Influence of hepatic taurine concentration on bile acid conjugation with taurine

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HARDISON, WILLIAM G. M., AND JOHN H. PROFFITT. Influence of hepatic taurine concentration on bile acid conjugation with taurine. Am. J. Physiol. 232(1): E75-E79, 1977. — In vitro, addition of taurine to liver homogenates increases the proportion of cholic acid conjugated with taurine. In the present study, the relation between hepatic taurine concentration and the proportion of infused sodium cholate conjugated with taurine was studied in the whole organ. The isolated perfused liver was studied to eliminate possible transfer of taurine to or from the large extrahepatic pools present in vivo. During cholate infusion, the proportion of taurocholate excreted in bile decreased, and the proportion of glycocholate increased, a complementary fashion. Infusion of taurine with cholate prevented these changes. Hepatic taurine concentration, calculated from measured hepatic taurine concentrations before and at the end of cholate infusion, fell. Fall in proportion of total bile acid excreted as taurocholate was most rapid at low hepatic taurine concentrations between about 1.4 and 0.65 μmol/g liver. Hepatic taurine concentration is a major determinant of the proportion of bile acid conjugated with taurine.

bile acids; taurine; rat liver

ABOUT 30-40% OF THE BILE ACID POOL IN MAN IS TAUROCONJUGATED BILE ACID (21). With cholestasis and biliary obstruction, the percentage is higher (9, 21). In situations characterized by increased conjugation requirements, such as during chenodeoxycholate therapy (8) or by high bile acid loss, such as during cholestyramine feeding (10) and during external biliary drainage (22), the percentage is lower. Feeding of taurine to patients with external biliary drainage abruptly increases the percentage of taurine-conjugated bile acids (22). Both human and rat liver homogenates exposed to equimolar concentrations of taurine and glycine in the incubation medium conjugate cholic acid predominantly with taurine (3, 12). Human liver homogenates, when exposed to high concentrations of glycine, conjugate cholic acid predominantly with glycine (12). The conclusion reached from these studies is that the percent of bile acid conjugated with taurine depends in some way on the relative concentrations of taurine and glycine available in the hepatocyte for conjugation. Since glycine is considered always available, easily synthesized and never depleted in vivo, the chief variable in regulation of percent bile acid conjugated with taurine is considered to be hepatic taurine concentration. Nonetheless, studies have not been performed which relate taurine concentrations in the intact liver to the percentage of excreted bile acid conjugated with taurine.

The present study defines this relation of hepatic taurine concentration to the percentage of excreted bile acid conjugated with taurine. The perfused liver was used in order to eliminate possible fluxes of taurine into and out of large extrahepatic taurine pools which exist in vivo (14).

METHODS

Liver perfusion. Under ether anesthesia, livers were removed from rats fasted for 16 h and placed in a liver perfusion apparatus (Medical Research Apparatus Corp., Boston). They were perfused for 6 h at 37°C with the following perfusate: washed bovine erythrocytes (bovine blood, a gift from Talone Packing Co., Escondido, Calif.) 33% in Ringer bicarbonate buffer containing 4% bovine serum albumin and 150 mg/dl glucose. The perfusate was oxygenated with 95% O2-5% CO2, and pH was adjusted to 7.4 initially and hourly by addition of 4% sodium bicarbonate.

During perfusion, all bile was collected hourly through a PE-10 polyethylene catheter inserted into the bile duct. Collection was into tared test tubes. After 1 h of perfusion and again at termination, about 0.6 g of liver was taken for taurine assay.

Beginning at 1 h an infusion was begun into the perfusate. Infusate composition with rates were as follows: sodium cholate, 0.46 μmol/min (10 livers); equimolar cholate and taurine, each 0.46 μmol/min (3 livers); and control infusion (4 livers). All infusates were made up in Ringer bicarbonate containing 1% bovine serum albumin.

Measurement of bile acids. Bile, 25 μl, was assayed for total bile acids by the hydroxysteroid dehydrogenase method of Talalay (23). Bile, 250 μl, was put into 1 ml absolute ethanol, heated, centrifuged, and the supernatant removed and evaporated to dryness. It was redissolved in 200 μl 70% ethanol and applied to a 20 x 20 cm thin-layer chromatography plate coated with silica gel H, 500 μm thick. Plates were developed in isopentyl acetate, propionic acid, n-propanol, and water (30:30:20:15 vol/vol) (10) and stained with iodine vapor. Bands corresponding to the applied standard cholic, glycocholic, and taurocholic acids were scraped and
eluted with methanol. Eluates were evaporated to dryness and total bile acid was measured as before. Each bile acid was calculated as percent of total bile salt eluted from the silica gel. Individual bile acid concentration was calculated by multiplying this percentage by total biliary bile acid concentration. No free bile acid was found in bile samples collected before sodium cholate infusion. No bile acids other than unconjugated cholate, taurocholate, and glycocholate were found in bile samples collected during sodium cholate infusion. For graphical representation, all mean biliary bile acid values for a given hour are plotted temporally at the end of the hour.

**Measurement of taurine.** Taurine was measured by a modification of the method of Wilbraham et al. (25). About 300 mg of liver tissue were homogenized in 2.0 ml water containing about 75,000 dpm of [14C]taurine (sp act 4.6 mCi/mmole) (New England Nuclear Corp., Boston). The homogenate was deproteinized by addition, dropwise with agitation, of 1.0 ml 60% trichloroacetic acid. The sample was centrifuged and the supernatant removed. The protein pellet was rehomogenized in 2.0 ml water, centrifuged, and supernatants were combined. Trichloroacetic acid was removed from this solution by four to five extractions with an equal volume of water saturated diethyl ether (11). The solution was then made slightly alkaline (pH 8) with 4% NaHCO3. Next, 2 ml of 3% ethanolic 2,4-dinitrofluorobenzene (Eastman Chemicals, Rochester) was added and the solution heated at 85°C for 45 min. A marble placed over the tube prevented excessive evaporation. The solution was then acidified to pH 1 with 2 N HCl and was extracted with 10-ml portions of chloroform. Extractions continued until the chloroform phase, when extracted with 1 N NaOH, yielded no yellow color in the NaOH phase (about 4-5 extractions). The remaining solution was evaporated to dryness, redisolved in 0.5 ml water, and 8 ml of acetone were added. The precipitated inorganic salts were removed by centrifugation, and the supernatant evaporated to dryness and redisolved in 200 μl water. A portion, 25 μl, of this solution was applied to a 2.5 cm x 7 cm cellulose polyacetate strip and subjected to electrophoresis (250 V, 45 min, pH 8.6 barbital buffer). The band corresponding to taurine was cut out, dried, weighed, and dissolved in 2 ml glacial acetic acid. Optical density was measured at 360 nm, corrected for the absorbance of cellulose polyacetate, and taurine concentration was calculated. One milliliter of this solution was added to 15 ml Bray’s solution (Burdick & Jackson Laboratories, Inc., Muskegon, Mich.) and counted in a Nuclear-Chicago model Mark II liquid scintillation counter. From this, recovery could be determined and hepatic taurine concentration, in micromoles per gram liver weight, calculated. Assays were performed in duplicate.

**RESULTS**

Figure 1 shows the excretion in nanomoles per gram liver per minute of total bile acids, unconjugated cholate, glycocholate, and taurocholate for the 10 perfused livers infused with cholate. Total bile acid excretion equalled infusion rate except in the last 2 h when excretion rate fell slightly below infusion rate. Excretion of unconjugated cholate rose to a constant rate very early in the perfusion. Excretion of total bile acid conjugates was nearly constant but fell slightly as did total bile acid excretion toward the end of perfusion. Major changes, however, occurred in the fractions of glycine- and taurine-conjugated bile acids making up the total conjugates. Taurocholate excretion declined as glycocholate excretion increased in a fashion complementary to the decline in taurocholate excretion. These changes were prevented by infusion of equimolar taurine and cholate. Figure 2 shows biliary composition at hour 4 for 10 livers infused with cholate and three livers infused with equimolar taurine and cholate. Although total bile acid and total conjugated bile acid excretion rates in animals infused with cholate were not significantly different from those animals infused with equimolar taurine and glycine, taurine infusion did prevent the in-
crease in glycocholate and decrease in taurocholate excretion in bile.

Cholate infusion caused a net loss of taurine as taurocholate from the livers. Figure 3 shows liver taurine concentrations before cholate infusion and after 5 h of cholate infusion. Initial hepatic taurine concentrations varied widely. Nonetheless, final hepatic taurine concentrations fell within a narrow range, suggesting that in the present study a limit exists for the degree to which a liver can be depleted by cholate infusion. Table 1 shows initial hepatic taurine content and the amount lost in bile as taurocholate for each liver. In most, more taurine was lost as taurocholate than could be accounted for by the decrease in hepatic taurine content; therefore, synthesis of taurine must have been occurring. The fact that hepatic taurine concentration in perfused livers not infused with cholate consistently increased indicates that taurine depletion is not necessary for synthesis to occur (Fig. 3).

The changes in the proportions of glycine- and taurine-conjugated bile acids excreted in bile were dependent on changes in hepatic taurine concentration. Hepatic taurine concentration at any time, $T_i$, may be calculated as follows

$$T_i = T_o + r \cdot t - \frac{(TC_o)}{W}$$

where $T_o$, $r$, $(TC)_o$, and $W$ are, respectively, initial taurine concentration, calculated taurine synthesis per gram liver per minute, total biliary taurocholate excreted at time, $t$, and liver weight, $W$. Calculation of $r$ is as follows

$$r = \frac{TC/W - (T_o - T_f)}{t_f}$$

where $T_f$ is final hepatic taurine concentration, $TC$ is total taurocholate excreted in bile, $W$ is liver weight, and $t_f$ is the duration of cholate infusion in minutes. Ratio of biliary taurocholate to glycocholate excretion at any time, $r$, may be determined from a graph of taurocholate to glycocholate excretion ratio versus time for each liver.

Figure 4 shows the relation between calculated hepatic taurine concentration and ratio of taurocholate to glycocholate excretion rate ($T/G$ ratio). Data were not consistently available at $T/G$ ratios less than 0.43, since hepatic taurine synthesis prevented taurocholate excretion rate from falling to zero. A source of error exists in calculation of hepatic taurine concentrations. Derivation of $r$ assumes constant taurine synthetic rate during cholate infusion. We tried to verify this assumption in two livers by obtaining an extra biopsy after 3 h of cholate infusion. Synthetic rates calculated for the final 2 h of infusion were 74 and 112% of rates calculated for the entire period of infusion. Because these terminal synthetic rates are calculated from small differences in hepatic taurine content and small increments of taurocholate excretion, measurement errors are magnified and the data are probably inaccurate. It is unlikely we can further verify our assumption by techniques used in these studies.

**DISCUSSION**

The data presented demonstrate that hepatic taurine concentration is a major determinant of the proportion of bile salt conjugated in bile.
portion of bile acid conjugated with taurine. Although it is tempting to try to relate this dependency to the probable enzymatic mechanisms involved, the process of bile acid conjugation is complex and at present poorly understood. The first step is conversion of the unconjugated bile acid to the bile acid-CoA derivative by a microsomal activating enzyme (4, 20). The second step is formation of an amidic linkage between this derivative and either taurine or glycine (4, 20). The question of whether one enzyme or separate taurine and glycine conjugating enzymes exist remains unanswered (4, 5). This is largely because the conjugating enzyme or enzymes have never been purified nor have they been studied with their natural substrate, bile acid-CoA. Recent studies suggest, however, that in the rat one conjugating enzyme exists with different affinities for taurine and glycine (P. Killenberg, personal communication). If this is true, one would expect both taurine and glycine concentrations in the hepatocyte to influence the percentage of bile acid conjugated with taurine. However, the reported normal range for hepatic glycine concentrations of 1.6-2.3 \( \mu \text{mol/g liver} \) (24) is small compared to that of measured taurine concentrations (1.9-12.5 \( \mu \text{mol/g liver} \)). We measured glycine concentration on a Beckman amino acid analyzer in one liver only and found that it did not change during cholate infusion. The relative constancy of hepatic glycine concentrations probably explains why we were able to identify a relation between hepatic taurine concentration and percentage of bile acid conjugated with taurine, even though we did not measure glycine concentrations. Variation of glycine concentrations from liver to liver, however, may explain some of the variation seen in Fig. 4.

If one considers taurocholate in terms of percentage of conjugated bile acid in bile, the greatest change in taurocholate percentage occurs over a relatively small range of hepatic taurine concentrations. Thus, taurocholate percentage changes from 33% at hepatic taurine concentration of 0.68 \( \mu \text{mol/g liver} \) to 80% at hepatic taurine concentration of 1.4 \( \mu \text{mol/g liver} \). At hepatic taurine concentrations above 2.5 \( \mu \text{mol/g liver} \), taurocholate would constitute over 90% of the conjugated bile salt. If one were to examine the relation between percent taurocholate in bile and hepatic taurine concentrations in livers with concentrations between, for instance, 3.0 and 12.5 \( \mu \text{mol/g liver} \), one would probably conclude that no relation existed.

We were unable consistently to achieve bile acid T/G ratios below 0.43. This is not simply a limitation imposed by the short viability of the perfused liver preparation, since a similar observation has been made in the bile fistula dog (16). As hepatic taurine concentration decreases, biliary taurocholate excretion rate decreases. When this rate becomes equal to taurine synthetic rate, hepatic taurine concentration becomes constant. It is not possible, then, totally to deplete hepatic taurine by infusion of unconjugated cholate into livers which synthesize taurine.

The pattern of bile acid excretion during infusion of sodium cholate raises some questions concerning the mechanisms of bile acid conjugation and excretion. The appearance of unconjugated cholate in bile well before hepatic taurine concentration falls and its continued excretion at a constant rate suggest that the capacity for bile acid conjugation and excretion is exceeded. Others have noted a similar phenomenon (17-19). However, one may not conclude that maximum conjugation capacity is exceeded. If formation of cholesteryl-CoA is the limiting reaction in conjugation, provision of more substrate in the form of infused cholate might accelerate the overall process of conjugation. Alternatively, excretion of unconjugated cholate may represent a pathway that a certain proportion of cholate takes, even if conjugating capacity is not exceeded. In two animals infused with cholate, we increased cholate infusion rate by 30% at h 3. Excretion of both conjugated and unconjugated cholate increased (unpublished observations). This suggests that excretion of unconjugated cholate in our perfusions did not indicate maximum conjugating capacity had been reached.

The excretion rate of total bile acid conjugates changes little in spite of the shift in percentages of glycine and taurine conjugates. At the beginning of sodium cholate infusion, taurocholate usually constituted over 90% of the total conjugated bile acid excreted and at the end less than 30%; nonetheless, total conjugated bile acid excretion rate fell only 10-15% over 5 h of cholate infusion. When taurine is provided in abundance, glycine conjugation is virtually eliminated, but total conjugate excretion is not significantly changed. Thus, the rat liver maintains conjugation rate in spite of a shift in substrate from predominantly taurine to predominantly glycine. Nonetheless, it is evident that taurine is the preferred substrate because taurocholate constitutes half of the conjugated bile acid when hepatic taurine concentration is only about one-half to one-fourth that of hepatic glycine concentration. Similar conclusions have been reached by other investigators working with rat liver homogenates (12, 19). When hepatic taurine concentration is 2.4 \( \mu \text{mol/liver} \), so that concentrations of hepatic taurine and glycine are about equal, taurocholate percentage exceeds 90%. This percentage is similar to the 80% reported by Gottfries, Schersten, and Ekdahl (12) when human liver homogenates were incubated with equimolar concentrations of glycine and taurine.

The rate of unconjugated bile salt infusion used in the present studies exceeds by greater than 10 times the approximate rate of bile acid conjugation in vivo (6, 15). Therefore, the demonstrated dependence of conjugated bile salt G/T ratio upon hepatic taurine concentrations may never be physiologically important in the rat. It may be important in other species such as man in whom bile salt G/T ratios are closer to unity than those in the rat. Such species may be more dependent on exogenous taurine or taurine from slowly equilibrating endogenous taurine pools. In the cat, a species in which hepatic taurine synthesis is either slow or absent and bile acid is exclusively taurine conjugated, taurine deprivation can induce taurine de-
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iciency and retinal degeneration not prevented by the usual metabolic precursors of taurine, methionine, and cysteine (13). Whether man is protected from taurine deficiency by his ability to conjugate bile salt with glycine is unknown. Studies in patients with ileal resection or on chenodeoxycholate therapy (7) may answer this question. Patients on 1.5 g/day of such therapy will require about 100 mg of taurine for bile acid conjugation even at a low G/T ratio of 5/1. Growing interest in taurine and its relation to a variety of neuromuscular disorders (1, 2), moreover, lend importance to such questions.

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REFERENCES