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CFTR Activation Raises Extracellular pH of NIH/3T3 Mouse Fibroblasts and C127 Epithelial Cells

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Abstract. Cystic Fibrosis (CF) is caused by mutations in the gene for CFTR, a cAMP-activated anion channel found in apical membranes of wet epithelia. Since CFTR is permeable to HCO_3^- and changes in extracellular fluid composition may contribute to CF lung disease, we investigated possible differences in extracellular pH (pHo) between CFTR-expressing and control cell lines. The Cytosensor™ Microphysiometer was used to study forskolin-stimulated extracellular acidification rates in CFTR-expressing and control mouse mammary epithelial (C127) and fibroblast (NIH/3T3) cell lines. Forskolin, which activates CFTR via raised cAMP, caused *decreased* extracellular acidification of CFTR-expressing NIH/3T3 and C127 cells by 15–35%. By contrast, forskolin caused *increased* extracellular acidification of control cells by 10–20%. Ionomycin, which may activate CFTR via PKC, also elicited this decreased extracellular acidification signal only in cells expressing CFTR. In control experiments, dideoxyforskolin had no effect on the acidification rates and osmotic stimuli were shown to equally stimulate all cell lines. These results suggest a role for CFTR in controlling pHo and complement recent evidence that HCO_3^- dependent epithelial secretion may be reduced in amount and altered in composition in CF.

Key words: Cystic fibrosis — Microphysiometry — Bicarbonate — HCO_3^- — Cytosensor

Introduction

CFTR is an apical membrane anion channel involved in epithelial fluid secretion and salt absorption (Kerem et al., 1989; Riordan et al., 1989; Rommens et al., 1989; Quinton, 1990). In cystic fibrosis (CF), disease pathology in many organs is attributed primarily to impaired Cl^- conductance in some wet epithelia (Fuller & Benos, 1992). However, manifestations of CF in some organs have been difficult to attribute solely to a defect in Cl^- conductance (Quinton, 1999). Recently, there has been renewed interest in aspects of pH regulation associated with CFTR (Poulsen & Machen, 1996; Lee et al., 1998; Lee et al., 1999*a*). CFTR expression has been shown to affect intracellular pH in a CF pancreatic cell line (Elgavish, 1991) and transfected fibroblasts (Barasch et al., 1991), and more recent studies have focused on cytosolic pH as well as the capacity of CFTR to conduct bicarbonate ions (Poulsen et al., 1994; Chan et al., 1996; Poulsen & Machen, 1996; Clarke & Harline, 1998; Lee et al., 1998; Ballard et al., 1999) or alter bicarbonate transport through other pathways (Gottlieb & Dosanjh, 1996; Seidler et al., 1997; Clarke & Harline, 1998; Ballard et al., 1999; Lee et al., 1999*b;* Shumaker et al., 1999). However, with few exceptions (Cheung, Wang & Chan, 1998), prior investigations examined intracellular pH, while the most important CF pathophysiology appears to arise from alterations in external fluids. For example, increased acidity of secretions containing enzymes or mucins can lead to premature activation of enzymes, increased thickness of mucus, and consequently ductal blockage and irritation (Quinton, 1999). Smith et al. (1996) also reported that the primary cause of CF lung disease may reside in the extracellular milieu, the airway surface fluid (ASF). Antimicrobial agents in the ASF maintain lung sterility (Lehrer, Lichtenstein & Ganz, 1993) and Smith et al. (1996) found that elevated extracellular salt concentration inhibits antimicrobial function.

To examine CFTR's effect on extracellular pH (pHo) more closely, we used a highly sensitive pH biosensor that allows real-time measurement of changes in

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pHo (Parce et al., 1989; McConnell et al., 1992). The silicon Cytosensor™ Microphysiometer detects changes in pHo as a result of transient acid/base fluxes such as those caused by short-lived transporter activity, alterations in metabolic rate and changes in intracellular pH (McConnell et al., 1992). It can assay (10^4 to 10^6 cells per channel) and detects subtle changes (~0.001 pH unit) in the rates at which cells acidify their environment (Parce et al., 1989; McConnell et al., 1992). At rest, an average cell in culture acidifies pHo at a rate of 10^8 protons $\cdot$ sec^{-1} $\cdot$ $cell^{-1}$ (Parce et al., 1989; McConnell et al., 1992). In addition, cells almost invariably alter acid/base export when stimulated. Changes in extracellular acidification rates result from alterations in production of acidic products of metabolism (principally lactic acid, H^+ and CO_2) and the mechanisms regulating cellular pH (principally Na^+/H^+ exchange, and HCO_3^- flux) (Parce et al., 1989; McConnell et al., 1992).

The present report represents the first use of microphysiometry to determine effects of CFTR expression and activation on pHo. In two cell lines (NIH/3T3 and C127) stably expressing CFTR, the cytosensor detected decreased acidification of the extracellular media following cAMP-mediated stimulation of CFTR-expressing cells *vs.* control cells. Decreased extracellular acidification could be caused either by increased base export or decreased acid export.

Materials and Methods

Materials

Unless otherwise indicated, chemicals were obtained from Sigma (St. Louis, MO) and culture media from GIBCO BRL (Grand Island, NY). Supplies for the Cytosensor™ Microphysiometer were obtained from the manufacturer, Molecular Devices Corporation (Sunnyvale, CA).

Cell Culture

C127 mouse mammary epithelial control cells, and cells stably expressing small amounts of wild-type CFTR (BPV, 2WT2 cells, respectively) were obtained from Genzyme Corporation (Seng Cheng), and characterized elsewhere (Denning et al., 1992; Dechecchi et al., 1993). They are stable cell lines transfected by the calcium phosphate precipitation method with the BPV vector (bovine papilloma-based vector with a neomycin resistance gene) alone (BPV cells), or the BPV vector with the cDNA for CFTR (2WT2 cells). NIH mouse fibroblast cell lines (NIH/3T3, 3T3/WT) were obtained from M.J. Welsh. They are untransfected parental cells (NIH/3T3) and a stable cell line cotransfected with a retroviral vector containing human CFTR cDNA and a neomycin resistance vector, pSV2neo (3T3/WT) (Anderson et al., 1991). NIH and C127 cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin and 0.5 mg/ml G418 and do not form tight junctions or polarize. The presence of functional CFTR was regularly assayed by cAMP-dependent activation of Cl^- channels in Iodide-125 efflux experiments (Venglarik, Bridges & Frizzell, 1990).

Measurement of Extracellular Acidification: General Operating Principles

The microphysiometer is based on a microflow chamber (a 1.4 μl cylindrical space of 50 um height and 6 mm diameter) in which cells are in diffusive contact with a semiconductor-based pH sensor, the light-addressable potentiometric sensor (LAPS) (Hafeman, Parce & McConnell, 1988; Parce et al., 1989). The LAPS determines the pH in a Nernstian fashion (61 mV per pH unit change at 37°C) from the sum of a continuously applied voltage and the voltage at the surface of the chip that changes relative to acidification (McConnell et al., 1992). Two fluid paths are associated with each cell chamber, and both data acquisition and fluid path selection is computer controlled. In this study, cell chambers were used in which adherent cells were grown on a membrane and during the assay retained between two microporous polycarbonate membranes. Extracellular acidification rates were determined as the rate of change of sensor output during periodic interruptions of fluid flow which causes transient acidifications of <0.1 pH unit. Acidification rates were obtained using a least-squares regression of data obtained during periodic interruptions of fluid flow (40–60 sec) and were reported in microvolts per second (μV/sec). One microvolt per second (μV/sec) corresponds closely to an acidification rate of 0.001 pH unit/min (at pH 7.4). Since acidification rate varies with absolute pH we always started the experiment at pH 7.4 and the total pH never varied by more the 0.1 pH units for the duration of the experiment. When rates of acidification were compared across different chambers in which the absolute number of cells (and thus basal acidification rate) varied, the data were normalized to account for variation in acidification levels between chambers. Normalization was performed by Molecular Devices' cytosensor software "Cytosoft 2.0.1." In normalization, steady state acidification rates prior to drug application were established as baseline and rate values (μV/sec) were converted to percentage (%) change. For detailed information including schematics and illustrations concerning the microphysiometer developed at Molecular Devices Corporation *see also* McConnell et al. (1991) and Owicki & Parce (1992).

Measurement of Extracellular Acidification

Specific Experimental Procedures

NIH (NIH/3T3, 3T3/WT) or C127 (BPV, 2WT2) cells were seeded into 12-mm diameter disposable 3.0 μm porous polycarbonate cell capsules at 3.0×10^5 cells/well in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. The cultures were incubated at 37°C in a humidified atmosphere of 95% air/5% CO_2 overnight (16–18 hr). To measure the rate of acidification, cultures in disposable cell capsules were loaded into the chambers of the microphysiometer following manufacturer's recommendations. To enhance the detection of subtle pH changes sensor chambers were perfused with nominally bicarbonate-free DMEM with a low buffering capacity (1.0 mM sodium phosphate and ~100 μM HCO_3^- from atmospheric CO_2). Concentration of bicarbonate can be estimated given a solubility of 0.592 ml CO_2/ml water and assuming room air is 740 mm Hg with 0.0314% CO_2 (Brookes & Turner, 1994). Media were also degassed/debubbled and warmed to 37°C before perfusing the cells. Adherent cells were not manipulated in any way during an experiment, but pHo was continually monitored. The medium also contained drugs where indicated. In tonicity experiments, to achieve the indicated level of hypotonicity running buffer was diluted with the appropriate amount of water (sodium phosphate was added to maintain buffer concentration). Reservoirs of medium were connected to the flow chamber by tubing

in which the flow was set to 100 μl/min. Directing flow from a given reservoir was done by software driven valves. Each cycle lasted 2 min for epithelia (C127 cell lines) or 1 min for fibroblasts (NIH cell lines) and consisted of a perfusion phase and an interruption phase. During the perfusion phase the medium was continuously passed through the chamber for an interval of 80 sec for C127 cells and 40 sec for NIH cells. During the next phase, the pumps were inactivated, during which the rate of acidification within the chamber was calculated, recorded and plotted by the software. Flow during this phase was interrupted for an interval of 40 sec for epithelia and 20 sec for fibroblasts. The flow was then resumed and the next cycle begun, with rates of acidification returning again to baseline.

Immunoblot

Cells grown to confluency in a T-75 flask were scraped free in PBS, centrifuged at 1200 rpm for 10 min, resuspended in lysis buffer (5 mM Tris-HCL at pH 7.4 plus 5 mM EDTA with 1% Triton X-100), and held on ice with frequent vortexing for 30 min. A sample was assayed for protein concentration using bicinchoninic acid and the remainder diluted with an equivalent volume of 2× sample buffer (1.25 M Tris-HCl, pH 6.8, 4% SDS, 25% glycerol, bromophenol blue and 0.7% mercaptoethanol) and stored −20°C until run (100 μg/lane) on a 7.5% polyacrylamide gel. Gels were blotted onto Hybond-ECL (nitrocellulose) membrane from Amersham using a Genie Blotter (24V for 2 hr); the membrane was then removed and blocked with 5% nonfat dry milk in TBST (20 mM Tris-base, 137 mM NaCl, 0.05% Tween-20) for at least 1 hr at 22°C before incubating overnight at 4°C with anti-CFTR monoclonal antibodies "α-1468" (1:10,000 dilution). "α-1468" antibody recognizes a cytoplasmic C-terminus region of CFTR. The α-1468 polyclonal antibody was prepared in collaboration with R. Kopito and H. Nguyen. Antibodies were raised in rabbits to oligopeptides consisting of the terminal CFTR sequence 1468–1480 conjugated to Keyhole Limpet hemocyanin. Antibodies were immunopurified from antiserum. After rinsing, the membrane was incubated @ 22°C for 1 hr in HRP rabbit anti-mouse IgG diluted at 1:1000 in TBST-1% BSA, and then washed for at least 1 hr in fresh TBST-1% BSA. Detection was done using an ECL Kit (Amersham) according to manufacturer's instructions.

Statistical Analysis

Except where noted, data are reported as means ± SEM. Statistical significance was assessed with two-tailed Student's *t*-test for samples with unequal variances as implemented in Excel (Microsoft Corporation) as appropriate. To clarify the origin of all data, in Figs. 2 and 3 the notation "$n = x(y)$" will be used where "x" is the traditional trial number. A single trial, "$n = 1$", represents data acquisition from a single capsule of cells in one experiment. The notation "y" is the number of multiple replications performed on one capsule of cells during one experiment. Standard procedure is that multiple curves would be averaged to represent the response from a single capsule of cells. For example, if a single capsule is tested twice, this is represented as $n = 1(2)$. For all figures and statistical calculations one capsule was represented by one averaged measurement and statistical tests were done using the number of capsules as trial number.

Results

Detection of Functional CFTR Expression

Mouse epithelial C127 and mouse fibroblast NIH/3T3 cell lines that were either untransfected, vector-transfected, or stably transfected with vector containing CFTR cDNA were studied. When assayed for expression of CFTR protein with a monoclonal antibody made *vs.* a cytoplasmic C-terminal epitope of CFTR (peptide 1468–1480), cells transfected with CFTR cDNA (2WT2 and 3T3/WT cells) expressed detectable levels of CFTR protein, but untransfected or vector controls did not (Fig. 1). Immunoblots densely stained a 170 kD band in 2WT2 and 3T3/WT cells (Fig. 1; lanes B, C, F, respectively), but no band was detected in untransfected C127 epithelia, untransfected NIH/3T3 fibroblasts, or vector transfected BPV epithelial cells (Fig. 1; lanes D, E, A, respectively). The presence of functional CFTR was regularly confirmed by cAMP-dependent activation of Cl^- channels in Iodide-125 efflux experiments (*data not shown*) (Venglarik et al., 1990).

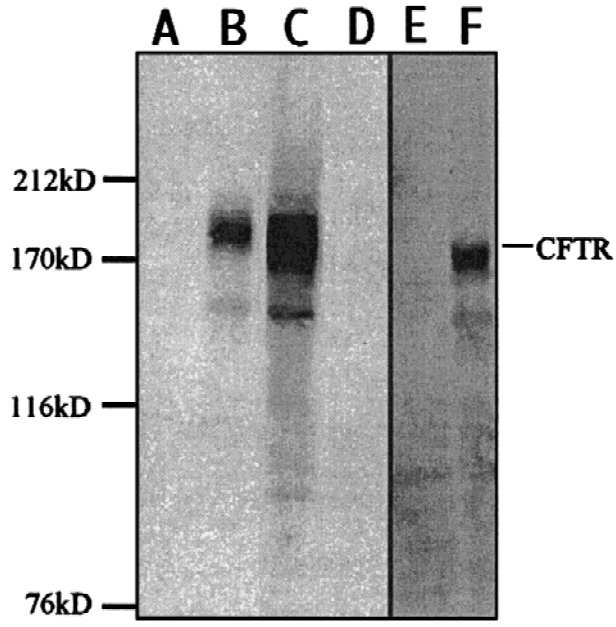

Fig. 1. Western blot analysis of CFTR protein expression levels in NIH and C127 cell lines. Cell homogenate (100 μg/lane) was run on a 7.5% polyacrylamide gel and the blot was probed with anti-CFTR 'α-1468' primary antibody. Lanes A–F are from two blots probed for CFTR protein expression. Blot 1 (left) has four lanes with protein from C127 cell lines while blot 2 (right) has two lanes with protein from NIH lines. The various CFTR glycosylation states stain as a diffuse band around 170 kD (indicated). CFTR protein is abundant in 3T3/WT (lane F) and 2WT2 (lanes B w/G418, C w/out G418) cells, yet undetectable in NIH/3T3 (lane E), C127 (lane D) or BPV (lane A) cells. Antibody "α-1468" is *vs.* CFTR's C-terminus. Molecular mass standards in kilodaltons (kD) are indicated at left; for cell line information see methods.

CFTR Activation Reduced Extracellular Acidification Rates

Stimulation of NIH/3T3 fibroblasts with 10 μM forskolin, which elevates $[cAMP]_i$, caused an increase in the

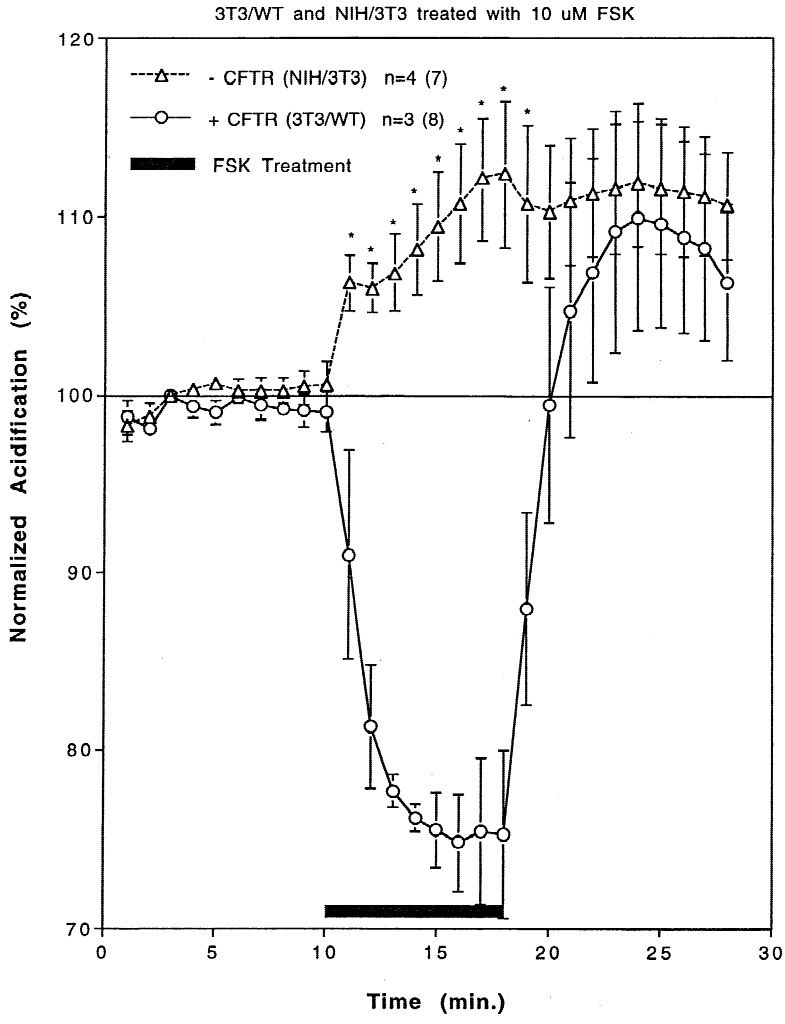

Fig. 2. Effect of 10 μM forskolin treatment on normalized acidification responses of CFTR-expressing (3T3/WT) and CFTR-deficient (NIH/3T3) NIH fibroblasts. Fibroblasts expressing CFTR showed greatly reduced acidification rates (extracellular alkalinization) in response to forskolin treatment while fibroblasts not expressing CFTR increase extracellular acidification. After forskolin washout, the CFTR-expressing cells continue to show a reduced acidification rate when compared to controls. (* indicates significance when compared to controls at $P < 0.05$; error bars as SEM.)

acidification rate of pHo (Fig. 2) as is expected for any stimulus that increases the cell's metabolism. The magnitude of the increase, 10%, represents an increased flux of ~10^7 protons · sec^{-1} · $cell^{-1}$ and is within typical ranges observed (Owicki & Parce, 1992). In marked contrast, the same forskolin stimulus produced a 25% decrease in the acidification rate of CFTR-expressing fibroblasts (3T3/WT) (Fig. 2). Forskolin activates CFTR via cAMP-dependent phosphorylation (Rommens et al., 1991), with a time course that is consistent with the alkaline signal that was observed. After forskolin washout, a strong increase in acidification temporarily equalized the acidification rates of CFTR-deficient and CFTR-expressing lines, but the reduced acidification rates of CFTR-expressing cells appear to be sustained beyond washout of forskolin. This is seen both in these studies with NIH cells as well as those with C127 cells (Figs. 2, 3).

To evaluate the possibility that the alkalinization signal might be specific to the clonally selected cells used in these experiments, but not linked to CFTR expression, we also measured pHo responses in C127 mouse mammary epithelial cell lines, using a 10-fold lower concentration of forskolin and a shorter exposure time to increase the possibility that any response observed would result from elevation of $[cAMP]_i$. Using these conditions C127 cells showed CFTR-dependent changes much like those seen in NIH fibroblasts (Fig. 3). Consistent results across these conditions argues for a specific effect. To confirm that the response to forskolin was mediated by $[cAMP]_i$, cells were treated with 10 μM of the forskolin analogue dideoxyforskolin which does not activate the PKA pathway. Dideoxyforskolin elicited no response (Fig. 4*A*). In a complementary experiment, application of 100 μM cpt-cAMP, which activates PKA without producing other effects of forskolin, produced responses that were equivalent in magnitude to those produced by forskolin (Fig. 4*B*).

To isolate the pHo responses to forskolin, the control (CFTR-deficient) cell acidification rates were subtracted from CFTR-expressing cell rates and the results replotted. As shown in Fig. 5, when plotted in this way responses in both fibroblasts and epithelial cells express-

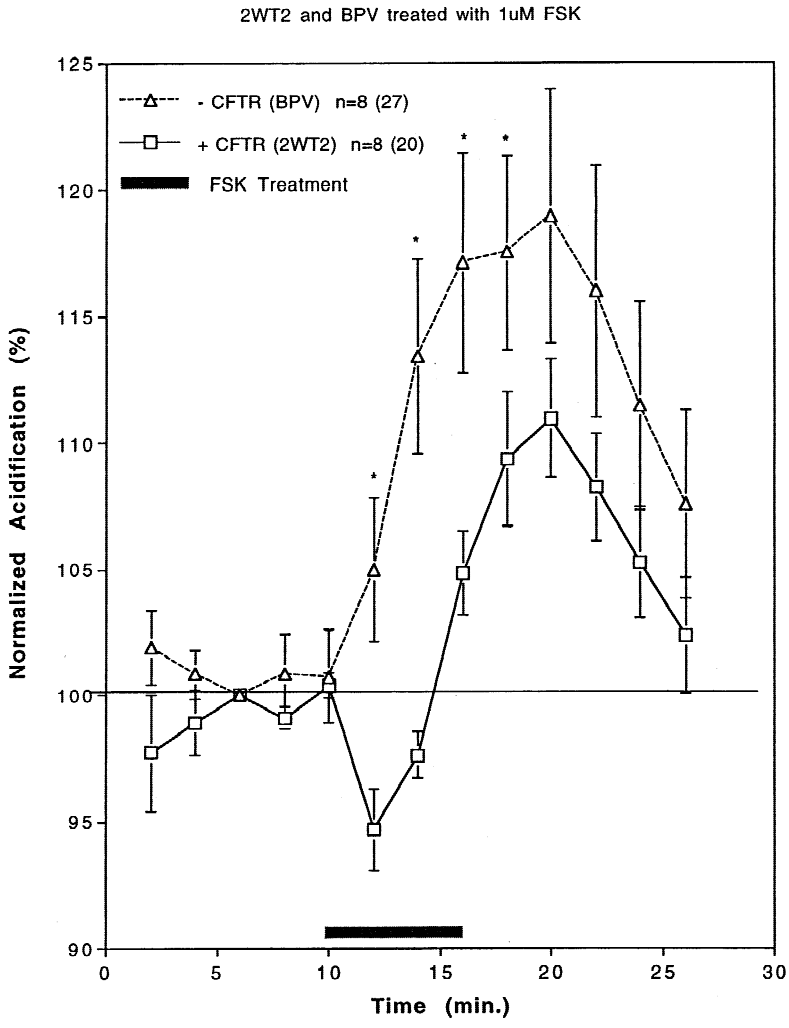

Fig. 3. Effect of 1 μM forskolin treatment on normalized acidification responses of CFTR-expressing (2WT2) and nonexpressing (BPV) C127 epithelia. Epithelial cells expressing CFTR showed reduced acidification rates (extracellular alkalinization) in response to forskolin treatment when compared to controls. As seen in fibroblasts (Fig. 2), there was a sustained suppression of acidification in CFTR-expressing epithelial cells following forskolin washout. (* indicates significance when compared to control at $P < 0.05$, error bars as SEM.)

ing CFTR are biphasic extracellular alkalinizations (Fig. 5). As indicated in Fig. 5, to overcome the background (metabolic) 10% increase in acidification and produce a 25% decrease, 10 uM forskolin must activate CFTR-expressing fibroblasts to generate a 35% increase in extracellular alkalinization.

Responses to other Stimuli

The CFTR-dependent responses we observed are most simply explained as a result of activation of CFTR. If so, responses to stimuli that do not activate CFTR would be expected to show little difference between CFTR-expressing and control cell lines. Two stimuli were tested that, at least in some cell lines, do not activate CFTR.

To test calcium-mediated pathways, 1.3 μM ionomycin (an antibiotic that greatly increases membrane permeability to Ca^{2+}) was applied. Unexpectedly, ionomycin, like forskolin, also reduced the rate of acidification in CFTR-expressing but not control C127 cells (Fig. 6*A*).

To test osmotic stimuli, dilutions in tonicity of 30, 50, and 70% were applied. These stimuli, which are expected to activate cell swelling and subsequent regulatory volume decreases, increased acidification rates in a dose-dependent manner equally in both CFTR-expressing and control C127 cell lines (Fig. 6*B*).

Discussion

A typical cell secretes ~10^8 protons/second at rest (Owicki & Parce, 1992). Superimposed on this background are fluctuations in acid/base transport that occur in response to any stimulus that alters metabolism. The increases of 10%, or 10^7 protons $\cdot$ sec^{-1} $\cdot$ $cell^{-1}$ observed in the control cell lines are evidence of a metabolic increase to forskolin. In contrast, CFTR-expressing cell lines responded with a marked decrease in the rate of

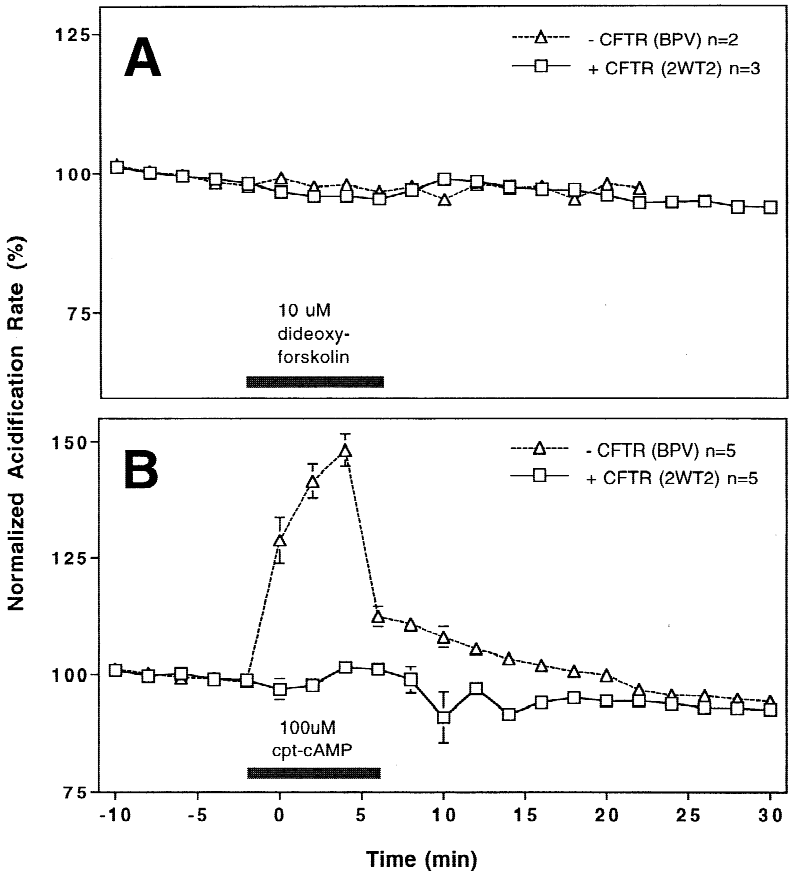

Fig. 4. Effect of dideoxyforskolin or cpt-cAMP treatment on normalized acidification responses of CFTR-expressing (2WT2) and nonexpressing (BPV) C127 cells. The protein kinase A agonist, cpt-cAMP, increases extracellular alkalinization from CFTR-expressing 2WT2 cells. (*A*) Application of 10 uM dideoxyforskolin (which does not activate PKA) has no effect on either cell line. (*B*) Application of 100 μM cpt-cAMP causes a CFTR-dependent extracellular alkalinization. Treatment periods are indicated by the gray bars.

extracellular acidification. The CFTR-dependent decrease in acidification rates could represent either a decrease of metabolic activity or the export of base that transiently masks the acidification signal.

Hypothesized Mechanism of the Response

In conceptually similar experiments using the same C127 cell lines and the pH-sensitive dye BCECF, Mastrocola, Porcelli and Rugolo (1998) provided evidence that regulation of cytosolic pH was due to stilbene-sensitive anion exchangers in all cell lines, and that anion exchanger activity was unaffected by CFTR expression. However, in C127 cells expressing CFTR, cAMP elevating agents stimulated HCO_3^- fluxes that were not inhibited by stilbenes, and they proposed that those fluxes were mediated by CFTR.

Analysis of our results leads us to the same conclusion. We hypothesize that the CFTR-dependent decrease in extracellular acidification rates represents, at least in part, the export of base that is superimposed on the metabolism-based increase in acidification. That is suggested by three observations. First, the biphasic nature of the response is consistent with two competing processes, export of acid (based on increased metabolism) and export of base (based on the opening of the CFTR conductance pathway for HCO_3^-). Second, the rebound observed when forskolin is withdrawn again suggests competing processes. Third, the magnitude of the response is most easily explained by the hypothesis that base as well as acid is being exported. It is difficult to propose a plausible mechanism whereby activation of CFTR would convert a 10% increase in metabolism into a 35% decrease. However, export of base in CFTR-expressing cells is consistent with current views of CFTR's function as a HCO_3^--permeant channel (Kopelman et al., 1988; Smith & Welsh, 1992; Poulsen et al., 1994; Chan et al., 1996; Poulsen & Machen, 1996; Clarke & Harline, 1998; Lee et al., 1998; Devor et al., 1999) or regulator of anion-exchangers (Illek, Fischer & Machen, 1998; Mastrocola et al., 1998).

The estimated base efflux via CFTR is 35% of 10^8 protons $\cdot$ sec^{-1} $\cdot$ $cell^{-1}$ or 3.5×10^7 base equivalents $\cdot$ sec^{-1} $\cdot$ $cell^{-1}$. To neutralize that amount of acid at a pH of 7.1 would require ~10:1 HCO_3^-:H^+ (with proportionately more or less HCO_3^- required at higher or lower pH). If that were entirely carried by a HCO_3^- current, it would represent a transient peak HCO_3^- current of

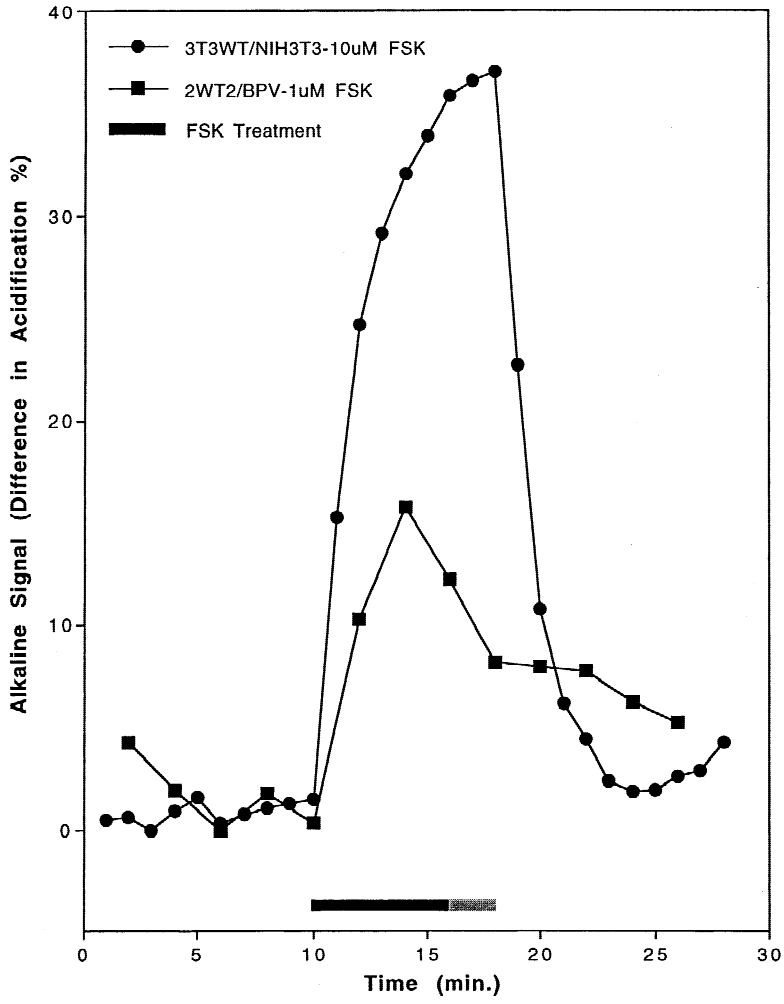

Fig. 5. Comparison of CFTR-dependent extracellular alkalinization in NIH and C127 cell lines. Forskolin response curves from CFTR-expressing cells were subtracted from CFTR-deficient cells to find the absolute value of the difference between the two responses. For NIH fibroblasts cell lines, 3T3/WT acidification rates were subtracted from NIH/3T3 rates (from Fig. 2). In C127 epithelia cell lines, 2WT2 rates were subtracted from BPV rates (from Fig. 3). C127 cells were stimulated with 1 μM forskolin for 6 min and NIH cells with 10 uM forskolin for 8 min. Treatment period(s) indicated by gray bar.

~60 pA per cell (1 pA = 6.24×10^6 ions per sec). Is that a reasonable number? CFTR-dependent whole cell currents in transfected 3T3 cells were ~1300 pA with 150 mM Cl^- and a driving force of 50 mV (Haws et al., 1992), so the required HCO_3^- current is only ~5% of that value. The difference can be attributed to the reduced conductance of CFTR for HCO_3^- (~25% of the Cl^- conductance (Poulsen et al., 1994)) and the reduced concentration of HCO_3^- in the cell. If the driving force for HCO_3^- were −50 mV, a cellular concentration of ~25 mM HCO_3^- would be needed to provide the observed currents. We propose the driving force is larger and the $[HCO_3^-]_i$ is smaller. A larger driving force is predicted because the only HCO_3^- in the buffer was from atmospheric CO_2, which is estimated to generated ~0.1 mM of HCO_3^- (Brookes & Turner, 1994). Because of the low extracellular HCO_3^- and CO_2, $[HCO_3^-]_i$ is either generated from metabolic production of CO_2 or is retained from prior conditions. Because 6 molecules of CO_2 are produced for each glucose molecule utilized, the metabolic source of CO_2 could be appreciable.

To summarize, CFTR provides a sufficiently large HCO_3^- conductance pathway to explain the reduced acidification response in CFTR-expressing cells.

Two stimuli were tested that, at least in some cell lines, do not activate CFTR. Yet, when these cells were stimulated with ionomycin and diluted buffer, ionomycin gave a response similar to forskolin, but the buffer dilution did not. These results could be interpreted to mean that only activators of CFTR elicit a decrease in the rate of acidification, if it is assumed that ionomycin activates CFTR in these cells (Dechecchi et al., 1993). However, a HCO_3^--conductance explanation of the pH changes we observed should apply to any HCO_3^- permeable channel. Thus our logic predicts that cells should show reduced acidification as a result of HCO_3^- flux through either calcium-activated or swelling-activated channels. We observed the expected response to ionomycin, but not to swelling. However if one reviews the magnitude of the cell's metabolic response to swelling (Fig. 6*B*) it is clear the enormous increase in metabolism swamps out the possibility of detecting any bicarbonate signal moving

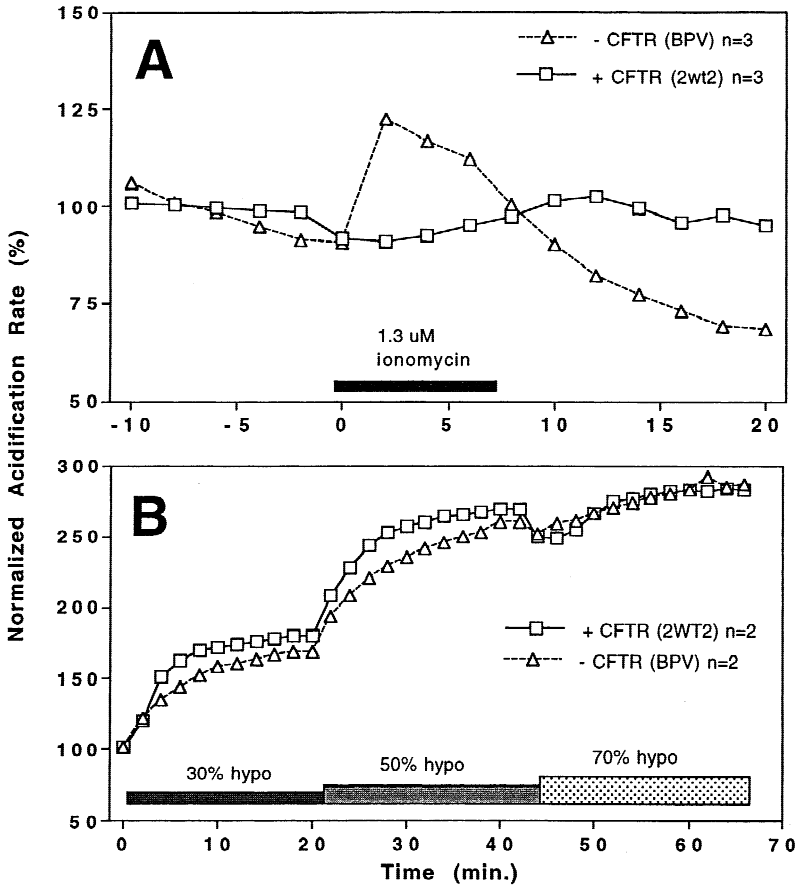

Fig. 6. Effect of ionomycin or hypotonicity treatment on normalized acidification responses of CFTR-expressing (2WT2) and nonexpressing (BPV) C127 cells. Ionomycin, a protein kinase C agonist, increases extracellular alkalinization of CFTR-expressing 2WT2 cells, yet hypotonicity does not. (*A*) 1.3 μM ionomycin (which activates PKC via Ca^{2+} elevation) causes a CFTR-dependent inhibition of acidification. (*B*) Osmotic stimuli (30, 50, 70% dilutions in tonicity), that do not directly activate CFTR, increased acidification rates equally in both cell types. Treatment periods are indicated by the gray bars.

through VSOAC (volume-sensitive organic osmolyte-anion channel; Jackson et al., 1994).

Alternative Mechanisms

Activation of CFTR will tend to move the cell voltage toward E_{Cl}. In most cells E_{Cl} is close to the resting potential, and the minor change in voltage is unlikely to explain the observed effects. It would have no effect on voltage-insensitive Na^+/H^+ exchangers, and because of the large chemical gradient for HCO_3^- it should also have rather minor effects of HCO_3^- export.

CFTR may activate anion exchangers (Lee et al., 1999*b*) and that could contribute to the responses we observe, but that interpretation is made unlikely by the experiments of Mastrocola et al. (1998). The experiments so far do not rule out the possibility that activated CFTR inhibits acid efflux via one or more Na^+/H^+ exchangers, which could also contribute to the responses observed.

Functional Significance and Usefulness as an Indicator of CFTR Function

CFTR plays multiple roles, even as an anion channel. In the sweat duct its primary function is clearly as a Cl^- conductance pathway (Quinton, 1990, 1999), but in fluid-secreting epithelia it is important for both Cl^- and HCO_3^- secretion (Quinton, 1999), and recent work has emphasized that CFTR-dependent changes in bicarbonate secretion may be of special import for understanding the pathophysiology of cystic fibrosis (Poulsen et al., 1994; Poulsen & Machen, 1996; Clarke & Harline, 1998; Lee et al., 1998; Ballard et al., 1999; Quinton, 1999; Devor et al., 1999). In the glandular structures where CFTR expression is prominent, such as pancreatic duct, vas deferens, and airway submucosal glands, and in the thin surface fluid of the airways themselves, the extracellular fluid is produced by the epithelia, and so changes in ion transport have dramatic effects on its composition.

The Cytosensor experiments demonstrate clear differences in extracellular pH of CFTR-expressing NIH/3T3 and C127 cells. Regardless of mechanism or functional significance, the distinctive, CFTR-dependent pH signal provides a robust and convenient monitor of CFTR function. As such it might prove advantageous for high throughput screening designed to identify agents that activate wild-type or mutant CFTR.

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