Mechanoregulation of intracellular Ca\(^{2+}\) concentration is attenuated in collecting duct of monocilium-impaired orpk mice

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Our findings implicate defects in structure/function of primary epithelial cells comprising this segment (19). Similarly, Praetorius and Spring (30) reported that bending of the ~2.5-μm cilium of the microperfused rabbit cortical collecting duct (CCD) stimulate release of Ca\(^{2+}\) from inositol 1,4,5-trisphosphate-sensitive internal stores and influx of Ca\(^{2+}\) from the extracellular space in both principal and intercalated cells. Principal cells, which reabsorb sodium and secrete potassium (16, 33), are characterized by a single apical cilium that projects into the lumen (5, 41). Intercalated cells, which primarily function in acid-base homeostasis but can reabsorb potassium under certain conditions (2, 35, 48), are decorated with a plethora of apical microvilli and microcilia (5). Within the kidney, all renal tubular cells except intercalated cells possess an apical cilium (17, 27, 34).

The primary cilium, a nonmotile structure projecting from the centriole, plays a critical role as a chemo- and/or mechanosensor in a variety of different cells (26). The physiological importance of structurally and functionally intact cilia in renal epithelial cells is underscored by the growing body of evidence that mutation of proteins localized to the cilium is associated with a renal cystic phenotype. These proteins include polycystins 1 (PC1) and 2 (PC2) and polyductin/bricrycin in autosomal dominant polycystic kidney disease (ADPKD) and ARPKD (23, 40), respectively, and polaris (46) and cystin (13) in the orpk and cpk mouse models of ARPKD.

The orpk mouse, which bears an insertional mutation in the Tg737 gene encoding the protein product polaris (21, 22), exhibits a renal disease phenotypically resembling ARPKD, as well as defects in left-right axis determination, retinal degeneration, and skeletal malformation. Of note is that the primary cilium in the kidney, retina, and embryonic node in mature orpk mice are short (25). From analyses of homologs in Chlamydomonas reinhardtii (IFT88) and Caenorhabditis elegans (osm-5), polaris is thought to be a component of the intraflagellar transport particle responsible for movement of proteins from the basal body at the base of cilia into and out of the cilia axoneme (9, 14, 25). As seen in the Tg737 mutant mouse, loss of IFT88 or osm-5 results in cilia assembly defects, suggesting a highly conserved function for this protein.

We have recently proposed that fluid shear acting on the apical membrane or hydrodynamic bending moments acting on the ~2.5-μm cilium of the microperfused rabbit cortical collecting duct (CCD) stimulate release of Ca\(^{2+}\) from inositol 1,4,5-trisphosphate-sensitive internal stores and influx of Ca\(^{2+}\) from the extracellular space in both principal and intercalated cells comprising this segment (19). Similarly, Praetorius and Spring (30) reported that bending of the ~8-μm cilium of cultured Madin-Darby canine kidney cells either directly with a micropipette or by increasing the rate of flow superfusing the apical surface of monolayers resulted in an increase in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)), a response attributed to external Ca\(^{2+}\) entry through mechanosensitive channels followed by intracellular Ca\(^{2+}\) release.
Recent studies by Nauli et al. (23) showed that PC1 and PC2, the products of the two genes implicated in ADPKD, participate in fluid-flow sensation by the primary cilium in renal epithelial cells. Cells isolated from embryonic transgenic mice lacking functional PC1 and grown as monolayers on coverslips formed cilia but did not exhibit a superfuse flow-induced Ca\(^{2+}\) influx as did age-matched control cells (23). The flow response of wild-type cells was abolished by pretreatment with blocking antibodies directed against PC2, a Ca\(^{2+}\)-permeable cation channel (6) that interacts with PC1 (8), as well as inhibitors of the ryanodine receptor (23). These data led to the speculation that conformational changes of PC1 within the apical cilium transduce a mechanical signal into a chemical response by activating associated PC2 Ca\(^{2+}\) channels and that the local Ca\(^{2+}\) influx in the cilium subsequently triggers internal Ca\(^{2+}\) release.

From the studies summarized above, we hypothesized that Ca\(^{2+}\) signaling pathways that are continuously regulated by urinary (i.e., tubular) flow through the collecting duct are dysregulated in ARPKD. To test this hypothesis, we examined urinary (i.e., tubular) flow through the collecting duct are dysregulated in ARPKD. To test this hypothesis, we examined the effect of increases in tubular fluid flow rate on [Ca\(^{2+}\)], in collecting ducts isolated from mutant orpk and age-matched control mice and microperfused in their native tubular geometry, to 1) confirm that it is functional integrity of the cilium that determines the fully differentiated hydrodynamic response to flow and 2) characterize the ontogeny and cellular basis of the mechatro-induced response.

**METHODS**

**Animals.** The generation and characterization of orpk mice has previously been described (21, 45). Mice were housed and bred in the Mount Sinai School of Medicine animal care facility. All mice utilized in this study were maintained on the inbred FVB/N background. Newborns were allowed to remain with their mothers until weaning. Animals were fed standard mouse chow and given free access to food and water.

Newborn animals homozygous for the Tg737°rpk insertional mutation (orpk mutants) were easily identified by their hindlimb polydactyly (47). Orpk mutants rarely survived beyond postnatal day 21 (P21), as previously described (21). Genotyping of all animals was performed by PCR analysis of DNA isolated from tail biopsies, performed at the time of death, using previously described conditions (45). Animals were killed by intraperitoneal injection of pentobarbital sodium (100 mg/kg). All experiments were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. Animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee.

**Scanning electron microscopy.** Kidneys for scanning electron microscopy (SEM) analyses were prepared as previously described (1). Samples were fixed in 2.5% glutaraldehyde and 0.1 M cacodylate buffer, cryoprotected in 70% ethanol, freeze fractured in liquid nitrogen to randomly expose internal kidney structures, postfixed in 1% OsO\(_4\), washed in distilled H\(_2\)O, dehydrated through an ethanol series, and washed twice in 100% ethanol. Kidney pieces were dried from liquid CO\(_2\), mounted on aluminum stubs, sputter coated, and viewed with a JEOL SEM.

**Isolation of single tubules.** Kidneys were removed via a midline incision, and single tubules were dissected freehand in cold (4°C) Ringer solution containing (in mM) 135 NaCl, 2.5 K\(_2\)HPO\(_4\), 2.0 CaCl\(_2\), 1.2 MgSO\(_4\), 4.0 lactate, 6.0 L-alanine, 5.0 HEPES, and 5.5 D-glucose, pH 7.4, 290 ± 2 mosmol/kgH\(_2\)O, as previously described (19). A single tubule was studied from each animal.

Mouse collecting duct segments were isolated as previously described (18). Because of the extremely short length of neonatal tubules, collecting ducts extending from the cortex to medulla were used for this study. In general, collecting ducts were dissected from the inner stripe of the outer medulla toward the cortex.

Each isolated tubule was immediately transferred to a temperature- and O\(_2\)- and CO\(_2\)-controlled specimen chamber. The base of the chamber was prepared using a no. 1 glass coverslip (VWR Scientific, Media, PA) previously painted with a 1-μl drop of Cell-Tak (Collaborative Biomedical Products, Bedford, MA) (19). Each collecting duct was mounted on concentric glass pipettes, cannulated and perfused, and bathed at 37°C with Burg perfusate containing (in mM) 120 NaCl, 25 NaHCO\(_3\), 2.5 K\(_2\)HPO\(_4\), 2.0 CaCl\(_2\), 1.2 MgSO\(_4\), 4.0 sodium lactate, 1.0 Na\(_3\) citrate, 6.0 L-alanine, and 5.5 D-glucose, pH 7.4, 290 ± 2 mosmol/kgH\(_2\)O (19). In some experiments, collecting ducts were perfused with Burg solution prepared without Ca\(^{2+}\) (Ca\(^{2+}\)-free perfusate) (19). During the 30-min equilibration period and thereafter, the perfusion chamber was continuously suffused with a gas mixture of 95% O\(_2\)-5% CO\(_2\) to maintain pH of the Burg solution at 7.4 at 37°C. The bathing solution was continuously exchanged at a rate of 10 ml/h using a peristaltic syringe pump (Razel, Stamford, CT).

**Measurement of flow rate.** Isolated collecting ducts were microperfused in vitro as previously described (19, 43). The flow rates corresponding to the “slow” and “fast” flow rates generated in the present study were measured in three tubules. Measurements were performed in the absence of transepithelial osmotic gradients, and thus water transport was assumed to be zero. Samples of tubular fluid were collected under water-saturated light mineral oil by timed filling of a precalibrated 30-ml volumetric constriction pipette. Three to four samples of tubular fluid were collected at each perfusion rate (slow and fast), and flow rates were calculated (in nl/min per mm tubular length). The flow rate was varied by adjusting the height of the perfusate reservoir.

**Measurement of [Ca\(^{2+}\)].** After equilibration, microperfused tubules were loaded with 20 μM fura 2-AM (Calbiochem, La Jolla, CA) added to the bath for 20 min. Using a Nikon Eclipse TE300 inverted epifluorescence microscope linked to a cooled Pentamax charge-coupled device camera (Princeton Instruments) interfaced with a digital imaging system (MetaFluor, Universal Imaging, Westchester, PA), we measured [Ca\(^{2+}\)] in individually identified fura 2-loaded cells visualized using a Nikon S Fluor ×40 objective (numerical aperture 0.9, working distance 0.3) as previously described (19). Autofluorescence was not detected at the camera gains utilized.

Collecting ducts were alternately excited at 340 and 380 nm, and images, acquired every 1 s (for the first minute after the increase in luminal flow rate) to 15 s, were digitized for subsequent analysis. At the conclusion of each experiment, an intracellular calibration was performed, using 10 μM EGTA-AM in a Ca\(^{2+}\)-free bath and then a 2 mM Ca\(^{2+}\) bath containing ionomycin (10 μM) (19). Standard equations were used to calculate experimental values of [Ca\(^{2+}\)]. At least four randomly chosen cells were analyzed in the wall of each collecting duct.

Several microperfused tubules were loaded with fura 2 and then, after a fluorescence photomicrograph was obtained, fixed with 2% paraformaldehyde before luminal perfusion for 5 min with 5 μg/ml fluorescein-conjugated Dolichos biflorus agglutinin or Texas red-conjugated peanut lectin (Vector Laboratories, Burlingame, CA) or for 20 min with a 1:50 dilution of an anti-vacular H\(^{+}\)-ATPase monoclonal antibody (provided by S. Gluck, Gainesville, FL) (10). In the latter studies, the primary antibody was visualized by subsequent application of an Alexa 488-conjugated donkey anti-mouse IgG (Molecular Probes). The fixed collecting ducts were again photographed to identify fura 2-labeled cells that stained with the cell-specific probes.

To assess the sources of Ca\(^{2+}\) contributing to the [Ca\(^{2+}\)], transient, the effect of flow was studied in some collecting ducts 3–5 min after luminal Ca\(^{2+}\) was removed (with or without 1 mM EGTA added to
the luminal perfusate) or after a 20-min pretreatment with basolateral thapsigargin (100 nM), an irreversible inhibitor of endoplasmic reticulum Ca\(^{2+}\)/H\(_{\text{ATPase}}\) that prevents refilling of intracellular Ca\(^{2+}\)/H\(_{\text{ATPase}}\) pools and leads to depletion of internal stores. Collecting ducts treated with thapsigargin were perfused with either the standard perfusate or the Ca\(^{2+}\)/H\(_{\text{ATPase}}\)-free perfusate, as indicated.

**Statistics.** All results are expressed as means ± SE; n equals the number of animals. Collecting ducts were harvested from at least five litters of animals. For functional studies, significant differences were determined by paired or unpaired t-tests, as appropriate, using the statistical software program SigmaStat (SPSS, Chicago, IL). Unpaired data that did not fit a normal distribution were analyzed using the nonparametric Mann-Whitney rank test. Significance was asserted if \(P < 0.05\).

**RESULTS**

Ultrastructural analyses of postnatal days 7 and 14 wild-type, heterozygous, and orpk homozygous mutant kidneys. Ultrastructural analyses of wild-type and orpk heterozygous kidneys at postnatal day 7 (P7; Fig. 1, A and B) and day 14 (P14; Fig. 1, D and E) did not reveal any apparent age-specific differences between the two genotypes, confirming the recessive nature of the orpk insertional mutation at the ultrastructural level. In contrast, orpk homozygous mutants at P7 (Fig. 1C) and P14 (Fig. 1F) exhibited short cilia. The frequency distribution of cilia height among the three genotypes at P7 and P14 is shown in Fig. 2. From this analysis, there appeared to be no difference in cilia height between +/+ and +/- collecting ducts in either age group. Cilia of <1 \(\mu\text{m}\) were detected only in the +/- segments. The observation that cilia length was always >2 \(\mu\text{m}\) in the P7 +/- and +/- collecting ducts, whereas at P14 there were large numbers of cilia in the 1- to 2-\(\mu\text{m}\) category, suggests that cilia length falls with advancing age. This finding extends a previous observation that cilia height normally decreases with increasing age in this animal model (1). In the latter study, P200 cilia in wild-type kidney were ~50% shorter (1–2 \(\mu\text{m}\)) than P14 cilia (3–4 \(\mu\text{m}\)). This age-dependent reduction in height of renal cilia likely reflects a developmental program of postnatal maturation of the renal collecting tubule.

For the purposes of modeling the forces acting on the cilia and apical membranes of control and mutant collecting ducts (see Table 2), we calculated an average cilia length for each
genotype at each age from the appropriate frequency distribution of cilia length.\(^1\)

Effect of flow on [Ca\(^{2+}\)]\(_i\), in collecting ducts from control mice. Collecting ducts loaded with fura 2 exhibited heterogeneity in the intensity of cytoplasmic fluorescent staining (Fig. 3B), similar to that described by our laboratory in the adult rabbit CCD (19, 43). The presence of high levels of carbonic anhydrase in fully differentiated H\(^+/\)HCO\(_3\)\(^-\) transporting intercalated cells, compared with that in principal cells, leads to the selective accumulation of functional fluorescent dyes, such as fura 2, when the acetoxymethyl ester of the probe is added to the medium bathing the tubules. Thus fura 2-loaded intercalated cells generally appear brighter than principal cells when viewed in epifluorescence illumination. However, we were unsuccessful in the present study in defining whether brightly fluorescent cells in 1- and 2-wk-old mouse collecting duct were esterase-rich intercalated cells. We found that peanut lectin, which binds to the apical surface of HCO\(_3\)\(^-\) -secreting β-intercalated cells in the rabbit CCD (43) and mouse intercalated cells (28), and an anti-H\(^+\) pump antibody labeled only a minority of bright cells in 2-wk-old collecting ducts (data not shown). Some peanut lectin-positive cells did not selectively accumulate fura 2 (data not shown).

We were thus unable to conclusively identify unique cell types in the mouse collecting duct. To the extent that the effect of flow on [Ca\(^{2+}\)]\(_i\), in the present study was measured in discrete bright and dull cells, the data are presented for each.

Baseline [Ca\(^{2+}\)]\(_i\) was measured initially at a flow rate of ~2.5 nl·min\(^{-1}\)·mm tubular length\(^{-1}\) and was similar in dull and bright cells at the two ages (Figs. 4 and 5), averaging 125.2 ± 6.4 (n = 4) and 111.9 ± 10.4 (n = 6) nM in tubules from 1- and 2-wk-old control animals, respectively. There was no significant difference between resting [Ca\(^{2+}\)]\(_i\) in wild-type (108.6 ± 9.8; n = 5) and heterozygous (125.9 ± 8.9 nM; n = 5) animals in the first 2 wk of postnatal life (P = 0.21).

An acute increase in tubular fluid flow rate to ~45 nl·min\(^{-1}\)·mm tubular length\(^{-1}\), sufficient to increase tubular diameter by 16.9 ± 0.6% (Table 1), increased [Ca\(^{2+}\)]\(_i\) in all dull and bright collecting duct cells (Figs. 4 and 5).\(^2\) Again, within each age group, there was no significant difference in peak [Ca\(^{2+}\)]\(_i\) achieved in response to flow between dull and bright cells (Figs. 4 and 5). However, the magnitude of the flow-induced increase in [Ca\(^{2+}\)]\(_i\) (Δ[Ca\(^{2+}\)]\(_i\)) was significantly lower in 1-wk-old (169.7 ± 23.4 nM; n = 4) compared with 2-wk-old (460.4 ± 38.0 nM; n = 6) control animals (P < 0.001). There was no significant difference in the flow-induced Δ[Ca\(^{2+}\)]\(_i\) between wild-type (518.4 ± 66.4 nM; n = 3) and heterozygous (402.3 ± 24.7 nM; n = 3) animals at 2 wk of age (P = 0.13). Because we studied only two wild-type and two heterozygous animals at 1 wk of age, statistical analyses of differences in Δ[Ca\(^{2+}\)]\(_i\) were not performed in this age group.

In five 3-wk-old control animals, the resting and flow-induced peak [Ca\(^{2+}\)]\(_i\) in dull cells (106.5 ± 7.0 and 467.9 ± 85.4 nM; Δ[Ca\(^{2+}\)]\(_i\) = 361.0 ± 89.8 nM) did not differ from that measured in bright cells (100.8 ± 8.3 and 454.1 ± 66.8 nM; Δ[Ca\(^{2+}\)]\(_i\) = 353.2 ± 73.0 nM; P = not significant). This observation suggests that it is unlikely that the similar flow-induced [Ca\(^{2+}\)]\(_i\) responses detected in dull and bright cells in younger animals was simply due to functional immaturity of the presumed different cell types.

Effect of flow on [Ca\(^{2+}\)]\(_i\), in collecting ducts from mutant orpk mice. Cells within dilated portions of cystic collecting ducts (Fig. 3A) failed to exhibit flow-induced increases in [Ca\(^{2+}\)]\(_i\). Given that the shear and drag forces on the cilia and apical membranes of the cystic cells in response to a given flow rate were expected to be less than those experienced by cells in the more proximal portions of normal diameter, our analyses focused on cells in segments of normal diameter just proximal to the cyst. The diameters of control and mutant orpk tubules in the regions in which [Ca\(^{2+}\)]\(_i\) measurements were actually performed under slow and high flow conditions are summarized in Table 1. There was no significant difference in tubule diameter between control and mutant 1-wk-old animals either at slow or fast flow rates. At 2 wk of age, the diameter of mutant tubules was slightly greater than controls at slow flow rates (but not different from 1-wk-old control or mutant collecting ducts perfused at slow flow rates). At fast flow rates, the diameter of mutant tubules tended to be greater than that measured at slow flow rates, although the difference did not achieve statistical significance, but was similar to that observed in 1-wk-old control and mutant segments perfused at fast flows.

Baseline [Ca\(^{2+}\)]\(_i\) was similar in dull and bright cells in mutant collecting ducts at 1- and 2-wk of age (Figs. 4 and 5), averaging 117.3 ± 4.6 (n = 4) and 103.4 ± 10.8 (n = 5) nM, respectively. An increase in tubular fluid flow rate led to an increase in [Ca\(^{2+}\)]\(_i\) in collecting duct cells in both 1- and

\(^1\) The average cilia height calculated from the interval data provided by the frequency distribution may be imprecise in that the underlying attribute being measured (height) in reality assumes an infinite number of real values (continuous variable).

\(^2\) We acknowledge that it is possible that experiments using a lower flow rate could have revealed a difference between the flow-induced increase in [Ca\(^{2+}\)]\(_i\) in 1-wk mutant and control animals. However, these experiments would be technically difficult to perform because our perfusion apparatus does not allow us to precisely grade the flow rates.
Fig. 4. Representative tracings of the effect of a sustained increase in luminal fluid flow rate on intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) in a dull (solid line) and bright (dotted line) cell in a microperfused collecting duct from a P14 control (left) and mutant (right) orpk mouse. [Ca$^{2+}$]$_i$ increased in all cells in response to flow, although the increase in [Ca$^{2+}$]$_i$, induced by flow was blunted in the mutant mice compared with control mice.

2-wk-old orpk mice (Figs. 4 and 5). At 1 wk of age, the flow-induced $\Delta$[Ca$^{2+}$]$_i$ in orpk mutants was similar to that in control animals, averaging 149.8 ± 24.9 nM ($P = \text{not significant}$) (Fig. 5B). However, the flow-induced $\Delta$[Ca$^{2+}$]$_i$ in collecting duct cells in 2-wk-old mutant mice (213.6 ± 54.7 nM) was significantly less than that detected in age-matched control tubules ($P < 0.05$) and was not different from that observed in mutant tubules at 1 wk of age ($P = \text{not significant}$).

Mathematical models for fluid shear stress and drag and torque on cilia. Because the diameter of control and mutant collecting ducts increased in response to flow, we sought to determine whether the changes in diameter would significantly affect the wall shear stress experienced by the cells and the drag and bending moment (torque) on the cilia, using our previous flow model (19). Figure 5 in Ref. 19 reveals that there is very little departure from a parabolic velocity profile in the lumen of the tubule for cilia $\leq 2.5 \mu$m. Furthermore, the velocity near the wall, extending to the tip of the cilia, closely follows a linear profile in the region of the cilia. The first observation allows us to apply the Poiseuille flow theory in relating the fluid shear stress ($\tau_w$) at the wall of the tubule to the tubule flow rate ($Q$; nl/min) and diameter ($D$; $\mu$m) of the tubule. The classical relation for Poiseuille flow in a circular tube is

$$Q = \frac{\pi D^4}{128 \mu} \cdot \frac{dp}{dx} \quad (1)$$

where $\mu$ is the fluid viscosity and $dp/dx$ is the local pressure gradient. One can also express the wall shear stress in terms of this pressure gradient:

$$\tau_w = -\frac{D}{4} \cdot \frac{dp}{dx} \quad (2)$$

Combining the above two equations yields

$$\tau_w = -\frac{32 \mu}{\pi} \cdot \frac{Q}{D^3} \quad (3)$$

This last result allows us to calculate the blunting effect of increases in diameter with increasing flow on wall shear stress and, hence, the hydrodynamic drag and torque on the cilia.

To calculate the drag and bending moment on the cilia, we note that the local drag per unit length acting on the cilia is proportional to the local relative velocity between the fluid flow passing around the cilia and the cilia's own local velocity due to its deformation in the flow. For steady flow, the cilia is stationary, but, even for a time-varying flow, the calculations in Ref. 19 show that the cilia velocity can be neglected because the deformations at physiological flow rates are at most 1% of its length for cilia $\leq 2.5 \mu$m. Thus the relative velocity is nearly the same as the local average fluid velocity, which, as indicated above, varies linearly with distance $y$ from the wall of the tubule. The local drag per unit length, $d$, on the cilia is proportional to this local average fluid velocity and is given by $d = \alpha y$ where $\alpha$ is a proportionality factor. Integration reveals...
that the total drag on the cilia $D_g = \alpha L^2/2$ and the total bending moment $T = \alpha L^3/6$, where $L$ is the cilia length. These two results can be combined as $T = 2/3 D_g L$ to show that the bending moment is the same as a concentrated force $D_g$ acting at $2/3$ the height of the cilia.

The theoretical model for determining the proportionality factor $\alpha$ is described in Guo et al. (7) and summarized in the appendix to Ref. 19. Using this model, we can write $\alpha$ as

$$\alpha = \frac{4 \pi \tau_w}{F(c)} \quad (4)$$

where $F(c) = \ln c^{1/2} - 0.745 + c - \frac{c^2}{4} \quad (5)$

is the hydrodynamic interaction function and $c$ is the fractional surface area occupied by the cilia in the cross-sectional plane. The function $F(c)$ is the denominator of Eq. A5 in Ref. 19. If the cilia form an hexagonal array

$$c = \frac{\pi a^2}{(2a + \Delta)^2 \cdot \sqrt{3}} \quad (6)$$

where $a$ is the cilia radius and $(2a + \Delta)$ is the center to center spacing of the cilia. Combining the foregoing approximations for $D_g$ and $T$ in the previous paragraph and results of Eqs. 3 and 4, one obtains

$$D_g = \frac{64 \mu}{F(c)} \cdot \frac{QL^2}{D^2} \quad (7)$$

$$T = \frac{64 \mu}{3 F(c)} \cdot \frac{QL^3}{D^3} \quad (8)$$

Equations 7 and 8 enable one to relate the drag and torque on cilia of different length $L$ in tubules with different diameter $D$ and flow $Q$. For a given tubule whose diameter is changing with flow, the hydrodynamic interaction function $F(c)$ must be recalculated because the solid fraction $c$ of the cilia changes with the tubule diameter. The surface area of each principal cell scales with the tubule diameter if the tubule length is unchanged.

Table 2 summarizes the calculations for wall shear stress, total shear force per cell, and drag and torque on the cilia present in 2-wk-old wild-type and mutant tubules. The results show the blunting effect of the increase in tubule diameter with flow on the drag and torque on the cilia. A similar 17% increase in diameter in wild-type and mutant tubules due to an 18-fold increase in flow from 2.5 to 45 nl/min leads to only a 10.6-fold increase in drag and torque on the cilia. The drag and torque on the wide-type cilia are 7 and 14 times, respectively, that on the shorter mutant cilia at both the low and high flow rates. Thus the observation that the peak $[\text{Ca}^{2+}]$, response in the 2-wk-old mutant is one-half that in the age-matched control suggests that the response does not scale with drag and bending moment on the cilia of blunted length. Also of note is that the drag force due to the fluid shear acting on the apical membrane of the principal cells is ~6.9 times the drag on the cilia in wide-type tubules and 30.2 times the drag on the mutant cilia at the slow flow rates. As presented in the DISCUSSION, we propose that this drag could play a role in the $[\text{Ca}^{2+}]$ response if the apical surface of the principal cell is covered with a surface glycocalyx.

**Table 1. Diameter of tubules studied at slow and fast flow rates in collecting ducts isolated from maturing control and mutant orpk mice**

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Control Animals</th>
<th>Mutant Animals</th>
<th>P Value (control vs. mutant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$D_\mu$, $\mu$m, at 2.5</td>
<td>$D_\mu$, $\mu$m, at 45</td>
<td>$D_\mu$, $\mu$m, at 2.5</td>
</tr>
<tr>
<td></td>
<td>1 nl/min $^{-1}$ mm$^{-1}$</td>
<td>1 nl/min $^{-1}$ mm$^{-1}$</td>
<td>1 nl/min $^{-1}$ mm$^{-1}$</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$ = no. of animals; $D_\mu$ diameter.

**Table 2. Calculated results of theoretical models for the perfused collecting duct of orpk mice**

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type (2 wk old)</th>
<th>Wild-Type (2 wk old)</th>
<th>Mutant (2 wk old)</th>
<th>Mutant (2 wk old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate ($Q$), nl/min</td>
<td>2.5</td>
<td>45.0</td>
<td>2.5</td>
<td>45.0</td>
</tr>
<tr>
<td>Tubule diameter ($D_\mu$), $\mu$m</td>
<td>18.5</td>
<td>21.7</td>
<td>21.7</td>
<td>25.5</td>
</tr>
<tr>
<td>Cilia length ($L$), $\mu$m</td>
<td>2.3</td>
<td>2.3</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Average velocity, mm/s</td>
<td>0.155</td>
<td>2.03</td>
<td>0.113</td>
<td>1.47</td>
</tr>
<tr>
<td>dp/dx, dyn/cm$^3$</td>
<td>1,450</td>
<td>138 $\times$ 10$^2$</td>
<td>766</td>
<td>7,230</td>
</tr>
<tr>
<td>$\alpha$, dyn/cm$^2$, $\alpha = 2D_g L^2$</td>
<td>2.34</td>
<td>25.0</td>
<td>1.45</td>
<td>15.4</td>
</tr>
<tr>
<td>Wall shear stress, dyn/cm$^2$</td>
<td>0.67</td>
<td>7.48</td>
<td>0.42</td>
<td>4.61</td>
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<tr>
<td>Shear force/cell, pN</td>
<td>4.29</td>
<td>55.9</td>
<td>2.66</td>
<td>34.5</td>
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<tr>
<td>Drag on cilia ($D_g$), pN</td>
<td>0.618</td>
<td>6.61</td>
<td>0.088</td>
<td>0.93</td>
</tr>
<tr>
<td>Torque on cilia ($T$), pN$\mu$m</td>
<td>0.948</td>
<td>10.1</td>
<td>0.064</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Viscosity (dyn·s/cm$^2$) is 0.01; principal cell surface area = $64 \mu m^2$ (36).
absence of luminal Ca
 superscript 2+ for a similar time period (Fig. 7). These data indicate that the flow-induced increase in [Ca
 superscript 2+] requires Ca
 superscript 2+ influx across the apical membrane.

**Contribution of internal Ca
 superscript 2+ stores to flow-induced [Ca
 superscript 2+] transients.** From our previous studies in rabbit CCD, we considered it likely that the flow-induced increase in collecting duct [Ca
 superscript 2+] was due, at least in part, to release of internal Ca
 superscript 2+ stores. We thus sought to examine whether the blunted flow-induced [Ca
 superscript 2+] response in mutant tubules reflected limited internal Ca
 superscript 2+ stores compared with controls.

Pretreatment of 2-wk-old control (n = 8) and mutant (n = 7) tubules with thapsigargin, an irreversible inhibitor of endoplasmic reticulum Ca
 superscript 2+-ATPase, in the presence of external Ca
 superscript 2+, led to an increase in [Ca
 superscript 2+] in both dull and bright cells as internal stores were emptied, followed by a decay to a plateau value that significantly exceeded resting [Ca
 superscript 2+] (Figs. 7, A and B, and 8). There was no significant difference between the thapsigargin-triggered peak [Ca
 superscript 2+] in control and mutant cells (P = 0.14 for dull and P = 0.32 for bright cells) in the presence of luminal Ca
 superscript 2+ (Fig. 8). An increase in tubular fluid flow rate ~20 min after exposure to thapsigargin elicited a significant, albeit minimal, increase in [Ca
 superscript 2+] in both dull (Δ[Ca
 superscript 2+] = 44.4 ± 18.5 and 70.5 ± 25.4 nM) and bright (Δ[Ca
 superscript 2+] = 37.7 ± 19.7 and 59.4 ± 33.3 nM) cells in control and mutant tubules, respectively (Figs. 7, A and B, and 8). There was no significant difference in this minimal flow-stimulated increase in [Ca
 superscript 2+] between control and mutant cells (P = 0.40 for dull and P = 1.0 for bright cells). It is likely that the blunted flow-induced [Ca
 superscript 2+] increase in thapsigargin-treated tubules reflects the well-described Ca
 superscript 2+-dependent inactivation of Ca
 superscript 2+ entry pathways (24) at the high plateau [Ca
 superscript 2+] values (200–250 nM), significantly exceeding baseline levels, measured just before flow was increased (Fig. 8).

Because the presence of external Ca
 superscript 2+ in the experiments described above might have contributed to the generation of the thapsigargin-triggered [Ca
 superscript 2+], transient, we performed additional thapsigargin experiments in control (n = 5) and mutant (n = 6) tubules perfused in the absence of luminal Ca
 superscript 2+. In these experiments, Ca
 superscript 2+ was removed from the luminal perfusate for 3–5 min before thapsigargin was added to the bathing solution (Fig. 7). We could not also remove Ca
 superscript 2+ from the bathing solution because epithelial cells began to slough into the lumen within 3 min after bath Ca
 superscript 2+ removal. As was observed in the presence of luminal Ca
 superscript 2+, there was variability in the thapsigargin-triggered [Ca
 superscript 2+] response in cells perfused in the absence of luminal Ca
 superscript 2+ (Fig. 8). However, there was no significant difference between the thapsigargin-triggered peak [Ca
 superscript 2+] in control and mutant dull cells (P = 0.32 for dull and P = 0.23 for bright cells) in collecting ducts perfused in the absence of Ca
 superscript 2+. To the extent that the peak [Ca
 superscript 2+], achieved in response to thapsigargin, in the absence of luminal Ca
 superscript 2+, reflects internal Ca
 superscript 2+ stores, these data suggest that a loss in function of polaris does not affect internal Ca
 superscript 2+ stores in mouse collecting duct cells.

In all cells studied in the absence of luminal Ca
 superscript 2+, [Ca
 superscript 2+], fell to baseline levels within 20 min of exposure to thapsigargin, an observation consistent with the notion that the sustained plateau elevation of [Ca
 superscript 2+] in thapsigargin-treated tubules reflects the well-described Ca
 superscript 2+-dependent inactivation of Ca
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 superscript 2+] values (200–250 nM), significantly exceeding baseline levels, measured just before flow was increased (Fig. 8).
The present study describes, for the first time, the flow-induced $[\text{Ca}^{2+}]_i$ responses of native renal epithelial cells in collecting ducts isolated from the orpk murine model of ARPKD and microperfused in vitro. We have previously shown that rapid increases in luminal fluid flow rate and circumferential stretch, leading to fluid shear at the apical membrane or hydrodynamic bending moments at the cilium, lead to increases in $[\text{Ca}^{2+}]_i$ in collecting duct cells (19). The results of the present study indicate that collecting duct cells in 2-wk-old orpk mutant animals, studied in their native tubular geometry, respond to an increase in tubular fluid flow rate with a blunted increase in $[\text{Ca}^{2+}]_i$, compared with the response of cells in segments isolated from age-matched control (wild-type and heterozygous) littersmates. Of note is the observation that abnormal mechano-induced $[\text{Ca}^{2+}]_i$ responses were detected in mutant collecting ducts in regions of normal diameter, contiguous with but proximal to dilated regions. Nauli et al. (23) also reported abnormal flow-induced $\text{Ca}^{2+}$ signaling in principal cells in “precystic” collecting ducts isolated from fetal PKD1 knockout mice. These data suggest that the aberrant $\text{Ca}^{2+}$ response to flow is not simply due to cystic dilatation but may be programmed in cells destined to develop into cysts.

The minimal $[\text{Ca}^{2+}]_i$ response in orpk mutant animals is not solely due to the blunted cilia length; the magnitude of the flow-induced increase in $[\text{Ca}^{2+}]_i$ was lower in 1-wk-old control animals with 2-wk-old control animals (5), although the cilia length in the former tended to be greater than that in the latter (Fig. 2). Similarly, we have previously reported that the magnitude of the flow-induced increase in $[\text{Ca}^{2+}]_i$ transient in principal cells in newborn rabbit CCDs, which by SEM possess cilia that are longer than those detected in the adult (5), is less than that measured in 5-wk-old animals (43). These observations underscore the notion that it is the functional and not structural (length) integrity of cilia that may be required to maintain the fully differentiated functional and morphological phenotype of renal epithelial cells.

Cumulative evidence now suggests that the flow-stimulated $[\text{Ca}^{2+}]_i$ response of the collecting duct is associated with hydrodynamic forces acting on the cilia. However, it should be noted that PC1 and PC2, proteins implicated in fluid flow sensation by the cilium in renal epithelial cells, are associated not only with the cilium but also with the adherens junction (20, 32). Thi et al. (37) recently demonstrated in rat fat-pad endothelial cells exposed to fluid shear that shear forces acting on the surface glyocalyx of endothelial cells produce a torque on the actin cortical web beneath the apical membrane, a force that is then transmitted to the adherens junctions. An equivalent apical glyocalyx may also be present in principal cells of the mammalian collecting duct (12). Because the drag due to fluid shear acting at the apical surface is 6.9- and 30.2-times greater than the drag on the cilia in wild-type and mutant cells, respectively (Table 2), at low shear (and even greater at high shear, it would not be surprising if the $[\text{Ca}^{2+}]_i$ response could also be elicited by a hydrodynamic torque acting at the level of the adherens junction.

**DISCUSSION**

Fig. 8. Summary of the effect of Tg and an increase in luminal flow rate on $[\text{Ca}^{2+}]_i$ in dull (A and B) and bright (C and D) cells in collecting ducts isolated from 2-wk-old control (A and C) and mutant (B and D) orpk mice in the presence (+$\text{Ca}^{2+}$,L) and absence ($-$-$\text{Ca}^{2+}$,L) of luminal $\text{Ca}^{2+}$.

$[\text{Ca}^{2+}]_i$ responses were detected in thapsigargin-treated tubules perfused with luminal $\text{Ca}^{2+}$, transient in any cell studied (Fig. 8), suggesting that the small flow-stimulated increase in $[\text{Ca}^{2+}]_i$ detected in thapsigargin-treated tubules perfused with luminal $\text{Ca}^{2+}$ was entirely dependent on apical $\text{Ca}^{2+}$ entry.

In the absence of luminal $\text{Ca}^{2+}$, an increase in tubular fluid flow rate ~20 min after exposure to thapsigargin failed to elicit a $[\text{Ca}^{2+}]_i$ transient in any cell studied (Fig. 8), suggesting that the small flow-stimulated increase in $[\text{Ca}^{2+}]_i$, detected in thapsigargin-treated tubules perfused with luminal $\text{Ca}^{2+}$ was entirely dependent on apical $\text{Ca}^{2+}$ entry.

The minimal $[\text{Ca}^{2+}]_i$ response of the collecting duct is associated with a blunted increase in $[\text{Ca}^{2+}]_i$ compared with the response of cells in segments isolated from age-matched control (wild-type and heterozygous) littersmate. Of note is the observation that abnormal mechano-induced $[\text{Ca}^{2+}]_i$ responses were detected in mutant collecting ducts in regions of normal diameter, contiguous with but proximal to dilated regions. Nauli et al. (23) also reported abnormal flow-induced $\text{Ca}^{2+}$ signaling in principal cells in “precystic” collecting ducts isolated from fetal PKD1 knockout mice. These data suggest that the aberrant $\text{Ca}^{2+}$ response to flow is not simply due to cystic dilatation but may be programmed in cells destined to develop into cysts.

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**Fig. 8.** Summary of the effect of Tg and an increase in luminal flow rate on $[\text{Ca}^{2+}]_i$ in dull (A and B) and bright (C and D) cells in collecting ducts isolated from 2-wk-old control (A and C) and mutant (B and D) orpk mice in the presence (+$\text{Ca}^{2+}$,L) and absence ($-$-$\text{Ca}^{2+}$,L) of luminal $\text{Ca}^{2+}$.

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In the present study, the blunting effect of the flow-induced increase in diameter on the hydrodynamic torque exerted on the cilia, which reduces an 18-fold increase in flow to an 10.6-fold increase in effective wall shear and cilia torque, is not nearly as great as the equivalent effect observed on the brush border microvilli of mouse proximal tubule (4). In the latter study, a fivefold increase in flow led to a 1.5-fold increase in diameter, with a much more sizeable blunting of the fluid shear stress and torque acting on the microvilli. In the latter application, a fivefold increase in flow produced only a twofold increase in torque. Furthermore, mathematical modeling that related changes in flow to changes in torque revealed that the rates of sodium and water reabsorptions in the proximal tubule were directly proportional to microvilli torque. This linear proportionality is not observed in the present [Ca\textsuperscript{2+}], response, which exhibits a highly nonlinear behavior with a possible threshold.

We observed that the flow-induced [Ca\textsuperscript{2+}], response in 1-wk-old mutant collecting ducts was comparable to that detected in age-matched controls. The hydrodynamic forces experienced by the short cilia in the orpk mutant mice should be significantly less than those experienced by cilia in control cells (19) and thus lead to a blunted [Ca\textsuperscript{2+}], response. We speculate that the similar mecano-induced responses in the two cells reflect, in part, functional immaturity of an as yet unidentified signal transduction pathway in the immediate neonatal period. Alternative explanations are that the cilia may not be the only “flow sensor” in the collecting duct, as suggested above, or that the [Ca\textsuperscript{2+}], transient detected at this age (and in the 2-wk-old mutant tubules) is not due to fluid flow per se but is due to another physical stimulus. Possible candidates are an increase in hydrostatic pressure, which has recently been reported to induce apical and basolateral nucleotide release (29), and/or circumferential stretch (19). As we have previously suggested (19), circumferential stretch of the magnitude elicited in the perfused tubules in the present study would not likely occur in the kidney with intact capsule under conditions of health. However, comparable increases in collecting duct diameter have been reported after ureteric obstruction (15, 42). It should be noted that the high fluid flow rate of ~45 nl·min\textsuperscript{-1}·mm length\textsuperscript{-1} was selected in the present study to give a percent increase in tubular diameter comparable to that examined by us in an earlier study in rabbit (19). The physiological range of urinary flow rates in vivo in the distal nephron of the young animal is unknown.

The [Ca\textsuperscript{2+}], transient induced by luminal flow in the phenotypically normal 2-wk-old mouse collecting duct appears to be critically dependent on luminal Ca\textsuperscript{2+} entry. In the absence of luminal Ca\textsuperscript{2+} (Fig. 6), an increase in flow elicited only a slight increase in [Ca\textsuperscript{2+}], in cells with presumably intact internal stores. In contrast to our previous study of the rabbit collecting duct response to flow (19), the results of the present study are not consistent with a major contribution of internal Ca\textsuperscript{2+} store release to the flow response in mouse collecting duct. Specifically, mouse collecting duct cells fail to exhibit a sustained plateau elevation in [Ca\textsuperscript{2+}], following the flow-induced peak in [Ca\textsuperscript{2+}], (Fig. 4), as was characteristic of the rabbit response (19). Given that store-operated Ca\textsuperscript{2+} entry is present in 2-wk-old mouse collecting ducts (Figs. 7 and 8), significant internal store release would have been expected to generate a sustained plateau elevation of [Ca\textsuperscript{2+}], as observed in rabbit (19). Pretreatment of mouse collecting ducts with basolateral thapsigargin also led to a marked diminution in the flow-induced [Ca\textsuperscript{2+}], response (Figs. 7 and 8). Although the latter blunted response may point to a requirement for internal store release in flow-stimulation of [Ca\textsuperscript{2+}], it more likely reflects Ca\textsuperscript{2+}-dependent inactivation of Ca\textsuperscript{2+} entry pathways (24) because the flow stimulus was presented to cells with a high [Ca\textsuperscript{2+}],

Mutations in the polycystins, which give rise to ADPKD, are associated with lower [Ca\textsuperscript{2+}], than that shown in controls (23, 31). In contrast, baseline [Ca\textsuperscript{2+}], in microperfused collecting ducts from orpk mutants did not differ from that measured in wild-type mice (Figs. 5 and 8). Furthermore, baseline [Ca\textsuperscript{2+}], in 1- and 2-wk old normal mouse collecting duct cells was similar, a finding that contrasts with the developmental increase in resting [Ca\textsuperscript{2+}], reported in rabbit collecting duct cells after the first week of postnatal life (43). In an analysis of purinergic-induced Ca\textsuperscript{2+} signaling in mouse ureteric bud (E14–P1) and neonatal CCD (P7 to P20) principal cells, Tschop et al. (38) observed similar resting [Ca\textsuperscript{2+}], during all developmental stages. Relevant to the present study was the observation by the latter investigators that, when cells were bathed in a Ca\textsuperscript{2+}-free solution, ATP induced similar increases in [Ca\textsuperscript{2+}], at all developmental stages (38), suggesting that the magnitude of internal Ca\textsuperscript{2+} stores did not change with development. In contrast, ATP-induced external Ca\textsuperscript{2+} entry, mediated by t-type Ca\textsuperscript{2+} channels, was acquired only after P7, when collecting duct morphogenesis was complete (38). These data led the authors to conclude that ontogeny of the collecting duct is associated with the developmental acquisition of functional conducting Ca\textsuperscript{2+} channels after P7 (38). Similarly, the tolerance of neonatal compared with mature proximal tubules to anoxia, which leads to an increase in [Ca\textsuperscript{2+}], to ~250 and ~975 nM in the two age groups, respectively, has been proposed to be due to a low activity and/or number of voltage- or ligand-gated Ca\textsuperscript{2+} channels (3) in these nephron segments early in life.

Based on these studies by others and the results of the present study, we propose that there exists a significant difference in expression and/or activity of an apical flow-activated Ca\textsuperscript{2+} entry pathway between control and mutant orpk collecting duct cells. The identity and ontogeny of this apical Ca\textsuperscript{2+} entry pathway activated by luminal flow has yet to be precisely identified. Immunodetectable endogenous PC2 is a nonspecific cation channel found in the cilium and plasma membrane of collecting duct cells (20, 44). At the molecular level, the epithelial Ca\textsuperscript{2+} channel, a channel that exhibits some molecular structural characteristics of the transient receptor proteins that mediates capacitative Ca\textsuperscript{2+} currents in response to depletion of internal Ca\textsuperscript{2+} stores, and the capsaicin receptor VR1 have been identified in the collecting duct (11). Relevant to the postnatal age at which murine models of ARPKD have been studied in this investigation is the atypical dihydropyridine-activated Ca\textsuperscript{2+} channel, unique to newborn rat, in the apical membrane of cultured CCD cells (39). Future efforts must be directed at more fully exploring the ontogeny and regulation of these and other Ca\textsuperscript{2+} entry pathways in health and disease.

In summary, collecting ducts isolated from the orpk murine model of ARPKD respond to increases in tubular fluid flow rate with increases in [Ca\textsuperscript{2+}],. The [Ca\textsuperscript{2+}], transient elicited by flow in control tubules (wild-type or heterozygous animals)
requires luminal Ca\textsuperscript{2+} entry. Based on data from the present study and from others (23, 38), we speculate that the diminished responsiveness of collecting duct cells to flow in ARPKD is due primarily to a limited capacity for mechano-induced luminal Ca\textsuperscript{2+} entry. The relationship of this process to genesis and progression of ARPKD has yet to be established.

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REFERENCES


