# Ibuprofen Inhibits Cystic Fibrosis Transmembrane Conductance Regulator–mediated Cl⁻ Secretion

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## Abstract

We evaluated the acute effects of ibuprofen and salicylic acid on cAMP-mediated Cl⁻ secretion ($I_{sc}$) in both colonic and airway epithelia. In T84 cells, ibuprofen inhibited the forskolin-dependent $I_{sc}$ in a concentration-dependent manner, having an apparent $K_i$ of 142 $\mu$M. Salicylic acid inhibited $I_{sc}$ with an apparent $K_i$ of 646 $\mu$M. We determined whether ibuprofen would also inhibit the forskolin-stimulated $I_{sc}$ in primary cultures of mouse trachea epithelia (MTE) and human bronchial epithelia (HBE). Similar to our results in T84 cells, ibuprofen (500 $\mu$M) inhibited the forskolin-induced $I_{sc}$ in MTEs and HBEs by $59\pm4\%$ ($n = 11$) and $39\pm6\%$ ($n = 8$), respectively. Nystatin was employed to selectively permeabilize the basolateral or apical membrane to determine the effect of ibuprofen on apical Cl⁻ ($I_{Cl}$) and basolateral K⁺ ($I_K$) currents after stimulation by forskolin. After forskolin stimulation, ibuprofen (500 $\mu$M) reduced both the $I_{Cl}$ and $I_K$; reducing $I_{Cl}$ and $I_K$ by 60 and 15%, respectively. To determine whether this inhibition of $I_{Cl}$ was due to the inhibition of CFTR, the effects of ibuprofen and salicylic acid on CFTR Cl⁻ channels in excised, inside-out patches from L-cells were evaluated. Ibuprofen (300 $\mu$M) reduced CFTR Cl⁻ current by $60\pm16\%$ and this was explained by a short-lived block ($\sim$ 1.2 ms) which causes an apparent reduction in single channel amplitude from $1.07\pm0.04$ pA to $0.59\pm0.04$ pA ($n = 3$). Similarly, salicylic acid (3 mM) reduced CFTR Cl⁻ current by $50\pm8\%$ with an apparent reduction in single channel amplitude from $1.08\pm0.03$ pA to $0.48\pm0.06$ pA ($n = 4$). Based on these results, we conclude that the NSAIDs ibuprofen and salicylic acid inhibit cAMP-mediated Cl⁻ secretion in human colonic and airway epithelia via a direct inhibition of CFTR Cl⁻ channels as well as basolateral membrane K⁺ channels. This may reduce their efficacy in conjunction with other therapeutic strategies designed to increase CFTR expression and/or function in secretory epithelia. (*J. Clin. Invest.* 1998. 102:679–687.) Key words: CFTR • chloride secretion • intestine • airway • ibuprofen

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## Introduction

Cystic fibrosis (CF)[1] is characterized by persistent *Pseudomonas aeruginosa* colonization of the conducting airways leading to the migration of inflammatory cells, including polymorphonuclear leukocytes (PMNs), into the airways of CF patients. It is known that PMNs release the potent chemokinetic and chemoattractant, leukotriene B during an inflammatory response (1) resulting in the further migration of inflammatory cells. Cromwell et al. (2) have demonstrated the existence of the leukotrienes in the sputum of CF patients. In addition to the migrating granulocytes themselves potentially causing damage to the airway epithelium, Metchnikoff first hypothesized in 1887 that the products released from the invading granulocytes might be capable of injuring the host tissue as well (3). More recently, the oxidative metabolites of arachidonic acid and the inflammatory cell-derived proteases have been implicated in the destruction and shedding of the airway epithelia observed in CF (3–7). Based on these observations, it has been proposed that anti-inflammatory drugs might be useful in CF therapy. The nonsteroidal anti-inflammatory drug (NSAID) ibuprofen is known to inhibit 5-lipoxygenase and hence leukotriene formation (8) suggesting that ibuprofen may be useful in the treatment of CF. Ibuprofen was initially shown to attenuate the inflammatory response to *P. aeruginosa* challenge in both a mouse (9) and rat (10) model without affecting the clearance or pulmonary burden of the pathogen. These results led to a clinical trial in CF patients where it was shown that high-dose (50–100 $\mu$g/ml plasma) ibuprofen slowed the annual rate of change in forced expiratory volume in one second (FEV₁) in patients with mild lung disease while having no apparent adverse effects (11). In a second clinical trial to assess pharmacokinetics and cohort therapy experience, plasma ibuprofen concentrations as high as 150 $\mu$g/ml were reported (12).

While NSAIDs are widely used over-the-counter medications, their use is known to result in intestinal inflammation and injury (13–15). This effect is believed to be caused by an increased mucosal permeability (16). Paradoxically, it has been speculated that this is due to the inhibition of the cyclooxygenase-dependent oxidation of arachidonic acid resulting in the depletion of endogenous prostaglandins (13). In addition to their effects on the cyclooxygenase- and lipoxygenase-dependent oxidation of arachidonic acid, the NSAIDs are structurally related to the known Cl⁻ channel blockers, diphenyl-

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1. *Abbreviations used in this paper:* 293B, trans-6-cyano-4-(*N*-ethylsulphonyl-*N*-methylamino)-3-hydroxy-2,2-dimethyl-chromane; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; FEV₁, forced expiratory volume in one second; HBE, human bronchial epithelium; HPC, human placental collagen; $I_{Cl}$, Cl⁻ currents; $I_K$, K⁺ currents; $I_{sc}$, short circuit current; MTE, murine tracheal epithelium; NSAID, nonsteroidal anti-inflammatory drug; PD$_{te}$, transepithelial potential difference; PKA, protein kinase A; PMNs, polymorphonuclear leukocytes.

amine-2-carboxylate and 5-nitro-2-(3-phenylpropylamino)-benzoate (17). Also, the NSAIDs, niflumic acid and flufenamic acid are known inhibitors of the $Ca^{2+}$-dependent $Cl^-$ channel in *Xenopus* oocytes (18). These results suggested that the structurally related NSAID ibuprofen may similarly affect ion transport across epithelia. Thus, we determined the effect of ibuprofen on $Cl^-$ secretion in both human colonic and airway epithelia. We demonstrate that ibuprofen, at a pharmacologically relevant concentration, inhibits cAMP-dependent $Cl^-$ secretion across human colonic and airway epithelia and that this inhibition is due at least in part to the blocking of the cystic fibrosis transmembrane conductance regulator (CFTR) $Cl^-$ channel.

## Methods

*T84 cell culture.* T84 cells were grown in DME and Ham's F-12 (1:1) supplemented with 15 mM Hepes, 14 mM $NaHCO_3$, and 10% FBS. The cells were incubated in a humidified atmosphere containing 5% $CO_2$ at 37°C. For measurements of short-circuit current ($I_{sc}$) T84 cells were seeded onto Costar transwell cell culture inserts (0.33 $cm^2$; Costar Corp., Cambridge, MA) and the culture media changed every 48 h. $I_{sc}$ measurements were performed on filters after 14–21 d in culture.

*L cell culture.* L cells, a murine fibroblast cell line stably expressing human CFTR (19), were grown in DME supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. For patch-clamp experiments the cells were plated onto human placental collagen (HPC, type VI; Sigma Chemical Co., St. Louis, MO)–coated plastic coverslips 24–48 h before use.

*Primary cultures of murine tracheal epithelium.* CD-1 mice (Charles River Laboratories, Wilmington, MA) were killed by cervical dislocation, the trachea was removed and cleaned of adherent tissues, and opened longitudinally. The tracheas were incubated overnight at 4°C in MEM containing 0.1% protease XIV, 0.01% deoxyribonuclease, and 1% FBS. The epithelial cells were isolated by centrifugation and resuspended in Ham's F-12 media supplemented with 1 μg/ml insulin, 7.5 μg/ml transferrin, 1 μM hydrocortisone, 30 nM 3,5,3′-triiodothyronine ($T_3$), 1 ng/ml cholera toxin, 2.5 ng/ml epidermal growth factor, and 10 ng/ml endothelial cell growth substance. This was combined 1:1 with 3T3 fibroblast conditioned DME containing 2% FBS. The cells were seeded onto Costar Corp. transwell cell culture filter inserts, and after 4 d in culture, the media bathing the apical surface was removed to establish an air interface. Measurements of $I_{sc}$ were performed after approximately 10 additional d in culture.

*Primary cultures of human bronchial epithelium.* Tissue was obtained from pathology specimens after lung transplantation for a variety of pathologic conditions including emphysema, chronic obstructive pulmonary disease, scleroderma, and $α_1$-antitrypsin disease under a protocol approved by the University of Pittsburgh Human Experimentation Committee. The mainstem bronchi were removed, cleaned of adherent tissues and opened longitudinally. The bronchi were incubated overnight at 4°C in MEM containing 0.1% protease XIV, 0.01% deoxyribonuclease, and 1% FBS. The epithelial cells were removed from the underlying musculature by blunt dissection, isolated by centrifugation and washed in MEM containing 5% FBS. After centrifugation, the cells were resuspended in bronchial epithelial growth media, supplemented with 26 μg/ml bovine pituitary extract, 10 μg/ml insulin, 1 ng/ml epidermal growth factor, 13 ng/ml $T_3$, 20 μg/ml transferrin, 0.2 ng/ml retinoic acid, 1 μg/ml hydrocortisone, 100 μg/ml gentamycin, and 100 ng/ml amphotericin (catalog No. CC-3170; Clonetics Corporation, San Diego, CA). The cells were then plated into HPC-treated t-25 tissue culture flasks. Upon reaching 80–90% confluence, the cells were trypsinized (0.1%), resuspended in MEM plus 5% FBS and seeded onto HPC-coated Costar Transwell filters (0.33 $cm^2$) at a density of approximately $2 \times 10^6/cm^2$. After 24 h, the media was changed to DME/F12 (1:1) plus 2% Ultroser G (Bio-

Sepra, Inc., Cedex, France) and an air interface at the apical membrane established. The media bathing the basolateral surface was changed every 48 h. Measurements of $I_{sc}$ were performed after ~10–20 additional days in culture.

*$I_{sc}$ measurements.* Costar transwell cell culture inserts were mounted in an Ussing chamber (Jim's Instruments, Iowa City, IA) and the monolayers continuously short-circuited (University of Iowa, Department of Bioengineering, Iowa City, IA). Transepithelial resistance was measured by periodically applying a 5-mV pulse, and the resistance calculated using Ohm's law. The bath solution contained (in mM): 120 NaCl, 25 $NaHCO_3$, 3.3 $KH_2PO_4$, 0.8 $K_2HPO_4$, 1.2 $MgCl_2$, 1.2 $CaCl_2$, and 10 glucose. The pH of this solution was 7.4 when gassed with a mixture of 95% $O_2$–5% $CO_2$ at 37°C. The effects of forskolin, ibuprofen, and glibenclamide on apical membrane $Cl^-$ currents ($I_{Cl}$) were assessed after permeabilization of the serosal membrane with nystatin (360 μg/ml), and the establishment of a mucosa-to-serosa $Cl^-$ concentration gradient. Serosal NaCl was replaced by equimolar Na-gluconate. Nystatin was added to the serosal membrane 15–30 min before the addition of drugs (20). Successful permeabilization of the basolateral membrane was based upon the recording of a negative $Cl^-$ current as described previously (20). The effects of forskolin, ibuprofen, and trans-6-cyano-4-(*N*-ethylsulphonyl-*N*-methylamino)-3-hydroxy-2,2-dimethyl-chromane (293B) on basolateral membrane $K^+$ currents ($I_K$) were assessed after permeabilization of the apical membrane with nystatin (180 μg/ml) for 15–30 min, and establishment of a mucosa-to-serosa $K^+$ concentration gradient. For measurements of $I_K$, mucosal NaCl was replaced by equimolar K-gluconate, while serosal NaCl was substituted with equimolar Na-gluconate. Chloride was removed from these solutions to prevent cell swelling that may be associated with the limited $Cl^-$ permeability of the nystatin pore. In all gluconate solutions the $CaCl_2$ was increased to 4 mM to compensate for the $Ca^{2+}$ buffering capacity of the gluconate anion. Forskolin, ibuprofen, salicylic acid, and glibenclamide were added to both sides of the monolayers at the indicated concentrations. Bumetanide and 293B were added only to the serosal bathing solution while amiloride was added only to the mucosal bathing solution. Changes in $I_{sc}$ are calculated as a difference current between the sustained phase of the response and respective baseline values.

*Single channel recording.* Channels were identified as CFTR based upon the channel amplitude at –80 mV (1.07±0.06 pA), a linear current–voltage relationship with a slope conductance of 11.8 pS (data not shown), stimulation of channel gating by forskolin when in the cell-attached configuration, stimulation of channel gating by the catalytic subunit of protein kinase A (PKA) when in the excised, inside-out configuration and the ongoing requirement for ATP subsequent to PKA-dependent activation. Such channels are routinely observed in cells transfected with the CFTR gene but not in parental or mock-transfected cells (19). Additionally, kinetic behavior of the channels in control conditions (see Figs. 4 and 5) was indistinguishable from our previous characterization of CFTR when evaluated in this system (21, 22).

Data were acquired from excised membrane patches and analyzed as described previously (21, 23) with minor modifications. Before changing solution composition at the cytosolic face of an excised membrane patch, CFTR channel gating was recorded for a control period in excess of 80 s to insure steady state kinetic activity. The 0.75-ml bath was refreshed at a rate of four bath volumes per minute and maintained at 34–37°C throughout all experiments. The pipette solution contained (in mM): 140 *N*-methyl-D-glucamine-HCl (NMDG-Cl), 1 $CaCl_2$, 2 $MgCl_2$, 10 1,3-bis[tris(hydroxymethyl)-methylamino]propane-HCl (BTP); pH was adjusted to 7.35 with HCl. The standard bathing solution contained (in mM): 150 NaCl, 2 $MgCl_2$, 10 NaF, 0.5 EGTA, 0.26 $CaCl_2$, and 10 BTP, pH 7.35 (adjusted with HCl). Free $Ca^{2+}$ concentration was calculated to be 100 nM (24). $F^-$ was included as a nonspecific inhibitor of phosphatases that might be active on patch excision and can lead to channel inactivation (25). The pH of bath solutions containing either ibuprofen or salicylic acid was adjusted as necessary with BTP before patch exposure.

Single channel amplitude ($i$), mean current ($I$), and number of channels ($N$) present in the patch were determined as described previously (21). For macroscopic current analysis (i.e., $I$ in the presence of various concentrations of NSAIDs expressed as a percent of $I$ in the absence of NSAIDs; $I/I_{con}$) and fluctuation analysis, recordings 85–170 s in length were analyzed for each control or experimental condition with Bio-Patch software (version 3.21; Molecular Kinetics, Inc., Pullman, WA) as described previously (21).

*Chemicals.* 293B was a generous gift from Dr. Rainer Greger (Albert-Ludwigs-Universitat, Freiberg, Germany). Nystatin was a generous gift from Dr. S. Lucania (Bristol Meyers-Squibb, Princeton, NJ). Bumetanide, ibuprofen, salicylic acid, acetylsalicylic acid and glibenclamide were obtained from Sigma Chemical Co. Forskolin was obtained from Calbiochem Corp. (La Jolla, CA). $Na_2ATP$ was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). The catalytic subunit of PKA was obtained from Promega Corp. (Madison, WI). Forskolin, bumetanide and ibuprofen were made as 1,000-fold stock solutions in ethanol. Salicylic acid was dissolved in the appropriate experimental buffer. Cell culture medium was obtained from GIBCO BRL (Gaithersburg, MD) unless otherwise noted above.

*Data analysis.* All data are presented as means±SEM, where $n$ indicates the number of experiments. Nonlinear curve fitting was completed using SigmaPlot (version 4.14; Jandel Scientific, San Rafael, CA). Statistical analyses were performed using Student's $t$ test. A value of $P < 0.05$ was considered statistically significant.

## Results

*Effect of ibuprofen and salicylic acid on cAMP-dependent Cl⁻ secretion in T84 cells.* In initial experiments, we determined the effect of ibuprofen on the model Cl⁻ secretory epithelium, T84. As shown in Fig. 1 (*top*), forskolin stimulated a sustained Cl⁻ secretory response ($I_{sc}$) in these cells. Subsequent to forskolin, ibuprofen (500 μM) caused a rapid and reversible inhibition of $I_{sc}$. After washout of ibuprofen and relief of associated inhibition, the $I_{sc}$ was inhibited by bumetanide, confirming the Cl⁻ secretory nature of the response. In seven experiments, forskolin (10 μM) increased $I_{sc}$ by 67±4 μA/cm² and this was inhibited 46±4% by ibuprofen (500 μM). A concentration–response curve for the ibuprofen-dependent inhibition of forskolin-induced $I_{sc}$ is shown in Fig. 1 (*bottom, circles*). Forskolin (10 μM) increased $I_{sc}$ 71±8 μA/cm² and this was subsequently inhibited in a concentration-dependent fashion with ibuprofen. The data were fitted by a Michaelis-Menten relation with an apparent $K_i$ of 142±32 μM ($n = 5$) and a predicted maximal inhibition of 68%. Similar inhibition was observed in the presence of bovine serum albumin (0.4 g/liter) suggesting that the effect of ibuprofen on epithelial Cl⁻ secretion would not be significantly affected in vivo by plasma protein binding (data

## T84 Cells
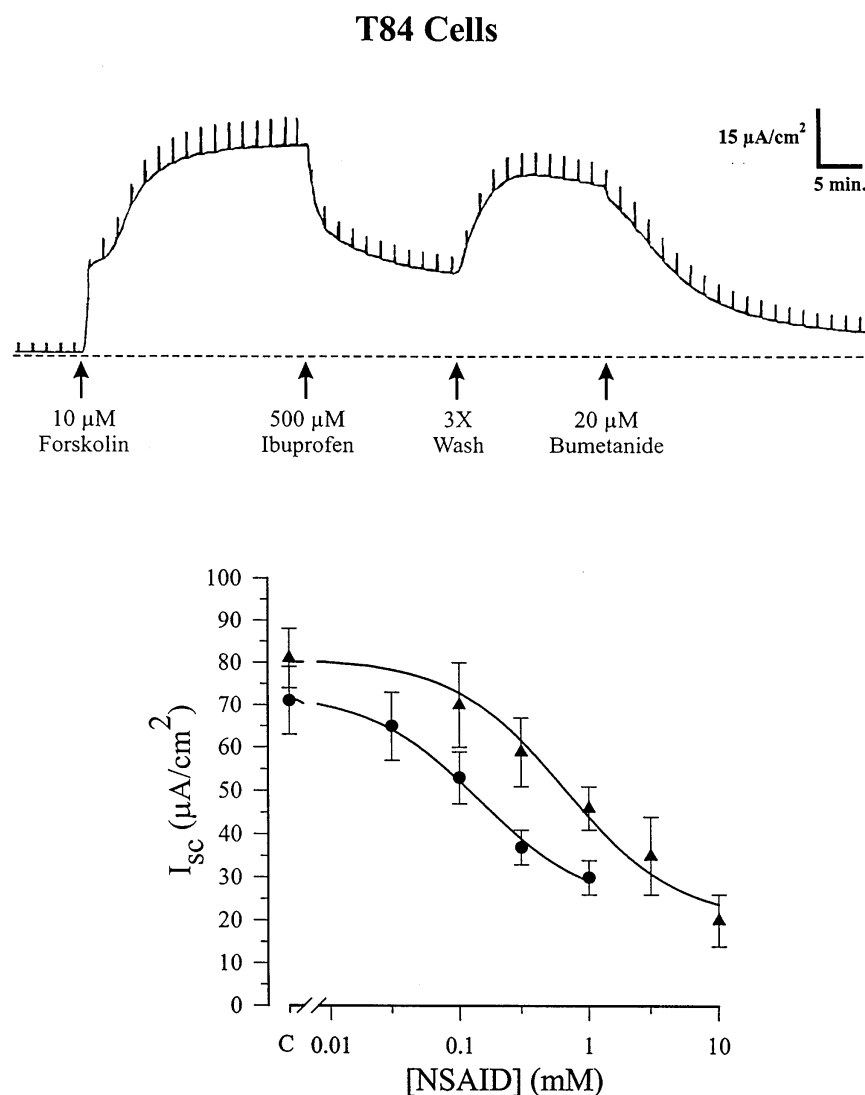




*Figure 1.* (*Top*) Effect of ibuprofen (500 μM) on a forskolin-stimulated Cl⁻ secretory response ($I_{sc}$) in T84 monolayers. Ibuprofen (mucosa and serosa addition) inhibited $I_{sc}$ and this was reversed upon washout of the ibuprofen (3× wash). Bumetanide (20 μM) inhibited the remainder of the $I_{sc}$. Current pulses represent response to voltage pulses of +5 mV (5 s duration) to measure tissue conductance. The dashed line indicates the zero (0) current level. (*Bottom*) Average concentration–response curve for inhibition of the forskolin-stimulated $I_{sc}$ by ibuprofen (*circles*; $n = 5$) and salicylic acid (*triangles*; $n = 5$). The data were fitted by a Michaelis-Menten function using a Hill coefficient of 1.0. Apparent $K_i$ values were 142±32 μM for ibuprofen and 646±32 μM for salicylic acid. *C*, control.
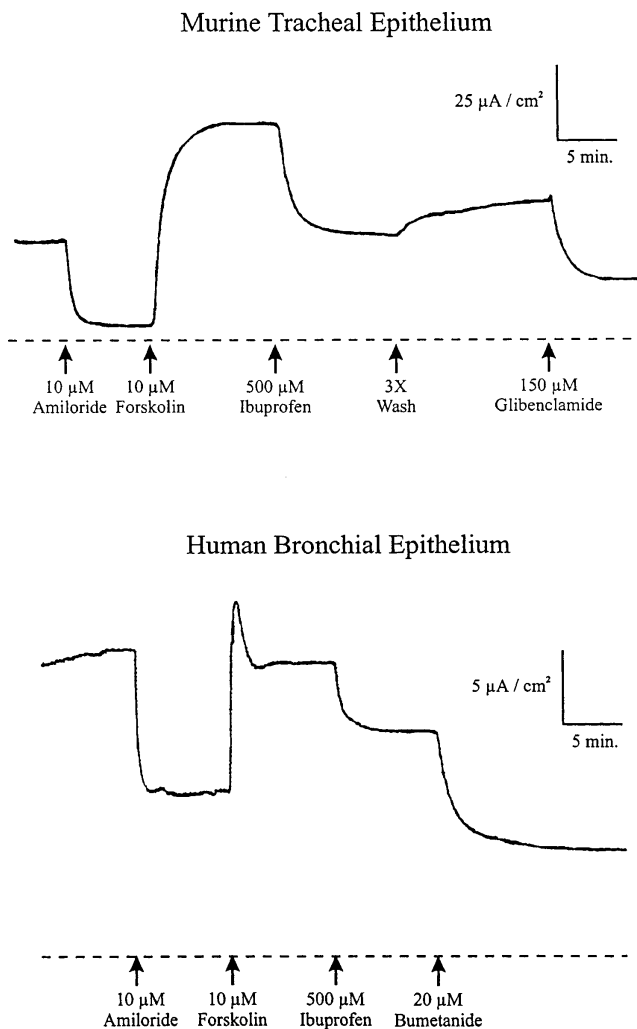
not shown). These data demonstrate that the NSAID ibuprofen inhibits cAMP-mediated Cl$^-$ secretion within the pharmacological range currently being tested in clinical trails for CF therapy (200–400 $\mu$M plasma levels; reference 11).

The effect of salicylic acid on the forskolin-induced $I_{sc}$ was also determined. Forskolin (10 $\mu$M) increased $I_{sc}$ 81$\pm$7 $\mu$A/cm$^2$ ($n = 15$) and this was inhibited by salicylic acid in a concentration-dependent manner with an apparent $K_i$ of 646$\pm$32 $\mu$M and a predicted maximal inhibition of 74% (Fig. 1, *bottom*, *triangles*). We also evaluated the effect of acetylsalicylic acid (aspirin) on forskolin-induced $I_{sc}$. At both 500 $\mu$M and 1 mM, aspirin was less efficacious, inhibiting $I_{sc}$ by only 15$\pm$1% ($n = 4$) and 23$\pm$2% ($n = 4$), respectively.

*Effect of ibuprofen on cAMP-dependent Cl$^-$ secretion in primary cultures of murine tracheal and human bronchial epithelia.* Our studies from colonic cells were extended to determine whether ibuprofen would similarly inhibit the forskolin-stimulated $I_{sc}$ in both primary cultures of murine tracheal epithelia (MTE) and human bronchial epithelia (HBE). As shown for one MTE monolayer in Fig. 2 (*top*), after inhibition of basal Na$^+$ absorption with amiloride (10 $\mu$M), forskolin (10 $\mu$M) induced a sustained increase in $I_{sc}$. The subsequent addition of ibuprofen (500 $\mu$M) induced a rapid inhibition of $I_{sc}$. Upon removal of the ibuprofen this inhibition was partially reversible, consistent with our data from T84 cells (Fig. 1). Glibenclamide (150 $\mu$M) inhibited the remaining current suggesting the current is being carried across the apical membrane via CFTR. In 11 monolayers the baseline $I_{sc}$ was 32$\pm$5 $\mu$A/cm$^2$ and this was reduced to 8$\pm$5 $\mu$A/cm$^2$ in the presence of amiloride. The subsequent addition of forskolin increased $I_{sc}$ by 29$\pm$4 $\mu$A/cm$^2$ and this was subsequently inhibited 59$\pm$4% by ibuprofen. Addition of glibenclamide returned $I_{sc}$ to baseline (8$\pm$2 $\mu$A/cm$^2$).

The effect of ibuprofen on forskolin stimulated $I_{sc}$ in HBE is shown in Fig. 2 (*bottom*). The monolayer was initially exposed to mucosal amiloride (10 $\mu$M) to inhibit basal Na$^+$ absorption. Subsequently, forskolin (10 $\mu$M) induced a transient increase in $I_{sc}$ followed by a sustained plateau. Ibuprofen (500 $\mu$M) partially inhibited this sustained $I_{sc}$ response with bumetanide (20 $\mu$M) further inhibiting $I_{sc}$. Bumetanide reduced $I_{sc}$ to below the amiloride-induced $I_{sc}$ level suggesting that a portion of the $I_{sc}$ remaining after amiloride is due to basal Cl$^-$ secretion as previously described (26). In eight monolayers, the basal $I_{sc}$ averaged 29.4$\pm$8.3 $\mu$A/cm$^2$ with an open-circuit transepithelial potential difference (PD$_{te}$) of $-10.5\pm3.7$ mV. This PD$_{te}$ is similar to what has been reported for in vivo measurements of nasal PD$_{te}$ (27). Amiloride reduced $I_{sc}$ to 16.8$\pm$4.5 $\mu$A/cm$^2$ with forskolin subsequently inducing an initial peak increase in $I_{sc}$ of 21.7$\pm$3.9 $\mu$A/cm$^2$ with the sustained plateau phase being 15.8$\pm$3.9 $\mu$A/cm$^2$ above the amiloride-induced baseline. Ibuprofen inhibited the sustained phase 39$\pm$6%. Bumetanide further inhibited $I_{sc}$ to below baseline (14.3$\pm$2.9 $\mu$A/cm$^2$). Taken together, our results from the MTEs and HBEs demonstrate that ibuprofen inhibits cAMP-dependent Cl$^-$ secretion across airway epithelia.

*Transepithelial Cl$^-$ and K$^+$ current measurements.* Our results suggest that ibuprofen is inhibiting Cl$^-$ secretion by directly altering the apical membrane Cl$^-$ conductance ($G_{Cl}$) or by altering the electrochemical driving force for Cl$^-$ secretion, an effect that might occur via modulation of a basolateral membrane K$^+$ conductance ($G_K$). To further resolve the conductance pathways inhibited, the pore-forming antibiotic nys-



Murine Tracheal Epithelium

25 $\mu$A / cm$^2$

5 min.

10 $\mu$M Amiloride   10 $\mu$M Forskolin   500 $\mu$M Ibuprofen   3X Wash   150 $\mu$M Glibenclamide



Human Bronchial Epithelium

5 $\mu$A / cm$^2$

5 min.

10 $\mu$M Amiloride   10 $\mu$M Forskolin   500 $\mu$M Ibuprofen   20 $\mu$M Bumetanide

*Figure 2.* (*Top*) Effect of ibuprofen (500 $\mu$M) on the forskolin-stimulated $I_{sc}$ in murine tracheal epithelia. Amiloride (10 $\mu$M; mucosal) was added to inhibit the basal Na$^+$ absorption in this tissue. Subsequently, forskolin (10 $\mu$M) increased $I_{sc}$ and this was reversibly inhibited by ibuprofen (mucosal and serosal). After washout of the ibuprofen, the current was inhibited by glibenclamide (150 $\mu$M; mucosal and serosal) suggesting that CFTR mediates Cl$^-$ secretion in this tissue. (*Bottom*) Effect of ibuprofen (500 $\mu$M) on the forskolin-stimulated $I_{sc}$ in human bronchial epithelia. Amiloride (10 $\mu$M; mucosal) was added to inhibit the basal Na$^+$ absorption in this tissue. Subsequently, forskolin (10 $\mu$M) increased $I_{sc}$ and this was inhibited by ibuprofen (mucosal and serosal). Bumetanide (20 $\mu$M; serosal) caused a further reduction in $I_{sc}$.

tatin was used to selectively permeabilize either the basolateral or apical membrane, and the appropriate transepithelial ion gradients established to measure $I_{Cl}$ or $I_K$, respectively (see Methods). In Fig. 3, the effects of ibuprofen on $I_{Cl}$ and $I_K$ after stimulation by forskolin are shown. After establishment of a mucosa-to-serosa Cl$^-$ gradient and permeabilization of the basolateral membrane with nystatin, forskolin stimulated an inward current, as expected for the imposed Cl$^-$ gradient (Fig. 3, *top*). Ibuprofen (500 $\mu$M) inhibited this cAMP-mediated $I_{Cl}$. The subsequent addition of glibenclamide (100 $\mu$M) further inhibited the current, confirming that CFTR was the conductive pathway at the apical membrane. Note that in the presence of glibenclamide, both the nystatin- and forskolin-induced
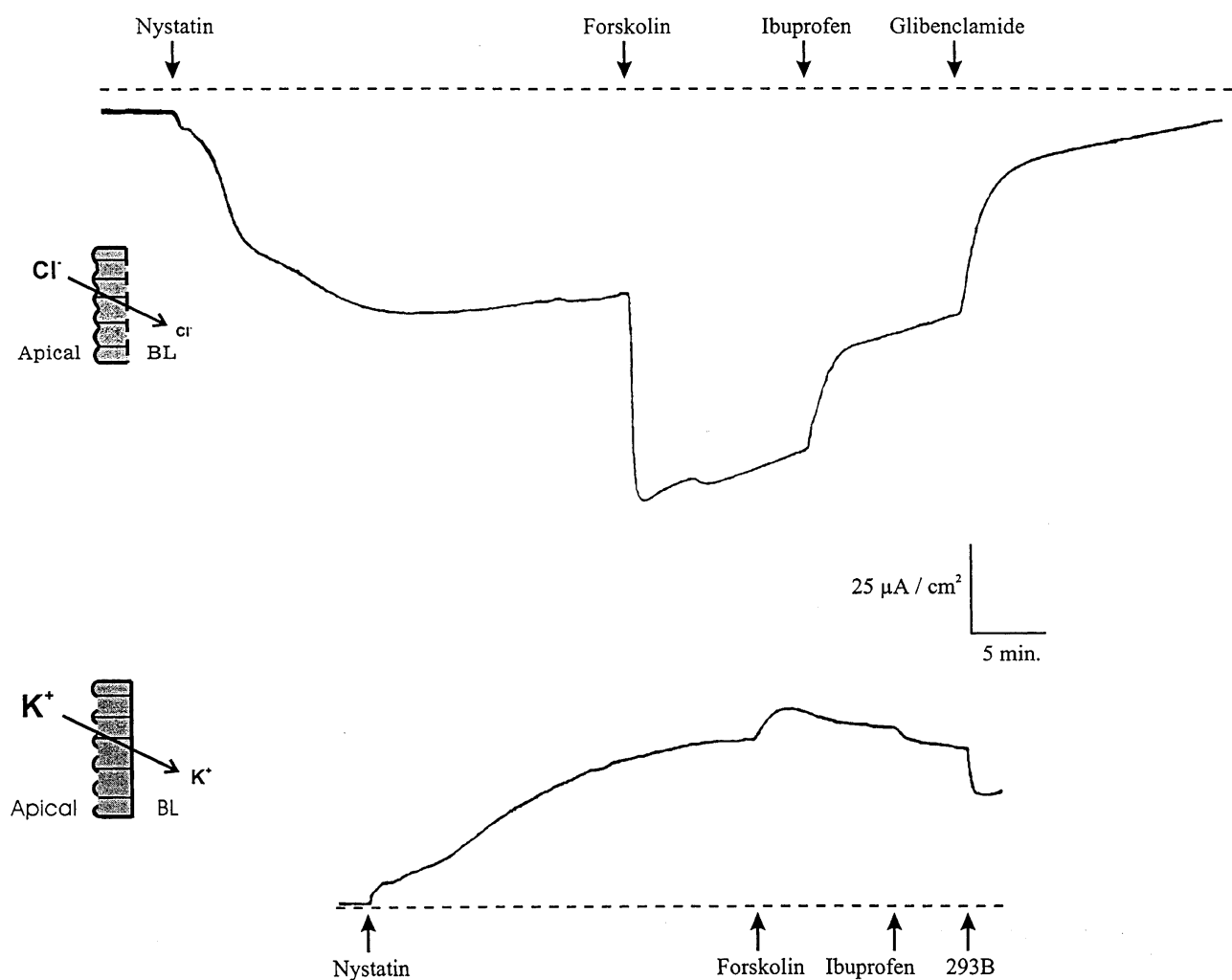
*Figure 3.* (*Top*) Effect of ibuprofen (mucosal and serosal) on the forskolin (10 μM)-induced apical membrane Cl⁻ current after establishment of a mucosa-to-serosa Cl⁻ gradient and permeabilization of the basolateral (*BL*) membrane with nystatin (see Methods). Forskolin induced an increase in inward current; consistent with an absorptive Cl⁻ flow. Ibuprofen (500) inhibited the $I_{Cl}$. The subsequent addition of glibenclamide (100 μM) further inhibited the $I_{Cl}$. (*Bottom*) Effect of ibuprofen (500 μM) on the forskolin-induced K⁺ current ($I_K$) after establishment of a mucosa-to-serosa K⁺ gradient and permeabilization of the mucosal membrane with nystatin (see Methods). Ibuprofen modestly inhibited the forskolin-induced K⁺ current while the subsequent addition of 293B (100 μM) further blocked the response. The monolayer illustrations indicates the direction of the ion gradient and the membrane that was permeabilized with nystatin (*dashed line* in monolayer illustration). The dashed line indicates the zero current level.

$I_{Cl}$ was inhibited suggesting that the basal conductance induced by nystatin is due to CFTR as well, similar to what we have reported previously (20, 26, 28). In five monolayers, nystatin increased $I_{Cl}$ an average of 45±3 μA/cm² and this was further increased 48±6 μA/cm² in the presence of forskolin (10 μM). The addition of ibuprofen reduced $I_{Cl}$ an average of 54±3 μA/cm² and this was further inhibited 35±6 μA/cm² by glibenclamide. In previous studies we found no evidence for either a Ca²⁺-activated Cl⁻ channel, an outwardly-rectifying Cl⁻ channel or ClC Cl⁻ channels in the apical membrane of confluent T84 cells suggesting that CFTR is the dominant conductance under our recording conditions (20, 28). This suggests that ibuprofen inhibits ∼ 60% of the CFTR Cl⁻ conductance in these experiments, in agreement with our $I_{sc}$ measurements.

After establishment of a mucosa-to-serosa K⁺ gradient and permeabilization of the mucosal membrane with nystatin, forskolin increased $I_K$ as reported previously (Fig. 3, *bottom*; ref-

erences 26, 29). Ibuprofen induced a small but reproducible inhibition of $I_K$. The subsequent addition of 293B further inhibited $I_K$. Lohrman et al. (30) previously characterized 293B as an inhibitor of the cAMP-dependent K⁺ conductance in rat colon. Further, we demonstrated that forskolin-dependent Cl⁻ secretion in T84 cells is inhibited by 293B and that 293B inhibits the forskolin-stimulated $I_K$ in these cells (29). Again, note that 293B reduced $I_K$ below the level induced by nystatin consistent with our previous report (29). This suggests that a portion of the nystatin-induced $I_K$ is due to the presence of a cAMP-dependent K⁺ conductance. In six experiments, nystatin increased $I_K$ 41±3 μA/cm² and this was further increased 12±3 μA/cm² by forskolin (10 μM). The addition of ibuprofen reduced $I_K$ by 5±1 μA/cm². The subsequent addition of 293B further reduced $I_K$ by 12±2 μA/cm². In the absence of forskolin, ibuprofen had no effect on $G_K$. We demonstrated previously that the nystatin-induced $I_K$ is blocked by both 293B and

Ba$^{2+}$ in an additive fashion (29) with both inhibitors reducing $I_K$ by an equal amount. If we assume that 293B inhibits $\sim$ 50% of the basal K$^+$ conductance then this suggests that ibuprofen inhibits $\sim$ 15% of the total basolateral K$^+$ conductance.

*Effect of ibuprofen on CFTR in excised, inside-out patches.* The results above demonstrate that ibuprofen inhibits CFTR-dependent Cl$^-$ current. To determine the mechanism of this inhibition, the effect of ibuprofen on CFTR Cl$^-$ channels in excised, inside-out patches from mouse L cells recombinantly expressing CFTR (19) was determined. We characterized previously the kinetics of nucleotide-dependent gating (21, 22) and sulfonylurea-dependent block of CFTR (23, 31) in these cells in detail. The results of one experiment with ibuprofen

are shown in Fig. 4. Ibuprofen (300 μM) dramatically reduced mean current (*I*) from 2.2 pA to 1.5 pA and this was associated with an apparent reduction in single channel amplitude from 1.07 pA to 0.52 pA (*i*; compare amplitude histograms for control and ibuprofen in Fig. 4). Increasing the concentration of ibuprofen to 1 mM further reduced *I* to 0.94 pA and transitions between open and closed states of the channels could no longer be resolved. In three patches, 300 μM ibuprofen reduced *I* by 60±16% while causing an apparent reduction in *i* from 1.07±0.04 pA to 0.59±0.04 pA. Note that, when filtered at 200 Hz, complete closures of the channels are not resolved in the presence of ibuprofen (Fig. 4, *top*) resulting in the appearance of a change in baseline. However, when filtered at
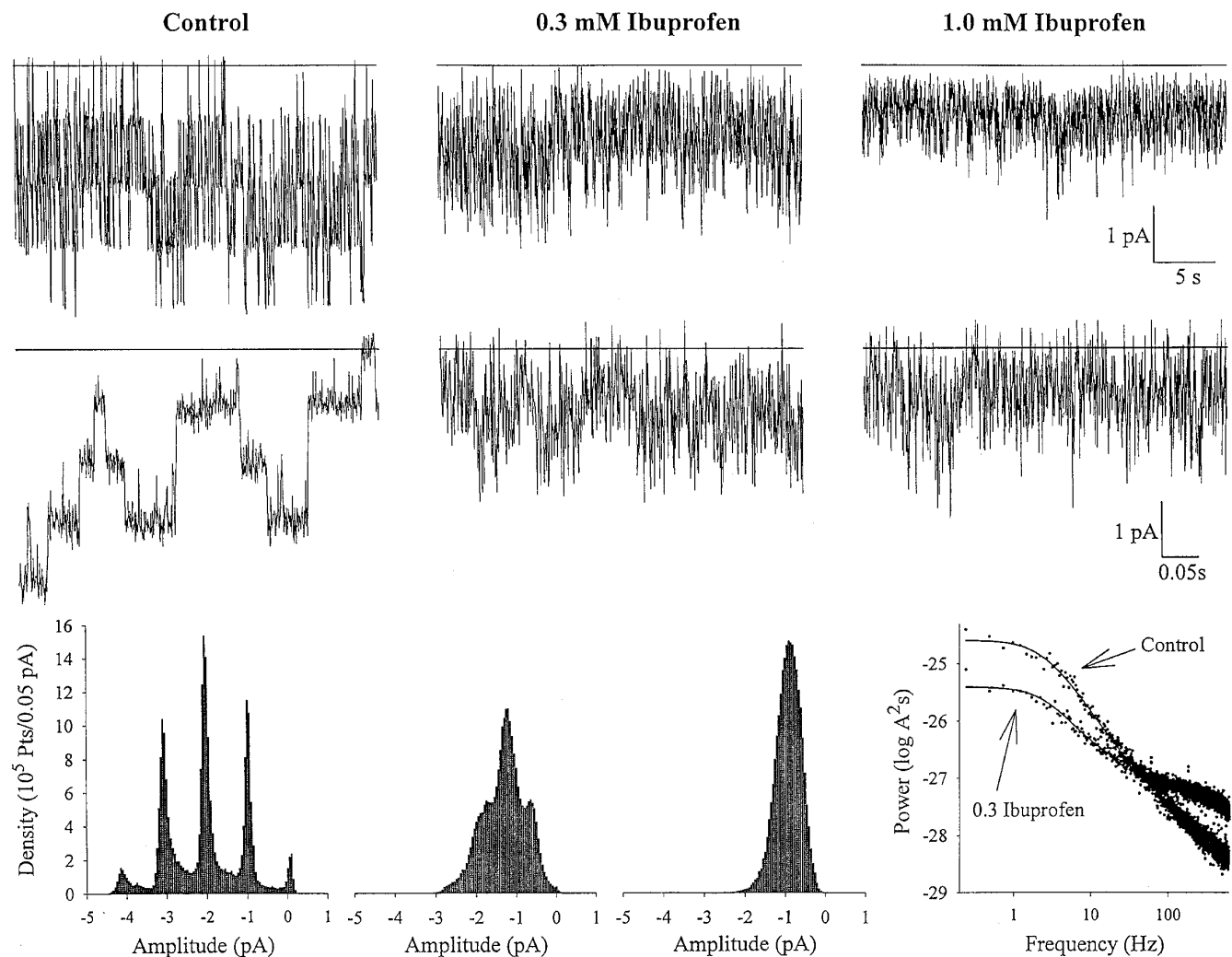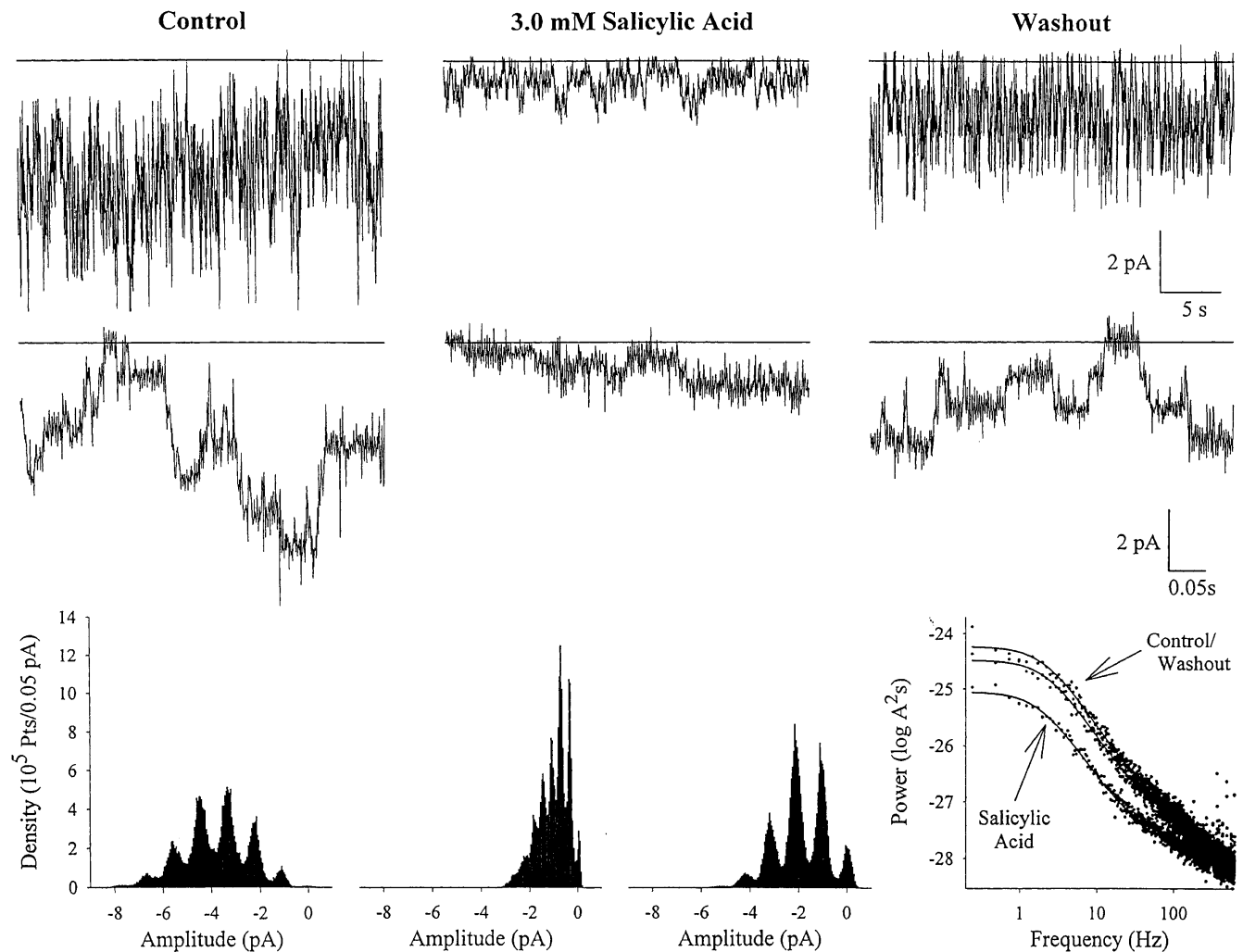


*Figure 4.* Ibuprofen inhibits CFTR-mediated Cl$^-$ current in membrane patches excised from L cells. (*Top*) The changes in gating characteristics and reduction in patch current caused by increasing concentrations of ibuprofen as indicated. Average current was 2.2, 1.5, and 0.9 pA in the three conditions, respectively. Current records were filtered (8 pole Bessel filter) at 100 Hz and plotted at 200 Hz. (*Middle*) Expanded records of current traces from the top panels. Records were filtered at 800 Hz and plotted at 2 kHz. (*Bottom left*) Amplitude histograms of the data presented in the top panels. In control conditions, the mean single channel amplitude was 1.06 pA. Single channel amplitude was reduced to 0.52 pA by 0.3 mM ibuprofen and could not be determined in the presence of 1.0 mM ibuprofen. 1 min of data, filtered at 50 Hz, were used to construct each histogram. (*Bottom right*) The power density spectra and associated multi-Lorentzian fits of current recordings made in presence or absence of 0.3 mM ibuprofen. The data show that ibuprofen introduces high-frequency transitions ($f_c$ = 410 Hz; $S_o$ = 5.9 × 10$^{-28}$ A$^2$s) not seen in the control conditions and causes a reduction in power and frequency associated with nucleotide-dependent gating (control, $f_{c1}$ = 2.4 Hz, $S_o$ = 2.6 × 10$^{-25}$ A$^2$s, and $f_{c2}$ = 87 Hz, $S_o$ = 4.5 × 10$^{-28}$ A$^2$s; ibuprofen, $f_{c1}$ = 2.7 Hz, $S_o$ = 3.7 × 10$^{-26}$ A$^2$s and $f_{c2}$ = 31 Hz, $S_o$ = 1.4 × 10$^{-27}$ A$^2$s). Data were filtered at 800 Hz and sampled at 2 kHz. 23 and 35 nonoverlapping segments (4.096 s) were used to construct control and ibuprofen power density spectra, respectively. Lorentzian components could not be resolved in this frequency domain in the presence of 1 mM ibuprofen.

800 Hz, the baseline was not affected by addition of ibuprofen (Fig. 4, *middle*). These results are consistent with a short-lived block of CFTR by ibuprofen. Further evidence of high frequency block is presented in the lower right panel of Fig. 4. Power density spectra were constructed from data recorded in the absence and presence of 300 μM ibuprofen. In control conditions, and similar to previous reports from our laboratory (21–23, 31), the data were well fitted by two Lorentzian components; a low frequency component ($f_c$ = 2.4 Hz) which has been associated with nucleotide-dependent bursts and a higher frequency component ($f_c$ = 87 Hz) associated with short-lived closures within a burst. Addition of ibuprofen resulted in a reduction of the power associated with each of these transitions and the introduction of a new set of transitions with $f_c$ = 410 Hz. If one assumes a simple open-blocked mechanism as we

previously reported for glibenclamide (23) and tolbutamide (31), these results suggest a $k_{on}$ of 5.8 μM$^{-1}$s$^{-1}$ and $k_{off}$ of 810 s$^{-1}$ resulting in a predicted $K_d$ of 140 μM. The predicted $k_{off}$ or $\tau_{block}$ (1.2 ms) is consistent with our ability to resolve blocked events when filtered at 800 Hz, but not at 200 Hz. More complex kinetic schemes could be envisioned; however, these predictions can account for all of the data presented in Fig. 4 and the concentration dependency reported in Fig. 1 (*bottom*).

Similar results were obtained from additional experiments to evaluate the effects of salicylic acid (3 mM) on CFTR channel activity. As shown in Fig. 5, salicylic acid dramatically and reversibly reduced CFTR-mediated Cl$^-$ current. In this patch, salicylic acid caused a reduction in $I$ from 3.5 to 1.4 pA and the apparent single channel amplitude was reduced from 1.1 to 0.4 pA. Fluctuation analysis revealed that salicylic acid reduced



*Figure 5.* Salicylic acid reversibly inhibits CFTR-mediated Cl$^-$ current in membrane patches excised from L cells. (*Top*) The reversible, salicylic acid–induced changes in gating characteristics and reduction in patch current caused by 3.0 mM salicylic acid. Average current was 3.5, 1.4, and 2.1 pA in the three conditions, respectively. Current records were filtered at 100 Hz (8 pole Bessel filter) and plotted at 200 Hz. (*Middle*) Expanded records of current traces from the top panels. Records were filtered at 800 Hz and plotted at 2 kHz. (*Bottom left*) Amplitude histograms of the data presented in the top panels. In control conditions and after washout, the mean single channel amplitude was 1.1 pA. Single channel amplitude was reduced to 0.38 pA by 3.0 mM salicylic acid. 1 min of data, filtered at 50 Hz, were used to construct each histogram. (*Bottom right*) The power density spectra and associated multi-Lorentzian fits of current recordings made in presence or absence of salicylic acid. The data show that salicylic acid causes a reduction in power and corner frequency associated with nucleotide-dependent gating (control, $f_{c1}$ = 1.9 Hz; $S_o$ = 5.8 × 10$^{-25}$ A$^2$s and $f_{c2}$ = 95 Hz, $S_o$ = 9.3 × 10$^{-28}$ A$^2$s; salicylic acid, $f_{c1}$ = 1.7 Hz, $S_o$ = 8.8 × 10$^{-26}$ A$^2$s and $f_{c2}$ = 93 Hz, $S_o$ = 2.5 × 10$^{-28}$ A$^2$s; washout, $f_{c1}$ = 2.1 Hz; $S_o$ = 3.3 × 10$^{-25}$ A$^2$s and $f_{c2}$ = 95 Hz, $S_o$ = 6.1 × 10$^{-28}$ A$^2$s). Data were filtered at 800 Hz and sampled at 2 kHz. 22 or more non-overlapping segments (4.096 s) were used to construct each power density spectra.

the power associated with nucleotide-dependent gating; however, a salicylic acid–induced Lorentzian component was not observed in this frequency domain. Upon removal of the salicylic acid, $I$ increased to 2.1 pA, $i$ returned to 1.1 pA and the power density spectra approximated that observed in control conditions. On average, CFTR Cl⁻ current was reduced by $50\pm8\%$ with an apparent reduction in single channel amplitude from $1.08\pm0.03$ pA to $0.48\pm0.06$ pA ($n = 4$). These results demonstrate that the NSAIDs ibuprofen and salicylic acid inhibit the CFTR Cl⁻ channel and that this inhibition likely explains the NSAID-dependent inhibition of cAMP-mediated Cl⁻ secretion in colonic and airway epithelia.

## Discussion

While two of the hallmarks of CF are chronic inflammation and persistent bacterial colonization, there remains the open question of whether inflammation precedes bacterial colonization in the etiology of the disease. Resolution of this question is important in determining the role of anti-inflammatory agents in CF therapy. Based on these findings, the use of anti-inflammatory agents has been proposed in CF (3, 5). Indeed, results from an initial clinical trial using prednisone demonstrated improved physical (e.g., height, weight) and respiratory (e.g., $FEV_1$) parameters (32). Unfortunately, a second, larger clinical trial uncovered adverse effects that resulted in a premature termination of the trial (33). However, these earlier results confirmed that anti-inflammatory agents may be useful in CF therapy. This led to the more recent clinical trials in which it was demonstrated that the NSAID ibuprofen resulted in a slowing of the rate of loss in $FEV_1$ in CF patients with mild lung disease (11, 34).

While the above results suggest that NSAIDs may be useful in CF therapy, they do not address the mechanism by which these compounds improve lung function. CF is caused by a mutation in the CFTR Cl⁻ channel resulting in an absent or diminished Cl⁻ secretory function of the airway in response to cAMP-mediated agonists. Thus, in an attempt to determine whether an improved Cl⁻ secretory function underlies the NSAID-induced improved respiratory function, we evaluated the acute effect of the NSAIDs ibuprofen, salicylic acid, and aspirin on Cl⁻ secretory current in colonic and airway epithelia. We demonstrate that these NSAIDs inhibit, rather than augment, cAMP-mediated Cl⁻ secretion in the human colonic cell line, T84 as well as in primary cultures of murine tracheal and human bronchial epithelia. In addition, we demonstrate that this inhibition is due to the ability of these NSAIDs to directly modulate CFTR Cl⁻ currents in excised membrane patches. This inhibition is due to the introduction of a short-lived blocked state that, when filtered at low frequency, appears as a reduction in single channel amplitude. However, given the ability of these NSAIDs to readily cross the plasma membrane, our studies do not allow us to determine whether the observed inhibition of CFTR is due to an interaction with the intracellular or extracellular side of the channel. Also, while we are unable to directly evaluate the potential pH effects of NSAIDs on Cl⁻ secretion, our intact epithelium and excised patch–clamp experiments demonstrate that the effect of ibuprofen and salicylic acid on CFTR occur at physiologically relevant pH (7.3–7.4). Finally, our results also indicate that ibuprofen induces a small inhibition of the cAMP-dependent basolateral membrane $K^+$ conductance in T84 cells. This

cAMP-dependent $G_K$ has not been identified at the single channel level in T84 cells. In total, our results demonstrate that the NSAID-dependent inhibition of Cl⁻ secretion primarily is due to inhibition of CFTR although a lesser effect on basolateral $G_K$ is also apparent.

Our results in Ussing chambers are similar to what has been reported (35) using primary cultures of canine trachea. However, these authors did not determine the effect of ibuprofen on Cl⁻ secretion in their studies. Also, Mochizuki et al. (35) found that aspirin had a higher affinity than salicylic acid for inhibition of cAMP-dependent Cl⁻ secretion. In contrast, we find that aspirin is less efficacious in inhibiting Cl⁻ secretion in the colonic cell line, T84. Currently, it is unclear why we observe these differences; however, this may be explained by either differences between colonic and airway epithelia or species differences in the CFTR protein. An additional possibility is that the NSAIDs may be interacting preferentially with a given regulated (kinetic) state of CFTR. As the canine trachea is primarily a Cl⁻ secretory epithelium whereas the human airway is primarily $Na^+$ absorptive, the regulation of CFTR in these two tissues, and hence the interaction with a blocker, may be somewhat different. In any event, our results confirm and extend those of Mochizuki et al. (35) by demonstrating that the NSAIDs acutely inhibit cAMP-dependent Cl⁻ secretion in human colonic and airway epithelia and that this inhibition of transepithelial current is due to block of CFTR Cl⁻ channels.

In addition to the acute effects detailed in this study, these NSAIDs may also have long-term effects that are more influential to the beneficial effect seen in CF. For example, salicylate is known to partially induce the heat shock response (36, 37) and heat shock proteins are known to act as chaperones for CFTR (38). Also, aspirin has been shown to induce the expression of eicosanoids, which are potent inhibitors of leukotriene $B_4$-mediated PMN migration (39), and is thus anti-inflammatory. Finally, both salicylate and aspirin have been shown to inhibit the activation of NF-κB, a transcription factor critical for the inducible expression of gene products involved in the inflammatory response (40). NF-κB has been shown to be activated as part of the endoplasmic reticulum overload response (41). As ΔF508 CFTR is trapped in the ER (42) this may lead to the activation of NF-κB. Indeed, we have found that in primary cultures of HBE homozygous for the ΔF508 CFTR mutation, there is an activation and nuclear translocation of NF-κB (Bradbury, N.A., W. Walker, and D.C. Devor, unpublished observations). Thus, it is important to determine the long-term effect of NSAIDs on CFTR. In a preliminary report, Legros et al. (43) found that NSAIDs, including ibuprofen, salicylic acid, and aspirin decreased CFTR mRNA expression. We have found that incubation of T84 cells in ibuprofen or salicylic acid (72 h) results in a reduced level of forskolin-stimulated $I_{sc}$ (Devor, D.C., unpublished observations). After ibuprofen exposure during cell culture, forskolin-stimulated $I_{sc}$ could be inhibited to a similar extent by the acute addition of ibuprofen (Devor, D.C., unpublished observations). These results suggest that chronic exposure to ibuprofen results in reduced CFTR expression whereas acute exposure results in reduced Cl⁻ conductance through CFTR.

In conclusion, we demonstrate that ibuprofen inhibits CFTR-dependent Cl⁻ secretion in both colonic and airway epithelia (see Figs. 1 and 2). This is due to both a direct inhibition of the CFTR Cl⁻ channel as well as a lesser effect on basolat-

eral $G_K$ (see Figs. 3–5). The concentrations used in this study were within the range of plasma levels previously reported to be beneficial in CF patients (11). These results coupled with the recent results of Edelman and colleagues (43) suggest that the beneficial effect of NSAIDs in CF therapy are unrelated to their effects on CFTR. However, the direct effects of NSAIDs on CFTR described herein are of critical importance because the efficacy of therapeutic strategies designed to increase either CFTR expression (e.g., gene therapy, chemical chaperones) and/or function (e.g., pharmacological openers of CFTR) in secretory epithelia may be adversely affected by concomitant NSAID treatment. Furthermore, the treatment of CF patients expressing mild mutations with NSAIDs may likewise be contraindicated because of an anticipated reduction in CFTR Cl⁻ conductance.

## Acknowledgments

## References

1. Ford-Hutchinson, A.W., M.A. Bray, M.V. Doig, M.E. Shipley, and M.J.H. Smith. 1980. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature.* 286:264–265.

2. Cromwell, O., M.J. Walport, H.R. Morris, G.W. Taylor, M.E. Hodson, J. Batten, and A.B. Kay. 1981. Identification of leukotrienes D and B in sputum from cystic fibrosis patients. *Lancet.* 164–165.

3. Hammerschmidt, D.E. 1983. Leukocytes in lung injury. *Chest.* 83:16S–20S.

4. Barton, A.D., K. Ryder, R.V. Lourenco, W. Dralle, and S.G. Weiss. 1976. Inflammatory reaction and airway damage in cystic fibrosis. *J. Lab. Clin. Med.* 88:423–426.

5. O'Driscoll, B.R.C., and A.B. Kay. 1982. Leukotrienes and lung disease. *Thorax.* 37:241–245.

6. Janoff, A. 1983. Proteases and lung injury. A state-of-the-art minireview. *Chest.* 83:54S–58S.

7. Zakrzewski, J.T., N.C. Barnes, J.F. Costello, and P.J. Piper. 1987. Lipid mediators in cystic fibrosis and chronic obstructive pulmonary disease. *Am. Rev. Respir. Dis.* 136:779–782.

8. Vanderhoek, J.Y., and J.M. Bailey. 1984. Activation of a 15-lipoxygenase/leukotriene pathway in human polymorphonuclear leukocytes by the anti-inflammatory agent ibuprofen. *J. Biol. Chem.* 259:6752–6756.

9. Sordelli, D.O., M.C. Cerquetti, G. El-Tawil, P.W. Ramwell, A.M. Hooke, and J.A. Bellanti. 1985. Ibuprofen modifies the inflammatory response of the murine lung to *Pseudomonas aeruginosa*. *Eur. J. Respir. Dis.* 67:118–127.

10. Konstan, M.W., K.M. Vargo, and P.B. Davis. 1990. Ibuprofen attenuates the inflammatory response to *Pseudomonas aeruginosa* in a rat model of chronic pulmonary infection. *Am. Rev. Respir. Dis.* 141:186–192.

11. Konstan, M.W., P.J. Byard, C.L. Hoppel, and P.B. Davis. 1995. Effect of high-dose ibuprofen in patients with cystic fibrosis. *N. Engl. J. Med.* 332:848–854.

12. Oermann, C.M., M.M. Sockrider, and D.J. Murry. 1996. One year's experience with high-dose ibuprofen therapy in CF. *Pediatr. Pulmonol.* 13(Suppl.):280–281.

13. Scheiman, J.M. 1996. NSAIDS, Gastrointestinal injury, and cytoprotection. *Gastroenterology Clin. of North America.* 25:279–298.

14. Davies, N.M. 1995. Toxicity of nonsteroidal anti-inflammatory drugs in the large intestine. *Dis. Colon Rectum.* 38:1311–1321.

15. Smalley, W.E., and M.R. Griffin. 1996. The risks and costs of upper gastrointestinal disease attributable to NSAIDS. *Gastroenterol. Clin. N. Am.* 25:373–398.

16. Jenkins, A.P., D.R. Trew, B.J. Crump, W.S. Nukajam, J.A. Foley, I.S. Menzies, and B. Creamer. 1991. Do non-steroidal anti-inflammatory drugs increase colonic permeability? *Gut.* 32:66–69.

17. Wangemann, P., M. Wittner, A. DiStefano, H.C. Englert, H.J. Lang, E. Schlatter, and R. Greger. 1986. Cl⁻ channel blockers in the thick ascending limb of the loop of Henle. Structure activity relationship. *Eur. J. Physiol.* 407:S128.

18. White, M.M., and M. Aylwin. 1990. Niflumic and flufenamic acid are potent reversible blockers of Ca²⁺-activated Cl⁻ channels in *Xenopus* oocytes. *Mol. Pharmacol.* 37:720.

19. Yang, Y., D.C. Devor, J.F. Engelhardt, S.A. Ernst, T.V. Strong, F.S. Collins, J.A. Cohn, R.A. Frizzell, and J.M. Wilson. 1993. Molecular basis of defective anion transport in L cells expressing recombinant forms of CFTR. *Hum. Mol. Genet.* 2:1253–1261.

20. Devor, D.C., A.K. Singh, R.J. Bridges, and R.A. Frizzell. 1996. Modulation of Cl⁻ secretion by benzimidazolones. II. Coordinate regulation of apical GCl and basolateral GK. *Am. J. Physiol.* 271:L785–L795.

21. Schultz, B.D., C.J. Venglarik, R.J. Bridges, and R.A. Frizzell. 1995. Regulation of CFTR Cl⁻ channel gating by ADP and ATP analogues. *J. Gen. Physiol.* 105:329–361.

22. Venglarik, C.J., B.D. Schultz, R.A. Frizzell, and R.J. Bridges. 1994. ATP alters current fluctuations of cystic fibrosis transmembrane conductance regulator: evidence for a three-state activation mechanism. *J. Gen. Physiol.* 104:123–146.

23. Schultz, B.D., A.D. DeRoos, C.J. Venglarik, A.K. Singh, R.A. Frizzell, and R.J. Bridges. 1996. Glibenclamide blockade of CFTR chloride channels. *Am. J. Physiol.* 271:L192–L200.

24. Brooks, S.P., and K.B. Storey. 1992. Bound and determined: a computer program for making buffers of defined ion concentrations. *Anal. Biochem.* 201:119–126.

25. Tabcharani, J.A., X.-B. Chang, J.R. Riordan, and J.W. Hanrahan. 1991. Phosphorylation-regulated Cl⁻ channel in CHO cells stably expressing the cystic fibrosis gene. *Nature.* 352:628–631.

26. Devor, D.C., A.K. Singh, A.C. Gerlach, R.A. Frizzell, and R.A. Bridges. 1997. Inhibition of intestinal Cl⁻ secretion by clotrimazole: direct effect on basolateral membrane K⁺ channels. *Am. J. Physiol.* 273:C531–C540.

27. Knowles, M.R., L.L. Clarke, and R.C. Boucher. 1991. Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. *N. Engl. J. Med.* 325:533–538.

28. Devor, D.C., A.K. Singh, R.J. Bridges, and R.A. Frizzell. 1997. Psoralens: novel modulators of Cl⁻ secretion. *Am. J. Physiol.* 272:C976–C988.

29. Devor, D.C., A.K. Singh, R.A. Frizzell, and R.J. Bridges. 1996. Modulation of Cl⁻ secretion by benzimidazolones. I. Direct activation of a Ca²⁺–dependent K⁺ channel. *Am. J. Physiol.* 271:L775–L784.

30. Lohrmann, E., I. Burhoff, R.B. Nitschke, H.J. Lang, D. Mania, H.C. Englert, M. Hropot, R. Warth, W. Rohm, M. Bleich, and R. Greger. 1995. A new class of inhibitors of cAMP-mediated Cl⁻ secretion in rabbit colon, acting by the reduction of cAMP-activated K⁺ conductance. *Eur. J. Physiol.* 429:517–530.

31. Venglarik, C.J., B.D. Schultz, A.D.G. de Roos, A.K. Singh, and R.J. Bridges. 1996. Tolbutamide causes open channel blockade of cystic fibrosis transmembrane conductance regulator Cl⁻ channels. *Biophys. J.* 70:2696–2703.

32. Auerbach, H.S., M. Williams, J.A. Kirkpatrick, and H.R. Colten. 1985. Alternate-day prednisone reduces morbidity and improves pulmonary function in cystic fibrosis. *Lancet.* 686–688.

33. Rosenstein, B.J., and H. Eigen. 1991. Risks of alternate-day prednisone in patients with cystic fibrosis. *Pediatrics.* 87:245–246.

34. Konstan, M.W., C.L. Hoppel, B. Chai, and P.B. Davis. 1991. Ibuprofen in children with cystic fibrosis: pharmacokinetics and adverse effects. *J. Pediatr.* 118:956–964.

35. Mochizuki, H., A. Morikawa, K. Tokuyama, T. Kuroume, and A.C. Chao. 1994. The effect of non-steroidal anti-inflammatory drugs on the electrical properties of cultured dog tracheal epithelial cells. *Eur. J. Pharmacol.* 252:183–188.

36. Jurivich, D.A., L. Sistonen, R.A. Kroes, and R.I. Morimoto. 1992. Effect of sodium salicylate on the human heat shock response. *Science.* 255:1243–1245.

37. Jurivich, D.A., C. Pachetti, L. Qiu, and J.F. Welk. 1995. Salicylate triggers heat shock factor differently than heat. *J. Biol. Chem.* 270:24489–24495.

38. Yang, Y., S. Janich, J.A. Cohn, and J.M. Wilson. 1993. The common variant of cystic fibrosis transmembrane conductance regulator is recognized by hsp 70 and degraded in a pre-Golgi nonlysosomal compartment. *Proc. Natl. Acad. Sci. USA.* 90:9480–9484.

39. Claria, J., and C.N. Serhan. 1995. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proc. Natl. Acad. Sci. USA.* 92:9475–9479.

40. Kopp, E., and S. Ghosh. 1994. Inhibition of NF-kB by sodium salicylate and aspirin. *Science.* 265:956–958.

41. Pahl, H.L., and P.A. Baeuerle. 1997. The ER-overload response: activation of NF-kB. *Trends Biochem. Sci.* 22:63–67.

42. Cheng, S.H., R.J. Gregory, J. Marshall, S. Paul, D.W. Souza, G.A. White, C.R. O'Riordan, and A.E. Smith. 1990. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell.* 63:827–834.

43. Legros, M., F. Brouillard, D. Tondelier, and A. Edelman. 1997. Aspirin inhibits CFTR expression in T-84 cells. *Pediatr. Pulmonol.* S14:229.