Secretin induces the apical insertion of aquaporin-1 water channels in rat cholangiocytes

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1Center for Basic Research in Digestive Diseases, Departments of Internal Medicine and Biochemistry and Molecular Biology, Mayo Clinic and Foundation, Mayo Medical School, Rochester, Minnesota 55905; and 2Departments of Biological Chemistry and Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Marinelli, Raúl A., Pamela S. Tietz, Linh D. Pham, Lisa Rueckert, Peter Agre, and Nicholas F. LaRusso. Secretin induces the apical insertion of aquaporin-1 water channels in rat cholangiocytes. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G280–G286, 1999—Aquaporin-1 (AQP1) water channels are present in the apical and basolateral plasma membrane domains of bile duct epithelial cells, or cholangiocytes, and mediate the transport of water in these cells. We previously reported that secretin, a hormone known to stimulate ductal bile secretion, increases cholangiocyte osmotic water permeability and stimulates the redistribution of AQP1 from an intracellular vesicular pool to the apical cholangiocyte plasma membrane. Nevertheless, the target plasma membrane domain (i.e., apical or basolateral) for secretin-regulated trafficking of AQP1 in cholangiocytes is unknown, as is the functional significance of this process for the secretion of ductal bile. In this study, we used primarily an in vivo model (i.e., rats with cholangiocyte hyperplasia induced by bile duct ligation) to address these issues. AQP1 was quantitated by immunoblotting in apical and basolateral plasma membranes prepared from cholangiocytes isolated from rats 20 min after intravenous infusion of secretin. Secretin increased bile flow (78%, P < 0.01) as well as the amount of AQP1 in the apical cholangiocyte plasma membrane (127%, P < 0.05). In contrast, the amount of AQP1 in the basolateral cholangiocyte membrane and the specific activity of an apical cholangiocyte marker enzyme (i.e., γ-glutamyltranspeptidase) were unaffected by secretin. Similar observations were made when freshly isolated cholangiocytes were directly exposed to secretin. Immunohistochemistry for AQP1 in liver sections from secretin-treated rats showed intensified staining at the apical region of cholangiocytes. Pretreatment of rats with colchicine (but not with its inactive analog β-lumicolchicine) inhibited both the increases of AQP1 in the cholangiocyte plasma membrane (94%, P < 0.05) and the bile flow induced by secretin (54%, P < 0.05). Our results in vivo indicate that secretin induces the microtubule-dependent insertion of AQP1 exclusively into the secretory pole (i.e., apical membrane domain) of rat cholangiocytes, a process that likely accounts for the ability of secretin to stimulate ductal bile secretion.

Cholangiocytes, as polarized epithelial cells, have their plasma membrane divided by tight junctions into two domains: 1) the apical domain facing the ductal lumen, which functions as the secretory pole for ductal bile formation, and 2) the basolateral domain facing adjoining cells and underlying connective tissue. Although these two cholangiocyte domains contain different proteins (23), the water channel aquaporin-1 (AQP1) is expressed on both (26) and mediates the osmotically driven movement of water (22, 29). AQP1 is also expressed in erythrocytes, some endothelial cells, and in fluid transporting epithelia throughout the body at sites of constitutive (not regulated) water transport (1). Nevertheless, as we recently reported using an in vitro experimental model (i.e., isolated cholangiocytes), secretin increases the osmotic water transport and causes the redistribution of AQP1 from an intracellular location to the cell surface (24). However, the targeting (i.e., basolateral vs. apical domain) of the secretin-induced trafficking of AQP1 in cholangiocytes as well as the functional relevance of this process for the elaboration of ductal bile are unknown. To address these issues we used in vivo and in vitro experimental models in the rat and found that secretin caused the insertion of AQP1 exclusively into the apical (secretory) domain of cholangiocytes, a mechanism that appears to be essential for the secretin-induced ductal bile secretion.

MATERIALS AND METHODS

Animal model for study of ductal bile secretion. Adult male Fisher rats after induction of cholangiocyte hyperplasia by bile duct ligation (BDL) were used in these studies. The BDL rat is a well-established experimental model for the study of secretin-stimulated ductal bile secretion (4). Under pentobarbital anesthesia (5 mg/100 g body wt ip) the rat common bile duct was cannulated with polyethylene PE-50 tubing and heat sealed. After 1 wk of bile duct obstruction, the cannula was externalized and clipped off to allow bile collection, and the intravenous access was established via the femoral vein using a PE-50 cannula. Forty minutes after the biliary obstruction was released, 10 ml of 10−7 M secretin (Peninsula Laboratories, Belmont, CA) or vehicle (PBS, pH 7.4) was administered over 20 min, and bile was collected in two 10-min periods. Rectal temperature was kept at 37°C, and bile volume was determined gravimetrically by assuming a bile density of 1.0 g/ml. Bile osmolality was measured by injecting 10 µl of sample into a Wescor 5100C vapor pressure osmometer (Logan, Utah). The isolation of cholangiocytes was begun immediately after secretin (or vehicle) administration as will be described.

SECRETIN IS A GASTROINTESTINAL hormone that stimulates ductal bile secretion via specific receptors on bile duct epithelial cells, or cholangiocytes (9, 23, 28).

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In separate experiments BDL rats were injected intravenously with 0.5 µmol/100 g body wt colchicine or β-lumicolchicine in PBS (14) 2 h before administration of secretin.

Preparation of cholangiocytes. Cholangiocytes were isolated from livers of BDL rats by enzymatic digestion and mechanical disruption and purified by sequential counterflow elutriation with a J2-21 centrifuge equipped with a J E-6B rotor (Beckman Instruments, Fullerton, CA). The final cell suspension contained ~75% pure cholangiocytes [based on staining with the cholangiocyte marker γ-glutamyltranspeptidase (γ-GT)], and the viability was >90% as assessed by trypan blue exclusion. With the technique employed, three different populations of cholangiocytes (i.e., small, medium, and large) can be separated (5, 6). Small cholangiocytes do not express either secretin receptor or key transporters involved in bile secretion (5, 6), and for this reason they were excluded from our final cell preparation. Most of the contaminating cells were sinusoidal endothelial cells (19), which do not express secretin receptor (7) or AQ1 (29) and therefore did not interfere in the studies.

In one set of experiments cholangiocytes from BDL rats receiving no treatment were exposed to secretin in vitro. Cells were incubated in Krebs-Ringer-HEPES buffer, pH 7.4, in the presence of 0 or 10^(-7) M secretin for 15 min at 37°C, washed, and frozen until the preparation of plasma membrane fractions. As described elsewhere (19, 31) morphological and biochemical cell polarity was maintain under these experimental conditions.

Isolation of apical and basolateral cholangiocyte plasma membrane domains. Apical and basolateral plasma membranes were prepared from isolated cholangiocytes as previously described by us (31). Briefly, cholangiocytes were washed and sonicated in 0.3 M sucrose containing 0.01% soybean trypsin inhibitor, 0.1 mM phenylmethylsulfonyl fluoride, and 0.1 mM leupeptin (Sigma, St. Louis, MO). The plasma membrane fraction (designated total cholangiocyte plasma membrane) was obtained by centrifugation at 200,000 × g for 60 min on a discontinuous 1.3 M sucrose gradient (24). An aliquot of these membranes was saved, and the remainder was further subfractionated by high-speed centrifugation through discontinuous sucrose gradients to obtain two subfractions enriched in either apical or basolateral cholangiocyte plasma membrane domains (31). Cholangiocytes exposed (in vitro or in vivo) to secretin and corresponding controls were processed in parallel, and the membrane enrichments did not differ between groups. The apical and basolateral plasma membrane preparations were essentially devoid of intracellular membranes (31). Nevertheless, according to the distribution of marker enzymes (31), a minor degree of contamination of the basolateral by the apical membrane (about 11%) was expected, which did not affect the interpretation of the results.

Protein concentration was determined by the fluorescamine method using BSA as standard (32). γ-GT activity was assayed as described (27), and the data were expressed as specific activity (µmol p-nitroaniline formed·min⁻¹·mg protein⁻¹).

Immunochemistry for AQ1 in liver sections. Rats (normal or 1 wk BDL) were handled and treated with secretin as previously mentioned, and then the livers were perfused via the portal vein with PBS to eliminate the blood, removed, sliced, and fixed by immersion with 4% paraformaldehyde. Paraffin sections (4 µm) were placed in 10 mM citrate buffer (pH 6.0) and microwaved twice for 2 min to improve staining by antigen unmasking. After the sections underwent washing and quenching of endogenous peroxidase, they were blocked and incubated with rabbit affinity-purified antibodies against AQ1 (2 µg/ml; Alpha Diagnostics International, San Antonio, TX) for 2 h at room temperature. The following steps were carried out by an immunoperoxidase procedure (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA). The peroxidase was visualized by reaction with diaminobenzidine and hydrogen peroxidase (Sigma) and counterstain with hematoxylin. Controls using nonimmune rabbit IgG (Vector Laboratories) or omission of primary or secondary antibody revealed no labeling.

Immunoblots for AQ1. Solubilized plasma membrane fractions from cholangiocytes as well as from erythrocytes (positive controls) and hepatocytes (negative controls) were subjected to SDS-PAGE and transferred to nitrocellulose sheets. After being blocked, blots were incubated overnight at 4°C with AQ1 antisera (17) diluted 1:500. The blots were then washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (Tago, Burlingame, CA), and bands were detected by the enhanced chemiluminescence detection system (ECL, Amersham). Autoradiographs obtained by exposing nitrocellulose sheets to Kodak XAR film were scanned and quantitated using appropriate software program (Molecular Analyst, Bio-Rad).

![Diagram](http://apjg.physiology.org/download)
RESULTS

Apical vs. basolateral insertion of AQP1 in secretin-treated cholangiocytes. To study the membrane domain involved in the secretin-induced insertion of AQP1 in cholangiocytes, we isolated fractions enriched in apical and basolateral plasma membranes from secretin-exposed isolated cholangiocytes. As we previously reported (24), the exposure of cells to secretin caused a significant increase of AQP1 in total cholangiocyte plasma membranes (189%, $P < 0.05$; Fig. 1), whereas the specific activity of the apical cholangiocyte markers $\gamma$-GT and alkaline phosphatase was unaffected (not shown). Further subfractionation of these membranes showed that the increase of AQP1 was almost exclusively associated with the apical membrane domain (120%, $P < 0.05$). In contrast, the amount of AQP1 in basolateral membranes was not significantly altered by secretin.

Effect of secretin on bile flow and cholangiocyte plasma membrane AQP1 in BDL rats. The above results were consistent with the concept that the insertion of AQP1 into the apical (secretory) domain of cholangiocytes is involved in the secretin-regulated mechanism of ductal bile secretion. To begin to investigate this issue, we studied the in vivo effect of secretin. The hormone was infused into BDL rats (a model for the study of ductal bile secretion), and bile flow and content of AQP1 in cholangiocyte plasma membranes.
were assessed. As shown in Fig. 2, secretin increased bile flow (78%, \( P < 0.01 \)) and the amount of AQP1 in the cholangiocyte plasma membrane (121%, \( P < 0.05 \)) without affecting the specific activity of \( \gamma \)-GT.

Apical vs. basolateral insertion of AQP1 in secretin-treated BDL rats. In agreement with the results in hormone-treated isolated cholangiocytes (see above), after infusion of secretin in vivo, AQP1 was predomi-

**Fig. 4.** Immunohistochemistry for AQP1 in 4-µm paraffin liver sections from vehicle-treated (A and C) or secretin-treated (B and D) rats. C and D are negative immunohistochemical controls (see MATERIALS AND METHODS). A: bile ducts from vehicle-treated rats showed diffuse staining throughout cytoplasm of cholangiocytes. Some cells also showed visible apical staining. B: bile ducts from rats treated with secretin exhibited intensified staining at apical edge of cholangiocytes (small arrows). Peribiliary vascular endothelia (large arrows) showed intense staining; no evident change was observed after secretin treatment. Sinusoidal endothelia were unstained. Magnification, \( \times 1,000 \).

**Fig. 5.** Effect of colchicine on secretin-induced bile flow and AQP1 increase in cholangiocyte plasma membranes. A: 1-wk BDL rats were intravenously injected with colchicine or lumicolchicine (5 µmol/kg body wt). After 2 h, secretin (1 ml, \( 10^{-7} \) M) or vehicle was administered intravenously for 20 min, and bile was simultaneously collected. Then cholangiocytes were isolated and plasma membranes were prepared and immunoblotted for AQP1. Amount of AQP1 was quantitated by densitometry. Data are means ± SE for 4–6 animals in each experimental group. *\( P < 0.05 \) and **\( P < 0.01 \) compared with corresponding (–) secretin values (Student’s \( t \)-test). B: immunoblot for AQP1 of cholangiocyte plasma membrane (10 µg total protein/lane) from 5 lumicolchicine- and 6 colchicine-treated rats all treated with secretin. Corresponding densitometric values are shown in A.
To achieve ductal bile secretion, we utilized the microtubule blocker colchicine, which we had previously shown inhibits secretin-induced insertion of AQP1 into the basolateral domain of cholangiocytes (24). As illustrated in Fig. 3, pretreatment of rats with colchicine abolished the increase of AQP1 in the apical plasma membrane and decreased the secretin-induced bile flow by 25 ml·min⁻¹·kg body wt⁻¹ (~54%). In contrast, under these conditions, bile osmolality increased by 6 mosmol/kg. The inactive analog β-lumicolchicine did not affect the action of secretin compared with rats receiving no treatment (data not shown).

**DISCUSSION**

The major findings reported here relate to the molecular mechanisms of water transport in the liver. Our results using an established in vivo rodent model for studying ductal bile secretion indicate that 1) the ductal choleresis induced by secretin is associated with an increase in the amount of AQP1 protein in the apical plasma membrane of cholangiocytes and 2) the disruption of cholangiocyte microtubules by colchicine inhibits both the secretin-induced choleretic cholangiocyte membrane. These observations, confirmed in vitro using an isolated cholangiocyte experimental model, provide a plausible molecular explanation for secretin-induced ductal bile secretion.

Bile is initially secreted at the canalicular membrane of hepatocytes and then modified by cholangiocytes during passage through bile ducts. Although this ductal bile secretion results from the osmotically driven transport of water across biliary epithelia, the regulatory and mechanistic details of this process are unknown (9, 22, 23, 28). However, in a recent study using isolated cholangiocytes, we showed that secretin (which stimulates ductal bile secretion) induces the insertion of functional AQP1 water channels into cholangiocyte plasma membranes. This finding was the first reported evidence for hormone-regulated membrane insertion of AQP1 in any epithelial cell and led us to propose that this process played an important role in secretin-induced ductal bile secretion. In the present study, we have extended those observations using primarily an in vivo rat model. The biochemical and morphological results reported here indicate that secretin causes the insertion of AQP1 into the apical (but not the basolateral) domain of cholangiocytes and that this process likely accounts for the ability of secretin to stimulate ductal bile secretion.

Although secretin is well known to stimulate ductal bile secretion by interacting with specific cAMP-coupled receptors on the basolateral domain of cholangiocytes (9, 23, 28), little as well as controversial information is available on the stimulus-secretion coupling. For example, it has been proposed that secretin induces exocytosis of vesicles containing bafilomycin A₁-inhibitable H⁺-ATPase into the basolateral domain of pig cholangiocytes. The H⁺ secretion would increase intracellular pH and, in turn, the secretion of HCO₃⁻ and water into bile. Nevertheless, rat and human cholangiocytes lack bafilomycin A₁-sensitive H⁺-ATPase activity (13, 30), indicating that the mechanisms involved in ductal bile secretion are not the same in all species. Based on recently published observations (9, 22, 23, 28) and those reported here, it can be proposed that the secretin-induced increase in cAMP triggers the exocytic insertion of AQP1 and the activation of apical cystic fibrosis transmembrane regulator Cl⁻ channels. The resulting efflux of Cl⁻ would cause the exit of HCO₃⁻ (via activation of apical Cl⁻/HCO₃⁻ exchanger) and the paracellular transport of sodium ions. The vectorial transport of these electrolytes would in turn drive the osmotic transcellular movement of water via AQP1 into the biliary space (Fig. 6). As we discussed elsewhere (22), no substantial fraction of the transepithelial water flow seems to be paracellular. Importantly, the identification of a growing number of new
apical transport proteins (3, 20, 21) raises the possibility that the movement of other osmotically active solutes are involved in hormone-regulated ductal bile formation; for example, it has been reported that the Na\(^+\)-coupled transport of bile salts may be regulated by secretin (2). Thus we would propose that on secretin stimulation AQ1 is targeted toward and inserted into the apical (secretory) domain of cholangiocytes to optimize efficient coupling between biliary transport of solutes and water.

Cholangiocytes are cells with an unusually high cholesterol content in their plasma membrane, i.e., cholesterol-to-phospholipid ratio $\geq$ 1 (31, 35). Although a physiological role for the high amount of cholesterol in the cholangiocyte plasma membrane is unknown, it has been well documented that cholesterol reduces membrane water permeability (16). These facts suggest that for cholangiocytes to maintain osmotic equilibrium under basal conditions they are required to express AQPs in their plasma membranes.

Our data indicate that under basal (nonsecretin-stimulated) conditions, cholangiocytes possess a greater density of AQ1 in the apical than the basolateral domain. If AQ1 were the only water channel protein present in cholangiocytes, there would be an imbalance in the membrane water permeability of these two domains. This apparent disparity in the polarized distribution of AQ1 strongly suggests that other members of the AQ family of proteins are also expressed in the basolateral domain of cholangiocytes. In this regard, we recently found that cholangiocytes contain mRNA for aquaporin-3 and aquaporin-4 (Marinelli, Pham, and LaRusso, unpublished data), two water channels that have been described to be present exclusively in the basolateral domain of the epithelial cells in which they are expressed (22). Nevertheless, the fact that AQ1 increases in the apical but not in the basolateral domain of cholangiocytes on secretin stimulation suggests that the apical domain is the limiting barrier for net transepithelial water transport in cholangiocytes.

Our finding that secretin only modulates the apical insertion of AQ1 suggests that AQ1 is constitutively inserted into the basolateral domain and/or that signaling pathways other than the CAMP cascade are involved. Evidence for the CAMP-mediated stimulation of apical targeting of vesicles containing other transporters has been shown in a number of epithelia, including colonocytes, hepatocytes, and kidney tubule cells (8, 10, 11, 25, 34). In kidney collecting tubule cells, for example, it has been well documented that vasopressin (via CAMP) induces the exocytic insertion of aquaporin-2 (an AQ1-related water channel) exclusively into the apical domain of these cells (25, 34).

We found that the in vivo administration of the microtubule depolymerizing drug colchicine inhibited the increase of AQ1 in cholangiocyte plasma membrane induced by secretin. These observations are in agreement with those previously reported by us using an in vitro model (i.e., isolated cholangiocytes), as well as with studies in kidney proximal tubule cells indicating that microtubules are required for insertion of AQ2 into the plasma membrane (18, 24). The finding that colchicine also inhibited the secretin-induced bile flow is in conflict with one previous report (15) describing the lack of inhibitory action of colchicine in a different model for the study of ductal bile secretion (i.e., $\alpha$-naphthyl-isothiocyanate-fed rats). The reason for this discrepancy is unclear, although differences in the experimental models as well as the doses of colchicine may be involved. On the other hand, our data do agree with those of Cho and Boyer (12) who provided direct evidence for the colchicine-induced inhibition of secretin-stimulated ductal bile flow using isolated rat bile duct units.

We believed that the inability of secretin to increase AQ1 in cholangiocyte plasma membranes in microtubule-disrupted rats prevented the ductular cholestasis. A possible colchicine-induced inhibition in the biliary transport of osmotically active solutes, as described for HCO\(_3\) in pig (33), may significantly contribute to the failure of secretin to stimulate bile flow. Nevertheless, the associated increase in bile osmolality is making this possibility uncertain.

In conclusion, the data presented in this study, together with our previous observations (24), support the concept that on secretin stimulation vesicles containing AQ1 are directed (via microtubules) to the secretory pole (i.e., apical membrane domain) of cholangiocytes to facilitate the osmotic movement of water and in turn, the elaboration of ductal bile. These results provide the first cohesive and comprehensive molecular explanation for hormone-induced ductal bile secretion (Fig. 6).

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