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Physiology

Ketoconazole activates Cl^- conductance and blocks Cl^- and fluid absorption by cultured cystic fibrosis (CFPAC-1) cells

ULRICH KERSTING*, DOROTHEE KERSTING†, AND KENNETH R. SPRING

National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

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ABSTRACT **The role of arachidonic acid metabolites in the regulation of apical cell membrane Cl^- conductance and transepithelial transport of fluid and Cl^- by cultured pancreatic cells from cystic fibrosis (CFPAC-1) and corrected (PAC-1) cell lines was evaluated by the use of inhibitors. CFPAC-1 cells did not exhibit an apical membrane Cl^- conductance, absorbed Cl^- and fluid, and did not respond to stimulation or inhibition of cAMP action. PAC-1 cells exhibited a cAMP-responsive apical Cl^- conductance, which was blocked by indomethacin, a cyclooxygenase inhibitor. Ketoconazole, an epoxygenase inhibitor, had virtually no effects on PAC-1 cell Cl^- conductance but caused CFPAC-1 cells to develop a cAMP-insensitive Cl^- conductance, blocked Cl^- and fluid absorption, and reduced transepithelial electrical resistance. Ketoconazole treatment effectively reversed the cystic fibrosis defect in these cultured cells.**

Although it is well established that the cystic fibrosis transmembrane conductance regulator (CFTR) is the protein that is defective in cystic fibrosis (CF), it is unclear whether this protein normally functions as a pump for some unknown solute and as a cAMP-sensitive Cl^- channel (1–5). Recently, it has been shown that although the CFTR constitutes a linear Cl^- channel, activation of the outwardly rectifying Cl^- channel by cAMP requires the presence of a functional CFTR (6). Thus the CFTR seems to be a Cl^- channel and to control the activity of another channel (7). CF cells are capable of activating other Cl^- channels since increasing intracellular Ca^{2+} activates a cAMP-insensitive Cl^- conductance (8–10), and a modest increase in ^{36}Cl efflux from CF cells was observed after exposure to A_1-adenosine-receptor antagonists (11).

The structure of the CFTR is unlike that of other ion channels and is strikingly analogous to transport proteins of the ATP-binding cassette superfamily (1). The close structural analogy with other transport proteins led to the proposal (12) that the primary function of the CFTR may be the excretion of arachidonic acid (Δ_4Ach) metabolites or related compounds. Δ_4Ach metabolites, produced by cyclooxygenase, lipoxygenase, or cytochrome P-450 monooxygenase, have been shown to be involved in the regulation of a number of transport processes in epithelia (13–16). Cyclooxygenase products (e.g., prostaglandins) induce Cl^- secretion via cAMP-stimulated protein phosphorylation by protein kinase A in various tissues (13, 14, 17). Lipoxygenase products (e.g., leukotrienes) may activate or inactivate Cl^- transport (18) or regulate Cl^- channels indirectly by stimulation (19, 20) or inhibition (20, 21) of prostaglandin formation. P-450 monooxygenase transfers one activated oxygen to Δ_4Ach thereby forming "epoxides" (14), which stimulate mucus secretion (22), regulate cell membrane transporters (13, 14, 23), and inactivate the Na^+/K^+-ATPase of the renal thick ascending limb of Henle (24, 25) and cornea (26).

We chose CFPAC-1 (4, 27), the CF pancreatic adenocarcinoma cell line, as a cell model to test whether Δ_4Ach metabolites were involved in regulation of apical Cl^- channels in CF cells and to gain further understanding about the function of CFTR. In our study we denote the PLJ-CFPAC-1 cells carrying the CF defect as CFPAC-1 cells and the corrected PLJ-CFTR-CFPAC-1 cells that serve as controls as PAC-1 cells. We show that abnormal absorption of Cl^- and fluid in CFPAC-1 cells is blocked by inhibition of the production of cytochrome P-450 metabolites.

MATERIALS AND METHODS

Cell Culture on Plastic Supports. Clone 6 CFPAC-1 cells and retrovirus-CFTR-gene-transfer-corrected PAC-1 cells, gifts from R. A. Frizzell (Gregory Fleming James Cystic Fibrosis Center, Birmingham, AL), were maintained as described (4). For dome measurements, cells were plated in multiwell flasks and domes were counted in six fields of view in each well at ×12 magnification. Three experiments with four wells per experiment were performed for each drug. Drugs were added to the culture medium and confluent monolayers were incubated for 3 days.

Cell Culture on Permeable Supports. CFPAC-1 cells were plated on transparent collagen membranes (Cellagen membranes, ICN) at a cell density of 5.7×10^7 cells per cm^2. PAC-1 cells did not grow well on Cellagen membranes and thus were plated with the same cell density on polycarbonate filters (Costar, tissue-culture-treated Transwell; pore size, 3 μm). CFPAC-1 cells failed to grow on polycarbonate filters. Confluency was confirmed by light microscopy and by transepithelial-resistance measurements. The culture medium of monolayers on permeable supports was exchanged for one containing ketoconazole (0.1 mM) 3 days before transepithelial resistance, apical and basolateral bath volumes, bath Cl^- concentrations, pH, and osmolality were analyzed.

For the determination of the rate of fluid transport by cells grown on permeable supports, a measured volume of cultured medium was added to each bath, the preparations were returned to the incubator, and the bathing solutions were aspirated at various time intervals thereafter (typically 1–3 h). Volume loss by evaporation and fluid sampling was determined in controls consisting of a support without cells, and correction factors were developed and applied to experimental measurements of the bathing solution volumes.

Cl^- concentrations were determined by colorimetry (chloride color reagent from Sigma) on a UV/visible spectrophotometer (Perkin–Elmer). Osmolality was measured using the freezing-point method (Micro-Osmette, Precision Systems, Natick, MA). pH was measured with microelectrodes (response time, 5 s; minimal sample volume, 5 μl; Micro-Com-

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Abbreviations: CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; Δ_4Ach, arachidonic acid; NDGA, nordihydroguaiaretic acid; (R_P)-cAMP[S], (R_P)-adenosine cyclic 3′,5′-phosphorothioate; GSH, glutathione.

*Present address: Department of Physiology, University of Würzburg, 8700-Würzburg, Germany.
†Present address: Department of Biochemistry, University of Würzburg, 8700-Würzburg, Germany.

bination pH probe, Microelectrodes, Londonderry, NH) in 1-ml vials, equilibrated with 5% CO_2/95% air.

Cl^- Conductance Measurements. Segments of permeable supports were mounted in a modified Ussing chamber that allowed rapid changes of the apical bath Ringer solution. The apical cell membrane potential was measured with microelectrodes; voltage deflections, measured 20 s after the apical bath Ringer was switched to a low Cl^- Ringer (4 mM Cl^-, gluconate substitution) solution, were proportional to the apical Cl^- conductance. Control Ringer solution was 120 mM NaCl/3.3 mM KH_2PO_4/0.83 mM K_2HPO_4/24 mM $NaHCO_3$/1 mM $MgCl_2$/1 mM $CaCl_2$. Low Cl^- Ringer solution was 114 mM sodium gluconate/3.3 mM KH_2PO_4/0.83 mM K_2HPO_4/24 mM $NaHCO_3$/1 mM $MgCl_2$/1 mM $CaCl_2$/6 mM calcium lactate, pH 7.4, gassed with 5% CO_2/95% air.

Compounds. Forskolin was purchased from ICN. (R_P)-Adenosine cyclic 3',5'-phosphorothioate {(R_P)-cAMP[S]} was purchased from Biolog (La Jolla, CA). Δ_4Ach, 5,8,11,14-eicosatetraynoic acid, indomethacin, ketoconazole, nordihydroguaiaretic acid (NDGA), and SKF-525A hydrochloride (proadifen) were purchased from Biomol (Plymouth Meeting, PA).

Statistics. Data are expressed as the mean ± SEM. Significances were calculated with the two-sample independent-group *t* test or with the paired-group *t* test, as indicated.

RESULTS

Inhibition of the Production of Δ_4Ach Metabolites Alters Apical Cl^- Conductance in PAC-1 and CFPAC-1 Cells. The apical membrane potential was −16.6 ± 1.26 mV (n = 38) in PAC-1 cells and −23.1 ± 2.48 mV (n = 45) in CFPAC-1 cells, comparable to previously published values for membrane potentials in airway epithelial cells (2, 10). Low Cl^- superfusion resulted in a depolarization (ΔE) in PAC-1 cells of 7.8 ± 0.45 mV, consistent with the existence of a Cl^- conductance (Table 1). ΔE in PAC-1 cells was ≈8-fold greater than that seen in CFPAC-1 cells (Fig. 1 and Table 1). These findings indicate, in agreement with previous studies (2–5), that PAC-1 cells express an apical Cl^- conductance that is virtually absent from CFPAC-1 cells.

After incubation with ketoconazole, CFPAC-1 cells developed an apical Cl^- conductance that was ≈2.5 times larger than that seen in the PAC-1 cells (Fig. 1 and Table 1). Ketoconazole did not change the apical Cl^- conductance in PAC-1 cells. Another inhibitor of the cytochrome P-450 pathway, SKF-525A, also activated an apical Cl^- conductance in CFPAC-1 cells of similar magnitude (Table 1). NDGA, a blocker of the lipoxygenase pathway, activated only a small apical Cl^- conductance in CFPAC-1 cells and had no effect on PAC-1 cells. Indomethacin, a blocker of the cyclooxygenase pathway, had no effect on apical Cl^- conductance in CFPAC-1 cells but completely inhibited it in PAC-1 cells. The ketoconazole-induced apical Cl^- conductance in CFPAC-1 cells was slightly reduced by indomethacin. When PAC-1 cells were treated with indomethacin to inhibit the endogenous Cl^- conductance, ketoconazole activated a Cl^- conductance that was not significantly different in magnitude from control.

Table 1. Apical cell membrane potential depolarizations (ΔE) induced by low Cl^-

Treatment	ΔE, mV	
	CFPAC-1	PAC-1
Control	0.93 ± 0.46 (13)	7.8 ± 0.45 (26)
Ketoconazole	14.9 ± 1.4 (11)*	7.9 ± 0.62 (23)
SKF-525A	13.07 ± 0.62 (12)*	—
NDGA	5.94 ± 0.52 (27)*	8.5 ± 0.34 (10)
Indomethacin	1.8 ± 0.64 (8)	−0.77 ± 1 (31)*
Indomethacin + ketoconazole	11.2 ± 0.7 (12)*	6.1 ± 0.46 (17)

Cl^- concentration was reduced from 124 mM to 4 mM in apical bath Ringer solution. Positive ΔE values indicate depolarization and negative values indicate hyperpolarization. Cells, grown on permeable supports for 5–12 days, were incubated in culture medium with drugs (0.1 mM) overnight prior to the experiment. Drugs were added to the superfusion Ringer solutions during the experiment. Number of experiments is shown in parentheses. *, $P < 0.01$ compared to control by the two-sample independent-group *t* test.

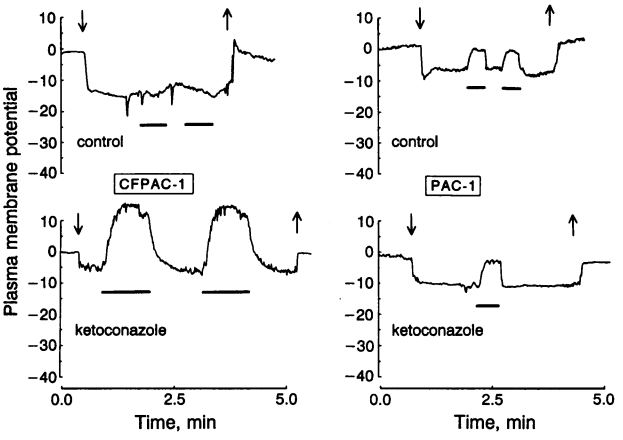

FIG. 1. Depolarizations of apical plasma membrane potential (ΔE), proportional to the relative apical Cl^- conductance, in CFPAC-1 and PAC-1 cells during superfusion (bars) with low Cl^- (4 mM) Ringer solutions. Arrows mark cell impalement and withdrawal of the microelectrodes.

Role of cAMP in Regulation of Apical Cl^- Conductance. Forskolin, an activator of cAMP-dependent processes, increased the endogenous Cl^- conductance in PAC-1 cells by 19% but failed to stimulate any Cl^- conductance in CFPAC-1 cells (Table 2). The relatively small conductance increase in PAC-1 cells indicated that the Cl^- conductance was nearly maximally stimulated by endogenous cAMP under control conditions. If the apical Cl^- conductance were nearly maximally activated in PAC-1 cells, it would be expected that (R_P)-cAMP[S], an inhibitor of cAMP-activated protein phosphorylation, would block it. As indicated by a very small ΔE, the apical Cl^- conductance in PAC-1 cells was virtually abolished after incubation with (R_P)-cAMP[S]. Ketoconazole had no effect on the abolition of the Cl^- conductance of PAC-1 cells by (R_P)-cAMP[S].

The ketoconazole-induced apical Cl^- conductance in CFPAC-1 cells was unaffected by forskolin or (R_P)-cAMP[S] (Table 2). Thus our data indicate that the ketoconazole-induced Cl^- conductance of CFPAC-1 cells is not under the control of cAMP.

Table 2. Effects of forskolin and (R_P)-cAMP[S] on apical plasma membrane depolarization (ΔE)

Treatment	ΔE, mV	
	CFPAC-1	PAC-1
Control	0.9 ± 0.46 (13)	7.8 ± 0.45 (26)
Forskolin	0.8 ± 0.3 (14)	9.3 ± 0.5 (12)†
(R_P)-cAMP[S]	—	1.1 ± 0.4 (16)*
Ketoconazole	14.9 ± 1.4 (11)*	7.9 ± 0.45 (26)
Ketoconazole + forskolin	14.4 ± 1.9 (19)*	—
Ketoconazole + (R_P)-cAMP[S]	16.8 ± 1.1 (20)*	1.6 ± 0.4 (17)*

Methods were as described in Table 1. Concentration of (R_P)-cAMP[S] and ketoconazole was 0.1 mM. Forskolin was added to a concentration of 0.02 mM. *, $P < 0.01$; †, $P < 0.05$ compared to controls by the two-sample independent-group *t* test.

Effects of Transepithelial Resistance (R_t). CFPAC-1 cells, grown on permeable supports, formed confluent monolayers with a high transepithelial resistance (R_t = 900 Ω·cm²) about three times higher than that in PAC-1 cells (Fig. 2*A*). Ketoconazole treatment reduced the R_t to 150 Ω·cm² in CFPAC-1 cells and had no effect on R_t in PAC-1 cells. The reduction in R_t of CFPAC-1 cells after ketoconazole treatment was consistent with the activation of a large Cl^- conductance in the apical membrane.

Regulation of Dome Formation. Domes, blisters between the epithelium and the solid support of the culture dish formed by salt and water absorption, were used to screen for alterations in the rate of fluid absorption with the expectation that secretion of salt and water should diminish the number of domes formed. The frequency of domes was ≈7 times higher in CFPAC-1 cells than in PAC-1 cells, indicating that fluid absorption is a feature of CFPAC-1 cells (Table 3). Ketoconazole and SKF-525A completely blocked dome formation by both PAC-1 and CFPAC-1 cells. NDGA and indomethacin did not change dome formation significantly. 5,8,11,14-Eicosatetraynoic acid, a blocker of all three Δ_4Ach pathways, blocked dome formation slightly in CFPAC-1 cells but had no effect in PAC-1 cells.

To test whether the frequency of dome formation was altered by cAMP, we incubated confluent monolayers with forskolin and/or (R_P)-cAMP[S]. Forskolin reduced dome formation by about half in CFPAC-1 cells and eliminated

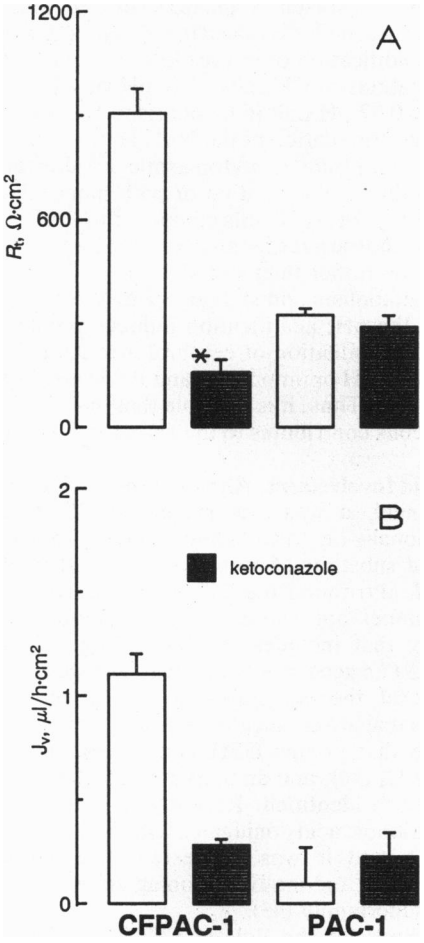

FIG. 2. Transepithelial resistances (R_t, n = 7) (*A*) and transepithelial fluid absorption (J_v, n = 9) (*B*) of CFPAC-1 and PAC-1 monolayers grown on permeable supports. Ketoconazole (0.1 mM)-treated cells were incubated with the drug for 3 days (shaded bars). Open bars represent control, untreated monolayers. n, Number of experiments. *, $P < 0.01$ by the two-sample independent-group t test.

Table 3. Relative number of domes

Treatment	Domes relative to control, %	
	CFPAC-1	PAC-1
Control	100 ± 7	14 ± 8
Ketoconazole	0*	0*
SKF-525A	0*	0*
NDGA	94 ± 4	14.5 ± 10
Indomethacin	95 ± 9	15 ± 6
ETYA	78 ± 6†	15 ± 7
Forskolin	57 ± 10*	0*
(R_P)-cAMP[S]	129 ± 9†	25 ± 5*
Ketoconazole + forskolin	0*	0*
Ketoconazole + (R_P)-cAMP[S]	0*	0*

Number of domes in CFPAC-1 cells incubated in control culture medium was designated 100% (269 ± 19 domes per cm²). Drugs (0.1 mM, except forskolin at 0.02 mM) were added to culture medium 5 days after cell plating when cell monolayers were confluent. Dome number was counted 2 days later. Number of experiments was 12. ETYA, 5,8,11,14-eicosatetraynoic acid. *, $P < 0.01$; †, $P < 0.05$ compared to controls with the two-sample independent-group t test.

domes in PAC-1 cells (Table 3). (R_P)-cAMP[S] increased dome formation by ≈30% in CFPAC-1 cells and by ≈80% in PAC-1 cells. Stimulation of dome formation by (R_P)-cAMP[S] in both cell lines was completely inhibited by ketoconazole. Domes also did not form in either cell line after treatment with forskolin plus ketoconazole. These results are consistent with the conclusion that the inhibition of dome formation by ketoconazole did not involve cAMP.

Fluid Absorption in CFPAC-1 Cells Is Blocked by Ketoconazole. The results of the dome formation observations were confirmed by direct measurements of transepithelial fluid transport of cells grown on permeable supports. CFPAC-1 cells absorbed apical bath culture medium when cultured on permeable support for 3 days. Fluid transport (J_v) in CFPAC-1 cells was 1.1 ± 0.1 μl/h·cm² (Fig. 2*B*). Fluid absorption in CFPAC-1 cells was reduced to 0.25 ± 0.02 μl/h·cm² by ketoconazole. PAC-1 cells did not exhibit significant fluid absorption and ketoconazole had no significant effects.

Transepithelial pH Gradients and Ketoconazole. Fig. 3*A* shows that the negative pH gradient in CFPAC-1 cells (mucosal bath culture medium more alkaline than serosal bath) was reversed after ketoconazole treatment. CFPAC-1 cells acidified both bathing solutions with a serosal pH of 7.18 ± 0.04 and a mucosal pH of 7.23 ± 0.04. Ketoconazole treatment resulted in further acidification of both mucosal (pH = 6.75 ± 0.03) and serosal (pH = 6.78 ± 0.03) baths. PAC-1 cells acidified both the mucosal (pH = 6.82 ± 0.04) and serosal (pH = 6.85 ± 0.05) baths. Acidification by PAC-1 cells was further increased after ketoconazole (mucosal pH = 5.85 ± 0.04; serosal pH = 6.1 ± 0.04).

Transepithelial Osmotic Gradients and Ketoconazole. Measurements of the osmolality in mucosal and serosal media showed that CFPAC-1 cells created a transepithelial osmolality gradient of 4 ± 0.65 milliosmoles/kg of H_2O (serosal osmolality minus mucosal; Fig. 3*B*). Considering the rate of transepithelial fluid absorption in CFPAC-1 cells (shown in Fig. 2*B*), this osmolality gradient indicates net solute absorption. Ketoconazole reversed the osmotic gradient (osmolality difference = −4 ± 1.14 milliosmoles/kg of H_2O; Fig. 3*B*) in CFPAC-1 cells. PAC-1 cells did not absorb solute, and ketoconazole had no effects on the osmotic gradients produced by these cells.

Transepithelial Cl^- and Ketoconazole. CFPAC-1 cells absorbed Cl^- and created a transepithelial Cl^- gradient, Cl^- = 9.4 ± 1.9 mM (serosal Cl^- minus mucosal; Fig. 3*C*). Keto-

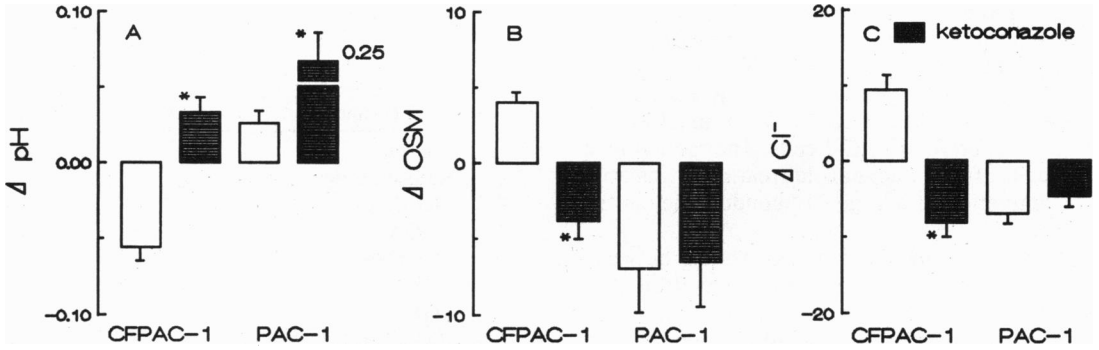

FIG. 3. (*A*) Transepithelial pH (ΔpH; $n = 8$). (*B*) Osmolality gradients (ΔOSM; milliosmoles/kg of H_2O; $n = 8$). (*C*) Cl^- concentration gradients (ΔCl; mM; $n = 9$). CFPAC-1 and PAC-1 monolayers were grown on permeable supports 5–12 days after plating. Control and monolayers with ketoconazole (0.1 mM) added to the culture medium were incubated for 3 days. n = Number of experiments. Significance of transepithelial gradients (paired-group t test) was as follows. (*A*) CFPAC-1 ΔpH, $P < 0.01$; PAC-1 ΔpH, $P < 0.01$. (*B*) CFPAC-1 ΔOSM, $P < 0.01$; PAC-1 ΔOSM, $P < 0.05$. (*C*) CFPAC-1 ΔCl^-, $P < 0.01$; PAC-1 ΔCl^-, $P < 0.01$. *, $P < 0.01$ for ketoconazole-induced changes calculated with the two-sample independent-group t test.

conazole reversed the transepithelial Cl^- gradient in CFPAC-1 cells and blocked Cl^- absorption. PAC-1 cells did not absorb Cl^- and the transepithelial Cl^- gradient (-6.8 ± 1.4 mM) was not significantly changed by ketoconazole.

DISCUSSION

The sensitivity of CFPAC-1 cells to specific inhibitors of Δ_4Ach metabolic pathways differed depending on the presence of a functional CFTR: the cAMP-sensitive Cl^- conductance in PAC-1 cells was eliminated by inhibition of the cyclooxygenase pathway, whereas a cAMP-insensitive Cl^- conductance was activated in CFPAC-1 cells by inhibition of the epoxygenase pathway. The two cell lines could not be grown on the same type of permeable support and comparisons of the characteristics of the two cell lines must be tempered by concerns about any effects of substrates on cell properties. These concerns may be somewhat allayed by the fact that the rates of dome formation by both cell lines grown on plastic (Table 3) were in excellent agreement with the conclusions obtained from the electrical measurements made on cells grown on permeable supports (Tables 1 and 2). The nature of the permeable support also should not influence comparisons of the response to the various drugs within a cell line.

Cl^- Conductance in PAC-1 Cells Is Regulated by cAMP and Cyclooxygenase Metabolites. (R_P)-cAMP[S] inhibited the Cl^- conductance in PAC-1 cells, whereas forskolin further increased the high baseline Cl^- conductance by 19%. PAC-1 cell Cl^- conductance was eliminated by indomethacin. Thus it seems clear that the Cl^- conductance in PAC-1 cells is under the control of cAMP, which is in turn regulated by cyclooxygenase metabolites. It is worthy of note that ketoconazole had virtually no effect on the Cl^- conductance of PAC-1 cells except in the presence of indomethacin. This result is consistent with the conclusion that epoxygenase products play no obvious role in the regulation of the apical membrane Cl^- conductance of normal cells but may be involved when other Δ_4Ach metabolic pathways are inhibited. PAC-1 cells exhibited demonstrable effects of ketoconazole in the absence of indomethacin as shown by increased bathing solution acidification and a decreased rate of dome formation. These results are consistent with some other regulatory roles for epoxygenase metabolites that do not involve changes in apical membrane Cl^- conductance.

Cl^- Conductance in CFPAC-1 Cells Is Induced by Inhibition of Epoxygenase Metabolites. Ketoconazole and SKF-525A, both specific blockers of P-450 (28–30), induced an apical Cl^- conductance in CFPAC-1 cells, whereas forskolin, (R_P)-cAMP[S], and indomethacin did not influence CFPAC-1 cell Cl^- conductance. NDGA, the lipoxygenase inhibitor, induced a small Cl^- conductance in CFPAC-1 cells. It has been proposed that some lipoxygenase products [e.g., 5-hydroxyeicosatetraenoic acid (5-HETE)] may also be linked to the P-450 pathway (13, 31), although ketoconazole was 20 times less potent than NDGA in inhibiting 5-HETE formation (31). We conclude that a defective CFTR markedly alters the sensitivity of PAC-1 cells to epoxygenase metabolites and that a cAMP-insensitive Cl^- conductance is produced by inhibition of the P-450 pathway.

pH and CF. Barasch *et al.* (32) showed that the pH in intracellular organelles is raised in CF cells and proposed that defective acidification of intracellular compartments causes the abnormalities in CF cells. The pH of CFPAC-1 cells is elevated by 0.37 pH unit in comparison to PAC-1 cells (33). The reported stimulation of the Na^+/H^+-antiport in CF cells (34) could contribute to cytoplasmic alkalinization. However, the reduced acidification of both mucosal and serosal bath media by CFPAC-1 cells compared to PAC-1 controls in the present study argues for an overall reduction of metabolic acid excretion rather than stimulation.

Δ_4Ach metabolism and stability of Δ_4Ach metabolites also depend on the pH; acidification induces prostaglandin formation and vasodilation of cerebral arteries (35, 36). P-450 has an alkaline pH optimum (36) and P-450 activity is blocked in acidosis (37). Thus, it is possible that the alkaline status of CFPAC-1 cells contributes to the activation of P-450 enzyme activity.

Fatty Acid Involvement. Although the CFTR gene encodes a Cl^- channel, it was also suggested (38) that CFTR is multifunctional—i.e., a Cl^- channel and a transporter for an unidentified substrate. From homology and model studies, Hyde *et al.* (39) found the CFTR protein to be unlike any known channel but similar to the ATP-binding cassette superfamily that includes the multi-drug-resistance transporter and *STE6* gene product of yeast. Ringe and Petsko (12) proposed that the substrates of CFTR could be Δ_4Ach metabolites that are conjugated with glutathione (GSH). Both an ATPase that pumps GSH conjugates, including GSH-leukotriene C_4 (40), and an active prostaglandin transporter (41) have been identified. Recently, it was shown that epoxyeicosatrienoic acid conjugates with GSH by GSH *S*-transferase (42), and it was suggested that the multi-drug-resistance pump and the GSH-conjugate pump are members of a gene superfamily (43).

A Hypothesis for the Role of CFTR. Our results can be explained if the CFTR functions both as a Cl^- channel and as an ATPase that excretes P-450 metabolites (e.g., epoxides), possibly conjugated to GSH. The CFTR could then constitute a "vacuum cleaner" to keep the concentration of these GSH-epoxides low in plasma membranes. PAC-1 cells, with a functioning CFTR, would be relatively insensitive to in-

hibitors of the production of epoxides because of rapid excretion by CFTR. Impaired removal of GSH-epoxides from CF cells by the defective CFTR should increase the concentration of free epoxides in CF cells. Subsequently, these free epoxides could selectively inhibit cell membrane transporters and enzyme activities (14).

Increase of GSH-epoxides in CF cells could also change the lipid composition of plasma membranes and thus the three-dimensional structure of membrane-bound proteins and their function (44, 45). The increase of GSH-epoxides signals oxidative stress and should trigger a compensatory increase in GSH formation. Increased extracellular GSH and increased GSH reductase activity have been reported in CF (46). Epoxides and the increase in the ratio of unsaturated fatty acids to saturated fatty acids stimulate phospholipase activity in plasma membranes (45, 47). Thus, a possible increase of phospholipase A_2 activity in CF cells explains observations that prostaglandin formation (48) and Δ_4Ach release are increased in CF (49). The increased liberation of Δ_4Ach from phospholipids by an activated phospholipase A_2 increases the fatty acid turnover that in turn may explain the fatty acid deficiency in CF (50). Essential fatty acid deficiency could also explain some of the impairments of antibacterial lung defense mechanisms in CF (51).

In summary, we propose a crucial role for CFTR in the excretion of Δ_4Ach metabolites in addition to its function as a Cl^- channel. Our observations show that inhibition of the production of P-450 metabolites effectively corrects the Cl^- transport defect of CF by activating an apical Cl^- conductance that is independent of cAMP. Correction of abnormal Cl^- and fluid transport by P-450 inhibitors makes ketoconazole, and related compounds, potentially valuable candidates for drug therapy of CF.

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1. Fuller, C. M. & Benos, D. J. (1992) *Am. J. Physiol.* **263**, C267–C286.
2. Quinton, P. M. (1990) *FASEB J.* **4**, 2709–2717.
3. Welsh, M. J. (1990) *FASEB J.* **4**, 2718–2725.
4. Schoumacher, R. A., Ram, J., Ianuzzi, M. C., Bradbury, N. A., Wallace, R. W., Tom Hon, C., Kelly, D. R., Schmid, S. M., Gelder, F. B., Rado, T. A. & Frizzell, R. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4012–4016.
5. Kraus, R. D., Bubien, J. K., Drumm, M. L., Zheng, T., Peiper, S. C., Collins, F. S., Kirk, K. L., Frizzell, R. A. & Rado, T. A. (1992) *EMBO J.* **11**, 875–883.
6. Egan, M., Flotte, T., Afione, S., Solow, R., Zeitlin, P. L., Carter, B. J. & Guggino, W. B. (1992) *Nature (London)* **358**, 581–584.
7. Higgins, C. (1992) *Nature (London)* **358**, 536.
8. Boucher, R. C., Cheng, E. H. C., Paradiso, A. M., Stutts, M. J., Knowles, M. R. & Earp, H. S. (1989) *J. Clin. Invest.* **84**, 1424–1431.
9. Welsh, M. J. & Liedtke, C. M. (1986) *Nature (London)* **322**, 467–470.
10. Willumsen, N. J. & Boucher, R. C. (1989) *Am. J. Physiol.* **256**, C226–C233.
11. Eidelman, O., Guay-Broder, C., van Galen, P. J. M., Jacobson, K. A., Fox, C., Turner, R. J., Cabantchik, Z. I. & Pollard, H. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5562–5566.
12. Ringe, D. & Petsko, G. A. (1990) *Nature (London)* **346**, 312–313.
13. Bonventre, J. V. & Nemenoff, R. (1991) *Kidney Int.* **39**, 438–449.
14. Fitzpatrick, F. A. & Murphy, R. C. (1989) *Pharmacol. Rev.* **40**, 229–241.
15. Smith, W. L. (1989) *Biochem. J.* **259**, 315–324.
16. Irvine, R. F. (1982) *Biochem. J.* **204**, 3–16.
17. Beitch, B. R., Beitch, I. & Zadunaisky, J. A. (1974) *J. Membr. Biol.* **19**, 381–396.
18. Schaeffer, B. E. & Zadunaisky, J. A. (1986) *Invest. Ophthalmol. Visual Sci.* **27**, 894–904.
19. Eling, T. E., Danilowicz, R. M., Henke, D. C., Sivarajah, K., Yankaskas, J. R. & Boucher, R. C. (1986) *J. Biol. Chem.* **261**, 12841–12849.
20. Leikauf, G. D., Ueki, I. F., Widdicombe, J. H. & Nadel, J. A. (1986) *Am. J. Physiol.* **251**, F47–F53.
21. Calderaro, V., Giovane, A., Simone, B. D., Camussi, G., Rossiello, R., Quagliuolo, L., Servillo, L., Taccone, W., Giordano, C. & Balestrieri, C. (1991) *Am. J. Physiol.* **261**, G443–G450.
22. Marom, Z., Shelhamer, J. H., Sun, F. & Kaliner, M. (1983) *J. Clin. Invest.* **72**, 122–127.
23. Furlong, T. J., Moriyama, T. & Spring, K. R. (1991) *J. Membr. Biol.* **123**, 269–277.
24. Carroll, M. A., Sala, A., Dunn, C. E., McGiff, J. C. & Murphy, R. C. (1991) *J. Biol. Chem.* **266**, 12306–12312.
25. Escalente, B., Erlij, D., Falck, J. R. & McGiff, J. C. (1991) *Science* **251**, 799–802.
26. Schwartzman, M. L., Balazy, M., Masferrer, J., Abraham, N. G., McGiff, J. C. & Murphy, R. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8125–8129.
27. Drumm, M. L., Pope, H. A., Cliff, W. H., Rommens, J. M., Marvin, S. A., Tsui, L. C., Collins, F. S., Frizzell, R. A. & Wilson, J. M. (1990) *Cell* **62**, 1227–1233.
28. Van den Bosche, H., Willemsens, G., Cools, W., Marichal, P. & Lauwerst, W. (1983) *Biochem. Soc. Trans.* **11**, 665–667.
29. Van den Bosche, H. & Willemsens, G. (1982) *Arch. Int. Physiol. Biochim.* **90**, B218–B219.
30. Capdevila, J., Gil, L., Orellana, M., Marnett, L. J., Mason, I., Yadagiri, P. & Falck, J. R. (1988) *Arch. Biochem. Biophys.* **261**, 257–263.
31. Beetens, J. R., Loots, W., Somers, Y., Coene, M. C. & De Clerk, F. (1986) *Biochem. Pharmacol.* **35**, 883–891.
32. Barasch, J., Kiss, B., Prince, A., Saiman, L., Gruenert, D. & Al-Awqati, Q. (1991) *Nature (London)* **352**, 70–73.
33. Elgavish, A. (1991) *Biochem. Biophys. Res. Commun.* **180**, 342–348.
34. Willumsen, N. J. & Boucher, R. C. (1991) *Am. J. Physiol.* **261**, C332–C341.
35. Leffler, C. W. & Busija, D. W. (1985) *Circ. Res.* **57**, 689–694.
36. Wagerle, L. C. & Mishra, O. P. (1988) *Circ. Res.* **62**, 1019–1026.
37. Kikuta, Y., Kusunose, E., Okumoto, T., Kubota, I. & Kusunose, M. (1990) *J. Biochem.* **107**, 280–286.
38. Higgins, C. F. & Hyde, S. C. (1991) *Nature (London)* **352**, 194–195.
39. Hyde, S. C., Emsley, P., Hartshorn, M. J., Mimmack, M. M., Gileadi, U., Pearce, S. R., Gallagher, M. P., Gill, D. R., Hubbard, R. E. & Higgins, C. F. (1990) *Nature (London)* **346**, 362–365.
40. Ishikawa, T. (1989) *J. Biol. Chem.* **264**, 17343–17348.
41. Bito, L. Z. (1973) *Exp. Eye Res.* **16**, 299–306.
42. Spearman, M. E., Prough, R. A., Estabrock, R. W., Falck, J. R., Manna, S., Leibman, K. C., Murphy, R. C. & Capdevila, J. (1985) *Arch. Biochem. Biophys.* **242**, 225–230.
43. West, I. C. (1990) *Trends Biochem. Sci.* **15**, 42–46.
44. Schaeffer, B. E. & Zadunaisky, J. A. (1979) *Biochim. Biophys. Acta* **556**, 131–143.
45. Sevanian, A., Muakkassah-Kelly, S. F. & Montestruque, S. (1983) *Arch. Biochem. Biophys.* **223**, 441–452.
46. Shapiro, B. L., Smith, Q. T. & Warick, W. J. (1975) *Pediatr. Res.* **9**, 885–888.
47. Sevanian, A., Stein, R. A. & Mead, J. F. (1981) *Lipids* **16**, 781–789.
48. Chase, H. P. (1978) *Lancet* **ii**, 236–238.
49. Carlstedt-Duke, J., Bronnegard, M. & Strandvik, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9202–9206.
50. Rogiers, V., Dab, I., Michotte, Y., Vercruysse, A., Crokaert, R. & Vis, H. L. (1984) *Pediatr. Res.* **18**, 704–709.
51. Harper, T. B., Chase, H. P., Henson, J. & Henson, P. M. (1982) *Am. Rev. Respir. Dis.* **126**, 540–547.
52. Capdevila, J., Parkhill, L., Chacos, N., Okita, R., Masters, B. S. & Estabrock, R. W. (1981) *Biochem. Biophys. Res. Commun.* **101**, 1357–1363.