Bile Acid Feeding Induces Cholangiocyte Proliferation and Secretion: Evidence for Bile Acid–Regulated Ductal Secretion

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Background & Aims: We have shown that taurocholate (TC) and taurolithocholate (TLC) interact in vitro with normal cholangiocytes, increasing DNA synthesis, secretin receptor (SR) gene expression, and adenosine 3′,5′-cyclic monophosphate (cAMP) synthesis. To further extend these in vitro studies, we tested the hypothesis that bile acids (BAs) directly stimulate cholangiocyte proliferation and secretion in vivo. *Methods:* After feeding with TC or TLC (1% for 1–4 weeks), we assessed the following in vivo: (1) ductal proliferation by both morphometry and immunohistochemistry for proliferating cell nuclear antigen (PCNA) and measurement of [^{3}H]thymidine incorporation; and (2) the effect of secretin on bile secretion and bicarbonate secretion in vivo. Genetic expression of H_3-histone and SR and intracellular cAMP levels were measured in isolated cholangiocytes. *Results:* After BA feeding, there was an increased number of PCNA-positive cholangiocytes and an increased number of ducts compared with control rats. [^{3}H]Thymidine incorporation, absent in control cholangiocytes, was increased in cholangiocytes from BA-fed rats. In BA-fed rats, there was increased SR gene expression (approximately 2.5-fold) and secretin-induced cAMP levels (approximately 3.0-fold) in cholangiocytes, which was associated with de novo secretin-stimulated bile flow and bicarbonate secretion. *Conclusions:* These data indicate that elevated BA levels stimulate ductal secretion and cholangiocyte proliferation.

Cholangiocytes that line the intrahepatic biliary tree secrete water and electrolytes in response to hormones.[1–3] Secretin stimulates ductal bile secretion by interacting with secretin receptor (SR) present only on cholangiocytes,[2] resulting in increases in intracellular adenosine 3′,5′-cyclic monophosphate (cAMP) synthesis.[3,4]

Cholangiocytes are normally in a quiescent state.[3,4] In experimental models of ductal hyperplasia, such as bile duct ligation (BDL) or 70% hepatectomy, cholangiocytes proliferate markedly, leading to enlargement of intrahepatic ductal mass.[1,3] Cholangiocyte proliferation is closely coupled with increased DNA synthesis, SR gene expression, secretin-induced cAMP synthesis, and secretin-stimulated bicarbonate-rich choleresis.[1–3]

Bile acids (BAs) interact with cholangiocytes and alter cholangiocyte secretion and growth.[4–8] To explain a bicarbonate-rich hypercholeresis after administration of certain BA, a "cholehepatic shunt pathway" has been proposed, suggesting that protonated BAs in the lumen of bile ducts are reabsorbed by cholangiocytes.[7] Toxic hydrophobic BAs accumulate in both the blood circulation and liver in patients with chronic liver disease. Previous studies have shown toxicity of hydrophobic BAs to hepatocytes[9,10] and bile ducts.[11] We have shown that, in vitro, both taurocholate (TC) and taurolithocholate (TLC) increase DNA synthesis, SR gene expression, and secretin-stimulated cAMP levels.[4] Furthermore, we and others[5,12] have shown functional and genetic expression of the sodium-dependent apical bile acid transporter (ABAT) in the cholangiocyte apical membranes. This Na^+-dependent ABAT probably plays an important role in the BA effects on cholangiocyte proliferation and secretion because BAs alter cholangiocyte function in vitro only in the presence of sodium, and ABAT in cholangiocytes has a Michaelis constant (K_m) for TC similar to the BA concentration at which the maximum effects of BA on cholangiocyte secretory and proliferative functions are observed in vitro.[4,12] Thus, we propose that uptake of BAs from bile by ABAT initiates signals that modulate cholangiocyte proliferation and secretion.[4,12]

In this study, we wanted to determine whether in-

Abbreviations used in this paper: ABAT, apical bile acid transporter; BA, bile acid; BDL, bile duct ligation; CK-19, cytokeratin 19; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GGT, γ-glutamyl transferase; γ-GT, γ-glutamyltranspeptidase; KRH, Krebs–Henseleit bicarbonate solution; SR, secretin receptor; TC, taurocholate; TLC, taurolithocholate.

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creased biliary BA concentrations could directly stimulate in vivo cholangiocyte proliferation and ductal secretion in the absence of cholestasis or hepatic injury. We experimentally increased biliary BA concentration by feeding rats TC (a physiologically relevant BA) or TLC (which has been shown to induce ductal hyperplasia in rodents[8]) for 1–4 weeks. There is enrichment[13] and a 2–3-fold increase in the BA pool[14] with the administrated BA in BA-fed rats. In rats fed TC or TLC, we found that BA concentrations were increased in bile but not in serum and that there was no biochemical or histological evidence of hepatic damage or cholestasis, yet there was cholangiocyte proliferation and increased ductal secretion.

Materials and Methods

Animal Model and Materials

Male Fischer 344 rats (125–150 g) from Charles River (Wilmington, MA) were used in the present studies. The animals were kept in a temperature-controlled environment (20–22°C) with alternating 12-hour light-dark cycles. The studies were conducted in rats fed 1% TC or 1% TLC, representing approximate doses of 275 and 260 μmol/day, respectively, for 1–4 weeks. Rat chow containing 1% TC, 1% TLC, or AIN 76 (control diet) was prepared by Dyets Inc. (Bethlehem, PA). Rats were fasted overnight before experiments. Before each protocol, rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). Reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated. *N*(γ-L-glutamyl)-4-methoxy-2-naphthylamide, the substrate for γ-glutamyltranspeptidase (γ-GT), was purchased from Polysciences (Warrington, PA). Both TC and TLC were purchased from Calbiochem-Novabiochem Corp. (La Jolla, CA).

Determination of Liver Inflammation and Hepatic Damage

After TC or TLC feeding, the degree of liver inflammation was assessed by the grade system described by Davis and Madri.[15] H&E-stained liver sections were graded for the degree of inflammation in a coded fashion as follows: 0, normal; +1, triad-based; +2, triad-based with focal parenchymal inflammation without necrosis; +4, triad-based with lobular inflammation with unicellular necrosis; and +5, confluent necrosis. To determine whether BA feeding induces liver damage, the serum levels of selected enzymes associated with hepatic damage (serum glutamic pyruvic transaminase [SGPT], serum glutamic oxalacetic transaminase [SGOT], γ-glutamyl transferase (GGT), alkaline phosphatase, and both total and direct bilirubin) were measured by use of commercially available kits (Sigma).

Purification of Cholangiocytes From Normal and BA-Fed Rats

Pure (by γ-GT histochemistry; results not shown) preparations of cholangiocytes from control and BA-fed rats were obtained as previously described.[2,3,16,17] Briefly, after standard collagenase perfusion,[17] a cholangiocyte-enriched fraction (40%–55% pure by γ-GT) was obtained from intact portal tracts, as described by Ishii et al.,[17] and subsequently purified by immunoaffinity separation.[3,17]

Assessment of Intrahepatic Ductal Mass in Normal and BA-Fed Rats

Intrahepatic duct mass was calculated by point count analysis[3,18] by determination of the fraction of total matrix of points overlapping bile ducts, stained for γ-GT,[19] in coded frozen liver sections (6 μm) from normal and BA-fed rats (n = 12). In each liver section, the entire area was examined by multiple photographs taken in a mosaic fashion. From the total number of points over hepatic tissue and the number of points over γ-GT–positive ducts, we calculated the volume percent of liver occupied by ducts.[3,18]

Indirect immunohistochemistry was performed to detect proliferating cellular nuclear antigen (PCNA) in proliferating bile ducts. Briefly, formalin-fixed liver sections were deparaffined, followed by microwave treatment (5 minutes in water at 500 W). After adequate blocking of endogenous peroxidase activity by methanol-peroxidase solution, nonspecific binding was blocked by incubation with normal goat serum for 30 minutes at room temperature. Sections were incubated with anti-PCNA antibody (clone PC-10; Oncogene Research Products, Cambridge, MA) at 4°C overnight. After several washes with cold phosphate-buffered saline, biotin-labeled secondary antibody at a dilution of 1:100 (Dako, Kyoto, Japan) was added for 1 hour at room temperature. For detection of this reaction, a peroxidase-labeled avidin-biotin complex with diaminobenzidine (Dojin Chemical Co, Kumamoto, Japan) was used as a substrate. Sections were counterstained with hematoxylin and examined with a microscope (Olympus Optical Co., BX 40, Japan); 300–400 cholangiocytes were analyzed for each interval.

In Vivo Measurement of [^{3}H]Thymidine Incorporation in Cholangiocytes

We measured DNA synthesis of pure cholangiocytes from control and BA-fed rats by measuring of [^{3}H]thymidine incorporation as described previously.[3]

Measurement of H_3-Histone and SR Gene Expression

Steady-state levels of H_3-histone and SR messenger RNAs (mRNAs) were measured by the lysate ribonuclease (RNase) protection assay (Direct Protect kit; Ambion Inc., Austin, TX).[3,4] The phenotypic characteristics of cholangiocytes from normal and BA-fed rats were assessed by hybridization with cytokeratin 19 (CK-19), a specific marker of cholangiocytes.[3] The comparability of the cell lysate used was determined by hybridization with glyceraldehyde-3-phosphate dehydrogenase (GAPDH).[2,4,16] Antisense mRNA probes were transcribed from selected complementary DNA (cDNA) linearized templates using the Maxiscript kit (Ambion Inc.).

Measurement of Intracellular cAMP Levels

After purification, cholangiocytes (1×10^5) were incubated for 5 minutes at 22°C[3] with 10^{-7} mol/L secretin (Peninsula Laboratories, Belmont, CA) or 1% bovine serum albumin (control), and intracellular cAMP levels were measured by radioimmunoassay (Amersham, Arlington Heights, IL) as described previously.[3,4,16]

Measurement of Spontaneous and Secretin-Stimulated Bile Flow

In control and BA-fed rats, bile secretion and bicarbonate secretion were measured as described.[1,3,16] Briefly, one jugular vein was incannulated with a PE 50 cannula (Clay-Adams, New York, NY) to infuse Krebs–Henseleit bicarbonate solution (KRH) or secretin dissolved in KRH. When steady-state bile flow was achieved, secretin (10^{-7} mol/L) was infused for 30 minutes followed by a final infusion of KRH for 60 minutes. Bile was collected at 10-minute intervals, placed in preweighed tubes, and immediately stored at −70°C. Bicarbonate concentration (measured as total CO_2) in bile was determined with a Natelson microgasometer apparatus (Scientific Industries, Bohemia, NY). Fasting animal blood was collected for determination of serum BA levels. The levels of total BA in bile or serum were determined by the 3α-hydroxysteroid dehydrogenase procedure[20] using a commercially available kit according to the instructions supplied by the vendor (Wako Chemicals USA, Inc., Richmond, VA). Aliquots of bile extracted with 4 volumes of isopropanol were analyzed for individual bile salt by reverse-phase high-performance liquid chromatography using an acidic isocratic phosphate buffer as previously described.[21]

Statistical Analysis

All data are expressed as means ± SE. Differences between groups were analyzed by the Student unpaired *t* test when 2 groups were analyzed or analysis of variance (ANOVA) when more than 2 groups were analyzed.

Results

Serum and Bile Concentration and Composition

There was a significant increase in total BA concentration in bile obtained from TC- or TLC-fed rats compared with control rats (Table 1). In contrast, no increase in serum total BA in TC-fed rats and a modest decrease in serum total BA in TLC-fed rats were observed (Table 1). The finding of no increase in serum total BA levels in BA-fed rats indicates that BA feeding with the amount chosen in our experiments does not induce cholestasis. BA composition of bile samples obtained from control and TC- and TLC-fed rats (Table 2) shows enrichment of bile with the fed BA.

Determination of Liver Inflammation and Hepatic Damage

Portal inflammation was not seen in rat liver sections after feeding with TC, TLC, or control diet. Hepatic inflammation scores were similar in rats fed TC, TLC, and control diet (results not shown). Serum levels of SGPT, SGOT, GGT, alkaline phosphatase, and both total and direct bilirubin were not significantly different in BA-fed rats and rats fed a control diet (results not

Table 1. BA Concentration in Bile and Serum and Basal and Secretin-Induced Bile Flow and Biliary Composition in Control, TC-Fed, and TLC-Fed Rats

	BA levels (mEq/L)		Bile secretion ($\mu L \cdot min^{-1} \cdot kg^{-1}$)		Bicarbonate concentration (mEq/L)		Bicarbonate secretion ($\mu Eq \cdot min^{-1} \cdot kg^{-1}$)	
Treatment	Bile	Serum	Basal	Secretin	Basal	Secretin	Basal	Secretin
Control	2.82 ± 0.22	0.056 ± 0.001	91.85 ± 14.48	86.99 ± 14.5	24.46 ± 1.98	27.00 ± 0.57	2.19 ± 0.21	2.36 ± 0.35
TC, 1 wk	11.23 ± 2.54[a]	0.049 ± 0.003	126.64 ± 16.21	136.90 ± 17.6	22.82 ± 0.68	32.07 ± 1.10[d]	2.71 ± 0.42	4.54 ± 0.54[e]
TC, 2 wk	8.76 ± 0.83[a]	0.045 ± 0.004	91.22 ± 2.25	105.49 ± 3.2[c]	28.32 ± 1.14	30.72 ± 0.75	2.58 ± 0.09	3.23 ± 0.08[e]
TC, 3 wk	8.73 ± 0.95[a]	0.064 ± 0.001	82.56 ± 7.09	102.68 ± 6.6[c]	30.15 ± 0.27	33.67 ± 0.33[d]	2.48 ± 0.30	3.45 ± 0.35[e]
TC, 4 wk	6.38 ± 0.26[a]	0.032 ± 0.002	79.97 ± 7.64	96.84 ± 6.8[c]	26.85 ± 1.35	31.96 ± 0.75[d]	2.15 ± 0.24	2.86 ± 0.21[e]
TLC, 1 wk	3.15 ± 1.02[b]	0.010 ± 0.002	85.35 ± 4.44	102.51 ± 3.3[c]	29.88 ± 0.22	34.68 ± 2.19[d]	2.54 ± 0.12	3.57 ± 0.30[e]
TLC, 2 wk	4.09 ± 0.57[a]	0.011 ± 0.001	65.09 ± 8.00	75.14 ± 7.9	29.87 ± 0.69	32.37 ± 2.19[d]	1.95 ± 0.24	2.42 ± 0.25[b]
TLC, 3 wk	3.43 ± 0.19[a]	0.006 ± 0.001	88.09 ± 3.50	100.93 ± 7.0[c]	22.67 ± 2.31	26.35 ± 0.70[d]	1.91 ± 0.21	2.66 ± 0.16[e]
TLC, 4 wk	3.79 ± 0.11[a]	0.009 ± 0.003	58.83 ± 3.83	72.95 ± 4.1[c]	30.80 ± 1.32	34.83 ± 1.56[d]	1.81 ± 0.14	2.52 ± 0.11[e]

NOTE. Data are means ± SE of at least 10 values from control rats and rats fed 1% TC or 1% TLC for 1–4 weeks. BA levels in bile and serum from control and BA-fed rats were measured as described in Materials and Methods using commercially available kits.

[a] $P < 0.05$ by the unpaired Student *t* test compared with the corresponding values of normal control rats. Values are mean ± SE for 6–10 rats and obtained at steady-state conditions of bile flow. After an equilibration period of 60 minutes with KRH solution, secretin was infused via a jugular vein for 30 minutes at 10^{-7} mol/L.

[b] Not significant.

[c] $P < 0.05$ vs. basal bile secretion.

[d] $P < 0.05$ vs. basal bicarbonate concentration of normal control rats.

[e] $P < 0.05$ vs. basal bicarbonate secretion.

Table 2. BA Composition in Bile Obtained From Control, TC-Fed, and TLC-Fed Rats

Treatment	Muricholic acid (*mmol/L*)	Taurocholic acid (*mmol/L*)	Taurochenocholic acid (*mmol/L*)	Taurodeoxycholic acid (*mmol/L*)	Taurolithocholic acid (*mmol/L*)
Control	2.16 ± 0.15	0.58 ± 0.06	0.05 ± 0.01	0.02 ± 0.005	Not detectable
TC, 1 wk	3.40 ± 0.87	6.03 ± 1.72[a]	0.22 ± 0.07	0.48 ± 0.18[a]	Not detectable
TC, 2 wk	2.96 ± 0.45	5.65 ± 0.24[a]	0.15 ± 0.06	0.14 ± 0.03[a]	Not detectable
TC, 3 wk	2.44 ± 0.22	5.87 ± 0.71[a]	0.10 ± 0.02	0.31 ± 0.08[a]	Not detectable
TC, 4 wk	1.66 ± 0.21	4.38 ± 0.26[a]	0.06 ± 0.006	0.24 ± 0.03[a]	Not detectable
TLC, 1 wk	1.65 ± 0.11	0.45 ± 0.09	0.09 ± 0.009	0.03 ± 0.006	0.46 ± 0.14[a]
TLC, 2 wk	2.32 ± 0.50	0.66 ± 0.11	0.29 ± 0.07[a]	0.028 ± 0.001	0.34 ± 0.03[a]
TLC, 3 wk	1.67 ± 0.24	0.86 ± 0.10	0.16 ± 0.02[a]	Not detectable	0.35 ± 0.04[a]
TLC, 4 wk	2.15 ± 0.05	1.11 ± 0.08	0.22 ± 0.03[a]	Not detectable	0.30 ± 0.05[a]

NOTE. Values are means ± SE of at least 6 rats and were obtained in the first 10 minutes of bile collection. BA composition in bile samples from control, TC-fed, and TLC-fed rats was measured by standard high-performance liquid chromatography.
[a]$P < 0.05$ vs. corresponding value of BA-fed control rats.

shown). The data suggest that BA feeding results in neither hepatocellular necrosis nor cholestasis.

Proliferative Activity of Cholangiocytes From Control and BA-Fed Rats

Only 2–3 bile ducts (stained for γ-GT or PCNA) were present in portal areas of liver sections from control rats (Figure 1). A marked increase in the number of bile ducts (stained for γ-GT or PCNA) per portal area was observed after TC or TLC feeding (Figure 1; at 1 week of feeding, representative experiments). In situ quantitative morphometric analysis showed a marked increase in the number of bile ducts (stained for γ-GT) after BA feeding for 1–4 weeks compared with control rats (Figure 2). Similarly, the percentage of PCNA-positive cholangiocytes was significantly increased with 1–4 weeks of BA feeding compared with controls (Figure 2). DNA synthesis of control cholangiocytes, measured by [^{3}H]thymidine incorporation, was very low (Figure 3). In contrast, [^{3}H]thymidine incorporation in purified cholangiocytes was markedly increased after TC or TLC feeding from 1–4 weeks (Figure 3). DNA synthesis, measured as H_3-histone gene expression, was very low in control cholangiocytes (Figure 4).[3] In contrast, after TC or TLC feeding for 1–4 weeks, there was a marked increase (15–20-fold) in H_3-histone gene expression compared with control cholangiocytes (Figure 4). Expression of both GAPDH and CK-19 mRNAs was similar among cholangiocytes from control and BA-fed rats (Figures 4 and 5).

Secretory Activity of Cholangiocytes From Control and BA-Fed Rats

Parallel to increased ductal proliferation observed in BA-fed rats, SR gene expression increased (2–3-fold) in

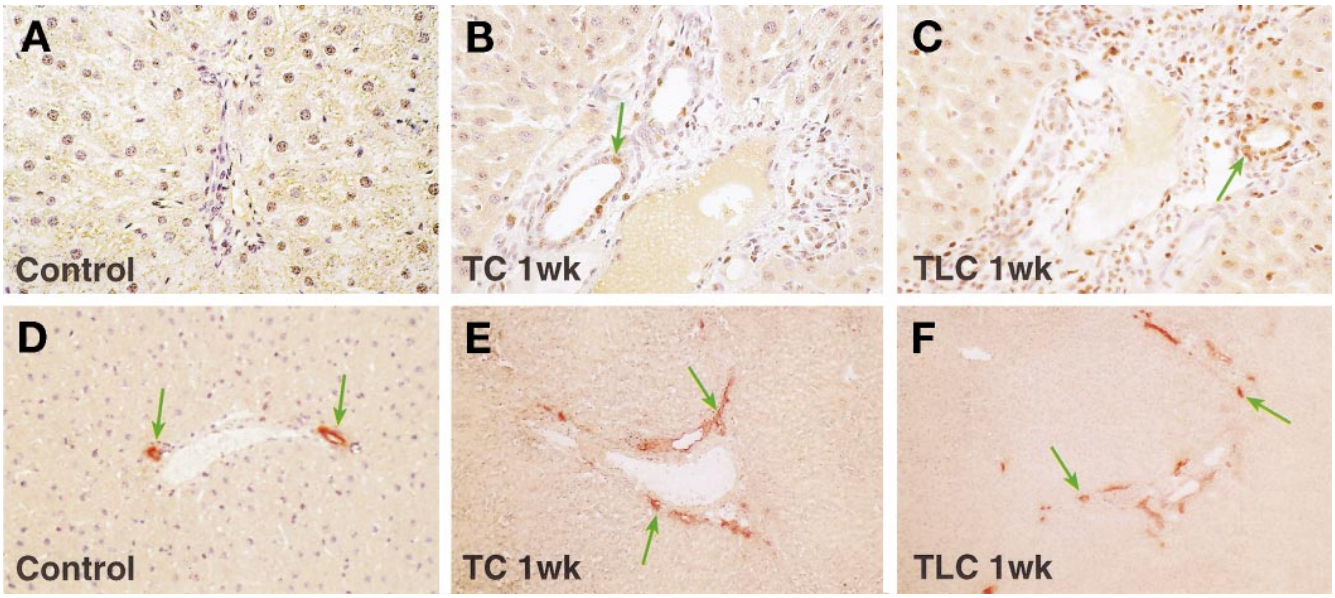

Figure 1. Staining for (*A–C*) PCNA and (*D–F*) γ-GT in sections from (*A* and *D*) control, (*B* and *E*) TC-fed, or (*C* and *F*) TLC-fed rats. After TC and TLC feeding (at 1 week, a representative experiment), there was a marked increase in the number of PCNA-positive cholangiocytes and bile ducts (original magnifications 125× and 160×; γ-GT staining in control rats). *Arrows* indicate cholangiocytes and bile ducts positive for PCNA or γ-GT, respectively.

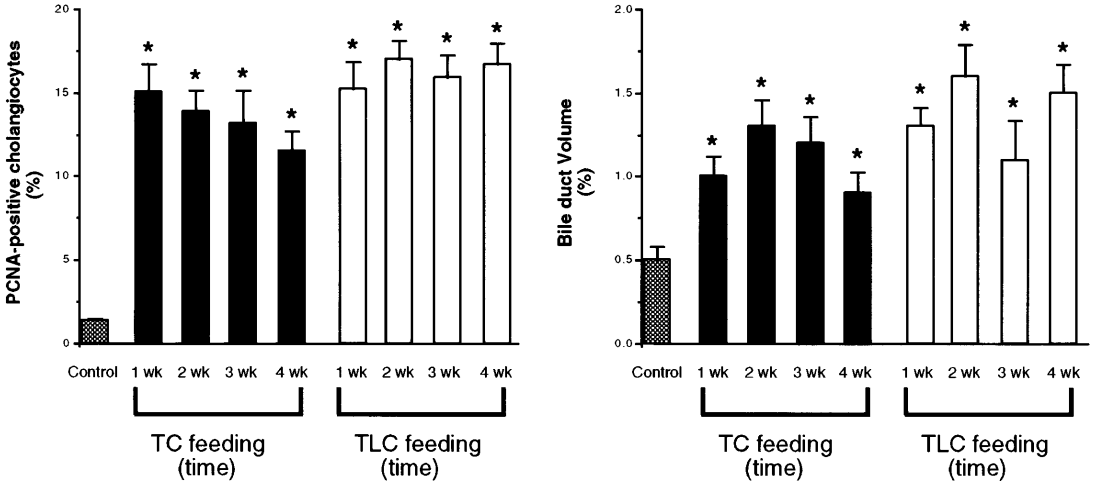

Figure 2. In situ quantitative morphometric analysis of the number of PCNA-positive cholangiocytes and number of bile ducts in liver sections stained for γ-GT (see Materials and Methods). For each interval, 300–400 cholangiocytes were analyzed. $*P < 0.05$ vs. control rats.

cholangiocytes from both TC- and TLC-fed rats compared with control cholangiocytes (Figure 5). Basal intracellular cAMP levels in cholangiocytes from TC- or TLC-fed rats were similar to those in control cholangiocytes (Figure 6). Although secretin significantly increased cAMP levels in cholangiocytes from rats fed a control diet, the increases in cAMP levels were significantly greater ($P < 0.05$) in cholangiocytes from rats fed TC or TLC for 1–4 weeks (see Figure 6). The effects of BA feeding on bile and both bicarbonate concentration and secretion are shown in Table 1. TC or TLC feeding resulted in increases in secretin-induced bile flow and both bicarbonate concentration and secretion (Table 1). The findings of increased SR gene expression, secretin-stimulated cAMP synthesis in cholangiocytes, and secretin-stimulated bile flow and bicarbonate secretion are consistent with increased duct secretion in BA-fed rats.

Discussion

Our studies show that after feeding, BAs in vivo interact with cholangiocytes and stimulate proliferative and secretory processes of these cells. There was an increase in the number of bile ducts in BA-fed rats compared with control rats with the absence of biochemical or histological evidence of hepatic injury or cholestasis. Parallel with changes in ductal mass, DNA synthesis, SR gene expression, and secretin-stimulated cAMP levels

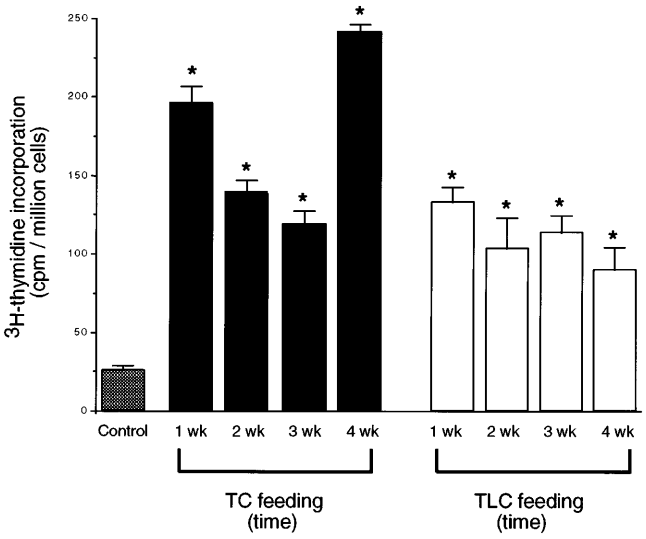

Figure 3. Ninety minutes after an intraperitoneal administration of 1 μCi/g body wt of [*methyl*-^{3}H]thymidine, the radioactivity incorporated into DNA was measured in cholangiocytes from control, TC-fed, and TLC-fed rats. $*P < 0.05$ compared with control cholangiocytes. Data are means ± SE of 3 experiments.

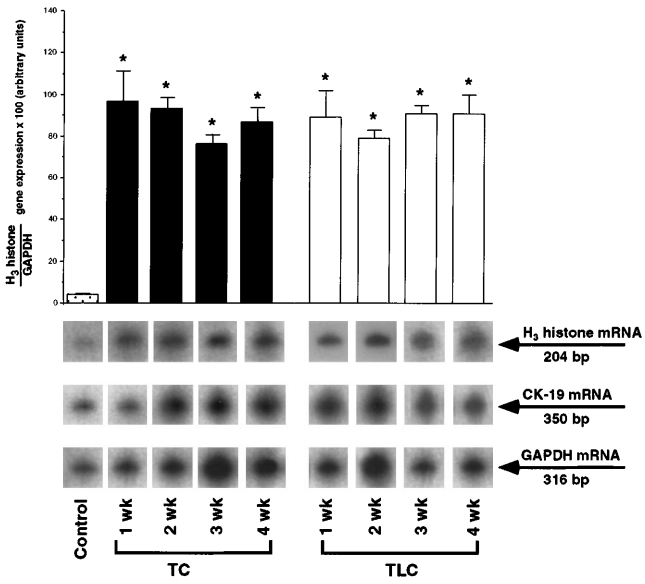

Figure 4. Expression of selected messages was determined by direct RNase protection assay using cell lysate samples, each containing 4.50×10^5 pure cholangiocytes from control and BA-fed rats. Autoradiograms (n = 3) were quantified by densitometry.

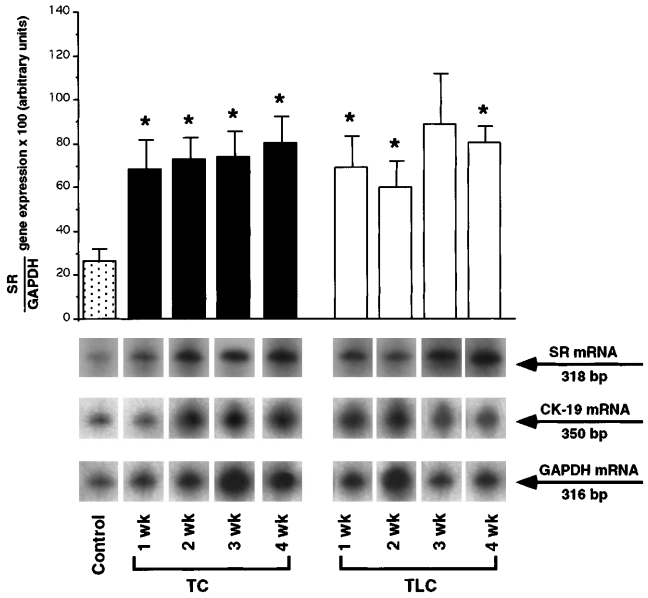

Figure 5. Genetic expression of SR in cholangiocytes from control and BA-fed rats. Molecular analysis was performed by RNase protection assays using cell lysate samples each containing 4.50×10^5 cholangiocytes. Data are mean ± SE of 3 experiments.

all markedly increased in cholangiocytes from BA-fed rats compared with control rats. In vivo, secretin markedly increased bile flow and both biliary bicarbonate concentration and secretion in BA-fed rats compared with absence of secretin-induced ductal secretion in controls. The data suggest that BAs modulate ductal bile secretion in the normal state and may induce ductal proliferation in the disease state (with BA accumulation).

When circulatory levels of BA are elevated with cholestasis, BA may be toxic to hepatocytes because of changes in hepatocyte Ca^{2+} and Mg^{2+}, as shown by Spivey et al.,[9] and adenosine triphosphate depletion.[10] In the BA-fed rat model, cholangiocytes respond with increased proliferation and secretion in the absence of biochemical or histological evidence of hepatocyte injury or cholestasis. Similar effects with both TC and the more potentially hepatotoxic TLC were noted in these studies, showing that BAs per se, not their toxicity potential, cause changes in the BA-fed model. Although short-term TLC administration produces severe cholestasis in rats[22] and cholestasis and fibrosis in rabbits,[23] our current study and a previous study[22] showed that long-term TLC administration in rats does not produce cholestasis or hepatic injury.[24] Progressive increases in proliferation and secretion were not observed after 1 week of BA feeding. Counterbalancing factors may come into play after 1 week that down-regulate cholangiocyte proliferation and ductal secretion. The present findings contrast with other models of ductal hyperplasia induced by injury, toxins, or cholestasis (e.g., 1-naphthylisothiocyanate feeding and BDL) in which cholangiocytes progressively proliferate up to 1 month.[1] Similar to our findings in cholangiocytes, BAs have been shown to stimulate hepatocyte proliferation associated with partial hepatectomy[25] and pancreatic acinar secretion.[26]

These data provide new insights into the physiological role of BA in the intrahepatic biliary ductal system. We have shown that BAs are absorbed by the apical membrane of cholangiocytes because of the presence of the Na^+-dependent BA transporter, ABAT,[12] and that Na^+-dependent BA transport in cholangiocytes is regulated by secretin.[27] Na^+-dependent BA uptake across the apical membrane of cholangiocytes appears to be required for BAs to alter cholangiocyte function because in vitro, BAs increase cholangiocyte proliferation and ductal secretion

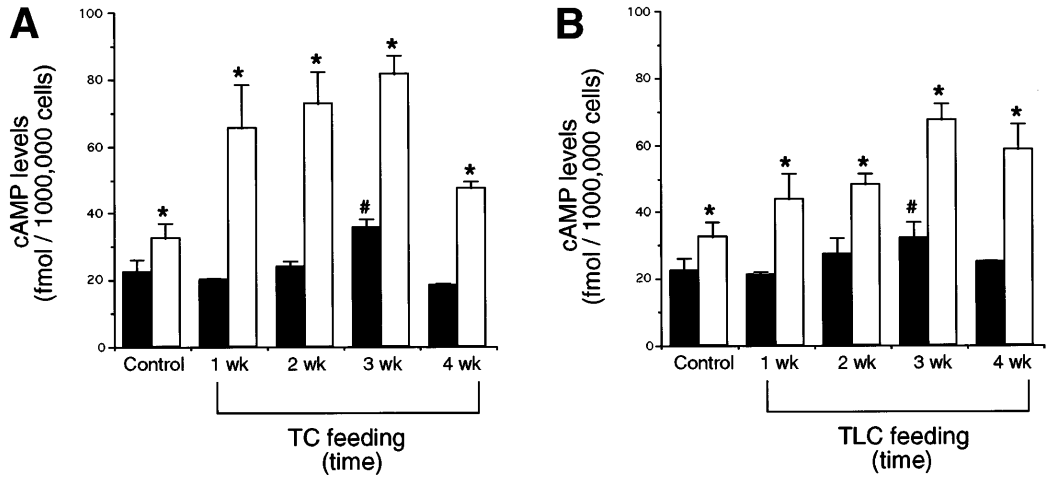

Figure 6. Intracellular cAMP levels in cholangiocytes from control and rats fed (*A*) 1% TC and (*B*) 1% TLC for 1–4 weeks. $^*P < 0.05$ vs. basal value. $^{\#}P < 0.05$, secretin-induced cAMP levels of cholangiocytes from BA-fed rats differing from secretin-induced cAMP levels of cholangiocytes from control rats. Data are mean ± SE of 6 experiments.

only in the presence of Na^+.[4] This study shows that exposure of increased BA levels to the apical (bile) but not basolateral (blood) cholangiocyte membrane stimulates cholangiocyte proliferation and ductal secretion. The studies do not exclude the possibility that high circulating BA levels, which occur in cholestasis, may also directly alter cholangiocyte function. Our previous experiments show that only certain-sized bile ducts (large but not small) respond to BA and that the distribution of ABAT resides in the same-sized ducts.[4] Similar correlation exists between the amount of solute load handled by different kidney tubule segments and the degree of tubule hyperplasia and hypertrophy.[28] Because it has been suggested that kidney tubule hyperplasia/hypertrophy compensates for the increased solute load,[28] we speculate that bile duct hyperplasia in BA-fed rats is compensating for increased biliary BAs. Additional studies showing increased transport activity of ABAT in cholangiocytes from BA-fed rats would support this concept. In addition to ABAT, another BA transport system has been demonstrated in cholangiocytes,[6] but the properties of this transporter make it an unlikely candidate target for BA effects on cholangiocyte function because it has a basolateral location and no dependence on Na^+.

The intracellular signaling mechanisms for BA-stimulated cholangiocyte secretion and growth have not been established. Because BAs directly stimulate cholangiocyte cAMP synthesis,[4] and elevated cholangiocyte cAMP levels are present in cholangiocyte hyperplasia,[16] cAMP is one likely signal. Consistent with this idea, preliminary data from our laboratory suggest that reduction of high biliary BA concentrations in BDL rats reduces cholangiocyte cAMP levels and reverses increased cholangiocyte proliferation.[29] Other preliminary studies from our laboratory show that ursodeoxycholic acid (UDCA), which does not alter cAMP levels, fails to increase cholangiocyte proliferation or secretion in normal cholangiocytes.[4] In contrast, we have observed that in UDCA-treated cholangiocytes from BDL rats and in UDCA-fed BDL rats,[30] the elevated cholangiocyte cAMP levels and increased cholangiocyte proliferation and secretion associated with BDL are ablated. Thus both up- and down-regulation of cholangiocyte proliferation and secretion by BAs are linked to cholangiocyte cAMP synthesis.

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