BILE ACIDS SECRETION AND SYNTHESIS BY ISOLATED RAT HEPATOCYTES

M. S. Anwer, R. Kroker and D. Hegner

Institut für Pharmakologie, Toxikologie und Pharmazie
Fachbereich Tiermedizin
der Universität München
Leiter: Prof. Dr. D. Hegner

Received March 24,1975

SUMMARY

Bile acids secretion and their distribution were studied in isolated rat hepatocytes. Bile acids secretion was linearly related with time for first three hours of incubation and the net secretion rate was 23.2 + 2.74 nmoles per g cells (wet weight) per minute. Isolated hepatocytes synthesized relatively more chenodeoxycholic acid than cholic acid compared to whole animal. These results suggest that isolated hepatocytes synthesize and secrete bile acids and thus provide experimental system to study the effect of drugs on bile acids secretion and synthesis at cellular level.

INTRODUCTION

Liver plays a major role in the uptake, synthesis, conjugation and excretion of bile acids. Changes in bile acids metabolism have been demonstrated in hepatic diseases (1) and by hepatotoxic agent (2) and drugs (3) in whole animal. Recently isolated hepatocytes have been used to study the metabolic and synthetic activities of liver at cellular level (4, 5). However, metabolism of bile acids in isolated hepatocytes has not been reported. Isolated hepatocytes could provide a suitable experimental system to study the effect of different drugs and hepatic diseases on bile acid metabolism at cellular level. To evaluate this possiblity, we have studied bile acid secretion and their

distrubution in isolated rat hepatocytes and compared them with those of whole animal.

MATERIALS AND METHODS

Male Wistar rats weighing 250 to 350 g maintained on standard diet with water were used in all studies. Bile was collected for five minutes after anesthetizing the rats with ether and cannulating the common bile duct.

Rat liver cells were prepared using a modification of the method of Berry and Friend (6). The liver was perfused for 20 minutes with a calcium free Hank's solution containing 0,1% hyaluronidase and 0,05% collagenase. Isolation of liver cells was then continued mechanically as described by Baur (7). Average cell yield was one gram cells (wet weight) per liver and 93 to 96% of the cells excluded 0,2% trypan blue. The cells retained their lactic dehydrogenase (LDH) activity and respired at a rate of 125 μMoles of O_2 per g cells per hour.

Eight to ten ml cell suspension (ca. 100 mg/ml) was incubated in 50 ml Erlenmayer flasks at 37°C with shaking (120 - 160 oscillation per min, amplitude 4 cm) in Tyrode buffer (pH 7,4) containing 3% bovine serum albumin fraction V and 1 mg% glucose. The aeration and pH were maintained by a slow stream of 95% O_2 - 5% CO_2. After 10 min of preincubation, the supernatants from cell suspension at different time intervals were assayed for bile acids. At intervals, an aliquot of cells was stained with trypan blue and the percent of cells excluding the dye was determined. Lactate, pyruvate and glucose in the supernatants were determined at the end of four-hour incubation periods.

Total bile acids in the supernatants and bile sampes were determined enzymatically with hydroxysteroid dehydrogenase

(Grade II, Sigma) by a modification of the method of Iwata and Yamasaki (8). Bile samples were diluted (1:10) with water. The reaction mixture contained 1,0 ml 1M glycine buffer containing EDTA (5.6 mmoles/1) and hydrazine sulfate (0,4 moles/1), 0,05 ml of ß-NAD solution (10 nmoles/1), 0,05 ml of hydroxy steroid dehydrogenase solution (5 units/ml) and 0,02 ml diluted bile or 0,1 ml supernatants. The reaction mixture was incubated at room temperature for one hour and the extinction measured at 366 nm against appropriate blanks. All determinations were performed in duplicates. A set of standards was always assayed with each set of determination. The total bile acids concentration was calculated from the molar extrinction coefficient of NADH at 366 nm and net increase in extinction. The coefficient of variation of duplicate determinations was 2-4%.

Individual bile acids in bile samples and supernatants were also determined enzymatically after thin layer chromatographic separation on silian gel G plates (9). Bile acids from one ml supernatant obtained after four hours of incubation were finally extracted in o,1 ml methanol (10) before chromatographic separation using solvent system II of Hoffmann (11). No free bile acids were detected in either the supernatants or bile. Recoveries of standard conjugated bile acids were 92-98%. Glucose was determined by glucose oxidase method (12). The net production of glucose during four hour incubation was calculated as the difference of four hour and zero hour glucose concentrations. Lactate and pyruvate were determined by the method of Boehringer, Mannheim Ltd.

RESULTS AND DISCUSSION

The intergrity and the metabolic state of the cells after four hours of incubation were evaluated by trypan blue exclusion,

lactate pyruvate ratio, glucose production rate and retention of LDH activity. Trypan blue was excluded by 80-85% of the cells. Lactate pyruvate ratio was 9, 3 ± 2, 58 (mean ± S. E. M.) and glucose production rate was 16 ± 4, 1 (mean ± S. E. M.) μmoles per g cells per hour. The liver cells retained 90% of the total LDH activity. Thus, cells were considered to be living and metabolically functional during four hours of incubation.

The liver cells continued to secrete bile acids in the incubation media throughout the incubation period (Fig. 1). No bile acid was detected in the samples taken after 10 min of preincubation. That bile acids were secreted and not released by the damaged cells was support by the following observation. a) No free bile acids were detected in the incubation media after four hours of incubation. Free bile acids would be expected to be present in the media if bile acids were released from the damaged cells. b) Bile acids appeared in the incubation media inspite of no cell damage (trypan blue exclusion) after one hour of incubation. c) Bile

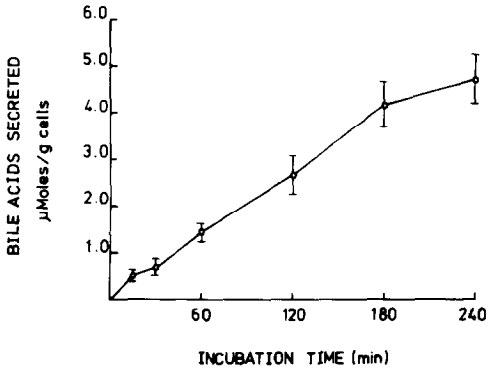

Figure 1: Time course of bile acids secretion by rat hepatocytes at 37°C and pH 7, 4. Each value represents mean of five studies and the vertical line represents standard error of mean. Bile acids were detected in two and three out of five studies in 15 and 30 min samples respectively. Cell weight represents wet weight.

acids could not be detected in the cell lysates (used to assay LDH activity) obtained by disrupting the cells by repeated freezing and thawing. Bile acids secretion appeared to be linearly related with time for three hours. Thus three-hour values were used to calculate the secretion rate which is actually the net secretion rate since part of the secreted bile acids would be taken up by the liver cells at the same time. The net secretion rate of bile acids (23, 2 $\pm$ 2, 74 nmoles/g cells/min) was lower than biliary excretion of bile acids (51, 8 nmoles/g liver/min) reported for male Wistar rats (9). The secretion rate of bile acids by isolated hepatocytes may be considered in the physiological range when the simultaneous uptake of bile acids by the liver cells are taken into consideration. Liver cells take up as much as 20% of added bile acid in 15 min (unpublished data).

The distribution of individual bile acids in the incubation media was significantly different from that of biliary bile acids (table 1). The ratio of taurine to glycine conjugates was significantly higher in the bile than in the incubation media. This may be explained by the availablity of substrate for conjugation in the liver cells. Taurine may be in short supply in the cells and as a result glycine was used to conjugate the bile acids. Similar change in conjugation is demonstrated in patients with illeal disorder resulting in taurine depletion (13). However, a metabolic alteration during the isolation procedure resulting in the preference of glycine conjugation, although unlikely, cannot be ruled out. The ratio of dihydroxy to trihydroxy bile acids was also significantly higher in the incubation media. This difference in the relative proportion of primary bile acids was considered to indicate bile acid synthesis and not secretion of preexisting bile acids by the hepatocytes. Biosynthesis of cholesterol, precursor of bile acids synthesis, has been demonstrated in isolated rat hepatocytes (14). Since the bile acids in the incubation media would be mostly primary bile acids, synthesis of chenodeoxycholic acid was relatively higher than that of cholic acid in the hepatocytes. A combination of an inhibition of 12 α-hydroxylase and a stimulation

Table 1: Bile acid distribution in bile and isolated rat hepatocytes.

	Bile	Hepatocytes
No. of animals/trials	4(8)[a]	5(10)
Taurocholic acid (%)[b]	58,4 ± 3,51[c]	12,6 ± 1,66
Glycocholic acid (%)	6,1 ± 0,94	23,0 ± 2,36
Tauro-dihydroxy bile acids (%)	28,8 ± 1,79	43,2 ± 1,81
Glyco-dihydroxy bile acids (%)	4,2 ± 0,95	10,9 ± 1,63
Unidentified bile acid (%)	2,7 ± 0,69	10,4 ± 1,61
Dihydroxy/trihydroxy bile acids	0,53 ± 0,063	1,58 ± 0,150
Taurine/glycine conjugates	10,6 ± 2,16	1,7 ± 0,15

[a]No. of determinations, [b]Percent of total bile acids, [c]Mean ± S.E.M.

All values were significantly different when compared between bile and hepatocytes by student's t-test at 95% significance level.

of microsomal 26-hydroxylase was suggested for similar increase in primary bile acids ratio in hyperthyroid rate (15). The liver cells would be expected to be devoid of any thyroid hormone following the isolation procedure. Thus, it is likely that factor (s) other than thyroid hormone may be responsible for relatively higher synthesis of chenodeoxycholic acid in the hepatocytes.

The present study showed that isolated rat hepatocytes synthesize and secrete bile acids and thus provide a suitable

experimental system for studying the effects of drugs and hepatic diseases on bile acids secretion and synthesis at cellular level. Relatively higher synthesis of chenodeoxycholic acid in the hepatocytes could be used to study the mechanism controlling the relative proportion of primary bile acids synthesis from cholesterol.

REFERENCES

1. Nair, P.P., and Kritchevsky, D. (1973) The Bile Acids, pp. 55-82, Plenum Press, New York.
2. Anwer, M.S.: Engelking, L.R., Gronwall, R., and Klentz, R.D. (1975) Res. Vet. Sci., in press.
3. Cronholm, T., Einarsson, K., and Gustafsson, J. (1974) Lipids 9, 844-849.
4. Crane, L.J., and Miller, D.L. (1974) Biochem. Biophys. Res. Commun. 60, 1269 - 1277.
5. Sundler, R., Akesson, B., and Nilsson, A. (1974) J. Biol. Chem. 249, 5102-5107.
6. Berry, M.N., and Friend, D.S. (1969) J. Cell. Biol. 43, 506-520.
7. Baur, H. (1971) Diplomarbeit an der Eberhard-Karl-Universität Tübingen.
8. Iwata, T., and Yamasaki, K. (1964) J. Biochem. (Tokyo). 56, 424-431.
9. Paumgartner, G., Horak, W., Prost, P., and Grabner, G. (1971) Naunyn-Schmiedeberg's Arch. Pharmacol. 270, 98-101.
10. Bruusgaard, A. (1969) Clin. Chim. Acta 28, 495-501.
11. Hoffmann, A.F. (1962) J. Lipid Res. 3, 127 -128.
12. Raabo, E., and Terkildsen, T.C. (1960) Scand. J. Clin. Lab. Invest. 12, 402-407.
13. Garbutt, J.T., Heton, K.W., Lack, L., and Tyor, M.P. (1969) Gastroenterology 56, 711-720.
14. Nilsson, A., Sundler, R., and Akesson, B. (1973) Eur. J. Biochem. 39, 613-620.
15. Björkhem, I., and Danielsson, H. (1974) Mol. Cell. Biochem. 4, 79-95.