Unimpaired osmotic water permeability and fluid secretion in bile duct epithelia of AQP1 null mice

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Mennone, Albert, Alan S. Verkman, and James L. Boyer. Unimpaired osmotic water permeability and fluid secretion in bile duct epithelia of AQP1 null mice. Am J Physiol Gastrointest Liver Physiol 283: G739–G746, 2002; 10.1152/ajpgi.00540.2001.—The mechanisms by which fluid moves across the luminal membrane of cholangiocyte epithelia are uncertain. Previous studies suggested that aquaporin-1 (AQP1) is an important determinant of fluid movement in rat cholangiocytes and that cyclic AMP mediates the movement of these water channels from cytoplasm to apical membrane, thereby increasing the osmotic water permeability. To test this possibility we measured agonist-stimulated fluid secretion and osmotically driven water transport in isolated bile duct units (IBDUs) from AQP1 wild-type (+/+) and null (−/−) mice. AQP1 expression was confirmed in a mouse cholangiocyte cell line and +/- liver. Forskolin-induced fluid secretion, measured from the kinetics of IBDU luminal expansion, was 0.05 fl/min and was not impaired in −/− mice. Osmotic water permeability (Pf), measured from the initial rate of IBDU swelling in response to a 70-mosM osmotic gradient, was 11.1 × 10−4 cm/s in +/- mice and 11.5 × 10−4 cm/s in −/− mice. Pf values increased by ∼50% in both +/- and −/− mice following preincubation with forskolin. These findings provide direct evidence that AQP1 is not rate limiting for water movement in mouse cholangiocytes and does not appear to be regulated by cyclic AMP in this species.

aquaporins; cholangiocytes; bile formation; adenosine 3',5'-cyclic monophosphate

BILE DUCT EPITHELIAL CELLS (cholangiocytes) play an important role in bile formation by modifying the primary secretions from hepatocytes by a number of secretory and absorptive transport mechanisms (4, 5). The primary secretory product of cholangiocytes is a bicarbonate-rich fluid that is secreted in response to secretin (3, 24), bombesin (9), vasoactive intestinal peptide (8), and other agonists (4). Absorption of fluid and solutes from the biliary lumen is facilitated by the action of the sodium/hydrogen exchanger (NHE)3 (26), bile salt transporter (ISBT) (2, 13), and glucose transporter (SGLT-1) (14). Water moves into bile from cholangiocytes in response to a small osmotic gradient created by these transport processes. However, it remains unclear whether water moves into and out of the biliary lumen through the cell membrane, via paracellular routes, or through specific ∼30-kDa protein channels known as aquaporins (AQP) (1, 17).

In epithelia in which there is a high rate of water flux, such as the kidney and gastrointestinal tract, water movement is facilitated by one or more of the ten AQP homologs identified in mammals (1, 17). Several AQP homologs have been described recently in rat hepatocytes (AQP 0, 3, 8, and 9) (10, 29), although water is thought to cross hepatocyte cell membranes primarily by pathways other than water channels (32). AQP homologs have also been described in rat cholangiocytes including AQP1, which is localized in the cytoplasm and the apical membrane (20, 27), and AQP4, which is restricted to the basolateral membrane (21). A recent preliminary report of RT-PCR studies describes eight different AQP homologs in cholangiocytes (29). Unlike all other cell types in which AQP1 is constitutively expressed, studies by Marinelli et al. (20, 21) suggest that the expression of AQP1 on the apical luminal membrane of the cholangiocyte is regulated by the insertion of AQP1-containing vesicles in response to increases in cellular cAMP. These findings are based on studies in which osmotic water permeability coefficients have been obtained in isolated rat cholangiocytes prepared from bile duct-ligated rats. In those studies, the distribution of AQP1 has been determined by Western blot analysis of apical and basolateral membrane preparations obtained from rat cholangiocyte preparations before and after the exposure of rat cholangiocytes to the hormone secretin, a stimulant of cAMP in this tissue. Immunohistochemical studies for AQP1 in liver sections from rats treated with secretin also showed intensified staining in apical regions of cholangiocyte (22).

In many tissues, AQPs are not rate limiting for transcellular water movement (23), and, despite prior studies, it remains unclear whether AQPs are rate limiting for transcellular water movement in the biliary epithelium. In biliary epithelium, the net flux of water is very small compared with epithelial tissues.
such as the kidney or salivary gland, in which these channels are necessary to facilitate the large fluxes of water that move across these epithelia (16, 28). In the present study, we have assessed water movement across isolated bile duct units (IBDUs) in the rat and mouse and used AQP1 knockout mice to de

across isolated bile duct units (IBDUs) in the rat and present study, we have assessed water movement that move across these epithelia (16, 28). In the

MATERIALS AND METHODS

Collagenase B was purchased from Roche Diagnostics (Indianapolis, IN). DNase, hyaluronidase, protease inhibitors, BSA, saponin, and forskolin were obtained from Sigma (St. Louis, MO). Pronase was from Calbiochem (La Jolla, CA), Matrigel was from Collaborative Biomedical, and MEM, α-MEM, gentamycin, and Trizol were from Gibco (Grand Island, NY).

Isolation of bile duct units. AQP1 wild-type (+/-) and null (-/-) mice (CD1 genetic background) were generated as described previously (18) and maintained in the Yale Univ. Medical School animal care facility before use. All mice were coded, and the genotype was unknown to investigators until completion of the study. In addition, in selected experiments, IBDUs were isolated from Sprague-Dawley rats (Charles River, Wilmington, MA). Experimental protocols were approved by the Yale Animal Care and Use Committee.

Bile duct units were isolated from livers as described previously from this laboratory (25, 26). Briefly, portal veins were cannulated, and the livers were perfused with Hank’s A buffer for 5 min followed by Hank’s B buffer containing 0.03% collagenase B. Once digested, the hepatocytes were removed by shaking the livers in 4°C L-15 media. Then the nonparenchymal tissue remnant was further digested with 0.036% pronase, 0.05% collagenase B, and 0.006% DNase in MEM media at 37 °C for 30 min. The tissue was then digested for an additional 30 min in the same solution except that 0.036% hyaluronidase replaced the pronase. The digested tissue was then sequentially passed through 100- and 30-μm mesh, where fragments of isolated bile ducts measuring ~30–100 μm in diameter were collected and plated on Matrigel-coated coverslip fragments and cultured in α-MEM for ~48 h.

Western blot analysis. Genotypes were confirmed by Western blot analysis from microsomal membrane preparations of mouse kidney with protease inhibitors by standard methods using an anti-AQP1 primary antibody diluted 1:2,500 overnight (rabbit polyclonal, Chemicon International, Temecula, CA) and an enhanced chemiluminescence (Amersham, Arlington Heights, IL) detection method. Western blot analyses were also determined in homogenates from a mouse cholangiocyte cell line, kindly provided by Dr. Y. Uneo (Sendai, Japan).

Immunofluorescence. Immunofluorescence microscopy was used to assess the presence of AQP1 in rat and mouse liver, rat kidney, and a mouse cholangiocyte cell line. Once removed, livers were cut into small cubes and quick frozen in liquid nitrogen-cooled freon before cryosectioning at 5 μm. Tissue sections as well as the cholangiocyte cell line grown on glass coverslips were fixed in −20°C acetone for 10 min, washed in PBS containing 0.05% saponin (PBS/S), then blocked with 1% BSA for 30 min. Rabbit anti-AQP1 antibody was incubated on the tissue or cells for 2 h at 5 μg/ml, washed in PBS/S for 1 h, then incubated in Cy2 or Cy3 anti-rabbit IgG secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h. After the slides were washed in PBS, they were mounted with Vectashield (Vector Laboratories, Burlingame, CA) and photographed using a Nikon E800 epifluorescence microscope and Hamamatsu charge-coupled device camera.

RT-PCR. RT-PCR was done on a mouse cholangiocyte cell line. The strong expression of AQP1 in liver endothelial cells prevented reliable interpretation of similar data from IBDUs and isolated cholangiocyte preparations. RNA was isolated using the Trizol method, and first-strand cDNA was amplified using the Superscript system (Gibco-BRL). The AQP1 specific primers 5′-CATGGCCAGTGAATCAGAAGAAGAC-3′ (forward) and 5′-TAGATGCCCAGGC CAGGCTCCCTC-3′ (reverse) were synthesized from the Yale Univ. Keck oligonucleotide facility. PCR was performed using 1.25 μM primers, 200 μM dNTP mix, 5 U Taq DNA polymerase, 1.5 mM MgCl2, 2 μl of first-strand DNA, and PCR buffer (20 mM Tris·HCl, 50 mM KCl). Samples were placed in a programmable thermal controller (M. J. Research), where they were heated to 94°C for 3 min to denature and annealed at 62°C and repeated 35 cycles. PCR products were confirmed by sequencing in the Keck Biotechnology Resource Laboratory, Yale Univ.
Experimental protocols. After 48 h in culture, bile duct fragments were transferred on glass coverslips to the stage of an inverted video microscope, where they were perfused at a rate of 1 ml/min at 37°C in a 36-µl thermostatically controlled chamber (Warner Instruments, Hamden, CT) to facilitate rapid perfusate exchange. Equilibration of perfusate under these conditions was achieved within 5 s (98%). IBDUs from AQP1 +/+ and −/− mice and normal rats were perifused with isotonic Krebs-Ringer bicarbonate (KRB; 300 mosM) for 10 min before initiating the specific protocol.

Cross-sectional images of IBDU lumens were obtained at a focal point representing the maximal cross-sectional area of the bile duct lumen as previously described from this laboratory. Lumen volumes were calculated as described (7, 25, 26), and changes in volume were plotted as a percent change in volume from time 0.

To measure osmotic water permeability, IBDUs were exposed to hypotonic KRB (230 mosM, 70 mM NaCl removed) after the 10-min preequilibration in isotonic media. Cross-sectional images of the expanding luminal area were obtained at 30-s intervals for up to 5 min. Osmotic water permeability coefficients (Pf, cm/s) were calculated from the rate of volume change over the first minute after exposure to hypotonic buffer when the initial rate of the volume change was linear, according to the equation (33)

\[
P_f = \frac{V_o}{S} \frac{d}{dt} \left[ \frac{S}{V_o} (\text{osm}_{\text{out}} - \text{osm}_{\text{in}}) \right]
\]

where osm_{out} is 230 mosM, osm_{in} is 300 mosM, V_o is initial IBDU lumen volume, V is IBDU volume 60 s after switching to hypotonic media, and S is initial surface area of the IBDU lumen immediately before the change in media, calculated from the maximal radius of the duct lumen (4πr²).

Fig. 2. Immunofluorescence of rat and mice liver using AQP1 antibodies. A: low magnification of wild-type mouse liver showing endothelial cell staining and weak, poorly reproducible cholangiocyte staining (inset). B: low magnification of rat liver demonstrating endothelial cell staining that is more pronounced in periportal regions and weak intracellular staining in cholangiocytes (inset, arrows). AQP1 +/+ (C) and −/− (D) mouse liver. Note the complete absence of staining in the AQP1 null liver. E: proximal tubules from rat kidney stained brightly for AQP1.
CHOLANGIOCYTE SECRETION IS NORMAL IN AQUaporIN-1 NULL MICE

RESULTS

Fig. 3. Effect of forskolin on luminal expansion of isolated bile duct units (IBDUs) from rat and AQP1 +/+ and −/− mice. Similar choleretic effects are observed between the 2 mouse groups; however, they were significantly lower than the rat (**P < 0.01).

The specific experimental protocols were as follows. 1) To measure forskolin-stimulated fluid secretion in IBDU from rat and AQP1 +/+ and −/− mice, IBDUs were perfused in isotonic KR 5 for 10 min and then with isotonic KR containing 10 µM forskolin for 30 min. Luminal images were obtained at 5-min intervals. 2) To measure the osmotic water permeability coefficient, IBDUs from rat and +/+ and −/− mice were perfused in isotonic KR for 10 min; then the perfusate was rapidly changed to hypotonic (230 mosM) media, and changes in luminal volume were measured at 30-s intervals for up to 90 s. 3) To determine whether an increase in cytoplasmic cAMP affects osmotic water permeability, IBDUs were pretreated for 15 min with either 10 µM forskolin or 50 nM secretin. IBDUs from AQP1 +/+ and −/− mice and rat were perfused in isotonic KR containing forskolin or secretin for 15 min before exposure to hypotonic media. Secretin was dissolved in 1% BSA before being infused; controls using BSA alone were used for comparison. To eliminate secretory effects due to cAMP-induced secretion in calculating Pt, forskolin-/secretin-induced changes in volume were calculated for the 90 s before exposure to hypotonic buffer and subtracted from the subsequent values induced by hypotonic media.

All results are expressed as means ± SE and tested for significant differences by unpaired t-tests, which compared observations between experimental and control measurements from each separate IBDU isolation.

RESULTS

Immunoblot, PCR, and immunofluorescence. Immunoblots of the crude membrane fraction from kidneys of +/+ mice showed bands at the predicted molecular sizes of nonglycosylated AQP1 (28 kDa) and glycosylated AQP1 (43 kDa; Fig. 1A). These bands were not detected in kidneys from AQP1 −/− mice. AQP1 was strongly expressed on endothelial cells both in rat and mouse liver (Fig. 2). Because these cells are present in the IBDU preparations, they prevented a direct assessment of AQP1 on cholangiocytes by Western blot analysis. However, Western blot of membranes from a mouse cholangiocyte cell line showed AQP1 protein expression (Fig. 1A, right) and transcript expression (Fig. 1B).

Immunofluorescence staining revealed strong expression of AQP1 on endothelial cells of +/+ mice (Fig. 2A) and rat liver (Fig. 2B) that contrasted with weaker and equivocal staining in cholangiocytes. The endothelial staining as seen in liver of AQP1 +/+ mice (Fig. 2C) was absent in the −/− mice (Fig. 2D). Proximal tubules from the rat kidney were strongly labeled for AQP1 where AQP1 is highly expressed (Fig. 2E).

AQP1 on mouse in rat and mice as shown in Fig. 3 and summarized in Table 1. There was no significant difference in the choleretic response to forskolin in IBDUs from AQP1 +/+ vs. −/− mice when adjusted for differences in the size of luminal space before the forskolin infusion. However, both the AQP1 wild-type and null mice responded to forskolin with lower rates of secretion compared with rat IBDUs [49 ± 9 fl·min⁻¹·pl⁻¹ baseline volume for +/+ (n = 61) and 39 ± 9 fl·min⁻¹·pl⁻¹ for −/− (n = 29) vs. 78 ± 12 fl·min⁻¹·pl⁻¹ (n = 10) for rat IBDUs]. These choleretic effects of forskolin confirm previously published observations on the effects of cyclic AMP-mediated secretion in rat and mice IBDUs (10, 25). The luminal diameters of IBDUs used for these studies averaged 28.7 ± 0.9 µm (n = 155) at 48 h. Given that the cross-sectional diameters of these ducts are nearly twice that value, these bile duct units are derived from the interlobular ducts that are known to be responsive to secretin and cyclic AMP-mediated secretion (12).

Nomarski video images of a representative mouse IBDU are illustrated in Fig. 4. A–C. Cell swelling in addition to luminal volume expansion can be seen in the 30-s image. The kinetics of IBDU lumen expansion in response to this rapid hypotonic challenge are shown in Fig. 5A. The accuracy of these measurements is dependent on a rapid exchange of fluid, which was accomplished using a low-volume perfusion chamber (36 µl) that enabled a 98% fluid exchange in <5 s. There was no significant difference in the increase in luminal volume

Table 1. Luminal volumes before forskolin infusions and forskolin-stimulated secretory rates in IBDUs from AQP1 +/+ mouse, AQP1 −/− mouse, and rat as shown in Fig. 3

<table>
<thead>
<tr>
<th>AQP +/+ mice</th>
<th>AQP −/− mice</th>
<th>Rat</th>
</tr>
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<tbody>
<tr>
<td>(n = 61)</td>
<td>(n = 29)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Luminal volume before forskolin infusion, pl</td>
<td>17.0 ± 2.7 a</td>
<td>10.7 ± 2.5 a</td>
</tr>
<tr>
<td>Secretory rate normalized to initial luminal volumes, fl·min⁻¹·pl⁻¹</td>
<td>49.0 ± 9.3</td>
<td>39.2 ± 9.1</td>
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</tbody>
</table>

Values are expressed as means ± SE; no. of isolated bile duct units (IBDU) in parentheses. *No significant difference in luminal volumes between aquaporin (AQP)-1 +/+ and AQP1 −/− mice, P = 0.15 or between the mice and rat luminal volumes, P = 0.12 and 0.34, respectively. †Secretory rates normalized to initial luminal volume were significantly greater in rat IBDUs than the −/− mouse IBDUs; P = 0.03.
following exposure to hypotonic media between the rat 
\((n = 31)\) and the AQP1 \(+/+\) \((n = 41)\) and \(-/-\) \((n = 34)\) 
mouse IBDUs. The time point of maximal increase over 
the first 60 s for each IBDU was determined and used to 
calculate \(P_f\) (Fig. 5C). Averaged \(P_f\) values were \(11 \pm 1.6 \times \) 
10\(^{-4}\) \((+/-\) mice, \(n = 41)\) and \(11.5 \pm 1.6 \times \) 
10\(^{-4}\) cm/s \((-/-\) mice, \(n = 34); \) differences not 
significant).

In a third set of experiments, IBDUs from rat and 
AQP1 \(+/+\) and \(-/-\) mice were preincubated with for-
skolin in isotonic KRB before switching to hypotonic 
KRB. Figure 5, B and C, shows the expansion of the rat 
and mouse IBDU lumens and calculated \(P_f\) following 
exposure to hypotonic media. No differences in luminal 
expansion or calculated values for \(P_f\) \((+/-\), 16.3 \pm 
2.0 \times 10\(^{-4}\) cm/s, \(n = 35)\) vs. \(-/-\), 18.8 \pm 3.3 \times 10\(^{-4}\) 
cm/s, \(n = 29)\) were seen between the wild-type and 
knockout mice following preincubation with forskolin. 
However, \(P_f\) values in IBDUs both from AQP1 \(+/+\) and 
\(-/-\) mice were somewhat higher \((P < 0.05)\) after pre-
incubation with forskolin compared with studies in 
which forskolin was not present. Thus this apparent 
increase in osmotic water permeability in mouse after 
forskolin does not involve AQP1. It is possible that 
forskolin may induce a change in IBDU morphology or 
increase water permeability in a parallel pathway. In 
contrast, \(P_f\) values in rat IBDUs were \(8.1 \pm 2.2 \times 10^{-4}\) 
cm/s \((n = 18)\) following forskolin and were not signifi-
cantly different from the unstimulated group \((9.6 \pm 
1.5 \times 10^{-4}\) cm/s, \(n = 18)\).

Calculations of osmotic water permeability following 
preincubation with secretin were also similar to control 
values with both rat and wild type mouse IBDUs (Fig. 
6). In rat, the \(P_f\) following secretin preincubation was 
\(8.0 \pm 2.8 \times 10^{-4}\) \((n = 18)\) vs. \(6.4 \pm 1.2 \times 10^{-4}\) cm/s \((n = 
19)\) for controls. In mouse IBDUs, \(P_f\) was \(22.0 \pm 4.4 \times \) 
10\(^{-4}\) cm/s \((n = 9)\) following secretin preincubation and 
\(17.0 \pm 2.3\) cm/s \((n = 8)\) for the controls.

**DISCUSSION**

The importance of AQPs in transcellular fluid move-
ment is tissue dependent. In general, AQPs are 
thought to facilitate fluid movement in tissues in which 
rapid osmotic fluid movement is required, such as in 
kidney tubules and secretory glands (1, 17, 31). Al-
though prior studies have identified AQP1 in rat 
cholangiocytes (10, 11, 20, 27), the role of AQPs as a 
rate-limiting determinant of net fluid secretion in in-
tact bile ducts has not been examined. In the present 
study, we have used AQP1 knockout mice to assess the 
role of AQP1 on water movement in mouse cholangio-
cyte IBDUs following agonist-induced secretion and 
osmotic gradients.

Our findings provide direct evidence that AQP1 is 
not an important determinant of \(P_f\) and thus fluid 
secretion in mouse cholangiocytes. First, the choleretic 
response to forskolin was not impaired in AQP1 null 
mice (Fig. 3 and Table 1). Second, \(P_f\) in IBDUs from 
AQP1 \(-/-\) mice was not different from that in IBDUs 
from \(+/+\) mice (Fig. 5). This finding is consistent with 
the equivocal-to-weak AQP1 immunostaining in 
cholangiocytes from \(+/+\) mice, with strongly positive 
control staining in kidney (Fig. 2). Possibly, other 
AQPs might be upregulated in this knockout model in 
the cholangiocyte, although this is unlikely given the 
low \(P_f\) of \(\sim 10^{-3}\) cm/s, suggesting lipid-mediated water 
transport, and because other AQPs are not upregulated 
in the kidney and other tissues in AQP1 null mice (31).

Previous studies have indicated that the expression 
of AQP1 on the luminal membrane is stimulated by 
cyclic AMP in rat cholangiocytes (22, 27). Regulated 
AQP1 vesicular transport is discrepant with studies of 
AQP1 regulation in other tissues where this water 
channel is constitutively expressed (17). In addition, 
protein kinase A phosphorylation sites have not been 
demonstrated in AQP1, in contrast to AQP2, which is 
highly regulated by cyclic AMP in other epithelia (17). 
Indeed in a transfected cell culture model, an AQP2 
chimera containing the AQP1 COOH terminus fails to 
undergo regulated vesicular transport (30). For these 
reasons, we examined the effects of forskolin and 
secretin pretreatment in rat and mouse IBDUs on the 
response to rapid changes in water movement induced 
by hypotonicity. Forskolin is a potent stimulant of 
the catalytic subunit of adenyl cyclase and is the strongest 
agonist for cyclic AMP-mediated signal-transduction 
events. Previous studies from our laboratory indicate...
that forskolin is a potent stimulus of cyclic AMP-dependent secretion in rat IBDUs (25). If cyclic AMP stimulated cytoplasmic to apical membrane movement of water channels in cholangiocytes, initial rates of volume expansion following hypotonic media-induced water movement should increase. However, we could find only a small, albeit significant, effect of forskolin pretreatment on these two parameters, and this was observed in both AQP1 +/+ and −/− mice but not rat. Because these differences in Pf values are small, it is unlikely that cyclic AMP would have a functionally significant effect on passive water movement across this epithelium. Previous studies demonstrated that secretin increased Pf in isolated rat cholangiocytes (20, 22). However, secretin did not increase Pf significantly in rat or mice IBDUs in our experiments compared with controls. In addition, we found that the Pf for rat IBDUs was consistently lower than values observed in mouse IBDUs. Although the reasons for these differences is not known, they could be explained by different levels of expression of AQPs between these two

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

![Graph D](image4)

**Fig. 5.** A: initial rates of luminal expansion following exposure of rat, wild-type, and AQP1 −/− IBDUs to hypotonic media were similar. B: initial rates of luminal expansion to hypotonic media were also similar in rat and wild-type and −/− IBDUs after preincubation with forskolin (10 μM; *P < 0.01). C: calculated osmotic water permeability (Pf) after exposure to hypotonic media without pretreatment with forskolin were the same in wild-type and AQP1 null mice and rats. Forskolin pretreatment showed no effect on rat Pf but resulted in a small but significant increase in Pf values in both wild-type and AQP null mice (*P < 0.05).
species. Indeed, net water fluxes across cholangiocyte epithelium vary considerably among species (6) but are particularly low in rodents. Bile is largely secreted from hepatocytes, and less than 10% of total bile productions or less than ~100 μl/g of liver tissue per hour originates from cholangiocytes in the rat. Thus rapid changes in water permeability across this epithelium would not be expected. Indeed, significant changes in net bile production have not been observed in the living anesthetized AQP1 null mouse (15). This contrasts markedly to the kidney epithelia and endothelia where fluid movement is several orders of magnitude larger (17) and where the loss of AQP1 results in rapid dehydration due to increased urine volumes.

Pf values in this study were significantly lower than previously reported for isolated rat cholangiocytes (20, 27). For this reason, we also measured the Pf in normal rat IBDUs when water movement was stimulated by exposure to hypotonic media to assess whether there were species differences between rat and mouse. However, we obtained a similar range of Pf measurements in the rat IBDUs (12.7 × 10⁻⁴ cm/s) as observed in the mouse model.

Differences in technique might account for the lower Pf values that we have observed. Our studies assessed movement of water across the cell and into the closed space of the IBDU lumen over a 60-s time period in response to the change in extracellular osmolarity, a process requiring water to cross several membrane barriers. Other studies measured Pf in single-cell preparations of cholangiocytes purified on immunomagnetic beads and exposed these cells to larger osmotic gradients for 5–30 s, in which more than one water channel must be contributing to the cell volume changes. Although we cannot exclude an influence from basolateral water channels on our measurements, luminal volume changes in response to hypotonicity should occur only by movement of water across the apical membrane or through paracellular pathways. We think it is unlikely that alterations in paracellular permeability account for the lower water permeability in our preparations, because Texas-red Dextran-40 is excluded from the lumens of most IBDUs (25) and higher, rather then lower, Pf values would be expected if leaky paracellular junctions had affected water movements. Thus our measurements would seem to directly reflect changes in apical water movement. It is also unlikely that our Pf values are affected by delays in fluid exchange in the chamber, because 98% of the perfusate fluid equilibrated within 5 s after the media was changed to the hypotonic buffer.

Together, our findings indicate that AQP1 is not a significant determinant of water permeability in the mouse cholangiocyte and is not rate limiting for forskolin-stimulated secretion. Furthermore, AQP1 is not regulated by cAMP in the mouse cholangiocyte. These observations are consistent with studies in other cell types. Nevertheless, we cannot exclude the possibility that other AQP homologs might be upregulated in the AQP1 −/− mouse and compensate for the absence of AQP1 in these experiments and mask a cyclic AMP effect. This possibility also seems unlikely, however, given the absence of such adaptations in other tissues such as the kidney.

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