Defective regulatory volume decrease in human cystic fibrosis tracheal cells because of altered regulation of intermediate conductance Ca^{2+}-dependent potassium channels

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The cystic fibrosis transmembrane conductance regulator (CFTR) protein has the ability to function as both a chloride channel and a channel regulator. The loss of these functions explains many of the manifestations of the cystic fibrosis disease (CF), including lung and pancreatic failure, meconium ileus, and male infertility. CFTR has previously been implicated in the cell regulatory volume decrease (RVD) response after hypotonic shocks in murine small intestine crypts, an effect associated to the dysfunction of an unknown swelling-activated potassium conductance. In the present study, we investigated the RVD response in human tracheal CF epithelium and the nature of the volume-sensitive potassium channel affected. Neither the human tracheal cell line CFT1, expressing the mutant CFTR-ΔF508 gene, nor the isogenic vector control line CFT1-LC3, engineered to express the βgal gene, showed RVD. On the other hand, the cell line CFT1-LCFSN, engineered to express the wild-type CFTR gene, presented a full RVD. Patch-clamp studies of swelling-activated potassium currents in the three cell lines revealed that all of them possess a potassium current with the biophysical and pharmacological fingerprints of the intermediate conductance Ca^{2+}-dependent potassium channel (IK, also known as KCNN4). However, only CFT1-LCFSN cells showed an increase in IK currents in response to hypotonic challenges. Although the identification of the molecular mechanism relating CFTR to the hIK channel remains to be solved, these data offer new evidence on the complex integration of CFTR in the cells where it is expressed.

CFTR | ΔF508 | chloride secretion | airways | hIKK

Defective epithelial ion transport is the hallmark of the genetic disease cystic fibrosis (CF; ref. 1). This abnormality is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR; ref. 2). CFTR is predominantly located in the apical membrane of epithelia, where it plays a central role in the transport of salt and water, but mislocated in the case of the most common CF mutation (ΔF508; ref. 3). The best-characterized function of the CFTR protein is as a Cl^- channel regulated by cAMP-dependent phosphorylation and ATP hydrolysis (4). However, CFTR has been suggested to have additional functions in epithelial cells, including the regulation of other ion channels (5).

Epithelial cells tightly regulate their volume (6). When exposed to hypotonic solutions, they rapidly swell. The response to this increase in cell volume is called regulatory volume decrease (RVD). RVD involves the activation of anion (7, 8) and cation channels (9), which permit the passive loss of inorganic ions and osmotically obliged water. By using a mouse model of CF bearing a null mutation that generates no CFTR protein, we have demonstrated that CFTR plays an important role in cell volume regulation in jejunal crypts (10, 11). Jejunal crypts of null and ΔF508 CF mice rapidly swelled when exposed to hypotonic solutions. However, they failed to undergo RVD and remained swollen in contrast to crypts of wild-type mice. The impaired RVD in jejunal crypts of null CF mice was not a consequence of dysfunction of the CFTR Cl^- channel, but instead was caused by defective K^+ channel activity (10). These data suggest that CFTR plays a regulatory role during RVD by small intestine crypts of the mouse.

In this work, we have studied the RVD and the volume-sensitive potassium channels in CF and rescued human tracheal cells transfected with wild-type CFTR. We have achieved two specific goals. First, we have shown that human CF airways present impaired RVD. Second, we have found that the swelling-modulated potassium channel defective in human CF airways is an intermediate conductance Ca^{2+}-dependent potassium channel (IK, also known as KCNN4).

Methods

Cells. The tracheal epithelial cell line CFT1 was generated from a CF ΔF508/ΔF508 patient (12). The vector control cell line CFT1-LC3 was obtained by transfection of CFT1 cells with a retroviral vector encoding β-galactosidase (βgal), and CFT1-LCFSN was obtained by transfection of CFT1 with wild-type CFTR (13). The three cell lines were grown in Ham's F-12 medium (GIBCO) supplemented with 10 μg/ml insulin (GIBCO), 0.5 μg/ml hydrocortisone (Sigma), 3.75 μg/ml endothelial cell growth supplement (Sigma), 25 ng/ml epidermal growth factor (Sigma), 3×10^{-8} M triiodothyronine (Sigma), 5 μg/ml transferrin (Sigma), and 10 ng/ml cholera toxin (Sigma; ref. 12). CFT1-LC3 and CFT1-LCFSN cell culture media also contained 150 μg/ml Neomycin B sulfate (GIBCO). Cells were incubated at 37°C in an atmosphere of 95% air–5% CO_2 and used within 48 h after being plated onto 35-mm plastic dishes.

Volume Measurements. Cell volume experiments were performed at room temperature. Cells were placed at the bottom of a recording chamber, bathed in isotonic Hanks' solution, and observed under phase contrast optics by using an inverted microscope [Leica (Deerfield, IL) DMIL]. The individual cell volume was calculated as described previously (8, 14) and normalized to that measured at time $t = 0$. The isotonic solution contained (mM): 140 NaCl, 2.5 KCl, 1.2 $CaCl_2$, 0.5 $MgCl_2$, 5 glucose, and 10 Hepes, pH 7.25 (osmolality, 302 ± 6 mOsm). The

Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; ENaC, epithelial Na^+ channel; hIK, human intermediate conductance Ca^{2+}-dependent K^+ channel; ICl_{swell}, swelling activated Cl^- current; ORCC, outwardly rectifying Cl^- channels; RVD, regulatory volume decrease.

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hypotonic solution (osmolality, 220 ± 5 mOsm) was prepared by removing 40 mM NaCl from the isotonic solution. For the study of the Ca^{2+} dependence of RVD, 1.2 mM $CaCl_2$ was replaced by 1 mM EGTA in the extracellular solution.

Electrophysiological Recordings. Ionic currents were measured by using the whole-cell recording mode of the patch-clamp technique (15). Cells were plated in 35-mm plastic dishes and mounted on the stage of an Inverted Olympus (New Hyde Park, NY) IX70 microscope, and the whole cell currents were recorded with an Axon (Axon Instruments, Foster City, CA) 200A amplifier. Cells were clamped at −80 mV and pulsed for 400 ms from −100 mV to +100 mV in 20-mV steps. The STRATHCLYDE electrophysiological software written by J. Dempster (University of Strathclyde, Glasgow, Scotland) was used for pulse generation, for data acquisition through an Axon Digidata A/D interface, and for subsequent analysis.

Whole-cell ionic currents were measured by using an intracellular solution containing (in mM): 140 KCl, 2 $MgCl_2$, 0.2 $CaCl_2$, 0.5 EGTA, and 10 Hepes, pH 7.25. The calculated intracellular free Ca^{2+} concentration was 100 nM, and the osmolality 290 mOsm. ATP and GTP were not added to the pipette solution to delay and reduce the activation of swelling-activated Cl^- channels (16, 17). The extracellular isotonic solution contained (in mM): 100 NaCl, 5 KCl, 0.5 $MgCl_2$, 1.2 $CaCl_2$, 10 Hepes, 5 glucose, pH 7.35. The osmolality was adjusted to 305 mOsm with 80 mM D-mannitol. The hypotonic solution used to investigate the swelling-activated ionic currents (28% hypotonic, 220 mOsm) was obtained by removing the D-mannitol from the isotonic solution. All chemicals were purchased from Sigma-Aldrich unless stated to the contrary. Iberiotoxin was obtained from Alomone Labs (Jerusalem, Israel).

Expression of hIK1 in Human Airway Cell Lines. To evaluate the expression of hIK1 in different cell lines, we generated a digoxigenin-labeled probe of 195 nucleotides by PCR (PCR DIG Probe Synthesis Kit, Boehringer Mannheim) by using human genomic DNA as the template. The probe was designed to recognize the region from nucleotides 377 to 570 of the hIK1 complete coding sequence (18), not shared with other known genes, confirmed by a BLASTN search. The specificity of the probe was double checked by performing a nested PCR from the first PCR product.

Total RNA was extracted from the different cell lines when they were 80% confluent by using the Nucleospin RNA II Kit (Macherey & Nagel). Thirty micrograms of RNA from each cell line were run through a 1% agarose-formaldehyde gel and transferred to positively charged nylon membranes as described previously (19). Blots were hybridized in Dig Easy Hyb solution (Boehringer Mannheim) overnight at 50°C with 20 ng/ml of the DIG-labeled hIK probe and washed with 0.5× SSC, 0.1% SDS at 68°C. The signal was detected by using a chemiluminescent alkaline phosphatase substrate and exposed to x-ray film at room temperature for 15 min.

Statistics. Results are expressed as means ± SEM of n independent experiments. To compare sets of data, we used Student's t test. Differences were considered statistically significant when $P < 0.05$.

Results

Characterization of the Secretory Phenotype in CFT1, CFT1-LC3, and CFT1-LCFSN Cells. Vectorial ion transport is greatly affected in many CF epithelia (1). CF cells, unlike normal epithelial cells, do not elicit a net Cl^- secretion when challenged with cAMP-elevating agents (20), whereas Cl^- secretion in response to agonists that elevate intracellular Ca^{2+} is preserved (20–23). To check the phenotypic characteristics of the original ΔF508 cells

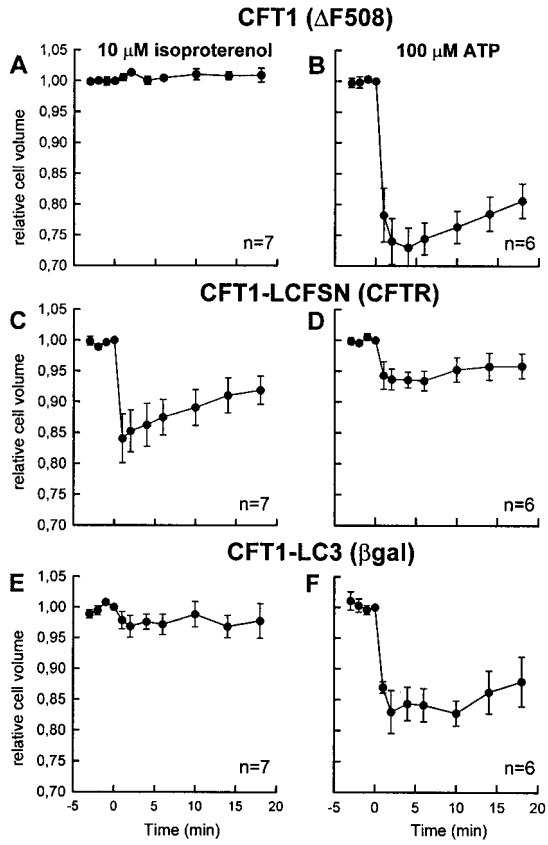

Fig. 1. Secretory responses of CFT1 (ΔF508), CFT1-LC3 (βgal), and CFT1-LCFSN (wild-type CFTR) cells. Relative changes in cell volume after the addition of 10 μM isoproterenol (*A*, *C*, and *E*) or 100 μM ATP (*B*, *D*, and *F*) to the solution bathing CFT1 (*A* and *B*), CFT1-LC3 (*E* and *F*), and rescued cells (CFT1-LCFSN) expressing wild-type CFTR (*C* and *D*). Volume was measured as described in *Methods*. The agonists were added at time = 0 min and remained in the bath for the duration of the experiment.

(CFT1) and the cells permanently transfected with βgal (CFT1-LC3) or wild-type CFTR (CFT1-LCFSN), we studied the secretory responses of these cells. Cells, triggered to secrete, decrease their cell volume as a consequence of the activation of ionic pathways, which leads to the loss of electrolytes and the osmotically obliged water (24, 25); therefore, to evaluate the secretory response, we measured the secretagogue-induced volume decrease of individual cells. Isoproterenol (10 μM), a cAMP-elevating, β-adrenergic agonist, failed to elicit statistically significant Cl^- secretion in both CFT1 (Fig. 1*A*; maximal change in volume: 1.01 ± 0.01; $P > 0.05$ when compared with preagonist cell volume) and CFT1-LC3 cells (Fig. 1*E*; maximal change in volume: 0.96 ± 0.02; $P > 0.05$). In contrast, CFT1-LCFSN cells, expressing wild-type CFTR, showed a 16% reduction of cell volume in response to isoproterenol (Fig. 1*C*; maximal change in volume: 0.84 ± 0.03; $P = 0.007$), suggesting the activation of the CFTR Cl^- channel. All three cell lines (Fig. 1 *B*, *D*, and *F*) responded with significant Cl^- secretion to 100 μM ATP, an agonist of purinergic receptors that elicits Cl^- secretion in tracheal cells (26). However, the response to ATP was more pronounced in CFT1 cells (maximal change in volume: 0.72 ± 0.03; $P < 0.001$) and CFT1-LC3 cells (maximal change in volume: 0.82 ± 0.02; $P < 0.001$) than in CFT1-LCFSN cells (maximal change in volume: 0.93 ± 0.01; $P = 0.004$), consistent with the compensatory increase in Ca^{2+}-dependent Cl^- channel activity described in CF airways (27, 28).

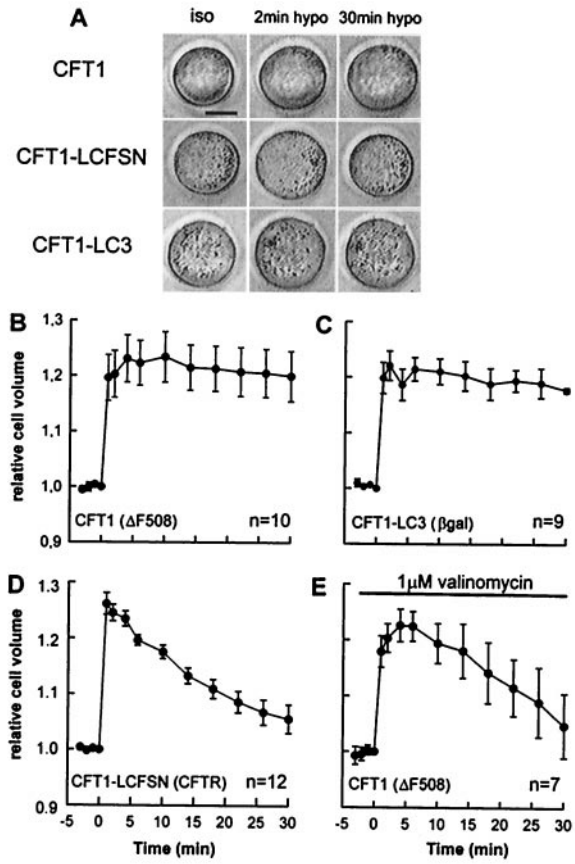

Fig. 2. Regulatory volume decrease in CFT1, CFT1-LC3, and CFT1-LCFSN cells. (*A*) Phase contrast images of CFT1 (*Top*), CFT1-LCFSN (*Middle*), and CFT1-LC3 (*Bottom*). Micrographs were taken in isotonic solution (*Left*), 2 min (*Center*), and 30 min (*Right*) after replacement of the isotonic solution with a hypotonic solution; bar = 10 μm. (*B*) Time course of volume changes in CFT1 cells. Peak volume 1.23 $\pm$ 0.04 and at 30 min 1.19 $\pm$ 0.04 (peak vs. recovery $P = 0.59$). (*C*) Time course of volume changes in CFT1-LC3 cells. Peak volume 1.22 $\pm$ 0.02 and at 30 min 1.17 $\pm$ 0.004 ($P = 0.17$). (*D*) Time course of volume changes in CFT1-LCFSN cells. Peak volume 1.26 $\pm$ 0.01 and at 30 min 1.05 $\pm$ 0.02 ($P < 0.001$). (*E*) Cell volume changes in CFT1 cells exposed to 1 μM valinomycin. Peak volume 1.22 $\pm$ 0.02 and at 30 min 1.04 $\pm$ 0.05 ($P = 0.02$). The drug was included in the bathing solutions during the period indicated by the horizontal line.

Regulatory Volume Decrease in CF and Rescued Airway Cells. After evaluating the presence of functional CFTR in all three cells lines, we studied their regulatory response to hypotonic shocks. Fig. 2*A* shows images of single CFT1 (*Top*), CFT1-LCFSN (*Middle*), and CFT1-LC3 (*Bottom*) cells. Images were taken under isotonic conditions (*Left*) and after 2 min (*Center*) and 30 min (*Right*) in a 28% hypotonic solution. Superfusion of the cells with the hypotonic solution resulted in a clear increase in the size of the cells. However, only the CFT1-LCFSN underwent RVD, recovering its initial size within 30 min of exposure to the hypotonic solution. Both CFT1 and CFT1-LC3 cells remained swollen throughout the hypotonic shock. Fig. 2 *B–D* shows the calculated changes in cell volume over the time after the exposure to 28% hypotonic solution. Of the three cell lines, only CFT1-LCFSN cells presented a marked recovery. To check whether a defective K^+ conductance might be the reason for the lack of RVD in the human CF cell lines, we exposed CFT1 cells to hypotonic shocks in the presence of 1 μM valinomycin (Fig. 2*E*), a K^+-selective ionophore. The addition of a potassium pathway permitted the regulatory response in CFT1 cells, agreeing with previous results obtained in intestinal crypts of null CF mice (10).

Identification of the Potassium Channel Activated by Cell Swelling. Potassium currents were recorded under isotonic conditions in CFT1 and CFT1-LCFSN cells dialyzed with high K^+ and 100 nM free Ca^{2+}-containing solutions (Fig. 3 *A* and *B*). The currents recorded were characterized by the presence of inward and outward current, saturation at positive potentials and the lack of marked voltage dependence. These characteristics resemble those of the IK channels (18, 29, 30). CFT1-LCFSN cells responded with a maximal increase in K^+ currents within 2 min of exposure to a hypotonic bathing solution (Fig. 3*B*). In contrast, no significant activation of currents was detected on CFT1 cells exposed to hypotonic shocks (Fig. 3*A*). The mean increase in current at different potentials in CFT1 and CFT1-LCFSN cells is shown in Fig. 3 *C* and *D*. Similar results to those obtained with CFT1 cells were observed in CFT1-LC3 cells permanently transfected with the βgal vector (maximal normalized current under isotonic and hypotonic conditions: 22 $\pm$ 6 and 25 $\pm$ 3 pA/pF; $n = 6$). Although CF cells did not respond with increases in IK activity to hypotonic shocks, both CFT1 and CFT1-LCFSN cells showed increased IK activity under isotonic conditions [35 $\pm$ 8 pA/pF ($n = 4$) and 34.5 $\pm$ 9 pA/pF ($n = 4$), respectively] when measured with a pipette solution containing 350 nM Ca^{2+}.

These experiments showed that comparable IK potassium channel activity was present under isotonic conditions in CFT1, CFT1-LC3, and CFT1-LCFSN cells. The expression of IK channels was also studied in the three cell lines to rule out the possibility of major changes in the channel expression between the three cell lines. Northern blots (Fig. 3*E*) showed that CFT1, CFT1-LCFSN, and CFT1-LC3 cells present a band around 2.1 kb, consistent with the expression of hIK in these cells (18). Moreover, no significant changes in the hIK mRNA levels were observed between the three cell lines (similar results were obtained in three other Northern blots).

The IK channel also shows a well-defined pharmacological profile (18, 29). One of the known inhibitors of IK channels, the imidazole derivative clotrimazole (18), proved to be an efficient blocker of the hypotonic-activated potassium channel in CFT1-LCFSN cells (Fig. 4*A*). The mean inhibition of CFT1-LCFSN IK currents by 100 nM clotrimazole was 66 $\pm$ 7% ($n = 3$; measured at +100 mV). To demonstrate that IK is a key player in the RVD response by CFT1-LCFSN cells, the drug was also used in cell volume experiments (Fig. 4*B*). Clotrimazole (100 nM) prevented the RVD response. On the contrary, iberiotoxin (100 nM) and apamin (5 μM), which are inhibitory toxins of the large (BK) and small (SK) conductance Ca^{2+}-dependent potassium channels (31), respectively, did not prevent the RVD response.

Ca^{2+} Dependence of the RVD Response. In several cell types, entry of extracellular Ca^{2+} is required for a complete RVD response. Typically, Ca^{2+} entry via stretch-activated cation channels after a hypotonic shock leads to the activation of the Ca^{2+}-dependent ion channels involved in the RVD response (32). To study whether the RVD response in CFT1-LCFSN cells requires extracellular Ca^{2+} entry, we monitored cell volume changes in cells exposed to a Ca^{2+}-free hypotonic solution. Fig. 5 shows that removal of Ca^{2+} from the extracellular hypotonic solution prevented the recovery of the initial cell volume.

Discussion

The CFTR protein, in addition to its function as a Cl^- channel, is able to modulate several cellular functions, including the regulation of epithelial Na^+ channels (ENaC) (33), different types of Cl^- channels (28, 34, 35), K^+ channels (36), membrane recycling (37), and ATP release (38). The interaction of CFTR with other ion cotransporters has also been postulated (39). We now show that CFTR is needed for an effective cell volume regulation in human airways exposed to hypotonic conditions.

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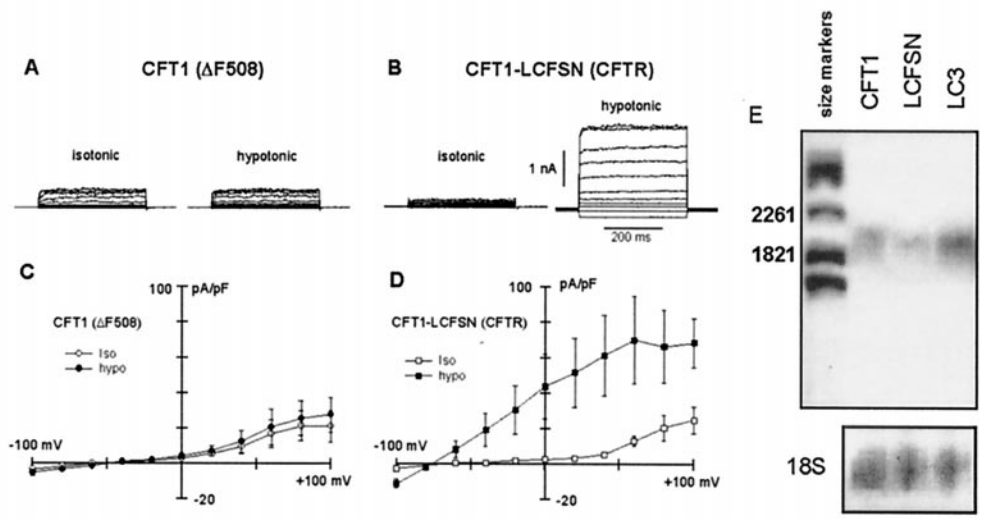

Fig. 3. Response of hIK channels to cell swelling in CFT1 and CFT1-LCFSN cells. Potassium currents were measured in both CFT1 cells (*A*) and CFT1-LCFSN cells (*B*) under isotonic and hypotonic solutions (3 min). (*C* and *D*) Current/voltage relationships obtained under isotonic conditions for CFT1 cells (○; $n = 7$) and CFT1-LCFSN cells (□; $n = 7$) and 3 min after exposure of CFT1 cells (●; $n = 7$) and CFT1-LCFSN cells (■; $n = 7$) to hypotonic solutions. (*E*) Northern blot analysis of hIK expression in CFT1, CFT1-LC3, and CFT1-LCFSN cells. Left lane shows the size markers. (*Bottom*) Ribosomic RNA shown as control for the amount of RNA in each lane.

Regulatory volume decrease in both murine (14) and human tracheal cells (this study) depends on the coordinated activity of Cl^- and K^+ channels. However, whereas the potassium channel involved in the murine cells is the complex KvLQT/IsK (also known as KCNQ1/KCNE3; ref. 14), IK channel (also known as KCNN4) is the main potassium channel involved in the RVD response in the human tracheal cells (this study).

Human IK channels belong to the family of Ca^{2+}-dependent potassium channels (31). The hIK channel gating depends on the concerted action of membrane potential and intracellular Ca^{2+}, and its single channel conductance is inwardly rectifying in symmetrical K^+ (18, 29). It is inhibited by several imidazole derivatives, such as clotrimazole (18), but it is insensitive to inhibitors of other members of the Ca^{2+}-dependent family of K^+ channels, such as iberiotoxin and apamin (18). The IK channel has been recently cloned from different species (18, 29, 40, 41). The activity of cloned IK channels expressed in *Xenopus* oocytes has also been shown to increase under hypotonic conditions, most likely as a consequence of an increase in intracellular Ca^{2+} induced by the cell swelling (41), and its role in RVD has been demonstrated in lymphocytes (30). Other intermediate conductance K^+ channels have been associated with cell-swelling responses in Ehrlich cells (42), although these channels are not the typical Ca^{2+}-dependent IK channels (43).

The fact that the ΔF508-CF cells (CFT1 and CFT1-LC3), unlike the rescued CFT1-LCFSN (CFTR) cells, showed IK activity but no swelling-induced activation pointed to a defect in the regulation of the channel protein, rather than alterations in its expression. The latter possibility was also ruled out by the Northern blot studies showing comparable expression of hIK in the three human airway cell lines.

The type of interaction between CFTR and hIK is not known at present. Several recent studies have provided insights into the molecular aspects of the CFTR regulation of outwardly rectify-

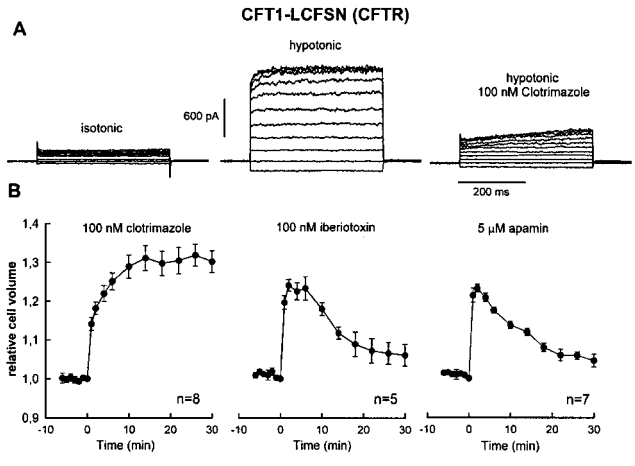

Fig. 4. Effect of potassium channel inhibitors on both hIK potassium currents and RVD in CFT1-LCFSN cells. (*A*) K^+ currents recorded under isotonic conditions and 1 min after exposure to a hypotonic solution. Addition of 100 nM clotrimazole blocked the swelling-activated potassium current (recorded 2 min after addition of clotrimazole to the hypotonic solution). (*B*) The indicated K^+ channel blocker was added 3 min before the addition (at 0 min) of the hypotonic solution, which also contained the appropriate blocker.

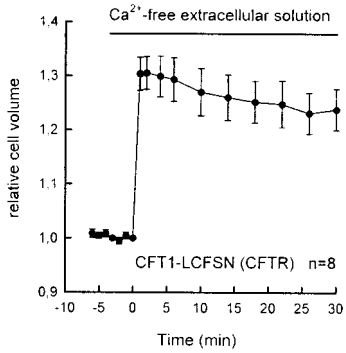

Fig. 5. Extracellular Ca^{2+}-dependence of RVD. Relative change in volume of CFT1-LCFSN cells, measured before and after replacement of the isotonic solution at time $t = -3$ min with a Ca^{2+}-free isotonic solution and at time $t = 0$ min with a Ca^{2+}-free hypotonic solution. Measurements were made on eight cells.

ing Cl^- channels (ORCC; ref. 44), ENaC (45), and ROMK2 potassium channels (36). Interestingly, these channels colocalize with CFTR to the apical membrane of the epithelia where they are expressed. All these studies point to the first half of the CFTR protein (or a part thereof, i.e., the first transmembrane domain, the first nucleotide binding domain, or the regulatory domain) as the domain/s involved in the regulation of the above mentioned channels. In the case of ENaC, a direct interaction between the two proteins was identified by the yeast two-hybrid technique (46) and by coimmunoprecipitation studies (47), although the cytoskeleton may also be involved in this interaction (48). The simple picture of a direct protein–protein contact is further complicated by the observation that CFTR modulation of several ion channels shows cAMP-dependent and cAMP-independent components (28, 47). As for the interaction of CFTR with ORCC channels, the current view postulates an indirect autocrine mechanism involving cAMP-mediated activation of CFTR-dependent ATP release, with subsequent activation of ORCC through the ATP binding to purinergic receptors (44).

With respect to the interaction between CFTR and IK channels, the fact that CFTR is localized to the apical membrane of secreting epithelia (49) and hIK channels to the basolateral membrane (50, 51) discards a direct physical contact between the two proteins. Therefore, a mediator molecule must link the regulation of hIK to the presence of CFTR.

One possible candidate is ATP. ATP efflux has been reported in cells under hypotonic conditions (52–54) and, in some cases, might be increased or facilitated by CFTR (55, 56). It was postulated that subsequent binding of extracellular ATP to a purinergic receptor increases the activity of swelling-dependent Cl^- channels (Cl_{swell}) and speeds up the RVD response (57). However, we have observed a more robust Cl_{swell} channel activity in CF cells than in rescued cells (M.N. and M.A.V., unpublished observations), in agreement with recent reports showing that expression of CFTR reduces ICl_{swell} (58) and questioning the regulation of Cl_{swell} by extracellular ATP (53, 56). Therefore, if extracellular ATP is the mediator molecule absent in human CF cells (CFT1 or CFT1-LC3) but present in the rescued CFT1-LCFSN cells, the swelling-dependent, CFTR-facilitated release of ATP should be linked to the activation of hIK during hypotonic shocks. No such association has been proved yet, although it is worth mentioning that activation of other Ca^{2+}-dependent K^+ channels by elevations in intracellular Ca^{2+} via purinergic pathways has been reported (59).

A second, although closely related, possibility might be the differential phosphorylation of hIK in control and CF cells. The regulation of heterologous hIK channels by phosphorylation has been described (60). In this line of thought, it is worth noting that the activation of phosphorylation processes in response to cell swelling (61, 62) has been associated to the release of ATP in an epithelial cell line (53). Alternatively, changes in the cytoskeleton could be mediating the swelling-dependent phosphorylation of channel proteins (61). This hypothesis brings us to a third possibility. Taking into account the close relation of epithelial hIK channels with the cytoskeleton (63) it might be possible that modifications of the cytoskeletal structure would activate the hIK channel. In this context, there is evidence of the interaction of CFTR with the cytoskeleton (64–66). Finally, the lack of response of hIK channels to cell swelling might be linked to alterations in Ca^{2+} mobilization in CF cells (67). It is well documented that entry of Ca^{2+} via stretch-activated channels leads to the activation of Ca^{2+}-dependent potassium channels (68), and our study shows that RVD in CFT1-LCFSN cells depends on the presence of extracellular Ca^{2+}. It might be possible that CFTR is needed for Ca^{2+} entry under hypotonic conditions. At present, all these hypotheses need to be explored to reveal the connection between CFTR and activation of hIK in response to hypotonic cell swelling.

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