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Collecting duct cells that lack normal cilia have mislocalized vasopressin-2 receptors

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Saigusa T, Reichert R, Guare J, Siroky BJ, Gooz M, Steele S, Fenton RA, Bell PD, Kolb RJ. Collecting duct cells that lack normal cilia have mislocalized vasopressin-2 receptors. Am J Physiol Renal Physiol 302: F801–F808, 2012. First published December 28, 2011; doi:10.1152/ajprenal.00253.2011.—Polycystic kidney disease (PKD) is a ciliopathy characterized by renal cysts and hypertension. These changes are presumably due to altered fluid and electrolyte transport in the collecting duct (CD). This is the site where vasopressin (AVP) stimulates vasopressin-2 receptor (V2R)-mediated aquaporin-2 (AQP2) insertion into the apical membrane. Since cysts frequently occur in the CD, we studied V2R and AQP2 trafficking and function in CD cell lines with stunted and normal cilia [cilia (−), cilia (+)] derived from the orpk mouse (hypomorph of the Tg737/H11002 gene). Interestsly, only cilia (−) cells grown on culture dishes formed domes after apical AVP treatment. This observation led to our hypothesis that V2R mislocalizes to the apical membrane in the absence of a full-length cilium. Immunofluorescence indicated that AQP2 localizes to cilia and in a subapical compartment in cilia (+) cells, but AQP2 levels were elevated in both apical and basolateral membranes in cilia (−) cells after apical AVP treatment. Western blot analysis revealed V2R and glycosylated AQP2 in biotinylated apical membranes of cilia (−) but not in cilia (+) cells. In addition, apical V2R was functional upon apical desmopressin (DDAVP) treatment by demonstrating increased cAMP, water transport, and benzamil-sensitive equivalent short-circuit current (Isc) in cilia (−) cells but not in cilia (+) cells. Moreover, pretreatment with a PKA inhibitor abolished DDAVP stimulation of Isc in cilia (−) cells. Thus we propose that structural or functional loss of cilia leads to abnormal trafficking of AQP2/V2R leading to enhanced salt and water absorption. Whether such apical localization contributes to enhanced fluid retention and hypertension in PKD remains to be determined.

aquaporin 2; epithelial Na channel; hypertension; polycystic kidney disease

POLYCYSTIC KIDNEY DISEASE (PKD) is a ciliopathy in which a loss in the structure/function of cilia affects cell polarity, differentiation, cell signaling, and fluid transport (1, 24, 31, 34, 37). In humans, loss of function in polycystin-1 and less frequently polycystin-2 causes autosomal dominant PKD (13, 18) whereas autosomal recessive PKD results from loss of function in the protein fibrocystin/polyductin (36). Both recessive and dominant forms of the disease cause changes in renal morphology including tubular dilation and cyst formation, which eventually results in renal failure. In addition to cystic disease, other comorbidities exist in PKD, including hypertension, although the mechanism for the increase in blood pressure has not been fully elucidated.

Elevated intracellular cAMP occurs in some forms of PKD and is known to contribute to cyst formation, in part, by stimulating proliferative activity of cyst epithelial cells (3, 11, 32). This may occur through cAMP-induced activation of the MAPK/ERK signaling cascade in PKD cells. However, in normal cells, cAMP may serve as an inhibitor of this pathway (33). One well-established regulator of cAMP level is the vasopressin-2-receptor (V2R), which is localized to the basolateral membrane of collecting duct cells (9). Vasopressin binding to V2R activates adenylyl cyclase and increases the cAMP level, resulting in aquaporin-2 (AQP2) translocation to the apical membrane of principal cells of the collecting duct (6, 12). Since the collecting duct is a predominant site for renal cystic development, recent studies have focused on analyzing the efficacy of V2R blockade on the rate of cystic progression (16, 30). In addition to its role in water transport, activation of the V2R-cAMP cascade also increases sodium reabsorption in the collecting duct through the epithelial sodium channel (ENaC) (22). Thus it is possible that enhanced activation of cAMP may lead to inappropriate salt and water reabsorption by the collecting duct and therefore contribute to the development of hypertension. The possibility of this scenario provides the impetus to further evaluate the V2R-cAMP-salt and water transport pathway in a PKD collecting duct cell model.

To investigate this hypothesis, we used a mouse immortalized collecting duct cell line (35) that is hypomorph for the intraflagellar protein polaris and has either no cilia or the cilia are severely stunted [designated as cilia (−) cells]. The control cell line was created by transfecting the Tg737 gene (now called ift88) back into the cilia (−) cell line, resulting in the expression of normal cilia [designated as cilia (+) cells]. Initial studies with vasopressin added to the apical cell surface resulted in fluid dome formation in cilia (−) cells, but not in cilia (+) cells, grown on nonpermeable supports. This phenomenon was unexpected since vasopressin should not have access to the

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V2R at the basolateral membrane in confluent cells grown on plastic. This finding suggests that there may be mislocalization of V2R to the apical membrane in collecting duct cells that do not have cilia. This suggestion is not without precedent; previous work has reported the mislocalization of the epidermal growth factor receptor from the basolateral membrane to the apical membrane in certain forms of PKD (8). Thus this work aimed to determine whether V2R is mislocalized to the apical membrane in PKD and to investigate the consequence of altered receptor localization on salt and water transport by the collecting duct.

MATERIALS AND METHODS

Cell culture. Studies were performed using a temperature-sensitive SV40 immortalized collecting duct cell line derived from the Oak Ridge Polycystic Kidney (orpk) mouse model that is hypomorphic for the ift88/Tg737 gene (35). Cilia are absent or severely stunted in this cell line, which we designated as cilia (-/H11002). The ift88/Tg737 gene was reintroduced into this cell line as a control and is designated as cilia (+/H11001). The cilia in these rescue cells have previously been shown to be functional. For example, elevated apical fluid flow can increase cytosolic Ca\(^{2+}\) concentration and regulate raf-1 kinase-inhibitory protein (28, 29). Immunofluorescence demonstrating the morphology of the monolayers of cilia (-) and cilia (+) cells are shown in Fig. 1A.

Note the absence of ciliary structures in cilia (-) cells, while cilia (+) cells exhibit normal cilia. Both cell lines were cultured in DMEM/F12 containing 5% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, 10 nM 3,3',5-triiodothyronine, 50 nM dexamethasone, 1.0 mg/ml insulin, 0.55 mg/ml human transferrin, 0.5 \(\mu\)g/ml sodium selenite, 12 U/ml IFN-\(\gamma\), and 500 \(\mu\)g/ml G418. Cells were cultured at the permissive temperature of 33°C in humidified air with 5% CO\(_2\). Cells were grown on either permeable filters (Transwell, 0.4-\(\mu\)m pore size, polyester 24-mm\(^2\) membranes, Corning Costar, Cambridge, MA) or in 10-cm plastic culture dishes until confluent. To promote differentiation, cells were incubated at 39°C in the absence of IFN-\(\gamma\) for 4–5 days. Only confluent monolayers of cells with a transepithelial resistance of >1,300 \(\Omega\)-cm\(^2\) were used for experiments. To determine the permeability of these cells to AVP, 0.5 \(\mu\)Ci of \(^{125}\)I-AVP (PerkinElmer) was added to the apical fluid in both cell lines grown on 24-mm\(^2\) permeable filters. After 30-min incubation, the isotope was counted in both apical and basolateral fluid.

Immunofluorescence. Confluent cilia (+) and cilia (-) cells grown on filters were serum starved for a total of 24 h before fixation. During this period, basolateral vasopressin (AVP; 10 nM, Sigma) was added to some of the filters starting 12 h before the application of apical or basolateral AVP for 30 min. Cells grown on nonpermeable supports were either not treated or were treated with AVP (10 nM) for 30 min before fixation. Cells were washed with cold PBS and fixed with 4% paraformaldehyde for 30 min on ice followed by permeabilization.
with 0.5% Triton X-100. Nonspecific binding was minimized by blocking for 30 min with 2% BSA followed by rinsing with PBS. Filters were detached from the filter holders and incubated with a goat polyclonal AQP2 antibody (1:100, Santa Cruz Biotechnology) or acetylated α-tubulin antibody (1:5,000, Sigma). In some studies, a rat monoclonal zonula occludens-1 antibody (1:100, Santa Cruz, Biotechnology) was used in combination with AQP2 antibodies. After three PBS washes, cells were incubated with secondary antibodies: green fluorescent Alexa 488-conjugated donkey anti-goat or donkey anti-rabbit antibody or Alexa 594-conjugated red fluorescent goat-anti-rat antibody (1:1,000) was used for 1 h. Filters were fixed on glass slides with nuclear stain (4,6-diamidino-2-phenylindole) containing mounting medium and examined by confocal laser microscopy (Leica, Wetzlar, Germany). Fluorescent intensity was analyzed by randomly selecting region of interest (ROI; n = 20).

**Membrane protein biotinylation and Western blot analysis.** Cilia (+) and cilia (−) were grown on filters and treated as described above. Apical cell surface biotinylation was performed by NHS-SS biotin (Pierce, Rockford, IL). In brief, cells were washed twice with ice-cold PBS and incubated for 30 min at 4°C under gentle agitation with 1 ml of 0.25 mM bis-sulfono-SS-biotin applied to the apical surface. The biotinylation reaction was quenched by a solution containing 50 mM NH₄Cl in PBS at 4°C for 5 min. Filters or cell culture dishes were then rinsed twice with cold PBS, and cells were scraped and transferred to Eppendorf tubes. After centrifugation for 3 min at 10,000 g, the supernatant was discarded and 500 μl of lysis buffer with protease inhibitors (Pierce) was added. The solution was then sonicated at low power followed by 30-min incubation on ice. Following the manufacturer’s instructions, NeutrAvidin-agarose was added to a spin column along with the cell lysate and gently rocked for 1 h at room temperature. After applying of wash buffer, 50 mM DTT was added and incubated for 1 h. After centrifuging at 1,000 g × 2 min, the biotinylated protein concentration of the eluted fraction was measured using the Bio-Rad protein assay method (Bio-Rad protein assay kit; Bio-Rad, Hercules, CA). Equal amounts of biotinylated protein were resolved on a 10–20% SDS-PAGE and transferred to nitrocellulose membranes (Invitrogen). The nitrocellulose membrane was blocked with 5% BSA followed by incubation with AQP2 antibody (1:500) or a rabbit-polyclonal V2R antibody 7251 AP [1:500, kindly provided by Dr. Robert Fenton (University of Aarhus, Aarhus, Denmark)]. After washing, the membrane was incubated with horseradish peroxidase (HRP)-conjugated anti-goat secondary antibody or with HRP-conjugated anti-rabbit secondary antibody (Millipore). AQP2 and V2R bands were visualized using chemiluminescence (ECL; Amersham International, Buckinghamshire, UK).

**Water permeability study.** Fluid flow across the confluent monolayer can be assessed by measuring the fluorescence intensity of a large impermeable fluorescence marker (Texas red dextran, 70 kDa, Molecular Probes) which was added at a concentration of 3 mM only to the apical solution. This method was similar to that developed by Kovbasnjuk and others (15) for measuring water transport in Madin-Darby canine kidney epithelial cells. Experiments were performed at 37°C in a humidified environmental chamber (Life Imaging Services) mounted on the confocal microscope. Fluorescence intensity was measured for over 60 min in both cilia (−) and cilia (+) cells after addition of AVP (10 nM) to the apical or basolateral chambers.

**Short-circuit current study.** Equivalent short-circuit current (Isc) was measured in both cell lines grown on filters. Transepithelial resistance (TER) and voltage were measured in the apical and basolateral chamber using an electrical resistance system (Millicell-ERS; Millipore). In a 37°C chamber, electrodes were carefully immersed in the extracellular solutions to avoid direct contact with the monolayer. The shorter electrode was immersed in the inner well (apical) and the longer electrode in the outer well (basolateral). Positive voltage indicates that the basolateral membrane is positive with respect to the apical membrane. TER and voltage were measured in the presence or absence of desmopressin (DDAVP; 10 nM, Sigma). Benzamil (10 μM) was added apically after DDAVP treatment in both cell lines. A PKA inhibitor (H89; 50 nM, Sigma) was added to the apical (20 nM) and basolateral (30 nM) solutions simultaneously 30 min before the addition of DDAVP. Isc = [adjusted voltage/adjusted TER (mA/cm²)] was calculated after TER and voltage were normalized by subtracting the background TER and voltage level.

**cAMP measurement.** A CAMP Enzyme Immunoassay Kit (Sigma) was used to measure cAMP levels in both cilia (−) and cilia (+) cells grown on permeable supports. Cells were lysed with 0.1 M HCl for 10 min then scraped, collected in a conical tube, and centrifuged at 2,100 g for 3 min, and the supernatant was used for assay. The supernatant (100 μl) was pipetted into a 96-well plate and incubated for 2 h with cAMP EIA antibody (50 μl) and alkaline phosphatase conjugate (50 μl). After washing of the wells with Tris-buffered saline three times, p-nitrophenyl phosphate substrate was added and incubated at room temperature for 1 h. Then, a stop solution (trisodium phosphate) was added and optical density at 405 nm was measured (Spectramax M5 plate reader, Molecular Devices). A standard curve was measured to calculate cAMP concentration, and sample results were normalized by protein concentration.

**Statistical analysis.** Results are shown as means ± SE or SD. The significance of the results were determined by ANOVA or unpaired t-tests. A value of P < 0.05 was considered to denote statistical significance and was marked with an asterisk in figures and tables.

### RESULTS

Cilia (−) cells grown in culture dishes form domes after AVP treatment. While studying the effects of AVP on cell volume regulation, we observed that cilia (−) cells but not cilia (+) cells formed domes after 30 min of AVP (10 nM) treatment (Fig. 1B) when the cells were grown on a solid surface. This suggests that apical administration of AVP stimulated salt and water transport in cilia (−) cells. It is possible that the effects of apical AVP in cilia (−) cells occurred through increased paracellular permeability to AVP and activation of basolateral receptors. To assess this possibility, we grew both cell lines on permeable supports and added 125I-AVP (PerkinElmer) to the apical fluid. As shown in Table 1, only an extremely small percentage of AVP passed from apical to basolateral solutions. Next, confocal microscopy was used to determine whether apical AVP was stimulating water transport by affecting the expression levels of AQP2. In cilia (−) and cilia (+) cells (Fig. 1C). Apical AVP (10 nM) treatment for 30 min increased AQP2 expression (in green in figure) in cilia (−) cells compared with cilia (+) cells. Fluorescent intensity of AQP2 was analyzed by randomly selecting regions of interest in both cell lines pre- and post-AVP treatment.

**V2R is present in apical membranes in cilia (−) cells but not in cilia (+) cells grown on permeable supports.** In the presence of apical AVP for 30 min, cilia (+) cells expressed AQP2 (in green in figure) mainly in the cytosolic compartment. There was little evidence for apical localization of AQP2 except for

| Table 1. 125I-AVP isotope: background-adjusted mean counts per minute |
|-----------------|-----------------|-----------------|
| Cilia (+)       | Cilia (−)       |
| 125I-AVP apical | 864,084 ± 33,447 | 877,610 ± 21,309 |
| 125I-AVP basolateral | 35.9 ± 9.92 | 66.4 ± 7.57 |

Values are means ± SD (n = 3. 125I-AVP was added to the apical side and the isotope was counted after 30 min. P < 0.05. Background = 8.7 counts/min. Basolateral counts were significant to background (P < 0.05).
the presence of this protein in primary cilium (Fig. 2A). In cilia
(−) cells, apical AVP (10 nM) led to AQP2 localization to the
apical and subapical membrane areas. There was also basolat-
eral expression of AQP2 in cilia (−) cells (Fig. 2A). AQP2
expression to apical AVP was similar to the results observed
by basolateral AVP treatment in cilia (−) cells (data not shown).
Western blot analysis was performed on biotinyl-
lated apical membrane fractions from both cilia (−) and cilia
(+) cells grown on permeable supports. As shown in Fig.
2B, apical membranes from cilia (−) cells demonstrated an
increased amount of glycosylated AQP2 (36 kDa) compared
with nonglycosylated AQP2 (29 kDa). In contrast, cilia (+)
cells expressed more of the nonglycosylated form of AQP2.
Western blot analysis of V2R of the apical membrane
fraction and total cell lysate are shown in Fig. 2, C and D.
There was an increased V2R band at 43 and 47 kDa in both
total cell lysate and apical membrane fractions in cilia (−)
cells while cilia (+) cells showed only minimal V2R ex-
pression (n = 3). This finding supports the presence of
apically mislocalized V2R in cilia (−) cells but not in cilia
(+). Densitometry of the apical V2R protein is shown in
the bar graph in the figure.

Stimulation of apical V2R in cilia (−) cells increases trans-
epithelial fluid transport. Water permeability studies were
performed to assess the function of apical V2R by monitoring
AVP-mediated transepithelial fluid transport in both cell lines.
Texas red-conjugated dextran (70 kDa) was added to the apical
solution, and fluorescence intensity was measured using a
confocal microscope. We first excluded the involvement of
evaporation by demonstrating stable fluorescence intensity
for over 20 min with no appreciable bleaching of the fluorescent
dextran. Both cell lines were then treated with 10 nM AVP
added only to the apical compartment. While the fluorescence
intensity remained unchanged after apical AVP treatment in
cilia (+) cells, there was significantly increased fluorescence
intensity in cilia (−) cells. This is indicative of continuous fluid
movement from the apical to the basolateral side (Fig. 3A).
As a control, basolateral AVP increased the dextran intensity in
both cilia (−) and cilia (+) cells (Fig. 3, B and C).

$I_c$ increases with apical AVP stimulation in cilia (−) cells
and is inhibited by a PKA inhibitor. Based on the results of
fluid transport study, we measured $I_{sc}$ to assess the presence of
ion transport (such as Na+) after DDAVP treatment. Cilia (−)
cells had a higher $I_{sc}$ before stimulation with AVP compared
with cilia (+) cells. As shown in Fig. 4A, there was a
significant increase in $I_{sc}$ ($\mu$A/cm$^2$) in cilia (−) cells com-
pared with cilia (+) cells after a apical DDAVP addition.
This increase in current was abolished by the addition of
benzamil (10 μM), suggesting that this increase in $I_{sc}$ was
through ENaC. Since ENaC is stimulated by cAMP, we
determined that a PKA inhibitor was effective in preventing
a DDAVP-induced increase in $I_{sc}$. Pretreatment of both
apical and basolateral membranes with the PKA inhibitor
H89 (50 nM) inhibited the effect of apical DDAVP in cilia
(−) and cilia (+) cells (Fig. 4B). As a control, basolateral
DDAVP was added to cilia (−) and cilia (+) cells and both
demonstrated an increase in $I_{sc}$, but the increase was higher
in cilia (−) cells compared with cilia (+) cells (n = 3, P <
0.05) (Fig. 4C). cAMP levels were measured in both cell
lines grown on filters before and after apical DDAVP (10
nM) treatment. As described in Fig. 4D, baseline cAMP
levels were similar in both cilia (+) and cilia (−) cells.

Fig. 2: A: examples of immunofluorescence xy plane images of cilia (−) and cilia (+) cells grown on permeable supports after 30 min of apical AVP
treatment. AQP2 is expressed in the ciliary shaft (arrows) and in the subapical compartment in cilia (+) cells (xz plane). Cilia (−) cells demonstrated
greater AQP2 expression after apical AVP compared with cilia (+) cells. Side view (xz plane) of cilia (−) cells indicates that, after apical AVP treatment,
AQP2 is expressed in the apical membrane, intracellular compartment, and to some degree in the basolateral membrane (n = 3). B: Western blot analysis of biotinyl-
lated apical membrane fractions demonstrated an increased amount of glycosylated AQP2 (37 kDa) compared with nonglycosylated AQP2 (29
kDa) in cilia (−) cells, whereas cilia (+) cells had a greater abundance of nonglycosylated vs. glycosylated AQP2 (n = 3). C and D: Western blotting of the vasopressin 2 receptor (V2R) indicated increased abundance in total lysates of cilia (−) cells compared with cilia (+) cells. In addition, V2R was
detected in the apical membrane fraction in cilia (−) cells but not in cilia (+) cells. Densitometry of apical V2R was significant in cilia (−) compared
with cilia (+) cells; n = 3. *P < 0.05.
However, upon apical DDAVP treatment, there was a significant cAMP increase in cilia (−) vs. cilia (+) cells (Fig. 4C).

**DISCUSSION**

**Orpk** mice are born with massive kidney cysts that resemble human autosomal recessive PKD (21). This mouse model is a hypomorph of the intraflagellar transport protein *ift88*. Thus this model is one reflecting the loss of both the structure and function of cilia. It is different from other autosomal dominant PKD mouse models, which exhibit a loss of ciliary function(s). While *ift88* is necessary for the assembly and maintenance of cilia, it is also known to affect cell cycle progression and membrane trafficking (10, 27). In this study, we examined the

**Fig. 3.** A: Texas red-conjugated dextran (70 kDa) was added to the apical solution, and serial fluorescence intensity was measured using a confocal microscope after apical AVP treatment in cells grown on permeable supports. Cilia (−) cells demonstrated a constant increase in dextran fluorescence intensity, indicating fluid movement from the apical to the basolateral side. There was no increase in fluorescence intensity in cilia (+) cells with apical AVP exposure (*n* = 10). B and C: with addition of basolateral AVP (10 nM), both cilia (−) and cilia (+) cells demonstrated an increase in relative fluorescence intensity (*n* = 10). D: scheme of the water transport experiment. Increased transepithelial fluid transport increases the fluorescence intensity in the apical solution.
role of primary cilium in AVP-mediated fluid transport using collecting duct cell lines with stunted cilia and collecting duct cells with full-length cilia (35). We found unusual the dome formation in cilia (−) cells grown to confluence in plastic culture dishes in response to apical AVP since V2R predominantly localizes to the basolateral membrane in principal cells of the collecting duct. Although apical V2R expression has been reported in cilia (17, 25), cilia (+) cells did not exhibit dome formation in response to apical AVP, suggesting that stimulation of the ciliary V2R receptors was not sufficient to activate significant transepithelial salt and water transport. These finding suggest that V2R might be mislocalized to the apical membrane in cilia (−) cells.

There is evidence that AQP2 expression may be altered, along with elevated renal cAMP, in PCK rats, an animal model similar to human autosomal recessive PKD (11). We examined the localization of AQP2 by indirect immunofluorescence in both cilia (+) and cilia (−) cell lines after administration of AVP. Our results indicate that AQP2 expression increased in cilia (−) cells but not in cilia (+) cells. Apical and basolateral AVP treatment triggered AQP2 insertion not only into the apical membrane but also into the basolateral membrane in cilia (−) cells. One interpretation of this finding is that cilia-related mechanisms lead to selective targeting of AQP2 to the apical membrane. Therefore, increased AQP2 expression at the apical membrane and evidence for mislocalized basolateral AQP2 in cilia (−) cells may contribute to enhanced AVP-mediated fluid transport in the cilia (−) cells. AQP2 protein is expressed in both glycosylated and nonglycosylated forms, yet this does not alter translocation of AQP2 to the plasma membrane nor affect protein function (2, 19). AQP2 is also expressed in both glycosylated and nonglycosylated forms, yet this does not alter translocation of AQP2 to the plasma membrane nor affect protein function (2, 19). AQP2 is also expressed in both glycosylated and nonglycosylated forms, yet this does not alter translocation of AQP2 to the plasma membrane nor affect protein function (2, 19). AQP2 is also expressed in both glycosylated and nonglycosylated forms, yet this does not alter translocation of AQP2 to the plasma membrane nor affect protein function (2, 19).

Further studies assessed the presence and distribution of V2R in these two cell lines. Increased total V2R in cilia (−) vs. cilia (+) cells was found in the surface membrane fraction of biotinylated membranes analyzed by Western blot analysis. In addition, we detected the presence of V2R in the apical membrane fraction from cilia (−) but not from cilia (+) cells. This directly supports our finding that apical AVP treatment causes rapid dome formation in cilia (−) cells through the activation of apical V2Rs.

To determine whether apical V2R was functional, we studied AVP-mediated fluid transport by measuring the intensity of fluorescein-labeled dextran applied to the apical chamber. One important finding was that apical application of AVP stimulated transepithelial water transport in cilia (−) cells. We further determined that there was almost no paracellular permeability to AVP in both cell types, which strongly suggests that AVP was increasing fluid transport in cilia (−) cells by activating apical receptors. In addition, the time course for water transport with basolateral AVP was different between cilia (−) and cilia (+) cells. Normally, agonist binding to V2R initiates a cascade of events that leads to internalization of this receptor from the basolateral membrane and desensitization to the hormone (26). This may explain the response of cilia (+) cells to basolateral addition of AVP, which resulted in a stimulation of water transport followed by decreased water transport over time. However, in cilia (−) cells, fluid transport continued to rise after basolateral AVP stimulation, which may reflect a lack of agonist-induced internalization of V2R in the absence of cilia.

The apical V2R response to DDAVP was also confirmed by measuring the equivalent Isc across the apical and basolateral membranes. Our results demonstrated that cilia (−) cells responded to apical DDAVP with elevated salt transport, as indicated by increased Isc, compared with the small change in Isc found in cilia (+) cells. Since the increases in Isc were sensitive to benzamil, we suggest that this is an ENaC-dependent current, as would be expected for a principal cell line derived from the collecting duct. The dose of benzamil (10 μM) used in this study was based on previous studies demonstrating that a 10 μM or higher concentration of benzamil was required to fully block Na+ current in these cell lines. This relative insensitivity to benzamil may reflect the contribution of the subunits that comprise the Na+ channel in these cells (23). Typically, AVP increases ENaC activity within the cell via the cAMP/PKA pathway (5, 22). We found that pretreatment with H89, a relatively nonspecific PKA inhibitor, significantly suppressed the DDAVP-mediated increase in Isc, suggesting an involvement of cAMP. In addition, cytosolic cAMP levels increased after apical DDAVP treatment in cilia (−) but not in cilia (+) cells. Thus apical V2R was able to stimulate cAMP production in a PKA-dependent manner, resulting in increased salt and water transport across cilia (−) cells (Fig. 5).

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**Fig. 5.** Scheme of cilia (+) and cilia (−) cells upon apical AVP treatment. Apical AVP stimulates the cAMP/PKA pathway, which increases salt and water movement in cilia (−) but not in cilia (+) cells.
Although plasma AVP levels can fluctuate in response to changes in water homeostasis, plasma AVP has been shown to be elevated in PKD patients with hypertension (7). It is also known that AVP is filtered freely by the glomerulus, and urinary AVP concentration can be up to 120 times higher than the plasma level (14, 20). Therefore, the presence of apical V2R and concurrent elevated urinary AVP may facilitate active fluid absorption in the collecting duct, possibly contributing to fluid retention and hypertension.

In summary, we have demonstrated that V2R is mislocalized to the apical membrane in cells that lack normal cilia. In the presence of apical AVP, the predominantly glycosylated AQP2 abundance increased and was detected at both the apical and basolateral membranes in cilia (−) cells. Apical V2R was shown to be functional since activation of this receptor with apical AVP increased salt and water transport through AQP2 and ENaC. Whether such apical V2R localization contributes to enhanced fluid retention and hypertension in PKD remains to be determined.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


