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

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Submitted 21 August 2015; accepted in final form 6 November 2015

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 (I_sc) measurements using human airway epithelial cells revealed that, unlike β-AR agonists, which increase I_sc, carvedilol decreases basal and β-(4-chlorophenyl)ethanol 3’,5’-cyclic monophosphate-stimulated current. The decrease in I_sc resulted from inhibition of the cystic fibrosis transmembrane conductance regulator (CFTR). The carvedilol effect was abolished by pretreatment with the β2-AR antagonistICI-118551, but not the β1-AR antagonist atenolol or the α1-AR antagonist prazosin, indicating that its inhibitory effect on I_sc 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 no 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-AR agonists; bias ligands; β-arrestin signaling; carvedilol

β2-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 antagonistICI-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 EB5P) 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 showed that the cytoplasmic ends of helices 6 and 7 assume distinct conformational states, depending on the activating ligand, with carvedilol primarily affecting the conformational states of helix 7 (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 pattern is more characteristic of CHF. The 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); 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. The gels were transferred onto nitrocellulose membranes and blocked in 5% milk-Tris-buffered saline with 0.2% Tween 20 (TBST) for 20 h at 4°C. Membranes were incubated overnight at 4°C with CFTR antibody (catalog no. 2269; 1:1,000 dilution), washed three times (5 min each wash) with TBST, and then incubated at room temperature for 1 h with goat anti-rabbit IgG-horseradish peroxidase (1:7,500 dilution). Membranes were washed three times (5 min each wash)

Fig. 2. Carvedilol inhibits 8-CPT-cAMP-evoked anion secretion. A: Isc trace showing the inhibitory effect of apically administered carvedilol on 8-CPT-cAMP-stimulated Isc. B: apical pretreatment with ICI-118551 completely blocked the effect of carvedilol on the 8-CPT-cAMP-induced increase in Isc. C: summary of Isc results in A and B (n = 6). *Significantly different from Basal. **Significantly different from 8-CPT-cAMP.
Filters were mounted on slides, and membrane localization of monolayers for 10 min and then removed by five washes with PBS. 6-Diaminido-2-phenylindole diluted in PBS was applied to the antibodies diluted in PBS for 45 min and washed five times with PBS. Monolayers were subsequently incubated in secondary Alexa Fluor saline solution containing (in mM) 130 NaCl, 6 KCl, 1.5 CaCl2, 1,000 M 8-CPT-cAMP, and the apical membrane currents were acquired using pCLAMP 9.2 (Molecular Devices). Current-voltage (I-V) relationships plotted in Fig. 3, B and C, were determined at 250 ms after initiation of the voltage step.

Statistical analysis. Statistical significance was determined using an unpaired, two-tailed t-test (for single comparisons) or an ANOVA followed by Bonferroni’s post test (for multiple comparisons). Results are expressed as means ± SE. P < 0.05 was considered significant.

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 \( I_{sc} \). 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 M salbutamol to CFTR-silenced Calu-3 (shCFTR) cells showed no increase in anion secretion. These results demonstrate that the increase in \( I_{sc} \) following stimulation with salbutamol requires activation of CFTR. Similarly, monolayers treated with 10 M 8-CPT-cAMP showed an increase in \( I_{sc} \) that was absent in shCFTR cells. Data in Fig. 1B show the inhibitory effect of 10 M carvedilol on the basal \( I_{sc} \) of WT Calu-3 cells. When monolayers were subsequently treated with 10 M 8-CPT-cAMP, no increase in \( I_{sc} \) was observed. In contrast, monolayers pretreated with 10 M ICI-118551 (a β2-AR-selective antagonist) did not exhibit a decrease in basal \( I_{sc} \) after apical addition of 10 M 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 $\beta_2$-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 CFTR$_{inh}$ 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 CFTR$_{inh}$ 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 and 8-CPT-cAMP. 

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**Fig. 4.** Microtubule disruption with nocodazole blocks carvedilol-dependent inhibition of 8-CPT-cAMP-stimulated anion secretion. **A**: images of Calu-3 cells 2 h after incubation with 30 µM nocodazole. Note loss of filament structure compared with untreated control cells. **B**: Western blot analysis of $\alpha$-tubulin protein expression in Calu-3 cells treated with nocodazole at 0 min (lane 0), 2 h (lane 1), 1 h (lane 2), and 0.5 h (lane 3). **C**: control $I_{sc}$ trace showing inhibition of the 8-CPT-cAMP response following treatment with 10 µM carvedilol. **D**: effect of carvedilol on the 8-CPT-cAMP-stimulated $I_{sc}$ after pretreatment for 2 h with nocodazole. **E**: effect of carvedilol on $I_{sc}$ in control and nocodazole-treated monolayers ($n = 6$). *Significantly different from Basal. **E**: effect of carvedilol on $I_{sc}$ in control and nocodazole-treated monolayers ($n = 6$). *Significantly different from 8-CPT-cAMP.
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-

![Merged](image1)

![β2-AR](image2)

![CFTR](image3)

**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.
DISCUSSION

In the present study, carvedilol acting at \( \beta_2 \)-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 \( \beta_2 \)-AR. The increase in anion secretion typically observed following 8-CPT-cAMP stimulation was restored in the presence of the \( \beta_2 \)-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 \( \text{Cl}^- \) 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.

![Fig. 6. Effects of carvedilol and epinephrine on apical membrane expression of CFTR. A: Western blot showing CFTR and \( \beta\)-tubulin protein in biotinylated membranes from Calu-3 cells treated with carvedilol or epinephrine (Epi) relative to untreated control cells. B: analysis of relative intensity of CFTR bands from 4 separate biotinylation experiments revealing a decrease in the level of CFTR protein following treatment with 10 \( \mu \)M carvedilol for 30 min. *Significantly different from Control.](http://ajplung.physiology.org/)

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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 results 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 CSE (44). Pretreatment with propranolol or ICI-118551 or silencing of CSE (44). Pretreatment with propranolol or ICI-118551 or silencing of CSE (44). Pretreatment with propranolol or ICI-118551 or silencing of CSE (44). Pretreatment with propranolol or ICI-118551 or silencing of CSE (44). Pretreatment with propranolol or ICI-118551 or silencing of CSE (44). Pretreatment with propranolol or ICI-118551 or silencing of CSE (44). Pretreatment with propranolol or ICI-118551 or silencing of CSE (44). Pretreatment with propranolol or ICI-118551 or silencing of CSE (44). Pretreatment with propranolol or ICI-118551 or silencing of CSE (44).

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.

REFERENCES


