Endothelial Cilia Are Fluid Shear Sensors That Regulate Calcium Signaling and Nitric Oxide Production Through Polycystin-1

Surya M. Nauli, PhD; Yoshifumi Kawanabe, MD, PhD; John J. Kaminski, MS; William J. Pearce, PhD; Donald E. Ingber, MD, PhD; Jing Zhou, MD, PhD

Background—When challenged with extracellular fluid shear stress, vascular endothelial cells are known to release nitric oxide, an important vasodilator. Here, we show that the ability of cultured endothelial cells to sense a low range of fluid shear depends on apical membrane organelles, called cilia, and that cilia are compartments required for proper localization and function of the mechanosensitive polycystin-1 molecule.

Methods and Results—Cells with the Pkd1null/null or Tg737orpk/orpk mutation encoded for polycystin-1 or polaris, respectively, are unable to transmit extracellular shear stress into intracellular calcium signaling and biochemical nitric oxide synthesis. Cytosolic calcium and nitric oxide recordings further show that fluid shear sensing is a cilia-specific mechanism because other mechanical or pharmacological stimulation does not abolish calcium and nitric oxide signaling in polycystin-1 and polaris mutant endothelial cells. Polycystin-1 localized in the basal body of Tg737orpk/orpk endothelial cells is insufficient for a fluid shear stress response. Furthermore, the optimal shear stress to which the cells respond best does not alter the apical cilia structure but modifies the responsiveness of cells to higher shear stresses through proteolytic modification of polycystin-1.

Conclusions—We demonstrate for the first time that polycystin-1 (required for cilia function) and polaris (required for cilia structure) are crucial mechanosensitive molecules in endothelial cells. We propose that a distinctive communication with the extracellular microenvironment depends on the proper localization and function of polycystin-1 in cilia. (Circulation. 2008;117:1161-1171.)

Key Words: blood flow ■ blood pressure ■ endothelium ■ endothelium-derived factors ■ physiology ■ polycystic kidney diseases

Primary cilia are protruding structures found on the apical membranes of many cell types. The structure and maintenance of these cilia are regulated by intraflagellate transport molecules, also known as intraflagella transport particles. Mutations in Tg737, an orthologous gene of Chlamydomonas flagella IFT88, results in cells with no cilia or shorter cilia.1,2 Mice with mutations in the Tg737 gene, encoding polaris, develop polycystic kidney disease (PKD).3 Mutations in Pkd1, encoding polycystin-1, also result in PKD.4 Polycystin-1 has been shown to localize in primary cilia and to mediate the sensitivity of kidney epithelial cells to fluid shear stress.5,6 With its large extracellular domain and remarkable mechanical strength,7 polycystin-1 has been proposed to be a mechanosensitive molecule.8–10

Although enlarged cystic kidneys are the most obvious phenotype in PKD, extrarenal manifestations also are frequent. These include, for example, aneurysms, mitral valve prolapse, and other abnormalities in the vasculature, pancreas, and liver.11 In particular, hypertension is observed in patients with PKD about a decade earlier than in the general population, even before the loss of renal function.12,13 Although the mechanism underlying hypertension-related cardiovascular defects in PKD is not completely understood, the aggressive control of hypertension in PKD patient is clinically necessary and practically relevant.12,13

Received April 18, 2007; accepted November 15, 2008.
From the Department of Pharmacology and Medicine, College of Pharmacy and Medicine, University of Toledo, Toledo, Ohio (S.M.N.); Department of Neurosurgery, Takatsuki Red Cross Hospital, Takatsuki, Osaka, Japan (Y.K.); Department of Biology, Boston University, Boston, Mass (J.J.K.); Department of Pharmacology, Loma Linda Medical School, Loma Linda, Calif (W.J.P.); Vascular Biology Program, Children’s Hospital, Harvard Medical School, Boston, Mass (D.E.I.); and Renal Division, Brigham and Women’s Hospital, Harvard Medical School, Boston, Mass (J.Z.).
Correspondence to Surya M. Nauli, PhD, University of Toledo, Department of Pharmacology, MS 607, Wolfe Hall Bldg, Room 2243, 2801 W Bancroft St, Toledo, OH 43606. E-mail SuryaNauli@UTeach.edu
© 2008 American Heart Association, Inc.
Circulation is available at http://circ.ahajournals.org

DOI: 10.1161/CIRCULATIONAHA.107.710111

Molecular Cardiology

Endothelial Cilia Are Fluid Shear Sensors That Regulate Calcium Signaling and Nitric Oxide Production Through Polycystin-1

Surya M. Nauli, PhD; Yoshifumi Kawanabe, MD, PhD; John J. Kaminski, MS; William J. Pearce, PhD; Donald E. Ingber, MD, PhD; Jing Zhou, MD, PhD
The endothelial cells that line blood vessels and the chambers of the heart have the ability to sense external mechanical forces such as fluid shear stress. Although shear stress can activate many biochemical and molecular pathways in endothelial cells, the exact mechanism of mechanical sensing and mechananochemical conversion is not known. It is known, however, that with changes in external fluid shear stress, endothelial cells exhibit an increase in cytosolic calcium followed by release of nitric oxide (NO) that is critical for control of vascular contractility.14 Furthermore, abnormalities in NO production or release have been associated with hypertension.15 In the present study, we show that primary cilia are mechanosensory organelles in endothelial cells. The mechanosensory function of cilia further depends on the structural polaris molecule and the mechanosensing polycystin-1 protein. We propose that primary cilia may play important and distinct roles in sensing and transducing extracellular fluid shear stress into intracellular signaling and biochemical responses in vascular endothelial cells.

**Methods**

**Embryonic Endothelial Cell Culture**

Time-pregnancies were generated by intercrossing mice double heterozygous for the Tg737orpk or Pkd1null allele and the temperature-sensitive SV40 large T antigen (Charles River Laboratories, Wilmington, Mass). Because both Pkd1null and Tg737orpk mice are lethal at the embryonic stage,3,16 aortas were isolated from 15.5-day embryos. Aortas from homozygous or wild-type embryos were dissociated with 1× trypsin/EDTA solution through a 1-cm 3 25G5⁄8 needle and plated in DMEM containing 2% fetal bovine serum, 0.75 g/L transferrin, 0.2 g/L ethanolamine, 36 ng/mL hydrocortisone, and 0.10 μmol/L 2,3,5-triido-L-thyronine, which were obtained from Sigma (St Louis, Mo). Unless otherwise indicated, cells were grown at 37°C in the absence of IFN-γ for 4 to 5 days before experiments.

**Fluorescence-Activated Cell Sorting**

After trypsinization, 106 cells/mL were incubated with 10 mg/mL of the endothelial marker intracellular adhesion molecule-2 (ICAM-2; Santa Cruz Biotechnology, Santa Cruz, Calif), fluorescein isothiocyanate (FITC)– conjugated ICAM-2 antibody was applied for 1 hour at room temperature at a dilution of 1:100 in PBS containing 1% fetal bovine serum to prevent any nonspecific binding of the antibody. After cells were washed 3 times to avoid nonspecific binding, they were analyzed with FACScan (Becton Dickinson, Franklin Lakes, NJ) at a wavelength of 525 nm (FITC, FL-1). Negative control cells were obtained exactly like cells from experimental groups except that FITC-conjugated anti-mouse antibody was used.

**Western Blotting and Immunoprecipitation**

Cells/tissues were lysed with 1× radioimmunoprecipitation assay buffer. Intracellular contents were collected by centrifugation at 100g for 10 minutes. Total cell lysate was analyzed by SDS-PAGE. In some experiments, cells were first challenged with different magnitudes of fluid shear stress (0, 1.1, or 7.2 dynes/cm²) for 10, 20, or 30 minutes. Cells were then rinsed vigorously with a small amount of lysis buffer composed of 10 mmol/L EGTA, 5 mmol/L NaF, and a tablet of protease inhibitor mixture (Roche Applied Bioscience, Basel, Switzerland) in phosphate buffer, pH 7.2. Cell lysate was then subjected to immunoprecipitation study with anti–polycystin-1 (1:5 dilution). For Western blot, anti-SV40 (1:400 dilution; Santa Cruz Biotechnology), anti-endothelial NO synthase (eNOS; 1:200 dilution; Abcam, Cambridge, Mass), anti-actin (1:300 dilution; Sigma), or anti-polycystin-1 (1:50; P-15, Santa Cruz Biotechnology) antibodies were incubated with the blots and then with a peroxidase-labeled secondary antibody (1:7500 dilution; Amersham Biosciences, Inc, Piscataway, NJ) for 1 hour each at room temperature.

**Immunolocalization Analysis**

Endothelial cells were grown to confluence and full differentiation. Cells were fixed with 4% paraformaldehyde in 2% sucrose solution for 10 minutes at room temperature. Cells were then incubated with anti-CD31 antibody (1:50 dilution; Sigma) for an hour followed by an FITC-labeled anti-mouse (1:500 dilution). For double labeling, an antibody to acetylated α-tubulin (1:10 000 dilution; Sigma) was premixed with a Texas Red–labeled anti-mouse (1:1000 dilution). The mixture was then added to the cells, and the cells were stained with 4′,6-diamidino-2-phenylindol (DAPI). To identify the expression patterns of polaris and polycystin-1, samples were incubated overnight at 4°C with anti–polycystin-1 antibody (1:200 dilution)
and subsequently with an FITC-labeled anti-rabbit (1:500 dilution) antibody for 1 hour at room temperature. An antibody to polaris 18 (1:100 dilution) was premixed with a Texas Red–labeled anti-rabbit antibody (1:1000 dilution) and incubated with the samples for 1 hour at room temperature.

**Calcium and NO Microfluorimetry**

A nonfluorescent, CO₂-independent medium for imaging was formulated (pH 7.3) that contained (in mmol/L) CaCl₂ 1.26, MgSO₄ 0.81, KCl 5.4, KH₂PO₄ 0.44, NaCl 137, Na₂HPO₄ 0.34, d-glucose 5.6, L-glutamine 2.0, sodium pyruvate 1.0, HEPES 20, and 2% serum. All chemicals were purchased from Sigma.

Cells were incubated for 30 minutes with the calcium-sensitive probe Fura 2AM (5 μmol/L) and/or NO-sensitive probe 4-amino-5-methyl-amin-2',7'-difluorofluorescein (DAF-FM; 20 μmol/L) at 37°C. These fluorescent probes were purchased from Invitrogen, Inc. Cells were then washed 3 times to remove excess probe. Cells were positioned under a Nikon TE2000 microscope equipped with a CoolSnap EZ cooled charge-coupled device monochrome digital camera using IPLab software for Macintosh (Scanalytics Inc, Billerica, Mass) or Metamorph/Metaflur software for PC (Molecular Devices Corp, Downingtown, Pa). Paired Fura images at excitation wavelengths of 340 and 380 nm were captured through an emission filter of 510 nm. DAF images at an excitation wavelength of 495 nm were captured through an emission filter of 515 nm. In some experiments in which both calcium and NO signals were captured sequentially, a long-pass emission filter was used.

For fluid shear stress experiments, cells were placed in a perfusion chamber that was 0.0254 cm thick and 1.0 cm wide (GlycoTech, Gaithersburg, Md). Before a shear stress of 0.7 to 50 dynes/cm² was applied to the monolayer cells, they were equilibrated in fluid shear stress of 0, 1.1, or 7.2 dynes/cm² for 30 minutes. For experiments with mechanical cell pressing, a glass micromanipulator needle was first coated with BSA. Once the distance of the needle from a single targeted cell was determined, the needle was programmed to that position on the apical membrane of the cell with an automated and motorized controller (Eppendorf, Westbury, NY). In other experiments, 1 μmol/L acetylcholine (Sigma) was added to the cell population.

**Statistical Analysis**

All quantifiable experimental values are expressed as mean±SEM, and values of P<0.05 were considered statistically significant. All comparisons among means were performed using ANOVA with post hoc comparisons via Fisher’s protected least-significant-difference test. All comparisons between 2 experimental groups were analyzed...
We confirmed that cells grown under permissive conditions completely shut down the SV40 gene. Cells under nonpermissive Tg737orpk/orpk promoter of which is regulated by temperature and IFN-γ. Furthermore, in the presence of IFN-γ, Aortic endothelial cells were isolated from wild-type, Pkd1null/null, and Tg737orpk/orpk embryonic mice. The purity of these endothelial cells was verified by confirming their expression of ICAM-2, which is a known endothelial marker involved in inflammation and angiogenesis (Figure 1). Primary cilia were identified with the ciliary marker acetylated α-tubulin. The presence of polycystin-1 in cilia also was observed. To further study the cellular and molecular functions of the cilia and polycystin-1, we isolated and characterized endothelial cells from Pkd1null/null and Tg737orpk/orpk mouse models.

Characterization of Endothelial Cells
Aortic endothelial cells were isolated from wild-type, Pkd1null/null, and Tg737orpk/orpk embryonic mice. The purity of these endothelial cells was verified by confirming their expression of ICAM-2, a well-established endothelial marker involved in inflammation and angiogenesis (Figure 2A). These endothelial cells were immortalized with the SV40 gene, the promoter of which is regulated by temperature and IFN-γ. We confirmed that cells grown under permissive conditions in the presence of 0.75 μg/L IFN-γ at 33°C express SV40 large T antigen regardless of the status of their confluence (Figure 2B). When switched to nonpermissive conditions in the absence of IFN-γ at 37°C, the endothelial cells completely shut down the SV40 gene. Cells under nonpermissive conditions were grown to differentiated state, and their expression of eNOS, a general marker for endothelial cells, was confirmed. Hyperproliferative cells under permissive conditions did not express eNOS (Figure 2C).

To further characterize the endothelial cells, we performed an immunofluorescence study by using CD31, another well-accepted endothelial marker. CD31, also known as platelet/endothelial cell adhesion molecule-1, is an important cell adhesion molecule involved in endothelial cell-cell contact, cell migration, angiogenesis, wound healing, and other processes. The study demonstrates that all endothelial cells were stained positive for CD31 (Figure 3). When the cells were characterized for the presence of cilia with acetylated α-tubulin as a ciliary marker, each wild-type and Pkd1null/null cell was shown to possess a single primary cilium (Figure 3). Note that some of the cilia were not visible because of a different focus level at different cell heights (z distance) relative to the apical membrane of neighboring cells. On the other hand, no cilia or short stubby cilia were observed in Tg737orpk/orpk cells.

Mechanosensory Functions of Polaris and Polycystin-1
We next performed immunofluorescence microscopic analyses on wild-type, Pkd1null/null, and Tg737 cells to confirm the cellular expression and localization of polaris and polycystin-1 (Figure 4A). Immunofluorescence study of wild-type endothelial cells confirmed that each cell possesses a single primary cilium on its apical surface and expresses both polycystin-1 and polaris in the cilia. Ciliary polycystin-1 is absent from the primary cilium of Pkd1null/null cells, although polaris is present. In Tg737orpk/orpk cells, no polaris is detected, whereas polycystin-1 appears to be concentrated in the basal body.

To test our hypothesis that cilia are mechanosensitive organelles, we challenged the cells with various magnitudes of fluid shear stress (Figure 4B). When cells were equilibrated for 30 minutes under static condition (0 dynes/cm²), we found that wild-type mouse embryonic endothelial cells responded best to optimal fluid shear stress of 7.2 dynes/cm² by increasing their cytosolic calcium. When cells were equilibrated for 30 minutes under a low shear stress (1.1...
Endothelial cells exposed to fluid shear stress for hours have also been reported that polycystin-1 can undergo proteolysis at certain shear stress magnitudes. A step increase in various magnitudes of fluid shear stress. Although wild-type cells respond to a range of shear stress, neither mutant cell shows any apparent response to different magnitudes of shear stress, n=3 to 6 for each time point in a given shear stress condition. Cumulative measurements of this response to fluid shear stress are shown in Figure 4.

**Fluid Shear Stress Induces Polycystin-1 Cleavage**

Endothelial cells exposed to fluid shear stress for hours have been shown to lose their cilia from the apical membrane. It also has been reported that polycystin-1 can undergo proteolytic cleavage, and this cleavage has been implicated to be associated with the mechanical fluid stimulus. To exam-
Cilia Play Distinct Roles in Fluid Shear Sensing

To validate ciliary roles in fluid sensing, we challenged the cells from static condition to a step increase in an optimal fluid shear stress of 7.2 dynes/cm² to induce increases in cytosolic calcium and NO. We performed simultaneous measurements of cytosolic calcium and NO levels (Figure 6A). Peaks of cytosolic calcium followed by NO production were seen in wild-type cells challenged with a shear stress of 7.2 dynes/cm², but no changes in cytosolic calcium or NO were observed in mutant cells. Changes in cytosolic calcium were plotted in line graphs from randomly picked cells (Figure 6B). Similarly, individual cells were randomly selected, and the amounts of cytosolic production of NO by the cells were averaged and plotted (Figure 6C). Peaks of NO observed in wild-type cells are the only statistically significant responses. These data indicate that both polycystin-1 and polaris are required for proper calcium signaling and NO production by endothelial cells in response to fluid shear stress.

To confirm the specificity of cilia function, we mechanically activated a single cell by gently touching its apical membrane with a micromanipulator and recording simultaneous measurements of cytosolic calcium and NO levels (Figure 7A). Calcium propagation as evidenced by the spreading of the calcium signal from a single touched cell to neighboring cells was observed in wild-type and mutant cells. Surprisingly, we did not observe signal propagation for NO. Only cells that had been touched and were immediately adjacent to the touched cell showed an increase in cytosolic NO. Ten cells within a population were randomly selected from the touched cell to the cells farther away from this cell, and their calcium and NO profiles were plotted (Figure 7B). The NO response of these 10 cells also was plotted (Figure 7C). The cell that was directly touched showed the highest increase in cytosolic calcium. Similarly, the touched cell had the highest NO production. To better visualize the data, cell populations were grouped on the basis of their distance away from the touched cell, and only their calcium and NO profiles were plotted (Figure 7D). No significant change in NO profile was observed within groups. In contrast, the farther the cells were from the point of force application, the smaller the changes were in cytosolic calcium. More important, no obvious differences were present in the calcium and NO patterns of wild-type and mutant cells, indicating that both polycystin-1 and polaris are not involved in this mechanotransduction response involving direct physical distortion of the apical membrane.

To further confirm that polycystin-1 and polaris are specific for the sensation of fluid shear stress through primary cilia, we challenged the cells with a pharmacological agonist, acetylcholine. Acetylcholine is known to induce an increase in cytosolic calcium through the muscarinic receptor, which in turn activates eNOS and results in NO release.²⁸ Treatment with 1 μmol/L acetylcholine increased both cytosolic calcium and NO in wild-type and mutant cells (Figure 8). Immediate increases in calcium and NO were observed in wild-type and mutant cells, and their levels remained high in all cell types, indicating that polycystin-1 and polaris mediate fluid shear stresses.
stress–dependent changes in calcium and NO via a distinct pathway.

**Discussion**

Our study provides strong evidence that the primary cilium, specifically its constituent polycystin-1 and polaris proteins, mediates a mechanism by which endothelial cells sense and respond to fluid shear stress. Using endothelial cells isolated from \( Pkd1 \) and \( Tg737 \) knockout mice, we showed that primary cilia are crucial organelles for fluid shear sensing in endothelial cells. We believe that primary cilia act as antennas to sense and transduce extracellular fluid shear into changes in biochemical signaling inside vascular endothelial cells. The absence of primary cilia in \( Tg737 \) or \( Pkd1 \) endothelial cells clearly abrogated the increases in cytosolic calcium and NO normally elicited by application of physiological levels of fluid shear stress. This indicates that the presence of polycystin-1 in the cells by itself is not sufficient to maintain the ability of the cell to sense shear stress. To act as a mechanosensing molecule, polycystin-1 has to be properly localized in the primary cilium, which may act like a large cantilever to amplify and concentrate stresses at the cell surface.

The presence of cilia in the cardiovascular system has been reported in cultured human umbilical vein endothelial cells and in developing chicken endocardium with low-shear-stress areas. In the present study, we show that cilia also can be found in aortic endothelia of embryonic mouse (Figure 1).

**Figure 6.** Fluid shear–induced calcium and transient NO require polaris and polycystin-1. A, Response of individual wild-type (wt), \( Pkd1 \) (Pkd1), and \( Tg737 \) (Tg737) cells from static condition to fluid shear stress of 7.2 dynes/cm\(^2\) is pseudocolored. The calcium (red) and NO (green) are superimposed. The color bar indicates the calcium (Ca\(^{2+}\)) or NO level relative to the corresponding baseline level. Black and blue represent a low (lo) level; red and green denote a higher (hi) level of calcium and NO, respectively. B, The average cytosolic calcium \([\text{Ca}^{2+}]_{\text{cyt}}\) response to shear stress. C, The average cytosolic NO \([\text{NO}]_{\text{cyt}}\) response. Fluid shear stress was applied after the baseline values of calcium and NO were obtained for at least 14 seconds as indicated by arrows. All the units of time are seconds. \( n=5 \). *Statistically significant responses from the baseline values.
by an innovative high-frequency Doppler ultrasound system of embryonic mouse dorsal aorta. These magnitudes of fluid shear stress have been used to induce differentiation of pluripotent embryonic stem cells to endothelium-like phenotypes. Together with the sensitivity of our cultured embryonic endothelial cells to a low fluid shear stress (Figure 4), we hypothesize that cilia might act as specialized low fluid shear–sensing organelles. Consistent with this idea, a much...
higher shear stress of 15 dynes/cm² on endothelial cells has been shown to disassemble cilia. 21

To enable us to study cilia function, we generated aortic endothelial cells derived from mouse embryos because both Pkd1null/null (Pkd1) and Tg737orpk/orpk (Tg737) mouse models are prenatally lethal.3,16 The division and differentiation of these cells are controlled by the SV40 gene, which is regulated by temperature and INF-γ (Figure 2). Cells undergoing division retract their cilia from the apical cell surface,5 and differentiated cells generate better-developed, functional cilia.5,8 Therefore, it is imperative for us to be able to control cellular proliferation and differentiation of cultured cells to study cilia function. These fully differentiated embryonic endothelial cells were characterized by positive expressions of eNOS, ICAM-2, and CD31 (Figures 2 and 3).

To study the molecular functions of polaris and ciliary polycystin-1, wild-type and mutant cells were first subjected to no (0 dynes/cm²), low (1.1 dynes/cm²), or high (7.2 dynes/cm²) fluid shear stress for 30 minutes. A step increase in a higher fluid shear stress was then applied to the cells (Figure 4). In all cases, mutant cells did not show any cytosolic calcium increase in response to a step increase in various magnitudes of fluid shear stress. This suggests that cilia and polycystin-1 are important mechanosensory components in endothelial cells. Furthermore, polycystin-1 is localized to the basal body in Tg737orpk/orpk endothelial cells (Figure 3). This further indicates that subcellular localization of polycystin-1 at designated microcompartments is indeed essential for fluid shear sensing in endothelial cells.

Interestingly, wild-type embryonic endothelial cells preexposed to different fluid shear stresses could modify their sensitivity in response to a step increase in fluid shear stress (Figure 4). The highest increase in cytosolic calcium is seen in endothelial cells in a step increase from static (no shear) to 1.1 and 7.2 dynes/cm²; no significant increase in cytosolic calcium was observed in cells preexposed to 7.2 dynes/cm².

**Figure 8.** Agonist-provoked calcium and NO signaling is independent of cilia. A, Response of individual wild-type (wt), Pkd1null/null (Pkd1), and Tg737orpk/orpk (Tg737) cells to 1 μmol/L acetylcholine is pseudocolored. The calcium (red) and NO (green) are superimposed. The color bar indicates the Ca²⁺ or NO level relative to the corresponding baseline level, with black and blue representing a low (lo) level and red or green denoting a high (hi) level. B, The average cytosolic calcium ([Ca²⁺]_cyt) response to acetylcholine. C, Plots of the average cytosolic NO ([NO]_cyt) response. Acetylcholine was applied to the cells for at least 14 seconds after the baseline values of calcium and NO were obtained as indicated by arrows. All time units are seconds. n=3.
Although cells with no shear (0 dynes/cm²) compared with those with an initial low shear (1.1 dynes/cm²) generated a higher cytosolic calcium increase in response to shear stress, cells preexposed to a low shear could respond to a broader range of fluid shear stresses. We have previously shown that the magnitude of shear stress that optimally increases cytosolic calcium varies with the developmental stage of the animal from which the cells were isolated and the cell type.9

Surprisingly, cells preexposed to a higher shear stress (7.2 dynes/cm²) became mechanosensitive to further increases in fluid shear stresses (Figure 4B). Our immunostaining studies ruled out the absence of cilia in cells equilibrated at 7.2 dynes/cm² (Figure 5). Consistent with previous studies,26,27 we suggest that polycystin-1 would undergo a proteolytic cleavage if cilia were activated. Although a shear stress of 1.1 dynes/cm² also would induce a secondary cleavage product of polycystin-1, cells equilibrated at 1.1 dynes/cm² would still maintain a high amount of functional full-length polycystin-1. This, in turn, would allow cells equilibrated at 1.1 dynes/cm² to remain responsive to further increases in fluid shear stress. These variations in shear sensing could possibly be parts of a complex mechanical behavior of cilia and the downstream mechanosignal transduction system that warrant thorough study.

Vascular endothelial cells have been known to release NO in response to fluid shear stress.14 To examine whether our mutant cells could possibly respond to shear stress by NO biosynthesis, we performed a simultaneous measurement of cytosolic calcium and NO (Figure 6). The increase in cytosolic calcium followed by NO was apparent in wild-type cells. In mutant cells, no increase in cytosolic calcium or NO was observed. This suggests that regardless of the readout measurements (calcium or NO), the mutant cells are mechanically insensitive to fluid shear stress. Although both Pkd1null and Tg737orpk/orpk cells fail to respond to fluid shear, these cells are still able to respond to other stimuli such as apical membrane distortion (Figure 7) and the pharmacological agent acetylcholine (Figure 8). This implies that polycystin-1 and polaris have a rather specific function to decipher extracellular fluid shear signal.

The increase in cytosolic calcium followed by NO was transient in wild-type cells. The transient increase in cytosolic calcium in response to fluid shear stress–induced cilia activation also was observed previously in mouse and human kidney epithelial cells.8–10 Cilium-specific calcium channels might open for only a short time. We and others have recently shown that mechanosensitive calcium channels such as polycystin-2 also may be involved in fluid shear sensing.8,32 At least in kidney epithelial cells, calcium entry through the polycystin-2 channel in response to fluid shear also is associated with intraorganellar calcium release.8,33 With regard to cardiovascular functions, it is possible that cilia were sensory organelles that responded by a short and transient pulse of cytosolic calcium increase and NO release, which might provide not only short but also longer physiological effects to surrounding cells.34

One interesting observation is that calcium and NO profiles are very different among the stimuli that we applied. Although calcium is always thought to be the mediator for eNOS activity, we demonstrate that cells have more complex ways to regulate the biosynthesis of NO. Depending on the stimulus, an endothelial cell could regulate the intracellular calcium in a way that allows a short, a prolonged, or no eNOS activity (Figures 6 through 8). We believe that such differences in calcium profiles also would alter the activity of many other calcium-dependent proteins and cascades, and it remains an interesting topic to pursue in the future.

Collectively, the findings of our study suggest that endothelial cells require functional mechanosensory cilia to respond to fluid shear stress. In response to an optimal shear stress, polycystin-1 is cleaved, resulting in nonresponsiveness of cells to further higher fluid shear. Cells with abnormal cilia function or structure are likely to fail to respond to fluid shear stress. Polycystin-1 for cilia function and polaris for cilia structure and maintenance play crucial roles in mediating fluid shear sensing in endothelial cells, as well as transduction of these mechanical signals into changes in calcium signaling and NO synthesis. We propose that cilia are mechanosensory microcompartments and that polycystin-1 is a sensory molecule that must be “housed” in the cilia to exert its mechanosensory role in endothelial cells.

Sources of Funding

This work was supported by awards from the American Heart Association (0630257N), National Institutes of Health (HL084451), deArce Memorial Endowment Fund, and the University of Toledo research program (Dr Nauli). This work was also supported, in part, by grants CA45548 (Dr Ingbear), HL64867 (Dr Pearce), and DK40703 and DK51050 (Dr Zhou).

Disclosures

None.

References

Patients with polycystic kidney disease exhibit an enhanced propensity for hypertension. Studies have revealed that mutations which result in abnormal ciliary proteins such as polaris and polycystin-1 lead to the development of polycystic kidney disease in mice. These findings suggest an association between ciliary function and the development of hypertension. Kidney cells that exhibit abnormal ciliary proteins fail to sense fluid shear stress. Within endothelial cells, this hemodynamic fluid flow can regulate blood pressure by altering calcium signaling and nitric oxide production. Here, we show that embryonic mouse aortic endothelial cells possess primary cilia, specialized organelles that sense and convert fluid shear stresses into changes in intracellular calcium and nitric oxide production. Polaris and polycystin-1 were shown to mediate these effects. In addition, prolonged activation of cilia by high shear stress would induce proteolytic cleavage of polycystin-1, thereby desensitizing endothelial cells to these mechanical stimuli. Overall, these findings suggest that dysfunction of endothelial cilia could interfere with normal shear-induced regulatory mechanisms that may contribute to abnormal vascular control in polycystic kidney disease patients and hence may lead to hypertension. Furthermore, hypertensive patients who exhibit ciliary desensitization resulting from continuous exposure to high levels of fluid shear might be unable to respond normally to small but damaging daily fluctuations in blood pressure. This might increase the likelihood of localized blood vessel injury, aneurysm, hemorrhage, edema, atherosclerosis, vascular ectasia, dissection, and other abnormalities. Greater insight into this novel mechanism of endothelial flow sensing by cilia may lead to advanced understanding of focal cardiovascular diseases and development of novel forms of “ciliary therapy” in the future.
Endothelial Cilia Are Fluid Shear Sensors That Regulate Calcium Signaling and Nitric Oxide Production Through Polycystin-1
Surya M. Nauli, Yoshifumi Kawanabe, John J. Kaminski, William J. Pearce, Donald E. Ingber and Jing Zhou

Circulation. 2008;117:1161-1171; originally published online February 19, 2008; doi: 10.1161/CIRCULATIONAHA.107.710111
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/117/9/1161

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/