Cytosolic potassium controls CFTR deactivation in human sweat duct

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Reddy, M. M., and P. M. Quinton. Cytosolic potassium controls CFTR deactivation in human sweat duct. Am J Physiol Cell Physiol 291: C122–C129, 2006. First published February 15, 2006; doi:10.1152/ajpcell.00134.2005.—Absorptive epithelial cells must admit large quantities of salt (NaCl) during the transport process. How these cells avoid swelling to protect functional integrity in the face of massive salt influx is a fundamental, unresolved problem. A special preparation of the human sweat duct provides critical insights into this crucial issue. We now show that negative feedback control of apical CFTR deactivation in human sweat duct, which provides a unique opportunity to manipulate the intracellular environment, including nucleotides and electrolytes such as NaCl—ion channels and pumps so that the salt influx at the apical membrane is compatible with the pump capacity. Several physiological strategies have been reported to be involved in this process, such as 1) metabolic coupling in which availability of ATP is linked to cystic fibrosis transmembrane conductance regulator (CFTR; see Refs. 1 and 20) and epithelial Na+ channel (ENaC) activity in the apical membrane (15) and K+ recycling in the basolateral membrane (36), 2) cross talk between Na+ channels in the apical membrane and K+ channels in the basolateral membrane (9, 12), and 3) permissive control of Ca2+-dependent activation of multiple Na+ transport pathways by intracellular Cl− (29). Recent evidence also suggests that cytosolic changes in Na+ and Cl− concentrations could control apical salt transport by regulating ENaC channels (8, 10). These observations suggest that this process of coordination of salt influx and efflux activity is complex and poorly understood.

Human reabsorptive sweat duct actively absorbs NaCl from lumen to blood from the isotonic sweat secreted by the secretory coil via CFTR and ENaC channels. Using this simple model system and based on the fact that most biological processes are regulated by negative feedback mechanisms, we sought to test the hypothesis that the transported electrolytes themselves are involved in a feedback regulation of salt influx (20). We note that, since salt is passively absorbed as electrical equivalents of cations and anions, salt uptake can be controlled by limiting either the apical membrane cation or the anion conductance during transport. Because CFTR is a major epithelial ion channel in the apical membrane of the highly active salt absorptive duct of the human sweat gland, we considered that this channel might be a strategic point to acutely regulate passive salt influx. We addressed this possibility with the basolaterally α-toxin-permeabilized native human sweat duct, which provides a unique opportunity to manipulate the intracellular environment, including nucleotides and electrolytes such as Na+, K+, and Cl− while assaying CFTR activity in situ in the apical membrane (22, 23, 26, 39). Because preliminary results indicated little effect of cytoplasmic Cl− on CFTR, we focused on the role of Na+ and K+ on CFTR activity.

METHODS

Sweat ducts were obtained from skin biopsies of young adult male volunteer subjects giving informed consent. Sweat ducts were dissected and microperfused as described previously. Segments of ducts ~1 mm long were microperfused with a double-barreled luminal micropipette that perfused and recorded transepithelial voltage through one barrel and passed constant current pulses through the other. The specific membrane conductance was calculated from the cable equation from the measured values of transepithelial voltage deflections in response to 50–100 nA transepithelial constant current pulses (500 ms), luminal diameter, and the specific conductance of the luminal perfusion solutions, as previously described (20, 22). Because the magnitude of transepithelial voltage deflections induced by constant current pulses is a function of lumen diameter and plasma membrane conductance [mainly CFTR Cl− conductance (CFTR-gCl)] in this case, small changes in lumen diameter will have significant impact on the conductance data. Hence, the lumen diameter was constantly monitored throughout the experiment so that the calculated values of conductance reflect occasional changes in lumen diameter. After confirming the integrity of the perfused tubule, we applied...
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RESULTS

Salt transport lowers intracellular K\(^+\) activity. [K\(^+\)]\(_i\) was measured in intact nonpermeabilized microperfused sweat ducts under nontransporting (blocked with amiloride) and actively transporting conditions (lumen and contraluminal bath contained 150 mM NaCl). [K\(^+\)]\(_i\), in nontransporting epithelial cells was 128 ± 3.0 mM. In contrast, [K\(^+\)]\(_i\) decreased to 76 ± 4.0 mM in the actively transporting epithelial cells (no amiloride; Fig. 1).

[K\(^+\)]\(_i\) specifically affected CFTR-gCl. Figure 2 shows that adding exogenous cAMP and ATP to the cytoplasmic bath in the presence of 140 mM K\(^+\) activated apical CFTR-gCl in permeabilized ducts. However, substituting K\(^+\) with equimolar Na\(^+\) deactivated CFTR in a dose-dependent manner and inhibited CFTR-gCl with half-maximal inhibition apparently at ~100 mM K\(^+\). This inhibition was completely reversible upon restoring [K\(^+\)]\(_i\) to 150 mM. To determine whether CFTR-gCl deactivation was caused by removal of K\(^+\) or addition of Na\(^+\), we substituted K\(^+\) with Li\(^+\), N-methyl-D-glucamine (NMDG\(^+\)), or Rb\(^+\). As shown in Fig. 3, cAMP- and ATP-activated CFTR-gCl was also deactivated by K\(^+\) substitution with either Li\(^+\) or NMDG\(^+\). However, like K\(^+\), Rb\(^+\) fully supported cAMP- and ATP-activated CFTR-gCl (Fig. 3, E and F).

[K\(^+\)] depletion has no effect on CFTR after irreversible phosphorylation. We examined whether K\(^+\) depletion interferes with the CFTR phosphorylation process. We irreversibly phosphorylated CFTR either by using ATP\(_7\)S as substrate (25) or by inhibiting the endogenous phosphatase activity by raising cytosolic pH (to 8.5) to prevent dephosphorylation of previously phosphorylated CFTR (we have previously shown that exposing the apical membrane preparations of the duct to cytosolic alkaline pH of 8.5 for ~10 min causes almost permanent deactivation of endogenous phosphatases responsible for deactivating CFTR so that the channel remains irreversibly phosphorylated; see Ref. 21). After irreversible phosphorylation, it was possible to activate CFTR-gCl by adding ATP alone without cAMP (24, 25). We then tested the effect of K\(^+\) depletion on irreversibly phosphorylated, ATP-activated CFTR-gCl. Under these conditions, K\(^+\) depletion had no effect on CFTR-gCl (Fig. 4A).

Okadaic acid prevents deactivation of CFTR. We tested whether [K\(^+\)]\(_i\) depletion activates an okadaic acid-sensitive endogenous protein phosphatase (PP) that dephosphorylates CFTR (3, 24). After addition of okadaic acid (10\(^{-10}\) to 10\(^{-8}\) M) to the cytosolic medium, cAMP + ATP normally activated CFTR-gCl even after complete substitution of cytosolic K\(^+\) with Na\(^+\) (Fig. 4, B and C).

Ca\(^{2+}\)-sensitive PP2B does not seem to dephosphorylate CFTR. It is known that Ca\(^{2+}\) is required for PP2B activity (33). Exploiting this property, we have investigated whether removing Ca\(^{2+}\) from the perfusate (5 mM EGTA was added to nominally Ca\(^{2+}\)-free perfusion solution) prevents dephosphorylation deactivation of cAMP-activated CFTR-gCl after K\(^+\) substitution with Na\(^+\). Figure 5 shows that CFTR-gCl was still normally deactivated after K\(^+\) substitution with Na\(^+\), even after removing Ca\(^{2+}\).

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1,000–5,000 U/ml of α-toxin from Staphylococcus aureus to the bath solution to selectively permeabilize the basolateral membrane (27). This procedure leaves the apical membrane intact but nonselectively permeabilizes the basolateral membrane, to molecules of up to ~5,000 molecular weight units. The integrity of all tissues was first confirmed by demonstrating the appearance of a large increase in CFTR-gCl after the addition of cAMP and ATP as shown in Fig. 2 before experimental manipulations were performed.

The luminal perfusion Ringer solutions contained (in mM) 150 NaCl, 5 K\(^+\), 3.5 PO\(_4\)-, 1.2 MgSO\(_4\), and 1 Ca\(^{2+}\), pH 7.4. Cl\(^-\)-free luminal Ringer solution was prepared by substitution of Cl\(^-\) with the impermeant anion gluconate. The cytoplasmic/bath solution contained (in mM) 145 K\(^+\), 140 gluconate, 3.5 PO\(_4\)-, 1.2 MgSO\(_4\), and 260 Ca\(^{2+}\) buffered with 2.0 mM EGTA (Sigma) to 80 mM free Ca\(^{2+}\), pH 6.8. ATP (5 mM K\(^+\) or Na\(^+\) salt), 5 mM adenosine 5’-O-(2-thiotriphosphate) (ATP\(_7\)S), 0.1 mM cAMP, 0.01 mM amiloride, and 10\(^{-10}\) to 10\(^{-6}\) M okadaic acid were added to the cytoplasmic bath as needed. Cytosolic K\(^+\) activity and the basolateral membrane potential were measured simultaneously using very sharp (tip diameter <0.1 μM) double-barreled K\(^-\)-sensitive Corning liquid ion exchanger-filled microelectrodes, as previously described (26). Cytosolic K\(^+\) concentration ([K\(^+\)]\(_i\)) was measured in an intact (nonpermeabilized) sweat duct microperfused with 150 mM NaCl Ringer solution in the lumen and contraluminal bath. Luminal amiloride was used to block apical Na\(^+\) transport in experiments designed to determine the effect of apical Na\(^+\) transport on [K\(^+\)]\(_i\). Data are presented both as representative examples of electrical traces and as means ± SE. Statistical significance was determined with Student’s t-test. A P value of <0.05 was taken to be significantly different.
**DISCUSSION**

Intracellular $[K^+]$ is a dynamic function of changing apical salt transport. Close coordination between the transport activities at the apical and basolateral membranes of epithelial cells is well known (9, 10, 12, 15, 36, 38). Even though such coordination is essential to maintaining the functional integrity of the cell in the face of changing rates of transepithelial salt transport, very little is known about the molecular mechanisms involved in this vital process. For example, it is known that an increase in apical salt transport activates basolateral K$^+$ conductance (gK; see Refs. 9, 12, 26, 36). However, the physiological significance of such an increase in gK during transepithelial salt transport is not fully understood. It has been the prevalent notion that the primary role of gK in epithelial transport is to provide a leakage pathway to balance the K$^+$ pumped into the cell by the Na$^+$-K$^+$ pump and to increase electrical driving force for transepithelial salt transport (26, 38). However, despite the fact that parallel activation of amiloride-sensitive ENaC and gK is expected to induce significant changes in the cytosolic monovalent cation composition that are well known to play a significant role in numerous physiological functions (4, 5, 35, 40), little is known about the potential role of such changes in regulating overall transepithelial transport activity.

Figure 1 shows an example of the rapid, reciprocal changes in cytoplasmic Na$^+$ and K$^+$ concentrations during salt absorption that follows activation of apical CFTR and ENaC in the native human sweat duct. Na$^+$ entering the cell through apical ENaC is apparently associated with increased basolateral gK, K$^+$ efflux, and lower $[K^+]_c$ during salt transport (Fig. 1). Blocking apical Na$^+$ entry and transport with amiloride revealed a rapid increase in $[K^+]_c$. These results demonstrate that, in the complete absence of neurohumoral influences, the transport activity at the apical membrane significantly alters $[K^+]_c$. Some feedback system seems essential to sustain balance between the limited pump capacity in the basolateral membrane to extrude Na$^+$ and the potentially massive salt diffusion in the cell across the apical membrane. Otherwise, cell swelling caused by excessive salt influx would inevitably compromise the integrity of the cell. Because monovalent cations play critical roles in numerous physiological functions and because transport activity at the basolateral membrane alters the cytosolic [Na$^+$] ([Na$^+$]$_c$)-to-[K$^+$]$_c$ ratio (4, 5, 35, 40), we sought to determine whether the increase in intracellular [Na$^+$]$_c$ or the decrease in [K$^+$]$_c$ mediates the change in CFTR activity in the apical membrane.

[Na$^+$]$_c$-to-[K$^+$]$_c$ ratios determine apical CFTR-gCl activity. We examined the effect of changing the [Na$^+$]$_c$-to-[K$^+$]$_c$ ratio on cAMP- and ATP-activated CFTR-gCl in α-toxin-permeabilized ducts (Fig. 2A). As shown in Fig. 2B, changes in [Na$^+$]$_c$, and [K$^+$]$_c$, similar to those observed during absorptive salt transport activity (Fig. 1) resulted in inhibition of CFTR-gCl that paralleled increases in the cytosolic Na$^+$-to-K$^+$ ratio. These results show that the cytosolic monovalent cationic environment should significantly influence the rate of salt entry by controlling the apical CFTR-gCl.

Reduced $[K^+]_c$, deactivates CFTR-gCl. The fact that increasing [Na$^+$]$_c$-to-[K$^+$]$_c$ ratios inhibited CFTR-gCl suggested the increase in [Na$^+$]$_c$, or the decrease in [K$^+$]$_c$, might mediate inhibition. Therefore, we substituted cytosolic K$^+$ with glu-
concentrations of Li⁺, NMDG⁺, or Rb⁺. As shown in Fig. 3, deactivation of CFTR-gCl previously activated with cAMP + ATP was inhibited when Li⁺ or NMDG⁺ replaced K⁺. Given the differences in size of Na⁺, Li⁺, and NMDG⁺, it seems unlikely that these cations are all inhibitory. In marked contrast, Rb⁺, well known to effectively substitute for K⁺ in many biological functions, like K⁺, did not inhibit CFTR-gCl. These results indicate that it is K⁺ depletion, and not Na⁺ accumulation, that mediates CFTR-gCl deactivation. Thus an apparent Kᵢ for CFTR inhibition appears to be in the range of ∼100 mM K⁺ (50 mM Na⁺ substitution; Fig. 2) and seems consistent with observed physiological values (Fig. 1).

K⁺ loss dephosphorylates CFTR. Because CFTR Cl⁻ channel activation requires phosphorylation (3, 34), [K⁺]ₑ depletion might conceivably directly affect CFTR protein function or indirectly alter its function by changing its phosphorylation state. We depleted [K⁺]ₑ after irreversibly thionophosphorylating CFTR with phosphatase-resistant ATPyS as the substrate for protein kinase A (PKA), as previously described (25). Figure 4A shows that completely substituting [K⁺]ₑ with Na⁺ had no effect on ATPyS phosphorylated CFTR, indicating that [K⁺]ₑ depletion probably does not affect CFTR, per se.

[K⁺]ₑ mediates phosphatase, not kinase, activity. The phosphorylation state of CFTR is the net result of kinase phosphorylation and phosphatase dephosphorylation rates (3, 11, 34). Thus we asked which of these two processes was affected by [K⁺]ₑ. After pretreating the ducts with okadaic acid, CFTR-gCl was activated by addition of cAMP + ATP in the cytoplasmic bath in the complete absence of K⁺ (Fig. 4, B and C). We also raised the cytosolic pH from 6.8 to 8.5 to irreversibly deactivate the endogenous phosphatase activity, as previously described (21). With either treatment, okadaic acid (Fig. 4, B and C) or high pH (results not shown) inhibition of endogenous phosphatase activity, cAMP plus ATP still fully activated CFTR-gCl in the complete absence of [K⁺]. These results indicate that, when endogenous phosphatase is inhibited with okadaic acid or high pH, [K⁺]ₑ has no effect on PKA phosphorylation, that is, neither PKA nor CFTR are affected by [K⁺]. Thus inactivation is most likely the result of an increase in phosphatase activity as [K⁺]ₑ decreases.

A simple interpretation of these results is that normal (non-transporting) [K⁺]ₑ tonically inhibits the endogenous phosphatase so that prior inhibition with okadaic acid or high pH prevents its activation when K⁺ concentration decreases and CFTR then remains phosphorylated and active (Fig. 4, B and C). This conclusion seems consistent with early observations that some protein phosphatases (PP2B and -C) are cation sensitive (6) and that K⁺ also regulates several other enzymes (14, 18, 35, 40). It is also intriguing that loss of cytosolic K⁺ is associated with activation of PP2A in apoptosis (37, 40).
We have considered the possibility that lowering $[K_+]_c$ might change CFTR conformation and simply prevent or slow dephosphorylation by preventing the phosphatase from interacting with CFTR. We reasoned that a $K^+$-dependent CFTR conformation change might well affect other regulatory properties of CFTR. We know that CFTR can be activated independent of phosphorylation by cytosolic glutamate (23). However, cytosolic glutamate activates CFTR-γCl in the complete absence of K$^+$ (results not shown). If neither kinase-dependent nor kinase-independent mechanisms of CFTR activation are affected by $[K_+]_c$, it seems less likely that a $K^+$-dependent conformational change in CFTR would explain the apparent inhibition of phosphatase activity seen here.

Which phosphatase? Most significantly, of known phosphatases, only PP2A ($K_0$ 0.2 nM) is blocked by the extremely low concentrations (as low as $10^{-10}$ M) of okadaic acid used here to block dephosphorylation of CFTR (3, 24, 32, 34). Preliminary phosphorylation studies in this laboratory also suggested that PP2A can dephosphorylate PKA-phosphorylated CFTR, which can be blocked by okadaic acid (results not shown). Furthermore, even though PP4 and PP5 can be inhibited by relatively low concentrations of okadaic acid (2 nM), these phosphatases are predominantly confined to the nucleus and are less likely to regulate ion channels in the plasma membrane (13). Okadaic acid also inhibits PP1 and PP2B, but at much higher concentrations. Moreover, PP1 probably does not dephosphorylate PKA-phosphorylated CFTR (34), and PP2B (calcineurin) requires Ca$^{2+}$ for its activity. Preliminary data showed that, in the complete absence of Ca$^{2+}$, deactivation of CFTR after K$^+$ depletion.

Fig. 4. Blocking dephosphorylation of CFTR prevents K$^+$ depletion-induced deactivation of CFTR-γCl. A: representative trace of an experiment showing the effect of cytoplasmic K$^+$ substitution by Na$^+$ before and after irreversibly phosphorylating CFTR with 5 mM adenosine 5′-O-(2-thiotriphosphate) (ATPγS; no physiological ATP was present during this time) as substrate to thiophosphorylate CFTR and render it resistant to dephosphorylation. Irreversible phosphorylation is indicated by the fact that CFTR can be activated by ATP alone in the absence of cAMP (A, right). Notice that K$^+$ deactivation deactivated CFTR before (left) but not after (right) irreversible phosphorylation. B: representative trace showing normal activation of CFTR-γCl after inhibiting endogenous phosphatase with okadaic acid, even in the absence of K$^+$ (150 mM Na$^+$) in the cytoplasmic bath. C: means, SEs, and significance of results collected from experiments similar to that shown in B. * $P < 0.001$, significantly different from phosphorylation-activated CFTR-γCl in either the presence of K$^+$ or in the presence of okadaic acid without K$^+$; n = 12.

Fig. 5. Role of Ca$^{2+}$-sensitive protein phosphatase (PP) 2B in dephosphorylating protein kinase A (PKA)-phosphorylated CFTR. This experiment was designed to test whether inhibiting PP2B by removing Ca$^{2+}$ can prevent cAMP- + ATP-activated CFTR after K$^+$ depletion. Notice that CFTR Cl$^-$ conductance is still inhibited by K$^+$ depletion in the complete absence of cytosolic Ca$^{2+}$, indicating that PP2B activation is not responsible for dephosphorylation deactivation of CFTR after K$^+$ depletion.
CFTR is dephosphorylated after K\(^+\) depletion so that PP2B cannot be the enzyme dephosphorylating CFTR in this tissue (Fig. 5). Based on these observations, one would suspect that PP2A itself could be the K\(^+\)-regulated phosphatase in this tissue. However, preliminary phosphatase assays with commercially available PP2A using \(p\)-nitrophenyl phosphate as the substrate indicated no direct affect of K\(^+\) on the enzyme activity (Dr. J. W. Hanrahan, personal communication). These surprising new results suggest either that the endogenous K\(^+\)-sensitive phosphatase may be distinctly different from PP2A or that the PP2A might be associated with some K\(^+\)-sensitive regulatory components in vivo. In fact, control of PP2A activity by protein inhibitor was previously shown (31). Further studies are required to identify the correct K\(^+\)-sensitive phosphatase involved in this CFTR regulation process.

**Implications.** It is often assumed that endogenous phosphatase activity is constitutive, serving merely to reverse the effects of kinases (2, 6, 7, 19, 32). However, the present results suggest that the activities of phosphatases are acutely regulated. These results are consistent with early observations indicating possible acute regulation of several phosphatases, including PP1, PP2A, and PP2B, by endogenous peptides, ATP, Ca\(^{2+}\), Mg\(^{2+}\), and other cations (31, 33). Furthermore, it is the relative activities of kinase phosphorylation and phosphatase dephosphorylation of functional proteins that ultimately determine the level of physiological responses (6, 32). Thus it seems reasonable to suggest that phosphatase activation rather than kinase inhibition is a principal component of ion transport regulation (and hence volume homeostasis) by epithelial cells. If the sweat duct can be taken as an example, we surmise that activation of PKA serves principally to stimulate transport, whereas activation of phosphatase serves to limit the rate of transport in meeting the demands of volume homeostasis. These results show for the first time that phosphatase dephosphorylation of CFTR is likely to be an important component of the physiological control of salt transport and that it is mediated by a heretofore unrecognized role of intracellular K\(^+\).

Because CFTR plays a significant role in both absorption and secretion, it seems important to understand whether similar [K\(^+\)]\(_e\)-dependent phosphatase regulation of CFTR dephosphorylation exists in other vital organs such as airways. The following observations suggest that this regulatory process may not be confined to this tissue alone. 1) It has been reported that raising basolateral K\(^+\) concentration, a maneuver known to increase [K\(^+\)]\(_e\) (26), activates a putative apical CFTR-gCl (35), 2) activation of gK with concomitant decline in [K\(^+\)]\(_e\) is a common physiological phenomenon associated with stimulation of transepithelial transport in absorptive and secretory epithelial cells that express CFTR (26, 38), and 3) okadaic acid-sensitive phosphatase was previously shown to dephosphorylate CFTR in absorptive and secretory epithelial cells (11, 24, 28, 34), suggesting that K\(^+\) control of CFTR activity may play a critical role in exocrine function in general.

Furthermore, CFTR and ENaC are two significant epithelial channels in the apical membranes of several epithelia, including airways, kidney, and sweat duct (30). Clear knowledge of functional interaction between these two principal ion channels is essential to better understand the epithelial transport in health and disease processes, such as cystic fibrosis, pseudohypoaldosteronism, and Liddle’s syndrome. Even though CFTR control of ENaC activity attracted much attention (30), the role...
of ENaC in regulating CFTR function is poorly understood. Studies on heterologous systems have indicated that coexpression of ENaC significantly enhanced CFTR activity in *Xenopus* oocytes (16). In contrast, the present studies in a native epithelial tissue indicated that increased activity of ENaC in fact causes a reciprocal decrease in CFTR activity (Fig. 2). These observations indicate that the in vivo functional interaction between these two channels could be much more complex and require further investigation.

In conclusion, this work provides the basis for a novel concept and mechanism of the homeostasis of cell volume during epithelial electrolyte transport when increased salt entry threatens cell volume with swelling. We surmise that the mechanism is comprised of a negative feedback system that controls salt entry in the cell of absorbing epithelia by limiting CFTR-gCl. We show that CFTR conductance in native cells of the human sweat duct is deactivated by an endogenous K+-sensitive phosphatase. Na+ enters the cell and replaces intracellular K+ during salt absorption. Lower K+ concentrations apparently relax tonic inhibition of phosphatase activity, which progressively deactivates CFTR by dephosphorylation. Deactivation of CFTR reduces Cl− conductance and limits salt entry until intracellular [K+] stabilizes. At this point, salt influx across the apical membrane matches the salt efflux driven by the Na+–K+ pump across the basolateral membrane so that cell volume is preserved (Fig. 6).

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