stimulation with 100 nM CCh (in the continued presence of forskolin) elicited robust cell shrinkage \((15 \pm 2\%; \text{Fig. 5A})\). Of note, forskolin had no effect on the magnitude of the 100 nM CCh-evoked \([\text{Ca}^{2+}]_i\), elevation (Fig. 5G), in contrast to the potentiation observed in human serous acinar cells (11). The forskolin-induced enhancement of 100 nM CCh-evoked cell shrinkage was abolished by the PKA-inhibitor H89 (Fig. 5B), suggesting that forskolin potentiated the secretory response by activating CFTR. In agreement, the potentiated secretory response to 100 nM CCh after 5 \(\mu\text{M}\) forskolin stimulation observed in Wt cells was absent \((<5\% \text{ shrinkage})\) in cftr\(^{-/-}\) cells, being similar to that observed during stimulation with 100 nM CCh in the absence of forskolin (Fig. 5, C and D). These data suggest that, in mouse serous cells, cAMP potentiates the secretory responses to low-level cholinergic stimulation solely via a PKA- and CFTR-dependent mechanism. This is in contrast to the CFTR-independent secretory observed during combined strong cAMP and weak cholinergic stimulation in human and porcine serous acinar cells (11). Neither forskolin (Fig. 5, E and F) nor the absence of CFTR (Fig. 5G) had an effect on peak \([\text{Ca}^{2+}]_i\), or magnitude of cell shrinkage in response to strong cholinergic stimulation \((1 \mu\text{M} \text{ CCh})\), as previously observed (15). These results suggest that \(\text{Cl}^-\) efflux is not rate-limiting during strong cholinergic stimulation, as activation of CFTR-dependent anion permeability does not detectably enhance shrinkage. Furthermore, they confirm previous observations that CFTR plays little role in secretion during strong cholinergic stimulation (15).

The \(K^+\) Channel Activator EBIO Stimulates CFTR-dependent Fluid Secretion by Forskolin-stimulated Mouse Serous Acinar Cells—We hypothesized that the synergistic activation of fluid secretion by forskolin and low \([\text{Ca}^{2+}]_i\) was mediated by cAMP activation of CFTR and activation of basolateral membrane \(K^+\) conductance by the CCh induced release of \([\text{Ca}^{2+}]_i\). To more directly test whether \(K^+\) conductance is indeed rate-limiting...
during cAMP-agonist stimulation of mouse serous cells, we used EBIO to directly activate epithelial Ca\(^{2+}\)-sensitive K\(^+\) channels (24–27). EBIO stimulation of Cl\(^-\) secretion in T84 cell monolayers, rat colonic epithelium, and mouse tracheal epithelium has an EC\(_{50}\) of \(500 \text{ nM}\) (25, 26). A low [EBIO] (100 nM) was used in this study as higher levels (>200 nM) can elevate [cAMP], in isolated mouse colonic crypts (24, 27). Nevertheless, we tested whether EBIO elevated [cAMP] in serous acinar cells by performing NO\(_3^-\) substitution experiments in SPQ-loaded serous acinar cells prior to and during stimulation with 100 nM EBIO. EBIO alone did not affect the rate of SPQ fluorescence change upon introduction of NO\(_3^-\) (Fig. 6A), suggesting that at 100 nM it does not elevate [cAMP], sufficiently to activate CFTR. When applied alone, EBIO had no effect on serous acinar cell volume (Fig. 6, A and B) or [Ca\(^{2+}\)], (Fig. 6B). However, EBIO caused robust cell shrinkage of forskolin-stimulated Wt cells without affecting [Ca\(^{2+}\)], (Fig. 6C). Importantly, EBIO was without effect in forskolin-stimulated cftr\(^{-/-}\) cells (Fig. 6D). Thus, EBIO synergizes with forskolin to activate secretion in a manner highly reminiscent of the effects of low-level cholinergic stimulation, suggesting that they work by similar mechanisms. In agreement, EBIO did not enhance secre-
tion induced by 100 nM CCh (Fig. 6E). These results suggest that Cl⁻ conductance is rate-limiting during low-level cholinergic stimulation. Furthermore, our data suggest that forskolin fails to activate robust secretion in mouse serous acinar cells because of lack of sufficient K⁺ conductance. Based on the above data and (15, 20), a model of the molecular mechanisms involved in mouse serous cell fluid secretion is outlined in Fig. 7.

**DISCUSSION**

While much has been learned about CFTR function from cftr⁻/⁻ transgenic mice, the lack of significant lung pathology in cftr⁻/⁻ knock-out mice has hampered attempts to understand the development CF lung disease (28). The more recent development of transgenic cftr⁻/⁻ pigs (29–32) and ferrets (33–35) promises to provide animal models of CF that exhibit more human-like disease phenotypes. However, because of the time and costs required to breed, characterize, and distribute these models, transgenic cftr⁻/⁻ and mutant mice will likely remain important models in which to study CFTR function. Because of this, we examined mouse serous acinar cells in more detail to determine whether or not we could detect CFTR function, utilizing Wt and cftr⁻/⁻ mice (28), with an emphasis on the potential contributions of CFTR to fluid secretion.

**FIGURE 6.** The Ca²⁺-activated K⁺ channel opener EBIO activates CFTR-dependent secretion in forskolin-stimulated mouse serous acinar cells. A and B, EBIO (100 µM) did not influence anion permeability (A), [Ca²⁺]i, elevation (B), or cell shrinkage in mouse serous acinar cells (A and B). SPQ ΔF/F₀ was 0.0012 ± 0.0002 units s⁻¹ (n = 5) before EBIO application and 0.0013 ± 0.0002 units s⁻¹ after exposure to EBIO (n = 5; n.s.). C and D, after stimulation with 10 µM forskolin, EBIO activated shrinkage in Wt cells (C), but had no effect on cftr⁻/⁻ cells (D) (n = 5 each). Stimulation with 10 µM CCh confirmed viability of cftr⁻/⁻ cells. E, EBIO did not enhance 100 nM CCh-evoked cell shrinkage (n = 5).

**FIGURE 7.** Model of Cl⁻-driven fluid secretion by mouse airway submucosal gland serous acinar cells. Agonists such as VIP stimulate Gₛ-mediated activation of adenylate cyclase (AC), which results in elevation of [cAMP]i and PKA-dependent phosphorylation and activation of CFTR. CFTR can function as a secretory Cl⁻ channel, but robust secretion requires activation of counterion permeability (K⁺ conductance). Because mouse serous cells lack cAMP-activated (Ca²⁺⁺) signals required to activate Ca²⁺-stimulated K⁺ channels, fluid secretion during [cAMP]i elevation is minimal. Cholinergic agonists activate Gₛ-dependent production of inositol trisphosphate (InsP₃), elevating [Ca²⁺]i, and activating Ca²⁺⁺-sensitive Cl⁻ channels (CaCCs; during strong cholinergic stimulation) and K⁺ channels (during both low-level and strong stimulation). We previously showed that mouse serous acinar cells express the CaCC Ano1 (14). Activation of both conductances is why strong cholinergic stimulation by itself can elicit a strong fluid secretion response. Fluid secretion is sustained by Cl⁻ uptake mediated by the Na⁺/K⁺Cl⁻ cotransporter (NKCC1). Model based on this study and Refs. 15, 20, as well as the generally accepted model of exocrine fluid secretion (12). Aquaporin localization based on Refs. 42, 43.
Another important goal was to shed light on functional differences observed between mouse and human serous cells and glands.

We found that while cAMP-elevating agonists activate robust CFTR-dependent anion permeability in mouse serous cells, a lack of cAMP-activated Ca\(^{2+}\) signaling prevents cAMP agonists from stimulating robust fluid secretion due to a lack of activation of K\(^{+}\) conductance. Activation of Ca\(^{2+}\)-activated K\(^{+}\) channels directly by 1-EBIO stimulated robust fluid secretion from forskolin-stimulated mouse serous cells. Despite a lack of significant fluid secretion generated by cAMP agonists alone, the cAMP/PKA-dependent activation of CFTR synergistically potentiates secretion during low-level cholinergic stimulation, suggesting that CFTR is an important component of the secretory pathway during times of combined cholinergic and cAMP stimulation. Our data suggest that, while the second-messenger-dependent regulation of fluid secretion differs between mouse and human or porcine serous cells, mouse serous cells are still a useful model in which to study CFTR-dependent secretion during combined cAMP and low-level cholinergic stimulation or exposure to Ca\(^{2+}\)-activated K\(^{+}\) channel activators.

**Elevation of [cAMP], Activates CFTR-dependent Ca\(^{2+}\)-independent Cl\(^{-}\) Permeability in Mouse Serous Acinar Cells**—Because we observed a lack of detectable serous cell fluid secretion by [cAMP]-elevating agonists, we utilized the SPQ NO\(_{3}^{-}\) substitution assay (11, 23) to determine if [cAMP], elevation caused an activation of CFTR. Because the SPQ assay tracks electroneutral substitution of Cl\(^{-}\) for NO\(_{3}^{-}\), it can measure changes in anion permeability in the absence of any cation counter-ion conductance. The SPQ assay revealed large increases in CFTR-dependent Cl\(^{-}\) permeability in response to experimental [cAMP], elevation with forskolin as well as in response to the peptide VIP and the \(\beta\)-adrenergic agonist isoproterenol. These results demonstrate functional expression of CFTR in mouse serous cells and confirm previous immunocytochemical detection of CFTR expression (15). Additionally, these data support the evidence that VIPergic and adrenergic stimulation can impact secretion from mouse submucosal glands (9) by having a direct stimulatory effect on serous acinar cell Cl\(^{-}\) conductance. However, it appears that these agonists by themselves cannot activate robust secretion as observed during cholinergic stimulation.

**Failure of [cAMP] -elevating Agonists to Activate Robust Fluid Secretion From Mouse Serous Cells Is Due to a Failure to Activate Ca\(^{2+}\)-activated K\(^{+}\) Channels Required for Sufficient Counterion Permeability**—[Ca\(^{2+}\)], responses were absent when mouse serous acinar cells were stimulated with cAMP agonists (this study and Ref. 15). In contrast, agonist-induced cAMP/ PKA-activated [Ca\(^{2+}\)], signals were observed in porcine bronchial and tracheal, human nasal (11), and ferret tracheal\(^{3}\) serous acinar cells. The mechanism(s) of the generation of these Ca\(^{2+}\) signals and the reason for their absence in mouse serous cells is not yet known, but Ca\(^{2+}\) appears to be a required component of the secretory response to cAMP-elevating agonists in the human, porcine, and ferret cells (11). Interestingly, the importance of Ca\(^{2+}\) signaling to cAMP-evoked fluid secretion is reflected in the increased ratio of the maximum rates of cAMP-evoked to cholinergic-evoked secretion in intact porcine (8, 18, 19), human (6, 7, 16), and ferret (36) glands compared with mouse glands (9, 10).

The restoration of cAMP-activated fluid secretion by EBIO suggests that cAMP-evoked [Ca\(^{2+}\)], signaling is required for activation of counterion K\(^{+}\) channels necessary for secretion. The concentration of EBIO used here had no independent effects on Cl\(^{-}\) permeability or [Ca\(^{2+}\)], in serous acinar cells, suggesting that it restored secretion by direct K\(^{+}\) channel activation. EBIO activates heterologously expressed and endogenous intermediate conductance Ca\(^{2+}\)-activated K\(^{+}\) (IK) channels with an EC\(_{50}\) of \(\approx 75–100\) \(\mu\)M (25, 26, 37, 38). EBIO is a weaker activator of small conductance Ca\(^{2+}\)-activated K\(^{+}\) channels SK1–4, with an EC\(_{50}\) of \(\approx 500\) \(\mu\)M (39). It is thus possible that the effects of 100 \(\mu\)M EBIO observed here occur through activation of IK channels. Nevertheless, the molecular identity of these channels is yet to be identified. Future electrophysiological studies are required to determine whether the channels activated by EBIO are the same as those activated during CCh-evoked [Ca\(^{2+}\)], elevation.

In addition, the threshold [Ca\(^{2+}\)], required for activation of the basolateral membrane K\(^{+}\) conductance remains to be defined. Fura-2 fluorescence changes track changes in global [Ca\(^{2+}\)], so [Ca\(^{2+}\)], in the localized vicinity of the basolateral membrane K\(^{+}\) channels is unknown. In mammalian salivary acinar cells, K\(^{+}\) conductance activation slightly preceded the observed rise of [Ca\(^{2+}\)], tracked by fura-2 (40), suggesting that elements of the receptor-mobilized intracellular Ca\(^{2+}\) were localized at or near the basolateral membrane resulting in a rapid localized increase in [Ca\(^{2+}\)], that exceeded the slower rise in global [Ca\(^{2+}\)]. The responses to low [CCh] in mouse (this study), pig, and human (11) cells suggest that a rise of [Ca\(^{2+}\)], is sufficient to activate the secretory basolateral K\(^{+}\) conductance. Coupled with the fact that forskolin and/or VIP fail to activate human or porcine cell secretion in the absence of Ca\(^{2+}\) signaling (e.g. in BAPTA-buffered conditions; (11)), our results strongly suggest that Ca\(^{2+}\) is the primarily second messenger responsible for K\(^{+}\) channel activation in human, pig, and mouse serous cells. Serous acinar cells from all three species appear to lack sufficient cAMP-activated K\(^{+}\) channel permeability to support secretion. The different secretory responses among these species stem solely from a lack of cAMP-induced Ca\(^{2+}\) signaling in mouse cells and not an intrinsic difference in the regulation of the basolateral K\(^{+}\) conductance. Future studies are necessary to provide detailed insights into the identity of the K\(^{+}\) channels and their regulation in human and mouse serous cells.

We previously showed that low-level cholinergic stimulation alone is insufficient to elevate [Ca\(^{2+}\)], high enough to activate robust CaCC-mediated fluid secretion in mouse, porcine, and human serous acinar cells. Here, we show that low-level cholinergic stimulation acts synergistically with cAMP stimulation to activate secretion in mouse serous cells. In forskolin-stimulated cells, the small [Ca\(^{2+}\)], elevation during subsequent stimulation with 100 nm CCh is associated with marked CFTR-de-
CFTR-dependent Fluid Secretion by Mouse Serous Acinar Cells

Serous acinar cells are involved in the secretion of mucus and electrolytes by the tracheobronchial airways, playing a crucial role in maintaining airway lubrication and preventing the accumulation of mucus plugs that can lead to respiratory distress. The activation of CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) is known to contribute to fluid secretion in these cells. However, the mechanisms underlying this process are complex and not fully understood.

In a recent study, the authors propose that therapeutics to enhance CFTR-dependent fluid secretion may offer a potential strategy for the treatment of cystic fibrosis (CF) airways disease. The study investigated the signaling pathways involved in CFTR-mediated fluid secretion, focusing on the role of cAMP (cyclic adenosine monophosphate) in activating CaCC (calcium-activated chloride channels) in mouse serous acinar cells.

The authors noted that the signaling mechanisms underlying fluid secretion exist between mouse and human serous cells, while fundamental differences in the signaling pathways underlying secretion exist between mouse and human serous cells. This study, therefore, highlights the importance of understanding the unique signaling pathways in different species to develop effective therapeutic strategies.

**Key Findings:***
- CFTR-dependent fluid secretion was observed in mouse serous acinar cells when stimulated with low-level cholinergic stimulation.
- Similar to porcine and human serous cells, CFTR-dependent fluid secretion was observed in mouse serous acinar cells.
- The study also observed a difference in fluid secretion between mouse and human serous cells, indicating the need for species-specific therapeutic approaches.

**Implications:**
This study provides valuable insights into the molecular mechanisms underlying CFTR-dependent fluid secretion, which could inform the development of targeted therapeutics for the treatment of CF airways disease. Further research is needed to better understand the signaling pathways involved in CFTR-mediated fluid secretion across different species.

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