Biochemical and Biophysical Research Communications **282,** 341–350 (2001)
doi:10.1006/bbrc.2001.4554, available online at http://www.idealibrary.com on IDEAL®

Polycystin-2 Is a Novel Cation Channel Implicated in Defective Intracellular Ca^{2+} Homeostasis in Polycystic Kidney Disease

Peter M. Vassilev,*[,1,2] Lei Guo,†[,1] Xing-Zhen Chen,†[,1] Yoav Segal,† Ji-Bin Peng,† Nuria Basora,† Hermik Babakhanlou,† Gabrielle Cruger,† Marie Kanazirska,* Chian-ping Ye,* Edward M. Brown,* Matthias A. Hediger,† and Jing Zhou†[,2]

†*Renal Division and* **Endocrine–Hypertension Division and Membrane Biology Program, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115*

Received February 19, 2001

Mutations in polycystins-1 and -2 (PC1 and PC2) cause autosomal dominant polycystic kidney disease (ADPKD), which is characterized by progressive development of epithelial renal cysts, ultimately leading to renal failure. The functions of these polycystins remain elusive. Here we show that PC2 is a Ca^{2+}-permeable cation channel with properties distinct from any known intracellular channels. Its kinetic behavior is characterized by frequent transitions between closed and open states over a wide voltage range. The activity of the PC2 channel is transiently increased by elevating cytosolic Ca^{2+}. Given the predominant endoplasmic reticulum (ER) location of PC2 and its unresponsiveness to the known modulators of mediating Ca^{2+} release from the ER, inositol-trisphosphate (IP_3) and ryanodine, these results suggest that PC2 represents a novel type of channel with properties distinct from those of the other Ca^{2+}-release channels. Our data also show that the PC2 channel can be translocated to the plasma membranes by defined chemical chaperones and proteasome modulators, suggesting that *in vivo,* it may also function in the plasma membrane under specific conditions. The sensitivity of the PC2 channel to changes of intracellular Ca^{2+} concentration is deficient in a mutant found in ADPKD patients. The dysfunction of such mutants may result in defective coupling of PC2 to intracellular Ca^{2+} homeostasis associated with the pathogenesis of ADPKD.

PC2 is an ~110-kDa integral membrane protein (Fig. 1a) that is homologous to an ~400-residue hydrophobic region of PC1 (1, 2), to voltage-activated Ca^{2+} channels, and transient receptor potential (TRP) channel subunits. PC1 and PC2 are expressed in kidney and in many other tissues but not always in the same structures or same cell types (3–7). PC2 appears to be able to oligomerize and to interact with PC1 *in vitro* (8, 9) but its physiological function is unknown. Homozygous mice with either PC1 or PC2 mutations die perinatally with massive cystic degeneration of the kidneys and pancreas (10, 11). Homozygous PC2 mice also develop heart defects (12). In the present study we describe the functional expression of PC2 in oocytes. We show that PC2 is a Ca^{2+}-permeable cation channel with properties distinct from any known intracellular or plasma membrane channels. Its regulation by changes in the level of intracellular Ca^{2+} is disturbed in a mutant found in patients with ADPKD.

METHODS

Pkd2 cDNA cloning and RNA preparation. The mouse Pkd2 cDNA was first isolated by RT-PCR mouse kidney RNA using degenerate human PKD2 primers. The most 5′ end of the cDNA was isolated from a genomic P1 clone. The R742X mutation was introduced by PCR-based, site-directed mutagenesis. The PC2 expression construct contains the coding region of mouse Pkd2 (nt 1–2091, GenBank NM_00861), in the pTLN2 vector. This construct was verified by multiple restriction digests and direct sequencing. A C-terminal c-myc epitope tag was introduced by PCR for antibody recognition. Capped RNA was synthesized from linearized templates using the mMessage mMachine *In Vitro* Transcription Kit (Ambion).

Oocyte preparation and expression of PC2 in oocytes. Oocytes at stage V–VI were extracted from *Xenopus laevis* and were defolliculated by treating for ~2 h at 18°C with 2 mg/ml collagenase (Boehringer Mannheim, Mannheim, Germany) in a Ca^{2+}-free solution containing in mM: 90 NaCl, 3 KCl, 0.82 $MgSO_4$, 10 Hepes, pH 7.5. Oocytes were injected on the same day (at least 4 h after defollicu-

[1] These authors contributed equally to this work.

[2] To whom correspondence may be addressed at Harvard Institutes of Medicine, Room 522, 77 Avenue Louis Pasteur, Boston, MA 02115. Fax: 617-525-5830. E-mail: (Peter Vassilev) pvassilev@rics.bwh.harvard.edu or (Jing Zhou) zhou@rics.bwh.harvard.edu.

0006-291X/01 $35.00

lation) or on the following day with 50 nl H_2O containing 50 ng cRNA of Pkd2 or R742X. Equal amounts of H_2O were injected into control oocytes. Injected oocytes were incubated at 18 or 14°C using Barth's solution containing (in mM): 90 NaCl, 2 KCl, 0.82 $MgSO_4$, 0.41 $CaCl_2$, 0.33 $Ca(NO_3)_2$, 10 Hepes, 10 units/ml penicillin and 10 μg/ml streptomycin, pH 7.5.

Immunocytochemistry. Oocytes were embedded in OCT (optimal cutting temperature) compound (VWR, Boston, MA), and were sectioned at 5 μm in a cryostat at −20°C and air-dried for 30 min. Sections were fixed in methanol at −20°C for 10 min and incubated with primary antibody, anti-c-myc (clone 9E10, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), diluted 1/50, for 1 h at room temperature. This was followed by 1 h incubation with secondary Fluorescein Anti-mouse IgG antibodies (Vector Laboratories, Inc., Burlingame, CA). Fluorescence was viewed using a MRI confocal microscope (Bio-Rad, Hercules, CA). No differences between the ion channel activities of c-myc-tagged and untagged PC2 were observed in control electrophysiological studies on oocytes injected with c-myc-tagged PC2.

Electrophysiological measurements. Patch-clamp methodology was employed for measurement of the properties of single ion channels using cell-attached or excised membrane patches (38). The studies on channel activities in intracellular membranes were carried out using a modification of a previously described method (14). After isolating the nuclei from the oocytes, segments of the surrounding membranes were excised using two pipets with large rough tips. Then one edge of each excised membrane segment was attached to the plastic dish by touching the bottom with one of the pipet tips while the rest of the membrane was left to float in the bath solution. This procedure allowed access to both sides of the membrane. Pipette solution contained in mM (unless otherwise specified): 100 KCl, 0.1 $CaCl_2$, 10 Hepes, pH 7.5. In some experiments 100 mM KCl was substituted with 100 mM NaCl or equal amounts of other salts as described in the figure legends. When filled with this external solution, the pipet tip resistances were 5–10 MΩ. Seals with resistances of >10 GΩ were employed in single channel experiments, and currents were measured with an integrating patch-clamp amplifier. Single channel currents were filtered at 3–10 kHz through an 8-pole Bessel filter. The bath solution contained, unless otherwise specified: 100 mM KCl, 0.1 μM $CaCl_2$, 10 mM Hepes, 5 mM EGTA, pH 7.5. The concentrations of Ca^{2+} in solutions containing low Ca^{2+} (0.1–1 μM) were adjusted according to a previous study (39).

Data acquisition and analysis. Voltage stimuli were applied and single channel currents digitized (20–150 μs per point) and analyzed using a PC, a Digidata converter, and programs based on pClamp (Axon Instruments, Foster City, CA). The baseline current was monitored frequently to ensure proper analysis of single channel currents. In all the figures shown, downward deflections represent negative inward currents. Po was calculated from 20- to 30-s segments of current records in patches containing apparently only one functioning channel, since only one current level was observed in these recordings. Several hundred or more events were analyzed using half-amplitude threshold criteria for generating each data point. The Goldman–Hodgkin–Katz (GHK) equation was used to calculate the permeability ratios between a defined cation (P_X) and K^+ (P_K): $E_{rev} = RT/F \ln[P_X/P_K]$, where E_{rev} is the change in reversal potential measured when K^+ is replaced by one of the monovalent cations, Na^+ or NH_4^+, in the pipette solution; R, T, and F, have their usual meaning. The Fatt–Ginsborg equation was used to determine the permeability of Ca^{2+} versus K^+: $E_{rev} = RT/2F \ln 4P_{Ca}[Ca]_o/P_K[K]_I$, where $[Ca]_o$ and $[K]_I$ are the concentrations of Ca^{2+} in the external pipet solution and K^+ in the internal bath solution in studies on inside-out patches, respectively. The experiments were carried out at 23°C. To estimate statistical differences, three or more experiments were performed for each condition.

RESULTS

We isolated and assembled full-length Pkd2 cDNA and expressed it in *Xenopus* oocytes. Ionic currents were measured in oocytes 3–5 days after injections with synthetic RNA (cRNA) encoding full-length PC2. Single channel studies using KCl in the pipette solution revealed the presence of a channel with distinctive bursts of frequent openings and closings in PC2-expressing oocytes (Fig. 1b). Such activity was absent in water-injected oocytes ($n = 45$) and oocytes expressing other proteins ($n = 67$). These channel activities, however, were observed in only 27 of 170 patches of PC2-expressing oocytes (~16%). To determine whether this was due to low levels of PC2 expression, we examined the levels and cellular distribution of PC2 in oocytes by immunocytochemistry with a specific antibody. PC2 was predominantly expressed in intracellular compartments with a relatively small amount on the plasma membrane (Fig. 2). This finding is consistent with a recent report that shows an intracellular localization of PC2, most likely in the ER in cultured mammalian cells (13). Since the outer nuclear membrane is continuous with the ER, we isolated nuclei from PC2- and water-injected oocytes to search for PC2 channel activities in intracellular membranes. Channel activities in their surrounding membranes were studied using a modification of a previously described method (14). The same type of PC2 channel activity observed in the plasma membranes was also found in intracellular membranes (Fig. 1c) but in larger proportion of patches (e.g., 18 of 48 or 38%). No channel activities with similar properties were found in any of the 35 patches obtained from intracellular membranes of water-injected oocytes.

PC2 appears also to be expressed near or on the cell surface in renal tubules *in vivo* (6, 7, 11). A plasma membrane localization would allow PC2 to interact with other polycystins, such as PC1 (3, 8, 9), as well as with other structurally homologous plasma membrane channels. Therefore we examined whether the PC2 protein could be translocated to the plasma membrane under defined conditions, and whether it functions similarly in both plasma and intracellular membranes. Such translocation would greatly simplify the characterization of the channel, since channels on the plasma membrane are more easily accessible for patch-clamping. We treated the oocytes with both chemical chaperone-like factors and proteasome inhibitors to establish optimal conditions for PC2 translocation. Similar approaches have been used successfully elsewhere (15–19). An increase in the level of PC2 expression in the plasma membrane was observed after treatment of oocytes for 24–36 h with the following agents: 5 μM lactacystin, 5 μM *N*-acetyl-leu-leu-norleucinal (ALLN), 10 mM trimethylamine-*n*-oxide (TMAO), and/or 1–7.5% glycerol. Glycerol alone was effective, but better

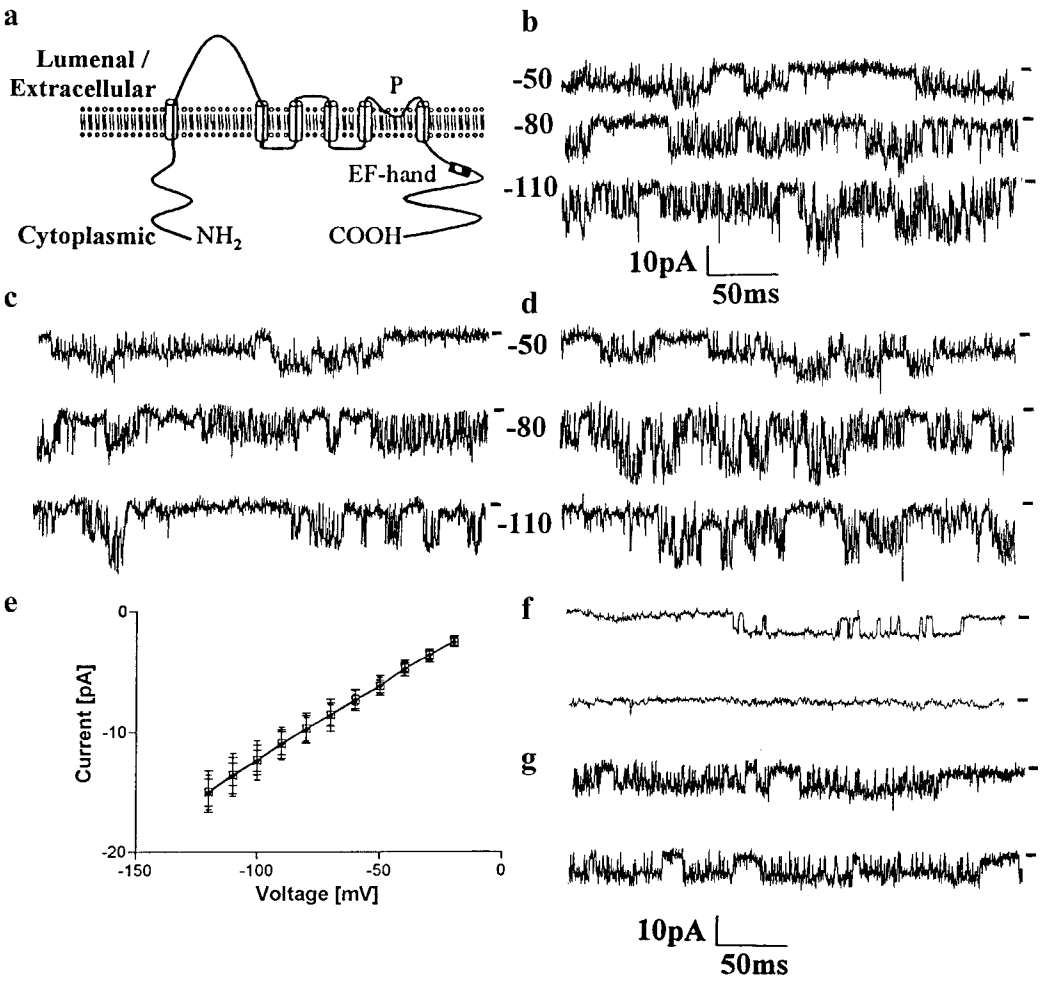

FIG. 1. Identification of PC2 channels in cellular and intracellular membranes. (a) Model of the putative membrane topology of PC2. P denotes the pore region. PC2-mediated channel activities were recorded in cell-attached patches of: (b) plasma membranes of untreated, PC2-injected oocytes; (c) intracellular ER-nuclear membranes of these oocytes; and (d) plasma membranes of PC2-injected oocytes treated for 36 h with a combination of 5% glycerol, 5 μM lactacystin, and 5 μM ALLN at 14°C. Varying voltages are indicated on the left sides of the current sweeps. Downward deflections represent negative inward currents. The dashes on the right sides of the current traces indicate the closed channel current levels. The pipet solution in b–d contained in mM: 100 KCl, 0.1 $CaCl_2$, and 10 Hepes, pH, 7.5.The calibration bars are the same for all the current traces in b–d. The current–voltage relations (e) were similar for the single channel activities measured in b–d. The values for b, c, and d are marked with filled circles, open squares, and crosses, respectively. Each data point in e represents mean ± SEM (n = 5). (f) Endogenous ER K^+ current traces taken at −60 mV in patches excised from ER/nuclear membranes of H_2O-injected oocytes before (top trace) and after addition of 0.5 mM bis-guanidino-*n*-decane (BGD) to the bath solution (bottom trace). (g) PC2 channel current traces taken at −60 mV in patches excised from ER/nuclear membranes of PC2-injected oocytes before (top trace) and after addition of 0.5 mM BGD to the bath solution (bottom trace). In f and g, the solutions on both sides of the patches contained the same concentration of KCl, 100 mM.

results were obtained when it was used in combination with the other agents, particularly with lactacystin. Oocytes were kept at a low temperature (14°C) which, as in earlier studies (18, 20), appears to promote channel translocation to the cell surface. Channel activities with large conductance and characteristic kinetics with frequent closings and openings were observed in 63 of 185 patches (34%) of PC2-injected oocytes treated in this way (Fig. 1d) but never in water-injected oocytes subjected to the same treatments (n = 52). The kinetics, conductance (Figs. 1b–1e), and other characteristics of PC2 channels in the oocytes treated with the agents described above were similar to those in intracellular membranes of untreated oocytes, showing that these procedures did not modify the channel properties. The translocation of PC2 protein to the cell surface after the treatments described above can be detected by immunocytochemistry (Fig. 2).

None of the endogenously expressed Cl^-, K^+ or other channels in the intracellular membranes displayed properties similar to those of the PC2 channels, including conductance and kinetics. For example, one of these channels was selective for K^+ and showed similarities to previously characterized K^+ channels in ER membranes but its conductance (72+/−6.3 pS, n = 4) was smaller than that of PC2 and it did not show the

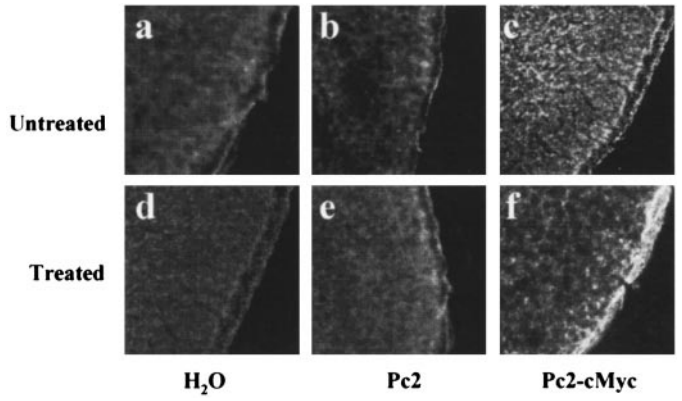

FIG. 2. Immunocytochemistry of H_2O- (a, d), PC2- (b, e), Myc-tagged (c, f) PC2-injected oocytes with and without treatment, with an anti-myc antibody. A predominant intracellular staining is seen in untreated oocytes injected with myc-tagged PC2 (c). A reduction in intracellular staining and an increase in cell membrane staining can be visualised in the treated oocytes (f).

same kinetics and other characteristics. This channel was blocked by an agent, bis-guanidino-*n*-decane (BGD) that has previously been used as an effective blocker of the ER K^+ channels[21] (Fig. 1f) but the same agent did not affect the activity of PC2 (Fig. 1g) confirming that PC2 represents a different type of channel. The conductances and the time-dependent behavior of some Cl^- channels were similar to those described in a previous study on nuclear membranes (22). We also treated the oocytes with actinomycin D (10 μg/ml), a well accepted approach to distinguish cloned channels from endogenous channels, and observed no effects on the PC2 channel activities in intracellular or plasma membranes. All these data support the notion that the observed channel activity does not result from upregulation or modulation of channels endogenous to the oocytes.

PC2-mediated channel activities were observed in the presence of both K^+ and Na^+ in the external pipette solution. The conductance in the presence of 100 mM K^+, however, was 123.8 ± 14.3 pS (mean ± SEM, n = 15), substantially higher than that observed with 100 mM Na^+ (23.4 ± 2.6 pS, n = 8) (Figs. 3a and 3b). The current–voltage relationships and the reversal potentials were determined under bi-ionic conditions (Na^+_o/K^+_i) in inside-out patches and it was found that the inward Na^+ currents are substantially smaller than the outward K^+ currents (Figs. 3b and d). The current–voltage relationships for K^+ were nearly linear from −120 to 60 mV, but there was a slight inward rectification from 70 to 120 mV (Fig. 3d). The outward currents, which are mediated by efflux of K^+, are similar in the presence of K^+ or Na^+ in the pipette solution. The reduced slope conductance for the inward Na^+ currents suggests that the channels may be less permeable to Na^+ than to K^+. The reversal potential was −50 mV and the calculated permeability ratio (P_{Na}/P_K = 0.14) shows that the channel is 7-fold more permeable to K^+ than to Na^+. Since the transport of ammonium is an important function of the kidney, the conductance and permeability of PC2 channel to NH_4^+ were determined. The amplitudes of the inward NH_4^+ currents were slightly smaller than those of K^+ but much larger than those of Na^+ (Fig. 3c). The outward currents due to K^+ efflux were similar in the presence of external K^+ or Na^+ in the pipet solutions. The conductance of NH_4^+, 93.4 ± 10.6 pS (n = 7), was substantially higher than that of Na^+ and slightly smaller than that of K^+ (Fig. 3d). Under bi-ionic conditions (100 mM $K^+_{ext.}$/100 mM $NH^+_{4\,int.}$) the reversal potential was only −8 mV and the calculated permeability ratio was P_{NH4+}/P_{K+} = 0.73.

The conductances of other monovalent cations, Li^+ and Rb^+, have also been determined and compared to those of K^+ and Na^+ in cell-attached patches of PC2-expressing oocytes. The conductance of Li^+ was similar to that of Na^+, 19.4 ± 2.3 pS (n = 3), while that for Rb^+ was at an intermediate level between those of K^+ and Na^+, 67.2 ± 8.6 pS (n = 4) (Figs. 3e–3g). In 11 patches of four separate batches of PC2-injected oocytes, we were unable to resolve any single channel currents with PC2-like characteristics in the presence of 100 mM CsCl in the pipette solution. It is possible that the Cs^+ current amplitudes are small and unresolvable at the single channel level.

To assess the contribution of anion fluxes to the PC2 channel currents we have varied the content of anions and cations in the solutions on both sides of the inside-out patches and found that Cl^- influx or efflux does not contribute substantially to the currents (data not shown).

Sequence analysis suggests the presence of EF hand motif in the C-terminal cytoplasmic tails of both PC2 (Fig. 1a) and polycystin-L (PCL), a close homologue, whose activity is modulated by an increase in the intracellular Ca^{2+} concentration (23). When we examined PC2 channel activity upon increases of intracellular Ca^{2+} concentration (Ca_i) from 0.1 to 1 μM, we observed a transient activation of the channel (Fig. 4a). Multiple channel activities can be seen after increasing the concentration of intracellular Ca^{2+} (Ca_i) from 0.1 to 1 μM in Fig. 4. The Ca^{2+}-induced channel activation includes superimposed channel openings to a second and third channel current levels. Although the probability of PC2 channel opening was substantial, even in the presence of low Ca_i (0.1 μM), it increased approximately 40% within 20–30 s after addition of 1 μM Ca_i (Fig. 4b). This effect, however, was transient and the Po decreased nearly to the basal level 3–5 min after the initial increase. These data show that the basal PC2 channel activity does not require high Ca_i but its sensitivity to changes in the level of Ca_i is substantial. The time course of the changes in Po was followed several minutes after the transient increase in Po to verify

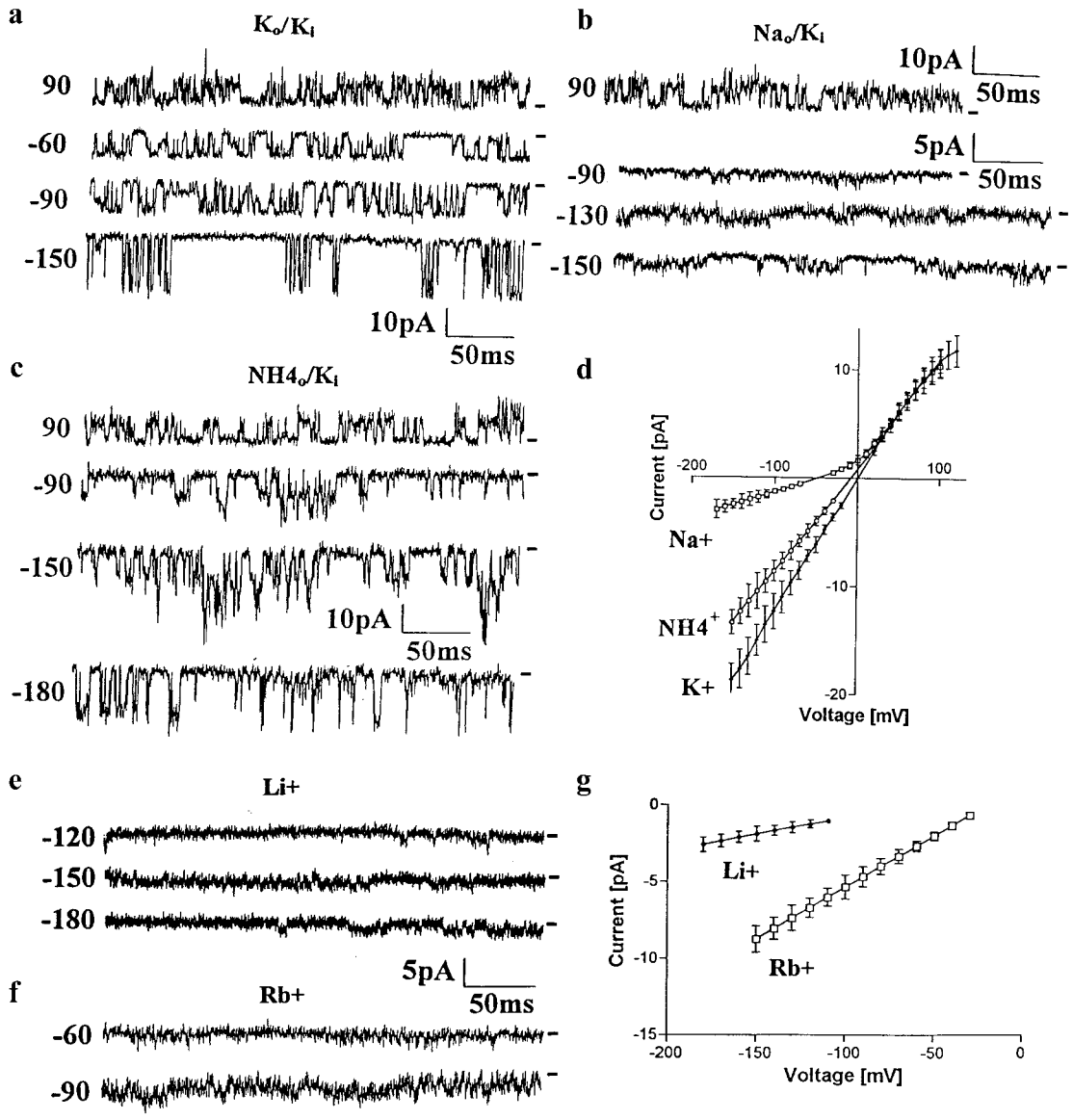

FIG. 3. Higher conductance for K^+ and NH_4^+ than for Na^+ and other monovalent cations of PC2 channels. Current traces were recorded in inside-out patches excised from PC2-injected oocytes in the presence of 100 mM KCl (a), 100 mM NaCl (b) or 100 mM NH_4^+ in the external pipet solution. The internal bath solution in a–c was the same, 100 mM KCl. The current records were taken at different voltages marked on the left side of each trace. Note that the small inward Na^+ currents in b are 2-fold expanded in comparison to the top trace with the outward K^+ currents, and all the traces in a. (c) Current traces taken at the indicated voltages in inside-out patches in the presence of 100 mM NH_4Cl in the external and 100 mM KCl in the internal solution. (d) Current-voltage relations estimated under the three different conditions in a (n = 6), b (n = 4), and c (n = 5). (e and f) Current traces taken in cell-attached patches of PC2-expressing oocytes in the presence of 100 mM LiCl or 100 mM RbCl in the external pipet solution, respectively. The voltages are marked on the left side of each current trace. The calibration bars between the two panels are the same for both e and f. The current–voltage relations for both Li^+ (n = 3) and Rb^+ (n = 4) are shown in g. The dashes on the right or left sides of the current traces indicate the closed channel current levels.

whether there are fluctuations in channel activity during this modulation of the channel. We observed a similar effect of Ca_i on PCL in our previous study (23). The transient increase in Po of the PCL channel, however, was more pronounced but several minutes later the channel activity decreased to a level below the basal level. We attributed this effect to channel inactivation or desensitization which may be related to defined oscillatory physiological processes inside the cells. No such desensitization of the PC2 channel, however, was observed.

We used a PC2 mutant originally identified from an ADPKD family (1605) in which arginine 742 was substituted with a stop codon, to further characterize the essential channel components and responsiveness of PC2 channel to intracellular Ca^{2+}. This mutant preserves all six putative transmembrane domains but lacks the C-terminus of PC2 that contains the EF-hand

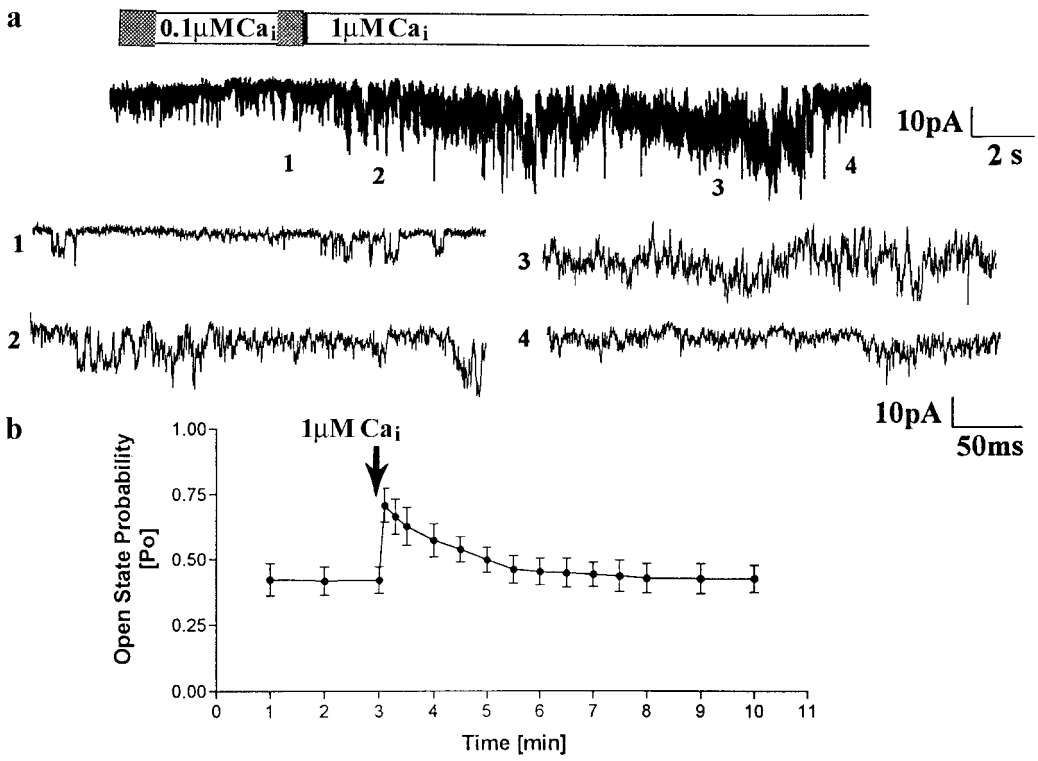

FIG. 4. Modulation of channel activity by intracellular Ca^{2+}. (a) Single channel activities at −90 mV in inside-out patches were initially recorded in the presence of 0.1 μM Ca_i. Increasing Ca_i to 1 μM elicited transient channel activation. Selected segments from the top trace marked with 1–4 are shown at the bottom at expanded time and current scales. (b) Time course of the changes in Po mediated by the increase in Ca_i. Po was calculated for 30-s segments of single channel current records taken before and after raising Ca_i ($n = 5$). The increase in Ca_i from 0.1 to 1 μM is marked with an arrow. The dashes on the right sides of the current traces indicate the closed channel current levels. The solutions on both sides of the patches contained the same concentration of KCl, 100 mM (see Methods for more details).

motif. We examined its unitary conductance, modulation by changes in the level of intracellular Ca^{2+} and other channel characteristics (Fig. 5). Its kinetics and current–voltage relationship (Figs. 5a and 5b) were similar to those of the wild-type PC2. Such channel activities were observed in 23 (24.5%) of 94 patches of oocytes expressing the PC2 mutant. The unitary conductance (116.2 ± 9.5 pS, $n = 6$) was slightly smaller than that of the wild-type PC2 but an increase of Ca_i from 0.1 to 1.0 μM failed to mediate significant activation of the PC2 mutant channel (Figs. 5c and 5d). The second current trace with higher basal activity shown in Fig. 5c confirms that at both low and higher basal levels of activity the mutant PC2 channel cannot be activated by elevation in Ca_i. This defective regulation by Ca_i is probably due to the missing Ca^{2+}-binding EF-hand at the C-terminus of PC2, and provides an example of how mutations in PKD2 cause disturbances in cation transport and Ca^{2+} signaling and homeostasis in ADPKD.

We also studied PC2 -mediated currents in the presence of divalent cations. The current amplitudes measured at defined voltages (Figs. 6a–6c) and the current–voltage relationships (Fig. 6d) for Ca^{2+} are slightly different from those for Sr^{2+} and Ba^{2+}. The I–V curve for Ca^{2+} was linear at low voltages, −30 to −80 mV, but it deviated from linearity at more negative voltages (Fig. 6d). This voltage dependence was less pronounced for the other divalent cations. The Ca^{2+} conductance, measured over the linear voltage range (−30 to −80 mV), 36.4 ± 3.8 pS ($n = 5$), was slightly smaller than those of Sr^{2+} (42.3 ± 3.5 pS, $n = 3$) and Ba^{2+} (46.5 ± 4.2 pS, $n = 4$). The permeability ratio, $P_{Ca^{2+}}/P_{K^+}$, determined from studies on inside-out patches, is 0.21, which is higher than that of P_{Na^+}/P_{K^+}. This permeability ratio and the conductances of the PC2 channels for divalent cations were similar in intracellular and plasma membranes. The Ca^{2+} conductance of the PC2 mutant was similar to that of the wild type PC2 (data not shown). The presence of millimolar concentrations of Ca^{2+} as well as of Mg^{2+} in the external pipette solution, however, reduced the PC2 channel currents with K^+ or other monovalent cations as charge carriers. The blocking effects of these divalent cations are similar to those of other types of Ca^{2+}-permeable channels and may play a role in preventing excessive Ca^{2+} influx through the channels *in vivo*. It should be pointed out that the relative permeabilities of Ca^{2+} and other monovalent cations to PC2 channel *in vivo* may be somewhat different from what we mea-

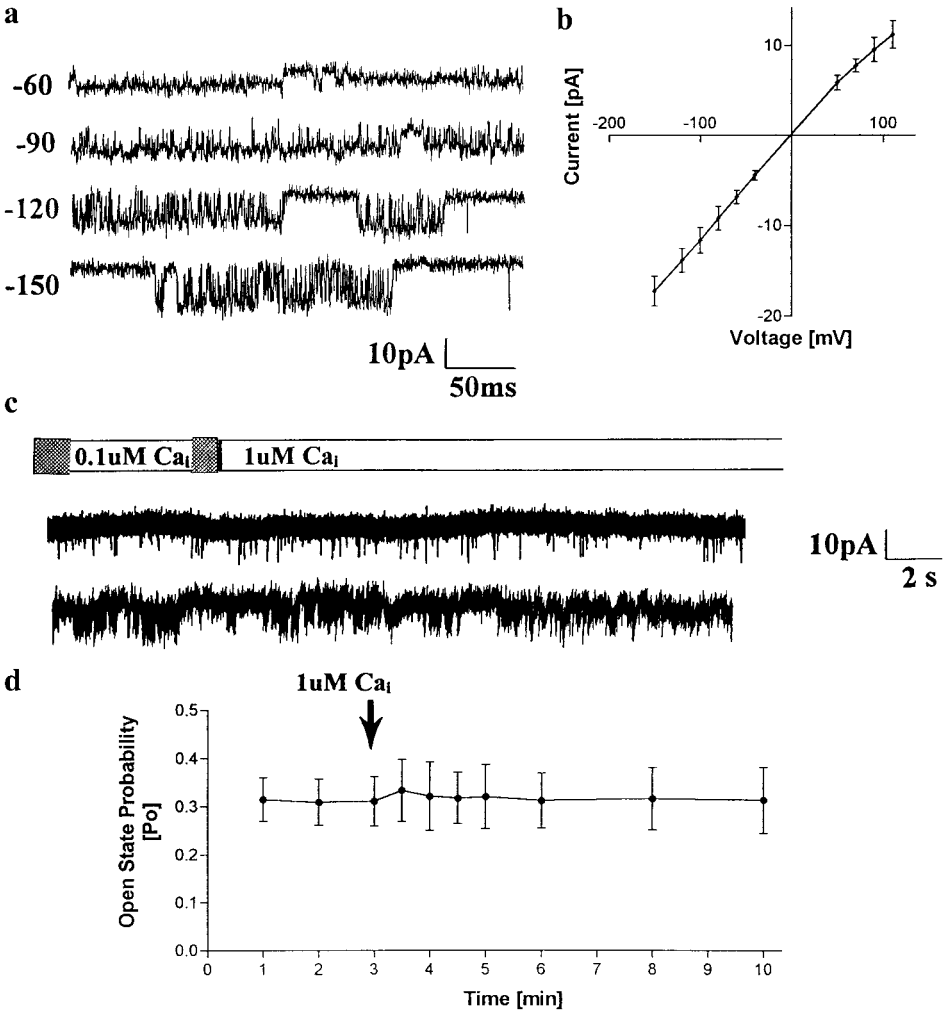

FIG. 5. Single channel properties of the PC2 mutant, R742X. (a) Current traces were recorded in inside-out patches excised from R742X-injected oocytes in the presence of 100 mM KCl on both sides of the membrane. The current records were taken at different voltages marked on the left side of each trace. (b) Current–voltage relations were determined under the conditions described in a (n = 6). (c) Two current traces with low (top trace) or higher (bottom trace) level of basal activity were taken in separate patches excised from R742X-injected oocytes. Single channel activities at −90 mV in inside-out patches were initially recorded in the presence of 0.1 μM Ca_i. Increasing Ca_i to 1 μM did not mediate substantial channel activation in both patches. (d) Po was calculated for 30-s segments of single channel current records taken before and after raising Ca_i (n = 4). The increase in Ca_i from 0.1 to 1 μM is marked with an arrow that refers to both top and bottom traces. The solutions on both sides of the patches contained the same concentration of KCl, 100 mM.

sured here since the high concentration of Ca^{2+} (50 mM) used to resolve the small currents at the single channel level probably additionally inhibits the channel. The open state probability of PC2 channel was also reduced by application of extracellular La^{3+} (0.2 mM).

We did not observe any effects of nifedipine, a blocker of the plasma membrane voltage-gated Ca^{2+} channels (Fig. 6e) as well as of ryanodine and IP_3 (Figs. 6f and 6g) on PC2 channel activity and properties suggesting that PC2 represents a different type of channel.

DISCUSSION

There is a preponderance of evidence for localization of PC2 within the ER in cultured cells (Ref. 13 and Fig. 1) as well as in some cell types in tissues[24]. These data distinguish PC2 from PCL not only by a characteristic kinetic behavior and relative permeabilities to cations but also in regard to membrane location. Given its location in the ER, and a Ca^{2+} conductance (36 pS) that is similar to IP_3 receptors (26–53 pS in 50 mM Ca^{2+}) (25, 26), PC2 likely contributes to Ca^{2+} release from intracellular stores as do the ryanodine and IP_3 receptors, the two known types of intracellular Ca^{2+} release channels. Because PC2 does not bear structural or topological similarities to these Ca^{2+} release channels and is not sensitive to IP_3 and ryanodine (this study), it may function as a novel type of Ca^{2+} release channel with distinct properties and may not only be sensitive to the levels of Ca_i but may also contribute to the regulation of intracellular Ca^{2+} homeostasis.

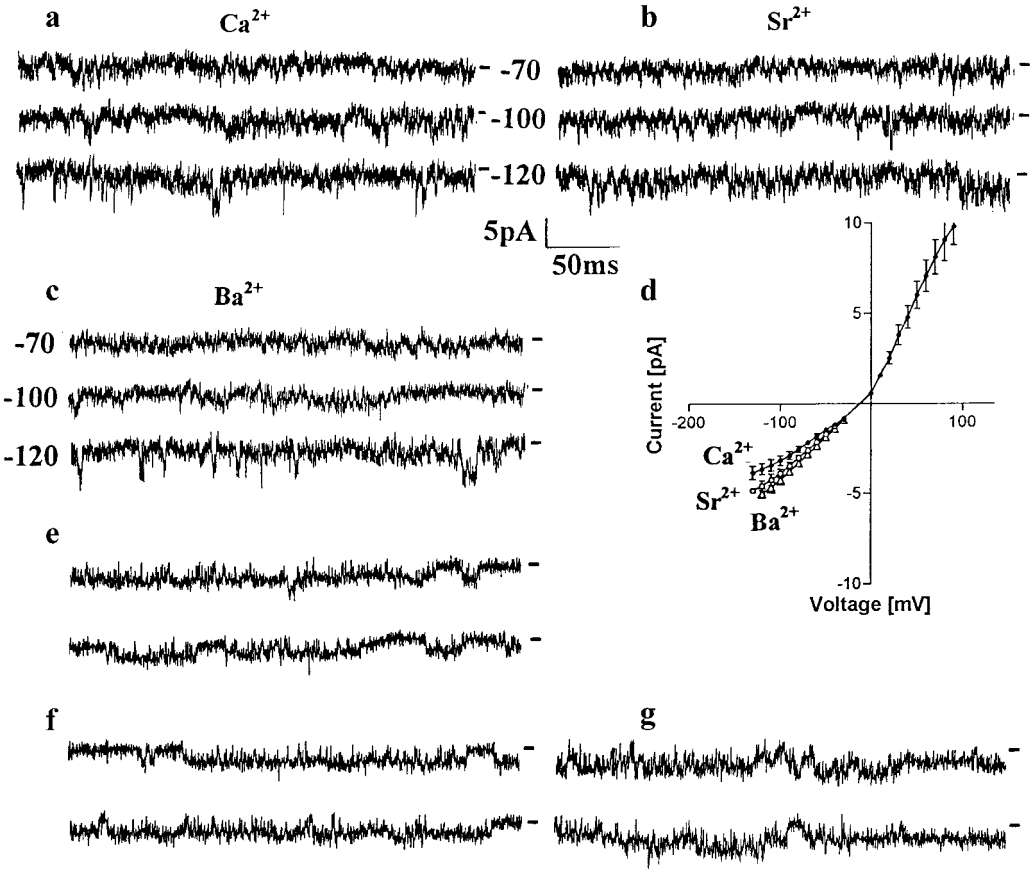

FIG. 6. Divalent cations as charge carriers. Single channel currents recorded in inside-out patches in the presence of 50 mM $CaCl_2$ (a), $SrCl_2$ (b), or $BaCl_2$ (c) in the external pipette solution, respectively. The calibration bars are the same for a–c and e–g. The dashes on the right or left sides of the current traces indicate the closed channel current levels. The current–voltage relations for Ca^{2+} ($n = 6$), Sr^{2+} ($n = 4$), and Ba^{2+} ($n = 4$) are shown in d. The data for Ca^{2+} including those for the outward currents were measured in inside-out patches in the presence of 50 mM $CaCl_2$ in the external solution and 100 mM KCl in the internal solution. (e) Current traces taken in the absence (top trace) or presence of 10 μM nifedipine (bottom trace) in patches formed on the plasma membranes of PC2-injected oocytes. (f) Current traces taken before (top trace) or after addition of 20 μM ryanodine (bottom trace) in patches of the ER/nuclear membranes of PC2-injected oocytes. (g) Current traces taken before (top trace) or after addition of 20 μM IP_3 (bottom trace) in patches of the ER/nuclear membranes. The traces in e–g have been taken at −110 mV.

PC2, IP_3 and ryanodine receptors all have higher conductances to K^+ than to Ca^{2+} (27) and are sensitive to changes in Ca_i. Both PC2 and ryanodine receptor are not modulated by IP_3 but can be activated by increases in Ca_i associated with physiological or pathophysiological processes. With its high K^+ conductance PC2 may also contribute to K^+ transport across the membranes of the Ca^{2+}-storing organelles. Recent reports support the importance of K^+ influx pathways in the ER that are coupled to Ca^{2+} release as part of a highly cooperative ion-exchange mechanism(28). Counter-currents carried by K^+ probably maintain the electroneutrality of the ER membrane system during Ca^{2+} release. PC2 channel may play a key role in such a process.

As an alternative possibility PC2 may not be highly functional in ER, if it is inactivated by defined intracellular factors but it may be involved in other ion transport functions after its translocation to the plasma membrane due to hormonal stimulation or binding to other proteins. Cytoplasmic and cell membrane localizations have both been reported for PC2 (6, 7, 11, 24, 29). There are precedents for channels residing in the ER that, under defined conditions, are translocated to and can function appropriately as plasma membrane channels. For example, the epithelial Na^+ channel (ENaC) is predominantly located on ER membranes, but depending on defined turnover mechanisms and other factors, it can play an important role in the plasma membranes of renal and other cells in mediating Na^+ reabsorption. Proteasome inhibitors and other agents that inhibit protein degradation have been used to promote its intermembrane trafficking (19). If PC2 is translocated by defined factors to the cell surface in some cell types, with its relatively high conductance for cations it may contribute substantially

to the regulation of mineral ion homeostasis and acid-base balance.

A channel (SVC) previously characterized in renal cortical tubule cell lines transformed with SV40 (30) displays remarkably similar properties to those of PC2. This cell line displayed features of thick ascending limb cells, the predominant site of PC2 expression in adult kidney (7). It is interesting to note that SVC was active only in SV40-transformed cells (30). Since the T/t common domain of SV40 can functionally substitute for defined molecular chaperones (31, 32), it is likely that the effect of SV40 transformation was to translocate a channel normally resident in the cytoplasm to the cell surface where its activity could be observed. An intracellular location of SVC provides a further point of similarity with PC2. That transgenic mice carrying SV40 large T antigen develop polycystic kidneys gives additional evidence that abnormal intracellular Ca^{2+} homeostasis may have a role in the pathogenesis of ADPKD.

Two very recent studies on polycystin-2 (PC2) functionally expressed in other membrane systems were described (40, 41). In the first study (40) only a whole-cell current configuration on CHO cells transiently transfected with polycystin 1 (PC1) and/or PC2 was employed. Large amplitude, cation-permeable currents were observed in cells co-transfected with both polycystins. These channels were permeable to Na^+, Ca^{2+} and other cations. These and other results are compatible with ours, but the single channel approach employed by us permitted a detailed comparison, for the first time, of the properties of PC2 in both intracellular and plasma membranes and enabled us to show defective regulation of the PC2 mutant by intracellular Ca^{2+}. We have also presented the first evidence that translocation of PC2 from ER to plasma membrane can be stimulated by modulators of proteasome and chaperone mechanisms. These mechanisms could potentially be regulated by various hormones and intracellular messengers and could be as important as PC1 in this regard, or they could act in concert with PC1 in regulating the distribution of PC2. Although at the present stage the expression patterns of PC1 and PC2 are somewhat controversial, there is ample evidence that PC1 and PC2 are not always expressed in the same structures or cell types (3, 5, 7, 29, 42, 43). The second study (41) employed quite different approaches from ours or those used in the other previously published study. The PC2 channels were characterized as Ca^{2+}-permeable cation channels. They were studied in membrane vesicles isolated from placental syncytiotrophoblasts which were reconstituted in artificial lipid bilayers as well as in SF9 insect cells transfected with PC2. One or two of the main conductances were similar to those of PCL that we described in our previous study (23) or to those of PC2 in our present study. Thus there are similarities but also differences between our findings and those described in this study. Some of the differences may be explained by the different approaches, experimental conditions and expression systems used in these studies. The advantages of our approaches allowed us to characterize for the first time: (1) the unique properties and operation of PC2 in both plasma and intracellular native membranes; (2) the stimulation of its translocation from ER to the plasma membranes by chemical chaperones and proteasome modulators; (3) the striking similarities between the sequence of cation conductances and other properties of PC2 and a previously described channel in renal thick ascending limb which could help identifying the physiological counterpart of PC2 in kidney; and (4) the defective function and regulation by Ca^{2+} of the R742X PC2 mutant.

The potential roles of PC2 in ion homeostasis probably differ from those of other types of ion channels such as CFTR and ENaC, mutations in which cause cystic fibrosis and Liddle's syndrome, respectively (33, 34). Via its Ca^{2+} permeability and sensitivity to changes in intracellular calcium concentration, PC2 may take part in the control of many cell functions including cell proliferation, differentiation and survival (35–37). Abnormalities in each of these cell functions are features of the pathogenesis of ADPKD. The deficient regulation by intracellular Ca^{2+} of the mutant form of PC2, described in the present study, suggests that dysfunctional coupling between PC2 activity and Ca^{2+} signaling may underlie the development of this common genetic disorder. Detailed analysis of PC2 channel and its mutant form in this study opens up novel avenues for the invention of therapeutic strategies for ADPKD.

ACKNOWLEDGMENTS

We thank Dr. Stephen T. Reeders for discussion and assistance in manuscript preparation. X.-Z.C. is recipient of the International Human Frontier Science Program, Long-Term Fellowship. This work is supported by the Polycystic Kidney Research Foundation, NARSAD and Stanley Foundation (to P.M.V.), NIH (to J.Z., E.M.B., and M.A.H). and the St. Giles Foundation (to E.M.B.).

REFERENCES

1. Mochizuki, T., *et al.* (1996) PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* **272,** 1339–1342.
2. Schneider, M. C., *et al.* (1996) A gene similar to PKD1 maps to chromosome 4q22: A candidate gene for PKD2. *Genomics* **38,** 1–4.
3. Geng, L., *et al.* (1996) Identification and localization of polycystin, the PKD1 gene product. *J. Clin. Invest* **98,** 2674–2682.
4. Geng, L., *et al.* (1997) Distribution and developmentally regulated expression of murine polycystin. *Am. J. Physiol* **272,** F451–F459.
5. Ward, C. J., *et al.* (1996) Polycystin, the polycystic kidney disease

1 protein, is expressed by epithelial cells in fetal, adult, and polycystic kidney. *Proc. Natl. Acad. Sci. USA* **93,** 1524–1528.

6. Ong, A. C., *et al.* (1999) Coordinate expression of the autosomal dominant polycystic kidney disease proteins, polycystin-2 and polycystin-1, in normal and cystic tissue. *Am. J. Pathol.* **154,** 1721–1729.
7. Markowitz, G. S., *et al.* (1999) Polycystin-2 expression is developmentally regulated. *Am. J. Physiol* **277,** F17-F25.
8. Qian, F., *et al.* (1997) PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nat. Genet.* **16,** 179–183.
9. Tsiokas, L., Kim, E., Arnould, T., Sukhatme, V. P., and Walz, G. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 6965–6970.
10. Lu, W., *et al.* (1997) Perinatal lethality with kidney and pancreas defects in mice with a targetted Pkd1 mutation. *Nat. Genet.* **17,** 179–181.
11. Wu, G., *et al.* (1998) Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell* **93,** 177–188.
12. Wu, G., *et al.* (2000) Cardiac defects and renal failure in mice with targeted mutations in Pkd2. *Nat. Genet.* **24,** 75–78.
13. Cai, Y., *et al.* (1999) Identification and characterization of polycystin-2, the PKD2 gene product. *J. Biol. Chem.* **274,** 28557–28565.
14. Mak, D. O., and Foskett, J. K. (1994) Single-channel inositol 1,4,5-trisphosphate receptor currents revealed by patch clamp of isolated *Xenopus* oocyte nuclei. *J. Biol. Chem.* **269,** 29375–29378.
15. Ward, C. L., Omura, S., and Kopito, R. R. (1995) Degradation of CFTR by the ubiquitin-proteasome pathway. *Cell* **83,** 121–127.
16. Jensen, T. J., *et al.* (1995) Multiple proteolytic systems, including the proteasome, contribute to CFTR processing. *Cell* **83,** 129–135.
17. Sato, S., Ward, C. L., Krouse, M. E., Wine, J. J., and Kopito, R. R. (1996) Glycerol reverses the misfolding phenotype of the most common cystic fibrosis mutation. *J. Biol. Chem.* **271,** 635–638.
18. Brown, C. R., Hong-Brown, L. Q., and Welch, W. J. (1997) Correcting temperature-sensitive protein folding defects. *J. Clin. Invest* **99,** 1432–1444.
19. Valentijn, J. A., Fyfe, G. K., and Canessa, C. M. (1998) Biosynthesis and processing of epithelial sodium channels in *Xenopus* oocytes. *J. Biol. Chem.* **273,** 30344–30351.
20. Denning, G. M., *et al.* (1992) Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive [see comments]. *Nature* **358,** 761–764.
21. Wang, J., and Best, P. M. (1994) Characterization of the potassium channel from frog skeletal muscle sarcoplasmic reticulum membrane. *J. Physiol.* **477,** 279–290.
22. Tabares, L., Mazzanti, M., and Clapham, D. E. (1991) Chloride channels in the nuclear membrane. *J. Membr. Biol.* **123,** 49–54.
23. Chen, X. Z., *et al.* (1999) Polycystin-L is a calcium-regulated cation channel permeable to calcium ions [see comments]. *Nature* **401,** 383–386.
24. Obermuller, N., *et al.* (1999) The rat pkd2 protein assumes distinct subcellular distributions in different organs. *Am. J. Physiol* **277,** F914-F925.
25. Maeda, N., *et al.* (1991) Structural and functional characterization of inositol 1,4,5-trisphosphate receptor channel from mouse cerebellum. *J. Biol. Chem.* **266,** 1109–1116.
26. Bezprozvanny, I., and Ehrlich, B. E. (1994) Inositol (1,4,5)-trisphosphate (InsP3)-gated Ca channels from cerebellum: conduction properties for divalent cations and regulation by intraluminal calcium. *J. Gen. Physiol* **104,** 821–856.
27. Coronado, R., Morrissette, J., Sukhareva, M., and Vaughan, D. M. (1994) Structure and function of ryanodine receptors. *Am. J. Physiol* **266,** C1485-C1504.
28. Nguyen, T., Chin, W. C., and Verdugo, P. Role of Ca^{2+}/K^{+} ion exchange in intracellular storage and release of Ca^{2+}. (1998) *Nature* **395,** 908–912.
29. Foggensteiner, L., *et al.* (2000) Cellular and subcellular distribution of polycystin-2, the protein product of the PKD2 gene. *J. Am. Soc. Nephrol.* **11,** 814–827.
30. Teulon, J., Ronco, P. M., and Vandewalle, A. (1994) Renal cells transformed with SV40 contain a high-conductance calcium-insensitive potassium channel. *Am. J. Physiol* **267,** C940–C945.
31. Kelley, W. L., and Georgopoulos, C. (1997) The T/t common exon of simian virus 40, JC, and BK polyomavirus T antigens can functionally replace the J-domain of the *Escherichia coli* DnaJ molecular chaperone. *Proc. Natl. Acad. Sci. USA* **94,** 3679–3684.
32. Stubdal, H. *et al.* (1997) Inactivation of pRB-related proteins p130 and p107 mediated by the J domain of simian virus 40 large T antigen. *Mol. Cell Biol.* **17,** 4979–4990.
33. Sheppard, D. N., and Welsh, M. J. (1999) Structure and function of the CFTR chloride channel. *Physiol Rev.* **79,** S23-S45.
34. Shimkets, R. A., *et al.* (1994) Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell* **79,** 407–414.
35. Berridge, M. J., Bootman, M. D., and Lipp, P. (1998) Calcium—A life and death signal [news]. *Nature* **395,** 645–648.
36. Calvet, J. P. (1993) Polycystic kidney disease: Primary extracellular matrix abnormality or defective cellular differentiation? *Kidney Int.* **43,** 101–108.
37. Woo, D. (1995) Apoptosis and loss of renal tissue in polycystic kidney diseases. *N. Engl. J. Med.* **333,** 18–25.
38. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391,** 85–100.
39. Tsien, R., and Pozzan, T. (1989) Measurement of cytosolic free Ca^{2+} with quin2. *Methods Enzymol.* **172,** 230–262.
40. Hanaoka, K., Qian, F., Boletta, A., Bhunia, A. K., Piontek, K., Tsiokas, L., Sukhatme, V. P., Guggino, W. B., and Germino, G. G. (2000) Co-assembly of polycystin-1 and –2 produces unique cation-permeable currents. *Nature* **408,** 990–994.
41. Gonzalez-Perrett, S., Kim, K., Ibarra, C., Damiano, A. E., Zotta, E., Batelli, M., Harris, P. C., Rasin, I. L., Arnaout, M. A., and Cantiello, H. F. (2001) Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca^{2+}-permeable nonselective cation channel. *Proc. Natl. Acad. Sci. USA* **98,** 1182–1187.
42. Griffin, M. D., O'Sullivan, D. A., Torres, V. E., Grande, J. P., Kanwar, Y. S., Kumar, R. (1997) Expression of polycystin in mouse metanephros and extra-metanephric tissues. *Kidney Int.* **52,** 1196–205.
43. Van Adelsberg, J., Chamberlain, S., D'Agati, V. (1997) Polycystin expression is temporally and spatially regulated during renal development. *Am. J. Physiol.* **272,** F602–609.