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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 Ca^{2+} and K^+ channels as functional biosensors, we found that full-length PC1 functioned as a constitutive activator of $G_{i/o}$-type but not G_q-type G-proteins and modulated the activity of Ca^{2+} and K^+ channels via the release of $G\beta\gamma$ 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), 3) 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 Ca^{2+}-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 Ca^{2+} 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 $G_{i/o}$ but not G_q G-proteins, independently from RGS protein activity. In addition, we show that the G-protein activating property of PC1 can be inhibited

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[1] The abbreviations used are: ADPKD, autosomal dominant polycystic kidney disease; PKD, polycystin kidney disease; PC, polycystin; LOH, somatic loss of heterozygosity; RT, reverse transcription; RGS, regulators of G-protein signaling proteins; GIRK, G-protein-activated inward rectifier potassium channel; FITC, fluorescein isothiocyanate; PTX, *Bordetella pertussis* toxin; NMDG, *N*-methyl-D-glucamine; NEM, *N*-ethylmaleimides.

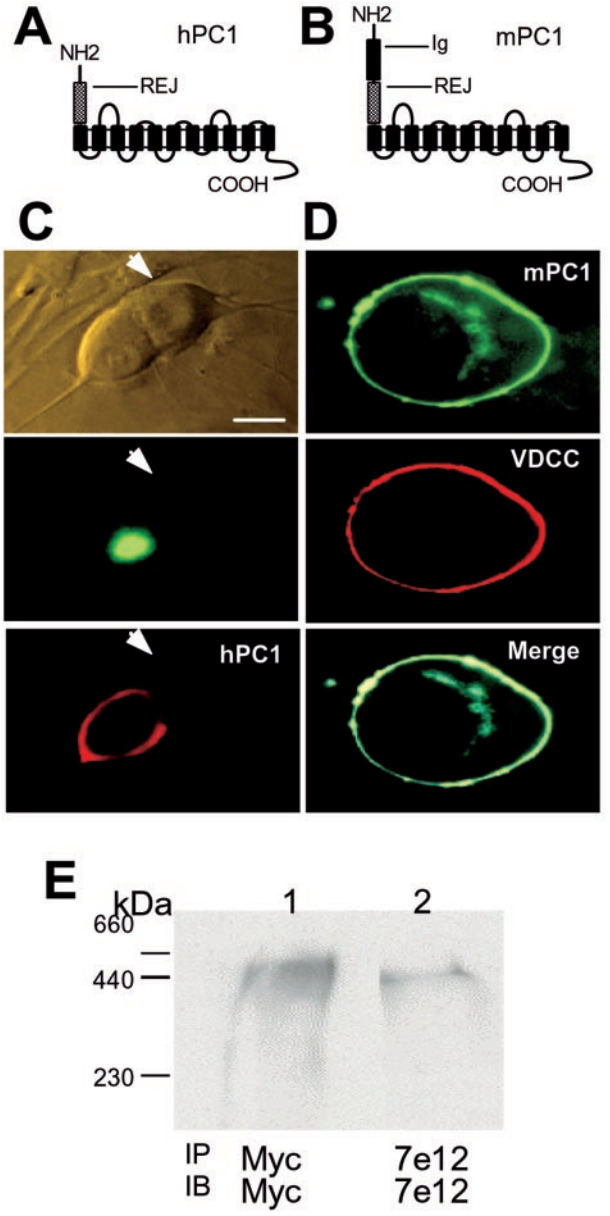

FIG. 1. **Cell surface expression of polycystin-1.** Proposed membrane topology for hPC1 (*A*) and mPC1 (*B*). The 11 TM topology is shown. PC1 is predicted to have an extensive, N-terminal extracellular region that contains several functional motifs and a small, C-terminal cytoplasmic tail known to interact with PC2. *REJ*: receptor for egg jelly; *Ig*: Ig-like repeat. *C*, corresponding images showing a rat-sympathetic neuron (*bright field*, *top panel*) intranuclearly injected with hPC1 cDNA together with a fluorescent marker (FITC, *middle panel*) and stained with the anti-hPC1 antibody MR3 (*bottom*). Note the strong labeling of the cell surface. The *arrow* indicates an uninjected neuron. *Bar scale*, 20 μm. *D*, confocal sections of a neuron microinjected with cDNA encoding Myc-tagged mPC1 and stained using anti-Myc antibody (*green*) and an antibody against voltage-dependent N-type Ca^{2+} channels (α1b) (*VDCC*, red). Antigens were stained after fixing. Note in the *overlay* that the two proteins co-localize at the plasma membrane. *E*, expression of full-length Myc-tagged mPC1 (*lane 1*) and untagged mPC1 (*lane 2*) in 5454 and Hek293 mammalian cell lines. Precleared cell lysates were immunoprecipitated (*IP*) with either monoclonal anti-Myc antibody (*lane 1*) or the monoclonal anti-mPC1 antibody, 7e12 (*lane 2*).

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 *Not*I 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, $GABA_BR1b$, $GABA_BR2$, Gβ_1, Gγ_2, and Gα-transducin cDNAs) or 40 μg ml^{-1} (G-protein α mutants). 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_2 G-protein subunits, retinal Gα transducin, Kir3.1/3.2 and $GABA_BR1b/R2$ subunits were described previously (25, 34, 38). PTX- and RGS-insensitive G-protein $\alpha_{oA/i1}$ 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 $G\alpha_{oA}$-G184S showed a >10-fold slower recovery from Ca^{2+} current inhibition by noradrenaline, confirming that $G\alpha_{oA}$ mutants are resistant to the action of endogenous RGS proteins.

Immunoblotting—Myc-tagged mPC1 and untagged mPC1 were transiently transfected into 5454 cells and Hek293 cells, respectively. Precleared 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. Immobilized 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 M-type K^+ 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 (bubbled with a 95% O_2-5% CO_2 mixture, pH 7.4). $GIRK_{1/2}$ currents were recorded using the same external solution, except that $[K^+]_o$ 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 Sylgard and had resistance of 2–3.5 MΩ. Internal solution for I_{Ca} was (mM): 120 NMDG, 14 CsCl, 10 Hepes, 11 EGTA, 0.5–1 $CaCl_2$, 0.1 phosphocreatine, 4 MgATP and 0.2 Na_3GTP (pH 7.3). Currents were measured with an Axopatch 200A amplifier, filtered at 1–5 kHz, and leak-subtracted using the leak subtraction procedure (*P*/6) of the pClamp software (Axon Instruments). Cell membrane capacitance and series resistance compensations were applied (75–85%). In experiments where GDP-β-S (3 mM) was added to the pipette solution, GTP was omitted. Ca^{2+} currents were evoked using a triple-pulse voltage protocol, and facilitation was calculated as the ratio (P_2/P_1) of Ca^{2+} current amplitude (see Fig. 3). The intracellular solution for $GIRK_{1/2}$ current consisted of (mM): KCl, 60; potassium acetate, 60; $MgCl_2$, 2.5; Hepes, 30; BAPTA (1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid), 10; Na_2ATP, 2; and Na_3GTP, 0.1 (adjusted to pH 7.2 with KOH and 290 mosmol l^{-1}). Currents were recorded with an Axoclamp 2B amplifier (discontinuous

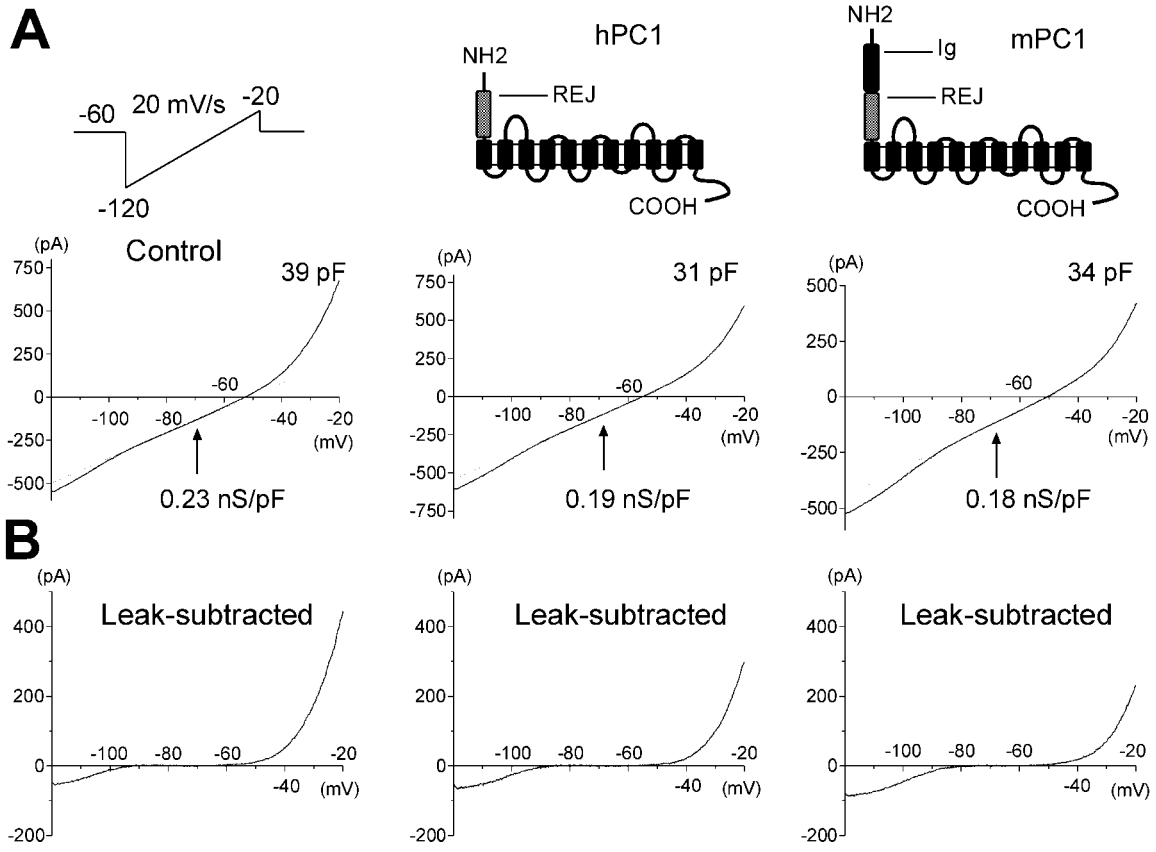

FIG. 2. **Polycystin-1 does not form cation channels in neurons.** *A*, representative current-voltage (*I-V*) relationships obtained in control neurons (injected with green fluorescent protein cDNA) and in neurons expressing hPC1 or mPC1. The thin lines (linear regression) represent the leak conductance extrapolated from the −60/−80 mV voltage region. Leak conductance is given normalized to the cell capacitance for comparison. *B*, *I-V* relationships minus leak currents, isolating inward and outward rectifications. Note that the reduction in the outward K^+ rectification in hPC1- and mPC1-expressing neurons results indirectly from the inhibition of Ca^{2+} channels (see below and Fig. 3).

mode), low-pass filtered at 1 kHz, and sampled at 6.67 kHz. $GIRK_{1/2}$ currents were typically evoked by 700 ms voltage ramps from −140 to −40 mV. The amplitude of $GIRK_{1/2}$ currents was measured as the Ba^{2+}-sensitive current (100 μM) 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; $MgCl_2$, 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 $\mu g\ ml^{-1}$. 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-α_{1B} 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 A/S, Denmark). Images were collected with an Axiophot fluorescence microscope (Zeiss, Germany) or a confocal microscope. Immunostaining was performed 2 days post-injection.

RESULTS

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. 1*C*). Further, Myc-tagged mPC1 was co-immunolabeled with the α_{1B} Ca^{2+} channel subunit, a typical plasma membrane protein. Confocal sections showed that Myc-tagged mPC1 co-localized with Ca^{2+} channels in the plasma membrane and was not retained intracellularly (Fig. 1*D*). 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. 1*E*).

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 ($PC1_{C1–226}$) in oocytes (29).

Polycystin-1 Modulates Voltage-dependent Ca^{2+} Channels and GIRK K^+ Channels—To assess the role of PC1 in signal

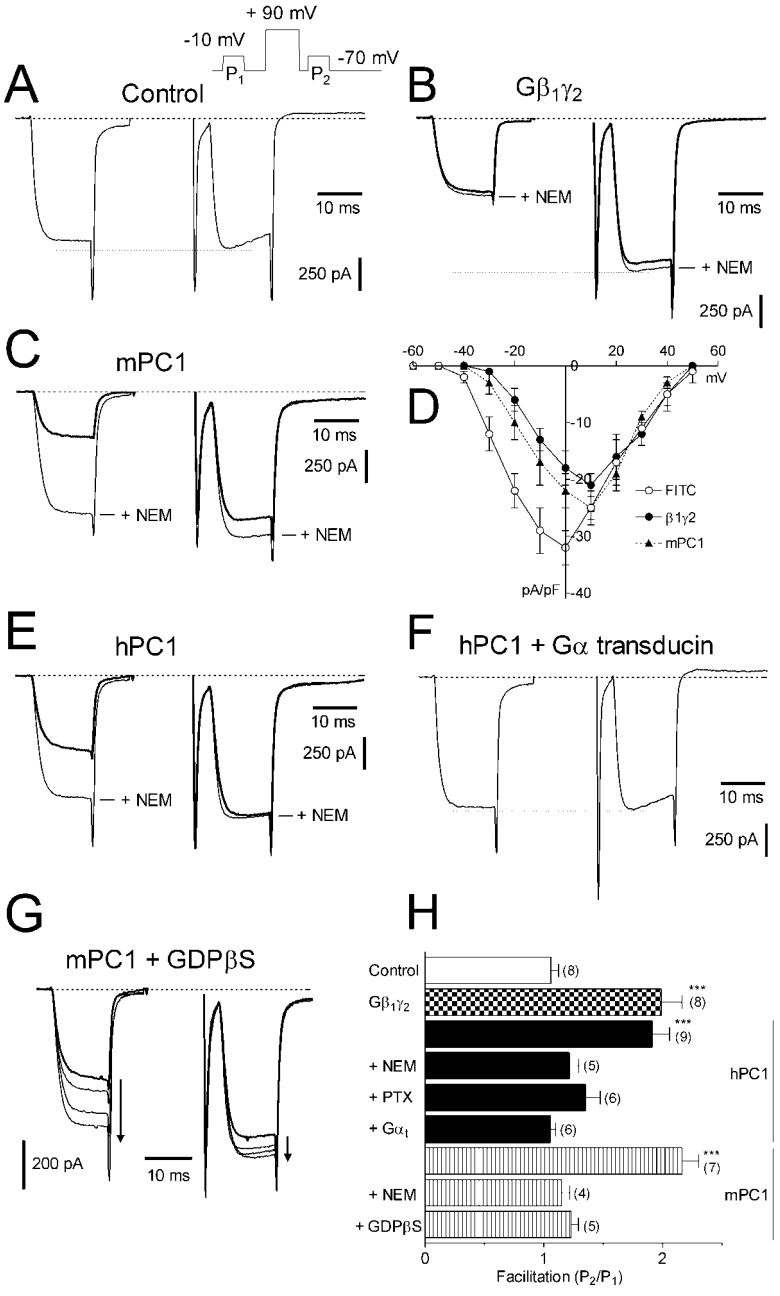

FIG. 3. **Polycystin-1 mimics Gβγ modulation of Ca^{2+} currents by activating $G_{i/o}$-type G-proteins.** Representative Ca^{2+} current traces recorded in an uninjected neuron (*A*) and in neurons expressing either $G\beta_1\gamma_2$ (*B*) or mPC1 (*C*). Ca^{2+} currents were evoked before (P_1) and 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 $G\beta_1\gamma_2$. *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 ($n = 6$), $G\beta_1\gamma_2$ 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 Gα transducin (*F*). Note that α transducin blocked hPC1-induced facilitation of Ca^{2+} currents. *G*, intrapipette diffusion of GDP-β-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-β-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; $G\alpha_t$, Gα transducin. ***, $p < 0.001$ *versus* Control. Facilitation was calculated as the ratio (P_2/P_1) of postpulse to prepulse currents.

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 Gβγ 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 $G\beta_1\gamma_2$, 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 Gβγ-mediated modulation of Ca^{2+} channels is the relief of inhibition by depolarizing voltages, a phenomenon termed facilitation (23) that results from the voltage-dependent dissociation of Gβγ from the Ca^{2+} channel (Fig. 3*B*). Like the action of Gβγ, 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 $G\beta_1\gamma_2$ (23 ± 4 pA/pF) (Fig. 4, *C* and *G*).

Polycystin-1 Modulates Ion Channels via $G_{i/o}$-protein Activation and Gβγ Release—Taken together, these results indicate that PC1-mediated effects were phenomenologically identical to those produced by Gβγ. To test whether PC1 was releasing Gβγ from endogenous G-protein heterotrimers or simply mimicking the action of Gβγ on ion channels, we expressed the Gβγ-sequestering agent Gα transducin (24–25, 34) together with PC1. Gα transducin fully abolished the action of hPC1 on both Ca^{2+} channels (Fig. 3, *F* and *H*) and $GIRK_{1/2}$ channels

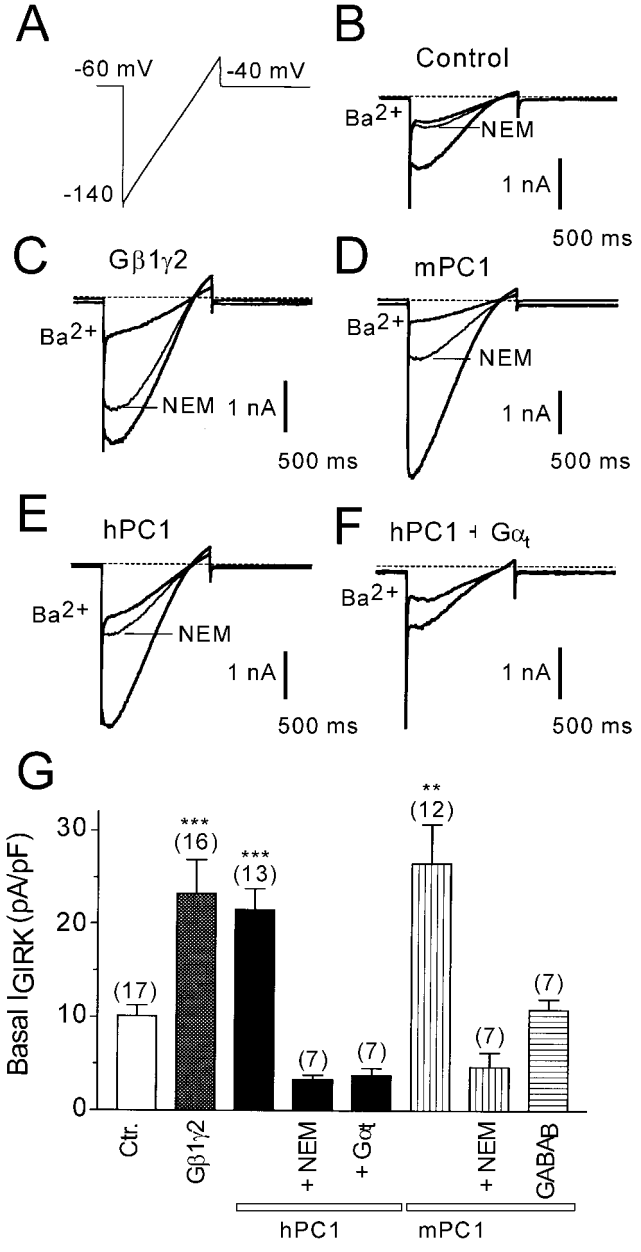

FIG. 4. **Gβγ-mediated activation of $GIRK_{1/2}$ channels by polycystin-1.** *A–F*, $GIRK_{1/2}$ currents in an uninjected neuron (*B*) and in neurons expressing $G\beta_1\gamma_2$ (*C*), mPC1 (*D*), hPC1 (*E*), and hPC1 together with Gα transducin (*F*). In each recording, $GIRK_{1/2}$ current was identified as the current blocked by 100 μM Ba^{2+}. Note again that NEM blocked the tonic activation of $GIRK_{1/2}$ by mPC1 and hPC1 but not by $G\beta_1\gamma_2$. The effects of NEM in control cells and in cells expressing $G\beta_1\gamma_2$ result from the basal turnover of endogenous G_i/G_o G-proteins. The voltage protocol is shown in *A*. *G*, summary of activation of $GIRK_{1/2}$ current observed in neurons under the different conditions indicated. **, $p < 0.01$; ***, $p < 0.001$ *versus* Control.

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

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

The properties of Ca^{2+} channel inhibition by PC1 as well as the modulation of $GIRK_{1/2}$ channels suggested that PC1 couples to $G_{i/o}$-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/$G_{i/o}$-protein interaction. After these treatments, mPC1 and hPC1 no longer modulated either Ca^{2+} (Fig. 3, *C*, *E*, and *H*) or $GIRK_{1/2}$ (Fig. 4, *D*, *E*, and *G*) channels. We confirmed that neither PTX nor NEM altered Gβγ interaction with ion channels (Figs. 3*B* and 4*C*). In an additional set of experiments, we tested whether PC1 could activate G_q-type G-proteins by testing its ability to inhibit M-type K^+ channels (36) (KCNQ channel family), which are selectively modulated via G_q-type G-proteins in rat-sympathetic neurons (37). There was no significant difference in the size of the M-current between uninjected 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_i/G_o-coupled $GABA_BR1b/R2$ receptors (38). In the absence of ligand, over-expression of $GABA_B$ receptors did not produce tonic modulation of either Ca^{2+} channels (facilitation 1.16, $n = 4$; not shown) or $GIRK_{1/2}$ channels (Fig. 4*G*). The successful expression of $GABA_B$ receptors was demonstrated by the 10-fold increased activation of $GIRK_{1/2}$ in response to baclofen (50 μM) (control cells: 7.5 ± 1.5 pA/pF, $n = 4$; cells expressing $GABA_BR1b/R2$: 60.3 ± 3.4 pA/pF, $n = 7$).

Activation of $G_{i/o}$-proteins by Polycystin-1 Is Independent on RGS Proteins—Recently, RGS7, a member of the RGS proteins that act as GTPase activating proteins for Gα 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 $G\alpha_{i/o}$ G-proteins. To address this, we used $G\alpha_{i/o}$ 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 $G\alpha_{i/o}$ G-proteins by PTX treatment. Fig. 5 shows that heterologous expression of PTX-insensitive (C351I) $G\alpha_{oA}$ or $G\alpha_{i1}$ mutants reconstituted mPC1 coupling to Ca^{2+} channels, $G\alpha_{oA}$ being slightly more efficient than $G\alpha_{i1}$ (Fig. 5, *B* and *C*). Expression of either double mutants $G\alpha_{oA}$ (C351I/G184S) or $G\alpha_{i1}$ (C351I/G183S) also restored mPC1-induced modulation of ion channel (Fig. 5, *C* and *D*), indicating that activation of $G\alpha_{i/o}$ subunits by PC1 was largely independent of RGS action.

Interaction of Polycystin-2 with Polycystin-1 via Their C Termini 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^{2+} currents was strongly repressed (Fig. 6, *A* and *B*). Consistently, basal activation of $GIRK_{1/2}$ 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^{2+} or GIRK channels (Fig. 6*C*).

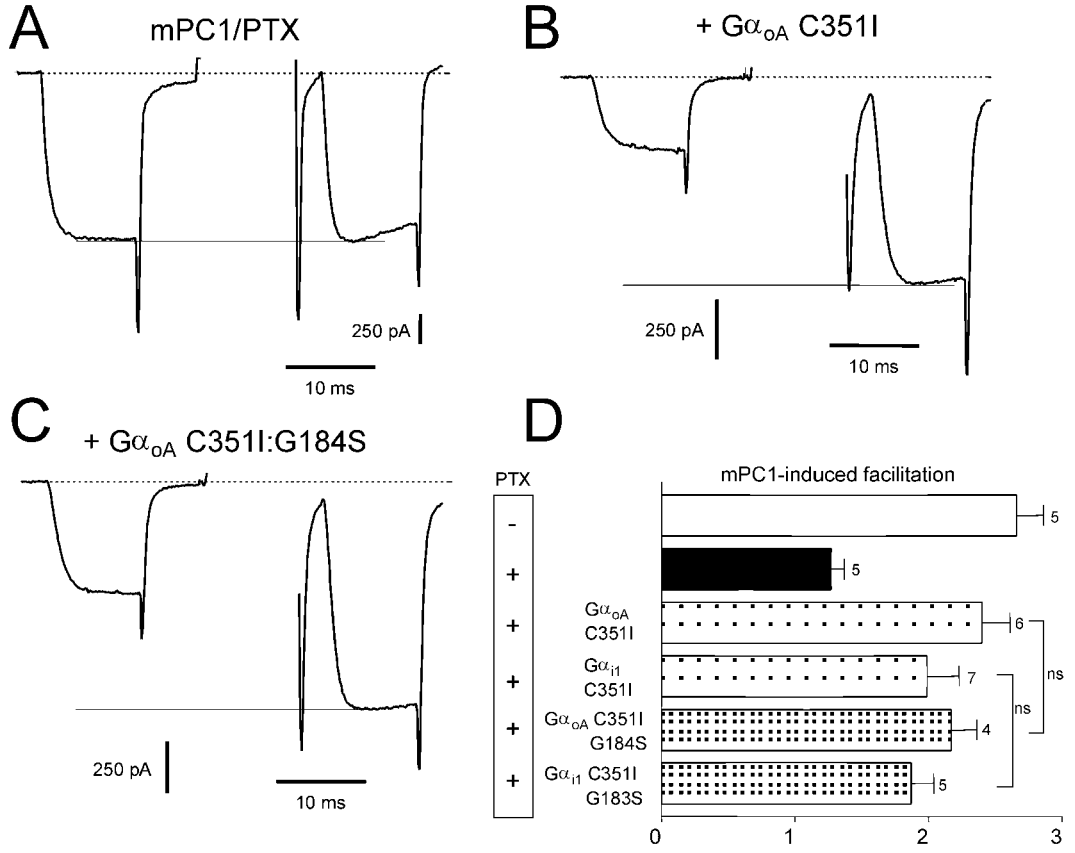

FIG. 5. **Stimulation of G-proteins by PC1 is independent of RGS proteins.** (*A–C*) neurons were pre-treated with PTX (500 ng/ml) for 24 h, and Ca^{2+} currents were recorded in neurons expressing mPC1 alone (*A*) or mPC1 together with PTX-insensitive $G\alpha_{oA}$ mutants (*B*) and PTX- and RGS-insensitive $G\alpha_{oA}$ mutants (*C*). Both mutants restore mPC1 signal. Neurons were microinjected with a ratio of cDNA (mPC1 *versus* $G\alpha$ mutant) of 1:0.2 to avoid possible $G\beta\gamma$ sink by the α subunits. *D*, summary of mPC1-induced facilitation in the presence or absence of PTX/RGS $G\alpha_{oA}$ and $G\alpha_{i1}$ mutants. *ns*, not significant.

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 (R742*X*), which includes the putative PC1 interaction domain. Expression of mPC2 R742*X* failed to occlude mPC1 modulation of either Ca^{2+} or GIRK1/2 currents (Fig. 6, *A*, *B*, and *D*). Electrophysiological recordings of ion channel activity (Fig. 6*A*, *inset*) confirmed that mPC2 R742*X* was targeted to the plasma membrane in accordance with previous reports (15, 40). Expression of mPC2 R742*X* alone did not modulate either Ca^{2+} 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 $GIRK_{1/2}$ or Ca^{2+} 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 $G_{i/o}$-protein-coupled receptor that regulates the activity of ion channels via $G_{i/o}$-proteins. Evidence that PC1 behaved as a G-protein-coupled receptor and directly activated G-proteins of the G_i/G_o family to release $\beta\gamma$ 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 $G\beta\gamma$-sequestering agent $G\alpha$ transducin. PC1 appeared to couple specifically to G_i/G_o proteins since it did not inhibit M-type K^+ channels, which are selectively modulated via G_q-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

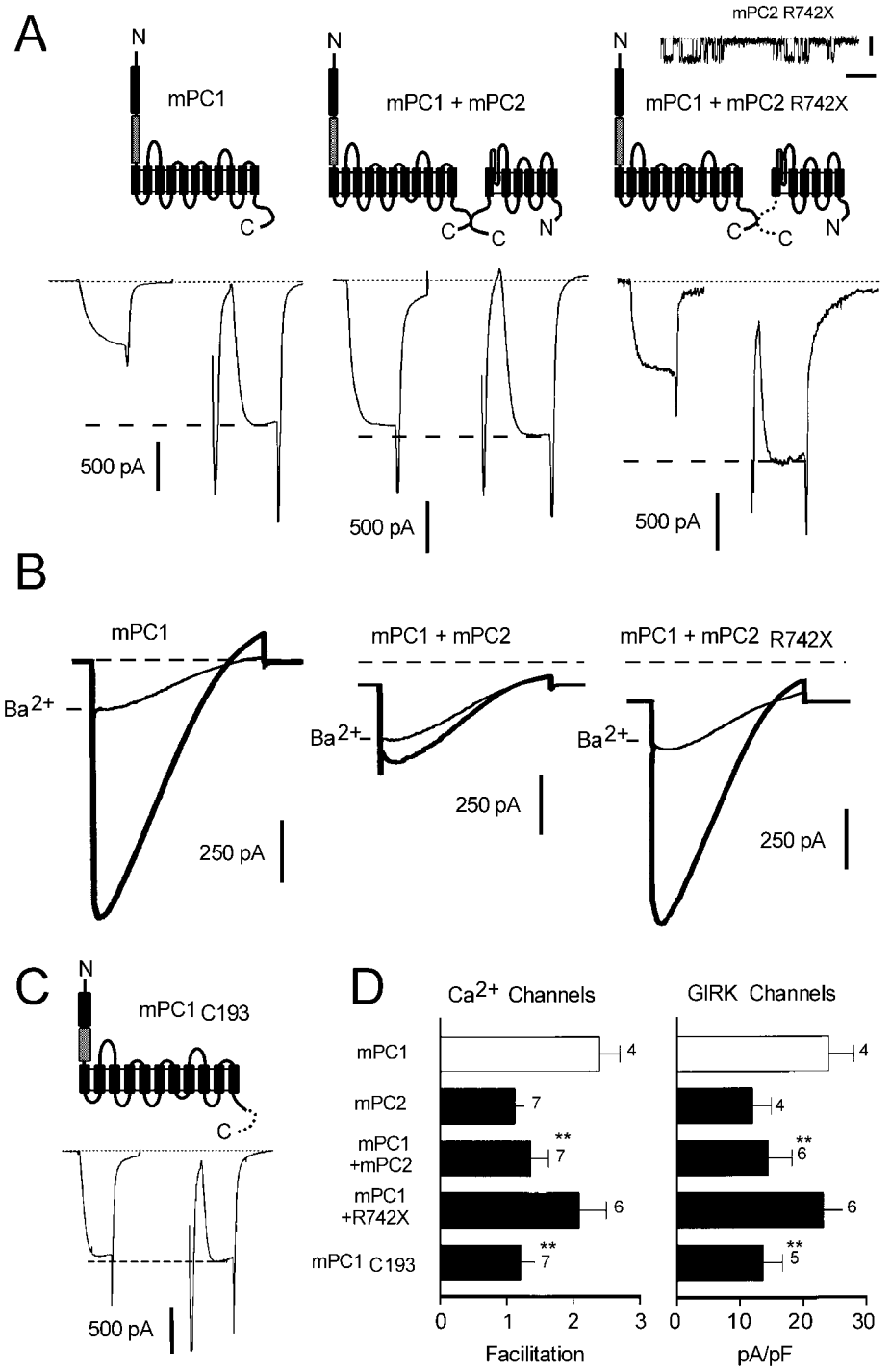

FIG. 6. **Co-expression of polycystin-2 repressed G-protein activation by PC1.** *A*, Ca^{2+} current facilitation in neurons expressing mPC1 alone (*left panel*) and mPC1 together with full-length mPC2 (*middle*) or mPC2 mutant R742*X* (*right*). Note the absence of facilitation in the cell co-expressing mPC1 and mPC2 and the strong facilitation in the cell expressing mPC2 R742*X*. The noise in the *right panel* resulted from the expression of the cation channel generated by mPC2 R742*X*, which is permeant to Ba^{2+} ($n = 5$) but not to $NMDG^+$ ($n = 8$) (data not shown). An example of mPC2 R742*X* channel activity recorded with a cell-attached patch (146 mM $Na^+_{pipette}$, $V_{pipette}$ 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^+ (isoosmolarly substituted by $NMDG^+$) and with Ba^{2+} (5 mM) as charge carrier. cDNAs: 200 ng/μl and for co-expression 200 ng/μl each. *B*, $GIRK_{1/2}$ current recording in neurons expressing mPC1 alone (*left panel*) and mPC1 together with full-length mPC2 (*middle*) or mPC2 R742*X* (*right*). Note the reduced basal activation of $GIRK_{1/2}$ channels in the cell co-expressing mPC2 and the normal activation in the cell expressing mPC2 R742*X*. 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 R742*X*. cDNA was as in *A*. *C*, expression of an mPC1 mutant lacking 193 C-terminal residues does not induce Ca^{2+} current facilitation. *D*, summary of the effects of mPC2 and mPC1 on Ca^{2+} current facilitation (*left*) and activation of $GIRK_{1/2}$ current (*right*). Recording conditions as in *A* and *B*. **, $p < 0.01$ *versus* mPC1.

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 immmunohistological 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 adenylate 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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