Na Transport in Autosomal Recessive Polycystic Kidney Disease (ARPKD) Cyst Lining Epithelial Cells

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Abstract. Autosomal dominant (ADPKD) and recessive (ARPKD) polycystic kidney disease are characterized by the progressive growth and expansion of cysts or ectatic collecting ducts, respectively, that ultimately destroy the normal renal parenchyma. Evidence from experimental models of ADPKD suggests that transepithelial Na and fluid secretion contribute to cyst growth, yet little is known about solute transport in ARPKD. This purpose of this study was to begin to characterize the expression and polarity of transport proteins involved in vectorial Na movement in ARPKD epithelium. Immunodetectable α1 and β2 subunits of the Na/K-ATPase localized to the apical membrane of collecting duct cysts in tissue sections of human fetal ARPKD nephrectomy specimens and conditionally immortalized cells derived from these cysts. Measurements of transepithelial 22Na transport performed on monolayers of ARPKD and age-matched collecting tubule (HFCT) cells grown on permeable supports revealed net Na absorption in both models. However, ARPKD cells absorbed Na at a rate approximately 50% greater than that of HFCT. Furthermore, Na absorption in ARPKD cells was partially inhibited by 100 μM apical amiloride or 1 mM basolateral but not apical ouabain. Northern blot analyses of ARPKD whole kidney and Western immunoblot of ARPKD cells showed approximately twofold greater expression of the α-subunit of the epithelial Na channel (ENaC) compared with age-matched controls. These results suggest that, despite the presence of apical Na/K-ATPase, ARPKD cyst-lining cells absorb Na by a pathway that is modestly amiloride-sensitive. Whether Na absorption is mediated by ENaC, perhaps of nonclassical subunit composition, or another amiloride-sensitive transporter remains to be determined.

Polycystic kidney disease (PKD) is a common genetic disease that is associated with a high morbidity and mortality. Autosomal dominant PKD (ADPKD) affects approximately 1:1000 individuals (1), whereas the incidence of autosomal recessive PKD (ARPKD) averages approximately 1:20,000 live births (2). Approximately 50% of patients with ADPKD develop end-stage renal disease (ESRD) by the sixth decade of life, whereas most infants with ARPKD that survive beyond the perinatal period develop chronic renal failure by adolescence. In ADPKD and ARPKD the progressive growth and expansion of cysts or ectatic collecting ducts, respectively, destroys normal renal parenchyma, ultimately leading to renal failure (3).

Evidence from experimental models of ADPKD and human disease suggest that cyst formation and expansion arise, at least in part, from transepithelial fluid secretion (4–9). Molecular, biochemical, and functional studies of cyst-lining epithelial cells in ADPKD in adults reveal that immunodetectable Na/K-ATPase, normally localized to the basolateral membrane of fully differentiated adult renal epithelial cells, is expressed but polarized to the apical membrane (9). In vivo and in vitro studies on immortalized cells derived from human ADPKD cysts demonstrate that apical α1 and β2 isoforms of Na/K-ATPase functionally transport Na from the basolateral to luminal compartments (9). This secretory flux was inhibited by ouabain, a specific inhibitor of Na/K-ATPase, when applied to the apical surface (9). In sum, these observations led to the hypothesis that cystic ADPKD renal epithelia secrete Na and water.

In ADPKD, cysts, which can arise from any tubular segment, “bud” off from the nephron and no longer communicate with the tubule from which they originate (3). In contrast, cysts in ARPKD are actually dilated ectatic collecting ducts that remain contiguous with the remaining nephron, allowing for urine to continue flowing through the collecting system (10). The collecting duct is a final site of renal regulation of Na and water balance. Within this segment, Na diffuses from the urinary space into principal cells through apical epithelial Na channels (ENaC) and is extruded at the basolateral membrane in exchange for uptake of K by the Na/K-ATPase (11). Murine models of ARPKD exhibit apical immunodetectable Na/K-ATPase in cystic collecting ducts (8), suggesting that cyst-lining epithelial cells in ARPKD may secrete Na, as in ADPKD. Few studies have systematically attempted to define the
transport characteristics of ARPKD cystic renal epithelia. The purpose of this study was to determine if ARPKD cystic collecting ducts are composed of absorptive or secretory epithelial cells with regard to Na, as well as to begin to identify the proteins that mediate this transport. These aims were accomplished using human fetal principal cell culture models generated from ARPKD and age-matched control kidneys.

Materials and Methods

**Cell Culture**

Conditionally immortalized cells of ARPKD renal cysts (pool and clone 5E from 19 wk fetal kidney) and age-matched normal fetal collecting tubule (HFCT; pool and clone 2C), prepared as described previously (12–14), were grown on type I collagen-coated translucent membrane inserts (Transwell-COL; pore size, 0.4 μm; 12 well inserts; Costar, Cambridge, MA) for immunofluorescence microscopy and measurement of transepithelial transport. Monolayers were grown to confluence at 33 °C in cell-type–specific supplemented medium containing 1% fetal bovine serum, 5 μg/ml human transferrin, and 10⁻⁸ M dexamethasone, as described previously (9,12). After the cells reached confluence, they were shifted to 37 °C for 11 to 15 d to maximize differentiation (13,15). All cells were placed in serum-free media for up to 24 h before fixation for immunofluorescence or analysis of transport. For some immunofluorescence studies, as indicated, cells were grown on glass coverslips precoated with type I rat collagen (BD Biosciences, Bedford, MA). Of note is that the HFCT clone 2C, as previously reported for HFCT clone 7F (3,15,16), expressed markers characteristic of differentiated principal cells, including the α1 subunit of Na/K-ATPase, the water channel aquaporin-2 (AQP-2), epidermal growth factor receptor (EGFR), and keratin, but it lacked intercalated cell-specific H⁺-ATPase and proximal tubule cell-specific aminopeptidase N (AMP-N) (Table 1).

**Western Immunoblot Analyses**

Cells grown to confluence in T25 flasks and differentiated at 37 °C for 14 d were washed with Tris-buffered saline (TBS) containing a comprehensive protease inhibitor cocktail and then extracted in 1% NP-40 and 0.5% Triton X-100 solution (15). After centrifugation at 14,000 rpm for 20 min, the supernatant was collected and the total protein in solution quantified using the BCA Protein Assay (Pierce, Rockford, CA). Equal aliquots of the protein, as indicated, were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA), and blocked with a rabbit polyclonal anti-α1 Na/K-ATPase antibodies have been fully described and characterized by Burrow et al. (18,19). A previously described rabbit polyclonal anti-AQP-2 antibody (19,20) and a commercially available mouse monoclonal anti-NHE-1 antibody (Pharmagen, San Diego, CA) were used. Control experiments were performed by substituting the primary antibody with preimmune serum and/or by omitting the primary antibody and labeling with the secondary antibody alone.

**Tissue Sections.** Paraffin-embedded 5 μM sections of normal human fetal, normal human adult, and ARPKD kidney were dewaxed, rehydrated, blocked, and labeled with the primary antibodies, applied as a 1:100 dilution for Na/K-ATPase or 1:500 dilution for AQP-2, in phosphate-buffered saline (PBS) containing 2% bovine serum albumin (BSA) for 45 min. Thereafter, the sections were washed three times in PBS and sequentially incubated with biotinylated anti-IgG (Vector Labs, Burlingame, CA) and avidin-biotin peroxidase (Vec-tastain Elite, Vector Labs), as described previously (18,19). A 1:25 dilution of the NHE-1 antibody was prepared and tissue labeled using a commercially available avidin-biotin labeling kit (Vector Labs, Burlingame, CA). Color development was accomplished with aminethylcarbazole (Vector Labs) as a substrate.

**Cell Monolayers.** For immunofluorescence studies, monolayers grown on permeable supports were labeled with 10% normal goat serum and incubated with 1:100 dilutions of the primary Na/K-ATPase antibodies added to both apical and basolateral compartments. After washing, a 1:100 dilution of a Texas red–conjugated secondary goat anti-rabbit IgG was applied. Apical and basolateral compartments were washed three times with PBS. Each filter was excised from its support and placed, basolateral surface down, on a slide to which 5 μl of Prolong Anti-Fade solution (Molecular Probes, Eugene, OR) had been applied. Each monolayer was examined by confocal laser scanning microscopy (TCS-SP UV microscope; Leica, Heidelberg, Germany). A similar protocol was followed for NHE-1 antibody labeling (1:25 dilution) except that the cells were grown on glass coverslips and an FITC-conjugated goat anti-mouse IgG secondary antibody (Sigma, St. Louis, MO) was used. Immunocytochemical labeling of cells fixed in 4% paraformaldehyde for AQP-2 expression was performed as discussed above for tissue sections.

**Table 1. Marker analysis of cell lines**

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<th>α1 Na/K-ATPase</th>
<th>β1 Na/K-ATPase</th>
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<th>EGFR</th>
<th>AQP-2</th>
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* EGFR, epidermal growth factor receptor; AMP-N, proximal tubule cell-specific aminopeptidase N; HFCT, fetal collecting tubule; ARPKD, autosomal recessive polycystic kidney disease.
Transepithelial Unidirectional Flux Studies

[14C]-inulin (50 μCi/ml; New England Nuclear, Boston, MA) was added to either apical or basolateral compartment to a final concentration of 0.67 μCi/ml, and 10-μl samples were collected from the opposite compartment at 1, 5, and 10 min for scintillation counting. Unidirectional fluxes were expressed as a percent of [14C]-inulin initially added to either the apical or basolateral compartment and then detected in the opposite compartment at the time intervals indicated, as described previously (9). Cell monolayers were considered tight if the [14C]-inulin leak to the contralateral compartment was less than 3% at 10 min (Amsler K, Wilson P; unpublished observations). Monolayers with significant baseline [14C]-inulin leaks (>3%) were not utilized for analysis of unidirectional 22Na fluxes. All inulin and 22Na (see below) flux studies were performed at 37°C with cells bathed in PBS with 5 mM D-glucose (9).

The 22Na transepithelial flux was determined by adding 22Na (100 μCi/ml; Amersham, Arlington Heights, IL) to a final concentration of 2 μCi/ml in either the apical or basolateral compartment and then collecting 10-μl aliquot samples from the opposite compartment at 1, 5, and 10 min. Unidirectional fluxes were expressed as described above. In each 12-well plate, multiple (3 to 4) determinations of each unidirectional flux were performed in the absence and presence of inhibitors.

Northern Blot Analyses of α-ENaC and GAPDH

α-ENaC and GAPDH probes were prepared by RT-PCR of total RNA from a 7-mo-old human nephrectomy specimen using a protocol identical to that previously reported by our laboratory (21,22). Gene-specific primers were synthesized by Gene Link (Thornwood, NY). The sequence of the α-ENaC sense primer was 5′-GGGATCCCTTGCYTGTCCARRA-3′, and that of the antisense primer was 5′-CGGAGCTCTTCACTGTYGWAAGAA-3′. The sequence of the sense GAPDH primer was 5′-GCTGAACGGGAAACTCACTG-3′, and that of the antisense primer was 5′-TCCACCACCTGTTGGCT-3′, and that of the antisense primer was 5′-GCTGAACGGGAAACTCACTG-3′, and that of the antisense primer was 5′-TCCACCACCTGTTGGCT-3′. Both PCR products (344 and 307 bp, respectively) were gel-purified (Qiagen Ltd, Surrey, UK) and labeled with deoxyctosine-5′-β[32P] triphosphate (Megaprime DNA-labeling system; Amersham, Arlington Heights, IL). Sequence analysis (Applied Biosystem 373 Fluorescence Sequencer) of the PCR products verified their identity.

Northern blots were prepared using total RNA extracted from homogenates of whole human kidney (fetal, postnatal, and ARPKD; Anatomical Gift Foundation and National Disease Research Interchange) using methods previously described (21,22). Briefly, 50 μg of total RNA from each kidney was size-fractionated by electrophoresis on a 1% agarose–3% formaldehyde gel and transferred overnight to a Hybond-N filter (Amersham, Arlington Heights, IL). Each filter was pre-hybridized for 2 h at 42°C with 50% formamide, 5× Denhardt reagent, 5× SSC, 40 mM sodium phosphate, pH 6.8, 0.1% SDS, and 200 μg/ml denatured salmon sperm DNA in DEPC-treated water. The RNA was hybridized overnight in the same solution with the 32P-labeled α-ENaC or GAPDH probe. Nonhybridizing radioactivity was removed by successive washing of the membranes with 1× SSC–0.5% SDS twice at room temperature for 30 min, and then 0.1× SSC–0.1% SDS three times at 50°C. The filter was then placed in an imaging cassette at room temperature for 48 h. The relative intensities of bands were analyzed using a phosphorimaging system (Storm PhorImager and ImageQuant, Molecular Dynamics). To compensate for differences in quantity of total RNA in each lane of the membrane, each densitometric value for α-ENaC was normalized to its respective value of GAPDH. These ratios, expressed as α-ENaC/GAPDH, were then normalized to the value obtained for the 7-mo-old kidney.

Statistical Analyses

Data are given as mean ± SEM (n = number of monolayers). For transport experiments, multiple determinations of each unidirectional flux in the absence and presence of inhibitors were averaged for each plate to provide a single value. Statistical analyses were performed using ANOVA with multiple-range test and Bonferroni inequality (SigmaStat version 2.03; SPSS Inc., San Rafael, CA). A value of P < 0.05 was used to assert statistical significance.

Results

Channel/Transporter Localization in Kidney Sections and Cell Culture

The expression and polarity of the α1 and β2 subunits of Na/K-ATPase were examined in sections of 19 wk fetal normal and ARPKD kidneys and their derived cell lines. ARPKD cyst lining epithelial cells exhibited apical immunodetectable α1 and β2 subunit isoforms of Na/K-ATPase (Figure 1, A through D). Additionally, modest α1 subunit labeling was noted at the basolateral membrane of the cystic epithelia (Figure 1, C and E). Collecting ducts in normal fetal kidney, as described previously by Burrow et al. (19), also exhibited immunodetectable apical α1β2 Na/K-ATPase (data not shown). Similar immunolocalization results for α1 and β2 subunits were obtained in a pediatric ARPKD kidney (Figure 1, E and F). Sections labeled with the preimmune control serum or with secondary antibody alone revealed no labeling.

Confocal analysis of indirect immunofluorescence labeling of ARPKD cells derived from the 19 wk fetal kidney shown in Figure 1 and grown on permeable supports revealed expression of both apical α1 and β2 subunits of Na/K-ATPase (Figure 2). No immunofluorescence above background was detected in control experiments performed using secondary antibody alone (data not shown).

To further confirm that the ARPKD cell line represents an appropriate model of the cystic epithelium from which the cells were derived, we also compared the expression of AQP-2 and NHE-1 between the two preparations. Immunodetectable AQP-2 was localized to the apical membrane of cysts in sections of human ARPKD kidney (Figure 3A) and was also present in immortalized ARPKD cells (Figure 3B). NHE-1 was expressed along the basolateral membrane of collecting ducts (identified by peanut lectin staining; data not shown) of normal adult human kidney (Figure 3C), but it was absent in cysts in human ARPKD kidney (Figure 3D; identical results were obtained in three ARPKD kidneys) and monolayers of ARPKD cells (data not shown).

The similar expression of the apical α1 and β2 subunits of Na/K-ATPase and AQP-2 and absence of immunodetectable NHE-1 in ARPKD monolayers and tissue sections, as well as extensive marker analyses (Table 1) (14), suggest that the ARPKD cell line represents a valid model of the human in vivo condition.
Transepithelial $^{22}$Na Transport in Cell Culture

Measurement of $^{14}$C-inulin leak across the monolayers was initially performed to determine whether confluent cells established a tight monolayer. There were no differences detected between absorptive and secretory inulin fluxes in either ARPKD ($1.5 \pm 0.3$ and $1.3 \pm 0.4\%$, respectively; $P = NS$) or HFCT ($0.8 \pm 0.1$ and $0.7 \pm 0.1\%$, respectively; $P = NS$) monolayers ($P = NS$ between ARPKD and HFCT absorptive or secretory fluxes).

The direction and magnitude of transepithelial $^{22}$Na transport were measured across monolayers of ARPKD and HFCT cells grown on permeable supports. To validate that the transport characteristics of the cell clones paralleled those of the pools from which they were derived, the unidirectional $^{22}$Na fluxes across both cell preparations were compared. As shown in Figure 4, the absorptive (apical to basolateral transport) fluxes for clonal and pooled cells were similar for each cell type, as was also true for the secretory (basolateral to apical transport) fluxes ($P = NS$). However, the absorptive flux significantly exceeded ($P < 0.05$) the secretory flux for pool and clone for both HFCT and ARPKD (Figure 4).

The $^{22}$Na absorptive flux was linear for both cell types over

Figure 1. Immunolocalization of $\alpha 1$ and $\beta 2$ subunits of Na/K-ATPase in human autosomal recessive polycystic kidney disease (ARPKD) cysts. Low-power (A and B) and high-power (C and D) light microscopy reveals that ARPKD cysts (*) in kidneys from which the immortalized ARPKD cell line used in this present study was derived possess immunodetectable apical (A, black arrows in C) and some basolateral (white arrow in C) $\alpha 1$ and apical $\beta 2$ (B, black arrows in D) Na/K-ATPase subunits. Similar immunolocalization of $\alpha 1$ (E; apical denoted by black and basolateral by white arrows) and $\beta 2$ (F) were observed in another pediatric ARPKD kidney.
10 min ($r^2 = 1.0$ and $0.99$ for ARPKD and HFCT, respectively; $P < 0.02$ for both cell types; Figure 5). At each time point, the $^{22}$Na absorptive flux for the ARPKD clone exceeded that of HFCT ($P < 0.03$; Figure 5).

To begin to explore the identity of the Na absorptive pathways, 100 $\mu$M amiloride was added to the apical compartments of monolayers of ARPKD and HFCT clonal cells grown on permeable supports. Amiloride inhibited $^{22}$Na absorption in ARPKD ($P < 0.03$; Figure 6) but not HFCT cells. Of note was that 10 $\mu$M amiloride had no effect on the unidirectional $^{22}$Na flux in either cell type (data not shown). Addition of 1 mM ouabain to the basolateral compartments of ARPKD clone cells inhibited Na absorption by 20.0 ± 7.7% in five of six experiments at 10 min. In a sixth experiment, ouabain inhibited the Na absorptive flux by 35.5% at 5 min. Application of 1 mM ouabain to the apical compartment reduced the $^{22}$Na absorptive flux by 0.04 ± 0.17% ($n = 3$), a value not significantly different than zero, suggesting that the apical Na/K-ATPase (specifically, the $\alpha_1\beta_2$ isoform) is nonfunctional with regard to Na reabsorption or insensitive to ouabain.

**Expression of Na Transport Proteins in Whole Human Kidney and Cell Monolayers**

The steady-state expression of $\alpha$-ENaC mRNA in maturing and ARPKD human kidneys was examined by Northern blot analyses. A single band of expected size for $\alpha$-ENaC (approximately 3.6 kb) (23) was detected in kidneys at all ages. Densitometric analyses revealed a progressive developmental increase in $\alpha$-ENaC expression in fetal kidneys between 24 and 40 wk ($r = 0.98$ by linear regression analysis; $P < 0.05$), with message abundance further increasing after birth. In two kidneys harvested from 1-mo-old infants with ARPKD, $\alpha$-ENaC expression was approximately twice that detected in age-matched control kidneys (Figure 7).

Western immunoblot analyses of homogenates of ARPKD cell lines showed that $\alpha$-ENaC protein was approximately twofold greater than that observed in normal HFCT cells (Figure 8). The channel subunit-specific antibody identified a single band at approximately 93 kDa in all cells studied.

**Discussion**

The present study demonstrates that the polarity of Na transport in fetal ARPKD cyst-lining principal cells is retained in the absorptive direction, an observation that contrasts with the reversal in polarity of vectorial transport reported in adult ADPKD cystic epithelial cells in primary culture (9). Furthermore, the rate of Na absorption in ARPKD cells is approximately 50% greater than that measured across age-matched control cells (Figure 5). The avid Na absorption detected in the ARPKD cell line is consistent with the low Na concentrations (5.0 ± 2.9 mEq/L) measured in 16 samples of cyst fluid freshly aspirated from two end-stage ARPKD nephrectomy specimens (24) but was unexpected given the presence of apical immunodetectable $\alpha_1$ and $\beta_2$ Na/K-ATPase subunit expression in ARPKD cysts (Figure 1) and the principal cells derived from cysts (Figure 2). Apical localization of functional enzyme would be predicted to mediate basolateral-to-apical vectorial solute and fluid transport, as has been reported in ADPKD (9), and thus contribute to collecting tubule cyst formation and enlargement. Our observations suggest that unlike the situation in ADPKD cyst lining cells derived from adult kidneys, the apical Na/K-ATPase $\alpha_1\beta_2$ complex in ectatic collecting ducts in fetal ARPKD kidney is ouabain-insensitive or nonfunctional.

Within the collecting duct, principal cells mediate Na absorption and K secretion, whereas intercalated cells are primarily involved in acid-base homeostasis (reviewed in reference 11). Na diffuses from the urinary fluid into the principal cell through apical ENaC and is extruded at the basolateral membrane by the Na/K-ATPase. ENaC is comprised of three subunits ($\alpha$, $\beta$, and $\gamma$). Coexpression of all three subunits constitutes a channel with ion selective permeability, gating properties, and a pharmacologic profile similar to the native channel (25,26). Whereas expression of the $\alpha$-subunit alone supports a small amiloride-sensitive current, $\beta$- and $\gamma$-subunits alone or together do not induce a Na current (25). The temporal relationship between ENaC $\alpha$-subunit expression and both short-circuit current in aldosterone-treated A6 kidney cells (27) and appearance of conducting apical amiloride-sensitive Na channels in the maturing rodent principal cell (28,29) provides additional evidence that the $\alpha$-subunit is essential for assembly...
targeting, and activation of apical amiloride-sensitive Na channels.

The unidirectional lumen-to-bath Na flux in ARPKD cells is mediated, in part and unlike control HFCT cells, by a pathway that is modestly sensitive to apical amiloride (Figure 6) and basolateral ouabain. The presence of relatively abundant α-ENaC message (Figure 7) and protein (Figure 8) in ARPKD kidneys and cell lines, respectively, compared with controls suggests that it is possible that Na absorption in ARPKD is mediated, at least in part, by this channel. However, the α,β,γ-ENaC complex is exquisitely sensitive to amiloride (K<sub>i</sub> = 150 nM) (25,28), yet inhibition of Na absorption across the

Figure 3. Immunolocalization of aquaporin 2 (AQP-2) and Na/H exchanger (NHE-1) in human fetal ARPKD cysts, normal human kidney, and ARPKD cells. Immunodetectable AQP-2 is present in both ARPKD renal epithelial cysts (A; along apical membrane) and cells (B). Immunodetectable NHE-1 is localized to the basolateral membranes of collecting ducts (peanut lectin positive, data not shown) in normal adult human kidney (C). However, ARPKD cysts (D) did not exhibit NHE-1 labeling above background.
ARPKD monolayers required significantly higher amiloride concentrations than those necessary to inhibit single-channel activity of the typical heterotrimeric channel (25,28). The amiloride sensitivity of ENaC is determined by subunit composition. McNicholas and Canessa (30) showed that the K_i of amiloride for the ENaC channel composed of αγ subunits was 0.13 ± 0.05 μM, whereas the K_i for amiloride for ENaC channels composed of α alone or αβ subunits was approximately 30-fold greater (4 ± 0.4 μM). Thus the relative insensitivity of Na absorption to apical amiloride in the present analysis of fetal principal cells may simply reflect the presence of a developmental stage and/or disease-specific ENaC channel of nonclassical composition (e.g., α alone or αβ subunits) (31,32), with ion selectivity, inhibitor sensitivity, and regulatory properties that differ from the channel expressed in the fully differentiated cell. Indeed, we and others have reported developmental changes in the electrophysiologic (28,29) and molecular (22,33) characteristics of ENaC in the collecting duct. Alternatively, the molecular correlate of the apical Na entry pathway in ARPKD cells may be a nonselective cation channel unrelated to ENaC (34–36). These possibilities are currently under investigation.

Also possible is that the modestly amiloride-sensitive Na absorption is mediated by an apical Na/H exchanger (NHE). Functional NHE activity is present at both the apical and basolateral plasma membranes of collecting duct cell lines (37), although the molecular correlates of the specific exchangers are unknown at present. Of the several NHE that have been identified in the kidney at the functional and molecular level, NHE-1 and NHE-3 have been best studied. NHE-1, expressed ubiquitously among mammalian cells, is localized at the basolateral membrane of polarized renal epithelial cells (38). NHE-3 is expressed along the brush-border of proximal tubule (39), but it is absent in collecting duct (37,40). The absence of immunodetectable NHE-1 in ARPKD cells suggests it unlikely that this exchanger participates in Na absorption in ARPKD. Once antibodies become available with high specificity for NHE-2 and -4, isoforms identified at the mRNA level in collecting duct cell lines (37), the expression of these proteins can be examined in ARPKD.

In fully differentiated renal epithelia, ouabain-sensitive basolateral Na/K-ATPase generates the driving force for transepithelial Na transport. Our detection of a modest (20%) reduction in Na absorption in ARPKD cells in response to basolateral application of 1 mM ouabain is compatible with the presence of functional basolateral pump. We speculate that the low sensitivity of transepithelial Na absorption to ouabain reflects a low abundance of pump and/or a limited sensitivity of resident basolateral pump to ouabain. In support of the former argument is the limited expression of basolateral compared with apical α1 subunit expression in sections of ARPKD kidneys and cyst-lining cells (Figure 1, C and E, and 2). The ouabain sensitivity of human fetal Na/K-ATPase is unknown. To the extent that species-specific differences exist for pump ouabain sensitivity and maturational changes in membrane composition/fluidity likely regulate transporter/channel function (41–44), we propose that the blunted ouabain sensitivity of the basolateral (and possibly apical) Na/K-ATPase may be due to unique characteristics of fetal membrane composition.

It is well established that Na/K-ATPase normally polarizes to the basolateral membrane of fully differentiated mature collecting duct cells and is transiently expressed along the apical membrane of these nephron segments in fetal development (19) and murine models of ARPKD (8). However, it is unknown whether the apical pump, comprised of α1 and β2 subunits, is functional in these settings. Our findings of modest bath-to-lumen (secretory) fluxes in principal cell cultures generated from ARPKD and age-matched HFCT suggest it is likely that apical Na/K-ATPase contributes little to net Na secretion in either model, a notion supported by the insignificant effect of apical ouabain on transepithelial Na transport in ARPKD cell monolayers. Again, these findings contrast directly with those in adult ADPKD epithelia, where α1β2 complexes are fully functional with regard to enzymatic catalytic activity as well as secretion of Na (9,18).

Little is known about the transport characteristics of ARPKD cystic epithelium, although emerging evidence suggests that some significant differences exist between ARPKD and ADPKD cell phenotypes. Principal cells isolated from the bpk murine model of ARPKD and grown as epithelial monolayers exhibit several features of principal cells in vivo, including amiloride-sensitive Na transport and AQP-2 expression.
Schweibert et al. (46) have recently shown that monolayers of collecting duct cells from the orpk mouse model of ARPKD exhibit a high transepithelial voltage that is sensitive to amiloride but not EIPA, a Na/H exchanger inhibitor. These preliminary results have been interpreted to reflect a heightened activity and/or expression of luminal amiloride-sensitive Na channels in the orpk collecting duct, a conclusion that is supported by the results of the present investigation of human ARPKD epithelium. Epithelial chloride secretion, mediated by an apical cAMP-regulated cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel, contributes to cyst formation and expansion in ADPKD (6,47). To discern whether CFTR-dependent chloride and fluid secretion contribute to cystogenesis in ARPKD, Nakanishi et al. (48) crossbred CFTR-deficient mice with mice homozygous for the cystic gene (bpk). In the resulting double-mutant mice, the lack of functional CFTR protein on the apical surface of cystic epithelium did not protect against cyst growth and renal failure, suggesting that CFTR is not essential for cyst development in this murine model of ARPKD.

Infants and children with ARPKD frequently present with hypertension, which appears well in advance of renal insufficiency (49). The mechanism underlying the early onset of hypertension in this disease has been proposed to reflect volume expansion (49), a speculation strengthened by our present results. Although our analyses of transport were performed in vitro in cells “protected” from the hormonal environment and local paracrine/autocrine environment prevailing in vivo, the parallels between the avid Na absorption in immortalized cells and low cyst fluid Na concentrations in freshly harvested infants and children with ARPKD frequently present with hypertension, which appears well in advance of renal insufficiency (49).
ARPKD nephrectomy specimens (24) suggest that ARPKD collecting duct cysts in vivo may be Na-reabsorptive epithelia. We do acknowledge, however, that the results of studies performed in a cell culture system may differ from the physiology of the in vivo condition. To the extent that the ARPKD cell line used in the present study is a satisfactory model of the native epithelium early in the course of disease, we speculate that dysregulated Na absorption across the cystic epithelium contributes to the morbidity of the disease.

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References


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