Constitutive Activation of G-proteins by Polycystin-1 Is Antagonized by Polycystin-2*

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Polycystin-1 (PC1), a 4,303-amino acid integral membrane protein of unknown function, interacts with polycystin-2 (PC2), a 968-amino acid α-type channel subunit. Mutations in their respective genes cause autosomal dominant polycystic kidney disease. Using a novel heterologous expression system and Ca2⁺ and K⁺ channels as functional biosensors, we found that full-length PC1 functioned as a constitutive activator of Gαi-type but not Gαq-type G-proteins and modulated the activity of Ca2⁺ and K⁺ channels via the release of Gi/γ subunits. PC1 lacking the N-terminal 1811 residues replicated the effects of full-length PC1. These effects were independent of regulators of G-protein signaling proteins and were lost in PC1 mutants lacking a putative G-protein binding site. Co-expression with full-length PC2, but not a C-terminal truncation mutant, abrogated the effects of PC1. Our data provide the first experimental evidence that full-length PC1 acts as an untraditional G-protein-coupled receptor, activity of which is physically regulated by PC2. Thus, our study strongly suggests that mutations in PC1 or PC2 that distort the polycystin complex would initiate abnormal G-protein signaling in autosomal dominant polycystic kidney disease.

Autosomal dominant polycystic kidney disease (ADPKD)1 (1–3), a common, potentially lethal disorder, results from mutations in either of two genes, PKD1 and PKD2, encoding polycystin-1 (PC1) and polycystin-2 (PC2), respectively (4–5). Multiple organ defects in homozygous mouse embryos deficient in PC1 or PC2 suggest a critical role for both proteins in embryogenesis (6–8), but the cellular mechanism(s) underlying these defects is unknown. ADPKD itself is characterized by focal formation and progressive development of fluid filled cysts originating from tubular epithelia in the kidney and liver (1, 2). The mechanisms of cystogenesis in ADPKD are unknown and confounded by at least four documented pathways: 1) somatic loss of heterozygosity (LOH) for PKD1 (9, 2) somatic LOH for PKD2 (10, 11) combined somatic loss of single and PKD2 alleles (11, and 4) over-expression of PKD1 (1, 12–13). Together these findings suggest that PC1 and PC2 interact in a complex signaling network, whose properties are not easily predicted from first principles.

PC2 is a 968-amino acid membrane protein and has recently been described as a Ca2⁺-permeable cation channel with potential roles in the endoplasmic reticulum (14–16) and/or on cell membrane (14–17). By contrast, PC1 is a 4303-amino acid plasma membrane protein (1, 12) composed of an ~2500-amino acid N-terminal extracellular domain with a number of adhesive domains, 7–11 transmembrane domains, and a small (~200 amino acids) C-terminal cytoplasmic domain (4) containing a coiled-coil structure known to interact with PC2 in vitro (18–19). Data on the function of the full-length PC1 is currently limited to its facilitation of PC2 translocation from the endoplasmic reticulum to the plasma membrane in Chinese hamster ovary cells (15). Studies of the short cytoplasmic tail of PC1 suggested a potential regulatory role of PC1 in G-protein signaling through stabilizing the regulator of G-protein-signaling protein, RGS7 (20) or direct binding to heterotrimeric G-proteins (21). Hitherto, the only reported potential ligand binding capacity of PC1 is that the 229-amino acid C-type lectin domain binds to carbohydrates and collagens I, II, and IV in vitro (22). One major obstacle hampering investigation of full-length PC1 function is the lack of an efficient expression system for its large size transcript (14 kb) and a read-out for its function(s).

Here, we have used rat sympathetic neurons as a novel expression system (23–25) to study the function(s) of the PC1 proteins. RT-PCR assays indicated that these cells do not normally express PC1 mRNA and hence provide a “tabula rasa” for assessing effects of heterologously expressed PC1. Well characterized Ca2⁺ and K⁺ channels (26) serve as specific read-outs for activated intracellular pathways. Our data demonstrate for the first time a biochemical function of full-length PC1. PC1 acts as a constitutive activator of Gαi but not Gαq G-proteins, independently from RGS protein activity. In addition, we show that the G-protein activating property of PC1 can be inhibited
by the cation channel PC2 via interactions of their C termini. Taken together, our data indicate that mutations in PC1 or PC2 may have a dosage effect such that mutations perturbing but not necessarily abrogating the polycystin complex initiate abnormal G-protein signaling pathways in cells that naturally co-express both PC1 and PC2.

**MATERIALS AND METHODS**

**DNA Constructs**—The human PKD1 expression construct was assembled from cDNA clones KG8, RT-PCR product puk3139, and a fragment of cDNA clone FK7 in pcDNA3.1/His-C (Invitrogen) through various digestion and ligation procedures. The final construct contains a partial 8428-bp cDNA of human PKD1, encoding the C-terminal 2492 amino acids of polycystin-1, with an "Xpress tag" at its N terminus. The full-length mouse PKD1 construct was assembled from cloned cDNA and RT-PCR products, sequence-verified for an open reading frame, and cloned into pcDNA3.1 at NotI site. The final construct encodes the entire mouse polycystin-1 (4303 amino acids). mPKD1-Myc tagged construct (pcDNA-PKD1-Myc) was generated by PCR-directed mutation of in-frame stop codons from pcDNA-PKD1. The mPC1 mutant was generated by a deletion of the last 193 amino acids of mouse polycystin-1. The C-terminal-deleted region includes a binding domain for heterotrimeric G-proteins, a G-protein activation peptide (21), and putative interaction site with PC2. The mPC2 mutant was generated as described (40) and subcloned into pcDNA3.1 by standard procedures. This mutant lacks the C-terminal 226-amino acids of PC2, which includes the EF-hand and the putative PC1 interaction domain.

**Primary Neuronal Cultures and Nuclear Microinjection**—Sympathetic neurons were dissociated from superior cervical ganglia of 15- to 19-day-old male Sprague-Dawley rats and cultured on laminin-coated glass coverslips as described (25). Plasmids were diluted in Ca**2+**/free Krebs solution (290 mosmol l\(^{-1}\), pH 7.3) containing 0.5% FITC-dextran (70 kDa, Molecular Probes) to a final concentration of 100 μg ml\(^{-1}\) (Kir3.1 and Kir3.2 cDNAs), 200 μg ml\(^{-1}\) (hPKD1, mPKD1, mPKD2, GABA\(_{A}Rb, GABA_{A}Rc, G_{i/L}_{3}, G_{i/L}_{4}, \) and G-transducin cDNAs) or 40 μg ml\(^{-1}\) (G-protein α subunits). Plasmids were pressure-injected into the nucleus of sympathetic neurons with sharp micropipettes (50–60 MΩ) using manual impalement (24) or automated microinjection (37) (Eppendorf, Hamburg, Germany). Neurons were maintained in culture for a further 2 days prior to recording or immunostaining. cDNAs for bovine β\(_{1}\) and γ\(_{i}\) G-protein subunits, retinal Gvaso transducin, Kir3.1/3.2 and GABA\(_{A}Rh/Rc2 subunits were described previously (25, 34, 38). PTX- and RGS-sensitive G-protein α\(_{i,i/L}\) subunits were generated by point mutation of Cys-351→Ile and Gly-183/184→Ser as detailed (39). All constructs were subcloned in cytomegalovirus promoter vectors and verified by automated DNA sequencing. Cells expressing Go\(_{i,i/L}\)G184S showed a >10-fold slower recovery from Ca**2+** current inhibition by noradrenaline, confirming that Go\(_{i,i/L}\) mutatns are resistant to the action of endogenous Gt8 proteins.

**Immunoblotting**—Myc-tagged mPC1 and untagged mPC1 were transiently transfected into 5454 cells and Hek293 cells, respectively. Pre-cleared cell lysates were incubated with an anti-Myc antibody (Santa Cruz) or the anti-PC1 antibody 7e12 (27) at 4 °C for 12 h. Immunocomplexes were then captured with protein A beads for 2 h. The beads were washed and bound proteins were then fractionated by SDS-PAGE followed by Western blot analysis. Western blots were probed with an anti-Myc polyclonal antibody (Zymed Laboratories Inc.) or 7e12 prior to their incubation with horseradish peroxidase-coupled antibody. Immunoblotted antibodies were detected by ECL.

**Patch Clamp Recording**—Neurons were recorded using the whole cell variants (patch-ruptured or perforated patch) of the patch clamp technique. For recording of Ca**2+** currents and leak and K-typed currents, the external solution was (mM): NaCl, 110; NaHCO\(_{3}\), 23; KCl, 3; MgCl\(_{2}\), 1.2; CaCl\(_{2}\), 2.5; Hepes, 5; glucose, 11; tetrodotoxin, 0.0005 μM; Na\(_{3}\)GTP, 0.1 (adjusted to pH 7.2 with KOH and 290 mosmol l\(^{-1}\)). GIRK 1/2 currents were obtained using patch-ruptured whole-cell recording. Pipettes were coated with Silgard and had resistances of 2–5 MΩ. Internal solution for I\(_{GIRK}\) was (mM): NaCl, 140; NaGlu, 10; Hepes, 11; tetrodotoxin, 0.0005 M; GTP\(_{1}\)b (95% O\(_{2}-5%\) CO\(_{2}\) mixture, pH 7.4). GIRK\(_{1/2}\) currents were recorded using the same external solution, except that [K\(_{1}^{+}\)] was 12 mM (isosmotically compensated for Na\(^{+}\)).

Ca**2+** and GIRK\(_{1/2}\) currents were obtained using patch-ruptured whole-cell recording. Pipettes were coated with Silgard and had resistances of 2–5 MΩ. Internal solution for I\(_{GIRK}\) was (mM): NaCl, 140; NaGlu, 10; Hepes, 11; tetrodotoxin, 0.0005 M; GTP\(_{1}^{\text{b}}\) (95% O\(_{2}-5%\) CO\(_{2}\) mixture, pH 7.4). GIRK\(_{1/2}\) currents were recorded using the same external solution, except that [K\(_{1}^{+}\)] was 12 mM (isosmotically compensated for Na\(^{+}\)).
Polycystin-1 Homomers Expressed in Neurons Do Not Form Ion Channels—Cultured sympathetic neurons were microinjected intranuclearly with cDNAs encoding full-length mPC1 or N-terminal-truncated hPC1 lacking the Ig-like repeat (Fig. 1, A and B), and somatic recordings were made 48 h later using the perforated patch clamp technique. Neurons so injected efficiently expressed PC1 in their outer membrane (Fig. 1, C and D). Immunostaining using an anti-hPC1 polyclonal antibody (MR3) (12) revealed immunoreactivity concentrated at the cell periphery in 78% of cells microinjected with hPKD1 cDNA (n = 26) (Fig. 1C). Further, Myc-tagged mPC1 was co-immunolabeled with the α₁B Ca²⁺ channel subunit, a typical plasma membrane protein. Confocal sections showed that Myc-tagged mPC1 co-localized with Ca²⁺ channels in the plasma membrane and was not retained intracellularly (Fig. 1D). Western blot analysis from mammalian cell lines transfected with DNA constructs for full-length mPC1 and Myc-tagged mPC1 revealed proteins of ~460 kDa, which is similar to the predicted size of PC1 encoded by the longest open-reading frame of PC1 (460 kDa) (Fig. 1E).

Electrophysiological recording in neurons revealed that, unlike PC2 (16–17) and polycystin-L (28), expressed PC1 protein did not function as a cation channel (n = 12; Fig. 2) in accordance with recent studies in Chinese hamster ovary cells (15); nor do they up-regulate endogenous cation-permeable channels in these cells, as recently reported for an expressed PC1 C-terminal fragment (PC1C1-226) in oocytes (29).

Polycystin-1 Modulates Voltage-dependent Ca²⁺ Channels and GIRK K⁺ Channels—To assess the role of PC1 in signal transduction, low-pass filtered at 1 kHz, and sampled at 6.67 kHz. GIRK₁₂ currents were typically evoked by 700 ms voltage ramps from −140 to −40 mV. The amplitude of GIRK₁₂ currents was measured as the Ba²⁺-sensitive current (100 μA) averaged between −125 and −130 mV. Leak currents were not subtracted.

Recording of passive membrane properties (Fig. 2) and M-current was made using the perforated patch clamp technique to avoid rundown (37). Patch pipettes were filled by dipping the tip for 40 s into filtered internal solution that comprised (mM): K⁺ acetate, 80; KCl, 30; Hepes, 40; MgCl₂, 3 (pH 7.35). Pipettes were then back-filled with the same internal solution containing 0.09 mg/ml amphotericin-B. After permeabilization, access resistances were 10–15 MΩ. Leak currents were not subtracted, otherwise mentioned (e.g. Fig. 2). Experiments were performed at 30–32 °C, and drugs were applied by using a gravity-fed perfusion system (10 ml min⁻¹). NEM (N-ethylmaleimide) was applied at 50 μM for at least 2 min, and PTX was incubated 12–24 h at 1 μg ml⁻¹. Data are expressed as means ± S.E. Statistical analysis was performed using Student’s t tests or two-way analysis of variance as appropriate.

Immunocytochemistry—Myc-tagged mPC1:cells were fixed with 4% paraformaldehyde for 20–25 min at room temperature followed by 5 min treatment with Triton (0.1%) to permeabilize membranes. After incubation with bovine serum albumin/phosphate-buffered saline, neurons were incubated with anti-Myc antibody for 1 h, washed, and then stained with FITC-conjugated anti-mouse antibody (1/100) for 35 min. Fluorescence images were obtained using a confocal microscope (Bio-Rad). The anti-human PC1 polyclonal antibodies MR3 (12) were used to examine the expression of the human PKD1 construct. After fixation with acetone for 5 min, cells were incubated for 2–3 h at room temperature with MR3 polyclonal antibodies (1:50). Polyclonal anti-α₁B subunit antibodies (ACC-002, Alomone) were used at 1/200. Bound antibodies were then detected using FITC- or tetramethylrhodamine isothiocyanate-conjugated secondary antibodies (1/100–200) (DAKO AS, Denmark). Images were collected with an Axioshot fluorescence microscope (Zeiss, Germany) or a confocal microscope. Immunostaining was performed 2 days post-injection.
transduction, we looked at the activity of N-type Ca\(^{2+}\) channels and inwardly rectifying GIRK\(_{1/2}\) K\(^+\) channels (Kir3.1,3.2 subunits), well known to be regulated by G-protein-coupled receptors (26, 30–31) through direct gating by G\(\beta\gamma\) subunits (23, 32–33). Fig. 3 shows the effects of hPC1 and mPC1 on the N-type Ca\(^{2+}\) channels present in these cells (34). Expression of PC1 produced a strong inhibition of their activity. In effect, hPC1 and mPC1 were as effective as over-expressing G\(\beta\gamma\), reducing Ca\(^{2+}\) current density at 0 mV from 33 ± 3 pA/pF in control neurons to 19 ± 4, 22 ± 3, and 17 ± 2 pA/pF, respectively (n = 10–14). A typical trademark of G\(\beta\gamma\)-mediated modulation of Ca\(^{2+}\) currents is the relief of inhibition by depolarizing voltages, a phenomenon termed facilitation (23) that results from the voltage-dependent dissociation of G\(\beta\gamma\) from the Ca\(^{2+}\) channel (Fig. 3B). Like the action of G\(\beta\gamma\), mPC1 or hPC1 mimicked the voltage-dependent facilitation of Ca\(^{2+}\) channels and produced a depolarizing shift of the I-V curves for Ca\(^{2+}\) channel activation (Fig. 3, C–E).

The results obtained on recording Ca\(^{2+}\) currents were qualitatively replicated using GIRK\(_{1/2}\) channels as biosensors for G-protein activation. GIRK\(_{1/2}\) channels were expressed heterologously in sympathetic neurons (35). In the presence of 12 mM external K\(^+\), these channels generate inwardly rectifying currents (Fig. 4). In cells expressing GIRK\(_{1/2}\) channels, both hPC1 and mPC1 significantly increased basal GIRK\(_{1/2}\) current from 10 ± 1 pA/pF in control neurons to 22 ± 2 and 27 ± 4 pA/pF, respectively (Fig. 4, D, E, and G). Here again, PC1 activation of GIRK\(_{1/2}\) currents was quantitatively comparable with that caused by G\(\beta\gamma\) (23 ± 4 pA/pF) (Fig. 4, C and G).

$\text{PC2 Inhibits G-protein Activation by PC1}$

**Fig. 3. Polycystin-1 mimics G\(\beta\gamma\) modulation of Ca\(^{2+}\) currents by activating G\(_{14/12}\)-type G-proteins.** Representative Ca\(^{2+}\) current traces recorded in an un.injected neuron (A) and in neurons expressing either G\(\beta\gamma\) (B) or mPC1 (C). Ca\(^{2+}\) currents were evoked before \(P_1\) and after \(P_2\) a depolarizing prepulse to +90 mV (top left inset). Note that NEM (50 μM), a G\(_i/o\)-G-protein inhibitor, blocked the voltage-dependent inhibition induced by mPC1 but not that by G\(\beta\gamma\). D, current-voltage relationships measured 7 ms after the start of the step for the Ca\(^{2+}\) currents in \(P_1\) in neurons microinjected with FITC (n = 6), G\(\beta\gamma\) cDNA (n = 12), and mPC1 cDNA (n = 14). Points are mean ± S.E. (E and F) representative Ca\(^{2+}\) current traces in neurons expressing hPC1 either alone (E) or together with G\(_\alpha\) transducin (F). Note that a transducin blocked hPC1-induced facilitation of Ca\(^{2+}\) currents. G, intrapipette diffusion of GDP-\(\beta\)-S reversed mPC1-induced modulation. Ca\(^{2+}\) currents were recorded 1 min after achieving the whole-cell configuration (tick line) in the presence of 3 mM internal GDP-\(\beta\)-S and then every 4 min (arrows). H, summary of facilitation (taken as an index of voltage-dependent inhibition) observed in neurons under the different conditions indicated. PTX, pertussis toxin; G\(_\alpha_1\), G\(_\alpha\) transducin. ***, p < 0.001 versus Control. Facilitation was calculated as the ratio \((P_2/P_1)\) of postpulse to prepulse currents. **PC2 Inhibits G-protein Activation by PC1**
the modulation of GIRK<sub>12</sub> channels suggested that PC1 couples to G<sub>i/o</sub>-type G-proteins, since similar receptor-mediated effects result from activation of this family of G-proteins (30–31). The effects of PC1 were therefore assessed after pre-treatment with agents (Pertussis toxin and NEM, see “Materials and Methods”) that are known to prevent receptor/G<sub>i/o</sub>-protein interaction. After these treatments, mPC1 and hPC1 no longer modulated either Ca<sup>2+</sup> (Fig. 3, C, E, and H) or GIRK<sub>12</sub> (Fig. 4, D, E, and G) channels. We confirmed that neither PTX nor NEM altered Gβγ interaction with ion channels (Figs. 3B and 4C). In an additional set of experiments, we tested whether PC1 could activate G<sub>q</sub>-type G-proteins by testing its ability to inhibit M-type K<sup>+</sup> channels (36) (KCNQ channel family), which are selectively modulated via G<sub>q</sub>-type G-proteins in rat-sympathetic neurons (37). There was no significant difference in the size of the M-current between un.injected neurons (4.4 ± 0.3 pA/pF, n = 7) and neurons expressing mPKD1 (3.9 ± 0.4 pA/pF, n = 5).

To test whether the tonic activation of G-proteins could result from over-expression of PC1 (or any G-protein-coupled receptor), we over-expressed G<sub>i/o</sub>-coupled GABA<sub>B</sub>R1b/R2 receptors (38). In the absence of ligand, over-expression of GABA<sub>B</sub> receptors did not produce tonic modulation of either Ca<sup>2+</sup> channels (facilitation 1.16, n = 4; not shown) or GIRK<sub>12</sub> channels (Fig. 4G). The successful expression of GABA<sub>B</sub> receptors was demonstrated by the 10-fold increased activation of GIRK<sub>12</sub> in response to bafilomycin (50 μM) (control cells: 7.5 ± 1.5 pA/pF, n = 4; cells expressing GABA<sub>B</sub>R1b/R2: 60.3 ± 3.4 pA/pF, n = 7).

**Activation of G<sub>i/o</sub>-proteins by Polycystin-1**

**Is Independent on RGS Proteins**—Recently, RGS7, a member of the RGS proteins that act as GTPase activating proteins for G<sub>i/o</sub> subunits, has been shown to bind to the C terminus segment of PC1 (20). Using RT-PCR, RGS7 transcripts along with many others (RGS2, 4–5, and 8) were detected in rat sympathetic neurons (data not shown). Although it is not known whether PC1/RGS7 complexes occur in vivo, we hypothesized that PC1 signals might be mediated via reduction of endogenous RGS-mediated GTPase activating protein activity. This would enhance the effects of any endogenously active G<sub>oA</sub> G-proteins. To address this, we used G<sub>oA</sub> mutants rendered insensitive to PTX and/or RGS proteins by point mutation of Cys-351 and Gly-183/184, which are sites for PTX-mediated ADP ribosylation and RGS binding, respectively (39). The actions of mutated G-proteins were isolated from those of native G<sub>oA</sub> G-proteins by PTX treatment. Fig. 5 shows that heterologous expression of PTX-insensitive (C351I) G<sub>oA</sub> or G<sub>oA</sub> mutants reconstituted mPC1 coupling to Ca<sup>2+</sup> channels, G<sub>oA</sub> being slightly more efficient than G<sub>oA</sub> (Fig. 5, B and C). Expression of either double mutants G<sub>oA</sub> (C351I/G184S) or G<sub>oA</sub> (C351I/G183S) also restored mPC1-induced modulation of ion channel (Fig. 5, C and D), indicating that activation of G<sub>oA</sub> subunits by PC1 was largely independent of RGS action.

**Interaction of Polycystin-2 with Polycystin-1 via Their C Terminal Inhibits G-protein Activation by PC1**—PC1 is thought to interact with PC2 through its C-terminal coiled-coil domain in vitro (18–19). This site is only few amino acids downstream from the putative G-protein activating region (21) and may well influence G-protein binding. We therefore tested whether in vivo heterodimerization of PC1 and PC2 alters G-protein activation by PC1. In cells co-expressing full-length mPC1 and full-length mPC2 (1:1 ratio) G-protein-induced facilitation of Ca<sup>2+</sup> currents was strongly repressed (Fig. 6, A and B). Consistently, basal activation of GIRK<sub>12</sub> current was reduced by ~85% in cells co-expressing mPC1 and mPC2 compared with cells expressing mPC1 homomers (Fig. 6, B and D). mPC2 alone had no effect either on Ca<sup>2+</sup> or GIRK channels (Fig. 6C).

![Fig. 4. Gβγ-mediated activation of GIRK<sub>12</sub> channels by polycystin-1. A–F, GIRK<sub>12</sub> currents in an uninjected neuron (B) and in neurons expressing Gβ<sub>1γ</sub>2 (C), mPC1 (D), hPC1 (E), and hPC1 together with Ga transducin (F). In each recording, GIRK<sub>12</sub> current was identified as the current blocked by 100 μM Ba<sup>2+</sup>. Note that Ga blocked the tonic activation of GIRK<sub>12</sub> by mPC1 and hPC1 but not by Gβ<sub>1γ</sub>2. The effects of NEM in control cells and in cells expressing Gβ<sub>1γ</sub>2 resulted from the basal turnover of endogenous Gα<sub>i/o</sub> G-proteins. The voltage protocol is shown in A. G, summary of activation of GIRK<sub>12</sub> current observed in neurons under the different conditions indicated. ***, p < 0.001; **, p < 0.01; versus Control.](image-url)

(Fig. 4, F and G), indicating that PC1 mediates its effects by releasing Gβγ complexes from their association with Ga subunits.

By analogy with G-protein-coupled 7TM receptors, we hypothesized that PC1 directly activates G-proteins and catalyzes GTP binding to the Ga subunit (21). We tested this by intracellularly dialyzing the non-hydrolyzable GDP analogue, GDP-β-S, in mPC1-expressing neurons. GDP-β-S (3 μM) reversed the effects of mPC1 on Ca<sup>2+</sup> channels by 74 ± 5% (n = 5) within 15–20 min of intracellular diffusion (Fig. 3, G and H).

The properties of Ca<sup>2+</sup> channel inhibition by PC1 as well as
These results suggest that co-assembly of PC2 with PC1 occludes G-protein binding/activation by PC1. To test whether these effects are dependent on an interaction at their respective C-terminal regions, we co-expressed the full-length mPC1 with an mPC2 mutant lacking the C-terminal 226 amino acids (R742X), which includes the putative PC1 interaction domain. Expression of mPC2 R742X failed to occlude mPC1 modulation of either Ca²⁺/H⁺ or GIRK1/2 currents (Fig. 6, A, B, and D). Electrophysiological recordings of ion channel activity (Fig. 6A, inset) confirmed that mPC2 R742X was targeted to the plasma membrane in accordance with previous reports (15, 40). Expression of mPC2 R742X alone did not modulate either Ca²⁺ or GIRK1/2 currents (current densities of 9 ± 2 pA/pF (n = 5) and 30 ± 4 pA/pF (n = 4), respectively; compare with control values above). Further, expression of an mPC1 mutant that lacks the C-terminal 193 amino acids, including the putative binding domain for G-proteins and the interaction site with PC2, lost its ability to activate G-proteins and modulate GIRK1/2 or Ca²⁺/H⁺ channels (n = 5–7) (Fig. 6, C and D).

**DISCUSSION**

In the present study, we have developed a mammalian expression system in which function of full-length polycystins can be tested. These cells (primary sympathetic neurons) are capable of rapidly expressing a wide variety of membrane receptors in functional form that need not be of neural origin and their complement of G-protein-regulated ion channels allows a convenient real-time readout of G-protein stimulation. Thus, though the downstream effectors we have used are largely neural-specific, and hence unlikely to be represented in cells that normally express polycystins, they provide valuable information regarding the primary events induced by polycystins that would be expected to apply to any cell.

From this viewpoint, our data clearly show that PC1 acts as a G<sub>i/o</sub>-protein-coupled receptor that regulates the activity of ion channels via G<sub>i/o</sub>-proteins. Evidence that PC1 behaved as a G-protein-coupled receptor and directly activated G-proteins of the G<sub>i/o</sub> family to release βγ subunits is provided by the facts that the effects of PC1 were reversed by: (a) dialyzing the cell interior with 3 mM of the non-hydrolyzable GDP analogue, GDP-β-S; (b) pretreatment with PTX or NEM, which prevents receptor/G-protein interaction; and (c) over-expressing the G<sub>i</sub>-sequestering agent G<sub>i</sub>transducin. PC1 appeared to couple specifically to G<sub>i/o</sub> proteins since it did not inhibit M-type K<sup>+</sup> channels, which are selectively modulated via G<sub>q</sub>-type G-proteins in sympathetic neurons.

These findings support the hypothesis that PC1 is involved in signal-transduction pathways, as suggested previously for the homologue of hPC1, LOV-1, in sensory neurons of Caenorhabditis elegans (41). PC1 may not require ligand binding to initiate G-protein signals since hPC1 lacking the N-terminal 1811 residues (extracellular domain) thought to be involved in cell-cell and/or cell-matrix interactions (22, 42, 43) replicated the effects of full-length mPC1 (though further experiments are needed to determine whether the REJ domain plays a role in G-protein activation). Our study also shows that co-assembly of PC2 with PC1 via their C termini inhibits the G-protein-activating properties of PC1. This is particularly important because in the kidney and many other tissues PC1 and PC2 are interacting partners, forming polycystin complexes (1). Thus, polycystin complex-disturbing mutations that result in either
PC1 over-expression or loss of PC2 would “re-activate” G-protein signaling pathways. Indeed, PC1 over-expression in renal cysts is a general finding in almost all ADPKD patients (1, 12, 44). The current study thus provides a key missing piece of a puzzle, and links a long-standing immunohistological observation with an important known signaling pathway. The results also offer a molecular explanation of the cystic kidney phenotype in transgenic mice over-expressing normal PC1 (13) and enlighten our understanding of the additive effects of the PKD1/PKD2 trans-heterozygous as a genetic basis for cystogenesis in ADPKD (11).

G-protein-mediated signaling pathways involving adeny- late cyclase and mitogen-activated protein kinases are known to control fluid secretion, cell proliferation, and differentiation. Abnormalities in these cell functions are central features of human ADPKD (1, 3). The identification of PC1 as a G-protein-coupled receptor potentially capable of activating such pathogenic pathways opens up new avenues for PKD research and provides a novel basis for the design of therapeutic strategies.

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FIG. 6. Co-expression of polycystin-2 repressed G-protein activation by PC1. A, Ca2+ current facilitation in neurons expressing mPC1 alone (left panel) and mPC1 together with full-length mPC2 (middle) or mPC2 mutant R742X (right). Note the absence of facilitation in the cell co-expressing mPC1 and mPC2 and the strong facilitation in the cell expressing mPC2 R742X. The noise in the right panel resulted from the expression of the cation channel generated by mPC2 R742X, which is permeant to Ba2+ (n = 5) but not to NMDG+ (n = 8) (data not shown). An example of mPC2 R742X channel activity recorded with a cell-attached patch (146 mM Na+, pipette, 0 mV) is shown in the inset. Scale: 2 pA, 0.5 s. All whole-cell recordings were made in the absence of external Na+ (isosmolarly substituted by NMDG+) and with Ba2+ (5 mM) as charge carrier. cDNAs: 200 ng/μl and for co-expression 200 ng/μl each. B, GIRKγ2 current recording in neurons expressing mPC1 alone (left panel) and mPC1 together with full-length mPC2 (middle) or mPC2 R742X (right). Note the reduced basal activation of GIRKγ2 channels in the cell co-expressing mPC2 and the normal activation in the cell expressing mPC2 R742X. Recordings were made in the presence of 146 mM external Na+. mPC2/mPC1 consistently caused a smaller increase in holding current at −60 mV than mPC2 R742X. cDNA was as in A. C, expression of an mPC1 mutant lacking 193 C-terminal residues does not induce Ca2+ current facilitation. D, summary of the effects of mPC2 and mPC1 on Ca2+ current facilitation (left) and activation of GIRKγ2 current (right). Recording conditions as in A and B. **, p < 0.01 versus mPC1.
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