CFTR-dependent and -independent swelling-activated K⁺ currents in primary cultures of mouse nephron

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The role of CFTR in the control of K⁺ currents was studied in mouse kidney. Whole cell clamp was used to identify K⁺ currents on the basis of pharmacological sensitivities in primary cultures of proximal (PCT) and distal convoluted tubule (DCT) and cortical collecting tubule (CCT) from wild-type (WT) and CFTR knockout (KO) mice. In DCT and CCT cells, forskolin activated a 293B-sensitive K⁺ current in WT, but not in KO, mice. In these cells, a hypotonic shock induced K⁺ currents blocked by charybdotoxin in WT, but not in KO, mice. In PCT cells from WT and KO mice, the hypertonicity-induced K⁺ currents were insensitive to these toxins and were activated at extracellular pH 8.0 and inhibited at pH 6.0, suggesting that the corresponding channel was TASK2. In conclusion, CFTR is implicated in the control of KCNQ1 and Ca²⁺-sensitive swelling-activated K⁺ conductances in DCT and CCT, but not in proximal convoluted tubule, cells. In KO mice, impairment of the regulatory volume decrease process in DCT and CCT could be due to the loss of an autocrine mechanism, implicating ATP and adenosine, which controls swelling-activated Cl⁻ and K⁺ channels.

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contrast, in PCT, CFTR does not control swelling-activated K⁺ conductance, inasmuch as these channels belong to the TASK2 family, the expression of which is not related to CFTR. Thus, in cftr−/− PCT cells, the lack of RVD is mainly due to the absence of regulation of the swelling-activated Cl⁻ currents.

MATERIALS AND METHODS

Animals

CFTR knockout mice were generated by the gene-targeting methodology described previously (28) at Centre de Développement des Techniques Avancées pour l’Expérimentation Animale (Orléans, France). This strain of mice was originally derived from ES129/Sv cells injected into C57BL/6 embryos. Mice were backcrossed with C57BL/6 mice for three generations and then intercrossed. They were allowed free access to food and water in a facility at 25 ± 1°C with a 12:12-h light-dark cycle. The 4- to 6-wk-old wild-type and cftr−/− mice belong to the TASK2 family, the expression of which is controlled by the light-dark cycle. The 4- to 6-wk-old wild-type and cftr−/− mice were killed by cervical dislocation, and the kidneys were removed. All experiments were performed in accordance with the guidelines of the French Agricultural Office and in compliance with the legislation governing animal studies.

Primary Cell Cultures

PCT, DCT, and CCT were microdissected under sterile conditions. Kidneys were perfused with Hanks’ solution (GIBCO) containing 700 kU/l collagenase (Worthington), cut into small pyramids that were incubated for 1 h at room temperature in the perfusion buffer (160 kU/l collagenase, 1% Nuserum, and 1 mM CaCl₂), and continuously aerated. The pyramids were then rinsed thoroughly in the same buffer devoid of collagenase. The individual nephrons were dissected by hand in this buffer under binoculars using stainless steel needles mounted on Pasteur pipettes. The criteria used to identify the nephron segments have been described previously (6). Briefly, PCT corresponded to the 1- to 1.5-mm segment of tissue located immediately after the glomerulus. The DCT portion was the segment between the macula densa and the first branching with another tubule [connecting tubule (CNT)]. The CNT segment was discarded. CCT was identified as the straight poorly branched portion resulting from voltage stimuli filtered at 1 kHz, sampled at 2.5 kHz, and stored directly on the computer hard disk. Cells were held at −50 mV, and 400-ms pulses from −100 to +120 mV were applied in 20-mV increments every 2 s.

Expression in Cultured Cells

The cDNA encoding CFTR was introduced into a polycistronic expression vector derived from the pRESneo plasmid (cytomegalovirus promoter; Clontech), in which the neomycin resistance gene had been replaced by cDNA encoding the chain of the human CD8 cell surface antigen. Distal cells were transfected using the DAC-30 method according to the manufacturer's instructions (Eurogentec, Herstal, Belgium). The 6-day-old cultured cells grown on a 35-mm-diameter petri dish were serum starved for 24 h before transfection. Transfected cells with 2 μg of CD8-CFTR cDNA was coexpressed PCT and CD8 at their plasma membrane and can be visualized using anti-CD8 antibody-coated beads (Dynabeads M-450, Dynal, Oslo, Norway) (13a). Cells were electrophysiologically tested 48 h after transfection.

Chemicals

5-Nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB; Calbiochem) was prepared at 100 mM in DMSO and used at 0.1 mM in final solutions. Forskolin and ionomycin were prepared at 10 and 2 mM, respectively, in ethanol and used at 10 and 2 μM, respectively, in bath medium. Tetraethylammonium (TEA) chloride, charybdotoxin (CTX), quinidine, 6-N,N-diethyl-β-γ-dibromomethylene-D-adenosine-5’-triphosphate trisodium (ARL-67156), apamin, forskolin, and ionomycin were obtained from Sigma (Saint Quentin Fallavier, France). 293B was prepared at 10 mM in DMSO and used at 10 μM in final solutions. Cloflikium was prepared at 10 mM in 50% DMSO-50% water and used at a final concentration of 10 μM. Cloflikium and 293B were gifts from Dr. Barhanin (UMR CNRS 6097).

Axon Instruments) was used to generate whole cell current-voltage (I-V) relationships, with the membrane currents resulting from voltage stimuli filtered at 1 kHz, sampled at 2.5 kHz, and stored directly on the computer hard disk. Cells were held at −50 mV, and 400-ms pulses from −100 to +120 mV were applied in 20-mV increments every 2 s.

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RESULTS

K⁺ Currents Activated by Forskolin

Experiments were performed in a hyperosmotic extracellular solution (350 mosmol/kgH₂O) to characterize K⁺ currents activated by 10 μM forskolin in PCT, DCT, and CCT cells. Under these conditions, volume-activated K⁺ currents could not be detected. Moreover, to eliminate the Cl⁻ currents, experiments were performed in the presence of 0.1 mM NPPB in the bath solution. In control cells not treated with forskolin, the voltage-step protocol elicited small currents that changed linearly with membrane potential in PCT, DCT, and CCT cells from kidneys of cftr+/− and cftr−/− mice (data not shown). In cftr+/+ mice, incubation of DCT and CCT cells with 10 μM forskolin for 10 min before the whole cell measurement induced a strong increase in membrane current amplitude (Fig. 1A). In DCT cells, the activated currents were linear over the duration of the onset pulse. They were outwardly rectifying, with a reversal potential (E_{rev}) of \(-81 ± 0.4 \text{ mV}\) and slope conductances of \(1.7 ± 0.2 \text{nS}\) at \(-60 \text{ mV}\) and \(15.8 ± 2 \text{nS}\) at \(100 \text{ mV}\) (n = 19 cells from 5 different mice). In CCT cells, the currents shared identical characteristics with DCT cells. E_{rev} was \(-80 ± 1.5 \text{ mV}\), and conductances were \(1.4 ± 0.4 \text{nS}\) at \(-60 \text{ mV}\) and \(12.2 ± 1.2 \text{nS}\) at \(100 \text{ mV}\) (n = 14 cells from 5 different mice). In contrast to DCT and CCT cells, PCT cells did not present significant forskolin-activated K⁺ currents. In these cells, the currents reversed at \(-42.8 ± 2.2 \text{ mV}\) with a slope conductance of \(2.6 ± 0.3 \text{nS}\) at \(100 \text{ mV}\) (n = 19 cells from 5 different mice). In cftr−/− mice, incubation with forskolin did not increase K⁺ conductance in any of the cultured segments under study. Taken together, these observations clearly indicated that, in DCT and CCT cells from

Fig. 1. A: forskolin-induced whole cell K⁺ currents in proximal convoluted tubule (PCT), distal convoluted tubule (DCT), and cortical collecting tubule (CCT) cells in primary culture of cftr+/+ and cftr−/− mice. Membrane voltage was held at \(-50 \text{ mV}\) and stepped to test potential of \(-100 \text{ to } -120 \text{ mV}\) in 20-mV increments. Whole cell currents were recorded after 10 min of incubation with 10 μM forskolin. B: effects of 10 μM 293B, 1 mM tetraethylammonium (TEA), and 0.5 mM quinidine on forskolin-induced whole cell K⁺ currents measured at \(+100 \text{ mV}\). Values are means ± SE; n, number of monolayers from 5 different mice.
cftr+/+ mice, the K⁺ conductance stimulated by forskolin was associated with CFTR.

The forskolin-sensitive K⁺ currents measured at +100 mV are compared in PCT, DCT, and CCT cells from cftr+/+ and cftr−/− mice in Fig. 1B. Only DCT and CCT from wild-type mice exhibited forskolin-activated K⁺ conductance. In these cells, application of 10 mM 293B, 1 mM TEA, and 0.5 mM quinidine blocked this current by 80 ± 2% (n = 10), 55 ± 5% (n = 18), and 85 ± 4% (n = 9), respectively.

K⁺ Currents Induced by Hypotonic Shock

To prevent development of Cl⁻ conductance, the effect of hypotonic swelling was tested in the presence of 0.1 mM NPPB in the bath solution. Control currents were recorded using an isotonic (290 mosmol/kgH₂O) free Ca²⁺ (5 mM EGTA) solution in the pipette and a hypertonic (350 mosmol/kgH₂O) solution containing 1 mM Ca²⁺ in the bath. In PCT, DCT, and CCT cells, the voltage-step protocol elicited small time-independent currents that changed linearly with the membrane voltage. The currents at +100 mV were 119 ± 14 pA (n = 24 cells from 5 different mice), 126 ± 16 pA (n = 20 cells from 5 different mice), and 104 ± 20 pA (n = 19 cells from 5 different mice) in PCT, DCT, and CCT cells, respectively. Because of their small amplitude, the nature of these currents was not analyzed further.

To produce a hypotonic shock, the monolayers were perfused continuously with a 290 mosmol/kgH₂O solution. In 75% of cftr+/+ cells, an increase in whole cell current was observed in 3 min. In all epithelial cell types, the currents reached a maximum in 4–5 min. Currents recorded in PCT, DCT, and CCT cells from cftr+/+ and cftr−/− mice during hypotonic shock are...
shown in Fig. 2A. In cftr+/+ cells, currents recorded in each cultured segment showed virtually no inactivation during the 400-ms pulse, and the channels involved in this conductance were activated at depolarized potentials. The slope conductances measured at +100 mV were 12–16 times the amplitude of those calculated at −60 mV. $E_{\text{rev}}$ was near the equilibrium potential for $K^+$ in all segments: $-73 \pm 2$ mV ($n = 9$ cells from 5 mice), $-80 \pm 4$ mV ($n = 13$ cells from 5 mice), and $-72 \pm 5$ mV ($n = 9$ cells from 5 mice) in PCT, DCT, and CCT, respectively. Regardless of the cell type, the swelling-activated $K^+$ currents were strongly blocked when the cells were reexposed to hypertonic solution (Fig. 2B).

For the cftr−/− mice, the effect of hypotonic shock was different between PCT and DCT or CCT cells. Hypotonic shock always induced swelling-activated $K^+$ currents in PCT cells ($n = 9$), whereas it did not significantly modify the $K^+$ conductance in DCT ($n = 19$) and CCT ($n = 9$) cells (Fig. 2). Current intensity measured at +100 mV for all types of cells from cftr+/+ and cftr−/− mice is shown in Fig. 2B. The
inability of hypotonic shock to trigger swelling-activated K⁺ currents in DCT and CCT cells was observed in 100% of the 28 trials. Overall, the results indicate that CFTR protein could be implicated in the control of swelling-activated K⁺ conductances in terminal, but not in proximal, segments of the nephron.

The results reported on DCT and CCT cells show that swelling-activated K⁺ conductances were roughly similar in these two cell types. For this reason, no further distinction was made between DCT and CCT in the following experimental series.

**Pharmacology of Swelling-Activated K⁺ Channels**

To further characterize the swelling-activated K⁺ currents, we tested the effect of various K⁺ channel blockers added separately to the bathing hypotonic solution. The swelling-activated outward K⁺ current measured at +100 mV in PCT cells from *cftr⁺/⁺* mice is shown in Fig. 3. Perfusion of 1 mM TEA, 10 nM CTX, and 10 μM 293B did not significantly modify the swelling-activated K⁺ currents. In contrast, 0.5 mM quinidine and 10 μM clofilium decreased K⁺ current amplitude by 64 ± 6% (n = 6) and 55 ± 5% (n = 5), respectively (Fig. 3, A and B). Similar results were obtained in PCT from *cftr−/−* mice, indicating that the swelling-activated K⁺ conductance was not related to CFTR in this segment.

The effects of the same channel blockers in DCT and CCT cells from *cftr−/−* mice are illustrated in Fig. 4. The noticeable difference from the PCT cells was the strong reduction of swelling-activated K⁺ currents in the presence of 0.5 mM quinidine, 1 mM TEA, 10 nM CTX, 10 μM 293B, 10 nM apamin, and 10 nM CTX + apamin. Currents were measured 200 ms after onset of pulse at +100 mV. Values are means ± SE of 15 cells from 5 monolayers.
of PCT cells led us to carry out a further experiment to obtain more information concerning the nature of this channel.

**Regulation of the K⁺ Conductance Induced by Hypotonic Shock in cftr⁻/⁻ PCT Cells**

**Role of extracellular pH.** To study the modulation of the swelling-activated K⁺ currents by extracellular pH (pHe), PCT cells were swollen in hypotonic solutions (270 mosmol/kgH₂O) adjusted to pH 6.0, 7.4, and 8.0. Trace recordings at three different pH values are shown in Fig. 5A. Compared with the control K⁺ currents measured at pHe 7.4, K⁺ currents at pHe 6.0 were reduced by 53 ± 3% (n = 9), whereas currents at pHe 8.0 were increased by 44 ± 10% (n = 9). The corresponding I-V curves are reported in Fig. 5B. Change in pHe did not significantly modify $E_{\text{rev}}$: $-73 ± 8, -73 ± 2, \text{and } -74 ± 4 \text{ mV at pHe 6.0, 7.4, and 8.0, respectively (n = 9).}$ Inhibition of the K⁺ current at acidic pH became significant for membrane potential of $-20 \text{ mV}$ [K⁺ current = 358 ± 75 (n = 6) and 174 ± 37 pA (n = 10) at pHe 8.0 and 7.4, respectively (unpaired t-test = 2.19, $P < 0.047$)].

**Role of extracellular Ca²⁺.** To eliminate the possibility that cytosolic Ca²⁺ is involved in the development of hypotonicity-induced K⁺ currents, experiments were generally performed using pipette solutions containing 5 mM EGTA without additional Ca²⁺. The effects of extracellular Ca²⁺ on the development of hypotonicity-induced K⁺ currents were also tested in cftr⁻/⁻ PCT cells. When the hypotonic shock was carried out in the absence of bath Ca²⁺, development of the K⁺ current was not significantly modified (Fig. 6, A and B). The channel implicated in this response was clearly blocked by hypertonicity and 0.5 mM quinidine (Fig. 6, C–E). These results, which were also obtained using cftr⁻/⁻ PCT cells (data not shown), indicate that Ca²⁺ is not involved in control of the swelling-activated K⁺ conductance measured in PCT cells in primary culture.

![Fig. 5. Effect of extracellular pH on development of hypotonicity-induced K⁺ currents in cultured PCT cells from cftr⁻/⁻ mice. Membrane voltage was held at −50 mV and stepped to test potential of −100 to +120 mV in 20-mV increments. A: whole cell currents recorded after 4–5 min of extracellular perfusion of a 30% hypotonic solution at pH 6.0, 7.4, and 8.0 in the presence of 5 mM EGTA and 5 mM MgATP in pipette solution and 100 μM NPPB in extracellular solution. B: average current-voltage (I-V) relationships measured 200 ms after onset of pulse in the same cell at rest (A) during hypotonic stimulation at pH 6.0, 7.4, and 8.0. Values are means ± SE of 9 cells from 5 monolayers.](http://ajprenal.physiology.org/)

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Regulation of the $K^+$ Conductance Induced by Hypotonic Shock in $cfr^{+/+}$ and $cfr^{-/-}$ DCT Cells

Role of external $Ca^{2+}$. As reported above, the experiments were carried out in the presence of 5 mM EGTA in the pipette solutions. In DCT cells from $cfr^{+/+}$ mice, the absence of bath $Ca^{2+}$ completely prevented hypotonicity from inducing $K^+$ currents (Fig. 7, A and B). Conversely, perfusion of a solution containing 1 mM free $Ca^{2+}$ restored the response to hypotonicity (Fig. 7C). As expected, this swelling-activated $K^+$ conductance was blocked by hypertonicity and by CTX + apamin (10 nM; Fig. 7, D and E). As previously described for swelling-activated $Cl^-$ currents in DCT cells (1), it appears that an influx of $Ca^{2+}$ is required to permit the development of swelling-activated $K^+$ currents in $cfr^{+/+}$ DCT cells. It was therefore interesting to study the role of extracellular $Ca^{2+}$ in $cfr^{-/-}$ DCT cells. Whole cell currents were recorded in the presence of 20 mM EGTA in the pipette solution and 1 mM free $Ca^{2+}$ in the bath (Fig. 8). In the absence of ionomycin in the bath solution, the hypotonic shock remained inefficient for triggering $K^+$ currents in $cfr^{-/-}$ cells (Fig. 8A). However, when the hypotonic shock was performed in the presence of 2 $\mu$M ionomycin, $K^+$ currents were activated within 5 min (Fig. 8B). These currents were clearly due to $K^+$ movements, because they were blocked by CTX + apamin and TEA (Fig. 8, C and D). Moreover, analysis of the I-V curves (Fig. 8E) indicated that the instantaneous outwardly rectifying currents reversed at $-79.5 \pm 1.0$ mV ($n = 10$). Finally, when the...
cells were reexposed to the hyperosmotic solution, the currents returned toward control level within 2–3 min. Overall, the ionomycin-induced K⁺/H⁺ currents developed during hypotonicity in DCT cells from cftr⁻/⁻ mice were quite similar to the swelling-activated K⁺/H⁺ currents measured in cftr⁺/+ mice.

Role of extracellular adenosine. The results described above strongly suggest that swelling-activated K⁺/H⁺ currents in DCT cells are regulated by an identical mechanism involving a Ca²⁺ influx. Inasmuch as we previously demonstrated that this Ca²⁺ influx could be due to stimulation of A₁ receptors by extracellular adenosine, experiments were performed to determine the role of adenosine in the K⁺ permeability of DCT cells from cftr⁺/+ and cftr⁻/⁻ mice. Adenosine (10 μM) activated an outwardly rectifying K⁺ conductance with $E_{rev}$ of $-82.1 \pm 4.1$ (n = 17 cells) and $-78.1 \pm 2.0$ mV (n = 18) for cftr⁺/+ and cftr⁻/⁻ DCT cells, respectively (Fig. 9, A and B). In the presence of adenosine, the maximal slope conductances reached $18.0 \pm 1.4$ (n = 17) and $18.7 \pm 1.0$ nS (n = 18) in cftr⁺/+ and cftr⁻/⁻ DCT cells, respectively. These adenosine-sensitive K⁺ currents were decreased in the presence of CTX (10 nM) by $84 \pm 3$% (n = 9) in both types of DCT monolayers. To further study the influence of external Ca²⁺ on adenosine-sensitive K⁺ currents, experiments were performed in the absence of bath Ca²⁺. As expected, removal of external Ca²⁺ completely prevented adenosine from inducing K⁺ currents (Fig. 10, A and B). Conversely, perfusion of a solution containing 1 mM free Ca²⁺ restored the response to adenosine (Fig. 10C).

Finally, adenosine mimicked the effect of hypotonic shock, and it remains to be understood how this nucleotide is produced during the hypotonic cell...
swelling. Experiments were therefore carried out to determine whether adenosine was generated by ATP degradation. For this purpose, DCT cells from cftr−/− mice were subjected to a hypotonic shock in the presence of the selective ecto-ATPase inhibitor ARL-67156. ARL-67156 (100 μM) completely blocked the swelling-activated K⁺ currents (Fig. 11A). Adenosine (10 μM) restored a swelling-activated K⁺ conductance, which displayed an outwardly rectified I-V plot with $E_{\text{rev}}$ of $-75.0 \pm 1.1$ mV ($n = 12$; Fig. 11B and D) and was strongly inhibited by CTX + apamin (Fig. 11C).

**K⁺ Currents Activated by an Osmotic Shock in Cultured DCT Cells From cftr−/− Mice Transfected With CFTR cDNA**

The cftr−/− DCT cells in primary culture were transfected with a plasmid pIres-CD8-cftr, allowing visualization of transfected cells using anti-CD8 antibody-coated beads. Whole cell currents were recorded 48 h after the transfection procedure. Currents recorded in labeled cells immediately (control) or 4–5 min after perfusion of the hypotonic solution are shown in Fig. 12A. As expected, the developed currents re-
versed close to the equilibrium potential of $K^+$ ($-75 \pm 9$ mV, $n = 8$) and were blocked by CTX + apamin and hypertonicity ($n = 3$; Fig. 12, A and C). In contrast, in the same culture, the nonlabeled cells did not respond to hypotonic shock. These results clearly demonstrate that the transitory transfection of cDNA encoding CFTR restored the ability of the cells to increase their $K^+$ conductance after a hypotonic shock.

**DISCUSSION**

In the majority of mammalian cells, an increase in cell volume activates $Cl^-$ currents. In renal tissue, we previously showed the presence of swelling-activated $Cl^-$ currents responsible for the RVD after a hypotonic shock (26, 27). Such currents were also found in primary cultures of PCT, DCT, and CCT from wild-type mice (1). Moreover, the $cfr$ knockout strongly decreased these currents in PCT, DCT, and cortical collecting duct cells (CCD). In this study, we were interested in the consequences of the $cfr$ knockout on the $K^+$ currents activated by cAMP and on swelling-activated $K^+$ currents activated by a hypotonic shock.

In the present work, we showed the presence of cAMP-activated $K^+$ currents in DCT and CCD cells from wild-type mice only. $K^+$ channel activity was observed in >90% of the trials only when the monolayers were treated with forskolin for 10 min before the seal formation. This could indicate that cAMP induced incorporation of new channels in the membrane of DCT or CCT cells. These $K^+$ currents were completely inhibited by the chromanol analog 293B. Because this antiarrhythmic is known to block mainly the KCNQ1

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**Fig. 9.** Effect of adenosine on development of $K^+$ currents in cultured DCT cells from $cfr+/+$ (A) and $cfr-/-$ (B) mice. Membrane voltage was held at $-50$ mV and stepped to test potential of $-100$ to $+120$ mV in $20$-mV increments. Whole cell currents were recorded after $3$ min of extracellular perfusion of $10$ mM adenosine in the presence of $5$ mM EGTA and $5$ mM MgATP in pipette solution and $1$ mM CaCl$_2$. CTX + apamin ($10$ nM) was perfused after development of $K^+$ currents. C: currents measured $200$ ms after onset of pulse at $+100$ mV in the same cell at rest. Values are means ± SE of $17$ cells from $6$ monolayers.
channels (15, 34), it is probable that the cAMP-sensitive K⁺ currents in mouse distal cells belong to the KCNQ1 family of channels. Activation of KCNQ1 channels by the cAMP pathway has been reported in epithelial cells from many tissues, including inner ear, colon, small intestine, and airways (1a, 8, 33). In the kidney, although the presence of KCNQ1 transcripts has been demonstrated in the CCD of the outer medulla and the DCT and CCT of the cortex (8), the role of the protein remains unclear. In our work, the putative presence of KCNQ1 in DCT and CCT cells raised the problem of its coexpression with a KCNE protein. In epithelia, KCNQ1 associates with the small regulatory β-subunit KCNE1 or KCNE3 to form a K⁺ channel that could be implicated in the cAMP-stimulated Cl⁻ secretion (1a). Using RT-PCR and Southern blotting experiments, we previously identified transcripts encoding the KCNQ1 and KCNE1 sequence in cultured PCT cells from wild-type mice (unpublished observations). In contrast, DCT cells also expressed KCNQ1, but not KCNE1, excluding a role for this subunit in the cAMP-sensitive K⁺ channel in this part of the nephron. Whether KCNQ1 expression in DCT and CCT cells required an additional subunit such as KCNE3 remains to be elucidated.

In previous studies (26), we clearly established that CFTR functions as a cAMP-activated Cl⁻ channel in the apical membrane of DCT cells. Although from these results the localization of the cAMP-sensitive K⁺ secretion cannot be specified, this channel might participate in the driving force of apical Cl⁻ secretion (15, 17). Our results show that forskolin did not activate K⁺ current in DCT and CCT cells from cftr+/−/− mice. Although the role of CFTR in the control of K⁺ channels is well established in various tissues (2, 16, 20), the mechanism of the interaction between CFTR and K⁺ channel protein is far from being completely understood. This mechanism probably depends on the nature of the K⁺ channel, because, in addition to KCNQ1, other types of channels, such as the ROMK family, could interact directly with CFTR (2, 11, 20). The data reported in the literature for the relation between KCNQ1 and CFTR are conflicting. Some studies lead to the conclusion that KCNQ1 currents were activated by the cAMP pathway, independent of the presence of CFTR (2, 18, 34); other studies demonstrate that CFTR directly activates KCNQ1 conductances (7, 17) and that this conductance was not detected in the epithelial cell line CFPAC expressing ΔF508-CFTR (16). This last finding corroborates our own observations and suggests that, in the distal tubule, KCNQ1 K⁺ currents are dependent on CFTR expression.

As expected, a hypotonic shock activated K⁺ currents in PCT, DCT, and CCD cells from wild-type mice. In the monolayers from cftr−/−/− mice, development of these K⁺ currents was completely impaired in DCT.
and CCT cells but was not modified in PCT cells. This suggests two different types of swelling-activated K⁺ currents in PCT and DCT cells. The pharmacological study performed on these channels confirmed this hypothesis. In PCT cells, the swelling-activated K⁺ conductance shared some properties with the K⁺ currents activated by cell swelling that have been reported in gallbladder epithelium (31) and in Ehrlich ascites tumor cells (12, 22). It was insensitive to TEA but was strongly blocked by quinidine and clofilium. Moreover, this K⁺ conductance was not affected by intracellular Ca²⁺ concentration but was sensitive to external pH variations, with activation at alkaline pH and inhibition at acidic pH. Finally, this pharmacological profile is consistent with TASK2 channels. Further experiments are required to definitively characterize the nature of the swelling-activated K⁺ channels in PCT. However, it is now well established that TASK2 is strongly expressed in the kidney (23) and, more precisely, in mouse proximal tubule, in situ or in primary culture (29).

In DCT and CCT cells, the swelling-activated K⁺ conductances exhibited a different pharmacology: they were inhibited by TEA and CTX and were dependent on extracellular Ca²⁺. The inhibition by CTX indicates that Ca²⁺-activated BK and intermediate K⁺ (IK) channels. Furthermore, a minor role of SK channels cannot be excluded, because this toxin inhibited the swelling-activated K⁺ currents by 27% and has been shown to be additive to the CTX effect.

These K⁺ conductances were activated by hypotonicity in the presence of a high concentration of EGTA in the pipette solution. The simplest interpretation of this finding could be that an increase in intracellular Ca²⁺ is not necessarily required to activate K⁺ currents and that Ca²⁺ influx is sufficient to increase the K⁺ conductance during hypotonicity (25, 27). This conclusion is supported by the recent data of Grunnet et al. (9), who reported that stimulation of BK and SK channels during swelling was not mediated by a change in intracellular Ca²⁺.

Although they belong to a distinct channel family, the K⁺ channels activated by hypotonicity in PCT and DCT cells of wild-type mice participate in RVD, together with swelling-activated Cl⁻ conductances (27). In both monolayers, the swelling-activated K⁺ currents were insensitive to the chromanol derivative 293B, indicating that KCNQ1 is not implicated in this conductance. This conclusion is at variance with that reached by Lock and Valverde (14), who proposed that the KCNQ1-KCNE1 complex was implicated in the maintenance of K⁺ secretion linked to RVD in murine airway cells. As we previously demonstrated (1), null mutation of CFTR strongly impaired the RVD in PCT and DCT cells. Obviously, in PCT cells this inhibition was due to a defect in

**Fig. 11.** Effects of ecto-ATPase antagonist (ARL-67156) on swelling-activated K⁺ currents in DCT cells from cfr⁻/⁻ mice. Membrane voltage was held at -50 mV and stepped to test potential of -100 to +120 mV in 20-mV increments. Whole cell currents were recorded after 4–5 min of extracellular perfusion of a 30% hypotonic solution in the presence of 5 mM EGTA and 5 mM MgATP in pipette and 100 μM ARL-67156 in extracellular bath (A), 10 μM adenosine (B), and 10 nM CTX + apamin (C). D: average I-V relationships measured 200 ms after onset of pulse in the same cell at rest. Values are means ± SE of 14 cells from 7 monolayers.
swelling-activated Cl\(^-\) conductances, because the accompanying activated K\(^+\) channels remained functional in cftr\(^{-/-}\) monolayers. In contrast, in DCT cells, swelling-activated Cl\(^-\) and K\(^+\) conductances were affected by the knockout of CFTR. At this step in our work, the question arose as to what extent the Cl\(^-\) and K\(^+\) channels in DCT cells were modulated by a common mechanism that was altered in the absence of CFTR. As demonstrated for swelling-activated Cl\(^-\) conductance, ionomycin in the presence of a high concentration of EGTA inside the cells restored the swelling-activated K\(^+\) currents in the DCT and CCT cells from cftr\(^{-/-}\) mice, confirming the role of Ca\(^{2+}\) entry in these cells. Moreover, adenosine mimicked the effect of ionomycin in the presence of external Ca\(^{2+}\) only. Therefore, in the case of the Cl\(^-\) channels, this Ca\(^{2+}\) influx might be due to stimulation of A\(_1\) receptors by adenosine generated by the degradation of ATP by membrane ectoenzymes. According to Braunstein et al. (3), lack of CFTR leads to impairment of ATP release during hypotonic shock (5, 10, 24). The final result is loss of the RVD process, because the first element (ATP release) of the cascade leading to activation of Cl\(^-\) and K\(^+\) channels is missing.

The results of these experiments are summarized in Fig. 13. In PCT and DCT cells, RVD after hypotonic challenge is dependent on CFTR. However, in PCT cells, only the swelling-activated Cl\(^-\) conductance is affected by this regulation. In DCT cells, swelling-activated Cl\(^-\) and K\(^+\) currents are regulated by CFTR during hypotonic shock. Although swelling-activated Cl\(^-\) currents share the same properties in PCT and DCT cells, this is not the case for
swelling-activated K⁺ currents: in PCT cells the K⁺ current could be due to TASK2 channels, whereas in DCT cells the K⁺ current could flow through Ca²⁺-dependent BK and SK channels. With regard to the cAMP-sensitive conductances, the data strengthen the hypothesis that CFTR mediates forskolin-activated Cl⁻ and K⁺ currents in DCT cells. The K⁺ conductance could be formed by KCNQ1-like channels. In contrast, forskolin-activated Cl⁻ and K⁺ currents are not detectable in PCT cells. Therefore, it appears that, in renal epithelium, CFTR not only functions as a cAMP-activated Cl⁻ channel but also participates in regulation of cell volume. These two functions are probably independent, because PCT exhibits only the latter, whereas DCT exhibits both functions. This suggests that the form of CFTR expressed in PCT is different from that in DCT. We have no evidence of such differences. However, it may be recalled that an alternative splice form of CFTR (TNR-CFTR) was found in inner medullary collecting duct, indicating a possible tissue-specific processing of CFTR (13).

The question arises of whether the inhibition of swelling-activated K⁺ and Cl⁻ currents in cftr⁻/⁻ has physiological consequences. The cftr⁻/⁻ mice exhibit drastic growth and intestinal dysfunctions and generally die 6 wk after birth. To optimize their lifetime, the animals are fed a special liquid diet. Despite these precautions, the animals remain very weak, and their renal function has not been investigated. Therefore, kidney function could be impaired, but the very poor physiological state of the animals could mask this
specific alteration. There is no apparent difference in growth between cell cultures from cftr⁻/⁻ and control cells from cftr⁺/+ mice, at least when they are kept in constant control conditions, such as an incubator. However, preliminary experiments (data not shown) indicate that a hypotonic shock stopped division of the cftr⁻/⁻, but not the cftr⁺/+, cells.

The situation is quite different in humans, because the dysfunction of CFTR was mainly due to ∆F508 mutation. However, according to Braunstein et al. (3), it is possible that the ∆F508 mutation resulted in an alteration of RVD. Thus we can suppose that the human kidney from CF patients will also exhibit altered RVD. The consequences of such a modification remain to be analyzed in terms of a renal role of CFTR.

REFERENCES


