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Expression of the polycystin-1 C-terminal cytoplasmic tail increases Cl^- channel activity in *Xenopus* oocytes

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Background. Cyst expansion in autosomal-dominant polycystic kidney disease (ADPKD) is characterized by active Cl^- secretion in excess of solute reabsorption. However, the connections between elevated epithelial Cl^- secretion and loss-of-function or dysregulation of either ADPKD gene polycystin-1 (PC1) or polycystin-2 (PC2) remain little understood.

Methods. Cl^- transport in *Xenopus* oocytes expressing the CD16.7-PKD1 (115-226) fusion protein containing the final 112 amino acid (aa) of the PC1 C-terminal cytoplasmic tail, or in oocytes expressing related PC1 fusion protein mutants, was studied by isotopic flux, two-electrode voltage clamp, and outside-out patch clamp recording.

Results. Expression in oocytes of CD16.7-PKD1 (115-226) increased rates of both influx and efflux of $^{36}Cl^-$, whereas CD16.7-PKD1 (1-92) containing the initial 92 aa of the PC1 C-terminal cytoplasmic tail was inactive. The increased Cl^- transport resembled CD16.7-PKD1 (115-226)-stimulated cation current in its sensitivity to ADPKD-associated missense mutations, to mutations in phosphorylation sites, and to mutations within or encroaching upon the PC1 coiled-coil domain, as well as in its partial suppression by coexpressed PC2. The NS3623- and 4, 4′-diisothiocyanatostilbene-2, 2′-disulfonic acid (DIDS)-sensitive $^{36}Cl^-$ flux was not blocked by injected ethyleneglycol tetraacetate (EGTA) or by the cation channel inhibitor SKF96365, and was stimulated by the cation channel inhibitor La^{3+}, suggesting that CD16.7-PKD1 (115-226)-associated cation conductance was not required for $^{36}Cl^-$ flux. Outside-out patches from oocytes expressing CD16.7-PKD1 (115-226) also exhibited increased NS3623-sensitive Cl^- current.

Conclusion. These data show that CD16.7-PKD1 (115-226) activates Cl^- channels in the *Xenopus* oocyte plasma membrane in parallel with, but not secondary to, activation of Ca^{2+}-permeable cation channels.

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Key words: autosomal dominant polycystic kidney disease, cation channel, isotopic flux, NS3623, coiled-coil domain.

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Autosomal-dominant polycystic kidney disease (ADPKD) accounts for ~10% of the dialysis population in North America. Eighty-five percent of ADPKD appears in families with heterozygous mutations in the *PKD1* gene on chromosome 16, encoding the 4303 amino acid (aa) polypeptide, polycystin-1. Most other ADPKD cases are attributable to heterozygous mutations in polycystin-2, a polycystin-1 binding protein thought to act in the same signaling-effector pathway. The hallmark pathology of ADPKD is formation and enlargement of epithelial-lined cysts arising in ~3% of nephrons (as well as in biliary and pancreatic ductules). Growth of these cysts in most parts of the kidney is associated with cyclic adenosine monophosphate (cAMP)-regulated proliferation and metaplasia of epithelial cells. This altered growth is accompanied by conversion from the normal tubular phenotype of net solute and fluid reabsorption to one of net solute and fluid secretion by the cystic epithelium. The net fluid secretion is believed to contribute to cyst expansion, compressing unaffected nephrons and leading ultimately to loss of renal function [1, 2].

Studies of intact cysts from ADPKD kidneys, of confluent monolayers of cyst epithelium grown on permeable supports, and of cysts formed by established lines of kidney epithelial cells suspended in matrix all display active electrogenic chloride secretion. This secretion is associated with basolateral membrane Cl^- loading by secondary active transporters and with apical Cl^- release via conductive pathways, including the cystic fibrosis transmembrane conductance regulator (CFTR) [3–5]. However, the mechanisms by which reabsorptive columnar epithelial cells of tubules become secretory cuboidal or flattened epithelial cells of cysts, and the roles of dysregulated or absent function of PC1 and/or PC2 in that phenotypic transition, remain unknown.

Overexpression of PC2 alone [6–8] or with PC1 coexpression [9] can induce Ca^{2+}-permeable cation channel activity in the plasma membrane. One longstanding model envisages a plasmalemmal complex of PC1 and PC2 interacting via C-terminal cytoplasmic coiled-coil

domains [10] in which PC1 acts as an extracellular ligand-gated, G-protein–coupled regulator of the PC2 cation channel. The PC1 interaction may also aid in trafficking PC2 from the endoplasmic reticulum to the plasma membrane. Although PC1 and PC2 can be coimmunoprecipitated, most cellular PC2 and PC1 are not colocalized, likely reflecting multiple, site-specific functions, perhaps mediated by site-specific binding partners. Indeed, PC1 can activate an endogenous cation channel distinct from homomeric PC2, if not itself function as a cation channel [11, 12].

Although CFTR is present in the apical membrane of a substantial fraction of ADPKD cyst cells [13, 14], and CFTR blockade prevents Madin-Darby canine kidney (MDCK) cyst growth in collagen gel, the clinical course of ADPKD in the few patients heterozygous or homozygous for CFTR ΔF508 leaves in question the role of CFTR-mediated Cl^- channel activity in cyst growth in patients [15]. Therefore, up-regulation of apical Ca^{2+}-activated Cl^- channel activity has also been proposed to explain cyst expansion in ADPKD. However, reconciliation of up-regulated Ca^{2+}-activated Cl^- channel activity with loss of PC1- or PC2-mediated cation channel function and its associated Ca^{2+} permeability would seem to require yet undefined intermediate signaling events.

As the 4303 aa human PC1 was initially not easy to overexpress, many functional studies of PC1 have focused on its C-terminal cytoplasmic tail, variably estimated at between 200 and 226 aa in length. This region expressed as a fusion protein in intact cells can activate protein kinase Cα (PKCα) and cJun-NH_2-terminal kinase-mediated, AP-1-dependent transcriptional events [16], in at least some cases mediated by G-protein activation [17]. Activation of AP-1 transcription may alternatively involve regulated intramembrane proteolytic liberation of the C-terminal tail and its translocation into the nucleus [18]. The PC1 C-terminal tail can also activate signaling via the Wnt/frizzled pathway, stabilizing the polycystin-1 binding protein β-catenin, and activating in turn transcription factors of the TCF/LEF family [19], but this finding has not been observed in all cases [20]. Two distinct regions of the C-terminal cytoplasmic tail have been shown to bind $G\alpha_i$ subunits [21] and the regulator of heterotrimeric G proteins, RGS7 [22] (which can in turn bind to the 14-3-3 family of protein phosphorylation regulators [23]). The presence of the $G\alpha_i$ binding site has recently been linked to $G\alpha_{i/o}$ activation and secondary activation of Gα-regulated K^+ and Ca^{2+} channels [24]. The ability of the PC1 C-terminal tail to bind Na^+,K^+-ATPase [abstract; Zatti A, et al, *J Am Soc Nephrol* 15:220A, 2004] is consistent with localization of some PC1 at the basolateral plasma membrane. The ability of the PC1 C-terminal tail to bind the intermediate filament protein vimentin [25] is consistent with localization of some PC1 at adherens junctions.

We showed previously that overexpression of the tripartite fusion protein CD16.7-PKD1(115-226) containing the C-terminal 112 polycystin-1 residues (aa 4192-4303) either in *Xenopus* oocytes [26] or in EcR-293 cells [27] increases activity of a novel endogenous Ca^{2+}-permeable cation channel. Currents mediated by this channel are attenuated by missense mutations associated with ADPKD, by missense mutations in PC1 serine and tyrosine phosphorylation sites or within the putative PC1 coiled-coil domain, and by truncations which encroach upon the coiled-coil domain [27]. A similar fusion protein carrying the cytoplasmic C-terminal tail of polycystin-1 enhanced adenosine triphosphate (ATP)-induced Ca^{2+} release from internal stores in transiently transfected human embryonic kidney (HEK)-293 and HeLa cells [28]. Yet another variant of this fusion protein conditionally expressed in stably transfected M1 mouse cortical collecting duct cells increased both magnitude and duration of ATP-induced intracellular $[Ca^{2+}]$ signaling, likely secondary to increased store depletion-induced Ca^{2+} entry. This was accompanied by enhanced, ATP-induced, DIDS-sensitive Cl^- conductance in these cells [29].

We tested the ability of CD16.7-PKD1 (115-226), the same fusion protein which activates Ca^{2+}-permeable cation channels in *Xenopus* oocytes [26] and EcR-293 cells [27], to regulate endogenous Cl^- transport in *Xenopus* oocytes. We found that expression of this fusion protein indeed up-regulated Cl^- transport, at least part of which is conductive. Moreover, the polycystin-1 mutations which reduced or abrogated cation current stimulation by the fusion protein similarly decreased or abolished Cl^- transport. However, the pharmacologic properties of the Cl^- transport suggested that its activation required neither elevation of intracellular Ca^{2+} nor activation of the cation conductance. Thus, activation of Cl^- transport may be an independent action of the C-terminal tail of polycystin-1.

METHODS

cDNA constructs

The cDNA encoding CD16.7-PKD1 (115-226) in the oocyte expression vector pXT7 has been described [26, 27]. All fusion protein constructs encoded the ectodomain from CD16, the transmembrane domain from CD7, and cytoplasmic domains comprising the indicated portions of the C-terminal cytoplasmic domain from human PC1 (PKD1). 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, as described previously [26, 27]. CD16.7-PKD1 (1-226), (1-92), (115-226), and the mutant variants of (115-226) generated for this study are depicted in the schematic

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