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A Regulatory Role of Polycystin-1 on Cystic Fibrosis Transmembrane Conductance Regulator Plasma Membrane Expression

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Key Words
Polycystic kidney disease • Cyst • CFTR • Secretion • Polycystin-1

Abstract
Autosomal dominant polycystic kidney disease (ADPKD) is caused by genetic mutations in either *PKD1* or *PKD2*, the genes that encode polycystin-1 (PC-1) and polycystin-2 (PC-2), respectively. ADPKD is characterized by the formation of multiple, progressive, fluid-filled renal cysts. To elucidate the mechanism of fluid secretion by ADPKD cysts, we examined the effect of PC-1 on the plasma membrane expression of cystic fibrosis transmembrane conductance regulator (CFTR), a key Cl^- secretory protein. Five stably transfected MDCK lines were used in this study: two transfected with empty vector (control cells) and three expressing human PC-1 (PC-1 cells). The cAMP-induced endogenous short circuit currents (I_{sc}) were smaller in PC-1 cells than in control cells. Compared to control cells, PC-1 cells transiently expressing pEGFP-CFTR showed significant reduction of whole cell cAMP-activated Cl^- currents. Cell surface biotinylation experiments also indicated a reduction in surface expression of CFTR in PC-1 cells compared to control. Furthermore, studies using CHO cells transiently expressing PC-1 and CFTR suggest the importance of the PC-1 COOH-terminus in the observed reduction of CFTR plasma membrane expression. No differences in either endogeneous K^+ currents or P2Y receptor responses were observed between PC-1 and control cells, indicating the specificity of PC-1's action. These results indicate that PC-1 selectively maintains low cell surface expression of CFTR. Moreover, these findings suggest that the malfunction of PC-1 enhances plasma membrane expression of CFTR, thus causing abnormal Cl^- secretion into the cyst lumen.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) presents with an incidence of 1 in every 1000 births and results in progressive renal cyst formation that distorts the renal parenchyma, leading to end-stage renal failure in 50 % of affected individuals [1, 2]. Cyst

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development is also observed in other epithelia, such as hepatic bile ducts, colon and pancreas [1]. Two genes associated with ADPKD, namely *PKD1* and *PKD2*, have been identified. Genetic studies demonstrated that ADPKD is genetically heterogeneous, with most families having mutations of either *PKD1* on chromosome 16 (85%) or *PKD2* on chromosome 4 (15%) [3, 4]. The *PKD1* gene encodes polycystin-1 (PC-1), which is thought to be a membrane protein involved in cell-cell or cell-matrix interactions, as well as possibly ciliary signaling [5-7]. The *PKD2* gene product is PC-2, a relatively non-selective cation channel [8-10]. Although the overall functions of PC-1 and -2 remain obscure, genetic and biochemical studies suggests that these two proteins participate in normal renal tubular development. Mice with homozygous targeted disruptions of either *PKD1* or *PKD2* show an embryonic lethal phenotype and massive cyst formation in the kidney [11-13]. These findings suggest that cyst formation is caused by loss of function in PC-1 or -2 and that these two proteins normally promote tubule formation. *In vitro* studies of renal epithelial cells also confirm that PC-1 converts the cystic phenotype to a tubular phenotype [14].

Cyst growth in ADPKD is driven by cell proliferation and fluid secretion. Based on two clues, a line of research has focused on the mechanisms involved in intracystic fluid accumulation [2]. First, most cysts lose up- and down-stream nephronal connections. Second, after drainage, cysts in ADPKD quickly fill again with liquid. Given that fluid secretion in normal secretory epithelia reflects primarily the balance of ion transport processes through ion channels and transporters, it is plausible that aberrant ion transport could result in intracystic fluid accumulation of ADPKD. PC-1 may work as a modulator of ion transport by regulating the subcellular localization of PC-2 [10, 15]. Therefore it is likely that the dysfunction of PC-1 in ADPKD causes intracystic fluid accumulation through changes in ion transport.

Agonists for intracellular cAMP production in cystic epithelial cells drive fluid secretion [2, 16] whereas inhibitors of the vasopressin-cAMP system remarkably reduced cystic phenotype in animal models [17]. These observations prompted us to explore the importance of cystic fibrosis transmembrane conductance regulator (CFTR)-mediated Cl^- secretion in ADPKD. CFTR is a cAMP-regulated Cl^- channel. Furthermore, although CFTR is downregulated in normal adult kidney [18], it has been identified in more than 50% of the epithelial cells lining the cysts from adult ADPKD kidneys [19, 20]. In addition, a less severe ADPKD phenotype has been reported in patients with coexisting ADPKD and cystic fibrosis [21, 22]. These data strongly suggest that CFTR contributes to the fluid secretion of ADPKD cysts.

In this study, we used electrophysiological and biochemical approaches on cells stably expressing PC-1 to examine the role(s) of PC-1 on the trafficking of CFTR to the plasma membrane. Our findings clearly show a role for PC-1 in regulating the expression of CFTR at the cell surface. These observations link dysregulation of PC-1 with abnormal, CFTR-mediated, Cl^- secretion into the cyst lumen.

Materials and Methods

Cell culture and transfection

We created a series of stable MDCK cell lines [14]. Five clones (G3 cells) and 6 subclones, (C8/68 cells and G7/36 cells), were created by transfection with either linearized pTRE-βPKD1 and pCIZeo, and shall be collectively referred to as PC-1 cells. Control cells (F2 and F6 cells) were established by transfection of pCIZeo alone. In a previous study, we showed that after culture in 3-dimensional collagen gels for 3 weeks, PC-1 cells developed striking tubular structures whereas the control cells formed cysts [14]. Thus, the detailed previous characterization guided our choice of G3, C8/68, and G7/36 cells as PC-1 cells, and F2 and F6 cells as control cells, in the present study. As previously described, the cells were maintained in DMEM, 100 U/ml penicillin, 100 µg/ml streptomycin, 500 µg/ml G418, 100 µg/ml Zeocin and 10% FCS.

Transient transfections of CHO and MDCK cells were performed with LipofectAMINE 2000 reagent (Invitrogen) according to the manufacturer's instructions. Each 100 mm dish of cells was treated with 6 or 10 µg of pEGFP-CFTR, 12 µg of pCI-PKD1-Flag, and/or 12 µg of pCI-R4227X-Flag as previously described [10].

Measurement of short-circuit current (I_{sc})

Short-circuit current (I_{sc}) measurements were performed in modified Ussing chambers constructed to accept Snapwell filters (Corning Costar Corp., #3407). I_{sc} was measured with an EC-825 voltage-current clamp amplifier (World Precision Instruments) in the voltage-clamp mode. Cells were cultured to confluence on Snapwell filters before measurement. The cell monolayers were bathed on both sides with a solution containing (in mM): 140 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 glucose and 10 Hepes (pH 7.4 with NaOH). The solution was subjected to constant recirculation, maintained at 37°C, and bubbled gently with air. Amiloride (10 µM) was added to the apical solution, and after stabilization, a cAMP cocktail (100 µM membrane-permeable cAMP analogue, 8-chlorophenyl-thio-cAMP (cpt-cAMP), 100 µM IBMX, and 20 µM forskolin) was applied to both apical and basolateral solutions. The cAMP cocktail-stimulated current was blocked by diphenylamine carboxylic acid (DPC; 0.1 mM).

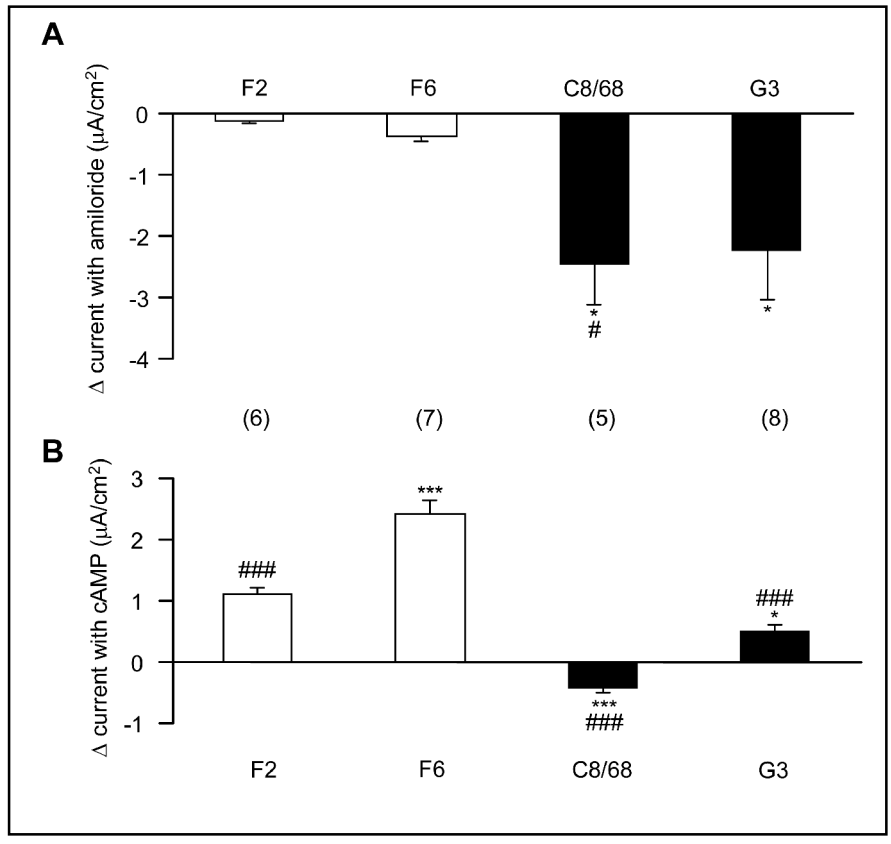

Fig. 1. Summary of changes in short-circuit current (I_{sc}) with the application of amiloride and cAMP cocktail to control (F2 and F6) and PC-1 (C8/68 and G3) cells. Changes (Δ current) were measured after the current responses to amiloride (A) or a cAMP cocktail (B) reached steady-state levels. The significance levels were $P < 0.001$ (***) and $P < 0.001$ (###) compared with F2 and F6 cells, respectively. The numbers of experiments are given in parentheses.

Whole Cell Patch-Clamp Recordings

Conventional tight seal, whole cell recordings [23] were performed to measure the cAMP-induced current responses of MDCK cells. The cells were grown on glass coverslips 1 day post-transfection with pEGFP-CFTR and the positive transfectants were identified by their GFP fluorescence under a fluorescent microscope (TE200, Nikon). After mild trypsinization (1-2 min), the cells were perfused with an extracellular solution (ES) containing (in mM) 150 NaCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 glucose, 20 sucrose, 0.1 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) and 10 Hepes (pH 7.4 with Tris). Sucrose and DIDS were included to prevent the activation of endogeneous currents (*eg*. volume-regulated channels). The pipette solution contained (in mM) 150 CsCl, 1 $MgCl_2$, 1 EGTA, $2Na_2ATP$ and 10 Hepes (pH 7.4 with Tris). A bolus of ES containing 250 μM cpt-cAMP was applied with an adjacently positioned pipette (30-35 μm diameter). Non-transfected cells did not respond to cpt-cAMP. All measurements were obtained at 31 - 34°C; compared to room temperature measurements, these conditions gave more consistent CFTR current activity. pClamp8 software (Axon Instruments) was used to sample and analyze data. To avoid significant aliasing, whole cell patch records were acquired at a 10 kHz sampling rate and filtered at 3 kHz through a 4-pole Bessel filter. The input capacitance was estimated from the decay time course of the capacitative surge and this value was used to normalize the current amplitude. Current-voltage (I-V) relationships were obtained at 100 msec of each voltage step from holding potentials of either 0 or +20 mV.

K^+-rich internal solution was used as an intrapipette solution to measure the endogenous K^+ currents at room temperature on MDCK cells grown on glass coverslips. The K^+-rich internal solution contained (in mM) 135 K·Gluconate, 15 KCl (pH 7.4 with Tris). Cells were perfused with an extracellular solution containing (in mM) 145 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 glucose, and 10 Hepes (pH 7.4 with Tris). I-V relationships were obtained at 100 msec of each voltage step from a holding potential of -80 mV.

Surface Biotinylation

Twenty four - 48 hours post-transfection with pEGFP-CFTR, cells were biotinylated in 1 mg/ml Sulfo-NHS-SS-Biotin (Pierce, 21331) at 4°C for 20 minutes. Cells were then lysed and the lysates incubated with immobilized NeutrAvidin beads (Pierce, catalog no. 53151). The bound proteins were eluted with 4X Laemmli sample buffer supplemented with 0.2 M DTT at 37°C for 60 minutes. The eluted proteins were immunoblotted as described previously [24]. GFP-CFTR was detected with monoclonal GFP antibody (1:600; Molecular Probes).

Results

Endogenous cAMP-stimulated short-circuit currents in control and PC-1 cells

In the presence of amiloride, an inhibitor of Na^+ reabsorption, the application of cAMP stimulates I_{sc} in

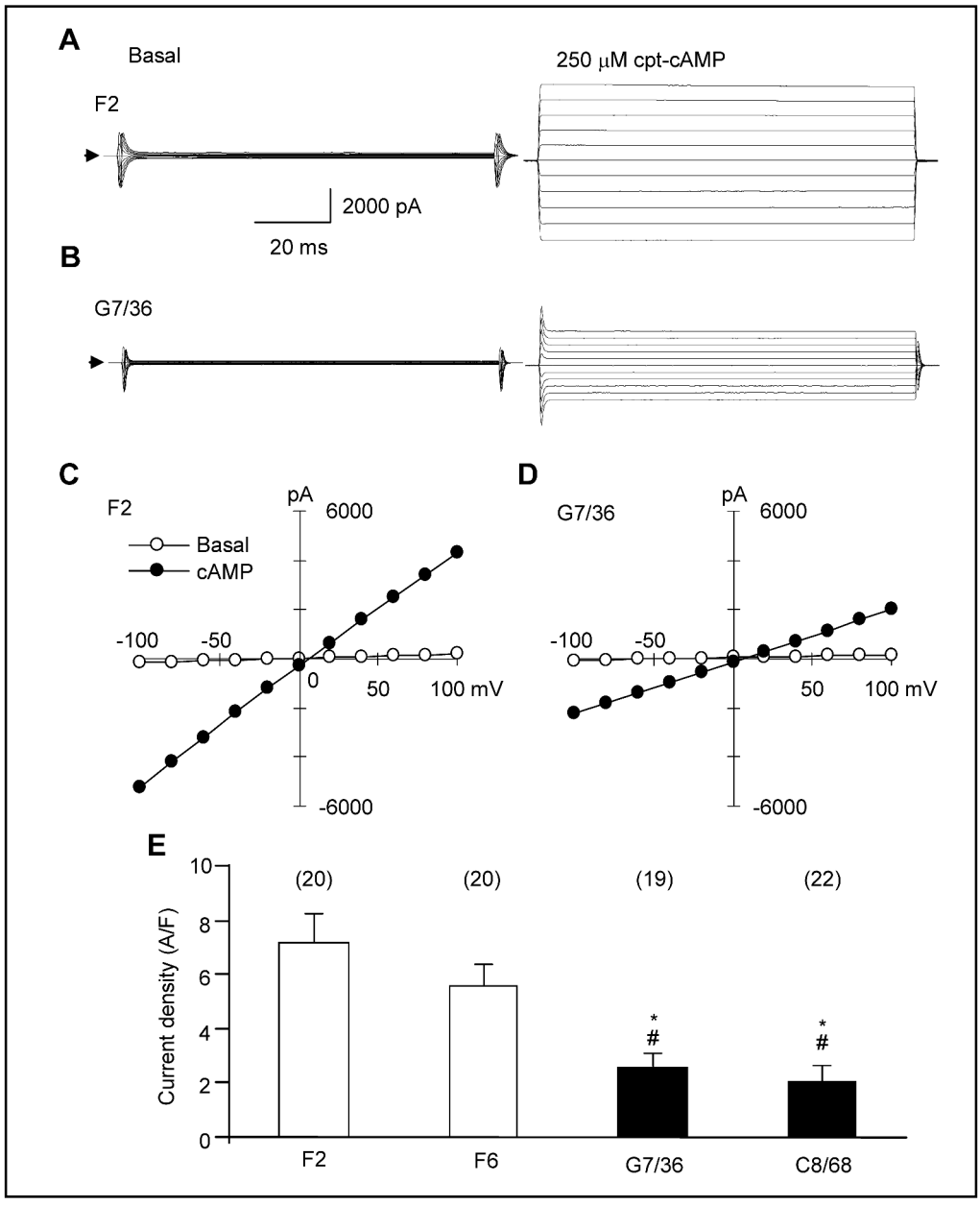

Fig. 2. PC-1 cells show reduced current activity in response to cAMP. Subconfluent MDCK cells were transfected with pEGFP-CFTR. Currents were measured by whole-cell patch clamp. Typical recordings from control F2 cell (A) and PC-1 G7/36 cell (B) in which application of cpt-cAMP (250 μM) evoked currents and the corresponding I-V relations (C; F2 cell, D; G7/36 cell) for steady-state currents are shown. The cells were held at 0 mV and 100 msec voltage steps applied. Arrowheads in (A) and (B) indicate the zero current levels under both basal and stimulated conditions. E, The bar graph shows the maximum deviations of the current from each basal level during stimulation with cpt-cAMP for 80 sec at a holding potential of +20 mV. Values are presented as mean ± SE. The numbers of experiments are given in parentheses. The symbols, # and *, represent the significance level of $P < 0.05$, compared with F2 and F6 cells, respectively.

MDCK cells, indicating transepithelial Cl^- secretion via endogenous CFTR channels [25]. We performed I_{sc} experiments to examine whether the expression of PC-1 affects this Cl^- secretion in MDCK cells. The stable lines used in our studies achieved adequate, basal transepithelial resistances to conduct these measurements. These values, shown as mean ± SE, were 181.3 ± 3.7 Ωcm^2 for F2 cells (n = 6), 164.3 ± 8.0 Ωcm^2 for F6 cells (n = 7), 94.4 ± 1.8 Ωcm^2 for C8/68 cells (n = 5), and 94.9 ± 1.2 Ωcm^2 for G3 cells (n = 8).

In PC-1 negative control cells, the addition of amiloride (10 μM) to the apical membrane caused a slight decrease in I_{sc} (Fig. 1A). After stabilization, a cAMP cocktail was applied to both apical and basolateral solutions to stimulate Cl^- secretion. This induced a swift increase in I_{sc} (Fig. 1B), which attained a maximum within 1-2 min, and then decreased to a plateau above basal I_{sc}. This response agrees well with the previous observations with MDCK cells [25]. In contrast, the addition of amiloride to the PC-1 cells, C8/68 and G3, prompted significantly greater decreases in I_{sc} than observed for the PC-1 negative control cells (Fig. 1A). The rapid response to amiloride in PC-1 cells reached a nadir within 30 s and was sustained at this level. Conversely, the cAMP cocktail-induced current responses were reduced dramatically in PC-1 cells compared to the control cells (Fig. 1B). These data suggest that the expression of PC-1 enhances amiloride-sensitive ion transport and inhibits CFTR channels.

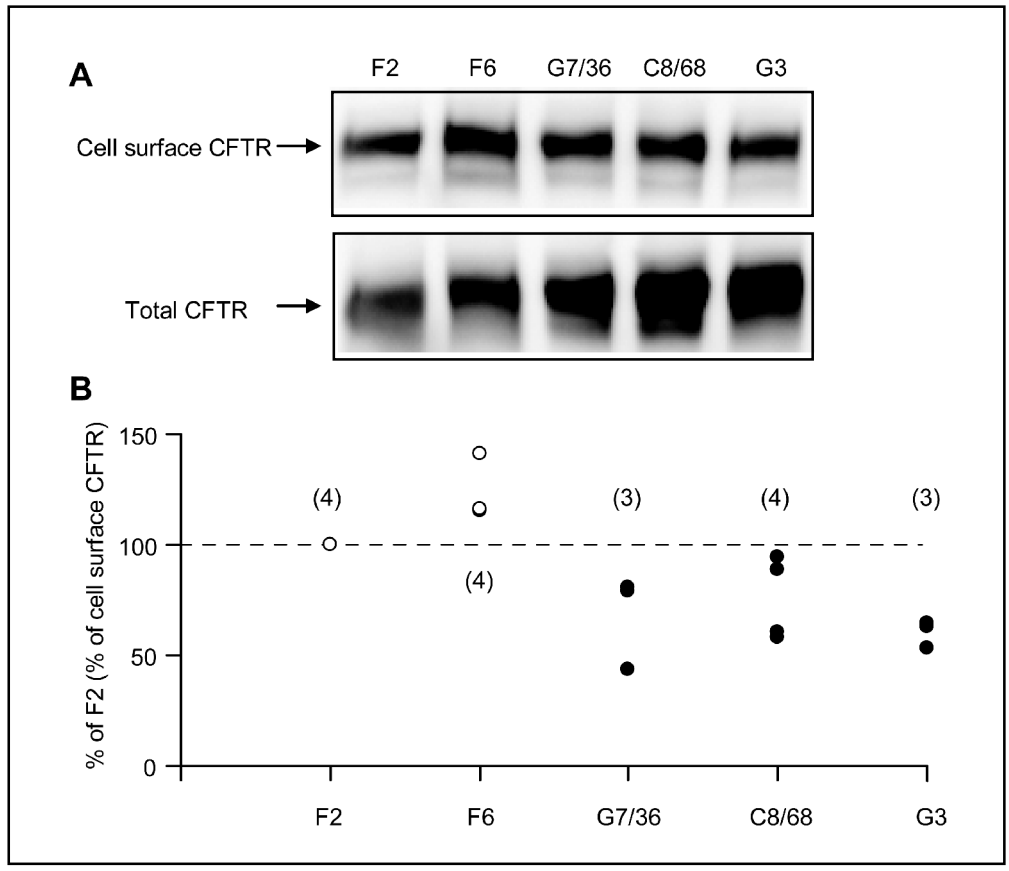

Fig. 3. PC-1 cells show reduced cell surface expression of exogenous CFTR protein. Subconfluent MDCK cells were transfected with pEGFP-CFTR. The biotin-labeled cell surface proteins, as well as the total lysates, were analyzed by western blot using GFP monoclonal antibody. F2 cells were used as a control in each experiment and the ratio of biotinylated to total CFTR was taken as an index of the cell surface expression level. The surface expression of CFTR in clones F6, G7/36, C8/68 and G3 was calculated similarly. Expressing these values as a ratio of that in F2 cells yields the relative biotinylation level; it is plotted here as the percentage of F2. Shown are a reperesentative blot (A) and the summarized quantified data (B). The numbers of experiments are given in parentheses.

cAMP-induced currents in control and PC-1 cells transfected transiently with pEGFP-CFTR

The reduced CFTR channel activity in PC-1 cells was examined by the patch-clamp technique. We initially tried to measure the endogenous cAMP-induced whole cell Cl^- current, but failed to do so. Others have reported very low endogenous CFTR levels in MDCK cells, despite the presence of measureable cAMP-induced I_{sc} [26, 27]. Therefore, as an alternative, we used MDCK cells transfected transiently with pEGFP-tagged, wild-type, human CFTR.

Figure 2 (panels A and B) shows typical current recordings from the GFP-expressing cells. In control F2 cells, whole cell currents were dramatically activated by the addition of cpt-cAMP and the I-V relationship was almost linear (Fig. 2, A and C), consistent with previous reports [24, 27]. On the other hand, PC-1 expressing G7/36 cells showed a reduced cAMP response compared to that observed in control F2 cells (Fig. 2, B and D). Figure 2E summarizes the results obtained from both of the control lines (F2 and F6) and from two of the PC-1 cell lines (G7/36 and C8/68). Again, these data indicate that the expression of PC-1 inhibits CFTR channels.

CFTR surface expression in the control and PC-1 cells transfected transiently with pEGFP-CFTR

The inhibition of cAMP-induced whole cell currents in PC-1 cells could be caused either by a direct inhibition of CFTR channel activity or by a reduction in CFTR channel number at the plasma membrane. To discriminate between these possibilities, we examined the plasma membrane CFTR protein expression by performing cell surface biotinylation studies.

Figure 3A is a representative blot showing that the biotinylated fractions of CFTR are greater for the F2 and F6 cells than for the G7/36, C8/68 and G3 cells. The signals corresponding to total and cell surface CFTR migrated slightly above ~ 200 kDa and were identified as band C in a previous study from our laboratory [24]. In a series of experiments, we used F2 cells as a control and the relative biotinylation level of CFTR to that in F2 cells was calculated. Figure 3B summarizes the quantitative data from this series of experiments. Lower levels of biotinylated CFTR was observed for PC-1 cells in all experiments. Because these biotinylation data roughly parallel the data from the patch-clamp studies (Fig. 2), it is likely that a reduction of cAMP-induced whole cell

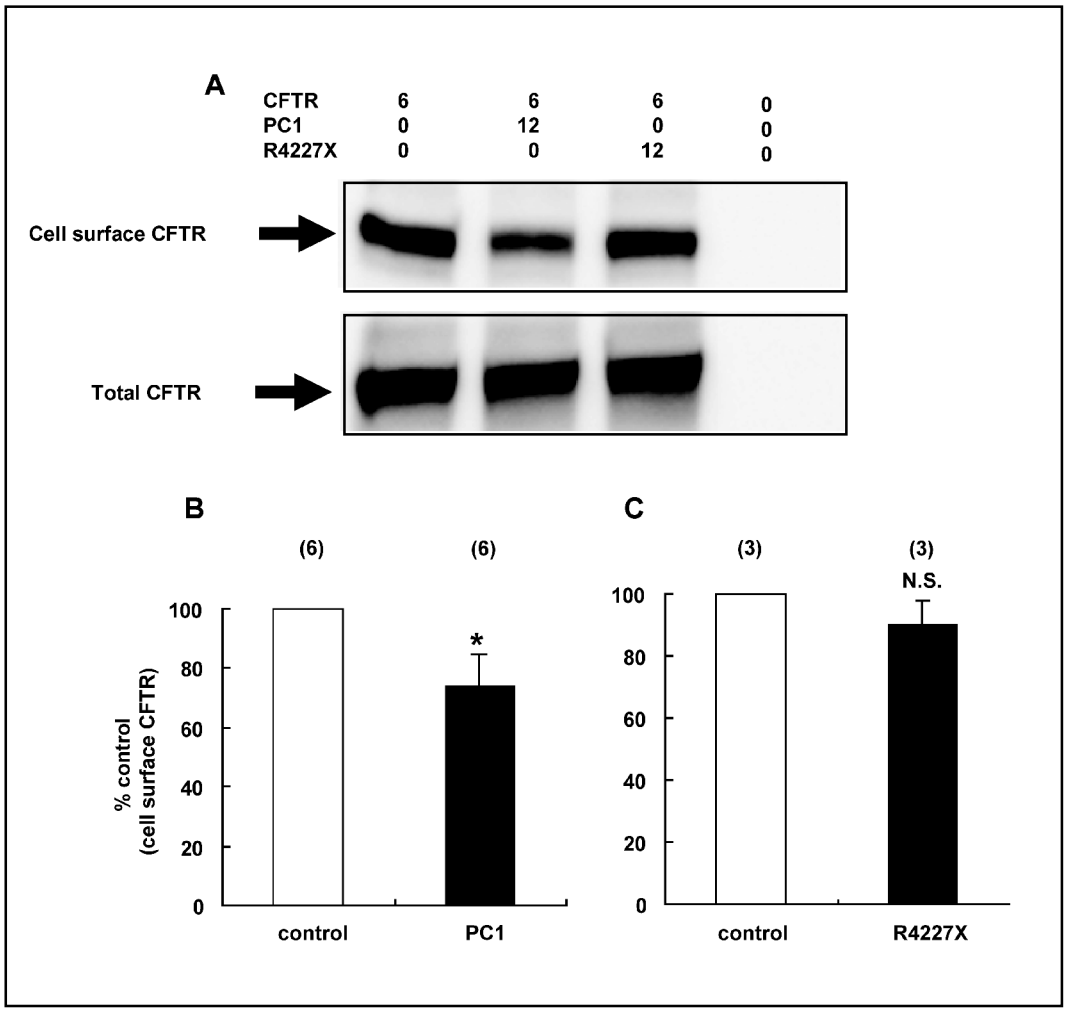

Fig. 4. A PC-1 mutant that lacks the COOH-terminal 76 amino acids (R4227X) has a modest effect on the plasma membrane expression of exogenous CFTR in CHO cells. Subconfluent CHO cells were transfected with pEGFP-CFTR (6 μg), pCI-PKD1-Flag (12 μg) and/or pCI-R4227X-Flag (12 μg). A representative blot is shown in A. Densitometric analysis of biotinylated CFTR is summarized in B. In each experiment, the percentage of control value was obtained by referencing the calculated surface expression levels to that from cells transfected only with pEGFP-CFTR (100%). The numbers of experiments are given in parentheses.

current in PC-1 cells is due to the decreased CFTR channel number at the cell surface.

A clinically relevant PC-1 mutant has a modest effect on the cell surface expression of CFTR

To address the question of which region of PC-1 is involved in the CFTR trafficking, we performed surface biotinylation experiments using CHO cells transiently co-expressing CFTR with either PC-1 or a clinically relevant PC-1 mutant, R4227X, which lacks the final 76 amino acids from the COOH-terminal. CHO cells were chosen for this purpose because previous studies indicate similar expression levels of PC-1 and R4227X in this system [10]. As can be seen in figure 4, CHO cells cotransfected with CFTR and PC-1 showed a remarkable reduction in the cell surface expression of exogenous CFTR compared to that seen with control CHO cells transfected with CFTR alone. Interestingly, and in contrast to wild-type PC-1, R4227X only slightly inhibited the cell surface expression of exogenous CFTR, and the effects did not achieve significance. These data indicate that the inhibitory effect of PC-1 on CFTR plasma membrane expression is at least partly due to the COOH-terminus of PC-1.

Endogenous K^+ currents and P2Y receptors in the control and PC-1 cells

MDCK cells endogenously express K^+ channels and P2Y receptors [28, 29]. To evaluate whether the effect of PC-1 on CFTR is specific, we asked if PC-1 expression affects the function of endogenous K^+ channels and P2Y receptors.

In F2 cells, time-dependent, outwardly rectifying currents were evoked by the voltage steps above 0 mV from a holding potential of -80 mV (Fig. 5A and B). The time-dependence identifies this current as the delayed-rectifier K^+ current reported previously by Lang and Paulmichl [28]. Figures 5A and B also show a similar I-V relationship for the G7/36 PC-1 cells. Figure 5C summarizes the amplitudes of the outward currents at

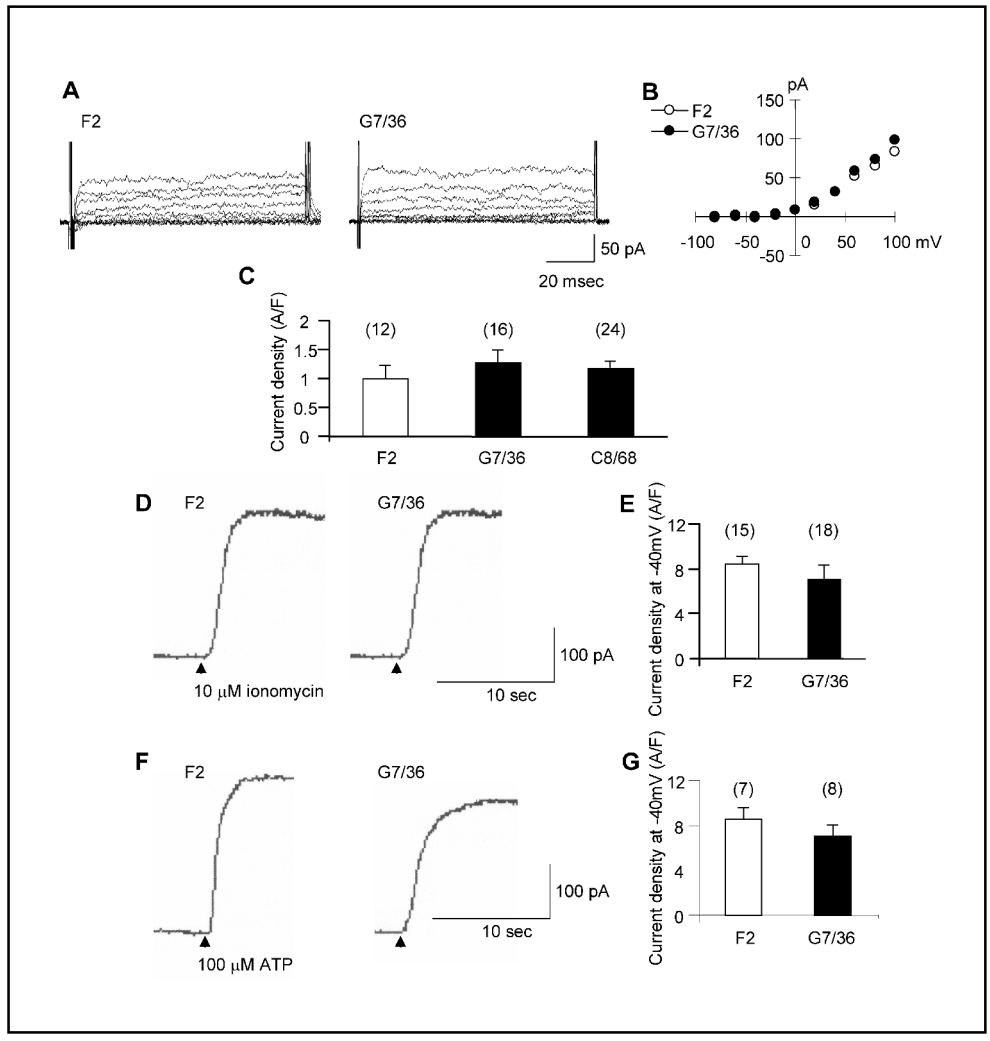

Fig. 5. The effect of PC-1 is specific for CFTR. Endogenous delayed-rectifier K^+ currents (A, B, and C), Ca^{2+}-activated K^+ currents (D and E), and P2Y receptor responses (F, G) do not differ between control and PC-1 cells. Panel A shows typical recordings from a control F2 cell (left) and a PC-1 G7/36 cell (right) in response to 100 msec voltage steps from a holding potential of -80 mV. Panel B shows the corresponding steady-state I-V relationships . Panel C summarizes the amplitudes of the steady state delayed-rectifier K^+ currents at +60 mV for the control F2 and two PC-1 MDCK lines, G7/36 as well as C8/68. Panel D shows typical recordings from a control cell (left) and a PC-1 cell (right) of currents evoked by the application of ionomycin (10 μM). The summarized data for steady-state currents at -40 mV are shown in E. Typical recordings from a control cell (left in F) and a PC-1 cell (right in F) show similar currents after application of ATP (100 μM). The reversal potentials for the currents shown in panels D and F were -70 and -80 mV, respectively (*data not shown*); these values are close to the predicted reversal potentials for K^+ currents under the specified recording conditions. The summarized data for ATP-evoked steady-state currents at a holding potential of -40 mV are shown in panel G. The values in bar graphs are presented as the mean ± SE. The numbers of experiments are given in parentheses.

+60 mV for the F2 and G7/36 cells, as well as for the PC-1, C8/68 cells. Thus, PC-1 had no significant effect on the delayed-rectifier K^+ currents in MDCK cells.

Two types of Ca^{2+}-activated K^+ channels, the maxi- and the intermediate conductance K^+ channels, have been found in MDCK cells [28]. We therefore checked the Ca^{2+}-activated K^+ channel activities in both control and PC-1 cells. Figure 5D shows that at a holding potential of -40 mV, the addition of ionomycin, a Ca^{2+} ionophore, activated an outward current in F2 cells that could reflect the activation of both these Ca^{2+}-activated K^+ channels. Similarly, current activation in response to ionomycin was observed in G7/36 cells. Figure 5E summarizes the quantified data of ionomycin-induced current responses at -40 mV in both F2 and G7/36 cells. Thus, as for the delayed rectifier K^+ current, PC-1 had no significant effect on the amplitudes of the Ca^{2+}-activated K^+ channels in MDCK cells.

MDCK cells express at least three different P2Y receptor subtypes, $P2Y_1$, $P2Y_2$, and $P2Y_{11}$ [29]. Stimulation of P2Y receptors with ATP results in the elevation of intracellular Ca^{2+}, thereby activating Ca^{2+}-activated K^+ channels. We also compared the P2Y responses to ATP in both control and PC-1 cells. At a holding potential of -40 mV, the addition of 100 μM ATP to either F2 or G7/36 cells activated outward currents (Fig. 5F). These responses were not significantly different (Fig. 5G).

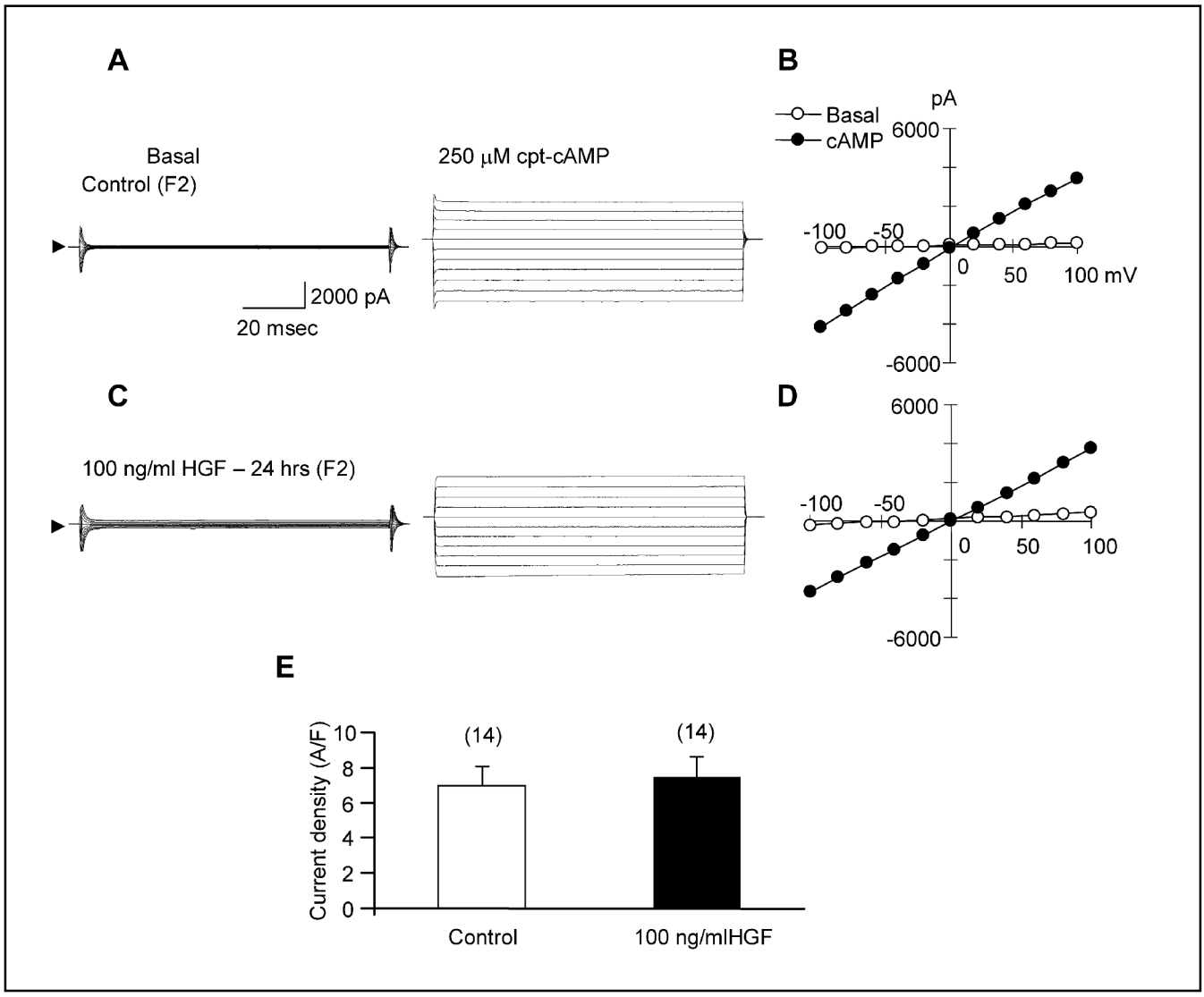

Fig. 6. Hepatocyte growth factor (HGF) does not replicate effects of PC-1-expression on CFTR currents. Subconfluent F2 cells were transfected with pEGFP-CFTR and were treated with (A and B) or without 100 ng/ml HGF (C and D) for 24 hours. Currents were measured by whole-cell patch clamp. Panels A and C show typical recordings in which application of cpt-cAMP (250 μM) evoked currents. The arrowheads indicate the zero current levels under both basal and stimulated conditions. B and D summarize the corresponding steady-state I-V relationships. The cells were held at +20 mV, and 100 msec voltage steps applied. E, The bar graph shows the maximum deviations of the current from each basal level during stimulation with cpt-cAMP for 80 sec at a holding potential of +20 mV. Values are presented as mean ± SE. The numbers of experiments are given in parentheses.

The effect of HGF on whole cell CFTR currents

Both HGF and PC-1 strongly induce tubulogenesis in MDCK cells [14, 30]. Thus, we checked the effect of HGF on CFTR-mediated whole cell currents in the control F2 cells transfected with pEGFP-CFTR. As shown in figure 6, pretreatment of cells with HGF for 24 hours had no significant effect on the CFTR current. These data suggest that the effect of PC-1 expression on CFTR is independent of HGF.

Discussion

In this study, we found that expression of PC-1 significantly inhibited cAMP-induced, CFTR-mediated I_{sc} in MDCK cells. The expression of PC-1 also reduced cAMP-activated CFTR channel activity in MDCK cells co-transfected with pEGFP-CFTR. This reduction by PC-1 paralleled a decrease in the cell surface expression of CFTR protein. Moreover, this effect was eliminated in studies utilizing the R4227X mutant of PC-1. These findings therefore suggest an important role of the PC-1 COOH-terminal in regulating CFTR surface expression. In contrast to its effect on CFTR, the expression of PC-1 exerted little effect on both the endogenous K^+ currents and P2Y receptors in MDCK cells. The treatment of MDCK cells with HGF did not mimic the effect of PC-1 on CFTR. Together, these results suggest that 1) the expression of PC-1 inhibits Cl^- secretion, 2) this inhibition is at least partly due to the reduction of CFTR channel number at the plasma membrane and 3) PC-1 specifically regulates CFTR by a mechanism that is unrelated to that of HGF.

As shown in Fig.1B, the expression of PC-1 almost completely inhibited the endogenous cAMP-induced I_{sc}. In MDCK cells transfected with pEGFP-CFTR, PC-1 significantly, but not completely, reduced CFTR channel activity (Fig. 2) as well its cell surface protein levels (Fig. 3).

Similarly, the effect of PC-1 was partial in CHO cells transiently co-expressing CFTR with PC-1. Although at present the reason for this discrepancy is not clear, the stronger effect of PC-1 in the I_{sc} experiments may be related to the lower expression level of endogenous CFTR protein in MDCK cells [26, 27]. Thus, we speculate that the incomplete inhibition of transfected pEGFP-CFTR by PC-1 may be due to extremely high CFTR expression levels in the over-expression studies.

Several lines of evidence suggest the importance of CFTR-dependent Cl^- secretion in the genesis and expansion of ADPKD cysts. Strong CFTR expression in the cyst-lining cells from ADPKD patients has been shown both immunohistochemically and electrophysiologically [19, 20]. An antisense oligonucleotide against human CFTR reportedly reduced fluid secretion by cells isolated from ADPKD patients [31]. The *in vitro* growth of ADPKD cysts is accelerated by cAMP agonists and inhibited by glybenclamide, an inhibitor of CFTR [16]. Recently, Torres et al [17] showed that vasopressin V2 receptor antagonist, which is expected to reduce the cellular cAMP level in vasopressin-stimulated epithelial cells, attenuated cyst growth in ADPKD animals. Although these data strongly suggest a role for CFTR in cystic Cl^- secretion, the mechanisms underlying regulation of CFTR expression in cyst-lining cells remain obscure. The loss of PC-1 function likely causes ADPKD [11-13]. We hypothesized that normal PC-1 function suppresses the apical membrane expression of CFTR. The findings of our present study suggest that the loss of PC-1 function leads to the enhancement of CFTR expression in cyst-lining cells.

So far, a COOH-terminal region of PC-1 has been shown to activate heterotrimeric G proteins [32], leading to the inhibition of adenylate cyclase. This suggests that PC-1 inhibits CFTR by decreased level of intracellular cAMP. In the present study, we observed an inhibition of cAMP-stimulated CFTR current responses that was associated with the expression of PC-1 (Fig. 1 and 2); this was accompanied by the decreased surface expression of CFTR (Fig. 3). Moreover, a disease-associated COOH-terminal PC-1 mutant diminished surface CFTR levels, suggesting the importance of this region in inhibition of the cAMP response. Taken together with the studies of Parnell [32], the present study suggests that a COOH-terminal region of PC-1 has a regulatory effect on CFTR through two independent mechanisms. The detailed molecular mechanism of the PC-1 regulation of CFTR expression remains to be clarified in future studies.

There have been conflicting reports concerning the phenotype of patients with coexisting ADPKD and CF. Compared to those without CF, O'Sullivan *et al* [21] have reported a less severe cystic phenotype for two patients affected by both ADPKD (without description of exact genotype) and CF (stop codon within exon3 and severe reduction in transcription for E60X). On the other hand, studies by Persu *et al* [33] of a patient affected by both ADPKD (without description of exact genotype) and CF (homozygous ΔF508 mutation) showed kidney involvement similar to that observed for patients with ADPKD without CF. Persu *et al* [33] provided several explanations for this apparent discrepancy, such as differences in CF mutations and age. However, a recent study by Torres *et al* [22] showed a milder cystic phenotype in a patient with coexisiting ADPKD and CF with a homozygous ΔF508 mutation, compared with an ADPKD sibling of similar age without CF. Clearly, observations in more families will be necessary.

PC-1 is known to be a large protein (460 - 520 kD) containing many motifs that are involved in cell-cell or cell-matrix interactions, as well as ciliary signaling. Many

different mutations spread throughout the PKD1 gene in ADPKD patients have been reported. The location of the mutation is thought to be a significant indicator of disease severity [34]. In this study, we showed that the COOH-terminus of PC-1 may potentially regulate CFTR trafficking. Interestingly, the immunohistochemical analysis by Persu *et al* [33] showed ΔF508-CFTR expression in the apical membrane of cyst-lining cells. It is well-known that the ΔF508-CFTR mutation results in its misfolding, ER retention and degradation. However, it also retains partial chloride channel function. It is possible that the patient reported by Persu *et al* [33] carries a PC-1 mutation that permits ΔF508-CFTR to evade the ER quality control machinery and traffick to the surface, thus contributing to chloride secretion and the unabated cystic phenotype.

We found the amiloride-sensitive I_{sc} in control cells to be negligible (Fig. 1). In contrast, PC-1-expressing cells showed a larger amiloride-sensitive I_{sc}. Amiloride inhibits the epithelial sodium channel (ENaC), as well as the Na^+/H^+ exchanger (NHE) and the Na^+/Ca^{++} exchanger (NCX and CALX). Of these three types of transporters, both ENaC and NHE are effectively inhibited by micromolar concentrations of amiloride [35-37], indicating that the amiloride-sensitive I_{sc} in PC-1 cells resulted from the inhibition of either ENaC or NHE. The rapid inhibition of I_{sc} shortly after the addition of amiloride to cells is most consistent with inhibition of ENaC, and not NHE, an electroneutral exchanger. Although functional ENaC is not thought to express well in MDCK cells, RNA encoding ENaC has been detected in this line [38]. The present findings suggest the presence of amiloride-sensitive ENaC in PC-1-expressing cells and that its activation may be favored by the expression of PC-1.

ENaC is inhibited by the activation of extracellular signal-regulated protein kinase (ERK 1/2) [39]. Several studies with cell lines from ADPKD patients suggest that abnormal ERK1/2 activation by epidermal growth factor and an uncommon cAMP-ERK1/2 signaling pathway contribute to PKD pathophysiology [40-42]. Thus, the inhibition of ERK1/2 by PC-1 may underly our observation that amiloride-sensitive I_{sc} is increased by PC-1. As demonstrated by Boletta and coworkers [14], MDCK cells constitutively express PC-2, a protein that interacts with PC-1 and is required for its function. The introduction of PC-1 to MDCK cells induces tubulogenesis in several clones. The present study shows that these cell lines also exhibit enhanced amiloride-sensitive sodium absorption, a finding that is consistent with a shift from a secretory, cystic phenotype to an absorptive phenotype favoring tubulogenesis. Additional study will be required to elucidate the mechanisms responsible for increased ENaC activity in cells expressed with PC-1

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