J. Membrane Biol. 134, 31–39 (1993)

The Journal of
Membrane
Biology


# F-Actin Network May Regulate a $Cl^-$ Channel in Renal Proximal Tubule Cells

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Received: 21 July 1992/Revised: 19 October 1992

**Abstract.** A variety of mechanisms have been proposed for the regulation of ion channel molecules. As integral membrane proteins, ion channels may interact with the cytoskeleton. Regulation of channels by the actin network may therefore be important. In the present study we used cytochalasin D and exogenous actin to test this possibility. The $Cl^-$ channel of the apical membrane of renal proximal epithelium was detected in its active state after prolonged depolarization. Within 6 sec after its addition, cytochalasin D (0.05 $\mu$g/ml) significantly decreased the number of open channels and mean open probability (*NPo*) of the $Cl^-$ channel. Colchicine (1 mM), which affects microtubules, did not influence channel activation. Cytochalasin D is known to not only disrupt the F-actin network but to inhibit polymerization of F-actin as well. The latter effect is also produced by DNaseI. Cytochalasin D, but not DNaseI, inactivated $Cl^-$ channels in cell-free membrane patches, suggesting that cytochalasin D inactivated the channel by disrupting the actin network. Cytochalasin D appeared to specifically affect the channel, as opposed to membrane permeability, since only the activated whole-cell $Cl^-$ currents were altered by cytochalasin D. Addition of actin polymer, but not actin monomer, reactivated the cytochalasin-D-depressed channel. Thus, repair of the disrupted F-actin network with actin polymer apparently restored the activity and number of open $Cl^-$ channels. We therefore conclude that the F-actin network interacts with and possibly regulates the $Cl^-$ channel of renal proximal tubule epithelia.

**Key words:** Ion channel—Cytochalasin D—Actin—Patch clamp—Cytoskeleton

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## Introduction

Chloride channels are known to play an important role in fluid movement across epithelia [5]. In respiratory epithelia, adrenergic hormones act through the second messengers, protein kinase A and protein kinase C, to phosphorylate and activate $Cl^-$ channels [8, 11]. Calmodulin is also able to open the $Cl^-$ channel in respiratory epithelia [31]. We have recently reported that the renal proximal tubule epithelium has an apically located $Cl^-$ channel of 33 pS which carries an outwardly rectifying current at positive membrane potentials [28]. The activation of this channel by parathyroid hormone also appears to involve phosphorylation by the second messengers, protein kinase A and protein kinase C [28]. In addition, this $Cl^-$ channel can be activated by prolonged depolarization [15]. The $Cl^-$ channel of the renal proximal tubule therefore possesses regulatory sites which are sensitive to both phosphorylation and voltage.

The primary structure of the *Torpedo marmorata* $Cl^-$ channel has recently been studied [12]. The cytoplasmic part of the molecule contains several sites possibly phosphorylated, which are important to the regulatory mechanism of channel activity. Although the structure may elucidate the manner by which phosphorylation activates the channel, channel regulation presumably also depends on more dynamic mechanisms. In particular, ion channel activity may be regulated by the movement of phospholipids within the membrane or by cytoskeletal-membrane interactions. The cytoskeleton is known to be involved in both the insertion of apical membrane components [16] and in vasopressin-induced water permeability in epithelial cells [30]. For example, the actin microfilament network has been shown to play a role in the stimulation

of secretion and in the vectorial delivery of apical membrane proteins in epithelial cells [16]. Actin and actin-binding proteins have been found to be linked to several proteins involved in ion transport, including the band 3 anion exchanger [4], the $\alpha$-subunit of the $Na^+$-$K^+$-ATPase [17], and $Na^+$ channels from brain [25]. Recently, studies by Cantiello et al. [3] have suggested that the activity of the epithelial $Na^+$ channel is regulated by F-actin.

Cytochalasins (Cyt.) are cell-permeable reagents which disrupt actin microfilaments by depolymerizing actin [24]. In this report, we tested the effects of Cyt. on the activity of the renal proximal tubule $Cl^-$ channel and then added actin back to the cytoplasmic face of the membrane. Our results suggest that the F-actin network plays a role in the activity of the $Cl^-$ channel.

## Materials and Methods

### Cells

Primary cultures were obtained from rabbit renal proximal convoluted tubules [28]. Cells derived from renal proximal tubules were grown in HAMF12 and DME (1 : 1) containing 20 mg/dl of penicillin, 15 mg/dl of streptomycin and 10% fetal calf serum (FCS). After the first few days in culture, the FCS-containing medium was replaced with serum-free medium containing 5 $\mu$g/ml human transferrin, $5 \times 10^{-8}$ M hydrocortisone, and 5 $\mu$g/ml bovine insulin to enhance the growth [27]. Cells plated in 60 mm dishes (Corning) reached confluence in 5–10 days, and were then transformed with the plasmid pSV-*neo3*. The plasmid DNA (1 mg/ml, DW) was enveloped by an equal volume of lipofectin (BRL, Gaithersburg, MD) at room temperature for 10 min. The cells were incubated in 60 mm dishes with 10 $\mu$g DNA in FCS-free media for 2 days. The transformed cells were then selected. This was done by incubating each dish of cells with 10% FCS and 4 mg/dl neomycin. After the first passage, the surviving cells were subcultured in neomycin-containing media, which were used in all subsequent passages. The subcultured, selected cells took two to three weeks to reach confluence. Cells of passages 3–5 were used in all experiments.

The transformed cells maintained the morphologic characteristics of primary cultured tubules, including apical microvilli and tight junctions. cAMP concentration was determined by radio immunoassay (Yamasa kit, Chiba, Japan). Cell production of cAMP was measured by the following hormones: control 62 ± 6.64; 1–34 parathyroid hormone $10^{-9}$ M 84.9 ± 15.8, $10^{-7}$ M 142 ± 24; salmon calcitonin $10^{-9}$ M 82.3 ± 18, $10^{-7}$ M 119 ± 18, and arginine vasopressin $10^{-7}$ M 70 ± 6.9 fmol/mg · protein [29].

### Plasmids

The plasmid pSV3-neo, which possesses the SV40 large T antigen (early region promoter) gene and is resistant to the aminoglycoside antibiotic G418 (neomycin), was obtained from the American Type Culture Collection (ATCC, Rockville, MD). *Escherichia coli* containing the plasmid were propagated, and the plasmid DNA was purified by the alkaline lysis method [21]. Plasmid DNA was digested with Bam HI from BRL, and was then analyzed by agarose gel electrophoresis.

### Patch-Clamp Experiments

Patch-clamp recordings were carried out according to the method described in previous papers [20, 28, 31]. Currents were recorded at room temperature (20–24°C) with an EPC-7 patch-clamp amplifier (List-Electronic, Germany) and the data were stored on a DAT recorder (DAT-200, Sony, Tokyo). Records were sampled at 2,000 points/sec and analyzed on a Sanyo MBC AX computer using the Axon ver. 5.5.1 software. The data obtained were transported to a Macintosh SE/30 computer and analyzed using Excel.2.2 software. Mean open probability ($NP_o$) was calculated as: $NP_o = \Sigma\, nt_n$, where $N$ is the number of functional channels in the patch, $P_o$ is the single channel open probability, $n$ represents the state of the channel (0 = closes, 1 = one open channel and so on), and $t_n$ is the length of time in state $n$. $N$ is the mean number of open channels, calculated as $S\, nt_n/t$, $t = \mathrm{S}\, t_n$.

Whole cells were bathed in Krebs-Henselite solution (mM: 125 NaCl, 1 $CaCl_2$, 1.23 $MgCl_2$, 1 $NaHPO_4$, HEPES and 3 $\alpha$-ketoglutarate, pH 7.4 by NaOH). The pipette contained a filtered solution of (mM) 130 tetraethylammonium (TEA) chloride, 1.5 $MgCl_2$, 1.0 $CaCl_2$ and 10 HEPES (pH 7.4, external solution). For inside-out patches, the bath contained a filtered solution of (mM) 130 TEACl, 1.2 $MgCl_2$, 1 $CaCl_2$, 5.5 ethyleneglycol-bis (-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), 3 Mg-ATP and 10 HEPES (pCa 7.0, pH 7.2 by Tris base, internal solution). The external and internal solutions were also used for whole-cell recordings. Reagents dissolved in internal or external solution were delivered by a separate glass pipette located near the patch-clamped cell. Cytochalasin D was usually delivered through this pipette over a period of 20 sec.

### Actin Polymerization

Monomeric actin (G-actin) was stored in a low ionic strength buffer containing (mM) 0.5 tris (hydroxymethyl) aminomethane HCl, 0.2 $CaCl_2$, 0.5 ATP, and 0.5 $\beta$-mercaptoethanol, pH 8.0, under liquid nitrogen. G-actin was dissolved in the internal solution, a high ionic strength buffer with Mg-ATP to rapidly polymerize into F-actin [14]. Molecular weight of G- and F-actin was measured by using HPLC gel-column. Running buffer was 0.1 M phosphate (pH 7.0) and elution was performed at 1 ml/min. To obtain different lengths of F-actin, monomeric actin was incubated in the internal solution for 2 min to form a short filament (dimer or trimer), or for 24 hr to form a long filament (MW over 200,000). The F-actin was added to the patches as rapidly as possible after polymerization.

### Visualization of Cytoskeletal Microfilaments

Cells grown on coverslips were exposed to Krebs-Henselite solution with or without Cyt. D (0.05 $\mu$g/ml) for 10 sec. They were immediately washed with phosphate buffer (pH 7.4), incubated with NBD-phallicidin ($10^{-8}$ M) for 1 hr and then fixed with alcohol [2]. Fluorescent microscopy (MC-100, Zeiss, Germany) was used to visualize the fluorescence.

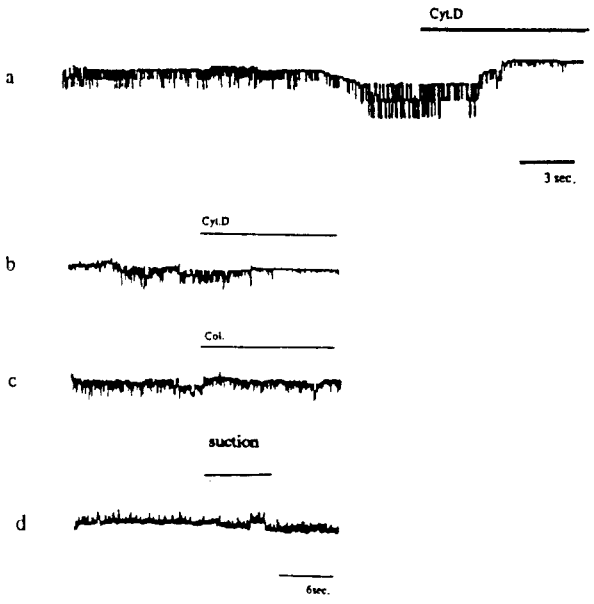


**Fig. 1.** Representative tracings of $Cl^-$ channels. A cell-attached patch clamp was performed with a pipette containing external solution. The $Cl^-$ channels were activated by depolarization to +60 mV for 2 min. We then added the following reagents, or carried out the procedures indicated: (*a*) Exposed to Cyt. D (0.05 μg/ml) in a cell-attached patch at +60 mV. (*b*) exposed to Cyt. D (0.05 μg/ml) in an inside-out patch at +80 mV and (*c*) exposed to colchicine (1 mM) in a cell-attached patch at +60 mV. (*d*) Connecting the pipette to a U-shaped tube to provide a suction of $-30$ $cmH_2O$ to the patch membrane.

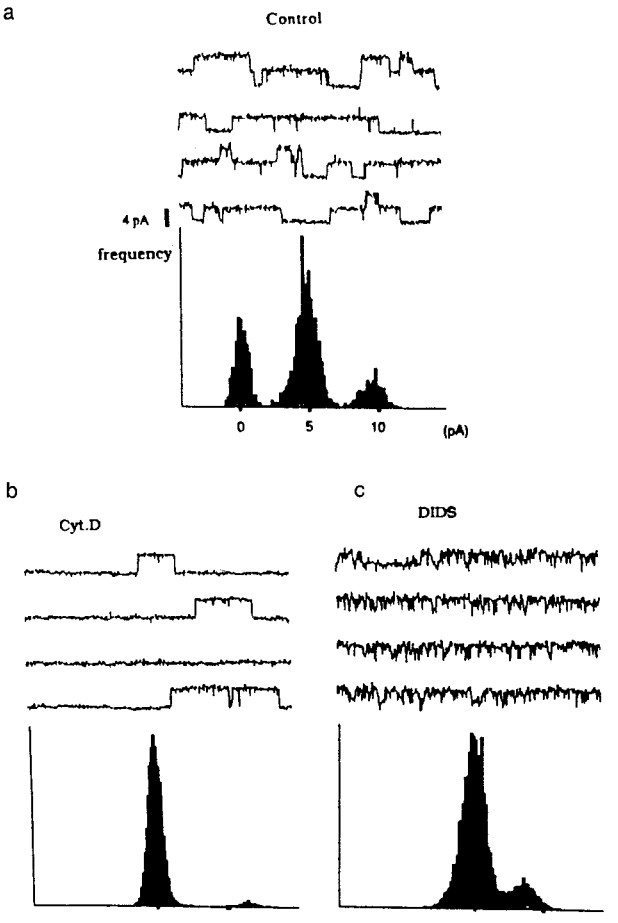


**Fig. 2.** Amplitude histograms of $Cl^-$ channel activity in cell-attached patches. Amplitude histograms were obtained from the representative tracings shown in Fig. 1. (*a*) Under control conditions and (*b*) 6 sec after treatment with Cyt. D (0.05 μg/ml). (*c*) Data obtained in a separate set of experiments, 1 min after treatment with DIDS ($10^{-4}$ M). The negative current is shown as an upper deflection.

### Materials

All chemicals were purchased from Sigma (St. Louis, MO).

### Statistics

The data were expressed as mean ± SE. Student's *t*-test was applied for results and $P < 0.05$ was considered significant. Chi-square test was used in one experiment.

## Results

### Effect of Cytochalasin D on $Cl^-$ Channel

Figure 1*a* shows a representative tracing of a $Cl^-$ channel in a cell-attached patch, which has been activated by prolonged depolarization, and to which Cyt. D (0.05 μg/ml) has been added. The $Cl^-$ channel was first detected after depolarization to +60 mV for 2 min. The activated $Cl^-$ channel continued to open for several minutes as indicated below (*see* Fig. 7). The channel was further activated and then blocked by Cyt. D. Since membrane stretch may alter the cytoskeleton modified by Cyt. D, we tested to see whether the activity of the $Cl^-$ channel was influenced by stretch. The activity of the $Cl^-$ channel was not influenced by either aspiration of $-30$ cm $H_2O$ (Fig. 1*d*, $NP_o$ (%): 210 ± 52 *vs.* 189 ± 46, $P > 0.1$, $n = 4$) nor by pipette manipulation (*data not shown*, $n = 4$). Thus, the $Cl^-$ channel is not activated or inactivated by stretch. Colchicine (1 mM), which blocks assembly of microtubules, did not affect $Cl^-$ channel activity (Fig. 1*b*) during the measured time interval. Cyt. B (0.5 mg/ml) was also found to inhibit channel activity ($n = 6$, *data not shown*). The channel had a conductance of 40 pS with a reversal potential of $-20$ mV, compatible with the characteristics of the $Cl^-$ channel recently described in renal tubules [28].

Figure 2 uses amplitude histograms to demonstrate the effect of Cyt. D on $Cl^-$ channel activity.

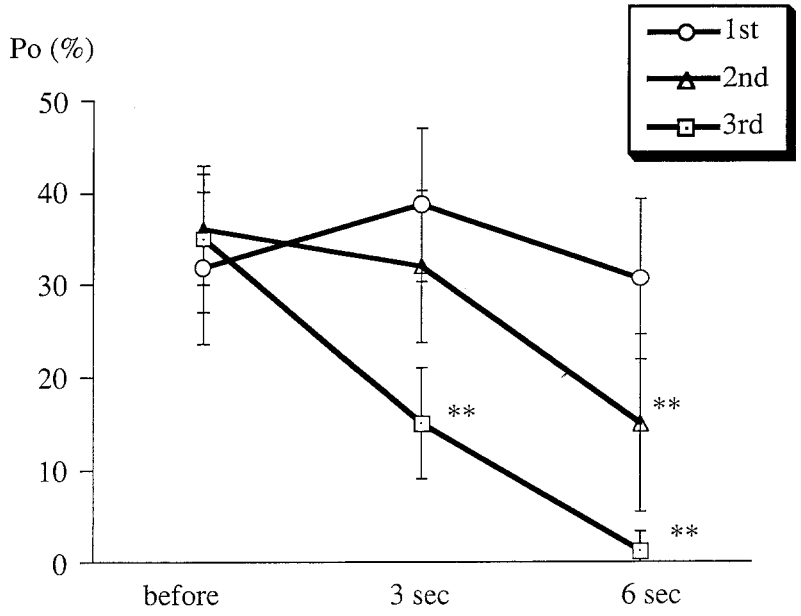


**Fig. 3.** Analysis of the effect of Cyt. D on the open probabilities of each opening channel. The effect of Cyt. D on the open probabilities of the first opening (circle), second opening (triangle) and third opening (square) channel are shown with eight determinants. Cell-attached patch clamp was performed with a pipette containing external solution. The $Cl^-$ channels were activated by depolarization to +60 mV for 2 min and the reagents were then added. One-second recordings at 0, 3 and 6 sec after addition of Cyt. D. * $P < 0.05$, ** $P < 0.01$.

The control patch contained two open channels, with clearly resolved current peaks (Fig. 2*a*). Following treatment with Cyt. D, one channel remained open, and maintained a constant baseline current (Fig. 2*b*). Diisothiocyanostilbene-2,2′-disulfonic acid (DIDS, $10^{-4}$ M), a competitive inhibitor of the $Cl^-$ channel, was added through another pipette to the cell. It reduced the amplitude and induced flickering of the current, resulting in an intermediate average current. Since such competitive antagonists are thought to block the entry of ions into the channel pore [33], the inhibitory effect of Cyt. D on the $Cl^-$ channel appeared to differ from that of the competitive inhibitors.

As shown in Fig. 2, the number of functional channels appeared to be reduced by Cyt. D. The open probability of each functional channel during the treatment with Cyt. D is summarized in Fig. 3. While the second and third gaps of current from eight determinants were significantly reduced over time, the first gap was not altered. Thus, the data might suggest that Cyt. D acts primarily to reduce the number of open channels rather than to affect the open-close lifetime of each channel in the patched membrane.

The changes in the mean channel number, $N$, and $NP_o$ of the $Cl^-$ channel by Cyt. D are illustrated in Fig. 4. Both parameters were obtained as the sum of a 1-sec analysis begun at the indicated time. Both the channel density and the $NP_o$ were significantly reduced. The differences were even more pronounced when the sum of a 10-sec analysis was determined: $NP_o$: 210 ± 56 to 57 ± 8.9%, $N$: 4.6 ± 0.6 to 1.4 ± 0.4/sec, both $n = 6$, $P < 0.001$.

## Effect of Cytochalasin D on $Cl^-$ Channel in Inside-Out Patches

The effect of Cyt. D (0.05 μg/ml) on the depolarization-induced activation of $Cl^-$ channels in inside-out patches was also examined (Fig. 1*b*). Cyt. D blocked $Cl^-$ current ($NP_o$ = 165 ± 17 *vs.* 20 ± 6.9%, $n = 8$, $P < 0.001$) in a manner similar to that observed in cell-attached patches (Fig. 1*a*). Inactivation of the $Cl^-$ channel by a higher dose of Cyt. D was also examined (Fig. 5). The effect of Cyt. D at a concentration of 0.05 μg/ml (filled bar) or of 5 μg/ml (hatched bar, $NP_o$ = 151 ± 40 *vs.* 16 ± 5.2%, $n = 8$, $P < 0.001$) reduced the number of open channels. Channel inactivation produced by a 20-sec treatment with 0.05 μg/ml, but not with 5 μg/ml of Cyt. D, was reversible. When recovery was defined as a return to the original number of open channels following washout of Cyt. D, recoveries occurred in five of eight experiments using 0.05 μg/ml, but in none of the eight experiments using a Cyt. D concentration of 5 μg/ml (significant by $\chi^2$ test). Bovine pancreous DNaseI binds the N terminus of most types of actin and inhibits the rate and extent of polymerization stoichiometrically [13]. DNaseI (10 μM) added to the activated $Cl^-$ channel for 20 sec did not alter the activity of the $Cl^-$ channel.

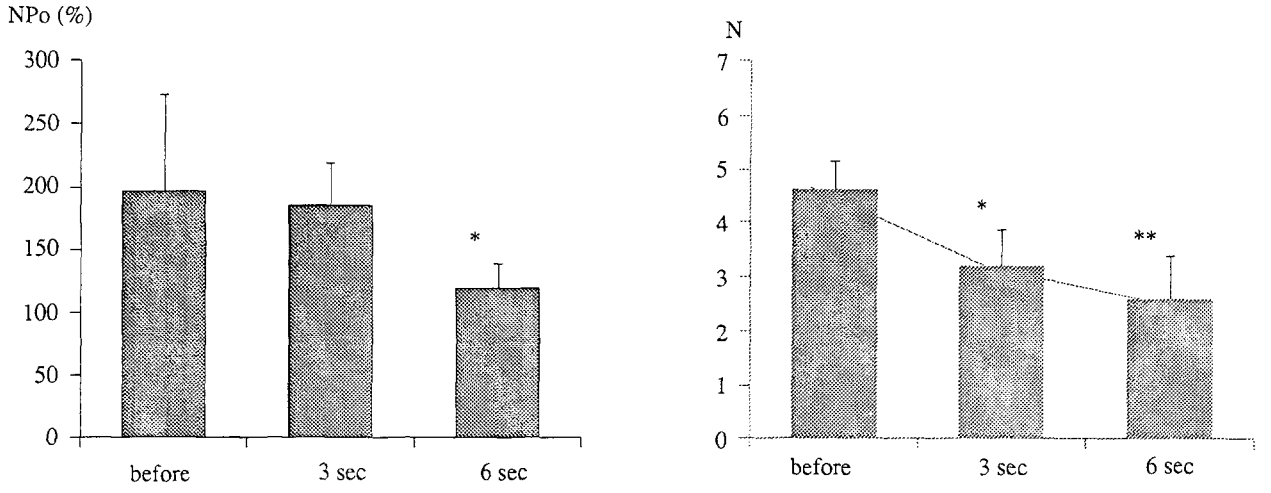


**Fig. 4.** Influence of Cyt. D on $NP_o$ and ion channel density in cell-attached patches. Cell-attached patch clamping was performed with a pipette containing external solution. $Cl^-$ channels were activated by depolarization to +60 mV for 2 min and then Cyt. D (0.05 μg/ml) was added. The effect of Cyt. D on $NP_o$ (left) and on the number of channels (right) was determined ($n$ = 6). * $P < 0.05$, ** $P < 0.01$.

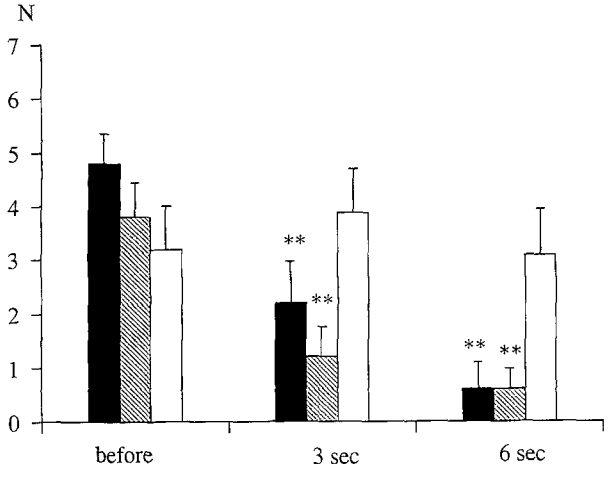


**Fig. 5.** Influence of Cyt. D on channel activity in inside-out patches. Inside-out patch clamping was performed with external solution in the pipette and internal solution in the bath. The $Cl^-$ channels were activated by depolarization to +60 mV for 2 min after detaching the membrane and reagents were then added. The effect of 0.05 μg/ml (filled bar, $n$ = 6), of 5 μg/ml (hatched bar, $n$ = 6) of Cyt. D and of 10 μM of DNaseI (open bar, $n$ = 3) on the number of open $Cl^-$ channels was determined. * $P < 0.05$, ** $P < 0.01$.

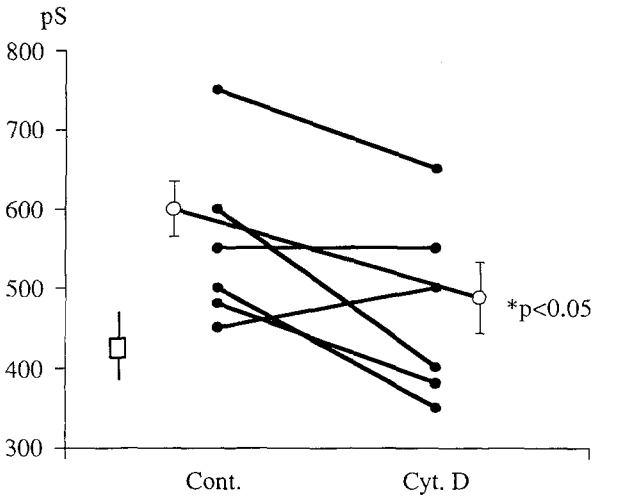


**Fig. 6.** Whole-cell conductance measured before and after Cyt. D treatment. Whole-cell patch clamping was performed with internal solution in the pipette and external solution in the bath. Conductance was calculated from currents and an applied voltage, 0 to +50 mV step at 1 Hz, in basal level (square). The voltage was maintained at +50 mV for 3 min, and the conductance was measured again (*Cont.*). Cyt. D was then added through another pipette, and conductance was measured for 2–4 min (*Cyt. D*). Each level of conductance is the sum of 20 recordings after stabilization of currents. * $P < 0.05$.

## Effect of Cytochalasin D on Whole-Cell Cl Current

To determine whether the effect of Cyt. D detached by patch clamping reflects a specific inactivation of the $Cl^-$ channel, whole-cell currents were measured (Fig. 6). As reported previously in cultured proximal tubule cells [28], reversal potential was zero without Cl gradient inside and outside of cell. *I-V* relation was outward rectified and 0 to +50 mV steps were used to measure Cl conductance. In the experimental ionic environment, whole-cell currents consisted essentially of the baseline $Cl^-$ and $TEA^+$ conductances and of a nonspecific leak. Holding the voltage

to −60 mV, depolarization increased Cl conductance for 2 min (*Cont.* in Fig. 6). Cyt. D reduced these conductances, but did not diminish the baseline conductances (open square in Fig. 6). This suggests that the reagent did not alter passive membrane permeabilities, but did specifically inhibit the activated component of the $Cl^-$ current.

### Reactivation of Cytochalasin-Suppressed $Cl^-$ Current by Actin

To clarify the role of actin filaments in $Cl^-$ channel regulation, and thereby explain the inhibitory effect of Cyt. D, we added various forms of actin (*see* Materials and Methods and [13]) to the cytoplasmic side of the patch membrane. As described above, Cyt. D of 5 μg/ml for 20 sec was used to inactivate the $Cl^-$ channel, since addition of actin washed out the reagent. Representative tracings are shown in Fig. 7. Once open, the $Cl^-$ channel remained in the activated state for a few minutes (control condition) but was rapidly inactivated by the addition of Cyt. D. Short actin partially, and long actin completely, reversed the Cyt. D-induced $Cl^-$ channel inactivation while G-actin did not. In contrast to the rapidity of inactivation by Cyt. D, it usually took 30 sec for actin to reactivate the $Cl^-$ channel. The number of open channels and the $NP_o$ were measured in 10-sec recordings under control conditions after addition of Cyt. D, and 1 min after addition of actin (Fig. 7). As shown in Fig. 7, both the number of open channels and the current ($NP_o$) driven by the $Cl^-$ channel were recovered following the addition of actin. DNaseI at low doses binds to actin ($K_d = 10^{-8}$ M) and inhibits further actin polymerization [18]. When DNaseI ($10^{-7}$ M) was added to the long actin mixture, the Cyt. D-induced inactivation of the $Cl^-$ channel was not reversed. Thus, active actin polymerization seemed required to reactivate the $Cl^-$ channel.

### Effect of Cyt. D on Distribution of Cytoskeletal Microfilaments

To examine the effect of Cyt. D, we stained actins by fluorescence-labeled phallicidin. Figure 8 (Top, left panel) shows the fluorescence images of the Cyt. D-untreated control cells and their microscopic view (Bottom, left panel). Actins localized subcellularly and to the border of the plasma membrane. The right panels of Fig. 8 show the fluorescence images of the Cyt. D-treated cells (Top) and their microscopic view (Bottom). Actins in the border of the plasma membrane were weakly stained, and partly disappeared.

## Discussion

The present study was undertaken to investigate the regulatory role of actin microfilaments in $Cl^-$ channel activations. Cyt. D was found to inhibit the opening of the $Cl^-$ channel present in the apical membrane of renal proximal tubule epithelia. Cyt. D is membrane permeable, and thus may cross the plasma membrane of cells and modify actin filaments. The effect of Cyt. D on the $Cl^-$ channel was also investigated using outside-out patches, and it was found to inhibit $Cl^-$ channel activation in a manner similar to that observed in inside-out patches (*data not shown*). This finding supports the notion that Cyt. D permeates the plasma membrane and modifies the actin network, thereby influencing the activity of the channel.

Cyt. D has a number of previously described effects on the cytoskeleton [6]. Cyt. D is a very potent toxin which affects both the depolymerization and polymerization of actin. In fact, Cyt. D elicits a fast and efficient polymerization of actin monomers [7]. Thus, the inhibitory mechanism of Cyt. D action might be due to an inhibition of actin polymerization within cells. However, because the inhibitory effect of Cyt. D was observed using inside-out patches in which cytoplasmic actin monomers are washed out, it is unlikely that Cyt. D inactivates $Cl^-$-channel-inhibiting actin polymerization. Recently, the membrane located on the patch pipette tip was carefully examined and even in these isolated membrane patches, the cytoskeletal microfilament network was maintained [22]. DNaseI inhibits polymerization of actin but does not disrupt the existing actin network. The finding that DNaseI did not inhibit the activity of the $Cl^-$ channel suggests that Cyt. D may exert its effect by disrupting the actin network. Studies in vitro have shown that polymeric but not monomeric actin is known to be in equilibrium with the actin network present on the cytoplasmic face of the plasma membrane [19]. We found that, when added to the cytosolic side of the membrane to repair the actin network, actin polymers reactivated the $Cl^-$ channels that had been suppressed by Cyt. D. The findings that this reactivation had a lag time and that addition of DNaseI to the actin polymers did not reactivate the $Cl^-$ channel, are both consistent with a mechanism of actin in which Cyt. D inactivates $Cl^-$ channels by disrupting the actin network. Therefore, the present study suggests that the actin network present on the cytoplasmic face of the apical membrane has regulatory effects on $Cl^-$ channels.

Another possible interpretation of our data is that actin filaments are required to insert cytoplasmic vesicles containing $Cl^-$ channels into the

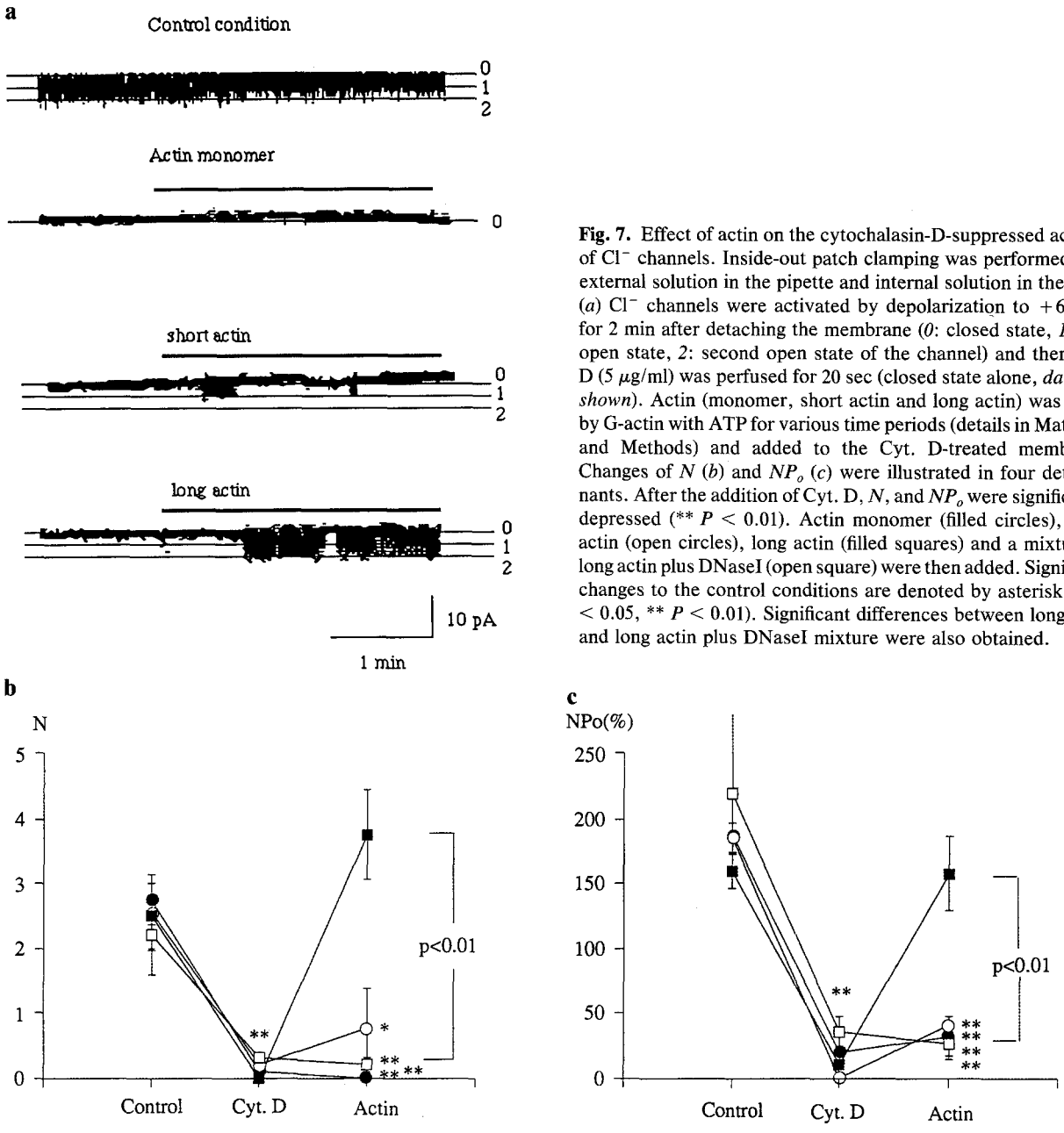


**Fig. 7.** Effect of actin on the cytochalasin-D-suppressed activity of $Cl^-$ channels. Inside-out patch clamping was performed with external solution in the pipette and internal solution in the bath. (*a*) $Cl^-$ channels were activated by depolarization to +60 mV for 2 min after detaching the membrane (*0*: closed state, *1*: first open state, *2*: second open state of the channel) and then Cyt. D (5 μg/ml) was perfused for 20 sec (closed state alone, *data not shown*). Actin (monomer, short actin and long actin) was made by G-actin with ATP for various time periods (details in Materials and Methods) and added to the Cyt. D-treated membrane. Changes of $N$ (*b*) and $NP_o$ (*c*) were illustrated in four determinants. After the addition of Cyt. D, $N$, and $NP_o$ were significantly depressed (** $P < 0.01$). Actin monomer (filled circles), short actin (open circles), long actin (filled squares) and a mixture of long actin plus DNaseI (open square) were then added. Significant changes to the control conditions are denoted by asterisks (* $P < 0.05$, ** $P < 0.01$). Significant differences between long actin and long actin plus DNaseI mixture were also obtained.

membrane, and that the Cyt. D causes the channel molecules to be endocytosed. Along these lines, cytoplasmic vesicles in the renal tubule have been shown to contain a $Cl^-$ channel and to be responsive to cAMP [1]. However, since Cyt. D had an inhibitory effect on the $Cl^-$ channel in isolated membrane patches, it seems unlikely that the observed Cyt. D effect involves the transport of cytoplasmic vesicles along microfilaments.

Acute changes in membrane conductance usually reflect changes in the $P_o$ of the channel [9], whereas chronic changes in membrane conductance are usually the result of a change in the number of opening channels in the membrane [32]. Acidification, however, rapidly blocks the $K^+$ channel in the diluting segment of the renal tubule, and also exerts its effect by altering channel density [10]. Actin filaments regulate epithelial $Na^+$ channel activity by changing the number of open channels [13]. Therefore, acute changes in the number of open channels might underlie the physiologic regulation of ion channels. Interestingly, F-actin itself has been shown to activate the $Na^+$ channel in renal epithelial cell line A6. In contrast to the findings described here, both Cyt. D and short actin were found to activate, and DNaseI to inhibit the $Na^+$ channel [13].

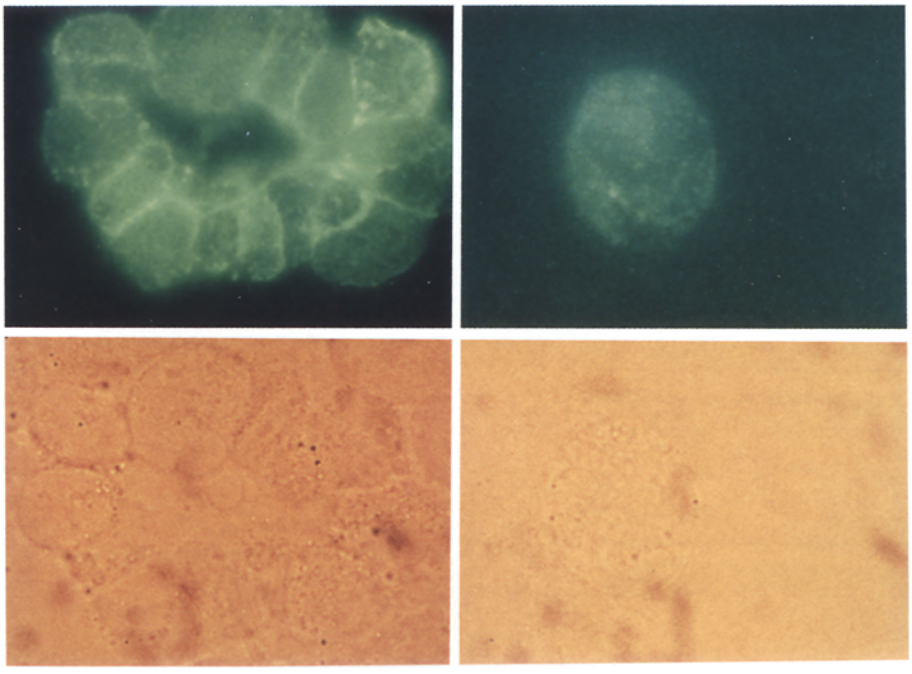

**Fig. 8.** Effect of cytochalasin D on cytoskeletal F-actins stained by NBD-phallicidin (400×). Phallicidin-stained F-actin networks extended to the peripheral margin of control. (Left: top, fluorescence; bottom, microscopy). The peripheral margin partly disappeared after exposure of the cells to Cyt. D for 10 sec (Right: top, fluorescence; bottom, microscopy). Although the right photograph contains at least two to three cells, only one cell was stainable.

The authors thus conclude that short actin directly regulated the activity of the $Na^+$ channel. Individual channels may, in fact, have distinct and diverse interaction with cytoskeletal microfilaments.

It has been postulated that the transport of $Cl^-$ is regulated by actin [26]. This hypothesis is based on the finding that the intestinal fluid secretion stimulated by cholera toxin is inhibited by cytoskeleton-disrupting agents [26]. Recently, a novel study by Shapiro et al. [23] revealed that stabilization of F-actin prevents cAMP-elicited secretion of $Cl^-$ in human intestinal epithelial cells. According to their observations, the depolymerization of actin inhibited the transport of $Cl^-$. However, in our study the depolymerization of actin by DNaseI did not inhibit the $Cl^-$ channel present in renal proximal tubule epithelia. Since in their investigation the cAMP-activated $Cl^-$ secretion occurred in the basolateral membrane of the intestinal cell, and we studied the $Cl^-$ channel present in the apical membrane of renal epithelia, it is possible that the mechanism of regulation of $Cl^-$ channel activation differs between apical and basolateral membranes.

There is growing evidence that actin polymerization is regulated by the second messengers, protein kinase A and diacylglyceride. It is therefore of interest that the regulation of the $Cl^-$ channel also appears to involve signal transduction. The possibility that both phenomena are connected needs to be addressed in future studies.

In conclusion, the $Cl^-$ channel present in the apical membrane of renal proximal epithelia interacts with the F-actin networks found on the cytoplasmic face of the plasma membrane. This interaction may be a physiologically or pathophysiologically important component of $Cl^-$ channel regulation.

We would like to thank T. Tamatsukuri for technical support. This study was presented to the American Society of Nephrology, Baltimore, 1991.

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