The isolated C-terminus of polycystin-1 promotes increased ATP-stimulated chloride secretion in a collecting duct cell line

Kimberly M. HOOPER*†, Robert J. UNWIN‡ and Michael SUTTERS*†

*Division of Renal Medicine, Johns Hopkins Bayview Medical Center, 4940 Eastern Avenue, Baltimore, MD 21224, U.S.A., †Gerontology Research Center, Laboratory of Cellular and Molecular Biology, Baltimore, MD 21224, U.S.A., and ‡Department of Nephrology & Physiology, Royal Free and University College Medical School, London, U.K.

ABSTRACT

Cyst expansion in autosomal dominant polycystic kidney disease (ADPKD) requires accumulation of fluid into the cyst lumen, which is probably driven by aberrant chloride secretion by the cyst lining epithelium. Extracellular ATP is a potent stimulus for chloride secretion in many epithelial systems, and provides a plausible mechanism for secretion in ADPKD. Therefore the link between polycystin-1 and ATP-stimulated chloride secretion was investigated in the M1 cortical collecting duct cell line. M1 cells were stably transfected with a glucocorticoid-inducible cytoplasmic C-terminal polycystin-1 construct fused to a membrane expression cassette. Induction of fusion protein expression was associated with augmentation of ATP-stimulated transepithelial chloride secretion. After nystatin-induced permeabilization of the basolateral membrane, it was determined that expression of the polycystin fusion protein modulated an ATP-responsive apical chloride conductance. It is concluded that up-regulation of ATP-stimulated chloride secretion might play a significant role in cyst expansion in ADPKD.

INTRODUCTION

In the majority of cases, autosomal dominant polycystic kidney disease (ADPKD) arises as a consequence of loss-of-function mutations in the PKD1 gene encoding polycystin-1 [1]. In ADPKD, progressive deterioration in renal function correlates closely with the degree of cyst expansion [2]. Fluid accumulation into cysts in ADPKD remains poorly understood, but it appears that the reabsorptive profile of the normal tubule epithelial cell becomes reversed to a secretory profile in transition to the cyst-forming phenotype. In view of the link between polycystin pathways and intracellular calcium homoeostasis [3], we reasoned that secretion into cysts in ADPKD might be driven by the up-regulation of calcium-mediated secretory pathways. Extracellular ATP is a potent stimulus for transepithelial fluid secretion, acting through stimulation of the purinergic P2Y-type receptor and elevation of the intracellular calcium concentration [4]. Furthermore, the components for chloride secretion stimulated by purinergic receptor activation have been demonstrated in epithelia from ADPKD kidneys [5]. Consequently, we decided to examine the relationship between polycystin-1 pathways and ATP-stimulated chloride secretion through the study of a novel system of transport-competent cortical collecting duct cells stably transfected to express a cytoplasmic C-terminal polycystin-1 fusion protein.

MATERIALS AND METHODS

Generation of stable cell lines

The M1 cell line [6] was selected as the parental source for stable transfections. A cDNA encoding the cytoplasmic C-terminal 193 amino acids of murine polycystin-1 was

Key words: ATP, autosomal dominant polycystic kidney disease, transepithelial transport.

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; DIDS, 4,4′-di-isothiocyanostilbene-2,2′-disulphonate; PPADS, pyridoxal phosphate-6-azophenyl-2,4′-disulphonic acid.

Correspondence: Dr M. Sutters (e-mail msutters@jhmi.edu).
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- **Immunofluorescent cytochemistry**
  Cells were grown to confluence on Matrigel (Becton-Dickenson)-coated glass slides and then treated with dexamethasone or vehicle for 6 h. To demonstrate lateral membrane cell-surface expression of the fusion protein, living confluent monolayers were incubated with an FITC-conjugated anti-human IgG Fc specific antibody at 4 °C for 2 h in calcium-free PBS to cause dissociation of apical tight-junctional integrity. After washes at 4 °C in PBS, the monolayers were fixed in 4% (v/v) paraformaldehyde, mounted in Immunofluore anti-quench (ICN) and imaged by epifluorescent microscopy.

- **Tissue culture and transepithelial transport**
  Cells were grown in Dulbecco’s modified Eagle’s medium/F12 supplemented with 5% (v/v) fetal calf serum. For transport studies, 250,000 cells were seeded on to Matrigel-coated 12 mm Snapwells (Costar) and grown for 7 days, with the final 2 days in the absence of fetal calf serum. Transepithelial transport was measured in standard Ussing chambers (Physiological Instruments) in a modified lactate Ringer buffer bubbled with 95% O2/5% CO2. All transport studies were performed under voltage-clamp conditions at 37 °C.

- **Nystatin permeabilization protocol**
  As transepithelial chloride secretion requires the coordinate activity of basolateral and apical transport proteins, modulation of the ATP-stimulated chloride current could result from effects upon one or more transporters at either location. We employed the nystatin permeabilization technique to permit analysis of ATP-responsive chloride conductance at the apical membrane [8]. After permeabilization of the basolateral membrane, the chloride ion conductance of the apical membrane was assessed by the creation of a transepithelial chloride gradient [119 mM NaCl substituted by sodium gluconate in one chamber, with 2-fold supplementation of calcium and magnesium (4 mM and 2.4 mM respectively) to adjust for the calcium-buffering activity of gluconate] and measurement of current arising from passive chloride diffusion across the apical membrane.

- **RESULTS**
  In the M1 and M1-derived cell lines, we found that apical ATP induced a secretory chloride current and simultaneously inhibited sodium reabsorption, as described by Cuffe et al. [10]. This response was blocked by apical administration of Reactive Blue 2 or DIDS, but was unaffected by suramin or PPADS (Figure 1a). Three slg7–PKD193-expressing cell lines (clones 2, 18 and 20) demonstrated dexamethasone-inducible expression of the C-terminal polycystin-1 fusion protein (Figure 1b) (albeit with some background leak in the absence of dexamethasone), and formed acceptably high-resistance monolayers. Cell-surface slg7–PKD193 was only seen in confluent non-permeabilized monolayers after cell–cell contacts were opened up by incubation in calcium-free buffer, indicating that the slg7–PKD193 fusion protein was localized to the lateral cell membranes (Figure 1c), as is native full-length polycystin-1 [9]. Three control lines were used in these experiments. The slg7 cell line expressed the membrane expression cassette alone. Clones p8 and p9 were derived from the vector-alone transfection and were maintained in G418 selection medium. Cell counts were performed by DAPI (4,6-diamidino-2-phenylindole) staining of nuclei on dexamethasone-treated monolayers after Ussing studies, and showed

- **Agonists and antagonists**
  The following (final concentrations in parentheses) were obtained from Sigma: dexamethasone (1 µM), amiloride (0.1 mM), ATP (100 µM), nystatin (300 µg/ml), pyridoxal phosphate-6-azophenyl-2,4′-disulphonic acid (PPADS; 250 µM), suramin (250 µM) and Reactive Blue 2 (100 µM). 4,4′-Di-isothiocyanostilbene-2,2′-disulphonate (DIDS; 300 µM) was obtained from Calbiochem.

- **Data analysis**
  The 15-min area under the time–current curve for each experiment was calculated using Sigma Plot, using the pre-ATP current as baseline. Multiple comparisons between cell lines were made by one-way ANOVA, with significance accepted at P < 0.05 by the unpaired two-tailed t test with the Bonferroni multiplicity adjustment, using Analyse-It software.
no difference between control and sIg7–PKD193-expressing cell lines (261 000 ± 8000 and 246 000 ± 12 000 respectively; three monolayers counted for each cell line).

In the presence of dexamethasone, in both intact and nystatin-permeabilized monolayers, the response to ATP was greater in each of the three sIg7–PKD193 cell lines compared with the control cell lines (Figures 2a and 2c). In the absence of dexamethasone, in intact monolayers, the response to ATP, although generally diminished in all cell lines, was no different between the control and sIg7–PKD193 cell lines (Figure 2b). Chloride secretory currents stimulated by basolateral 10 μM forskolin [mediated by cAMP activation of the CFTR (cystic fibrosis transmembrane conductance regulator)] were no different between cell lines (5 min area under the curve: control, 12.3 ± 2.3 μA · min; PKD193, 15.2 ± 1.3 μA · min).

**DISCUSSION**

It is unlikely that clonal variability could have accounted for the present findings, since multiple cell lines showed consistent responses. Furthermore, the difference between sIg7–PKD193-expressing and control cell lines was not apparent in a background of low-level expression of polycystin C-terminal fusion protein in the absence of dexamethasone. It should be noted that dexamethasone
Figure 2 Responses to ATP of intact monolayers of sIg7–PKD193 and control cell lines (a, b) and of nystatin-permeabilized monolayers (c)

(a) In the presence of 1 μM dexamethasone, the responses to ATP of each of the sIg7–PKD193 cell lines were greater than those of each of the control lines (stars; \( P < 0.05 \) by ANOVA with the Bonferroni correction; \( n = 10 \) for each cell line).

(b) In the absence of dexamethasone, the responses to ATP of all of the control and sIg7–PKD193 cell lines were indistinguishable (\( n = 7 \) for each cell line).

(c) Data from permeabilized monolayers with apical-to-basolateral chloride gradients. As was seen in the intact monolayer series, the sIg7–PKD193 cell lines 2, 18 and 20 showed a greater response to ATP compared with control lines. ATP responses in the sIg7–PKD193 cell lines 18 and 20 significantly exceeded the responses in all of the control lines (stars; \( P < 0.05 \) by ANOVA with the Bonferroni correction; \( n = 4 \) for each cell line).

Itself augmented ATP-stimulated chloride secretion in all cell lines (compare Figures 2a and 2b), suggesting a permissive effect. However, the increased response to ATP of the sIg7–PKD193 cell lines was not the consequence of generally enhanced sensitivity to the effects of dexamethasone, since dexamethasone-induced sodium reabsorption was the same in both groups of cell lines (control, 20.4 ± 1.3 μA; sIg7–PKD193-expressing, 24.7 ± 1.8 μA). The nystatin studies indicated that dexamethasone-induced expression of the cytoplasmic C-terminus of polycystin-1 increased ATP-stimulated transepithelial chloride secretion through modulation of chloride channels in the apical cell membrane. Our observations could result from interactions between the sIg7–PKD193 fusion protein and (1) heterotrimeric G-proteins [11,12], (2) intracellular calcium homeostasis [3] or (3) chloride channel activity or abundance. We have not excluded the possibility of concurrent effects on basolateral or other apical transport pathways.

The C-terminal cytoplasmic polycystin-1 fusion protein, by promoting a secretory phenotype, would appear to be acting in a dominant negative fashion. Other investigators have demonstrated the potential for interaction of the isolated cytoplasmic C-terminus of polycystin-1 with multiple signalling pathways [11–14], but it is not yet clear whether such studies indicate augmentation or interruption of the function of native full-length polycystin-1. In keeping with a possible dominant negative capacity of the isolated cytoplasmic C-terminal polycystin-1 fusion protein, an earlier study of the cell lines used in the present study [7] demonstrated that fusion protein expression was associated with transformation of the cAMP effect on proliferation from normal (inhibition) to an ADPKD pattern (stimulation). On the other hand, expression of the isolated cytoplasmic C-terminus of polycystin-1 appears to reproduce some of the effects of overexpressed full-length polycystin-1 in an inner medullary collecting duct cell line [15]. It may be that the effects of expression of the isolated cytoplasmic C-terminus of polycystin-1 will vary depending on the cell line, expression level, activity of endogenous polycystin-1 pathways and functional readout. As defined functions for full-length polycystin-1 emerge [16], it will become possible to characterize more precisely the effects of the isolated cytoplasmic C-terminal construct with respect to the activity of the full-length protein.

Our data are consistent with the suggestion that loss of polycystin pathways might augment the secretagouge activity of extracellular ATP. The antagonist profile suggested that ATP was acting via apical purinergic \( P_{	ext{Y}} \) receptors, and that the resultant current was mediated by an apical, calcium-activated chloride channel [17]. Purinergic signalling appears to be well placed to promote cyst expansion through synergistic effects to stimulate chloride secretion and inhibit sodium reabsorption [10]. It is even possible that purinergic mechanisms might contribute to disease progression in ADPKD through effects on cell proliferation and apoptosis [18]. Thus further studies are needed to determine the \( P_{	ext{Y}} \) receptors involved in this response, to establish the underlying mechanisms and to clarify the role of purinergic signal transduction in renal cystic disease.

ACKNOWLEDGMENTS

This work was funded by Satellite Research, The Poly-cystic Kidney Disease Foundation and the National Kidney Foundation of Maryland. M.S. is a recipient of the Johns Hopkins Clinician Scientist award. R.J.U.
acknowledges the St. Peter’s Trust (Les Clark Fund) for support.

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Received 10 September 2002/22 October 2002; accepted 5 December 2002