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Carvedilol binding to β₂-adrenergic receptors inhibits CFTR-dependent anion secretion in airway epithelial cells

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Peitzman ER, Zaidman NA, Maniak PJ, O’Grady SM. Carvedilol binding to β₂-adrenergic receptors inhibits CFTR-dependent anion secretion in airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 310: L50–L58, 2016. First published November 13, 2015; doi:10.1152/ajplung.00296.2015.—Carvedilol functions as a nonselective β-adrenergic receptor (AR)/α₁-AR antagonist that is used for treatment of hypertension and heart failure. Carvedilol has been shown to function as an inverse agonist, inhibiting G protein activation while stimulating β-arrestin-dependent signaling and inducing receptor desensitization. In the present study, short-circuit current (Isc) measurements using human airway epithelial cells revealed that, unlike β-AR agonists, which increase Isc, carvedilol decreases basal and 8-(4-chlorophenyl)thio]adenosine 3’5’-cyclic monophosphate-stimulated current. The decrease in Isc resulted from inhibition of the cystic fibrosis transmembrane conductance regulator (CFTR). The carvedilol effect was abolished by pretreatment with the β₂-AR antagonist ICI-118551, but not the β₁-AR antagonist atenolol or the α₁-AR antagonist prazosin, indicating that its inhibitory effect on Isc was mediated through interactions with apical β₂-ARs. However, the carvedilol effect was blocked by pretreatment with the microtubule-disrupting compound nocodazole. Furthermore, immunocytochemistry experiments and measurements of apical CFTR expression by Western blot analysis of biotinylated membranes revealed a decrease in the level of CFTR protein in monolayers treated with carvedilol but not significant change in monolayers treated with epinephrine. These results demonstrate that carvedilol binding to apical β₂-ARs inhibited CFTR current and transepithelial anion secretion by a mechanism involving a decrease in channel expression in the apical membrane.

β₂-adrenergic receptor (AR) agonists function as bronchodilators that are used for treatment of pulmonary diseases, including asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis (23, 26, 28, 29, 32). Their mechanism of action on airway smooth muscle cells involves stimulation of adenylyl cyclase, leading to an increase in cAMP production and activation of protein kinase A (PKA), which is capable of phosphorylating multiple proteins that are critical for regulating airway smooth muscle tone (26). Similarly, these agonists also act on β₂-ARs on airway epithelial cells to stimulate anion secretion (4, 28). Previously, experiments with 16HBE14o− cells (immortalized human bronchial epithelial cells) and Calu-3 cells (adenocarcinoma cell line derived from human airways) demonstrated that both cell lines express β₂-AR mRNA and that stimulation with isoproterenol increased cAMP accumulation that was inhibited by pretreatment with the β₂-AR-selective antagonist ICI-118551, but not CPG 20712A, a β₁-AR-selective antagonist (1, 5). Furthermore, stimulation of Calu-3 cells with isoproterenol or epinephrine increased cystic fibrosis transmembrane conductance regulator (CFTR)-dependent anion secretion by raising intracellular cAMP concentration and mobilizing intracellular calcium from internal stores (4, 19, 31). Additionally, the short-acting β₂-AR-selective agonist salbutamol and the long-acting agonist salmeterol have been shown to modulate paracellular permeability of normal human bronchial epithelial cells (39). These agonists are able to cross the epithelium primarily through the paracellular pathway and, therefore, can regulate their own transport into the submucosa by modulating tight junction permeability.

Earlier investigations of β₂-AR regulation of anion secretion and CFTR function in airway epithelial cells revealed that the receptor and CFTR form a signaling complex in the apical membrane (21). Biochemical analyses showed that CFTR and the β₂-AR bind to the PDZ domains of Na⁺/H⁺ exchanger regulatory factor (NHERF, or EBP50) and that β₂-AR activation appears to increase the association between CFTR and NHERF (34). Also present within the signaling complex is ezrin, an anchoring protein (AKAP) for PKA, which phosphorylates CFTR to activate the channel (21, 33). Phosphorylation of the CFTR regulatory (R) domain was previously shown to inhibit formation of the β₂-AR-CFTR signaling complex by inhibiting binding of the COOH terminus of CFTR to NHERF (21). Thus, CFTR phosphorylation by PKA appears to provide a mechanism for uncoupling the β₂-AR from CFTR once the channel has been activated.

β-Blockers have been widely used for treatment of hypertension for several decades, and significant pharmacological and physiochemical heterogeneity is known to exist between various classes of β-AR antagonists (17). One example is carvedilol, a third-generation vasodilating β-blocker that is used as a cardioprotective compound for treatment of chronic heart failure (CHF). Unlike some earlier-generation β-AR antagonists, carvedilol maintains cardiac output and reduces hypertension by decreasing vascular resistance. Carvedilol has been shown to inhibit agonist binding at β₁- and β₂-ARs and also to block norepinephrine binding to α₁-ARs to reduce vasoconstriction (25, 30, 37). As a result, the hemodynamic effects of carvedilol are similar to those of renin-angiotensin system blockers and significantly greater than those of conven-
tional β-AR antagonists such as atenolol (15, 17). Furthermore, carvedilol has been shown to function as an inverse agonist by blocking G protein activation while stimulating β-arrestin-associated signaling pathways (14). Structural studies involving the use of site-specific 19F-nuclear magnetic resonance labels within the β2-AR (18). Moreover, crystal structures of the β1-AR bound to carvedilol showed additional interactions with helices 2, 3, and 7 and extracellular loop 2 compared with other β-AR blockers, and these changes may underlie the G protein-independent signaling characteristics of carvedilol and, potentially, other inverse agonists (41).

A significant number of individuals afflicted with CHF also exhibit pulmonary disease (3). Reversible airway obstruction has been described in acute heart failure patients, while a restrictive respiratory pattern occurs as a consequence of enlargement of the heart, increased accumulation of fluid in the lung, and diminished alveolar gas exchange (16). Studies of patients with CHF and COPD treated with carvedilol revealed no significant limitations in reversible airflow, indicating that carvedilol was well tolerated in these patients (2, 16). A possible mechanism that could explain why treatment with a β-blocker like carvedilol does not exacerbate bronchial obstruction may be its antagonist actions on α1-ARs. In COPD, α1-AR activation is thought to partially mediate the reduction in airflow; thus, blocking these receptors may compensate for some of the reduced bronchodilation effect associated with β-AR blocker therapy. However, in patients with asthma, carvedilol treatment was contraindicated. Results from a small group of asthma patients showed that carvedilol was poorly tolerated in 50% of these patients, as evidenced by signs of wheezing, lethargy, or hypotension. This finding suggests that the α1-AR effect was unable to compensate for the bronchoconstriction produced by β-AR antagonism (16). Similarly, in an ovalbumin-induced murine model of asthma, acute administration of carvedilol or nadolol significantly increased methacholine-evoked peak airway resistance (6, 22, 35). Chronic treatment with these antagonists, however, decreased peak resistance following methacholine challenge and significantly increased β-AR densities in membranes obtained from lung tissue. In contrast, alprenolol, a β-AR antagonist without inverse agonist activity, did not reduce airway hyperresponsiveness, mucous metaplasia, or the presence of inflammatory cells within the lung. These findings were consistent with the conclusion that β2-AR signaling was involved in the development of asthma in an allergen-driven mouse model.

The objective of the present study was to investigate the effects of inverse agonism associated with carvedilol binding to the β2-AR on anion secretion in an airway epithelial cell model where CFTR and β2-AR interactions have been well characterized. The results showed that, unlike the β2-AR-
selective antagonist ICI-118551, carvedilol was capable of inhibiting basal and 8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate (8-CPT-cAMP)-evoked anion secretion. Moreover, exposure to carvedilol reduced the apical surface expression of CFTR. The results demonstrate a unique interaction between carvedilol and the β2-AR that facilitates down-regulation of CFTR in airway epithelial cells.

MATERIALS AND METHODS

Materials. Eagle’s minimum essential medium with Earl’s salts (MEM), fetal bovine serum, nonessential amino acids, penicillin-streptomycin, phosphate-buffered saline (PBS), 4',6-diamidino-2-phenylindole, and SlowFade reagent were obtained from Life Technologies (Carlsbad, CA); trypsin from Lonza (Allendale, NJ); the Western blot protein markers Magic Mark and See Blue, nitrocellulose membranes, 10% Bis-Tris gels, and enhanced chemiluminescence (ECL) reagent from Life Technologies (Carlsbad, CA); Restore Western blot stripping buffer and cell surface protein isolation kits from Thermo Scientific (Rockford, IL); bovine serum albumin, carvedilol, epinephrine, 8-CPT-cAMP, salbutamol, and nocodazole from Sigma Aldrich (St. Louis, MO); the β2-AR-selective antagonist ICI-118551 from Tocris Bioscience (Bristol, UK); CFTR antibody (catalog no. 2269) used for Western blotting from Cell Signaling (Danvers MA); β-tubulin primary antibodies and secondary goat anti-rabbit and goat anti-mouse antibodies from Santa Cruz Biotechnology (Dallas, TX); anti-α-tubulin for Western blotting from Millipore (Billerica, MA); and antibodies used for immunocytochemistry, including the β2-AR antibody (ab40834) and anti-CFTR antibody (ab2784), from Abcam (Cambridge, MA).

Cell culture. Human airway adenocarcinoma (Calu-3) cells (American Type Culture Collection) were maintained in MEM containing 10% fetal bovine serum, 1% nonessential amino acids, and 1% penicillin-streptomycin in a humidified 5% CO2 incubator at 37°C. Similarly, cells expressing shRNAs targeting CFTR (identified as shALTR cells) were maintained in culture as described above with the addition of 4 μg/ml puromycin (24).

Measurement of polymerized tubulin. The effect of nocodazole on tubulin content within the polymerized tubulin-containing fraction of cell lysates was measured in wild-type (WT) Calu-3 cells following a previously published protocol (29). Cells were lysed in tubulin extraction buffer [in mM: 20 PIPES, 140 NaCl, 1 MgCl2, 1 EGTA, 0.5 Nonidet P-40 (NP-40), and 0.5 PMSF (pH 6.8)], and the lysates were centrifuged at 13,000 g for 10 min at 23°C. The pellet from this centrifugation step represented the NP-40-insoluble fraction containing polymerized tubulin. Pellets were suspended in sample buffer and boiled for 10 min, and proteins were separated by SDS-PAGE. The relative amount of a tubulin within the NP-40-insoluble fraction after 30, 60, and 120 min of exposure to 30 μM nocodazole was determined by Western blotting.

Apical membrane biotinylation and Western blot analysis. Calu-3 cells were grown on T-75 flasks until they reached 90% confluence. Cells were treated and membrane proteins were isolated using a cell surface protein isolation kit (Thermo Scientific). Briefly, the procedure involved treatment of cells with 10 μM epinephrine or carvedilol for 30 min. Cells were then washed in ice-cold PBS, and surface proteins were labeled with biotinylation reagent (sulfo-NHS-SS-biotin). Labeled protein was harvested and affinity-purified using NeuTrAvidin agarose resin. Proteins were eluted using 50 mM DTT in RIPA buffer, placed on a heat block for 5 min at 95°C, and spun at 1,000 g for 2 min. Isolated cell surface protein was subjected to Western blot analysis.

Biotinylated membrane proteins were loaded onto 10% Bis-Tris gels with a protein marker lane containing See Blue and Magic Mark.
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before incubation in ECL reagent for 1 min. After X-ray film exposure, blots were stripped for 5 min in Restore stripping reagent and reblocked in 5% milk-TBST overnight at 4°C. Membranes were then incubated in β-tubulin antibody (1:500 dilution) overnight at 4°C and washed three times in TBST before incubation with secondary goat anti-rabbit IgG-horseradish peroxidase antibody for 1 h at room temperature. Thereafter, blots were placed in ECL reagent for 1 min and exposed to X-ray film.

**Immunocytochemistry.** Cells were grown on Snapwell filters until they reached confluence. Monolayers serving as untreated controls or following treatment with carvedilol (10 μM) or epinephrine (10 μM) were fixed with 4% paraformaldehyde for 30 min and then incubated with 0.1% Triton X-100 for 20 min. The cells were washed three times between steps, blocked in 3% bovine serum albumin for 2 h, and exposed to X-ray film.

**Electrophysiology.** Transepithelial resistances were measured using an EVOM epithelial voltmeter attached to Ag/AgCl "chopstick" electrodes (World Precision Instruments, New Haven, CT). CFTR activity was measured using monolayers (~1,000 Ω·cm²) that were mounted in Ussing chambers and bathed on both sides with standard saline solution containing (in mM) 130 NaCl, 6 KCl, 1.5 CaCl₂, 1 MgCl₂, 20 NaHCO₃, 0.3 NaH₂PO₄, and 1.3 Na₂HPO₄ (pH 7.4), which was maintained at 37°C and bubbled with 95% O₂-5% CO₂. CFTR was determined by confocal microscopy (×60 oil immersion) using an Olympus FV1000 confocal microscope.

**RESULTS**

Figure 1A shows the effects of β₂-AR stimulation with salbutamol, a β₂-AR-selective agonist, and the effects of the cell-permeable cAMP analog 8-CPT-cAMP on Isc. The increase in current produced by apical application of salbutamol and 8-CPT-cAMP on WT Calu-3 and shALTR Calu-3 cells, respectively, reflects stimulation of transepithelial anion secretion. Addition of CFTRinh 172 caused nearly complete inhibition of salbutamol-evoked anion secretion. Conversely, apical addition of 10 μM salbutamol to CFTR-silenced Calu-3 (shCFTR) cells showed no increase in anion secretion. These results demonstrate that the increase in Isc following stimulation with salbutamol requires activation of CFTR. Similarly, monolayers treated with 10 μM 8-CPT-cAMP showed an increase in Isc that was absent in shCFTR cells. Data in Fig. 1B show the inhibitory effect of 10 μM carvedilol on the basal Isc of WT Calu-3 cells. When monolayers were subsequently treated with 10 μM 8-CPT-cAMP, no increase in Isc was observed. In contrast, monolayers pretreated with 10 μM ICI-118551 (a β₂-AR-selective antagonist) did not exhibit a decrease in basal Isc after apical addition of 10 μM carvedilol. Moreover, ICI-118551 rescued the effect of 8-CPT-cAMP on anion secretion (Fig. 1C), whereas the β₁-AR antagonist atenolol and the α₁-AR antagonist prazosin did not prevent
inhibition of the 8-CPT-cAMP-evoked $I_{sc}$ response by carvedilol (Fig. 1D).

The effect of carvedilol on the increase in $I_{sc}$ evoked by 8-CPT-cAMP is shown in Fig. 2A. At the peak of the 8-CPT-cAMP response, 10 μM carvedilol was applied to the apical surface of the monolayers (27). Unlike ICI-188551, carvedilol produced a sustained decrease in $I_{sc}$. However, pretreatment of monolayers with 10 μM ICI-118551 blocked the effect of carvedilol on 8-CPT-cAMP-evoked anion secretion (Fig. 2B). Inhibition of the carvedilol response by ICI-118551 indicated that the effect of carvedilol was dependent on interaction of the antagonist with the β2-AR. Peak current responses from these experiments are summarized in Fig. 2C.

Figure 3 shows the results of experiments designed to investigate whether the effect of carvedilol on $I_{sc}$ was due to inhibition of CFTR activity. Apical membrane anion currents were measured in Calu-3 monolayers after the basolateral membrane resistance was eliminated following treatment with the pore-forming antibiotic amphotericin B (15 μM). For these experiments, the basolateral surface of the monolayer was bathed in intracellular solution and the apical membrane was bathed in physiological saline solution. The apical membrane was held at 0 mV and stepped from −30 to +30 mV in 5-mV increments for measurement of CFTR conductance. Representative traces showing the CFTRinh 172-sensitive currents from this voltage-step protocol are shown in Fig. 3A. CFTR was stimulated by treatment of the cells with 8-CPT-cAMP, which evoked a significant increase in the slope of the CFTRinh 172-sensitive $I-V$ relationship (Fig. 3B). Addition of 10 μM carvedilol inhibited the 8-CPT-cAMP-evoked current (Fig. 3A) and significantly reduced the CFTR conductance as determined from the slope of the $I-V$ relationship: 3.84 ± 0.36 mS (n = 5) in the basal state, 12.20 ± 0.53 mS (n = 4) in cells treated with 10 μM 8-CPT-cAMP, and 2.63 ± 0.13 mS (n = 5) in cells...
treated with 10 μM carvedilol. In contrast, no significant increase in apical current or conductance was observed in shCFTR cells (Fig. 3C): 2.43 ± 0.20 mS (n = 5) in the basal state, 3.56 ± 0.22 mS (n = 5) in cells treated with 10 μM 8-CPT-cAMP, and 2.58 ± 0.15 mS (n = 5) in cells treated with 10 μM carvedilol. These results indicate that the inhibitory effect of carvedilol on 8-CPT-cAMP-stimulated anion secretion in Calu-3 cells was the result of a decrease in CFTR activity.

Previously, protein phosphatase 2A was shown to colocalize with CFTR in the apical membrane of Calu-3 cells, where it functions in regulating the phosphorylation state and functional activity of the channel (36). We tested the possibility that the inhibitory effect of carvedilol on 8-CPT-cAMP-dependent anion secretion was due to stimulation of phosphatase activity by pretreating Calu-3 monolayers for 20 min with 200 nM okadaic acid, a potent inhibitor of this phosphatase (IC50 = 0.1–1 nM). The magnitude of carvedilol-dependent inhibition of the Isc response evoked by 10 μM 8-CPT-cAMP was 10.7 ± 1.1 μA (n = 4) for control monolayers and 10.3 ± 1.0 μA (n = 8) after okadaic acid pretreatment. No significant difference was observed between control and okadaic acid treatment condi-

![Fig. 5](image_url)  
**Fig. 5.** Immunocytochemistry showing colocalization of β2-AR and CFTR before (top) and after treatment with 10 μM carvedilol (middle) or 10 μM epinephrine (bottom). β2-AR, green; CFTR, red; merged images, yellow; nuclear staining, blue.
tions, suggesting that the inhibitory effect of carvedilol did not involve an increase in phosphatase activity.

To determine if the decrease in CFTR conductance was associated with changes in the apical membrane expression of CFTR, Calu-3 cells were exposed to 30 μM nocodazole for 2 h to disrupt microtubule-dependent insertion and retrieval of the channel from the apical membrane. Cells treated with nocodazole showed microtubule disruption, as indicated by a decrease in tubulin-containing filaments (Fig. 4A). Furthermore, Western blot analysis revealed that cells treated with nocodazole for 0.5, 1, and 2 h exhibited a decrease in α-tubulin associated with the NP-40-insoluble fraction (Fig. 4B), indicating an increase in microtubule depolymerization (40). The effect on $I_{sc}$ was then determined using monolayers pretreated with 30 μM nocodazole prior to stimulation with 10 μM 8-CPT-cAMP and subsequent treatment with carvedilol. cAMP caused an increase in CFTR-dependent $I_{sc}$ in control and treated cells; however, the effect of carvedilol on $I_{sc}$ of nocodazole-treated cells was significantly reduced compared with the effect on $I_{sc}$ of untreated monolayers (Fig. 4, C and D). The $I_{sc}$ results are summarized in Fig. 4E.

Immunocytochemistry data presented in Fig. 5 show a decrease in CFTR and β2-AR labeling following treatment with 10 μM carvedilol. To quantitatively determine the effect of carvedilol and epinephrine on CFTR expression in the apical membrane, a set of apical membrane biotinylation experiments were performed following treatment with 10 μM carvedilol or 10 μM epinephrine for 30 min. Carvedilol treatment showed a significant decrease in apical CFTR protein expression compared with control cells, whereas no significant decrease was observed with epinephrine (Fig. 6A). In Fig. 6B, however, some variability in the densitometry measurements associated with the epinephrine response may explain the apparent reduction in labeling following epinephrine treatment in Fig. 5. Overall, however, these results reveal that carvedilol exposure produced a decrease in apical CFTR protein expression.

**DISCUSSION**

In the present study, carvedilol acting at β2-ARs was shown to inhibit basal CFTR-dependent anion secretion and 8-CPT-cAMP-evoked CFTR channel activity, responses consistent with inverse agonism associated with the compound. We used 8-CPT-cAMP to activate CFTR independently of any upstream receptor signaling pathway to measure changes in the level of channel activity. The unexpected finding from these experiments was that carvedilol treatment inhibited the 8-CPT-cAMP-evoked CFTR current, which would not be expected if carvedilol was only blocking agonist binding to the β2-AR. The increase in anion secretion typically observed following 8-CPT-cAMP stimulation was restored in the presence of the β2-AR-selective antagonist ICI-118551, demonstrating that carvedilol does not directly inhibit CFTR or other ion transport pathways involved in cAMP-evoked anion secretion. Experiments with okadaic acid, testing for a possible stimulatory effect of carvedilol on phosphatase activity to explain the decrease in CFTR function, yielded negative results, suggesting that dephosphorylation of the channel was not responsible for the inhibitory actions of carvedilol.

Since receptor internalization is known to be dependent on microtubules, we used a well-established approach to block internalization: treatment with nocodazole, a cell-permeable, microtubule-depolymerizing agent. We independently confirmed that, at the concentration and conditions we used, nocodazole caused microtubule depolymerization, which blocked the actions of carvedilol on 8-CPT-cAMP-induced CFTR channel activity. Earlier investigations of anion secretion in rat distal colon showed that CFTR-dependent anion secretion was reduced by microtubule inhibitors, but not by agents that disrupt microfilaments (11, 13). Similarly, transepithelial Cl− transport in renal epithelial (A6) cells was also shown to be inhibited with the microtubule inhibitor nocodazole, whereas forskolin-induced CFTR recruitment into the apical membrane of cultured human colonic epithelial (T84) cells was blocked.
by nocodazole, but not by cytochalasin D, an F-actin-disrupting agent (20, 38). These results demonstrate that the inhibitory effect of carvedilol on anion secretion was dependent on an intact microtubule network and suggest that the decrease in CFTR activity was associated with a decrease in channel expression within the apical membrane.

Apical membrane biotinylation experiments following carvedilol exposure showed a reduction in CFTR protein expression with no significant decrease in response to epinephrine treatment compared with control monolayers. This change in the level of CFTR expression coincided with immunocytochemistry data showing a decrease in apical CFTR labeling after carvedilol treatment. These findings indicate that at least a portion of the decrease in CFTR function associated with carvedilol exposure resulted from removal of CFTR protein from the apical membrane. Earlier studies with Calu-3 cells showed that the signaling complex formed by the β2-AR, CFTR, and associated scaffolding proteins dissociates following PKA-dependent phosphorylation of CFTR (21). Moreover, carvedilol was shown to induce β2-AR ubiquitination and internalization independently of G protein activation and β-arrestin recruitment by a mechanism involving interactions with MARCH2-E3 ligase, thus regulating apical expression and signaling of β2-ARs (14). Previous investigations suggest that the effect of carvedilol on CFTR surface expression observed in the present study may result from internalization of the β2-AR and CFTR, since carvedilol binding to the β2-AR does not induce G protein signaling and subsequent PKA-dependent phosphorylation of CFTR, which appears to be necessary for uncoupling of the β2-AR-CFTR signaling complex (21).

In a recent study, β2-AR activation by cigarette smoke extract (CSE) produced an increase in mucin 5AC (MUC5AC) expression and mucus hypersecretion in human airway epithelial cells and in a rat model where the airways were exposed to CSE (44). Pretreatment with propranolol or ICI-118551 or silencing of the β2-AR by RNAi blocked the effects of CSE on MUC5AC expression and mucus hypersecretion. However, inhibition of cAMP-PKA signaling induced by β2-AR activation failed to block CSE-evoked MUC5AC expression and secretion, whereas silencing of β-arrestin-2 significantly decreased ERK/MAPK phosphorylation required for CSE stimulation of MUC5AC production and goblet cell metaplasia. These results suggest the conclusion that the mechanism of CSE-induced mucus hypersecretion by the airway epithelium involves activation of the β-arrestin signaling pathway coupled to the β2-AR and that β-AR antagonists may be useful in limiting mucus hypersecretion and goblet cell metaplasia in smoking-associated COPD.

Acute and chronic cigarette exposure is also known to cause rapid and sustained inhibition of CFTR-dependent anion secretion in vitro and in vivo (7–10, 12). The decrease in secretion was found to be due to rapid internalization of CFTR, which involved trafficking from the plasma membrane to a low-solubility, perinuclear compartment without lysosomal degradation (9). Internalization was also dependent on CSE-induced activation of the ERK/MAPK pathway, and treatment with N-acetylcysteine was shown to block the increase in ERK phosphorylation, suggesting a role for oxidative stress in downregulation of CFTR (12, 43). The results of the present study and the study of Zhou et al. (44) described above suggest an additional possibility linked to the activation of β2-ARs and β-arrestin signaling that may also contribute to smoking-induced CFTR internalization and subsequent dehydration of airway surface liquid.

In summary, the results of this study show that treatment of a model airway epithelium with the inverse agonist carvedilol results in inhibition of basal and 8-CPT-cAMP-stimulated anion secretion, distinct from the actions of the β2-AR-selective antagonist ICI-118551, which has no effect on basal secretion and does not block the effects of 8-CPT-cAMP. Moreover, the inhibitory effect of carvedilol on anion secretion was linked to a decrease in the apical expression of CFTR, indicating that carvedilol induces the internalization of the β2-AR-CFTR complex following binding to the receptor. We propose that the failure of carvedilol binding to induce conformational changes in the β2-AR required for G protein activation prevents uncoupling between CFTR and the receptor, even under conditions where cAMP is available to stimulate CFTR phosphorylation. We also suggest that stimulation of β-arrestin signaling following smoking-induced activation of β2-ARs may also contribute to CFTR internalization in smoking-associated COPD.

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