Channel-mediated water movement across enclosed or perfused mouse intrahepatic bile duct units

AI-YU GONG, ANATOLY I. MASYUK, PATRICK L. SPLINTER, ROBERT C. HUEBERT, PAMELA S. TIETZ, AND NICHOLAS F. LARUSSO

Center for Basic Research in Digestive Diseases, Division of Gastroenterology and Hepatology, Mayo Medical School, Clinic, and Foundation, Rochester, Minnesota 55905

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Gong, Ai-Yu, Anatoly I. Masyuk, Patrick L. Splinter, Robert C. Huebert, Pamela S. Tietz, and Nicholas F. LaRusso. Channel-mediated water movement across enclosed or perfused mouse intrahepatic bile duct units. Am J Physiol Cell Physiol 283: C338–C346, 2002; 10.1152/ajpcell.00162.2001.—We previously reported the development of reproducible techniques for isolating and perfusing intact intrahepatic bile duct units (IBDUs) from rats. Given the advantages of transgenic and knockout mice for exploring ductal bile formation, we report here the adaptation of those techniques to mice and their initial application to the study of water transport across mouse intrahepatic biliary epithelia. IBDUs were isolated from livers of normal mice by microdissection combined with enzymatic digestion. After culture, isolated IBDUs sealed to form intact, polarized compartments, and a microperfusion system employing those isolated IBDUs developed. A quantitative image analysis technique was used to observe a rapid increase of luminal area when sealed IBDUs were exposed to a series of inward osmotic gradients reflecting net water secretion; the choleretic agonists secretin and forskolin also induced water secretion into IBDUs. The increase of IBDU luminal area induced by inward osmotic gradients and choleretic agonists was reversibly inhibited by HgCl2, a water channel inhibitor. With the use of a quantitative epifluorescence technique in perfused mouse IBDUs, a high osmotic water permeability (Pf = 2.5–5.6 × 10−2 cm/s) was found in response to osmotic gradients, further supporting the presence of water channels. These findings suggest that, as in the rat, water transport across intrahepatic biliary epithelia in mice is water channel mediated.

epithelial cells; aquaporins; perfusion

CHOLANGIOCYTES, THE EPITHELIAL cells that line intrahepatic bile ducts, actively modify the volume and composition of bile initially secreted from hepatocytes; indeed, through the vectorial transport of water, electrolytes, and solutes, cholangiocytes may contribute up to 40% of total bile flow (1, 4, 8, 26, 27). Biliary epithelia selectively express a wide array of transport, exchanger, and channel proteins on their apical and basolateral membranes that provide them with an enormous capacity to transfer molecules across the biliary epithelial barrier spontaneously or in response to choleretic hormones (e.g., secretin). For example, biliary epithelia express aquaporins (AQPs), a family of regulated and constitutively expressed channel proteins that serve as selective pores for osmotically driven, passive water movement by epithelia engaged in rapid transport of large volumes of water (3, 17, 25). Using purified cholangiocytes isolated from rat livers, we previously demonstrated that secretin promotes water transport in biliary epithelia by inducing the microtubule-dependent targeting of AQP1-containing vesicles to the plasma membrane via a cAMP second messenger pathway (12, 18). However, a paucity of information on the molecular mechanisms of ductal bile secretion exists in part because of the lack of suitable experimental models.

To overcome some of these problems, we and others have developed several novel experimental approaches in the last decade, including freshly isolated and cultured cholangiocytes, intact, enclosed intrahepatic bile duct units (IBDUs), and IBDUs that can be micropunctured or microperfused (10, 20, 22, 32). Although these models have contributed greatly to our understanding of ductal bile formation, for the most part they are limited to the rat. Given the availability of gene-specific transgenic and knockout mice, adaptation of these techniques to mice could provide new and valuable insights into the mechanisms of ductal bile secretion.

In this report, we describe the isolation and perfusion of IBDUs prepared from mouse liver. Using these novel techniques, we provide evidence that water movement across the mouse biliary ductal barrier is water channel mediated and can be regulated by choleretic agents, suggesting a key role for AQP water channels in ductal bile formation in the mouse.

MATERIALS AND METHODS

Materials. Forskolin, collagenase XI, DNase, hyaluronidase, poly-L-lysine hydrobromide, dibutyryl cAMP (DBcAMP), fluorescein diacetate, and propidium iodide were purchased from Sigma (St. Louis, MO). 2-Mercaptoethanol and secretin were obtained from Peninsula Laboratories
(Belmont, CA). Fluorescein sulfonate [FS; fluorescein-5(6)-sulfonic acid, trisodium salt] was obtained from Molecular Probes (Eugene, OR).

Animals. Balb/c mice (6–8 wk old) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and maintained on a standard diet with free access to water. All the experimental procedures were approved by the Animal Use and Care Committee of the Mayo Foundation.

Solutions. The composition of isotonic (290 mosM) HEPES-buffered saline (HBS) was (in mM) 140 NaCl, 5.4 KCl, 0.8 Na2HPO4, 25 HEPES, 2.5 glucose, 2 CaCl2, and 0.8 MgSO4 (pH 7.4). The composition of isotonic (290 mosM) Krebs-Ringer bicarbonate (KRB) buffer was (in mM) 120 NaCl, 5.9 KCl, 1.2 Na2HPO4, 1 MgSO4, 1.25 CaCl2, 5 glucose, and 25 NaHCO3. Hypotonic and hypertonic KRB solutions were prepared by decreasing the concentration of NaCl (hypotonic) or adding sucrose (hypertonic) in isotonic KRB. The precise osmolality of solutions was determined with a freezing-point osmometer (Osmentte S, Precision System, Natick, MA).

Preparation of IB Dud s from mouse liver. The methods for isolation of IB Dud s from mouse liver was adapted from techniques for IB Dud s in rats previously described by us in rats (22). Briefly, normal Balb/c mice (6–8 wk old) were anesthetized with pentobarbital sodium (50 mg/kg ip). The portal vein was cannulated with a 20-gauge intravenous catheter (Becton Dickinson), and the liver was perfused in situ at 6°C with pentobarbital sodium (50 mg/kg ip). The portal vein was cannulated with a 20-gauge intravenous catheter (Becton Dickinson), and the liver was perfused in situ at 6–8 °C for 10 min with ice-cold saline. Subsequently, 2–3 ml of liquid trypan blue agar were injected into the portal vein. The liver was then removed and immersed in ice-cold, preoxygenated HBS. After removal of the hepatic capsule and surface hepatocytes, intrahepatic bile ducts were dissected under a dissection microscope, with the trypsin blue agar-filled portal vein used as a reference. The dissociated bile duct was digest by shaking at 37°C for 10 min in an enzyme solution (containing RPMI 1640 medium, supplemented with 0.032% collagenase XI, 0.016% DNase, and 0.03% hyaluronidase). Further microdissection was performed at higher magnification to remove residual hepatocytes, components of the residual portal veins and hepatic arteries, and excess connective tissues. The isolated 25- to 100-μm luminal diameter intrahepatic bile ducts were cut into 0.5- to 1.5-mm segments and transferred to eight-well culture chambers that were coated with poly-L-lysine hydrochloride, and 0.03% hyaluronidase. Further microdissection was performed at higher magnification to remove residual hepatocytes, components of the residual portal veins and hepatic arteries, and excess connective tissues. The isolated 25- to 100-μm luminal diameter intrahepatic bile ducts were cut into 0.5- to 1.5-mm segments and transferred to eight-well culture chambers that were coated with poly-L-lysine hydrochloride, and 0.03% hyaluronidase. Further microdissection was performed at higher magnification to remove residual hepatocytes, components of the residual portal veins and hepatic arteries, and excess connective tissues. The isolated 25- to 100-μm luminal diameter intrahepatic bile duct segments were sealed and formed enclosed IB Dud s.

For morphological studies, IB Dud s cultured overnight were directly observed under phase-contrast light microscopy or fixed, processed, and viewed as described elsewhere (22) for ultrastructural observation by transmission electron microscopy. Viability was assessed by trypan blue exclusion and fluorescence staining employing fluorescein diacetate (50 μM, 5 min), which stains viable cells green (11), and propidium iodide (20 μM, 5 min), which stains dead cells red (11).

Immunofluorescence for cytokeratin-19 and AQP1 in isolated IB Dud s. After culture overnight, IB Dud s were fixed with ice-cold absolute methanol for 10 min, permeabilized with Triton X-100, and incubated for 2 h at 37°C with primary antibodies to cytokeratin-19 (CK19; Amersham Life Science) or AQP1 (Alpha Diagnostics International). After they were washed, the slides were incubated for 1 h at 37°C with secondary antibodies, Texas red-conjugated goat anti-mouse IgG (Molecular Probes), or FITC-conjugated goat anti-rabbit IgG (Sigma), respectively. The slides were then mounted with mounting medium (H-1000, Vector Laboratories, Burlingame, CA), and fluorescence localization was detected by confocal laser scanning microscopy (model LSM-510, Carl Zeiss). Staining specificity was confirmed with control slides by incubation with nonimmune serum and without primary antibodies.

Microperfusion of isolated IB Dud s. An epifluorescence microperfusion system was adapted to mouse IB Dud s as described previously by us in rats (20) (Fig. 1). Briefly, viable individual 50- to 100-μm luminal diameter 1.0- to 1.5-mm-long IB Dud s were placed in a specially designed, temperature-controlled chamber mounted on the stage of an inverted fluorescence microscope (Eclipse TE300, Nikon). With a microperfusion apparatus built to specification by the Mayo Division of Engineering (Rochester, MN), one end of an individual IB DU was drawn into the tip of a glass holding pipette by gentle suction, and the lumen of the IB DU was then cannulated with a concentric perfusion pipette that was connected to a fluid-exchange pipette system with a variable-speed syringe pump and a Hamilton 1-ml gastight syringe. With the opposite end open and free, the IB Dud s were then perfused with perfusion solution containing nonabsorptive fluorescence marker at 20–100 nl/min. The external surface of the perfused IB DU was simultaneously bathed in a buffer that was oxygenated with a 95% O2-5% CO2 and changed

Fig. 1. Quantitative epifluorescence microperfusion system to measure water transport across mouse intrahepatic bile duct units (IB Dud s). A: schematic representation of IB DU microperfusion instrumentation. IB DU was perfused through its lumen at constant rates (nl/min) and positioned in the bath of a perfusion chamber on the stage of an inverted epifluorescence microscope. IB DU was immobilized by the holding pipette and perfused with the concentric perfusion pipette. Luminal fluorescence signal was detected from a measuring spot at the distal end of IB DU by a photomultiplier (PMT), and data were recorded and analyzed by computer. B: photomicrograph of a perfused mouse IB DU with 1 mM fluorescein sulfonate (FS), a cell membrane-impermeant fluorophore volume marker. Inset: measuring spot.
continuously with a pump (model P-1, Pharmacia) at an exchange rate of 1.0–1.5 ml/min. With a fluorescence illuminator attached to the epifluorescent microscope, the fluorescence intensity of the fluorescence marker at the distal end of the lumen of the perfused IBDU was detected in a small measuring spot (50–100 μm) by a photomultiplier (model H5784, Hamamatsu Photonics, Bridgewater, NJ), and the signal was digitized by a data-acquisition board using a personal computer (GX1p, Dell, Austin, TX). Data sample rate was set at 5–10 samples/s. Postprocessing of the raw data was done as necessary in Microsoft Excel. The system was checked for potential leakage by using trypan blue dye or FS. Our studies showed no detectable leakage of solutions from lumen to bath and from bath to lumen. At the end of each experiment, the viability of perfused IBDUs was assessed with trypan blue, and experiments in which cells failed to exclude dye were disregarded.

Quantification of water movement across the biliary epithelial layer in enclosed mouse IBDUs assessed by an image analysis technique. Water movement into (secretion) the luminal side of mouse IBDUs in response to osmotic gradients and choleretic agonists was determined from the changes of luminal area by a quantitative image analysis technique, a methodology previously validated by us in the rat (23). Briefly, serial photographs of the same enclosed IBDU before and after treatment were digitized, and the luminal area of IBDUs was measured with an image analysis software program. The results were expressed as percent changes in luminal area from basal values (without treatment). After 15 min of incubation in an isotonic KRB buffer, enclosed and polarized IBDUs were treated by 1) 10, 20, and 30 s of exposure to KRB buffers of various osmolalities (30, 100, 200, and 290 mosM) or 2) 30 min of exposure to 0–10^{-6} M secretin or 50 μM forskolin in an isotonic (290 mosM) KRB solution.

To investigate the role of AQP water channels in water transport across biliary epithelia, water movement across IBDUs induced by osmotic gradients (hypotonic) and choleretic agonists (secretin and forskolin) was observed in the presence or absence of an AQP water channel inhibitor, HgCl2 (16, 17, 29). After 15 min of incubation in the isotonic KRB buffer, the enclosed and polarized IBDUs were treated by 1) 10, 20, and 30 s of exposure to 100 mosM KRB solution or 30 min of exposure to 10^{-7} M secretin or 50 μM forskolin in an isotonic KRB buffer, 2) 10 min of preincubation with 15 μM HgCl2 followed by 10, 20, and 30 s of exposure to 100 mosM KRB solution or 30 min of exposure to 10^{-7} M secretin or 50 μM forskolin in an isotonic KRB buffer, and 3) 10 min of pretreatment with 15 μM HgCl2 followed by 10 min of incubation with 5 mM 2-mercaptoethanol and then 10, 20, and 30 s of exposure to 100 mosM KRB solution or 30 min of exposure to 10^{-7} M secretin or 50 μM forskolin in an isotonic KRB buffer. Changes in luminal area of IBDUs were assessed by the quantitative image analysis technique.

Quantification of water movement across the biliary epithelial layer in mouse IBDUs assessed by microperfusion technique. An epifluorescence microperfusion technique applied for quantification of water movement across mouse IBDU was adapted as previously described and validated by us in rats (20). Briefly, IBDUs were perfused with an isotonic buffer containing 1 mM FS (a cell-impermeant fluorophore) as a volume marker. FS fluorescence intensity was detected at the distal end of the lumen of perfused IBDUs, and the signal was digitized. Net water flow ($J_w$, nl·mm^{-1}·min^{-1}) was calculated from the perfusion rate and initial (C0) and collected (Ct) osmolalities of the perfusate as follows: $J_w = V_o /L(C_t / C_0 - 1)$ (13, 15), where $V_o$ is perfusion rate (nl/min), $L$ is length of bile duct units perfused (mm), $C_t$ is osmolality of collected fluid, and $C_0$ is osmolality of perfusate. $C_t$ was determined from the product of $C_0$ and the ratio of fluorescence intensities in the presence and absence of a transepithelial osmotic gradient. Osmotic water permeability coefficient ($P_f$, cm/s) was calculated from the fluorophore concentration, perfusion flow, lumen and bath osmolalities, and length and surface area of perfused IBDU as follows:

$$P_f = -\frac{V_o(C_t - C_b)}{V_w}A[\{C_0 - C_b\}m(C_tC_b - C_vC_b)] + C_b \cdot \text{ln}[(C_t - C_b)C_0/(C_b - C_0)]$$

where $V_o$ is the initial lumen perfusion rate (cm³/mol), $C_b$ is the osmolality of perfusate, $C_t$ is the collected fluid osmolality as described above, $C_b$ is osmolality of the bath solution, $A$ is the luminal surface area, and $V_w$ is the partial molar volume of water (18 cm³/mol). $A$ was calculated as $A = \pi DL$, where $D$ is the inner diameter and $L$ is the length of IBDUs perfused.

To further assess the role of AQP water channels in water transport across biliary epithelia, IBDUs were perfused under the following conditions: 1) perfusion with an isotonic KRB buffer at 21–81 nl/ml in a hypotonic (70 mosM) bath, 2) perfusion with an isotonic KRB buffer at a constant perfusion rate of 21 nl/ml in 70–530 mosM bath solutions, and 3) 10 min of perfusion with an isotonic KRB buffer at a constant perfusion rate of 21 nl/ml in an isotonic bath containing 100 μM DBcAMP or carrier alone followed by perfusion in a hypotonic (160 mosM) bath. FS fluorescence intensities under those conditions were measured, and $J_w$ and $P_f$ were calculated as described above.

Statistical analysis. Values are means ± SE. The unpaired Student’s t-test was used for statistical analysis.

RESULTS

Characterizations of isolated IBDUs. The isolation method described above yielded a pure preparation of intact, intrahepatic bile duct fragments from mouse liver with luminal diameters of 25–100 μm; hepatocytes and components of the portal vein and hepatic artery were not present. After culture overnight, the ends of IBDUs sealed and formed enclosed compartments. Under the light microscope, the IBDUs appeared intact, with a lumen surrounded by a single layer of columnar or cubical epithelia and a thin outer layer of connective tissue (Fig. 2A). Transmission electron microscopy demonstrated that the isolated IBDUs retained their in situ morphological polarity with abundant microvilli at the apical surface. The basal domain of IBDU epithilia contained multiple folds and interdigitations that lay adjacent to extracellular components. The epithelial cells that surrounded the IBDU lumen present typical ultrastructural characteristics of cholangiocytes in situ, including a basally located, irregularly shaped nucleus, a high nucleus-to-cytoplasm ratio, fewer mitochondria than in hepatocytes, and many vesicular structures especially at the apical cytoplasm. Tight junctions between adjacent cells are also apparent (Fig. 2D). Viability studies by trypan blue and fluorescence staining (fluorescein diacetate to stain live cells and propidium iodide to stain dead cells; Fig. 2B) showed that no dead cells were present in IBDUs. Viability and integrity of these IBDUs were consistently >95% by trypan blue exclusion and were not affected by incubation with HgCl2, forskolin, secretin, and 2-mecaptoethanol at the doses used in the study in KRB buffers of different osmolalities. More-
over, those IBDUs showed strong reaction in the luminal epithelial layer to antibodies against CK19 (Fig. 2E), a specific marker for biliary epithelial cells (22), and AQP1 (Fig. 2F), one of the major AQPs expressed in rat biliary epithelial cells in vivo (23). These histological and morphological observations suggest that the IBDUs isolated from normal mouse liver are viable, intact, and polarized and retain the phenotype of bile ducts in vivo.

Quantitation of secretory response of IBDUs to osmotic gradients and choleretic agonists (secretin and forskolin). Quantitative image analysis (Fig. 3) showed that IBDU luminal area rapidly increased after exposure to hypotonic KRB solutions of various osmolalities (200, 100, and 30 mosM). IBDU luminal area increased in a progressive, time- and osmotic gradient-dependent manner for 30 s (Fig. 3B), and then no further significant expansion was found (data not shown). In contrast, IBDUs in isotonic (290 mosM) buffer showed no change. These data suggest that water moved rapidly from bath to IBDU lumen induced by inward osmotic gradients, indicating that IBDUs isolated from mouse liver are capable of water transport.

To evaluate the secretory response to choleretic agonists, the effects of secretin and forskolin on IBDU luminal area were observed. After incubation of IBDUs with secretin for 30 min at 37°C in the absence of an osmotic gradient (isotonic buffer), the luminal area of the IBDUs increased in a dose-dependent manner, with a significant increase at $10^{-8} - 10^{-6}$ M ($P < 0.01$ vs. 0 M) and a maximal increase of 33% at $10^{-7}$ M (Fig. 4A). Similarly, exposure of IBDUs to 50 μM forskolin for 30 min in isotonic buffer at 37°C resulted in a 21% increase of luminal area ($P < 0.01$ vs. 0 μM; Fig. 4B). These data suggest that fluid secretion into the mouse IBDU lumen was induced by choleretic agonists.

HgCl₂ inhibited osmotic- and choleretic agonist-stimulated water movement across biliary epithelia. To investigate the role of AQP water channels in water transport across biliary epithelia, water movement across mouse IBDUs stimulated by osmotic gradients and choleretic agonist (secretin and forskolin) was studied in the presence or absence of an AQP water channel inhibitor, HgCl₂. Preincubation of IBDUs with HgCl₂ significantly blocked the increase in luminal area due to a hypotonic buffer (Fig. 5A), secretin (Fig.
were independent of lumen perfusion rate in the range

2-mercaptoethanol for 30 min in the absence of ago-

 bromide (KRB) buffer at 0 or 30 s. Changes of luminal area of mouse IBDUs were assessed by quantitative image analysis technique. *P < 0.05 vs. 290 mosM at the same time point.

IBDUs. IBDUs were incubated with isotonic KRB buffer in the

HgCl2. The effect of HgCl2 was reversible: addition of

HgCl2 2 mM forskolin (Fig. 7C), or forskolin (Fig. 5C; P < 0.05 vs. absence of HgCl2). The effect of HgCl2 was reversible: addition of 2-mercaptoethanol blocked the inhibitory effect of HgCl2 on water movement (Fig. 5). Exposure of IBDUs in an isotonic KRB buffer with 15 μM HgCl2 or 5 mM 2-mercaptoethanol for 30 min in the absence of agonists does not result in significant changes in luminal area of IBDUs (data not shown). HgCl2 and 2-mercaptoethanol at the doses used for the study did not significantly affect morphology and viability of IBDUs. These data show that water movement across polarized cholangiocytes into the lumen of enclosed IBDUs in response to osmotic gradients and choleretic agonist is blocked by HgCl2 presumably via inhibition of AQP water channels.

Water transport in perfused IBDUs. To directly quantitate water transport across mouse IBDUs (i.e., Jw and Pf), a quantitative epifluorescence micropuffusion technique was adapted from a technique in rats previously validated by us (20). In the absence of an osmotic gradient, FS fluorescence intensity did not change, reflecting no water movement across IBDU. Perfusion with a hypotonic buffer resulted in a prompt fluorescence decrease in <30 s, and reperfusion with an isotonic KRB buffer promptly returned fluorescence to the original level (Fig. 6A). Calculated Pf values were independent of lumen perfusion rate in the range 21–81 nl/ml, indicating absence of lumen unstirred layers in the perfused IBDU Pf measurements.

When an inward (secretory) osmotic gradient was established across IBDU (per fusate osmolality = 290 mosM, bath osmolality = 70 mosM), the FS fluorescence intensity decreased. Calculated Jw showed a negative value (−24.63 ± 2.26 nl·mm⁻¹·min⁻¹), indicating net water secretion (Fig. 7A). When an outward (absorptive) osmotic gradient was established across IBDU (per fusate osmolality = 290 mosM, bath osmolality = 530 mosM), the FS fluorescence intensity increased. Calculated Jw showed a positive value (13.05 ± 2.02 nl·mm⁻¹·min⁻¹), indicating net water absorption (Fig. 7A). These data suggest that mouse biliary epithelia can transport water in both directions (from lumen to bath and from bath to lumen). When IBDUs were perfused at a constant lumen flow in hypotonic bath buffers of different osmolalities (70–160 mosM), a relatively high Pf (4.2–5.6 × 10⁻² cm/s) was obtained (Fig. 7B). Moreover, the calculated Pf was independent of osmotic gradients. These findings are consistent with the presence of water channels (9, 30). However, when IBDUs were perfused in a hypertonic bath buffer (530 mosM), a lower Pf (2.5 × 10⁻² cm/s) was found (Fig. 7B). Furthermore, in the presence of DBcAMP in the bath solution, a significant increase of Pf was observed compared with the control (Fig. 7C),
suggesting an enhanced water permeability of the perfused IBDUs in the presence of DBcAMP.

**DISCUSSION**

The major findings reported here relate to the development of techniques for the isolation and microperfusion of IBDUs from normal mouse liver and their application to the study of water transport across biliary epithelia. Our data demonstrate that intact, polarized, and functional IBDUs can be reliably and reproducibly isolated from normal mouse liver. Using an image analysis technique employing enclosed IBDUs and an epifluorescent microperfusion system to assess water movement in perfused IBDUs, we found that water traverses the biliary epithelial barrier in mouse IBDUs in response to osmotic gradients and physiologically relevant stimuli by a transcellular pathway and a water channel-mediated mechanism. We believe that these techniques can provide valuable tools to study biliary epithelial physiology, can significantly enhance our understanding of the mechanisms of water, solute, and ion transport in bile ducts, and are suitable for use in transgenic and knockout mice.

Over the last decade, in vitro models for exploring water and ion transport and their regulation in biliary epithelia have been developed, including isolated IBDUs from pig (31) and rat (22) and a microperfusion technique very recently established by us for isolated rat IBDUs (20). However, the paucity of suitable disease models affecting bile ducts in the pig or rat has limited their usefulness. To take advantage of recently developed transgenic and knockout mice models for the study of hepatobiliary physiology and pathophysiology, we now have adapted these experimental models to mice. We have successfully isolated IBDUs from mouse liver by hand-microdissection combined with enzymatic digestion, an approach similar to that employed in the rat described previously by us (22). These isolated IBDUs are medium and large in size, are viable,
and retain their in situ morphology with a lumen surrounded by a single layer of epithelial cells. The presence of apical microvilli and apically oriented vesicles strongly suggests that IBDUs are capable of absorptive and secretory activities. After overnight culture, the ends of these mouse IBDUs seal and form enclosed functional units with a single epithelial layer positive to CK19 and AQP1, allowing assessment of the secretory responses of biliary epithelia by measuring the changes in luminal area. These findings resemble previous morphological descriptions of isolated rat cholangiocytes (10) or rat IBDUs (22) and confirm a single recent study demonstrating isolation of functional enclosed polarized bile duct units from mouse liver (6). However, our work substantially extended that study in two ways: 1) we have successfully applied these functional IBDUs to a microperfusion system for direct study of water transport across an epithelial barrier, and 2) we have demonstrated that water transport across mouse biliary epithelia is mainly water channel mediated.

The microperfusion technique was originally developed in rabbit isolated kidney tubules (5) and then successfully applied to study tubules from other species and other epithelia such as rat colonic crypts (24), rat intralobular pancreatic ducts (35), and guinea pig distal airways (9). Very recently, this approach has been successfully adapted to isolated rat IBDUs by us and demonstrated to be an excellent model to study water, solute (e.g., bile acids), and ion (e.g., HCO$_3^-$) transport (20). Compared with the enclosed IBDU model, the microperfusion model allows the luminal contents (i.e., manipulation of the perfusate) and the basolateral milieu (i.e., manipulation of the bathing buffer) to be independently and simultaneously modified. The procedure for microperfusion of mouse IBDU is quite similar to that for microperfusion of rat IBDU. Because the diameter of mouse IBDUs is smaller than that of rat IBDUs, a smaller pipette was used. With this minor modification, the IBDUs from mouse liver, with inner diameters of 50–100 μm, have been successfully perfused while remaining viable for ~4 h.

It is generally accepted that ductal bile formation accounts for ~40% of total daily bile output, although there is considerable species variation (1, 4, 8, 26, 27). How water, the major component of bile, is transported across biliary epithelia is still unclear. We previously demonstrated that rat cholangiocytes express multiple AQPs and transport water mainly via an AQP-mediated mechanism (19, 23). Here we show that mouse IBDUs strongly express AQP1, one of the major mercury-sensitive AQPs expressed in rat biliary epithelia (23), and transport water in response to osmotic gradients via an HgCl$_2$-sensitive and, presumably, AQP-mediated mechanism. When enclosed mouse IBDUs were exposed to inward (from bath to lumen) osmotic gradients, a rapid time- and osmotic gradient-dependent increase in ductal luminal area was observed and a maximal increase of ~30% of the luminal area over wide osmotic gradients was obtained. Although the magnitude of this luminal expansion may be less than expected and is consistent with mechanical limitation to swelling, probably as a result of the outer layer of connective tissue, which remains in IBDUs, it is very comparable to our previous reports in rat IBDUs (7, 23). Furthermore, HgCl$_2$, a well-known AQP water channel inhibitor that induces conformational changes in AQP proteins by binding to cysteine residues critical to the AQP pore structure and, therefore, blocks water transport, inhibited water transport by ~50% compared with control, strongly suggesting that the AQPs expressed in mouse IBDUs are mercury-sensitive AQPs.

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It is generally accepted that ductal bile formation accounts for ~40% of total daily bile output, although there is considerable species variation (1, 4, 8, 26, 27). How water, the major component of bile, is transported across biliary epithelia is still unclear. We previously demonstrated that rat cholangiocytes express multiple AQPs and transport water mainly via an AQP-mediated mechanism (19, 23). Here we show that mouse IBDUs strongly express AQP1, one of the major mercury-sensitive AQPs expressed in rat biliary epithelia (23), and transport water in response to osmotic gradients via an HgCl$_2$-sensitive and, presumably, AQP-mediated mechanism. When enclosed mouse IBDUs were exposed to inward (from bath to lumen) osmotic gradients, a rapid time- and osmotic gradient-dependent increase in ductal luminal area was observed and a maximal increase of ~30% of the luminal area over wide osmotic gradients was obtained. Although the magnitude of this luminal expansion may be less than expected and is consistent with mechanical limitation to swelling, probably as a result of the outer layer of connective tissue, which remains in IBDUs, it is very comparable to our previous reports in rat IBDUs (7, 23). Furthermore, HgCl$_2$, a well-known AQP water channel inhibitor that induces conformational changes in AQP proteins by binding to cysteine residues critical to the AQP pore structure and, therefore, blocks water transport, inhibited water transport by ~50% compared with control, strongly suggesting that the AQPs expressed in mouse IBDUs are mercury-sensitive AQPs.
transport (2, 14, 21, 29), inhibited osmotic gradient-induced water movement into IBDUs; this inhibitory effect was reversed by the sulphydryl reducing agent (14) 2-mercaptoethanol. These data are consistent with our studies in the rat and provide the first data in mice supporting the concept that, in the basal (without hormone stimulation) state, osmotically induced water movement across intrahepatic biliary epithelia is mainly water channel mediated. Given the large variation in ductal bile secretion among species and the absence of published data on the mechanisms of ductal water transport in mice, our studies provide baseline results necessary to interpret future experimental findings on ductal bile secretion in knockout and transgenic mice.

Channel-mediated water movement across the biliary epithelial barrier was further confirmed using microperfused mouse IBDUs. Net water secretion (negative $J_w$) and absorption (positive $J_w$) across the mouse biliary epithelial barrier in perfused IBDUs were observed in response to inward (from bath to lumen) and outward (from lumen to bath) osmotic gradients, respectively, suggesting that mouse intrahepatic biliary epithelia are capable of bidirectional water transport. Moreover, the relatively high osmotic water permeability coefficients ($P_f = 2.5–5.6 \times 10^{-2} \text{ cm/s}$) observed in perfused mouse IBDUs in the nonstimulated state further support the concept that water channels are involved in basal water transport in mouse biliary epithelia. Although the $P_f$ values we found ($2.5–5.6 \times 10^{-2} \text{ cm/s}$) were slightly lower than those in perfused kidney tubes ($5.5–25 \times 10^{-2} \text{ cm/s}$) (28), they were very comparable to our previous findings in studies using rat bile ducts ($1–5 \times 10^{-2} \text{ cm/s}$) (20) and consistent with the concept that $P_f > 0.01 \text{ cm/s}$ is consistent with the presence of water channels (9, 28, 30, 34). Interestingly, the calculated $P_f$ values for water transport generated by inward osmotic gradients were higher than those generated by outward osmotic gradients. Similar findings were reported in microperfused kidney collecting tubules (15) and rat IBDUs (20), while in tissues such as airways (9) and colon (33), the calculated $P_f$ is independent of the magnitude and direction of the osmotic gradients. It is possible that functional differentiation in various tissues with a polarized epithelial barrier (e.g., principally secretion or absorption) may influence water permeability in different directions via AQP$s$. If this is so, the fact that the intrahepatic biliary tree is principally a secretory organ may somehow account for the higher $P_f$ response to inward osmotic gradients.

Secretin is known to stimulate ductal bile secretion by binding to its receptor on the basolateral domain of cholangiocytes, a ligand-receptor interaction that activates cAMP and increases Cl$^-$ secretion. The increase of ion/solute transportation to the lumen generates an inward osmotic gradient and, thus, drives water secretion to the lumen. Secretin has also been demonstrated to induce AQP1-containing vesicles’ exocytic insertion into cholangiocyte plasma membranes (18). Our data show that secretin and forskolin induced fluid movement into the lumen of enclosed IBDUs. Interestingly, we have also found that fluid secretion into enclosed IBDUs induced by these choleric agonists can be blocked by the water channel inhibitor HgCl$_2$, suggesting that induced water movement is also water channel mediated, presumably driven by an osmotic gradient generated by stimulated active ion transport. However, the interpretation of these data requires caution, because HgCl$_2$ may affect not only water channels but also other channels and transporters involved in active ion transport (5). Moreover, a significant increase of $P_f$ in microperfused IBDUs was found in the presence of DBCAMP in the bath solution, which presumably represents an increase in the insertion of AQP1 molecules into the plasma membrane induced by DBCAMP, a phenomenon previously reported by us in isolated rat cholangiocytes (18), providing further evidence that water channels are involved in stimulated water transport in mouse biliary epithelia.

In conclusion, we have 1) developed physiological models of enclosed and microperfused IBDUs prepared from normal mouse liver and 2) demonstrated using these novel techniques that water movement across the mouse biliary epithelial layer is mainly water channel mediated in the basal state and after exposure to choleric agents. Our data suggest a key role for AQP water channels in regulated secretory processes, leading to ductal bile formation in the mouse. These techniques should facilitate the study of biliary epithelial transport activity using recently developed transgenic and knockout mice.

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