Comparative Regulation of Hepatic Sterol 27-Hydroxylase and Cholesterol 7α-Hydroxylase Activities in the Rat, Guinea Pig, and Rabbit: Effects of Cholesterol and Bile Acids

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The regulation of the classic and alternative bile acid synthetic pathways by key hepatic enzyme activities (microsomal cholesterol 7α-hydroxylase and mitochondrial sterol 27-hydroxylase, respectively) was examined in bile acid depletion and replacement and cholesterol-feeding experiments with rats, guinea pigs, and rabbits. The bile acid pool was depleted by creating a bile fistula (BF) and collecting bile for 2 to 5 days, and it was replaced by intraduodenal infusion of the major biliary bile acids (taurocholic acid [TCA], glycochenodeoxycholic acid [GCDCA], and glycocholic acid [GCA] in the rat, guinea pig, and rabbit, respectively) at rates equivalent to the measured hepatic flux of the bile acids. To study the effects of cholesterol, the animals were fed for 7 days on a basal diet with and without 2% cholesterol. Cholesterol 7α-hydroxylase and sterol 27-hydroxylase activities, measured by isotope incorporation assays, were related to bile acid output and composition and hepatic cholesterol concentrations. Intraduodenal infusion of bile acids increased the output of the tested bile acids, but did not significantly change hepatic cholesterol concentrations and had no effect on sterol 27-hydroxylase activity. Neither bile acid depletion nor replacement affected sterol 27-hydroxylase activity when three different substrates (cholesterol, 5β-cholestane-3α,7α-diol, and 5β-cholestane-3α,7α,12α-triol) were tested. In contrast, feeding 2% cholesterol increased hepatic cholesterol concentrations in rats, guinea pigs, and rabbits threefold, twofold, and eightfold, respectively, and increased hepatic mitochondrial sterol 27-hydroxylase activity (conversion of cholesterol to 27-hydroxycholesterol) in all three animal models. The stimulation and feedback inhibition of cholesterol 7α-hydroxylase activity by bile acid depletion and replacement were observed in all three animal models, whereas the effect of cholesterol feeding was species-dependent (cholesterol 7α-hydroxylase activity increased in the rat, did not change in the guinea pig, and was inhibited in the rabbit). Thus, in contrast to sterol 27-hydroxylase, which was upregulated by cholesterol but not affected by bile acid depletion and replacement in all three animal models, cholesterol 7α-hydroxylase activity was controlled consistently and inversely by the hepatic flux of bile acids, but was species-dependent in its response to a 1-week feeding with 2% cholesterol.

IT IS WELL ESTABLISHED that cholesterol 7α-hydroxylase (EC 1.14.13.17), which catalyzes the conversion of cholesterol to 7α-hydroxycholesterol in the smooth endoplasmic reticulum of the liver, is the rate-limiting step in the classic bile acid synthetic pathway. This enzyme is regulated inversely by the return of bile acids to the liver, as well as various hormones and cytosolic factors.[1-6] Recently, it was suggested that bile acid synthesis from cholesterol could also be initiated by a mitochondrial enzyme, sterol 27-hydroxylase (EC 1.14.13.15), which catalyzes the conversion of cholesterol to 27-hydroxycholesterol in an alternative bile acid synthetic pathway that bypasses microsomal cholesterol 7α-hydroxylase.[7-13] It has been suggested, but not proven, that the same mitochondrial sterol 27-hydroxylase also catalyzes the side-chain hydroxylation of intermediates in the classic synthetic pathway initiated by cholesterol 7α-hydroxylase, ie, the conversion of 5β-cholestane-3α,7α-diol and 5β-cholestane-3α,7α,12α-triol to 5β-cholestane-3α,7α,27-triol and 5β-cholestane-3α,7α,12α,27-tetrol, respectively.[14,15] These triol and tetrol intermediates undergo further side-chain hydroxylation and cleavage to yield chenodeoxycholic acid and cholic acid, respectively. Similar responses of sterol 27-hydroxylase activity to various experimental treatments, whether cholesterol, 5β-cholestane-3α,7α-diol, or 5β-cholestane-3α,7α,12α-triol as substrate, would provide further evidence that the same enzyme catalyzes the 27-hydroxylation of all three substrates.

Despite the emerging evidence of the important role of sterol 27-hydroxylase in cholesterol homeostasis, the regulation of sterol 27-hydroxylase by its substrate (ie, cholesterol) or end products (ie, bile acids) is not well documented. Conflicting reports of the effects of bile acids on sterol 27-hydroxylase have been published and attributed to species differences.[3,16-19] The rat and rabbit synthesize mostly cholic acid but have different susceptibility to diet-induced hypercholesterolemia and atherosclerosis. An examination of the regulation of cholesterol 7α-hydroxylase and sterol 27-hydroxylase in these two species may provide further insight into the relative importance of the classic and alternative bile acid synthetic pathways in cholesterol homeostasis in these animal models. Furthermore, previous reports suggested that the sterol 27-hydroxylase–initiated alternative pathway leads to the exclusive formation of chenodeoxycholic acid,[7,9] whereas more recent study has demonstrated the formation of substantial amounts of cholic acid from 27-hydroxycholesterol.[10] Since the guinea pig synthesizes mostly chenodeoxycholic acid, the study of sterol 27-hydroxylase activity in this animal model, as compared with animals synthesizing predominantly trihydroxy bile acids (rats and rabbits), will determine whether the regulation of sterol 27-hydroxylase is associated only with the formation of the dihydroxy bile acid.

The objectives of this study are (1) to compare the effects of

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Submitted February 9, 1999; accepted June 1, 1999.

Supported in part by US Public Health Service Grant No. DK26756, a Grant-in-Aid from the American Heart Association, New Jersey Affiliate, and the Veterans Affairs Research Service.

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0026-0495/99/4812-0016$10.00/0

bile acid depletion and replacement on hepatic mitochondrial sterol 27-hydroxylase (conversion of cholesterol to 27-hydroxycholesterol) and microsomal cholesterol 7α-hydroxylase activities in the rat, guinea pig, and rabbit; (2) to compare sterol 27-hydroxylase activities measured under the same hepatic bile acid fluxes but with different substrates (cholesterol, 5β-cholestane-3α,7α-diol, and 5β-cholestane-3α,7α,12α-triol); (3) to examine the effects of cholesterol feeding on hepatic sterol 27-hydroxylase and cholesterol 7α-hydroxylase activities in these three animal models; and (4) to relate changes in the two bile acid synthetic enzyme activities to changes in hepatic cholesterol concentrations.

MATERIALS AND METHODS

Animals

In the first set of experiments to study the effect of bile acids, the bile acid pool was depleted from rats, guinea pigs, and rabbits via a bile fistula (BF), followed by 24-hour duodenal infusion of the major conjugated bile acid at a rate experimentally determined to be equivalent to the normal bile acid flux to the liver in the studied species. All animals were adult males, and at least five animals were included in each group. Rats, guinea pigs, and rabbits normally have different bile acid pool sizes, and the time required for complete bile acid pool depletion was determined experimentally (measuring bile acid output at timed intervals) to be 2, 3, and 5 days, respectively.

Rat. Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 200 to 250 g were fed and libitum with rodent chow (Purina Mills, St Louis, MO). BFs were established in the rats as described previously.[1] Bile collected during the first half-hour was used for the determination of biliary bile acid output and composition. When the bile acid pool was depleted after 48 hours of bile diversion, the rats were infused intraduodenally (2.4 mL/h) with either a Ringer-Tyrode buffer supplemented with 25 μEq/h $NaHCO_3$ (BF group) or the same buffer containing taurocholic acid ([BF + TCA group] sodium salt, >98% pure with <0.1% taurodeoxycholate; Calbiochem, San Diego, CA). TCA was infused for 24 hours at 27 μmol/100 g rat/h (equivalent to the normal bile acid flux through the liver).[20] A group of sham-operated animals with intact interohepatic bile acid circulation served as controls.

Guinea pig. Male Hartley guinea pigs (Charles River, Wilmington, MA) weighing 890 to 1,250 g and maintained on a basal guinea pig diet (Purina Mills) were anesthetized with an intramuscular injection of ketamine 30 to 40 mg/kg body weight and xylazine 3 mg/kg body weight. A 0.86-mm ID, 1.27-mm OD polyethylene catheter was placed in the common bile duct. A PE-50 catheter (0.58-mm ID and 0.95-mm OD) was placed in the duodenum, and lactated Ringer's solution was infused at a rate of 10 to 15 mL/h with a peristaltic pump (model 77; Harvard Apparatus, South Natick, MA). All tubing was passed through a dorsal incision so that the animals did not need to be restrained during the experiments. Bile was collected the first half-hour to determine baseline bile acid output and composition, and then every 4 hours for 3 days to deplete the bile acid pool. Chenodeoxycholic acid (Aldrich Chemical, Milwaukee, WI) was conjugated with glycine according to the method of Tserng et al.[21] Glycochenodeoxycholic acid ([GCDCA] sodium salt) was dissolved in saline and infused intraduodenally for 24 hours, 3 days after construction of the BF. Animals with biliary obstruction during bile diversion (about three of 10) were excluded from the study. Control sham-operated guinea pigs had intact enterohepatic bile acid circulation.

Rabbit. Male New Zealand white rabbits (Hazleton Laboratories, Denver, PA) weighing 2.7 to 3.7 kg were fed ad libitum on rabbit chow (Purina Mills). Anesthesia was induced by intramuscular administration of ketamine 40 to 50 mg/kg + xylazine 4 to 5 mg/kg and acepromazine 0.5 mg/kg. Through the median incision, the common bile duct was cannulated with polyethylene tubing (1.19-mm ID and 1.70-mm OD) and the gall bladder was resected. The duodenum was punctured by a needle, and polyethylene tubing (0.58-mm ID and 0.97-mm OD) was inserted into the lumen and fixed by purse-string suture. The femoral vein was cannulated with polyethylene tubing (0.58-mm ID and 0.97-mm OD). All tubing was exteriorized through a subcutaneous channel in the back of the animal. Intravenous tubing was immediately connected to the infusion set, and lactated Ringer's solution with 5% dextrose was administered to prevent dehydration. The infusion rate was adjusted according to the loss of body fluid (usually about 12 mL/h), including loss from the BF. Bile was collected the first half-hour and every 8 hours thereafter in a plastic bag attached to the animal. The animals usually began eating and drinking 24 hours after the operation, and the intravenous infusion was then replaced by 0.9% NaCl 10 mL/h. Cholic acid (Aldrich Chemical) was conjugated with glycine,[21] and the sodium salt of glycocholic acid ([GCA] >98% pure by thin-layer chromatography) was infused intraduodenally for 24 hours after 5 days of bile depletion. The infusion rate was adjusted to be equal to the animal's hourly bile acid flux (determined by measuring the bile acid output within 30 minutes after construction of the BF). Sham-operated control rabbits had intact enterohepatic bile acid circulation.

In a second set of experiments to study the effect of feeding 2% cholesterol for 1 week, the amount of cholesterol to be added to the diet was dissolved in ether (about 100 mL/kg chow) and mixed thoroughly with the basal ground chows (same as already described for each species), followed by evaporation of the ether under a hood overnight. The control chows were similarly treated with ether without the addition of cholesterol. Animals with food intake and weight gain that differed by more than 10% from the average of the control group were excluded from the study (about one of 10 animals fed cholesterol, regardless of species). At least five animals were included in each group.

All animals were killed by exsanguination or anesthesia overdose. The livers were quickly frozen on dry ice and stored at −80°C until needed for sterol analysis and enzyme assays. Determinations of enzyme activities with freshly prepared microsomes/mitochondria from fresh liver specimens showed no significant change with storage. Although the same number of animals were included in each group at the start, the number of animals measured for each group varied because of failure to eat and gain the proper weight, bile obstruction during bile collection, or pooling of the same measurements from more than one group (ie, baseline bile acid output and composition measured with bile collected during the first half-hour include both the BF group and the bile acid–infused BF group before bile acid pool depletion and replacement). The experimental protocols were approved by the Institutional Animal Care and Use Committees of the University of Medicine and Dentistry of New Jersey–New Jersey Medical School and Veterans Affairs Research Service.

Enzyme Assays

Liver specimens were homogenized in 4 vol buffer (250 mmol/L sucrose, 10 mmol/L Tris, and 0.1 mmol/L disodium EDTA, pH 7.2) with a Potter-Elvehjem homogenizer. Aliquots of whole homogenates (200 to 500 μL) were saved for the determination of sterol concentrations, and microsomal and mitochondrial fractions were prepared from the remaining homogenates by differential ultracentrifugation (2,000 to 9,000 × *g* for mitochondria and 10,000 to 100,000 × *g* for microsomes). Protein concentrations in the liver fractions were measured by the method of Lowry et al.[22] Measurements of microsomal cholesterol 7α-hydroxylase and mitochondrial sterol 27-hydroxylase activity were performed by isotope-incorporation methods[1,3] using [4-^{14}C]cholesterol (New England Nuclear-Dupont, Boston, MA) as substrate. The substrate cholesterol was solubilized in either β-cyclodextrin (Molecusol; Pharmatec, Alachua, FL) or Triton X-100 (Sigma Chemical, St Louis, MO). β-Cyclodextrin was used in most of the study, but was replaced by

Triton X-100 in the rabbit cholesterol-feeding experiment because it was no longer manufactured by then. As reported previously by Petrack and Latario,[23] cholesterol 7α-hydroxylase activity was higher with β-cyclodextrin as a solubilizer. However, in each experiment, control and treated animals were measured by the same method. In cholesterol-fed animals in which hepatic cholesterol concentrations increased, cholesterol 7α-hydroxylase activity was measured in a reconstituted system after removal of endogenous cholesterol by acetone treatment and compared with similarly measured control microsomes.[2] In the bile acid infusion experiments, sterol 27-hydroxylase activity was also determined with 5β-cholestane-3α,7α,12α-triol (all three animal species) and 5β-cholestane-3α,7α-diol (guinea pig) as substrates. Unlabeled 5β-cholestane-3α,7α,12α-triol was prepared by electrolytic coupling of cholic acid with isovaleric acid according to Bergström and Krabisch[24] and specifically labeled with ^3H according to the method of Björkhem and Gustafsson.[25] The labeled triol was 92% pure by gas-liquid chromatography and had a specific activity of 2.38 cpm/pmol. 5β-Cholestane-3α,7α-diol was prepared by electrolytic coupling of chenodeoxycholic acid and isovaleric acid[25] and labeled with ^3H according to the method of Björkhem and Gustafsson[25]; it was 89% pure and had a specific activity of 1.70 cpm/pmol. 5β-Cholestane-3α,7α(25R&25S)27-triols and 5β-cholestane-3α,7α,12α(25R&25S)27-tetrols were prepared for use as product markers by the reduction of 3α,7α-dihydroxycoprostanoic acid and 3α,7α,12α-trihydroxycoprostanoic acid,[26] which are isolated from the bile of *Alligator mississippiensis*.[27] Products from the reactions with [^3H]5β-cholestane-3α,7α-diol were separated on Silica Gel 60 plates (EM Science, Gibbstown, NJ) developed with chloroform:acetone:methanol (70:50:7 vol/vol/vol). Products from the reactions with [^3H]5β-cholestane-3α,7α,12α-triol were separated on Silica Gel 60 plates developed twice with chloroform:acetone:methanol (70:50:8 vol/vol/vol). The bands of $Rf_{0.43}$ containing 5β-cholestane-3α,7α,12α(25R&25S)27-tetrols and 5β-cholestane-3α,7α,12α,24S-tetrol were eluted with 30 mL ethyl acetate:methanol (85:15 vol/vol), evaporated, redissolved in 50 μL methanol, and then separated on neutral alumina plates that were developed with benzene:ethyl acetate:methanol (90:20:14 vol/vol/vol). All labeled products were quantified by liquid scintillation counting. The formation of 27-hydroxylated products, expressed as picomoles per milligram of protein per minute, included both the 25R and 25S epimers.

Hepatic Cholesterol and Biliary Bile Acid Concentrations

Cholesterol in liver whole homogenates was extracted with hexane after saponification in 1N ethanolic NaOH and determined by gas-liquid chromatography as previously reported.[28] Biliary bile acids were deconjugated and methylated, and the methyl ester trimethylsilyl ether derivatives were quantified by capillary gas-liquid chromatography as described previously.[2] Tauroursodeoxycholic acid was used as an internal standard for the rat, and glycoursocholic acid for the guinea pig and rabbit.

Statistical Analysis

Differences between mean values in different treatment groups were analyzed by one-way ANOVA and a comparison of confidence intervals for the means.[29]

RESULTS

Effects of Bile Acid Depletion and Replacement

Table 1 shows biliary bile acid output and composition in BF animals with depleted bile acid pools and BF animals infused with the major bile acids at rates equivalent to the hepatic bile acid flux for the rat, guinea pig, and rabbit. At baseline, biliary bile acids included both primary bile acids (cholic acid and chenodeoxycholic acid) and secondary bile acids formed via gut microflora 7-dehydroxylase activity. Upon depletion of the bile acid pool (2 to 5 days of bile diversion through the BF), only bile acids formed endogenously in the liver were detected in the bile of BF animals and biliary bile acid output was significantly lower than the baseline level ($P < .01$) and equivalent to new synthesis. The major bile acid detected in the BF rat and rabbit was the trihydroxy bile acid, cholic acid, whereas in the BF guinea pig, the predominant bile acid was the dihydroxy bile acid, chenodeoxycholic acid. When the major bile acids were reinfused in the BF animals to replace the depleted bile acid pool, bile acid output was expanded to baseline levels and the infused bile acids became predominant.

Depletion and replacement of the bile acid pool with TCA, GCDCA, and GCA in the rat, guinea pig, and rabbit, respectively, did not change hepatic mitochondrial sterol 27-hydroxylase (conversion of cholesterol to 27-hydroxycholesterol) activity (Fig 1). The same lack of effect of bile acids on sterol 27-hydroxylase activity was observed in all three animal models when sterol 27-hydroxylase activity was assayed with 5β-cholestane-3α,7α,12α-triol as substrate. Furthermore, in the guinea pig, where the dihydroxy bile acid chenodeoxycholic

Table 1. Biliary Bile Acid Output and Composition in BF Animals With and Without Bile Acid Replacement

Animal	Treatment	Total Bile Acid Output (mg/h)	Bile Acid Composition (%)						
			CA	CDCA	DCA	UCA	UDCA	MCA	7KLCA
Rat	Baseline	18.2 ± 1.9	67	11	5	4	—	13	—
	BF (2 d)	4.2 ± 1.0*	63	26	—	—	—	11	—
	BF (2 d) + TCA (24 h)	22.5 ± 4.7	92	3	—	—	—	4	—
Guinea pig	Baseline	12.9 ± 0.4	—	58	—	—	7	—	35
	BF (3 d)	1.0 ± 0.1*	—	90	—	—	1	—	9
	BF (3 d) + GCDCA (24 h)	9.9 ± 1.7		84	—	—	2	—	14
Rabbit	Baseline	33.9 ± 3.1	10	—	90	—	—	—	—
	BF (5 d)	6.8 ± 0.4*	98	—	2	—	—	—	—
	BF (5 d) + GCA (24 h)	37.0 ± 5.5	95	—	5	—	—	—	—

NOTE. Output is expressed as the mean ± SEM. Four to 9 animals were measured in each group at baseline (bile collected the first 30 minutes after creating BF), after complete bile depletion by collecting bile for 2 to 5 days via the BF, or after complete bile depletion and intraduodenal infusion of the major bile acids for the tested animals.

Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; UCA, ursocholic acid; UDCA, ursodeoxycholic acid; MCA, α-muricholic acid and β-muricholic acid; 7KLCA, 7-ketolithocholic acid.

*$P < .01$ *v* baseline.

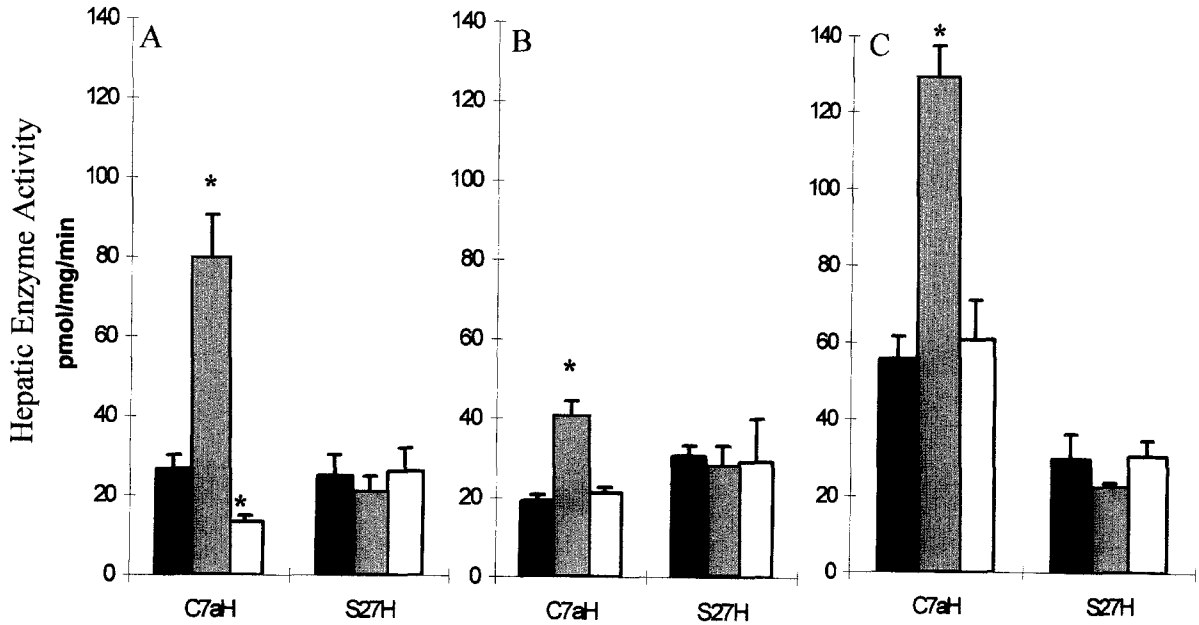

Fig 1. Effects of bile acid depletion and replacement on hepatic cholesterol 7α-hydroxylase (C7aH) and sterol 27-hydroxylase (S27H) activities in the rat (A), guinea pig (B), and rabbit (C). Both enzymes were measured with cholesterol as substrate. Animals either were sham-operated (intact enterohepatic bile acid circulation, ■), had the bile acid pool depleted via BF (▨), or had bile acid depletion followed by intraduodenal infusion of the major bile acids (TCA, GCDCA, and GCA for the rat, guinea pig, and rabbit, respectively, □). *Significantly different *v* sham-operated animals ($P < .01$).

acid is the major bile acid synthesized, sterol 27-hydroxylase activity did not change with various bile acid manipulations when the enzyme was assayed with 5β-cholestane-3α,7α-diol (Table 2). Sterol 27-hydroxylase activity was fourfold and twofold higher with 5β-cholestane-3α,7α-diol and 5β-cholestane-3α,7α,12α-triol as substrates versus cholesterol, but remained constant when the bile acid pools were depleted and replaced with the major bile acids. In contrast, cholesterol 7α-hydroxylase activity was regulated by hepatic bile acid flux: in all three animal models, cholesterol 7α-hydroxylase activity was significantly upregulated by bile acid pool depletion in BF animals, and was depressed to the lower levels observed at baseline when the bile acid pool in BF animals was replaced with the infused bile acids (Fig 1).

Effects of Cholesterol Feeding

The effects of dietary cholesterol on cholesterol 7α-hydroxylase and sterol 27-hydroxylase activities are shown in Fig 2. Sterol 27-hydroxylase activity, measured in the rat, guinea pig, and rabbit with cholesterol as substrate, was stimulated about twofold by feeding 2% cholesterol for 1 week. Cholesterol feeding for 1 week significantly increased cholesterol 7α-hydroxylase activity (+42%, $P < 05$) in the rat, produced no change in the guinea pig, and was associated with

Table 2. Sterol 27-Hydroxylase Activity in Bile Acid–Infused BF Animals Measured With Different Substrates

Animal	Treatment	Sterol 27-Hydroxylase Activity (pmol/mg/min)		
		Cholesterol	5β-Cholestane-3α,7α-Diol	5β-Cholestane-3α,7α,12α-Triol
Rat	Sham	25.1 ± 5.3	NA	70.1 ± 4.3*
	BF (2 d)	21.0 ± 4.0	NA	73.4 ± 7.6*
	BF (2 d) ± TCA (24 h)	26.4 ± 5.9	NA	83.3 ± 8.5*
Guinea pig	Sham	30.6 ± 2.6	136.1 ± 21.0*†	71.3 ± 8.9*
	BF (3 d)	27.8 ± 5.4	137.7 ± 24.2*‡	63.6 ± 4.7*
	BF (3 d) ± GCDCA (24 h)	29.1 ± 10.9	131.2 ± 22.6*†	69.5 ± 13.6
Rabbit	Sham	29.6 ± 6.6	NA	36.4 ± 2.9
	BF (5 d)	22.2 ± 1.2	NA	36.2 ± 4.0*
	BF (5 d) ± GCA (24 h)	30.3 ± 4.2	NA	41.3 ± 4.7

NOTE. Results are the mean ± SEM. Three to 7 animals were measured in each group. The animals either were sham-operated, had bile acid pools depleted via BF, or had bile acid pool depletion followed by intraduodenal infusion of the major bile acids for the tested animals.

Abbreviation: NA, not available.

*Significantly higher *v* catalytic activity with cholesterol as substrate, $P < .01$.

†Significantly higher *v* catalytic activity with 5β-cholestane-3α,7α,12α-triol as substrate, $P < .05$.

‡Significantly higher *v* catalytic activity with 5β-cholestane-3α,7α,12α-triol as substrate, $P < .01$.

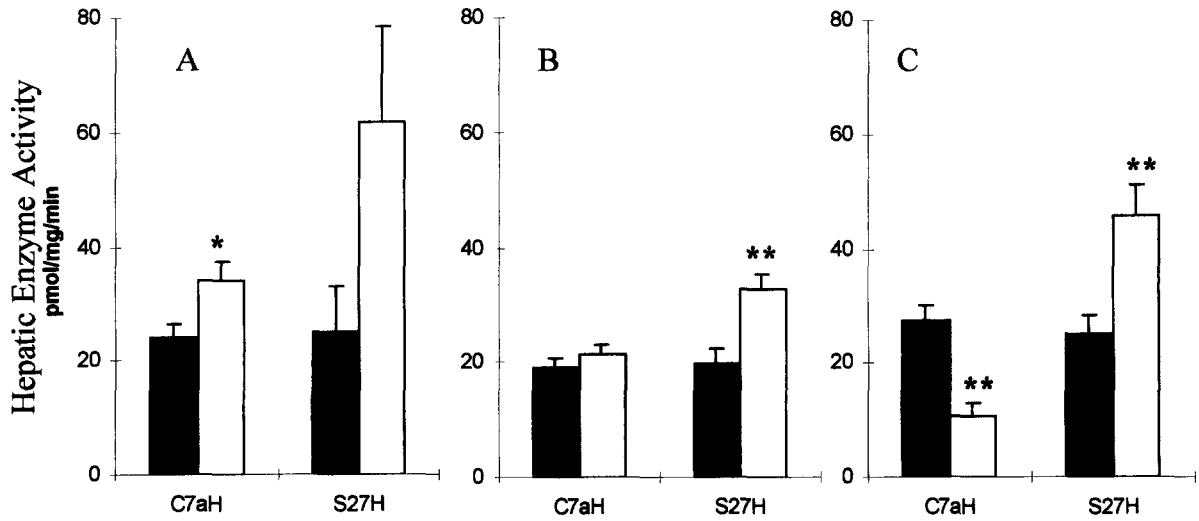

Fig 2. Effects of dietary cholesterol on hepatic cholesterol 7α-hydroxylase (C7aH) and sterol 27-hydroxylase (S27H) activities in the rat (A), guinea pig (B), and rabbit (C). Both enzymes were measured with cholesterol as substrate. Animals were fed for 1 week with either a control chow (■) or the same chow supplemented with 2% cholesterol (□). Significantly different *v* control animals: *$P < .05$; **$P < .01$.

an inhibition of cholesterol 7α-hydroxylase activity (−62%, $P < .01$) in the rabbit.

Hepatic Cholesterol Concentrations

Bile acid pool depletion and replacement produced no significant change in hepatic cholesterol concentrations. In contrast, feeding cholesterol significantly increased hepatic cholesterol, relative to baseline levels in control animals ($P < .01$). Feeding 2% cholesterol for 1 week increased hepatic cholesterol concentrations in the rat, guinea pig, and rabbit threefold, twofold, and eightfold, respectively, compared with control chow-fed animals (Table 3).

DISCUSSION

The results of this study demonstrate that (1) sterol 27-hydroxylase activity did not change with bile acid depletion and replacement in the rat, guinea pig, and rabbit, in contrast to cholesterol 7α-hydroxylase activity, which was regulated by the hepatic bile acid flux; (2) sterol 27-hydroxylase activity remained constant with bile acid depletion and replacement whether cholesterol, 5β-cholestane-3α,7α-diol, or 5β-cholestane-3α,7α,12α-triol was used as substrate; (3) feeding 2% cholesterol for 1 week increased hepatic cholesterol concentrations in all three animal models, especially guinea pigs and rabbits; and (4) increased hepatic cholesterol levels were associated with stimulated conversion of cholesterol to 27-hydroxycholesterol in all three animal models, but either increased, did not affect, or were associated with inhibited cholesterol 7α-hydroxylase activity in cholesterol-fed rats, guinea pigs, and rabbits, respectively.

The recent demonstration that cholesterol 7α-hydroxylase knockout mice can produce bile acids and survive[30] suggests that an alternative bile acid synthetic pathway that bypasses cholesterol 7α-hydroxylase may be of critical importance. It has been estimated that up to 50% of bile acids may be formed via the alternative bile acid synthetic pathway initiated by sterol 27-hydroxylase.[18,31] Conflicting reports have appeared on whether bile acids regulate sterol 27-hydroxylase activity by a feedback mechanism. Stravitz et al[17] and Twisk et al[18] reported feedback inhibition of sterol 27-hydroxylase activity by bile acids in rat hepatocyte cultures, and Vlahcevic et al[16] reported the regulation of this enzyme in rats by bile acid and cholestyramine feeding. In contrast, Shefer et al[3] and Araya et al[19] reported no change in sterol 27-hydroxylase activity and mRNA levels in bile acid–treated rats and rabbits, respectively. In this study, three different animal models with a different composition of biliary bile acids showed no effect of hepatic bile acid

Table 3. Effects of Bile Acids and Cholesterol on Hepatic Cholesterol Concentrations

Animal	Treatment	Hepatic Cholesterol (mg/g liver)
Infusion experiments		
Rat	Sham	2.34 ± 0.20
	BF (2 d)	2.35 ± 0.20
	BF (2 d) + TCA (24 h)	2.52 ± 0.16
Guinea pig	Sham	1.68 ± 0.23
	BF (3 d)	1.97 ± 0.17
	BF (3 d) + GCDCA (24 h)	1.68 ± 0.15
Rabbit	Sham	2.53 ± 0.84
	BF (5 d)	2.49 ± 0.38
	BF (5 d) + GCA (24 h)	2.74 ± 0.30
Feeding experiments		
Rat	Control	2.36 ± 0.23
	2% cholesterol	7.03 ± 1.31*
Guinea pig	Control	1.90 ± 0.15
	2% cholesterol	3.47 ± 0.30*
Rabbit	Control	2.53 ± 0.84
	2% cholesterol	19.20 ± 1.22*

NOTE. Results are the mean ± SEM. Four to 9 animals were measured in each group. The animals either were sham-operated, had bile acid pools depleted via BF, or had bile acid pool depletion followed by intraduodenal infusion of the major bile acids for the tested animals.

*$P < .01$ *v* control.

flux on sterol 27-hydroxylase activity, whether assayed with cholesterol, 5β-cholestane-3α,7α-diol or 5β-cholestane-3α,7α,12α-triol as substrate. It should be noted that the three animal models examined differed not only in their susceptibility to atherosclerosis (rabbits are more susceptible than rats) but also in the predominant bile acids present in the enterohepatic circulation (trihydroxy bile acids in rats and rabbits and dihydroxy bile acids in guinea pigs). Thus, the failure of bile acids to regulate sterol 27-hydroxylase activity was observed with different chemical structures and fluxes of bile acids through the liver (no bile acid flux in BF *v* regular flux in sham or bile acid–infused animals). Since all three tested bile acids (TCA, GCDCA, and GCA) produced no change in sterol 27-hydroxylase activity even under widely varying rates of bile acid flux through the liver, it is likely that this enzyme is not regulated by feedback inhibition by bile acids.

It should be noted that sterol 27-hydroxylase activities are about fourfold and twofold higher with 5β-cholestane-3α,7α-diol and 5β-cholestane-3α,7α,12α-triol as substrates, respectively, as compared with the activity with cholesterol as substrate. This observation is consistent with the belief that sterol 27-hydroxylase catalyzes the 27-hydroxylation of 5β-cholestane-3α,7α-diol and 5β-cholestane-3α,7α,12α-triol in the classic bile acid synthetic pathway in non–rate-limiting reactions. Support for this contention was provided by Björkhem et al,[32] who showed more efficient 27-hydroxylation of 5β-cholestane-3α,7α-diol and 5β-cholestane-3α,7α,12α-triol versus cholesterol in human liver mitochondria.

In contrast to the lack of a regulatory effect of bile acids, cholesterol feeding produced a marked stimulation of 27-hydroxylation of cholesterol, especially in guinea pigs and rabbits. Increases in sterol 27-hydroxylase activity were coupled with significantly higher hepatic cholesterol concentrations. These results are consistent with prior reports on cultured rat hepatocytes[17] and baboon liver.[33,34] Stravitz et al[17] found that the use of 2-hydroxypropyl-β-cyclodextrin to mobilize the cholesterol substrate significantly increased sterol 27-hydroxylase activity in cultured hepatocytes, and the delivery of exogenous cholesterol increased it further. Hasan and Kushwaha[33] and Kushwaha et al[34] reported that baboon hepatic sterol 27-hydroxylase activity and mRNA were induced by a high-cholesterol and high-fat diet. The stimulation of sterol 27-hydroxylase activity with a dietary-induced accumulation of tissue cholesterol suggests a significant role of this enzyme in cholesterol homeostasis, ie, to divert excess tissue cholesterol into a bile acid synthetic pathway.

The effect of cholesterol feeding on cholesterol 7α-hydroxylase is species-dependent, producing a significant increase in the rat, no change in the guinea pig, and a significant decrease in catalytic activity in the rabbit. Rudel et al[35] reported that cholesterol-fed African green monkeys developed plasma hypercholesterolemia associated with elevated hepatic cholesterol concentrations and reduced cholesterol 7α-hydroxylase activity, and a similar observation was made by Horton et al[36] in the hamster. Recently, Xu et al[37] suggested that the inhibition of cholesterol 7α-hydroxylase activity in cholesterol-fed rabbits was due to a cholesterol-induced enlargement of the bile acid pool, which resulted from induction of the sterol 27-hydroxylase–initiated alternative bile acid synthetic pathway but significantly downregulated cholesterol 7α-hydroxylase activity in the classic pathway. The different effects of cholesterol on cholesterol 7α-hydroxylase and bile acid synthesis observed in various studies may be due to the size of the bile acid pool and expression of the bile acid transporter in the tested animals. The presence of the gallbladder may allow the expansion of the bile acid pool in the rabbit and guinea pig, but not in the rat. Thus, the apparent different responses of the rat, guinea pig, and rabbit to cholesterol feeding (increase, no change, and inhibition of cholesterol 7α-hydroxylase) may be due to the net effects of the response of each species to both the substrate stimulatory effect (ie, increased cholesterol 7α-hydroxylase activity with cholesterol availability) and product feedback inhibition (ie, inhibited cholesterol 7α-hydroxylase activity once the bile acid pool has been expanded). The inhibited cholesterol 7α-hydroxylase activity in the rabbit after 1 week of feeding 2% cholesterol may reflect the overpowering effect of the feedback inhibition by bile acids which masks any stimulatory effect of the substrate cholesterol.

Thus, the observation that all three tested bile acids produced no change in sterol 27-hydroxylase activity in all three animal species under widely varying rates of flux to the liver suggests that it is unlikely that this enzyme is regulated by feedback inhibition by bile acids. On the other hand, the stimulation of sterol 27-hydroxylase associated with increased hepatic cholesterol concentrations in all animals tested suggests that this enzyme may have a significant role in controlling tissue cholesterol concentrations by diverting excess amounts to the alternative bile acid pathway that it initiates. In contrast, cholesterol 7α-hydroxylase consistently plays a regulatory role in the classic bile acid synthetic pathway via feedback inhibition by bile acids, but shows species-dependent responses to feeding excess cholesterol for 1 week.

ACKNOWLEDGMENT

The authors thank Eva Paroulek, Susan Hauser, and Bibiana Pcolinsky for valuable technical assistance.

REFERENCES

1. Shefer S, Nguyen LB, Salen G, et al: Feedback regulation of bile-acid synthesis in the rat: Differing effects of taurocholate and tauroursocholate. J Clin Invest 85:1191-1198, 1990

2. Shefer S, Nguyen LB, Salen G, et al: Differing effects of cholesterol and taurocholate on steady state hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase activities and mRNA levels in the rat. J Lipid Res 33:1193-1200, 1992

3. Shefer S, Kren BT, Salen G, et al: Regulation of bile acid synthesis by deoxycholic acid in the rat: Different effects on cholesterol 7α-hydroxylase and sterol 27-hydroxylase. Hepatology 22:1215-1221, 1995

4. Nguyen LB, Shefer S, Salen G, et al: Cholesterol 7α-hydroxylase activities from human and rat liver are modulated in vitro posttranslationally by phosphorylation/dephosphorylation. Hepatology 24:1468-1474, 1996

5. Ness GC, Pendleton LC, Zhao Z: Thyroid hormone rapidly increases cholesterol 7α-hydroxylase mRNA levels in hypophysectomized rats. Biochim Biophys Acta 1214:229-233, 1994

6. Kushwaha RS, Born KM: Effect of estrogen and progesterone on the hepatic cholesterol 7-alpha-hydroxylase activity in ovariectomized baboons. Biochim Biophys Acta 1084:300-302, 1991

7. Javitt NB: Bile acid synthesis from cholesterol: Regulatory and auxiliary pathways. FASEB J 8:1308-1311, 1994

8. Martin KO, Budai K, Javitt NB: Cholesterol and 27-hydroxycholesterol 7α-hydroxylation: Evidence for two different enzymes. J Lipid Res 34:581-588, 1993

9. Princen HMG, Meijer P, Wolthers BG, et al: Cyclosporin A blocks bile acid synthesis in cultured hepatocytes by specific inhibition of chenodeoxycholic acid synthesis. Biochem J 275:501-505, 1991

10. Ayaki Y, Kok E, Javitt NB: Cholic acid synthesis from 26-hydroxycholesterol and 3-hydroxy-5-cholestenoic acid in the rabbit. J Biol Chem 264:3818-3821, 1989

11. Toll A, Shoda J, Axelson M, et al: 7α-Hydroxylation of 26-hydroxycholesterol, 3β-hydroxy-5-cholestenoic acid and 3β-hydroxy-5-cholenoic acid by cytochrome P-450 in pig liver microsomes. FEBS Lett 296:73-76, 1992

12. Lee C, Martin KO, Javitt NB: Bile acid synthesis: 7α-Hydroxylation of intermediates in the sterol 27-hydroxylase metabolic pathway. J Lipid Res 37:1356-1362, 1996

13. Axelson M, Shoda J, Toll A, et al: Enzymatic conversion of 27-hydroxycholesterol to 7α- and 7β-hydroxylated bile acid precursors in human liver microsomes and mitochondria, in Paumgartner G, Stiehl A, Gerok W (eds): Bile Acids and the Hepatobiliary System: From Basic Science to Clinical Practice. Hingham, MA, Kluwer Academic, 1993, pp 50-57

14. Dahlbäck H: Characterization of the liver mitochondrial cytochrome P-450 catalyzing the 26-hydroxylation of 5β-cholestane-3α,7α,12α-triol. Biochem Biophys Res Commun 157:30-36, 1988

15. Dahlbäck-Sjoberg H, Björkhem I, Princen HMG, et al: Selective inhibition of mitochondrial 27-hydroxylation of bile acid intermediates and 25-hydroxylation of vitamin D_3 by cyclosporin. Biochem J 293:203-206, 1993

16. Vlahcevic ZR, Jairath SK, Heuman DM, et al: Transcriptional regulation of hepatic sterol 27-hydroxylase by bile acids. Am J Physiol 33:G646-G652, 1996

17. Stravitz RT, Vlahcevic ZR, Russell TL, et al: Regulation of sterol 27-hydroxylase and alternative pathway of bile acid biosynthesis in primary cultures of rat hepatocytes. J Steroid Biochem Mol Biol 57:337-347, 1996

18. Twisk J, Eli CMW, Princen HMG: Suppression of sterol 27-hydroxylase mRNA and transcriptional activity by bile acids in cultured hepatocytes. Biochem J 305:505-551, 1995

19. Araya Z, Wikvall K: Different effects on the expression of cyp7 and cyp27 in rabbit liver by cholic acid and cholestyramine. Biochem Biophys Res Commun 216:868-873, 1995

20. Heuman DM, Hernandez CR, Hylemon PB, et al: Regulation of bile acid synthesis. I. Effects of conjugated ursodeoxycholate and cholate on bile acid synthesis in chronic bile fistula rats. Hepatology 8:358-365, 1988

21. Tserng KY, Hackey DL, Klein PD: An improved procedure for the synthesis of glycine and taurine conjugates of bile acids. J Lipid Res 18:404-407, 1977

22. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951

23. Petrack B, Latario BJ: Synthesis of 27-hydroxycholesterol in rat liver mitochondria: HPLC assay and marked activation by exogenous cholesterol. J Lipid Res 34:643-649, 1993

24. Bergström S, Krabisch L: Preparation of some hydroxy coprostanes, 3α,7α- and 3α,12α-dihydroxycoprostane. Acta Chem Scand 11:1067, 1957

25. Björkhem I, Gustafsson J: ω-Hydroxylation of steroid side chain in biosynthesis of bile acids. Eur J Biochem 36:201-212, 1973

26. Dayal B, Shefer S, Tint GS, et al: Synthesis of 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24,25-pentol. J Lipid Res 17:74-77, 1976

27. Tint GS, Dayal B, Batta AK, et al: Biliary bile acids, bile alcohols, and sterols of *Alligator mississippiensis.* J Lipid Res 21:110-117, 1980

28. Xu G, Salen S, Shefer S, et al: Unexpected inhibition of cholesterol 7α-hydroxylase by cholesterol in New Zealand white and Watanabe heritable hyperlipidemic rabbits. J Clin Invest 95:1497-1504, 1995

29. Gardiner MJ, Altman DG: Calculating confidence intervals for regression and correlation, in Gardiner MJ, Altman DG (eds): Statistics With Confidence. London, UK, British Medical Association, 1989, pp 34-39

30. Schwarz M, Lund EG, Setchell KDR, et al: Disruption of cholesterol 7α-hydroxylase gene in mice. II. Bile acid deficiency is overcome by induction of oxysterol 7α-hydroxylase. J Biol Chem 271:18024-18031, 1996

31. Princen HMG, Meijer P, Wolthers BG, et al: Cyclosporin blocks bile acid synthesis in cultured hepatocyte by specific inhibition of chenodeoxycholic acid synthesis. Biochem J 275:501-505, 1991

32. Björkhem I, Gustafsson J, Johansson G, et al: Biosynthesis of bile acids in man: Hydroxylation of the C_{27}-steroid side chain. J Clin Invest 55:478-486, 1975

33. Hasan SQ, Kushwaha RS: Differences in 27-hydroxycholesterol concentrations in plasma and liver of baboons with high and low responses to dietary cholesterol and fat. Biochim Biophys Acta 1182:299-302, 1993

34. Kushwaha RS, Guntupalli B, Rice KS, et al: Effect of dietary cholesterol and fat on the expression of hepatic sterol 27-hydroxylase and other hepatic cholesterol-responsive genes in baboon (*Papio* species). Arterioscler Thromb Vasc Biol 15:1404-1411, 1995

35. Rudel L, Deckelman C, Wilson M, et al: Dietary cholesterol and downregulation of cholesterol 7α-hydroxylase and cholesterol absorption in African green monkeys. J Clin Invest 93:2463-2472, 1994

36. Horton JD, Cuthbert JA, Spady DK: Regulation of hepatic 7α-hydroxylase expression and response to dietary cholesterol in the rat and hamster. J Biol Chem 270:5381-5387, 1995

37. Xu G, Salen G, Shefer S, et al: Increased bile acid pool inhibits cholesterol 7α-hydroxylase in cholesterol-fed rabbits. Gastroenterology 113:1958-1965, 1997