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CHANGES OF THE PATTERN OF BILIARY BILE ACIDS DURING ISOLATED RAT LIVER PERFUSION

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In spite of the extensive use of isolated perfused liver systems for bile acid related studies, the composition of biliary bile acids during liver perfusion is not well known. Using recently developed bioluminescence assays for 3α-OH-, 7α-OH-, and 12α-OH- bile acids we studied the hydroxylation pattern of bile acids in bile during 90 minutes perfusion of isolated rat liver without added bile acid load. At the beginning 7α-hydroxylated bile acids comprised more than 50% of total bile acids from male livers and more than 90% from female livers, this percentage declined rapidly during the first 40-50 minutes of perfusion to values between 10 and 20%. 12α-hydroxylated bile acids comprised between 15 and 30% of the total at beginning of the perfusion and decreased to about 10% after 40 minutes. Sex differences as well as the influence of the duration of perfusion should be considered when the isolated perfused rat liver is used for bile acid related studies.

The perfused rat liver is a well accepted system to study liver metabolism and secretion (1,2,3). In spite of its use for bile acid secretion and uptake studies (4,5,6,7) little is known about the pattern of bile acids secreted during perfusion. This may, however, be of importance for the known decrease of bile acid secretion and bile flow during perfusion (7,8). This knowledge may also shed some light on the bile acid synthesis of the rat liver during perfusion without bile acid load. Since sex differences of hepatic bile acids in rats have also been reported (9), it seems to be necessary to be aware of possible differences in the excretion of different bile acids from livers of male and female rats.

Recently, bioluminescence assays have been developed for 3α-OH-, 7α-OH- and 12α-OH- bile acids, thus allowing their differentiation by the position of the hydroxy groups within the molecule (10,11). Using simple equations it is

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also possible to calculate the rates of excretion of some individual bile acids (12). Using these assays we have studied the bile acid secretion pattern during isolated rat liver perfusion.

MATERIALS AND METHODS

Materials:

Male and female rats (Fisher 344) with a body weight of 200-300 g were used. Rats were fed a standard diet (Purina Rat Chow, St. Louis, MO). Chemicals of analytical grade were purchased from Mallinckrodt (Paris, KE), lactic acid, acetoacetic acid and β-hydroxybutyric acid from Sigma (St. Louis, MO), Pyruvic acid, NAD, NADH, NADP and other biochemicals from Boehringer Mannheim (West Germany). Enzymes for the bioluminescence assays were partly supplied by Sigma (7α-hydroxysteroid dehydrogenase, 3α-hydroxysteroid dehydrogenase), and from Boehringer (bacterial diaphorase). The remaining enzymes were not commercially available and were supplied by Dr. Marlene DeLuca, Department of Chemistry, University of California, San Diego, CA (bacterial luciferase (13)) and by Dr. Ian A. MacDonald, Department of Medicine, Dalhousie University, Halifax, Canada (12α-hydroxysteroid dehydrogenase (15)).

Methods:

Perfusion: Livers were perfused as described by others (3,15,16) in a nonrecirculating system. The perfusate consisted of Krebs Ringer bicarbonate buffer (KBR, pH 7.4, gassed with carbogen) containing glucose (5 mM), lactate (2.1 mM), pyruvate (0.3 mM), acetoacetate (0.1 mM) and β-hydroxybutyrate (0.08 mM). Bile was collected after cannulation of the common bile duct with a PE-50 polyethylene catheter at 5-10 minute intervals during 90 minutes of perfusion. Perfusate samples were obtained at 10 minute intervals to assess metabolic parameters. O_2 uptake and pH of the liver outflow were recorded as well as pressure and flow rate (4ml/g/min) of the perfusate. After 90 minutes livers were fixed by perfusion with a mixture of paraformaldehyde (1.5%) and glutaraldehyde (0.25%) in phosphate buffer (pH 7.3), embedded in JB4 and examined by light microscopy.

Assay methods: Lactate, pyruvate, acetoacetate, β-hydroxybutyrate and glucose were measured as described (17). Urea was determined with aid of a test kit of Boehringer, LDH with a test kit of Sigma. Bile acid analysis was performed using the appropriate triple of enzymes coimmobilized on Sepharose 4B (10,11,18). A test tube with 350 µl of sodium phosphate buffer (0.1 M, pH 7.0) containing decanal (0.0001%), 10 µl of FMN (0.5 mM), 10 µl of the immobilized enzyme system, and 10 µl sample (bile, diluted 1:10-1:20) was inserted into an Aminco Glow Chem photometer. By injection of 100 µl NAD(P) (0.5 mM) the reaction was started and the resulting light emission peak recorded. The assay systems are described in detail elsewhere (10,11). By comparing the light emission with a standard curve bile acid concentrations were obtained for 3α-OH-, 7α-OH-, and 12α-OH- bile acids. Recovery studies with standards added to bile revealed recoveries between 90 and 105%, thus confirming the applicability of the assay on bile.

RESULTS

The general perfusion parameters were in good agreement with those reported in the literature (1,2,7, 15,16). The livers were also morphologically intact, as judged by light microscopy.

Bile flow as well as total bile acid excretion as measured by the 3α-assay decreased during perfusion as expected. There was a good correlation of bile acid excretion and bile flow either when eight perfused animals were compared or when the average at each time point of perfusion was used (Figure 1). There was no difference between male and female rats concerning bile flow (0.57±0.14 vs. 0.60±0.08 mg/min/g liver) and total bile acid excretion average (2.5±1.2 vs. 2.8±0.4 nmoles/min/g liver) although the females excreted slightly greater amounts in the first 30 minutes of perfusion than the males.

The percentage of 7α-hydroxylated bile acids of the total bile acids (chenodeoxycholic, α-muricholic and cholic) decreased from 64 to 18% during 50 minutes of perfusion (Figure 2). There was no significant change after this time. The decrease was less pronounced in males (51 to 14%) than in females (91 to 27%). The latter had almost all bile acids 7α-hydroxylated at the beginning of the perfusion period (Figure 2). They also had a higher percentage

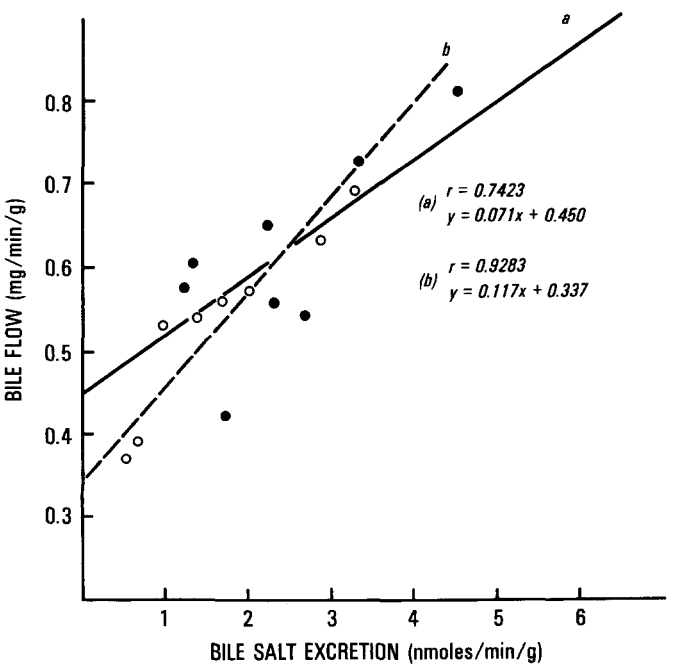

Figure 1. Correlation between bile salt excretion and bile flow.
a. Comparison of the average excretion and flow for each rat, closed circles.
b. Comparison of the average excretion and flow calculated every 10 minutes during perfusion, open circles.

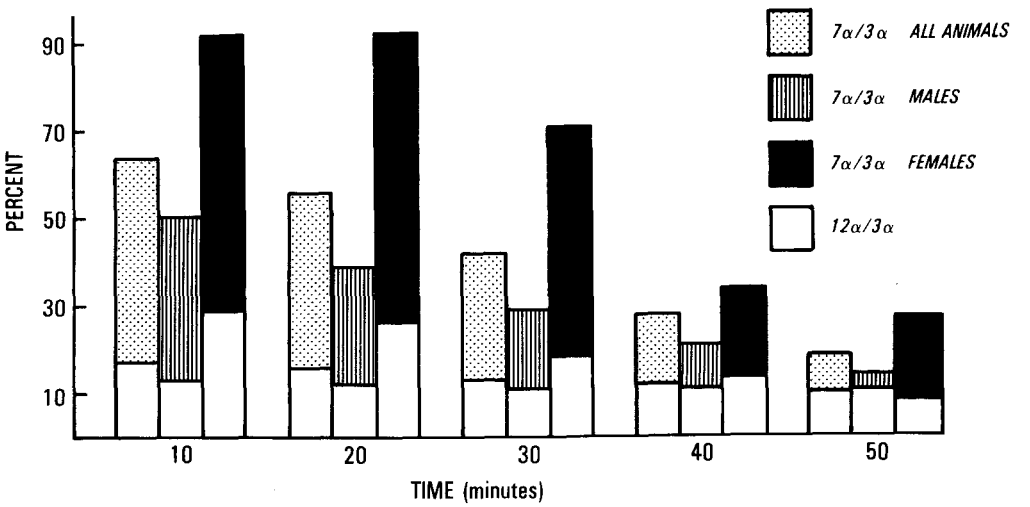

Figure 2. Percentage of total bile acids as 7α-hydroxylated or 12α-hydroxylated at several time points during perfusion for all animals (n=9), males (n=6) and females (n=3).

(27%) of 12α-hydroxylated bile acids than the males at the beginning which then decreased during the subsequent 50 minutes while no decrease occurred in males. After 50 minutes of perfusions the ratios of 7α/3α and 12α/3α were almost identical in males and females. Using simple equations (12) neglecting deoxycholic and lithocholic acid, since they have been shown to be present in rat bile in trace amounts, and assuming that bile acids neither 7α- nor 12α-hydroxylated were β-muricholic or hyodeoxycholic acid, we calculated the excretion of bile acid groups given in Table 1. Females had a large amount of chenodeoxycholic + α-muricholic acids at the beginning and also a higher

TABLE 1

Bile acids	all rats		males		females	
	20'	40'	20'	40'	20'	40'
Cholic	0.59	0.24	0.51	0.20	0.90	0.34
β-muricholic + hyodeoxycholic	1.51	1.48	2.02	1.50	0.30	1.60
α-muricholic + chenodeoxycholic	1.40	0.33	0.72	0.20	2.33	0.51
Total	3.50	2.05	3.25	1.90	3.53	2.45

Biliary bile acid excretion in nmoles/minute/g liver at 20 and 40 minutes of perfusion for males (n=6) and females (n=3) as calculated from the results of the three assays assuming that bile acids having neither a 7α- nor a 12α-hydroxy group were β-muricholic or hyodeoxycholic acid.

amount of cholic acid than males which, in turn, had predominantly 6- and 7β-hydroxylated bile acids. These differences, however, almost disappeared after 40 minutes of perfusion.

DISCUSSION

Methodology:

The pattern of bile acids in bile during isolated rat liver perfusion has rarely been studied since no simple and rapid methods were available. The bioluminescence assays are rapid and sensitive and, as such, make sequential monitoring of bile acid concentrations during perfusion feasible. Since they are also very sensitive, they can be used in similar studies with isolated hepatocytes where only small amounts of bile acids are present as has been recently described using a radioimmunoassay (20). Accuracy and specificity are also very good (10,11,12) thus making the results very reproducible. The addition of an assay using a 7β-hydroxysteroid dehydrogenase currently under investigation will make their application on experimental systems using rats even more useful.

Bile acid pattern:

Since the perfusion parameters as well as bile flow and total bile acid excretion were rather similar to the data described by others (3,4,5,7,8,16), the changes observed in the bile acid pattern in bile do not seem to be the result of perfusion irregularities.

The correlation obtained between bile flow and bile acid excretion was also in good agreement with that found by others (20,21,22). The pattern of bile acids in bile observed at the beginning of perfusion resembled that found in vivo (23,24). However, the amount of 6- or 7β-hydroxylated bile acids was somewhat greater and that of cholic acid smaller than reported. This may be due to species and sex differences. The sex difference found in our small number of animals resembled that recently reported by others (9) very closely, chenodeoxycholic and α-muricholic acid being the predominant bile acids in female livers. Since this sex difference disappears during perfusion it may be caused by systemic factors (e.g. hormones, bacterial flora) in the animals and not by differences in hepatic metabolic pathways.

During the perfusion period no enterohepatic circulation is present. Therefore, the bile acids excreted are thought to be derived mainly from de novo synthesized pools with progression of perfusion time. It has been shown that the perfused rat liver reduces 7 keto bile acids considerably less than 3 keto bile acids to α-hydroxy bile acids. Only 30% of 7-keto bile acids are reduced to 7α-OH acids. The amount of β-hydroxy acids could not be determined in that study (7). Also a decrease of 7α-hydroxylated bile acids in bile during starvation, a condition in some aspects similar to the isolated perfusion, has been reported (23). We cannot decide if the small amount of 7α-hydroxylated bile acids after 50 minutes of perfusion is due to an almost complete transformation of chenodeoxycholic into muricholic acids (25), to the use of other cholesterol pools (26), or to pathways from cholesterol to bile acids bypassing 7α-hydroxylation (27,28,29,30,31). The data are also in accordance with the results of others showing the rate of synthesis for cholic acid to β-muricholic acid to be 1:3 in rats (24,32).

In summary, it is important to realize that the pattern of biliary bile acids changes during isolated rat liver perfusion, and that sex differences also exist. This might also be true for intracellular bile acids in perfused livers and isolated hepatocytes as well. Monitoring of bile acid excretion during the experiment with the aid of bioluminescence assays may help to understand the changing pattern further and may facilitate the use of the perfused liver and presumably isolated hepatocytes as well in bile acid related studies.

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