Regulation of bile acid synthesis in the rat: relationship between hepatic cholesterol 7α-hydroxylase activity and portal bile acids

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Abstract Bile acid biosynthesis has been believed to be regulated by negative feedback control; however, recent experiments have cast considerable doubts on the concept. The aim of the study was to examine the consensus of the negative feedback regulation of bile acids by clarifying the correlation between the portal bile acids and the rate-limiting enzyme of bile acid biosynthesis, hepatic microsomal cholesterol 7α-hydroxylase. We measured the enzyme activity and the portal bile acids in male Wistar rats that were orally administered three different bile acids or cholestyramine for 2 weeks. The serum level of 7α-hydroxycholesterol was also determined to verify whether it would be a parameter of bile acid synthesis rate in the rat. The activity of cholesterol 7α-hydroxylase increased about threefold in rats treated with cholestyramine when compared with controls. On the other hand, in rats fed ursodeoxycholic, chenodeoxycholic, and deoxycholic acids, the enzyme activities decreased to 40%, 26%, and 28%, respectively. Treatment with cholestyramine had no significant effect on the portal bile acid concentration. Administration of ursodeoxycholic and chenodeoxycholic acids resulted in a significant increase in the concentration of portal bile acids, whereas deoxycholic acid feeding did not significantly affect it. In the control group, conjugated cholic acid constituted a major part of the portal bile acids while the administered bile acid and its metabolites became predominant in each bile acid feeding group. Treatment with ursodeoxycholic acid made the portal bile acids more hydrophilic, but, by contrast, administration of chenodeoxycholic, deoxycholic acids, and cholestyramine made the portal bile acids more hydrophobic. There was no significant correlation between the enzyme activities and either concentrations, composition, or overall hydrophobicity indices of the portal bile acids. Serum concentrations of 7α-hydroxycholesterol significantly correlated with the activities of the cholesterol 7α-hydroxylase ($r = 0.860$, $P < 0.01$). We conclude that bile acid synthesis is not regulated directly by the portal bile acids returning to the liver and that the serum level of 7α-hydroxycholesterol would be a good indicator of bile acid biosynthesis rate in the rat.—**Fukushima, K., H. Ichimiya, H. Higashijima, H. Yamashita, S. Kuroki, K. Chijiiwa, and M. Tanaka.** Regulation of bile acid synthesis in the rat: relationship between hepatic cholesterol 7α-hydroxylase activity and portal bile acids. *J. Lipid Res.* 1995. **36:** 315–321.

Supplementary key words hydrophobicity • serum 7α-hydroxycholesterol

Bile acid synthesis is a major pathway for the elimination of cholesterol and its regulation plays an important role in maintaining cholesterol and bile acid homeostasis. The first and rate-limiting step in the major pathway for conversion from cholesterol into bile acids is catalyzed by hepatic microsomal cholesterol 7α-hydroxylase (EC 1.14.13.17) (1). Several investigators have reported that depletion of bile acids by biliary drainage or dietary administration of cholestyramine increases the enzyme activity and bile acid synthesis (2–6). In contrast, administration of cholic acid (CA) (4, 7, 8) or chenodeoxycholic acid (CDCA) (4, 9) decreases both enzyme activity and bile acid synthesis. It has been the belief that the activity of cholesterol 7α-hydroxylase is regulated by bile acids returning to the liver.

However, recent studies in vitro and in vivo are not in agreement with the classical concept of negative feedback of bile acid and regulation of bile acid synthesis has become an area of controversy. For example, several groups of investigators could not demonstrate the inhibition of bile acid synthesis in bile fistula rats (10, 11), suggesting that bile acids may not regulate cholesterol 7α-hydroxylase directly. Pandak et al. (12) recently reported that administration of bile acid intraduodenally, but not intravenously, down-regulated cholesterol 7α-hydroxylase activity. On the other hand, Åkerlund and Björkhem (13) found that thoracic duct drainage led to up-regulation of bile acid synthesis, suggesting that a factor in lymph

Abbreviations: UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; HDCA, hyodeoxycholic acid; α-MCA, α-muricholic acid; CA, cholic acid; β-MCA, β-muricholic acid; ω-MCA, ω-muricholic acid; GLC, gas–liquid chromatography; GLC–MS, gas–liquid chromatography–mass spectrometry; DMES, dimethylethylsilyl; TEAP-LH-20, triethylaminohydroxypropyl-Sephadex LH-20.

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might regulate the cholesterol 7α-hydroxylase activity in vivo. In addition, increased bile acid synthesis in bile duct-ligated rats, despite high concentration of bile acids in the liver, is difficult to explain the negative feedback mechanism of bile acid synthesis (14, 15). In in vitro studies, absence of negative feedback control of bile acid biosynthesis in cultured or freshly suspended hepatocytes has been reported (16–18), whereas more recent studies have shown down-regulation of cholesterol 7α-hydroxylase activity, m-RNA levels, and transcriptional activity by bile acids in primary rat hepatocytes (19, 20).

To our knowledge, there is little information about the relationship between the portal bile acids and bile acid synthesis rate in the rat; nevertheless, this rodent has been extensively used for the investigation of bile acid metabolism and regulation. In the present study, we measured the hepatic cholesterol 7α-hydroxylase activity and the concentration and composition of bile acids in the portal vein in rats orally administered three kinds of bile acids or cholestyramine to determine whether the enzyme activity is regulated by the portal bile acids. In addition, a relationship between the enzyme activity and serum level of 7α-hydroxycholesterol was examined.

MATERIALS AND METHODS

Materials

All solvents used were analytical grade or distilled prior to use. Triethylaminohydroxypropyl-Sephadex LH-20 (TEAP-LH-20) was prepared as described by Axelson, Mörk, and Sjövall (21). Cholestyramine (Questran®) was obtained from Bristol-Myers Squibb Co., Ltd., Tokyo, Japan. Deoxycholic acid (DCA), CA, and CDCA were purchased from Sigma (St. Louis, MO). Ursodeoxycholic acid (UDCA) was from Tokyo Tanabe Co., Ltd., (Tokyo, Japan). Bile acids used in this study were checked for purity by thin-layer chromatography on precoated silica gel G plates (thickness, 0.2 mm; Merck, Darmstadt, West Germany) and by gas–liquid chromatography (GLC) as described below. Purities of the bile acids were better than 96%. 7α-Hydroxycholesterol and 5α-cholestane-3β,7β-diol were synthesized as described previously (22). TMSI-H (hexamethyldisilazane-trimethylchlorosilane-pyridine, 2:1:10) was purchased from Gasukuro Kogyo (Tokyo, Japan) and dimethylethylsilyl (DMES)-imidazole was from Tokyo Kasei Kogyo (Tokyo, Japan). NADPH was obtained from Kojin (Tokyo, Japan) and dithiothreitol (DTT) was from Sigma (St. Louis, MO). Bond-Elut silica cartridges (500 mg of silica) were purchased from Analytichem International (Harbor City, CA).

Animal experiments

Male Wistar rats (Kyudo, Fukuoka, Japan), 8 weeks old, weighing between 250 and 300 g, were used. The animals were kept in individual cages and had free access to water and standard laboratory chow (Oriental Yeast, Tokyo, Japan) under a controlled 12 h light–dark cycle. After 1 week of acclimation period, the animals were divided into five groups and fed for 2 weeks *1),* commercial powder chow (control group, n = 10); *2),* chow supplemented with 2% cholestyramine (cholestyramine-fed group, n = 8); *3),* 0.5% UDCA (UDCA-fed group, n = 8); *4),* 0.5% CDCA (CDCA-fed group, n = 8); or *5),* 0.1% DCA (DCA-fed group, n = 8). As 0.5% DCA feeding induced severe hepatotoxicity, this bile acid was fed in a lower concentration. The animals were killed in the mid-dark period. In order to examine the diurnal rhythm, seven animals were killed in the mid-light period (mid-light group). To minimize the variations arising from blood sampling (23), the abdomen was opened under ether anesthesia and 4 ml of portal blood was quickly aspirated by puncture. The liver was excised after exsanguination. The experiments followed the institution's guideline for the care and use of laboratory animals in research.

Equipment

A Shimadzu GC-15A gas–liquid chromatograph (GLC), equipped with a flame ionization detector, a van den Berg's solventless injector, and a data processing system (Chromatopack C-R3A; Shimadzu, Kyoto, Japan), and a Shimadzu Auto GC-MS 9020DF system with a data processing system (SCAP 1123) were used. A fused-silica capillary column (33 m × 0.2 mm I.D.) coated with a 0.25-μm layer of cross-linked methyl silicon (HiCap CBP1; Shimadzu) was used. Conditions for GLC were as follows: column oven temperature 285°C, injection port temperature 305°C, detector temperature 305°C, flow rate of helium carrier gas 2.7 ml/min. Conditions for determination of 7α-hydroxycholesterol by GLC–MS were the same as described previously (22).

Determination of hepatic microsomal cholesterol 7α-hydroxylase activity

Cholesterol 7α-hydroxylase activity was determined as described previously (22). Liver homogenate was prepared in 50 mM Tris-HCl buffer (pH 7.4) containing 0.3 M sucrose, 10 mM DTT, and 10 mM EDTA. The homogenate was centrifuged at 20,000 *g* for 15 min. The microsomal fraction was obtained by centrifugation of 20,000 *g* supernatant fluid at 100,000 *g* for 1 h. The pellet was suspended in homogenizing medium lacking DTT and recentrifuged at 100,000 *g* for 1 h. The resulting microsomal pellet was resuspended in 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA. A small aliquot of microsomal suspension was used for protein determination by the method of Lowry et al. (24). The standard assay system consisted of 0.5 ml of the microsomal preparation corresponding to 0.5–1.0 mg of protein and 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA and 1

mM NADPH in a total volume of 1.0 ml. The enzyme reaction was conducted for 15 min at 37°C. The reaction was terminated by addition of 10 ml chloroform-methanol 2:1 (v/v). 5α-Cholestane-3β,7β-diol was added as an internal standard. After extraction with chloroform-methanol 2:1 (v/v), the organic phase was evaporated to dryness under a stream of nitrogen. After purification with a Bond-Elut silica cartridge column (22) and as TMS derivatization, actual mass of 7α-hydroxycholesterol was analyzed by GLC-MS. In the selected ion monitoring mode, the ion at *m/z* 456 (M-90) was scanned for the TMS ether derivative of 7α-hydroxycholesterol and at *m/z* 458 (M-90) for that of the internal standard.

Assay of serum 7α-hydroxycholesterol

Serum concentration of 7α-hydroxycholesterol was determined by GLC-MS as described previously (25). In brief, 200 pmol 5α-cholestane-3β,7β-diol dissolved in 50 μl ethanol was added to 0.2 ml serum as the internal standard. After addition of 0.7 ml 0.9% NaCl solution and 1.8 ml ethanol, sterols were extracted with n-hexane. The collected n-hexane layer was evaporated to dryness under a stream of nitrogen. Sample was then hydrolyzed in 2.0 ml 3% KOH in 90% ethanol at 55°C for 45 min. After addition of 1.2 ml 0.9% NaCl solution, the sterols were extracted with n-hexane and the solvent was evaporated under nitrogen. After the purification and derivatization of the sample (26), its TMS derivative was analyzed by GLC-MS as described above.

Determination of the portal bile acids

Portal bile acids were determined according to the method previously described (27) with a minor modification. Briefly, 1 ml serum was diluted with one volume aqueous triethylamine sulfate (pH 7.0), followed by extraction on a octadecylsilane-bonded silica column at 64°C. After washing with water, bile acids were eluted with 95% aqueous methanol. The extract was passed through a column of a lipophilic anion exchange, TEAP-LH-20 in bicarbonate form. After washing to remove sterols and neutral lipids, free bile acids were eluted with 4 ml 0.15 M acetic acid in 95% aqueous methanol and then conjugated bile acids (glycine and taurine conjugated bile acids) were eluted with 7 ml 0.30 M acetic acid in 95% aqueous methanol (pH 6.6). The free bile acids were esterified with 5% ethanolic hydrochloric acid and silylated with DMES-imidazole (28). Conjugated bile acids were subjected to enzymatic hydrolysis (29) and then derivatized as mentioned above. Bile acid composition in each fraction was determined by GLC. The analysis of bile acid methyl ester TMS ethers on the widely used nonpolar phased column was not convenient for the present study, because the profile of bile acids in the rat plasma was complicated and the base line separation of the major bile acids derivatives was not obtained. The retention indices of the bile acid ethyl ester DMES ethers determined using C32-C40 n-alkanes were as follows: lithocholic acid (LCA), 3346; DCA, 3484; CDCA, 3514; hyodeoxycholic acid (HDCA), 3532; UDCA, 3543; α-muricholic acid (α-MCA), 3591; CA, 3632; β-muricholic acid (β-MCA), 3690; ω-muricholic acid (ω-MCA), 3773.

TABLE 1. Hepatic cholesterol 7α-hydroxylase activity

Group	n	Cholesterol 7α-Hydroxylase Activity
		pmol / min mg protein
Control	10	45.3 ± 2.7
Mid-light	7	20.9 ± 3.1[a]
Cholestyramine	8	139.7 ± 10.6[a]
UDCA	8	27.0 ± 2.6[a]
CDCA	8	33.4 ± 4.3[b]
DCA	8	32.5 ± 2.5[a]

Animals were killed at mid-dark, except mid-light group. Data are expressed as mean ± SEM.
[a] $P < 0.01$ vs. Control.
[b] $P < 0.05$ vs. Control.

TABLE 2. Concentration of the portal bile acid

Group	n	Free	Conj	Total
		μmol/l	*μmol/l*	*μmol/l*
Control	10	2.4 ± 0.4	13.1 ± 3.0	15.6 ± 3.0
Mid-light	7	15.9 ± 10.7	22.0 ± 7.1	37.8 ± 13.1
Cholestyramine	8	2.2 ± 0.3	17.8 ± 4.6	20.0 ± 4.8
UDCA	8	13.0 ± 0.8[a]	13.6 ± 1.9	26.6 ± 2.2[b]
CDCA	8	11.9 ± 1.7[a]	48.7 ± 15.5[b]	60.5 ± 16.6[a]
DCA	8	5.0 ± 0.6[a]	34.5 ± 12.5	39.5 ± 13.0

Free, free bile acid; Conj, glycine- and taurine-conjugated bile acid. Data are expressed as mean ± SEM.
[a] $P < 0.01$ vs. Control.
[b] $P < 0.05$ vs. Control.

Hydrophobicity index of the portal bile acids

Hydrophobicity indices of the portal bile acids were calculated using the values reported by Heuman (30).

Statistical analysis

Data are presented as mean ± SEM. The statistical differences were analyzed by unpaired Student's *t*-test. Linear regression analysis was performed as described previously (31).

RESULTS

Animals

There were no significant differences in initial and final body weights and food intake among the groups.

Hepatic microsomal cholesterol 7α-hydroxylase activity

Activities of the cholesterol 7α-hydroxylase are shown in **Table 1.** In the control group, the cholesterol 7α-

TABLE 3. Composition of individuals bile acid in the portal blood

Group	LCA	DCA	CDCA	HDCA	UDCA	α-MCA	CA	β-MCA	ω-MCA
Control									
Free	0.5 ± 0.1	6.3 ± 2.5	0.2 ± 0.1	5.3 ± 1.8	0.1 ± 0.1	0.3 ± 0.2	4.2 ± 1.7	N.D	0.4 ± 0.4
Conj	N.D	4.1 ± 0.5	4.1 ± 0.5	2.2 ± 0.3	0.6 ± 0.1	1.9 ± 0.3	51.5 ± 4.2	6.4 ± 1.3	0.2 ± 0.1
Mid-light									
Free	0.3 ± 0.1	3.5 ± 0.6	1.6 ± 1.2	4.1 ± 1.3	0.3 ± 0.2	0.9 ± 0.6	17.3 ± 8.2	0.2 ± 1.3	0.2 ± 0.1
Conj	N.D	2.3 ± 0.5	2.1 ± 0.3[b]	2.5 ± 0.8	0.7 ± 0.2	1.2 ± 0.3	44.2 ± 9.5	7.5 ± 1.9	0.3 ± 0.2
Cholestyramine									
Free	3.4 ± 1.1[a]	6.2 ± 1.6	0.7 ± 0.5	0.7 ± 0.3[a]	N.D	N.D	2.5 ± 0.8	N.D	3.3 ± 0.9[b]
Conj	0.1 ± 0.1	1.7 ± 0.2[b]	9.8 ± 1.1[b]	0.3 ± 0.2[b]	0.6 ± 0.1	3.3 ± 0.5[b]	59.4 ± 3.2	2.1 ± 0.5[b]	N.D
UDCA									
Free	9.5 ± 1.1[a]	2.1 ± 0.3	0.6 ± 0.1[b]	1.4 ± 0.2	32.9 ± 2.4[a]	0.1 ± 0.1	0.2 ± 0.1[a]	0.3 ± 0.1	0.2 ± 0.1
Conj	1.2 ± 0.2[a]	1.8 ± 0.2[b]	2.4 ± 0.2[b]	0.4 ± 0.2[b]	29.6 ± 3.0[a]	0.5 ± 0.1[b]	5.1 ± 0.5[a]	2.2 ± 0.4[b]	0.1 ± 0.1
CDCA									
Free	3.1 ± 1.9[a]	1.0 ± 0.3	19.2 ± 4.0[a]	0.2 ± 0.1[a]	1.1 ± 0.3[b]	0.7 ± 0.2	0.2 ± 0.1[a]	1.0 ± 0.3	0.2 ± 0.1
Conj	1.2 ± 1.0	0.9 ± 0.1[b]	36.8 ± 2.8[a]	0.1 ± 0.1[b]	4.2 ± 0.5[a]	9.7 ± 1.7[b]	3.6 ± 0.3[a]	8.8 ± 1.8	0.1 ± 0.0
DCA									
Free	0.5 ± 1.0	9.0 ± 2.0	0.1 ± 0.1	2.4 ± 0.5	N.D	0.1 ± 0.1	1.6 ± 0.5	0.2 ± 0.1	N.D
Conj	N.D	19.8 ± 0.8[a]	2.8 ± 0.5	2.0 ± 0.5	0.5 ± 0.4	1.5 ± 0.1	47.1 ± 2.2	4.5 ± 0.7	0.1 ± 0.1

Free, free bile acid; Conj, glycine- and taurine-conjugated bile acids; N.D, not detected. Data are expressed as % and mean ± SEM.
[a] $P < 0.01$ vs. Control.
[b] $P < 0.05$ vs. Control.

hydroxylase activity was 45.3 ± 2.7 pmol/min mg protein. The mean enzyme activity in the mid-light group was about 50% of that in the control group (mid-dark). Cholestyramine feeding increased the enzyme activity about threefold and administration of UDCA, DCA, and CDCA decreased the enzyme activities by 40%, 28%, and 26%, respectively.

Portal bile acid analysis

Results of portal bile acid analyses are summarized in **Table 2** and **Table 3.** There were no significant differences in the portal bile acid concentration between the control and cholestyramine-fed groups. Concentration of the portal bile acids was increased significantly in the UDCA-fed and CDCA-fed groups compared with the control group. Administration of DCA increased concentration of the portal bile acids, although there was no statistical difference. In the control rats, conjugated CA constituted major part of the portal bile acids. The administered bile acids and their metabolites were significantly increased in the UDCA-fed, CDCA-fed, and DCA-fed groups.

Hydrophobicity indices of the portal bile acids

Hydrophobicity indices of the portal bile acids are shown in **Table 4.** No significant difference was observed between the mid-dark and mid-light. Treatment with UDCA made the portal bile acids more hydrophilic and the portal bile acids became significantly hydrophobic in the cholestyramine-, CDCA-, and DCA-treated groups.

Cholesterol 7α-hydroxylase activity and the portal bile acids

There was no significant correlation between the cholesterol 7α-hydroxylase activities and either concentrations (**Fig. 1,** n = 49, $r = 0.156$, $P = 0.284$), or overall hydrophobicity indices (**Fig. 2,** n = 49, $r = 0.121$, $P = 0.407$) of the portal bile acids.

Cholesterol 7α-hydroxylase activity and serum 7α-hydroxycholesterol

The relationship between the activities of cholesterol 7α-hydroxylase and the serum levels of 7α-hydroxycholesterol is depicted in **Fig. 3.** A significant positive correlation (y = 49 × −663, n = 49, $r = 0.860$, $P < 0.01$) was observed between the two parameters.

TABLE 4. Hydrophobicity index of the portal bile acid

Group	n	Hydrophobicity Index
Control	10	−0.020 ± 0.027
Mid-light	7	−0.029 ± 0.021
Cholestyramine	8	0.097 ± 0.030[b]
UDCA	8	−0.108 ± 0.023[b]
CDCA	8	0.169 ± 0.054[a]
DCA	8	0.153 ± 0.017[a]

Data are expressed as mean ± SEM.
[a] $P < 0.01$ vs. Control.
[b] $P < 0.01$ vs. Control.

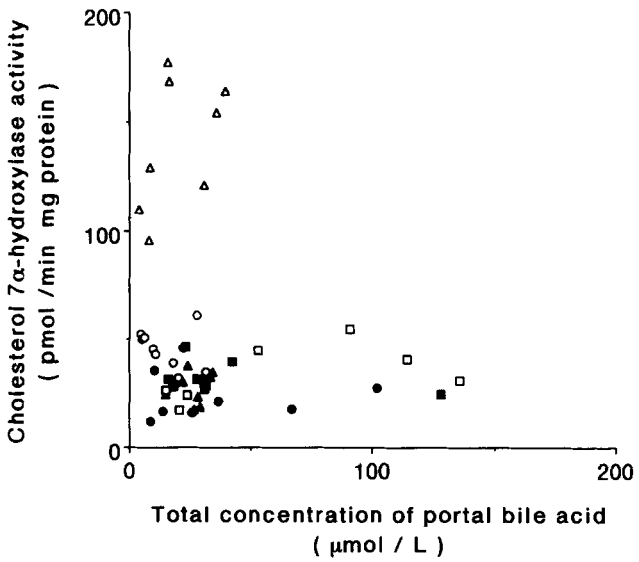

Fig. 1. Correlation between cholesterol 7α-hydroxylase activity and total concentration of portal bile acids. Symbols indicate control group (○), mid-light group (●), cholestyramine-fed group (△), UDCA-fed group (▲), CDCA-fed group (□), and DCA-fed group (■). There was no significant correlation between cholesterol 7α-hydroxylase activity and total concentration of portal bile acids; n = 49; r = 0.156; P = 0.284.

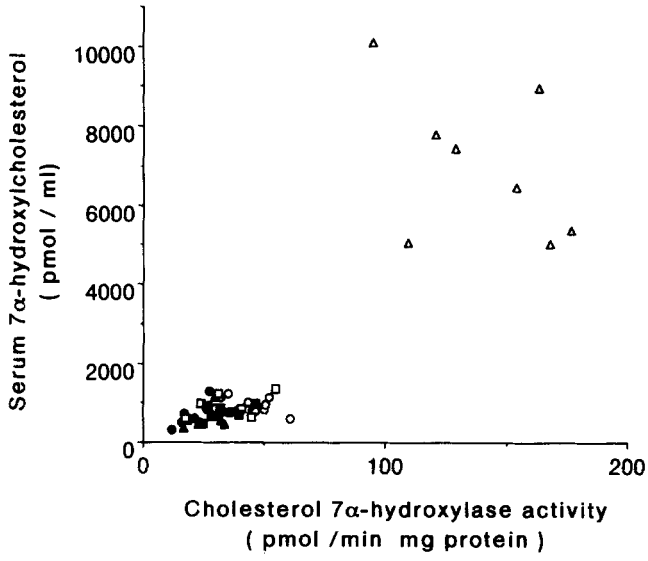

Fig. 3. Correlation between cholesterol 7α-hydroxylase activity and serum level of 7α-hydroxycholesterol. Symbols indicate control group (○), mid-light group (●), cholestyramine-fed group (△), UDCA-fed group (▲), CDCA-fed group (□), and DCA-fed group (■). There was a significant positive correlation between the two parameters; y = 49 × −663; n = 49; r = 0.860; P < 0.01.

DISCUSSION

In the present study, treatments with different bile acids or cholestyramine, and the light–dark cycle affected the activities of cholesterol 7α-hydroxylase and the portal bile acids in rats. The enzyme activity of cholesterol 7α-hydroxylase in the mid-dark group was twofold higher than that in the mid-light group. This was in agreement with other reports that bile acid synthesis exhibits a marked diurnal rhythm with a maximum occurring at midnight and a minimum at midday in normal (6, 32), cholestyramine-fed (6), bile fistula (6), or bile acid-treated (33) rats. However, there were no significant differences in the portal bile acid concentration and composition between mid-light and mid-dark.

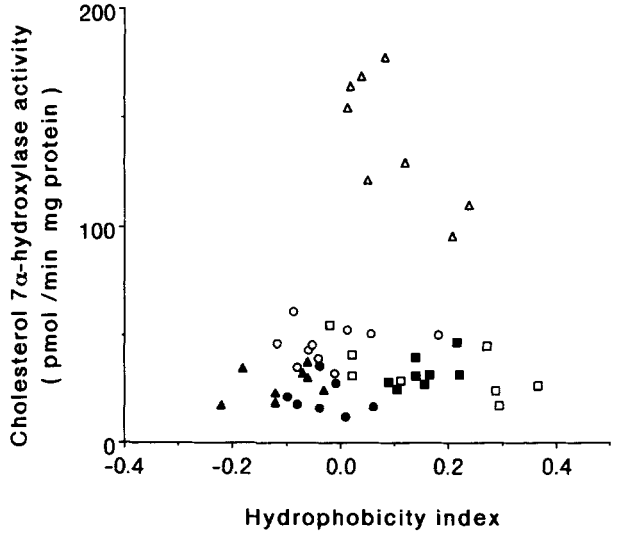

Fig. 2. Correlation between cholesterol 7α-hydroxylase activity and hydrophobicity index. Symbols indicate control group (○), mid-light group (●), cholestyramine-fed group (△), UDCA-fed group (▲), CDCA-fed group (□), and DCA-fed group (■). There was no significant correlation between cholesterol 7α-hydroxylase activity and hydrophobicity index; n = 49; r = 0.121; P = 0.407.

Our present study demonstrated a threefold increase in the enzyme activity after the cholestyramine feeding in agreement with previous studies (6, 32). The amount of bile acids in the portal vein was assumed to be low according to the classical concept of the negative feedback regulation of bile acid synthesis. However, the concentration of the portal bile acids was not significantly different from that in the control animals. Furthermore, the portal bile acids became significantly hydrophobic. These findings were consistent with previous studies in rats (23) and humans (34, 35) and suggest less significance in direct regulatory effects of portal bile acids on bile acid synthesis.

Cholesterol 7α-hydroxylase activity was suppressed by administration of the bile acids as shown in Table 1. CDCA and DCA have been reported to be the most potent inhibitors of bile acid synthesis (9). The effect of oral administration of UDCA on bile acid synthesis is still controversial (36). In a recent study, Reihnér et al. (35) found no significant changes in cholesterol 7α-hydroxylase activity when 15 mg/kg per day of UDCA was administered for 3 to 4 weeks in patients with gallstones while there was a decrease in this enzyme activity when CDCA was administered. Heuman, Hylemon, and Vlahcevic (9) have

reported similar results in Sprague-Dawley rats fed 1% UDCA for 2 weeks. In the present study, UDCA feeding resulted in a decrease in the enzyme activity in Wistar rats, which was in agreement with the results of Shefer, Zaki, and Salen (37). The discrepancy could be explained by the strain differences in bile acid biosynthesis and metabolism between Sprague-Dawley rats and Wistar rats (38). It was also reported that oral administration of UDCA or CDCA significantly increases muricholates in the Sprague-Dawley rat (8, 9). This is not the case for the Wistar rat (37).

Heuman et al. (9) observed a negative correlation between hydrophobicity indices of the biliary bile acids and the cholesterol 7α-hydroxylase activities, although the portal bile acids were not examined in their study. In the present study, however, the enzyme activities did not correlate with the overall hydrophobicity indices of the portal bile acids. The composition of portal bile acids might be different from that of biliary bile acids, because efficacy of hepatic extraction of bile acids from the portal circulation varies depending on their degree of hydroxylation, state of conjugation, and extent of protein binding (39). In addition, some bile acids undergo biotransformation in the liver such as conjugation, hydroxylation, and epimerization. As the bile acid composition in bile is fairly similar to that in hepatocytes (40), the regulatory role of bile acids in hepatocytes on the cholesterol 7α-hydroxylase activities might not be excluded. In the present study, we did not find any correlation between either concentrations, composition, or hydrophobicity indices of the portal bile acids and the activities of cholesterol 7α-hydroxylase, suggesting the lack of the direct down-regulatory effect on bile acid synthesis by the portal bile acids.

We recently demonstrated a significant positive correlation between serum levels of 7α-hydroxycholesterol and hepatic cholesterol 7α-hydroxylase activities in patients with cholelithiasis (25, 41). We have also shown that the serum 7α-hydroxycholesterol values reflected in vivo bile acid production in patients with obstructive jaundice after percutaneous transhepatic biliary drainage (42, 43). From these results, we have proposed that the serum level of 7α-hydroxycholesterol would be a good indicator of the hepatic bile acid synthesis rate in humans and the proposal was also verified in the rat in the present work.

In summary, we determined the hepatic microsomal cholesterol 7α-hydroxylase activity and portal bile acids in rats orally administered three different bile acids or cholestyramine. There was no correlation between the enzyme activities and either concentrations, composition, or hydrophobicity indices of portal bile acids, suggesting that bile acid synthesis is not regulated directly by portal bile acids returning to the liver. Serum levels of 7α-hydroxycholesterol reflected the hepatic enzyme activity in the rat.

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