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Secretin Activation of the Apical Na^+-Dependent Bile Acid Transporter Is Associated With Cholehepatic Shunting in Rats

Gianfranco Alpini,[1,3,4] Shannon Glaser,[2] Leonardo Baiocchi,[3] Heather Francis,[2] Xuefeng Xia,[5] and Gene LeSage[1,5]

The role of the cholangiocyte apical Na^+-dependent bile acid transporter (ASBT) in bile formation is unknown. Bile acid absorption by bile ducts results in cholehepatic shunting, a pathway that amplifies the canalicular osmotic effects of bile acids. We tested in isolated cholangiocytes if secretin enhances ASBT translocation to the apical membrane from latent preexisting intracellular stores. *In vivo*, in bile duct–ligated rats, we tested if increased ASBT activity (induced by secretin pretreatment) results in cholehepatic shunting of bile acids. We determined the increment in taurocholate-dependent bile flow and biliary lipid secretion and taurocholate (TC) biliary transit time during high ASBT activity. Secretin stimulated colchicine-sensitive ASBT translocation to the cholangiocyte plasma membrane and ^{3}H-TC uptake in purified cholangiocytes. Consistent with increased ASBT promoting cholehepatic shunting, with secretin pretreatment, we found TC induced greater-than-expected biliary lipid secretion and bile flow and there was a prolongation of the TC biliary transit time. Colchicine ablated secretin pretreatment-dependent bile acid–induced choleresis, increased biliary lipid secretion, and the prolongation of the TC biliary transit. In conclusion, secretin stimulates cholehepatic shunting of conjugated bile acids and is associated with increased cholangiocyte apical membrane ASBT. Bile acid transport by cholangiocyte ASBT can contribute to hepatobiliary secretion *in vivo*. (HEPATOLOGY 2005;41:1037-1045.)

Bile formation is a series of events. The first stage, started by canalicular secretion of bile acids and electrolytes is followed by a second stage where canalicular bile is modified as it passes down the biliary tree.[1] Secretin stimulates cholangiocyte secretion by increasing adenosine 3′,5′-monophosphate; 1,4,5-triphosphate (cAMP) synthesis, activation of Cl^- channels, and increased Cl^-/HCO_3^- exchanger activity.[2]

The primary focus on bile acid transport in the liver has been on sinusoidal uptake and canalicular secretion.[1] The second stage of bile formation (ductal phase) is not thought to involve bile acids under physiological conditions.[3] Yet there is much experimental evidence that suggests bile acids significantly interact with biliary epithelium. The exchange of bile acids between cholangiocytes and hepatocytes by the "cholehepatic shunt pathway"[4] may explain the bicarbonate-rich hypercholeresis following administration of unconjugated bile acids. The unconjugated biliary bile acids absorbed by cholangiocytes circulate back to hepatocytes by the peribiliary plexus and subsequently are excreted for the second time across the canalicular membrane. Each time a molecule passes through the cholehepatic shunt, it produces a bicarbonate ion as it is absorbed in protonated form.[4] Multiple passes of bile acids through the canalicular membrane leads to an increased bile acid biliary transit time, increased choleretic efficiency of bile acids (*e.g.*, increased bile flow for each bile acid molecule secreted), and increased biliary lipid secretion.[5]

We have characterized the transport activity of the apical Na^+-dependent bile acid transporter (ASBT) originally described in the ileum in isolated rat cholangio-

Abbreviations: cAMP, adenosine 3′,5′-monophosphate; 1,4,5-triphosphate; ASBT, apical Na^+-dependent bile acid transporter; ^{3}H-TC, ^{3}H-taurocholate; BDL, bile duct ligation; K_m, Michaelis constant; V_{max}, maximal velocity.

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cytes.[6] In addition, we have shown with immunofluorescence studies that ASBT is present in the apical membrane of large cholangiocytes and large (greater than 15 μm in diameter) isolated intrahepatic bile duct units.[6] In isolated bile duct fragments, cholangiocytes absorb bile acids.[7] Previous studies in bile duct fragments do not address the fate of bile acids after absorption (*i.e.*, entering cholehepatic shunt) and likely have limited predictability for the potential of bile acids absorption *in vivo*.

Membrane-based transporters are regulated by the exchange of transporters between the plasma membrane and internal cytoplasmic vesicles.[8] Shuttling from internal to external sites occurs by membrane exocytosis and movement from external to internal sites by membrane endocytosis.[8] The cellular distribution of transporters is controlled by circulating hormones (*e.g.*, aquaporin or water-channel distribution in bladder epithelial cells).[9] Aquaporin channels and cystic fibrosis transmembrane conductor regulation (CFTR) insertion onto the apical membrane of cholangiocytes are associated with increases of water and Cl^- transport in cholangiocytes.[10,11]

In these studies, we experimentally tested whether ASBT translocates to the cholangiocyte apical membrane and if increased ASBT activity (due to translocation) results in enhanced cholehepatic shunting of conjugated bile acids. Immunofluorescence microscopy and subcellular fractionation studies show that ASBT is intracellular. Following secretin stimulation, immunofluorescence microscopy shows that ASBT redistributes to the plasma membrane of cholangiocytes and ^{3}H-taurocholate (^{3}H-TC) uptake in purified cholangiocytes increases. Consistent with ASBT promoting cholehepatic shunting of conjugated bile acids, we found that TC induced greater-than-expected biliary lipid secretion and bile flow and that there was a prolongation of TC biliary transit time when cholangiocyte ASBT was increased. Translocation of ASBT is required for the bile acid–induced hypercholeresis and prolongation of biliary bile acid transit time, since both were blocked by pretreatment with the microtubule inhibitor colchicine. Together these findings show that secretin may acutely stimulate cholehepatic shunting of conjugated bile acids by ASBT translocation to the cholangiocyte apical membrane. The studies demonstrate the cooperation between hepatocytes and cholangiocytes in hepatobiliary transport of bile acids and biliary lipids.

Materials and Methods

Materials

Reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated. Porcine secretin was purchased from Peninsula (Belmont, CA).

Animal Model

Male Fisher 344 rats (250-300 g) were obtained from Charles River (Wilmington, MA), housed in a temperature-monitored environment (20-22°C) with a 12-hour light-dark cycle, and fed *ad libitum* with standard rat chow. To study bile ductal secretion *in vivo*, we employed the 1-week bile duct ligation rat model, which we have previously established as a model of cholangiocyte hyperplasia and secretin-stimulated ductal bile secretion.[12] The common bile duct was cannulated with PE-50 tubing and the end sealed with heat. After 1 week of obstruction, the end of the tubing was exteriorized and the tip cut off to allow for bile collections. Saline was administered via femoral vein. After setting up steady-state basal bile flow, rats received an infusion of taurocholate (5 μmol/min/kg body weight) for 30 minutes followed by normal saline for 30 minutes. Subsequently, secretin (100 nmol/L, Peninsula Laboratories, Belmont, CA) was infused for 30 minutes, then normal saline for 30 minutes followed by a second taurocholate infusion for 30 minutes. Rats were injected intraperitoneally with colchicine 1 mg/kg or normal saline 2 hours before the start of bile collection.

Biliary Transit Time. Biliary transit time was measured before and after secretin treatment using bile duct ligated (BDL) bile fistula rats. A single bolus dose of ^{3}H-TC (2 μmol) was administered into the portal vein and bile was collected in 2-minute aliquots for 60 minutes. Secretin was administered intravenously for 30 minutes followed by normal saline for 30 minutes. Then a second bolus dose of ^{3}H-TC was administered and bile was collected in 2-minute aliquots for 60 minutes. ^{3}H-TC secretion in bile was determined by scintillation counting, and biliary transit time was calculated from the halftime of biliary appearance of the administered dose of ^{3}H-TC. More than 95% of the administered ^{3}H-TC was recovered in bile.

Purification and Phenotypic Characterization of Cholangiocytes From Rat Liver. Cholangiocytes from BDL rats were isolated as previously described.[13,14] The purity of cholangiocytes was assessed by histochemistry for γ-glutamyl transferase, a specific marker of cholangiocytes subpopulation of liver cells.[13,14] Cell number and viability were assessed by Trypan blue exclusion.

Taurocholate Uptake in Isolated Cholangiocytes. Taurocholate uptake was determined in isolated cholangiocytes, and estimation of the Michaelis constant (K_m) and maximal velocity (V_{max}) were determined as we previously described.[6] Na^+-independent uptake was calculated by subtracting the background ^{3}H-TC uptake measured at zero time incubation from uptake in the presence of a Na^+-free (equal molar choline substitution) incubation buffer. Na^+-dependent uptake was calculated

by subtracting the uptake in the presence of choline from the total uptake in the presence of Na^+. Estimates of K_m and V_{max} were determined using a weighted least-squares fit of the sigmoidal curve according to the method of Vaughn et al.[15] Statistical significance was analyzed using Student unpaired *t* test.

Since ASBT is the only known bile acid transporter in cholangiocytes that mediates Na^+-dependent bile acid uptake, this determination was assumed to be reflective of ASBT activity in cholangiocytes.

ASBT Gene Expression. We measured gene expression for ASBT employing lysate RNase protection assay (Direct Product Kit, Ambion Inc., Austin, TX) and a complementary DNA ASBT probe (gift from B.L. Shneider, Mount Sinai School of Medicine, New York, NY) as previously described by us.[6]

ASBT Content in Cholangiocyte Apical and Microsomal Membranes. Cholangiocyte plasma and microsomal membranes were isolated as previously described.[16] Cholangiocytes were sonicated in 0.3 mol/L sucrose containing 0.01% soybean trypsin inhibitor, 0.1 mmol/L phenylmethylsulfonyl fluoride, and 0.1 mmol/L leupeptin. The plasma membrane fraction was obtained by centrifugation at 200,000*g* for 60 minutes on a discontinuous 1.3 mol/L sucrose gradient. The gradient was diluted to 0.3 mol/L sucrose, centrifuged at 17,000*g* for 30 minutes and then the supernatant was centrifuged at 200,000*g* for 60 minutes to yield the microsomal membrane fraction. The purity of the plasma membrane purified from both control and BDL rats was assessed by the enzyme content for alkaline phosphatase by a commercially available enzyme kit (Sigma). ASBT content in cholangiocyte microsomal and plasma membrane fractions was determined by immunoblotting as we previously described.[17]

Immunofluorescence Studies of ASBT Distribution in Cholangiocytes. Normal control or BDL rats received an infusion of normal saline or secretin for 30 minutes, then they were sacrificed and their livers were sectioned using a Zeiss cryostat. The sections were processed for immunofluorescence as we previously described.[6] Apical membrane and intracellular fluorescence were measured by a cooled change-coupled device camera (Roper Scientific, Tucson, AZ) and quantified by computed morphometry using software from ISee (Inovision Corp., Durham, NC). The apical membrane of bile ducts was visually identified and outlined by hand as a region of interest. Intracellular ASBT was measured in a region of interest defined by the area 10 μm outside the apical membrane. The apical region of interest was dilated to a width of 90% of the point spread function of the microscope objective so to include the great majority of fluorescence associated with the apical membrane. The relative amounts of fluorescence in the apical and intracellular region of interest were determined with the ISee software. Total fluorescence is the sum of the apical and intracellular.

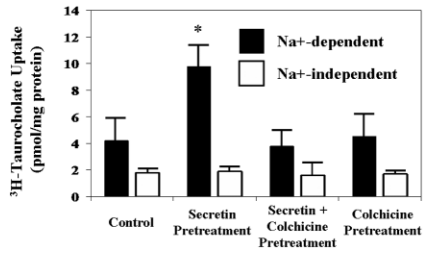

Fig. 1. The effect of secretin and colchicine on ^{3}H-TC uptake in isolated cholangiocytes. Cholangiocytes isolated from BDL rats were incubated in the absence (control) and the presence of secretin (100 nmol/L for 15 minutes) and colchicine (50 μmol/L for 1 hour at 37°C). Subsequently, Na^+-dependent and -independent uptake was determined in isolated cholangiocytes. Data are mean ± SE in 6 to 9 independent experiments. *$P < .05$ compared to control. ^{3}H-TC, ^{3}H-taurocholate; BDL, bile duct ligation.

Results

^{3}H-TC Uptake in Isolated Cholangiocytes. Na^+-dependent TC uptake in isolated cholangiocytes (4.6 + 0.8 pmol/mg protein) is similar to our previous report.[6] Pretreatment of cholangiocytes with secretin for 30 minutes before the uptake studies increases Na^+-dependent ^{3}H-TC uptake by 2-fold (Fig. 1) but did not alter Na^+-independent ^{3}H-TC in isolated cholangiocytes. There is a significant increase in V_{max} for Na-dependent ^{3}H-TC uptake in secretin-treated cholangiocytes (295 + 32 pmol/min/mg protein vs. 165 + 27 pmol/min/mg protein in controls, $P < .01$). However, the K_m for Na^+-dependent ^{3}H-TC uptake is not changed by secretin (52.1 + 7 and 56.3 + 9 μmol in secretin-treated and control cholangiocytes, respectively). The kinetics for Na^+-dependent ^{3}H-TC uptake in control and secretin-treated cells is shown in Fig. 2. The increase in V_{max} but not K_m is consistent with an increased number of transporters present in secretin-treated cholangiocytes. Pretreatment of cholangiocytes with colchicine ablates the secretin-stimulated Na^+-dependent ^{3}H-TC uptake (Fig. 1), but colchicine alone does not alter Na^+-dependent ^{3}H-TC uptake. The data show that secretin increased Na^+-dependent TC uptake in cholangiocytes is microtubule dependent and thus likely involves ASBT translocation.

Secretin Increases Plasma Membrane ASBT. To test whether the increase in ^{3}H-TC uptake in secretin treated cholangiocytes is due to ASBT translocation, we isolated plasma and microsomal membranes from secre-

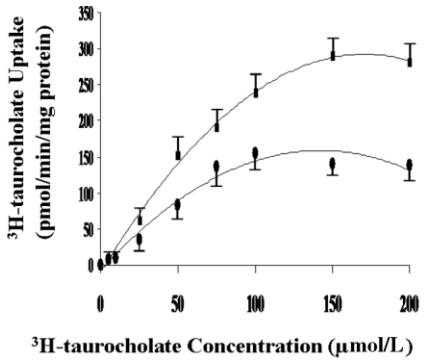

Fig. 2. Kinetics of Na^+-dependent ^{3}H-TC uptake. Na^+-dependent uptake in control cholangiocytes (**circles**) and cholangiocytes pretreated with secretin (**squares**) was determined as described in Fig. 1. ^{3}H-TC, ^{3}H-taurocholate.

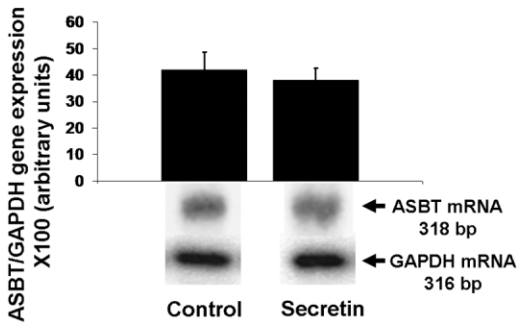

Fig. 4. ASBT genetic expression. Isolated rat cholangiocytes from BDL rats were treated with secretin or vehicle (control) as described in Fig. 1, and ASBT and GAPDH gene expression was determined by lysate ribonuclease protection assay. ASBT, apical Na^+-dependent bile acid transporter; BDL, bile duct ligation; mRNA, messenger RNA.

tin-treated cholangiocytes isolated from normal and BDL rats. ASBT in cholangiocyte plasma membrane increases and microsomal membrane ASBT decreases in secretin-treated cholangiocytes compared with controls (Fig. 3). Protein expression (data not shown) and gene expression for ASBT (Fig. 4) remain unchanged following secretin treatment, showing that secretin selectively increases plasma membrane ASBT from a preexisting pool. Consistent with a microtubule-dependent redistribution of ASBT, secretin does not increase plasma membrane ASBT in cholangiocytes pretreated with colchicine (Fig. 3). Purity of the isolated plasma membrane (assessed by alkaline phosphatase specific activity) was similar in control, secretin, secretin plus colchicine, and colchicine-treated cells from normal rats (13 ± 2.2, 15 ± 1.9, 11 ± 2.5 and 13 ± 2.3 μmol/mg/hr, respectively) and BDL rats (16 ± 2.7, 15 ± 2.8, 13 ± 2.1 and 17 ± 2.0 μmol/mg/hr, respectively). Employing immunofluorescence studies of ASBT distribution in liver sections from control and BDL rats infused with secretin and/or colchicine, we found that secretin increases apical membrane ASBT (Fig. 5A-B). Quantification of intracellular fluorescence and apical membrane fluorescence (Fig. 5C-D) shows a significant increase in apical fluorescence in secretin-treated normal and BDL rats but not in secretin plus colchicine treated animals.

Secretin Pretreatment Increases TC-Dependent Bile Flow and Biliary Lipid Secretion. We measured bile acid-dependent bile flow and biliary lipid secretion during basal ASBT activity and during increased ASBT activity (due to pretreatment with secretin 10^{-7} mol/L for 30 minutes) in BDL rats. The TC-stimulated choleresis is 3-fold greater with secretin pretreatment compared with the control period (47.1 vs. 15.6 μL bile/μmol bile acid, $P < .001$, Fig. 6). The increased TC-induced bile flow following secretin pretreatment is not because of the direct choleretic effect of secretin, since the TC infusion was started 30 minutes after completion of secretin pretreatment when basal bile flow was reestablished. Total bile acid output was similar during the control period and during the period after secretin pretreatment (5.8 ± 0.8 vs. 6.1 ± 0.5 μmol/min/kg body weight, respectively).

TC-induced biliary cholesterol and phospholipid secretion increase with secretin pretreatment (Fig. 6). The choleric efficiency of TC on biliary phospholipid increased from 0.15 ± 0.01 μmol phospholipid/μmol bile acid during the control period to 0.28 ± 0.01 μmol phospholipid/μmol bile acid ($P < .05$) with secretin pretreatment. The choleric efficiency of TC on biliary cholesterol was 0.031 ± 0.008 μmol cholesterol/μmol bile acid during the control period and 0.049 ± 0.007 μmol cholesterol/μmol bile acid ($P < .05$) with secretin pretreatment.

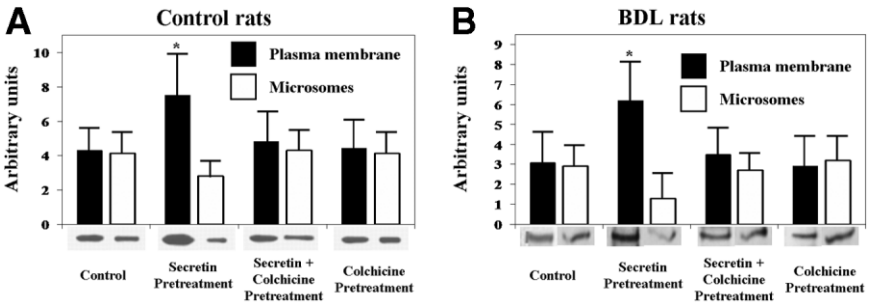

Fig. 3. Membrane distribution of ASBT. Plasma and microsomal membranes were isolated from control, secretin- and colchicine-treated cholangiocytes from (A) normal rats and from (B) BDL rats. ASBT content was determined by Western immunoblots (20 μg protein per lane). Data are mean ± SE in 3 to 5 independent experiments. *$P < .05$ compared with control. ASBT, apical Na^+-dependent bile acid transporter; BDL, bile duct ligation.

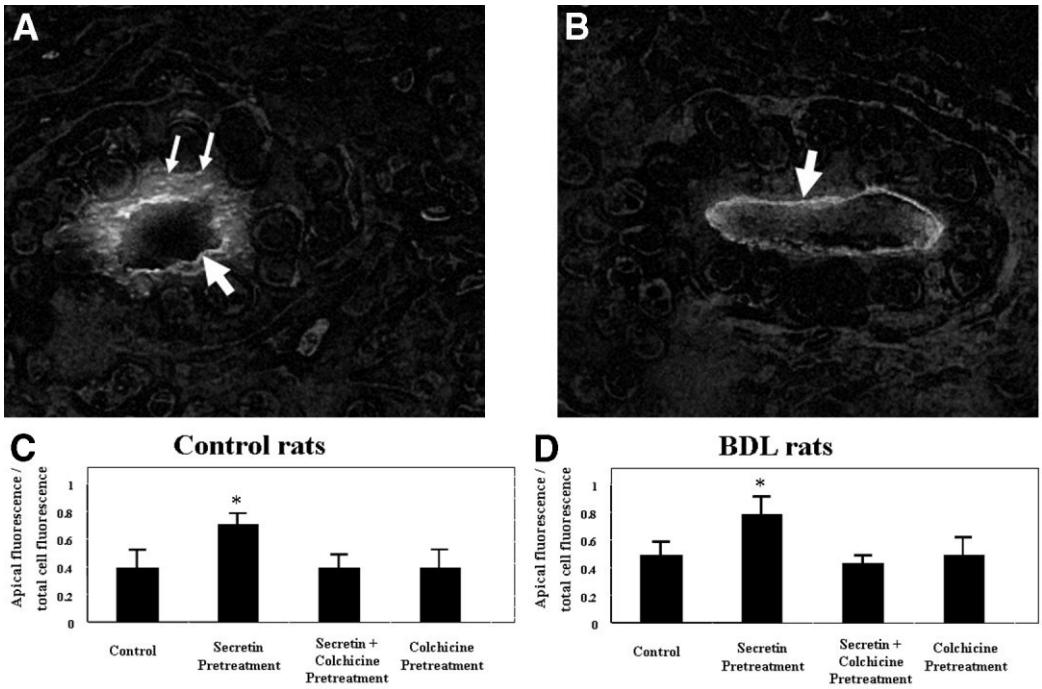

Fig. 5. Immunofluorescence localization of ASBT in cholangiocytes. Frozen sections from BDL rat liver were processed for ASBT immunofluorescence. (A) In control liver, ASBT is both intracellular (**small arrows**) and on the apical membrane (**large arrow**). (B) In a BDL rat pretreated with secretin infusion (100 nmol/L for 30 minutes) before sacrifice, ASBT distribution is chiefly in the apical membrane (**large arrow**). In (C) control and (D) BDL rats, apical membrane fluorescence was measured as a ratio to total fluorescence. Data are mean ± SE in 5 to 9 experiments. *$P < .05$ compared with controls infused with saline. ASBT, apical Na^+-dependent bile acid transporter; BDL, bile duct ligation.

Secretin Pretreatment Increases ^{3}H-TC Biliary Transit Time. Biliary transit time, measured from the appearance of ^{3}H-TC in bile (Fig. 7A), increases with secretin pretreatment (Fig. 7B). The biliary transit time calculated from the time to recovery of 50% of the administrated ^{3}H-TC significantly increased with secretin pretreatment (20.2 ± 3.4 minutes) compared with the transit time during the control period (15.4 ± 2.4 minutes).

Colchicine Blocks Secretin Pretreatment-Dependent TC Choleresis and Increases Lipid-to-Bile Acid Ratios and TC Transit Time. Since colchicine blocks secretin-induced increased ASBT activity in cholangio-

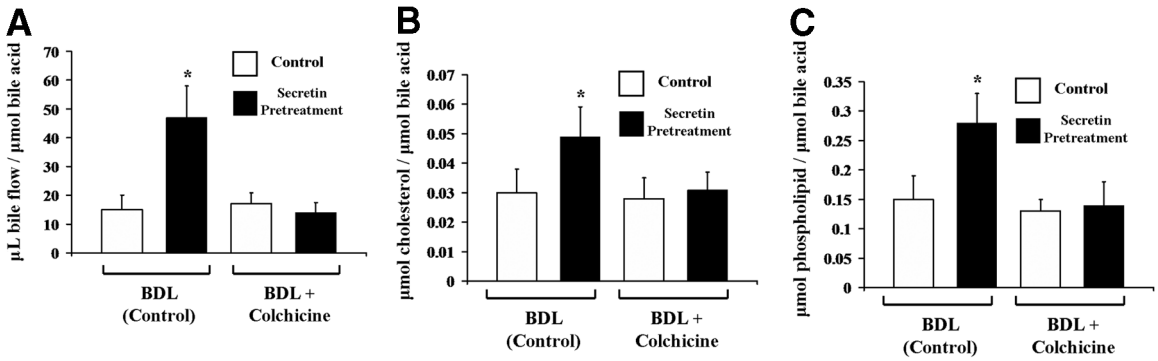

Fig. 6. The effect of secretin and colchicine on TC-induced choleresis, phospholipid, and cholesterol secretion in BDL rats. After setting up steady-state bile flow, BDL control and BDL + colchicine rats received serial infusions of each of the following for 30 minutes: TC (5 μmol/min/kg body weight), saline, secretin (100 nmol/L), saline, and then TC. Bile was collected during the first TC infusion (control) and during the second TC infusion (secretin pretreatment) and bile acids, phospholipid, and cholesterol output was determined. BDL + colchicine rats were pretreated with colchicine (1 mg/kg) 2 hours before starting bile collections. Bile flow cholesterol (B) and phospholipid (C) output are expressed as a ratio to total bile acid output. Data are mean ± SE in 6 to 12 experiments. *$P < .01$ compared with control. TC, taurocholate; BDL, bile duct ligation.

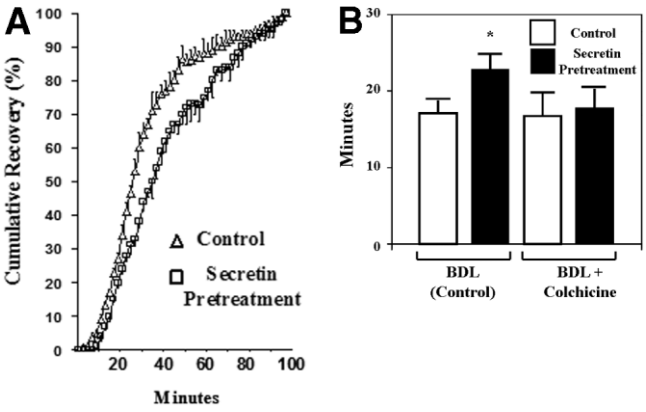

Fig. 7. The effect of secretin and colchicine on ^{3}H-TC biliary transit time. (A) ^{3}H-TC was injected into the portal vein of BDL rats and bile was collected in 2-minute aliquots for 60 minutes to measure the cumulative ^{3}H-TC biliary secretion for the control period (△). Then rats were pretreated with secretin (100 nmol/L for 30 minutes), then saline for 30 minutes and ^{3}H-TC transit time was determined again (□). (B) Biliary transit of ^{3}H-TC was calculated from the halftime of biliary appearance of the administered ^{3}H-TC. Data are mean ± SE in 5 to 10 experiments. *$P < .05$ compared with control. ^{3}H-TC, ^{3}H-taurocholate; BDL, bile duct ligation.

cytes (Fig. 1), we expected that colchicine *in vivo* would inhibit the TC-induced hypercholeresis associated with secretin pretreatment. In rats pretreated with colchicine without secretin pretreatment, TC induces a similar increase in total bile flow (Fig. 6A), and biliary lipid secretion (Fig. 6B-C) compared with control animals. These results are similar to previous studies[18,19] that showed colchicine has little effect on hepatocyte bile acid secretion, biliary lipid secretion, and bile acid–lipid coupling. Thus the effect of colchicine on TC-induced hypercholeresis with secretin pretreatment is not due to primary changes in hepatocyte TC or lipid transport. Yet, amplification of TC-stimulated bile flow, cholesterol, and phospholipid secretion with secretin pretreatment are absent in colchicine-treated rats (Fig. 6). In addition, colchicine ablates the prolonged TC biliary transit time associated with secretin pretreatment (Fig. 7). Thus, consistent with a requirement for ASBT translocation in cholangiocytes to augment TC cholehepatic shunting, we found colchicine ablates the secretin pretreatment-dependent TC choleresis and increased lipid-to-bile acid ratios and TC transit time.

TC-Induced Biliary Bicarbonate Secretion. In the control period, TC had little effect on biliary bicarbonate secretion, but with secretin pretreatment, TC increases biliary bicarbonate secretion 2-fold (Fig. 8). The increased TC-induced bicarbonate secretion following secretin pretreatment was not because of the direct choleretic effect of secretin, since prior to starting the TC infusion, bicarbonate secretion returned to basal levels. Colchicine blocked the secretin pretreatment-dependent TC-induced biliary bicarbonate secretion (Fig. 8).

Discussion

The studies examine bile acid absorption by ASBT in cholangiocytes, mechanisms that acutely control ASBT activity, and the role of ASBT in bile formation. We show that secretin causes reciprocal ASBT gain in cholangiocyte plasma membrane and ASBT loss in cholangiocyte intracellular membranes. Secretin increases TC uptake (V_{max} but not K_m) in isolated cholangiocytes. Immunofluorescence studies show that secretin increases ASBT in the cholangiocyte apical membrane *in vivo*. Translocation of ASBT to the plasma membrane requires microtubules since we find that the microtubule inhibitor colchicine prevented both redistribution of ASBT and the increase in TC uptake in cholangiocytes. Collectively, the data are most consistent with translocation of internal ASBT to the cholangiocyte apical membrane as the mechanism for secretin-stimulated bile acid transport in cholangiocytes. We next tested if the secretin-induced increases in ASBT activity in cholangiocytes results in cholehepatic shunting of bile acids. We employed previously established models of cholehepatic shunting that detect more than one pass of bile acid molecules through the canalicular membrane.[4] In BDL rats, we show secretin pretreatment causes increased TC choleresis, increased lipid-to-bile-acid ratios, and increased TC biliary transit time. These findings are consistent with secretin pretreatment causing multiple bile acid transits through the canalicular membrane and cholehepatic shunting. Colchicine ablated the increased TC choleresis, increased lipid-to-bile-acid ratios, and increased TC biliary transit time associated with secretin pretreatment. These studies show evidence for acute regulation of ASBT in cholangiocytes by transporter translocation and evidence for bile acid cholehepatic shunting,

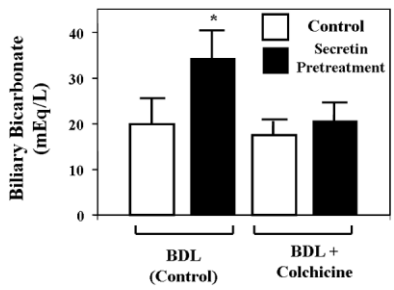

Fig. 8. The effect of secretin and colchicine on TC-induced biliary bicarbonate secretion. TC was infused intravenously in BDL rats before and after an infusion of secretin. Bile was collected during the first TC infusion (control) and during the second TC infusion (secretin-pretreatment) and bicarbonate output was determined. *$P < .001$ compared with control. TC, taurocholate; BDL, bile duct ligation.

which correlates with ASBT absorption of conjugated bile acids from bile.

ASBT Translocation. Our studies show that secretin causes redistribution of ASBT to the plasma membrane of cholangiocytes and causes a corresponding increase in TC uptake in cholangiocytes. Consistent with the idea the increased apical membrane ASBT originates from a preexisting pool in cholangiocytes, we found that secretin did not alter gene expression for ASBT. Rather, our Western analysis of plasma membranes from secretin-treated cholangiocytes shows selective increases in plasma membrane ASBT content and depletion of intracellular ASBT. With secretin pretreatment, Na^+-dependent TC uptake in cholangiocytes increased in parallel with apical membrane ASBT. The increase in V_{max} but not K_m for Na^+-dependent TC uptake in cholangiocytes is also consistent with increased number of transporters rather than a change of ASBT specific activity. We suggest, based on these data, that secretin increases apical membrane ASBT by redistribution of preexisting ASBT from vesicle-based intracellular stores to the apical membrane by exocytotic insertion onto the apical membrane. Also, we showed the microtubule inhibitor colchicine, which prevents vesicle-mediated translocation, ablates the secretin-induced increase in apical membrane ASBT. These data are consistent with previous studies showing that secretin increases vesicle traffic toward the cholangiocyte apical membrane and increases exocytosis in cholangiocytes.[10,11] Recent studies have shown that secretin increases cholangiocyte aquaporin and CFTR by microtubule-dependent insertion of aquaporins and CFTR into the cholangiocyte apical membrane.[10,11] Since CFTR and aquaporins translocate together in the same vesicle population,[10,11] the translocation of ASBT may be associated with other cholangiocyte apical membrane transporters. Hormone-stimulated insertion of transporters in renal tubule cells and intestinal epithelial cells regulate secretion.[9,20,21] Similar to cholangiocytes, cAMP regulates membrane-based trafficking of these transporters.[9,20,21] It is likely that ASBT distribution in cholangiocytes is also regulated by cAMP, since it is the principle second messenger for secretin.[1,13] Identical with our findings in cholangiocytes, increased intracellular cAMP stimulates ASBT activity in the ileum.[22]

Cholehepatic Shunting. Bile acid secretion at the hepatocyte canalicular membrane occurs because of the presence of facilitated and active transport. Unconjugated bile acids undergo passive nonionic absorption by bile ducts.[23] Since bile contains little unconjugated bile acids,[23] and exogenous bile acids that might induce cholehepatic shunting do not undergo cholehepatic shunting in man,[23] the physiological importance of cholehepatic shunt pathway, so far, has remained a curiosity. The method to show cholehepatic shunting in this study depends on measuring physiological sequela of multiple passes of bile acids through the canalicular membrane. Although these methods don't directly show the transfer of bile acids from bile to the peribiliary plexus, the use of three independent methods, all with results that are consistent with cholehepatic shunting, strongly supports our conclusions. Besides, secretin can not increase TC choleric efficiency by directly altering canalicular transporters. Rather, since cholangiocytes only express the secretin receptor in the liver,[13,24] the marked increase in choleric efficiency of TC following secretin treatment is because of altered transport in cholangiocytes. While our studies are consistent with secretin pretreatment increasing TC-dependent bile flow and biliary lipid as a consequence of increased ASBT transport, our studies do not provide a direct mechanistic link between cholehepatic shunting and secretin enhanced ASBT activity. Further studies employing the ASBT knockout mouse[25] might provide additional evidence for a role of ASBT in cholehepatic shunting of bile acids.

Consistent with TC cholehepatic shunting, we demonstrated secretin pretreatment increases TC biliary transit time from 15 to 20 minutes. Considering biliary dead space in BDL rats is 1070 μL,[12] TC transit from canalicular membrane to the end of bile fistula is 10 minutes. Thus the transit time from blood to canalicular membrane would be around 5 minutes during the control period and 10 minutes during the period following secretin pretreatment. The 2-fold increase in blood-to-canalicular membrane transit time would be consistent with each TC molecule passing through the canalicular membrane 2-fold greater in the period following secretin pretreatment. There is roughly a linear correlation between biliary bile acid and lipid secretion.[5] Thus, a doubling of TC flux through the canalicular membrane is consistent with the observed almost 2-fold increase in TC-dependent biliary secretion of cholesterol and phospholipid following secretin (Fig. 6).

We found that with secretin pretreatment, TC markedly increases biliary bicarbonate secretion. Although cholehepatic shunting induced by unconjugated bile acids increases biliary bicarbonate,[26] the mechanism for TC-dependent increases in biliary bicarbonate in this study is likely different. Conjugated bile acids absorbed by ASBT, are not protonated and thus should not increase biliary bicarbonate secretion by generating bicarbonate molecules in bile.[26] We have previously shown, however, that TC enhances basal and more so secretin-stimulated Cl^-/HCO_3^- exchanger activity in isolated cholangiocytes.[27] Based upon this previous

data, we propose that TC-induced bicarbonate secretion following secretin pretreatment is because TC potentiates secretin-stimulation of the Cl^-/HCO_3^- exchanger. Yet, it is unlikely the increases in bicarbonate alone account for the TC-induced hypercholeresis following secretin. The maximum expected increase in ductal bile flow for each bicarbonate anion secreted (8.5 μL bile flow for each μEq bicarbonate in BDL rats[12]) is considerably less than that the extra bile flow for each bicarbonate anion secreted (15.7 μL bile flow for each μEq bicarbonate) when TC was administrated following secretin in this study. Our recent studies show tauroursodeoxycholate increases biliary bicarbonate greater in BDL than compared with normal control rats, thus tauroursodeoxycholate is likely increasing bicarbonate secretion by a ductal mechanism.[28] Yet the mechanism for tauroursodeoxycholate- and TC-induced bicarbonate differs, since our studies showed tauroursodeoxycholate inhibits rather than increases the Cl^-/HCO_3^- exchanger.[28]

Secretin causes an increase in apical ASBT in both normal and BDL rats (Fig. 3). Secretin pretreatment *in vivo* does not cause TC hypercholeresis, increased lipid-to-bile-acid ratios and prolonged TC transit time in normal rats (data not shown). Secretin-receptor upregulation[13,29] and the increased numbers of ducts providing more bile acid absorptive area[13,29] explain secretin-enhanced TC cholehepatic shunting in BDL but not normal rats. Until biliary physiology studies were done in BDL rats, bile duct contribution to overall bile formation in rodents remained unrecognized.[12] Thus, the lack of evidence does not exclude the presence of cholehepatic shunting in normal rats; rather, the absence may be due to the limits of the current *in vivo* models of bile duct function.

In summary, the data presented in this study support the idea that secretin stimulates ASBT in cholangiocytes by microtubule-dependent translocation of ASBT protein to the cholangiocyte apical membrane. The increased ASBT results in increased absorption of biliary bile acids and augmentation of bile acid cholehepatic shunting. In addition, secretin pretreatment increases TC-dependent biliary bicarbonate secretion possibly through TC stimulation of the cholangiocyte Cl^-/HCO_3^- exchanger.[27] The data provide *in vivo* evidence that ASBT in bile ducts can contribute to bile formation.

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