Taurocholate is more potent than cholate in suppression of bile salt synthesis in the rat

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Abstract Synthesis of bile salts is regulated through negative feedback inhibition by bile salts returning to the liver. Individual bile salts have not been distinguished with regard to inhibitory potential. We assessed inhibition of bile salt synthesis by either cholate or its taurine conjugate in bile fistula rats. After allowing synthesis to maximize, baseline synthesis was determined by measuring bile salt output in four consecutive 6-hr periods. Next, sodium cholate (+[^{14}C]cholate) or taurocholate (+[^{14}C]taurocholate) was infused into the jugular vein for 36 hr and bile was collected in 6-hr aliquots. Hepatic flux of exogenous bile salt was determined by measuring output of radioactivity in bile divided by specific activity of the infusate. Synthesis was determined during the last four 6-hr periods of infusion by subtracting exogenous bile salt secretion from the total bile salt output. Thirteen studies using cholate and 13 using taurocholate were performed. Hepatic flux of infused bile salt varied from 1 to 12 μmol/100 g per rat per hr. Percent suppression of synthesis varied directly with hepatic flux of exogenous bile salt for both cholate and taurocholate in a linear fashion ($r = 0.66$, $P < 0.01$ and $r = 0.87$, $P < 0.0005$, respectively). Slope of the taurocholate line was 7.82 (% suppression/μmol per 100 g per hr), while slope of the cholate line was 3.66 ($P < 0.05$), indicating that taurocholate was approximately twice as potent as cholate in suppression of synthesis. At fluxes of 10–12 μmol/100 g per hr, taurocholate suppressed synthesis 84 ± 8 (SEM) % while cholate suppressed synthesis only 42 ± 12% ($P < 0.02$). The x-intercept of the taurocholate line was 0.65 (μmol/100 g per hr), while that of the cholate line was −1.01 (NS) suggesting that the threshold for initial suppression of synthesis did not differ for these two bile salts. We conclude that taurocholate is a more effective inhibitor of hepatic bile salt synthesis than cholate, and that intestinal deconjugation of bile salts may play a role in the regulation of synthesis.—**Pries, J. M., A. Gustafson, D. Wiegand, and W. C. Duane**. Taurocholate is more potent than cholate in suppression of bile salt synthesis in the rat. *J. Lipid Res.* 1983. **24:** 141–146.

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Synthesis of bile salts accounts for about 40% of cholesterol catabolism and provides a micellar solubilization system that facilitates absorption of dietary lipids and transports cholesterol down the biliary tree (1). The most important regulator of synthesis is feedback inhibition by bile salts returning to the liver (2). Much is known about this inhibitory mechanism, however a major question remains unanswered: are all bile salts equally as potent for suppression of synthesis? During normal enterohepatic cycling, the human liver extracts a variety of different bile salts from portal blood including cholate, chenodeoxycholate, deoxycholate, lithocholate, and ursodeoxycholate, all of which exist as conjugates of taurine or glycine as well as in the unconjugated form. Judging from studies in which individual bile salts have been fed to subjects or animals with intact enterohepatic circulations, some of these bile salts have unique effects on bile salt synthesis. In the study of Shefer et al., feeding taurocholate to rats reduced the activity of 7α-hydroxylase, the rate-limiting enzyme in bile salt synthesis, by about 65%, while feeding taurochenodeoxycholate had no effect on the activity of this enzyme (3). Schoenfield, Bonorris, and Ganz (4), on the other hand, found that feeding either cholate or chenodeoxycholate (both unconjugated) to hamsters significantly reduced activity of 7α-hydroxylase. If anything, chenodeoxycholate was the more potent suppressor of activity. Moreover it is well known that feeding unconjugated cholate, chenodeoxycholate, or ursodeoxycholate results in different effects on hepatic sterol secretion and synthesis in human subjects (5, 6).

Unfortunately, when feeding a bile salt to an organism with an intact enterohepatic circulation, it is impossible to ascribe observed changes directly to that bile salt. Whatever the form of the fed bile salt, it will exist in at least two forms in the enterohepatic circulation: conjugated and unconjugated. If a primary bile salt, such as cholate or chenodeoxycholate, is being fed, it will be partially degraded by intestinal bacteria to secondary bile salts. Moreover, since oral bile salt will alter absorption of cholesterol in an unpredictable manner, and since increased cholesterol delivery to the liver, at least in many species, exerts *positive* feedback stimulation of bile salt synthesis (1), effects observed during bile salt feeding cannot even be directly attributed to bile salt. Finally, the degree of suppression of synthesis is a function of the flux of bile salt across the liver. However,

in an animal with an intact enterohepatic circulation, this hepatic flux is difficult to measure and therefore cannot be rigorously taken into account.

In the present study these problems have been overcome by studying effects of intravenously infused bile salts in bile fistula rats. An additional advantage of this experimental design is that synthesis can be measured directly as output of bile salt from the fistula. Moreover, by including radiolabeled bile salt in the infusate, hepatic flux of exogenous bile salt can be directly determined. This study was designed to ask the question: do the bile salts, taurocholate and cholate, have different feedback potency? We chose these particular bile salts because taurocholate is the major bile salt synthesized by rat liver.

MATERIALS AND METHODS

Bile fistula rat model

Male Sprague-Dawley rats (Biolab Corporation, White Bear Lake, MN) weighing 220–300 g were maintained in a light-controlled environment and fed standard rat chow for at least 3 days before use. A bile fistula was made as previously described (7). Briefly, rats were anesthetized with ether and, under sterile conditions, PE-10 and PE-20 cannulas were inserted into the common bile duct and jugular vein, respectively. The cannulas were run subcutaneously and brought out through a small interscapular incision. Each animal was attached to a spring harness. Both cannulas were passed through the spring which was brought out through a small hole in the top of a metabolic cage and secured. The animals were allowed food and water ad libitum. The venous cannula was connected to an infusion pump (Harvard Apparatus Co., Inc., Dover, MA) set at an infusion speed of 17.71 ml per day. Bile was collected in a fraction collector at 6-hr intervals.

Materials

Sodium cholate and sodium taurocholate were purchased from Calbiochem, Los Angeles, CA. Thin-layer chromatography of 100 μg of each yielded a single band of sulfuric acid charring. [^{14}C]Taurocholate and [^{14}C]cholate, both labeled at the C-24 position, were purchased from New England Nuclear Corporation, Boston, MA. Radiochemical purity of both bile salts was assessed by thin-layer chromatography on silica gel G using a moving phase of isooctane–ethylacetate–acetic acid 5:5:1 (v/v) for cholic acid and butanol–water–acetic acid 10:1:1 (v/v) for taurocholic acid. The plates were divided into 7-mm strips which were quantitatively transferred to counting vials containing 10 ml of Aquasol plus 1.0 ml of water. In both cases >97% of the radioactivity on the plate moved with an R_f value identical to the corresponding authentic bile acid.

Analytical methods

Each 6-hr bile collection was quantitatively transferred to a 50-ml volumetric flask using 70% methanol to volume. A 10-ml aliquot was extracted with 20 ml of petroleum ether. Duplicate 2-ml aliquots of the methanol–water phase were removed and dried. The residue was taken up with a known volume of 0.01 N NaOH for determination of total bile salt by the enzymatic technique of Talalay (8) modified as previously described (9). In addition, duplicate 1.0-ml aliquots were removed for liquid scintillation counting. These were decolorized in sunlight and dried. Ten ml of a 10:1 mixture of Aquasol–water (New England Nuclear, Boston, MA) was added to the residue and the samples were counted on a Packard Tri-Carb model 3330 to an accuracy of at least 1%. Internal standardization with [^{14}C]toluene was used to correct for quenching.

Total bile acids and ^{14}C radioactivity were determined from 100-μl aliquots of infusate for each study. During sodium cholate infusion complete conversion to taurocholate was confirmed by TLC of an aliquot of bile.

Experimental design

After preparation of the bile fistula, animals were allowed to recover and bile salt synthesis was allowed to maximize for 72 hr before each experiment. Rats not active and eating at least 15 g/day of chow at this point were not used for study. In the rats accepted for study, four 6-hr samples of bile were collected to determine baseline bile salt synthesis. Next, either sodium cholate (+ 6 μCi/dl [^{14}C]cholate) or sodium taurocholate (+ 6 μCi/dl [^{14}C]taurocholate) in 2% bovine albumin–saline solution was infused into the jugular vein (0.0123 ml/min) for 36 hr. Concentration of unlabeled bile salt was adjusted to vary hepatic flux from 1–12 μmol/100 g per hr. During the infusion, six 6-hr bile samples were collected. The last four samples were used to calculate bile salt synthesis during infusion, as well as hepatic flux of exogenous bile salt.

Calculations

Bile salt synthesis (μmol/hr) during infusion was calculated from the specific activity of the infusate (SA_i), the specific activity of individual bile samples (SA_o), and hourly output of radioactivity in the bile (R) according to the formula:

$$\text{Synthesis} = (R)\left(\frac{1}{SA_o} - \frac{1}{SA_i}\right).$$

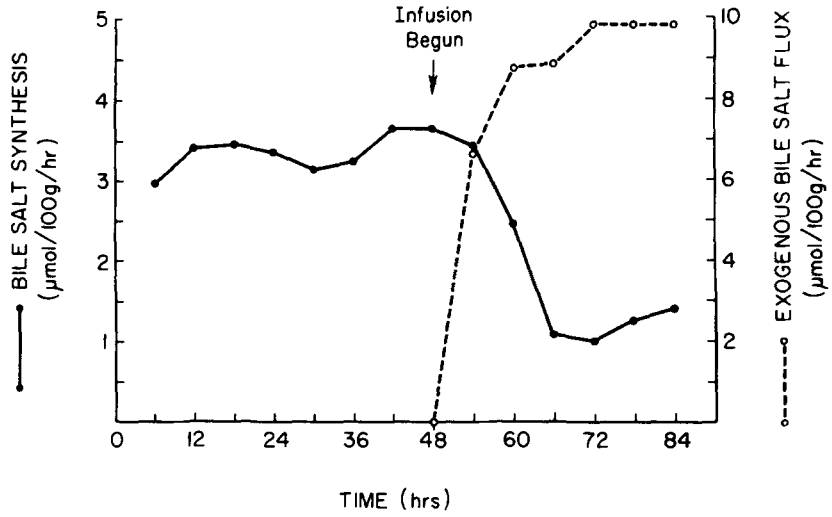

Fig. 1. Bile salt synthesis and exogenous bile salt flux for a typical taurocholate infusion study. Suppression of bile salt synthesis was determined by comparing the synthetic rate during hours 66 through 84 with hours 24 through 48.

Synthesis rates both before and during infusion were corrected for body weight of the rat. Hepatic flux of exogenous bile salt was calculated by multiplying R times the specific activity of bile salt in the infusate. Linear regression analysis was performed by method of least squares and comparison made by standard two-sided *t*-test (10).

RESULTS

The results from a representative experiment are shown graphically in **Fig. 1.** Before infusion, bile salt synthesis was equivalent to rate of output from the bile fistula, and varied diurnally as previously described (7). Near maximal suppression of bile salt synthesis was observed by the third 6-hr period of infusion.

The data from the sodium cholate and sodium taurocholate infusions are presented in **Table 1** and **Table 2.** Some variation in baseline maximal synthesis was observed among the animals. However, no significant differences in maximal synthesis were observed between the cholate and taurocholate groups as a whole. For each study an estimated rate of bile salt infusion (infusion pump speed × infusate bile salt concentration) was calculated and compared to the rate of exogenous

TABLE 1. Sodium taurocholate

	Infused Bile Salt		Synthesis		
Study	Hepatic Flux	Recovery[a]	Pre-Inf	Post-Inf	Suppression
	μmol/100 g per hr	%	*μmol/hr*		%
1	3.75	79	5.12	3.49	30.8
2	6.73	109	4.35	2.61	40.1
3	7.56	99	6.66	1.55	82.0
4	8.70	96	8.15	5.99	37.0
5	9.6	82	7.43	2.84	61.0
6	11.9	103	8.39	0.00	100.0
7	10.1	70	9.83	0.84	91.4
8	10.92	78	8.97	3.22	64.0
9	2.87	90	5.48	5.13	6.4
10	12.7	110	7.08	0.17	98.0
11	1.04	120	6.33	6.45	0.0
12	6.39	67	7.26	2.60	64.0
13	10.62	94	6.87	2.45	64.0
M ± 1 SD		92.1 ± 16.2	7.07 ± 1.55		

[a] For studies 1–6 the pump's infusion rate was calibrated after the experiments. For studies 7–13 the pump's infusion rate was estimated from the manufacturer's calibrations.

TABLE 2. Sodium cholate

Study	Infused Bile Salt		Synthesis		
	Hepatic Flux	Recovery[a]	Pre-Inf	Post-Inf	Suppression
	μmol/100 g per hr	%	*μmol/hr*		%
1	3.5	85	9.71	7.00	27.0
2	4.1	94	6.16	5.78	6.2
3	5.4	109	9.32	6.37	31.7
4	7.0	87	7.53	6.65	11.7
5	10.3	87	5.77	3.75	35.0
6	10.4	82	8.20	3.12	62.0
7	11.4	110	8.13	7.17	11.8
8	8.7	110	9.64	5.76	40.3
9	12.2	97	10.49	4.32	58.8
10	1.1	89	3.44	3.46	0.0
11	1.7	91	6.61	8.24	0.0
12	1.7	96	5.77	4.52	21.7
13	7.9	96	6.84	3.16	53.8
M ± 1 S.D.		94.8 ± 9.6	7.51 ± 2.0		

[a] For studies 1–6 the pump's infusion rate was calibrated after the experiment. For studies 7–11 the pump's infusion rate was estimated from the manufacturer's calibration. For studies 12 and 13 the pump's infusion rate was calibrated during the experiment.

bile salt secretion into bile. This estimated recovery of infused bile salt varied from 67% to 120% (Tables 1 and 2), but no significant differences existed between cholate and taurocholate groups. In addition, there was no correlation between rate of bile salt infusion and recovery.

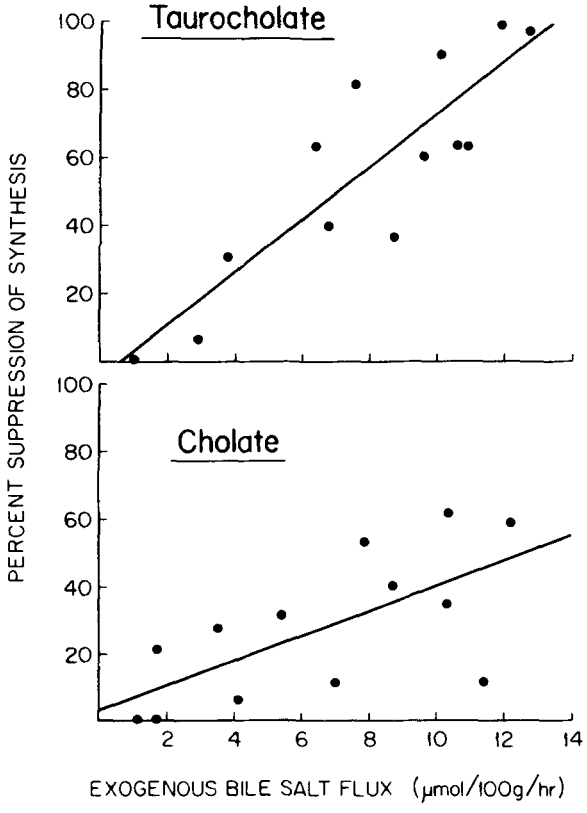

Fig. 2. Percent suppression of bile salt synthesis at varying exogenous bile salt fluxes for taurocholate and cholate infusions.

As shown in **Fig. 2,** percent suppression of bile salt synthesis varied directly with the hepatic flux of exogenous bile salt for both cholate ($r = 0.66$) and taurocholate ($r = 0.87$). The slope of the cholate line was 3.66 (percent suppression per μmol/100 g per hr) while the slope of the taurocholate line was 7.82 ($P < 0.05$). Viewed another way, when hepatic flux of exogenous bile salt was 10–12 μmol/100 g per hr, percent suppression of synthesis averaged 42 ± 12 (SEM) for cholate and 84 ± 8 for taurocholate ($P < 0.02$). To be certain that experiments with poor recovery of infused bile salt (Tables 1 and 2) had not adversely affected our results, we performed linear regression analysis, omitting studies with a recovery of ≤80% or ≥120%. This changed the slope of the taurocholate line to 8.80. Slope of the cholate line remained unchanged at 3.66 and the two slopes remained significantly different ($P < 0.05$). In any case, taurocholate was approximately twice as potent in suppressing bile salt synthesis as cholate.

Finally, the x-intercept of the cholate regression (−1.01 μmol/100 g per hr) was not significantly different from that of the taurocholate regression (0.65), suggesting that the threshold for initial suppression of synthesis was the same for these two bile salts.

DISCUSSION

The present study shows for the first time that two bile salts differ in their capacity to suppress bile salt production by the liver. Previous studies comparing the

effects of two or more bile salts on synthesis have done so by measuring activity of 7α-hydroxylase, the rate-limiting enzyme in bile salt synthesis (3, 4). However, it is well-known that activity of this enzyme does not necessarily reflect actual production of bile salt, which under certain conditions may be a function of substrate availability (11). Moreover, these earlier studies have largely been observations of the effects of feeding bile salt to animals or humans with an intact enterohepatic circulation, a situation in which the fed bile salt may alter cholesterol absorption and be altered itself by gut bacteria. Although these variables make rigorous interpretation of such studies difficult, it is interesting to note that Shefer et al. (3) found orally administered taurocholate to be more potent than an equivalent dose of either taurochenodeoxycholate or taurodeoxycholate in suppression of 7α-hydroxylase activity. Our data also indicate that taurocholate is particularly potent for suppression of actual bile acid production, exceeding the potency of cholate by a factor of approximately two.

The reason that taurocholate differs from cholate in feedback potency cannot be understood until more is known about the feedback inhibition mechanism itself. A bile salt may inhibit its own synthesis by binding to an intracellular protein with this complex, then acting in the nucleus to shut down synthesis of 7α-hydroxylase. Evidence supports such a chain of events for the action of estrogens, for example (12). In this context taurocholate might either bind to this protein better than cholate or the taurocholate-protein complex might be more effective in communicating the signal for shutdown of enzyme synthesis. Another alternative is that bile salts returning to the liver interact directly with 7α-hydroxylase as suggested by Shefer, Hauser, and Mosbach (13). If so, taurocholate might simply interact with the enzyme more effectively than cholate. It is also possible that inhibition of synthesis by bile salt is mediated by accumulation, through mass action, of an intermediate between cholesterol and bile salt, rather than by bile salt itself. However, our observation that taurocholate is more potent than cholate in suppression of bile acid production is evidence against this possibility since cholate is one step closer to cholesterol than taurocholate.

In the study of Shefer et al. (2), feedback inhibition of bile salt synthesis in the rat was shown to be complete at a hepatic flux of approximately 14 μmol of taurocholate/100 g per hr. The hepatic flux of taurocholate causing complete inhibition of bile salt synthesis in our studies (12 μmol/100 g per hr) was approximately the same (Fig. 2). However, our data demonstrate a relationship between hepatic flux of bile salt and percent suppression of synthesis that is roughly linear and approximately intersects the origin, suggesting that feedback control is continuous and gradual. This stands in contrast to the findings of Shefer et al. (2) in rats and also to data obtained in the rhesus monkey with controlled interruption of the enterohepatic circulation (14, 15). The findings in both cases suggest that percent suppression of synthesis remains near zero until hepatic flux of bile acid reaches a certain critical level ($\sim$12 μmol/100 g per hr in the rat, and roughly 3–5 μmol/100 g per hr in the monkey). The explanation for this discrepancy is not immediately apparent, but there are many differences in experimental design between our study and these previous studies. One difference is that Shefer et al. (2) provided their animals with a duodenal cannula into which a liquid diet was infused. Since we allowed our animals to eat rat chow ad lib, it is possible that they did not receive the amount of caloric supplementation provided in Shefer's study. However, it seems unlikely that this difference was important since we used only fistula animals that were eating at least 15 g of chow per day. Moreover, using this identical experimental design, we have previously demonstrated a reduction in bile acid synthesis when food is withdrawn. This indicates that our animals maintain adequate nutrition during the time of study.

Another difference in experimental design is that we infused bile acid into a peripheral vein, while in these previous studies (2, 14, 15) it was infused into the duodenum. The disadvantage of our approach is that an artificial elevation of the concentration of bile acid in peripheral venous blood is generated. However, there is no evidence to suggest that this elevated peripheral venous blood bile acid would itself affect the feedback system. On the other hand, with intraduodenal infusion, an unknown fraction of infused taurocholate would be deconjugated, which according to our data would reduce its potency for feedback inhibition. Moreover, bile acid infused into the duodenum would to some extent increase absorption of cholesterol, which could provide an off-setting stimulus to bile acid synthesis. Finally, bile acid delivered into the duodenum would not necessarily provide a constant hepatic flux of bile acid. Rather, changes in small bowel transit induced by eating or other factors would change the rate of delivery of bile acid to the terminal ileum resulting in a tidal, rather than constant, hepatic flux of bile acid. It is possible that a given amount of bile acid delivered tidally to the liver might have a much different effect on overall bile acid synthesis than the same amount of bile acid delivered continuously.

Our finding of a gradual and continuous relationship between hepatic flux of bile acid and suppression of synthesis is supported by the work of Bergström and Danielsson (16). Studying bile fistula rats and infusing taurochenodeoxycholic acid into the duodenum, they

showed a progressive suppression of synthesis of cholate at infusion rates ranging from 2–10 μmol/100 g per hr. They concluded that there was ". . . a fairly good correlation between amount of NaTCD (sodium taurochenodeoxycholate) absorbed and the magnitude of suppression of cholic acid formation."

Finally, Uchida et al. (17) found that the normal rat with an acutely interrupted enterohepatic circulation initially secretes bile salt at a rate of approximately 7 μmol/100 g per hr. Virtually identical rates were obtained by Wilson, Bentley, and Crowley (18), assuming the rats in their study weighed between 200–250 g. Since bile acid synthesis in the normal rat is roughly 15% of the maximal level achieved after prolonged interruption of the enterohepatic circulation, these data indicate that under physiologic conditions, a hepatic flux of bile acid intermediate between 0 and 12 μmol/100 g per hr results in considerable suppression of bile acid synthesis.

Hepner, Hofmann, and Thomas (19), using bile salt labeled on both amino acid and steroid moieties, have shown that in individual subjects up to 30% of the bile salt pool may be deconjugated by gut bacteria in a single enterohepatic circulation. The different efficacy of taurocholate versus cholate in suppression of synthesis suggests that such active deconjugation may be an important determinant of bile salt synthesis and therefore pool size. Different sensitivities of the feedback inhibition mechanism for individual bile salts might also help to explain the observation of Mok, von Bergmann, and Grundy (20) that human subjects with similar rates of bile salt synthesis nevertheless may differ greatly with respect to hepatic bile salt secretion.

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