Cation channel regulation by COOH-terminal cytoplasmic tail of polycystin-1: mutational and functional analysis

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The 4,303-amino acid PKD1 polypeptide has a large, NH2-terminal multi-domain extracellular region, a polytopic transmembrane domain of uncertain topographical disposition often modeled with 9 to 11 transmembrane spans, and a 226-amino acid COOH-terminal cytoplasmic domain (1, 20). The PKD1 transmembrane domain exhibits limited sequence relatedness with voltage-dependent Ca2+-channel (CaV) and transient receptor potential (TRP) channel polypeptides, but so far has not been found itself to mediate channel activity (1, 11, 47). When full-length human PKD1 (hPKD1) was coexpressed and associated with hPKD2 in CHO cells, a novel nonspecific cation current was observed in whole cell recordings, whereas neither polypeptide alone promoted expression of this current (18). However, functional surface expression of PKD2 in insect cells (16) or, in select conditions, in oocytes (43) did not require coexpressed heterologous PKD1. Complementation and cofractionation evidence has supported proposals that native PKD1 exists in complexes with β-catenin (19) and/or, in different cell growth conditions, with α2β1-integrin (46). Moreover, although their direct interaction has not been demonstrated, overexpression of tuberin has been shown to control lateral membrane localization of PKD1 (27).

Although putative ligands for the various extracellular domains remain largely undefined, the structure of an Ig-like PKD1 repeat domain is known (7), and evidence for homophilic interaction of Ig-like repeat domains has been presented (21). In contrast, studies with fusion proteins containing the COOH-terminal cytoplasmic domain have identified numerous binding and signaling functions. This COOH-terminal domain forms a complex with the COOH-terminal cytoplasmic tail of PKD2 (35, 39), which binds in turn to TRPC1 (40). The terminal portion of this cytoplasmic domain, PKD1-(115–226), also binds and stabilizes RGS7 (25),

Autosomal dominant polycystic kidney disease (ADPKD) accounts for ~10% of end-stage renal disease in the North American population. Mutations in the gene encoding polycystin-1 (PKD1) cause 85% of ADPKD. Most of the remaining cases are accounted for by mutations in the gene encoding polycystin-2 (PKD2). The dominant phenotype of ADPKD is the generation and gradual expansion of renal cysts, leading to compression of adjacent normal renal tissue (1, 47). Cysts have been postulated to arise in individuals with one mutant germ line allele from clonal expansion of tubular epithelial cells that have undergone somatic mutation in the germ line wild-type PKD1 allele (36). PKD1 mutations also cause cystic disease of the hepatobiliary tract and up to 10% of all cerebral arterial aneurysms (1).


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which binds, in turn, to 14-3-3 (5). The COOH-terminal domain also activates PKCα and c-Jun-NH₂-terminal kinase-mediated, AP-1-dependent transcriptional events (2, 3), and activates signaling via the Wnt/frizzled pathway. This latter event stabilizes the polycystin-1 binding protein β-catenin, which activates in turn TCF/LEF family transcription factors (26). Additional reported activities include activation of Goα and Goi proteins (32) and phosphorylation on serine and threonine residues (15, 29).

We have previously reported that expression in Xenopus oocytes of the fusion protein CD16.7-PKD1-(115–226), encoding the last 112 amino acids of the PKD1 COOH-terminal cytoplasmic tail, increased the magnitude of nominally endogenous Ca²⁺-permeable cation currents. These currents were accompanied by unitary sodium conductance events of 18–20 pS and unitary calcium conductance events of 11 pS (41). These currents differ from the larger unitary sodium conductances reported for PKD2 in insect cells and in lipid bilayers (16) or for PKD2 (43) or the homologous polypeptide PKDL in Xenopus oocytes (8). Interestingly, however, PKD2 has exhibited numerous and complex apparent subconductance states (16, 43), and unitary conductance of PKD2 was observed to change in the setting of the disease-associated COOH-terminal cytoplasmic tail truncation, 742X (9).

We have performed additional experiments to assess the specificity and relevance of PKD1-(115–226)-associated currents to ADPKD and to the physiological function of intact PKD1. We now report that introduction into CD16.7-PKD1-(115–226) of either of two missense mutations associated with ADPKD attenuates or abolishes upregulation of cation current in Xenopus oocytes. Missense mutations predicted to disrupt the putative coiled-coil domain of PKD1 also abrogate upregulation of cation current. Although none of these mutants exhibits a dominant negative phenotype, CD16.7-PKD1-(115–226)-associated cation current is suppressed by coexpression of a similar fusion protein encoding the PKD2 COOH-terminal cytoplasmic tail. We also demonstrate that similar cation channels are upregulated in 293-Ecr cells acutely induced to over-express CD16.7-PKD1-(115–226). This upregulation is associated with increased intracellular free calcium concentration ([Ca²⁺]ᵢ) in response to step increase in bath [Ca²⁺]. The current findings reinforce the physiological relevance of the signaling pathways invoked in the upregulation of Xenopus oocyte cation current by CD16.7-PKD1-(115–226) and extend their import to mammalian cells in culture.

**METHODS**

**Materials.** 3G8 mouse monoclonal anti-CD7 antibody was from Meditech (Madison, NJ) or was purified by protein G chromatography from supernatants of 3G8 hybridoma grown in serum-free conditions (cells gift of O. Mandelstam). Affinity-purified rabbit polyclonal antibody MAL-BD3 (to hPKD1 aa 4097–4032) was described previously (22). All other drugs and salts were from Sigma (St. Louis, MO).

**Mutations.** The cDNA encoding CD16.7-PKD1-(115–226) in the oocyte expression vector pXT7 has been described (41). All fusion protein constructs encoded the ectodomain from CD16, the transmembrane domain from CD7, and cytoplasmic domains comprising the indicated portions of the COOH-terminal cytoplasmic domain from hPKD1. Mutations were generated by four-primer polymerase chain reaction (PCR) techniques. Integrity of the PCR products and of their ligation junctions was confirmed by DNA sequencing and in some cases by in vitro translation of polypeptides of predicted size. CD16.7-PKD1-(1–226), (1–92), (–115–226), and the mutant variants of CD16.7-PKD1-(115–226) generated for this study are depicted in the schematic Fig. 1. Numbering of amino acids in full-length PKD1 polypeptide is as presented for the 4303aa sequence in GenBank entries AAC50128 and P98161. CD16.7-PKD2-(1–289) encoding the COOH-terminal cytoplasmic domain of polycystin-2 and CD16.7-PKD2-(63–192) encoding an internal segment of that domain (39) were each subcloned into pXT7.

**Oocyte preparation, cRNA expression, ⁴⁵Ca⁺² influx, and two-electrode voltage clamp measurement of oocyte current.** cRNA transcription and Xenopus oocyte isolation, culture, and microinjection were as previously described (41). Oocytes were injected with 12–25 ng total cRNA in a volume of 50 nl and studied 2–4 days after injection. For measurement of ⁴⁵Ca⁺² uptake into Xenopus oocytes, nominally Ca²⁺-free ND-96 influx medium was supplemented with 2 μCi ⁴⁵Ca⁺² (165 μM Ca²⁺) for 1 h prior to washing and scintillation counting (41).

Two-electrode voltage clamp recording in ND-96 bath of currents between holding potentials of −100 mV and +60 mV revealed the previously described ohmic currents in oocytes expressing CD16.7-PKD1-(115–226) (41). In divergent cation selectivity experiments, 50 mM MCl₂ substituted for 90 mM NaCl, where M indicates the divergent cations tested. In this paper, current magnitudes measured at holding potentials of −100 mV are presented.

**CD16.7-PKD1-(115–226)-transfected cell lines.** EcoR-293 cells (Invitrogen) stably expressing ecdysone receptor were grown in DMEM supplemented with 10% calf serum and 400 μg/ml Zeocin. The CD16.7-PKD1-(115–226) cDNA was subcloned into the pINT (Invitrogen) under the control of the ecdysone response element, then transformed by lipofection into EcoR-293 cells. Stable transfected populations were selected with G418. Clonal transfected lines were isolated and maintained under combined Zeocin/G418 selection. CD16.7-PKD1-(115–226) expression was induced by treatment with 1 μM muristerone for 18–20 h. Clonal lines 6 and 3 exhibited sustained inducible expression as detected by immunocytochemistry and immunoblot. These two lines were expanded in the presence of continued Zeocin/G418 selection for use in further studies.

**Electrical recording from EcoR-293 cells.** Cells stably transfected with CD16.7-PKD1-(115–226) were plated onto poly-L-lysine-coated coverslips, allowed to attach in the presence of Zeocin and G418. Subconfluent cells on coverslips incubated in the presence of 1 μM muristerone were incubated 1 h at room temperature with 3G8 anti-CD16, then 30 min with Cy5-conjugated goat anti-mouse anti-Ig. Muristerone-induced cells identified by fluorescence microscopy as expressing high levels of surface exposed CD16.7-PKD1-(115–226) were selected for whole cell and outside-out patch clamp recording. Control recordings were made from cells in parallel cultures untreated with muristerone.

Pipettes were pulled from borosilicate glass to a resistance of 5–8 MΩ. Currents were measured with an Axopatch 1D amplifier interfaced with a Digidata 1200 analog-to-digital/
digital-to-analog board to a HP Vectra computer. Data was acquired at 1 kHz and digitized at 5 kHz. For whole cell recording, the pipette solution was (in mM) 130 potassium aspartate, 10 HEPES, pH 7.40, 1 EGTA, 0.15 CaCl₂, and 0.15 MgCl₂; and the bath solution was 140 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, and 10 HEPES. For outside-out patch recording, the pipette solution was (in mM) 128 cesium aspartate, 10 Cs-HEPES, pH 7.40, 12 EGTA, and 0.7 CaCl₂; and the bath solution was 150 sodium methanesulfonate, 10 Na-HEPES, pH 7.40, and 10 H-EDTA. At negative pipette potentials in these outside-out patch conditions, almost all inward current was carried by Na⁺.

**Immunofluorescence microscopy.** Oocytes expressing various CD16.7-PKD1 constructs were immunostained with 3G8 antibody as previously described (41). Unfixed oocytes were incubated with 3G8, rinsed, postfixed in 3% paraformaldehyde, rinsed, quenched with 50 mM glycine in PBS, and rinsed again. Fixed oocytes were incubated with Cy5-conjugated goat anti-mouse Ig, rinsed, cleared, and imaged with a Bio-Rad model MRC1024 confocal immunofluorescence microscope.

Immunostaining of cell surface CD16 on unfixed EcR-293 cells with 3G8 was as described above. Fixed and permeabilized EcR-293 cells on coverslips were immunostained with rabbit polyclonal anti-PKD1 antibody raised against the COOH-terminal cytoplasmic tail amino acids numbered as previously (41). Terminal residues of PKD1-derived domains are also denoted by amino acids of full-length polycystin-1 within parentheses. The putative coiled-coil domain is shaded.

**RESULTS**

**Effect of ADPKD1 disease missense mutations on upregulation of cation current by CD16.7-PKD1-(115–226).** As previously reported and evident in Fig. 2A, CD16.7-PKD1-(115–226) expression is associated with substantial inward current measured at a holding potential of −100 mV. This current is ohmic, and ion substitution experiments demonstrated a substantial component of cation current. Outside-out patch record-
ing showed that this current correlates with substantially increased open probability of Ca\(^{2+}\)-permeable cation channels in the oocyte plasma membrane (41). This cation current correlated with surface expression of the CD16 epitope 3G8 (Fig. 2B). Moreover, \(^{35}\)S-methionine-labeled CD16.7-PKD1-(115–226) was immunoprecipitated from oocyte lysates with antibody to CD16 as well as with antibody to the PKD1 COOH-terminal cytoplasmic tail (Fig. 2D).

At least two missense mutations have been found in the COOH-terminal cytoplasmic tail of PKD1 in families with ADPKD, Q4225P (Q148P) and R4276W (R199W) (4). Cation current was nearly abolished in oocytes expressing the Q148P mutant of CD16.7-PKD1-(115–226) and was severely attenuated in oocytes expressing the R199W mutant (Fig. 2A). Surface expression of CD16 was unchanged by the R199W mutation and only partly reduced by the Q148P mutation (Fig. 2B). Thus introduction into CD16.7-PKD1-(115–226) of either of two human ADPKD missense mutations reduces or abolishes upregulation of cation current in Xenopus oocytes. These mutations also abolish upregulation of \(^{45}\)Ca\(^{2+}\) influx into Xenopus oocytes by CD16.7-PKD1-(115–226) (Fig. 2C). As shown in Fig. 3, neither missense mutation, when coexpressed with the fusion protein containing the wild-type PKD1-(115–226) sequence, exhibited a dominant negative phenotype.

**Effect of missense mutations predicted to disrupt the coiled-coil domain of PKD1.** Human PKD1 contains a putative coiled-coil domain predicted by the Coils program (30) to extend between residues 4222–4246, and perhaps extending further toward the NH\(_2\) terminus (Fig. 1), although a more stringent program (6) does not strongly support this prediction. The double mutation L152P/A155P in this region of the PKD1 COOH-terminal tail interfered with the ability of a PKD1 COOH-terminal tail fusion protein to coprecipitate with a PKD2 COOH-terminal tail fusion protein (35).

We have tested the effect of these mutations on the ability of CD16.7-PKD1-(115–226) to upregulate cation current in Xenopus oocytes. Figure 4 shows that not...
only the double proline substitution, but both individual proline substitution mutations reduce cation currents to the background level observed in oocytes that express CD16.7-PKD1-(1–92). In contrast, the L152P and A155P single mutants exhibit only modest reduction in surface expression of CD16 ecto-epitope. This data suggests that integrity of the putative coiled-coil domain is required for upregulation of cation currents in Xenopus oocytes by CD16.7-PKD1-(115–226).

We next examined the requirement for regions extending beyond the putative coiled-coil domain within PKD1-(115–226). We showed previously that fusion constructs encoding PKD1-(1–226), -(1–189), and -(115–189) were equally able to upregulate cation current, whereas those encoding PKD1-(1–92), -(1–115), and -(1–155) did not increase oocyte current. Figure 5 shows that any further truncation tested compromised or abolished upregulation of cation current. Thus removal of residues 115–129 at the NH2 terminus of the PKD1 component of CD16.7-PKD1-(115–226) or removal of residues 178–189 of the PKD1 component greatly reduced cation current. However, these reductions in current were not accompanied by apparent reduction in surface expression of the CD16.7-PKD fusion proteins (Fig. 5B). Thus integrity of regions extending beyond either end of the putative coiled-coil domain of the PKD1 COOH-terminal cytoplasmic tail is required for upregulation of cation current in Xenopus oocytes. Within this region is also found the src-like kinase phosphorylation site Y160 (4237). Integrity of this coiled-coil domain may, however, suffice to allow accumulation of CD16.7-PKD1 fusion protein at the oocyte surface. These results are compatible with the presence of ADPKD in families heterozygous for the mutations R4227X (33) and Q4236X (34), which encode polypeptides terminating at residues 150 and 159 of the COOH-terminal tail within the putative coiled-coil domain.

Effect of coexpression of other CD16.7-PKD fusion proteins. No evidence has been presented for biochemical interaction between CD16.7-PKD1-(115–226) with CD16.7-PKD1-(1–92). Figure 6 shows that the inactive CD16.7-PKD1-(1–92) fusion protein indeed does not express a dominant negative phenotype when coexpressed with the active CD16.7-PKD1-(115–226) polypeptide. Fusion proteins expressing either the entire PKD2 COOH-terminal cytoplasmic tail [CD16.7-PKD2-(1–289)] or a subfragment encompassing the PKD2 coiled-coil domain [CD16.7-PKD2-(63–192)] themselves did not induce cation current in Xenopus oocytes (Fig. 7 and not shown). However, coexpression of the itself inactive CD16.7-PKD2-(1–289) led to suppression of PKD1-(115–226)-associated current, whereas surface expression of the CD16 antigen was not reduced to a comparable extent. As noted previously (35, 39), the cytoplasmic domains of PKD1 and PKD2 can associate in vitro.

Exofacial liganding of CD16.7-PKD1-(115–226) modestly activates cation current. The multiple structural domains of polycystin-1 include several to which adherence and binding functions have been attributed, including the leucine-rich repeats, the C-type lectin domain (45), the REJ (“receptor-for-egg-jelly”) module (31), and the Ig-like PKD repeats (7, 21). The Ig-like PKD repeats exhibit homomeric interaction, and antibody to the repeats disrupts cell-cell interaction (21). In addition, the ability of polycystin-1 to complex with focal adhesion complex proteins in conditions in which cell-matrix interactions predominate, and with E-cadherin-catenin complexes in conditions in which cell-cell interactions predominate (15), further suggest that polycystin-1 recognizes extracellular ligands.

The ectodomain of CD16.7-PKD1-(115–226) afforded an opportunity to test the consequences to cation current of extracellular liganding of the fusion protein. As shown in Fig. 8A, the elevated current measured in oocytes expressing CD16.7-PKD1-(115–226) was further activated by bath exposure to 65 nM 3G8 murine monoclonal anti-CD16 (38). 3G8 had no effect on water-injected oocytes or oocytes expressing the inactive CD16.7-PKD1-(1–92) (Fig. 8B). Since the CD16 component of the PKD1 fusion proteins encodes the ectodomain of the human IgG Fc receptor FcyRIII, we showed that oocyte exposure to chicken IgY did not increase...
Fig. 4. Mutations which disrupt the putative coiled-coil domain of polycystin-1 abolish cation currents without abrogation of fusion protein surface expression. A: current recorded at −100 mV in oocytes expressing CD16.7-PKD1-(115–226), the corresponding mutant fusion proteins A155P and L152P, the double mutant A155P/L152P, and the negative control CD16.7-PKD1-(1–92). B: surface expression of CD16 in representative oocytes previously injected with water or with the indicated cRNAs corresponding to the bar graph in A. Surface CD16 was detected in 8 of 9 oocytes injected with A155P cRNA and in 7 of 8 oocytes injected with L152P.

Fig. 5. Truncations of the fusion protein polycystin-1 domain abolish cation currents without apparent diminution of fusion protein surface expression. A: current recorded at −100 mV in oocytes expressing CD16.7-PKD1-(115–226) and the polycystin-1 domain truncations PKD1-(115–177), PKD1-(115–171), PKD1-(130–189), and PKD1-(140–189). **P < 0.01 compared with all other conditions. B: surface expression of CD16 in representative oocytes previously injected with water or with the indicated cRNAs corresponding to the bar graph in A. Strong surface staining was observed in 10 of 10 oocytes injected with PKD1-(115–177) cRNA, in 5 of 7 oocytes injected with PKD1-(115–171), in 9 of 9 injected with PKD1-(130–189), and in 11 of 12 injected with PKD1-(140–189).
cation current under conditions where 3G8 did so (Fig. 8C). We next asked whether liganding via the Fc domain or via variable site interaction with the 3G8 antigen was important. Figure 8D shows that pooled rabbit IgG was as effective as 3G8 in increasing oocyte current, as was monoclonal anti-AE2 anion exchanger (not shown). This data is compatible with the recent localization of the 3G8 binding site (38) very close to the Fc binding sites (37). Interaction with the Fc binding site, likely in a 1:1 stoichiometry (37), sufficed to stimulate cation current. Comparison of divalent F(ab')2 and monovalent Fab fragments devoid of the Fc domain showed that cross-linking the fusion protein was not necessary for stimulation of cation current by these ligands.

**Divalent cation selectivity of CD16.7-PKD1-(115–226)-associated current.** We have reported previously that the CD16.7-PKD1-(115–226)-associated cation current is nonselective among monovalent cations, and conducts Ca2+ (41).

Recently, an endogenous, nonselective cation current of *Xenopus* oocytes has been described (28). We therefore compared the divalent cation selectivity of PKD1-(115–226)-associated current with that of the newly reported endogenous current and with the cation selectivities reported for PKD2 and PKDL1. Oocytes preincubated overnight in Cl−/H2O-free medium were injected with 5 mM final estimated EGTA, and recordings were carried out in a bath devoid of Ca2+ to minimize the contribution to inward current of Ca2+-gated Cl− current. As shown in Fig. 9, the conductance sequence was Sr2+ > Ca2+ > Mn2+ > Mg2+ > Ba2+. Mg2+ (P < 0.01) and Ba2+ (P < 0.05) currents measured at −100 mV were significantly lower than Na+ current. Change from Na+ to divalent bath shifted reversal potential to more negative values by 15–18 mV, showing that so-

**Fig. 7.** The inactive fusion protein CD16.7-PKD2-(1–289) exhibits dominant negative suppression of cation current when coexpressed with CD16.7-PKD1-(115–226). A: inward currents measured at −100 mV holding potential. *P < 0.05 and **P < 0.01, compared with PKD1-(115–226). B: surface expression of CD16 in representative oocytes previously injected with water or with the indicated cRNAs corresponding to the bar graph in A. Surface CD16 was detected in 0 of 6 oocytes injected with PKD2-(1–289) and in 4 of 5 coinjected with PKD1-(115–226) and PKD2-(1–289).
medium permeability exceeds that of divalents. In oocytes expressing CD16.7-PKD1-(115–226), clamped in the Na⁺/H⁺ bath, reversal potential and conductance were similar to those reported previously (reversal potential of −2 mV and slope conductance of 8.7 S, figure 1 in Ref. 41; water-injected oocytes exhibited reversal potential of −31 mV and slope conductance of 2 S in Na⁺ bath).

Weak blockers of CD16.7-PKD1-(115–226)-associated current. We previously reported a partial pharmacological characterization of the PKD1-(115–226)-associated current in Xenopus oocytes (41). Recently, the inositol-triphosphate receptor (IP3R) inhibitor 2-aminophenylborate (2-APB) was shown also to block plasmalemmal Ca²⁺ entry associated with heterologous expression of TRP3 (42) and of CaT1 (44). As shown in Fig. 10, 2-APB inhibited currents in oocytes expressing CD16.7-PKD1-(115–226). Inhibition measured at −100 mV was 18% (n = 5, P < 0.05). In contrast to inhibition by La³⁺, inhibition by 2-APB was independent of membrane potential. The effects of additional cation current blockers were also tested in separate oocytes. Ruthenium red at 10 and 100 μM inhibited current 9% and 13%, respectively (n = 5, P < 0.01). Clotrimazole at 10 μM inhibited current measured at −100 mV holding potential by 11% (n = 4, P < 0.03). Nifedipine at 1 μM inhibited current by 18% (n = 7, P < 0.01), but higher concentrations were not more effective. Diltiazem at 1 μM inhibited current by 9% (n = 6, P < 0.01).

CD16.7-PKD1-(115–226) increases cation currents and channel activity in EcR-293 cells. CD16.7-PKD1-(115–226) induced cation channel activity not only in Xenopus oocytes but also in EcR-293 cells. EcR-293 cells stably transfected with CD16.7-PKD1-(115–226) under the control of an ecdysone-responsive promoter were treated overnight with or without 1 μM muristerone. As shown in Fig. 11A, muristerone treatment induced accumulation of CD16.7-PKD1-(115–226) polypeptide. This accumulation was accompanied by the surface expression of the CD16 epitope in muristerone-treated cells.
Increased cation currents in muristerone-induced cells correlated with increased \([\text{Ca}^{2+}]_i\) changes in response to step increases in bath \([\text{Ca}^{2+}]_o\). Resting \([\text{Ca}^{2+}]_i\) of 105 ± 2 nM was indistinguishable in induced and uninduced cells in 1 mM \([\text{Ca}^{2+}]_o\). A step increase of bath \([\text{Ca}^{2+}]_o\) to 10 mM resulted within 2 min in a maximum \([\text{Ca}^{2+}]_i\) increase of 13 ± 1 nM in uninduced cells \((n = 3\) coverslips\)), but increased \([\text{Ca}^{2+}]_i\) to 35 ± 2 nM in muristerone-treated cells induced to express CD16.7-PKD1-(115–226) \((n = 5\) coverslips, mean ± SE, \(P < 0.02\)).

**DISCUSSION**

Most ADPKD is associated with heterozygous germ line mutations in the genes encoding either polycystin-1 or polycystin-2. However, the mechanisms by which the mutant polypeptides contribute to cystogenesis and cyst enlargement remain unknown. Indeed, although heterologous expression of polycystin-2 is associated with \([\text{Ca}^{2+}]_o\)-permeable cation channel function either independently \((16, 43)\) or with coexpression of full-length polycystin-1 \((18)\), the link between cation current and pathology remains poorly understood. ADPKD-1 is assumed to be a loss-of-function disease, but the commonly observed increased level of polycystin-1 polypeptide in polycystic kidneys is not yet reconciled with this assumption. Thus the presumed somatic missense mutations in the second allelic copy of the polycystin-1 gene might through loss-of-function (or, less likely, gain-of-function) either inhibit or disinhibit a downstream regulatory target of polycystin-1. This target could be either polycystin-2 itself, other ion channels, or other signaling polypeptides.

The COOH-terminal cytoplasmic tail of polycystin-1 and its subfragments themselves regulate multiple signaling pathways when expressed as transmembrane fusion proteins in host cells. Among the consequences of CD16.7-PKD1-(115–226) overexpression in *Xenopus* oocytes is upregulation of endogenous

(FIG. 11B) but not in untreated cells (FIG. 11B). Neither EcR-293 cells nor the parental HEK293 cells express endogenous polycystin-1 polypeptide detectable with our anti-PKD1 antibodies by immunoblot or immunocytochemistry (not shown), although endogenous PKD1 mRNA is present at low level in 293 cells \((23)\).

Muristerone-induced and uninduced cells grown on small polylysine-coated coverslips were incubated with 3G8 antibody and Cy5-coupled Ig, then washed. Fluorescent-tagged cells on induced coverslips were compared with randomly selected cells on uninduced coverslips. As shown in Fig. 12, A and B, whole cell cation currents of ohmic pattern measured in \(\text{Cl}^-\) bath were increased in induced cells. \(\text{La}^{3+}\)-sensitive inward current was increased fivefold \((n = 6, P = 0.02)\). Unitary cation conductance events observed in outside-out patch records (Fig. 12C) were nonrectifying at negative pipette potentials (Fig. 12D). \(NP_o\) was 6.5-fold higher in patches from induced \((n = 10)\) than from noninduced \((n = 6)\) cells (Fig. 12E, \(P < 0.01\)), and the elevated \(NP_o\) was inhibited 57% by 1 mM \(\text{La}^{3+}\) (Fig. 12F, \(n = 4, P < 0.01\)).

![Fig. 10. 2-Aminophenylborate (2-APB) inhibits CD16.7-PKD1-(115–226)-associated current. 2-APB is an inhibitor of PKD1-(115–226)-associated current \((n = 5)\).](http://physiolgenomics.physiology.org/)

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Ca<sup>2+</sup>-permeable cation channel activity (41). In the current paper, we provide several lines of evidence to support the hypothesis that the observed cation channel upregulation is relevant to the pathogenesis of ADPKD1. First, oocytes expressing CD16.7-PKD1-(115–226) fusion proteins with ADPKD1-associated missense mutations exhibit greatly reduced or absent cation currents without corresponding reduction of fusion protein surface expression (Fig. 2). Second, missense mutations and truncations predicted to disrupt the putative coiled-coil domain whose integrity is required for the interaction of the COOH-terminal cytoplasmic tails of polycystin-1 with that of polycystin-2 do not upregulate cation current (Figs. 4 and 5). Third, the upregulated cation current is suppressed by coexpressed PKD2 fusion protein (Fig. 6) with which it

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**Fig. 11.** Inducible expression of CD16.7-PKD1-(115–226) in EcR-293 cells. A: muristerone-induced CD16.7-PKD1-(115–226) expression in two stable cell lines, detected by immunoblot with antibody to PKD1 COOH-terminal tail; values on left indicate Mr. B: surface immunostaining of CD16 in uninduced cells. C: surface immunostaining of CD16 in muristerone-induced cells. Incubation of 3G8 anti-CD16 antibody with unfixed cells was followed by fixation, permeabilization, and incubation with Cy5-labeled secondary antibody.

**Fig. 12.** Cation channel activity in EcR-293 cells induced to express CD16.7-PKD1-(115–226). A: I-V relation of currents recorded in whole cell mode in uninduced (control, n = 4) and muristerone-induced cells (n = 6). B: mean inward La<sup>3+</sup>-sensitive currents recorded at −100 mV holding potential. *P < 0.05. C: traces from outside-out patch records from an uninduced cell and an induced cell before and after exposure to 1 mM La<sup>3+</sup>. D: I-V relation of unitary currents from representative outside-out patch pulled from induced cells. E: NP<sub>o</sub> of channels in outside-out patches from uninduced (n = 6) and induced cells (n = 10). *P < 0.01. F: NP<sub>o</sub> of channels in outside-out patches from induced cells before and after exposure to 1 mM La<sup>3+</sup> (n = 4, P < 0.01).
binds and which contains an endoplasmic reticulum retention signal (43). Fourth, cation channel upregulation by CD16.7-PKD1-(115–226) capable of elevating \([\text{Ca}^{2+}]_{i}\) is not limited to *Xenopus* oocytes but is observed also in the human embryonic kidney cell line EcR-293 (Fig. 12).

The multiple structural subdomains of the very large extracellular domain of polycystin-1 likely bind to matrix-associated or circulating ligands, and such binding might transduce signals via the COOH-terminal cytoplasmic tail or other portions of the polycystin-1 polypeptide. The unknown ligand of the extracellular REJ domain has received particular interest in this regard, since binding of the corresponding domain of the sperm receptor for egg jelly is associated with the \([\text{Ca}^{2+}]_{i}\)-mediated acrosome reaction (31). We hypothesized that antibody binding to the CD16 exofacial portion of the transmembrane CD16.7-PKD1-(115–226) fusion protein might similarly modulate PKD1-(115–226)-associated current in oocytes. This was indeed the case. Divalent and monovalent ligands were equally effective, though modest, upregulators of oocyte current, consistent with the known 1:1 stoichiometry of CD16:Fc interaction (10, 37, 38).

The ionic signal transduced by the putative PKD1/PKD2 plasma membrane complex has been proposed to be \([\text{Ca}^{2+}]_{i}\) (17). \([\text{Ca}^{2+}]_{i}\) permeation has now been shown in whole cell recording studies of CHO cells coexpressing PKD1 and PKD2 (18). In lipid bilayers, PKD2 alone allowed calcium permeation (16). We have shown PKD1-(115–226)-associated \([\text{Ca}^{2+}]_{i}\) permeation in oocytes by three methods (41). In the present study EcR-293 cells expressing CD16.7-PKD1-(115–226) exhibited sufficiently increased \([\text{Ca}^{2+}]_{i}\) permeability to result in increased \([\text{Ca}^{2+}]_{i}\), under conditions of acutely elevated bath \([\text{Ca}^{2+}]_{i}\). The present study also documented broad divalent cation selectivity of this current in *Xenopus* oocytes. The ability of this current to pass \(\text{Mn}^{2+}\) and \(\text{Ba}^{2+}\), as well as the previously demonstrated activity at depolarized potential and inhibition by acid pH, all distinguish it from the endogenous hyperpolarization- and acid-activated nonselектив cation current of oocytes (28). However, the significant permeation of \(\text{Sr}^{2+}\) and \(\text{Ba}^{2+}\) does resemble that observed by Chen et al. (8) in oocytes expressing PKD1.

We further characterized the pharmacological properties of the CD16.7-PKD1-(115–226)-associated current. 2-APB was recently shown to block activity of Trp3 and of CaT1 (42, 44). 2-APB also partially blocked the cation current associated with PKD1-(115–226) expression. Ruthenium red, nifedipine, clotrimazole, and diltiazem also modestly inhibited the cation current stimulated by CD16.7-PKD1-(115–226). Ruthenium red inhibits the nonselective cation channel that mediates capsaicin sensitivity (14). Nifedipine has long been used to block L-type calcium channels but also has been found to block novel nonselектив store-operated cation conductance responsible for filling calcium stores in smooth muscle (12). Imidazoles and diltiazem have been found to be blockers of maitotoxin-stimulated calcium influx in cultured cells (13). Thus the pharmacological profile presented, although nonspecific, does resemble that of other nonspecific cation channels, including some of TRP superfamily. It is possible in both the oocyte and EcR-293 cells that CD16.7-PKD1-(115–226) upregulates activity of polycystin-2 or PKDL, or a related ion channel. Alternatively, the PKD1 fusion protein might regulate distinct cation channel(s) and perhaps additional ion transport pathways.

In conclusion, we have demonstrated that disease-associated mutations in the cytoplasmic tail of PKD1 attenuate or abrogate regulatable nonspecific cation currents and \([\text{Ca}^{2+}]_{i}\) permeability associated with overexpression of the polycystin-1 fusion protein, CD16.7-PKD1-(115–226). We suggest that the data presented support the relevance of these findings for our growing understanding of polycystin-1 function as a signal transducer and modulator of ion channel function.

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