Expansion of the bile acid pool changes the biliary transport characteristics of centrizonal hepatocytes

J. Reichen and M. Le

Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3010 Berne (Switzerland), and the Division of Gastroenterology, Department of Medicine, University of Colorado School of Medicine, Denver (Colorado, USA)
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Summary. We investigated whether acinar differences in taurocholate transport are responsible for the increased maximal secretory rate observed after expansion of the bile acid pool. The bile acid pool was expanded by cholate feeding for four days. Periportal and centrizonal hepatocytes were then probed by ante- and retrograde liver perfusion, respectively. In control animals, secretory rate constant (α_1) averaged 0.439 ± 0.123 and 0.104 ± 0.035 min^{-1} during ante- and retrograde perfusion, respectively, in the absence of exogenous taurocholate. These values did not significantly change when taurocholate was infused. In cholate-fed animals, α_1 was comparable during antegrade perfusion but was significantly reduced (0.038 ± 0.035, $p < 0.05$) during retrograde perfusion in the absence of exogenous taurocholate, presumably owing to induction of cytosolic bile acid binding proteins. During loading with exogenous taurocholate, by contrast, α_1 was significantly accelerated (0.252 ± 0.026; $p < 0.01$) in centrizonal hepatocytes from bile-acid fed rats. Expansion of the bile acid pool is able to change the bile salt secretory characteristics of centrizonal hepatocytes toward those of periportal ones.
Key words. Taurocholate; acinus; liver perfusion; extraction; excretion; periportal; bile flow; oxygen consumption.

Hepatic uptake and biliary excretion of bile acids are main determinants of canalicular bile formation [1]. A marked lobular gradient for bile acids has been demonstrated autoradiographically [2]. While periportal and centrizonal hepatocytes take up bile acids equally well [3, 4], centrizonal hepatocytes are less efficient at biliary excretion [4].

Expansion of the bile acid pool alters the excretory capacity of the liver [5–8]. It has been suggested that this is due to recruitment of hepatocytes [5, 6] or an increase in bile acid carriers [8]. It is unknown, however, whether expansion of the bile acid pool alters the bile acid transport characteristics of the different acinar zones.

To answer this question, the effects of bile acid feeding on hepatic uptake and biliary excretion of taurocholate were studied in the rat. The investigations were performed in the in situ perfused liver during ante- and retrograde perfusion to probe periportal and centrizonal hepatocytes, respectively [4].

Materials and methods. Male Sprague-Dawley rats, body weight 269 ± SD 27 g, were obtained from Charles River Breeding Laboratories, Wilmington, MA. They were allowed free access to standard rat chow and tap water. They were kept in temperature and humidity controlled animal quarters under a 12-h light-dark cycle.

Taurocholic and cholic acids were obtained from Calbiochem, La Jolla, CA. Both bile acids were > 95% pure by thinlayer and gas liquid chromatography [9].

^{14}C-taurocholate (sp. act. 40.3 mCi/mmol) was obtained from New England Nuclear, Boston, MA. NAD was from Boehringer-Mannheim Biochemicals, Indianapolis, IN, 3-hydroxysteroid dehydrogenase from US Biochemical Corp., Cleveland, OH, and fatty acid-poor bovine serum albumin from Calbiochem.

To increase the bile acid pool, rats received 100 mg/kg b.wt of sodium cholate by gavage daily for four consecutive days [8]. Control animals received an equal volume of normal saline by gavage. The day after the last treatment, the animals were anesthetized with pentobarbital (50 mg/kg i.p.). The common bile duct was cannulated with PE 10 tubing. Bile was collected for 10 min to determine bile flow and bile acid secretion in vivo.

Thereafter, the animal was prepared for in situ rat liver perfusion as previously described [9]. Liver perfusion was carried out using a semi-synthetic perfusion medium consisting of washed human erythrocytes (20% v/v), bovine serum albumin (2% w/v) and glucose (0.1% w/v) in Krebs-Ringer-bicarbonate buffer.

Endogenous bile acids were drained from the liver during a 30-min period in 4 cholate and 4 control animals. Then, antegrade perfusion was carried out, followed by retrograde perfusion as described by Groothuis et al. [4]. Preliminary experiments had shown that the order of ante- or retrograde perfusion did not affect taurocholate uptake or excretion. During ante- and retrograde perfusion, 0.1 μCi of ^{14}C-taurocholate was injected as a bolus into the portal or hepatic vein, respectively. Total outflow was collected for 2 min to determine hepatic extraction efficiency and to prevent recirculation of the indicator. Bile was collected at 15-s intervals, under water, into liquid scintillation vials to minimize the effect of drop size [10]. Then, the direction of perfusion was reversed and taurocholate extraction and excretion determined again.

In another set of four control and four cholate fed animals, a taurocholate infusion of 2 $\mu moles \cdot min^{-1} \cdot kg^{-1}$ was given after a 20-min equilibration period. Thirty minutes thereafter, taurocholate extraction and excretion were determined during ante- and retrograde perfusion as described above.

Viability of the perfused organ was assessed using ALAT and potassium release and oxygen consumption as previously described [9, 10].

^{14}C-radioactivity was determined by liquid scintillation counting using Budget-solve^{R} (Research Products International, Elk Grove, IL) as scintillator and external standardization for quench correction. Bile acids in bile were determined by the 3-hydroxysteroid dehydrogenase assay as previously reported [9]. Bile flow was determined gravimetrically during 10-min periods assuming a density of 1 g/ml.

Taurocholate extraction E was determined as

$$E = \left(\frac{D - \Sigma dpm_{hv}}{D}\right) \cdot 100 \qquad (1)$$

where D is the radioactivity injected and Σdpm_{hv} the cumulative recovery in hepatic vein outflow.

The decline in radioactivity in bile y (t) was fitted to a mono- or biexponential equation

$$y(t) = \sum_{i=1}^{1,2} A_i e^{-\alpha_i \cdot t} \qquad (2)$$

by a non-linear least square fitting routine [11]. An F-test was used to differentiate between mono- and bioexponential decline of radioactivity. All retrograde perfusion experiments exhibited monoexponential decay. A second exponential (α_2) was observed during antegrade perfusion only; since α_2 contributed less than 5% to excretion it will not be reported. Results are reported as mean ± 1 SD. Means of two groups were compared by Student's t-test [12]. Multiple means were compared by a modification of Peritz's F-test [13]. Paired data were compared by the paired t-test [12]. $p < 0.05$ was considered statistically significant.

Results. Cholate fed animals gained weight at a rate comparable to control animals (11 ± 9 vs 15 ± 12 g/4 d; $p > 0.50$). There was no difference in liver weight between the two groups (11.6 ± 1.9 vs 11.8 ± 1.2 g; $p > 0.90$). Bile flow in vivo was increased by 59% in cholate fed rats (3.4 ± 0.32 vs 1.98 ± 0.29 $\mu l \cdot min^{-1} g liver^{-1}$; $p < 0.001$). Bile salt secretion rate was increased threefold (233 ± 31 vs 73 ± 26 nmoles $\cdot min^{-1} g liver^{-1}$; $p < 0.001$).

All experiments met the viability criteria set out in the method section. There was no difference in ALAT or potassium release during ante- or retrograde perfusion or between cholate-fed and control animals (data not shown). There was no difference in portal pressure or flow between ante- and retrograde perfusion or between control and cholic acid fed animals (table 1). By contrast, oxygen consumption was increased in livers from cholic acid fed animals during perfusion by either route; oxygen consumption was not affected by the direction of perfusion (table 1).

Bile flow under the different experimental conditions is reported in table 2. Bile flow tended to be higher in cholic acid-fed animals both in the absence and in the presence of exogenous taurocholate but this did not reach statistical significance. By contrast, bile flow during retrograde perfusion was, with one exception, always higher than during the corresponding antegrade perfusion regardless of treatment (fig. 1; $p < 0.005$ by paired t-test).

Taurocholate extraction did not differ during ante- and retrograde perfusion and was not affected by bile acid feeding (table 3). Extraction decreased slightly during the taurocholate infusion but again, no difference was noted between control and cholate-fed animals. The taurocholate excretion rate constant was higher during ante- than during retrograde perfusion (table 3). In the absence of exogenous taurocholate, it was significantly lower during retrograde perfusion in cholate-fed as compared to control rats ($p < 0.05$). When taurocholate was added, however, it was significantly higher in cholate-fed animals during retrograde perfusion ($p < 0.01$).

Discussion. The present study demonstrates that expansion of the bile acid pool by cholate feeding markedly alters the secretory characteristics of centrizonal hepatocytes as tested by retrograde perfusion. In the presence of a moderate exogenous taurocholate load centrizonal hepatocytes of cholate-fed animals excrete taurocholate as rapidly as periportal hepatocytes. In the absence of exogenous taurocholate, however, these hepatocytes excrete taurocholate even more slowly than periportal hepatocytes or centrizonal hepatocytes of control animals.

Ante- and retrograde perfusion is an established method to probe the different acinar zones in the liver. The technique has been successfully employed to study acinar differences in intermediate metabolism [14], drug metabolism [15] and taurocholate transport [4], and even to prepare hepatocytes from the different regions [16]. Gumucio et al. using region-specific toxins, demonstrated that centrizonal and periportal hepatocytes take up bile acids equally well [3]. Groothuis and her

Table 1. Characteristics of perfused rat liver during ante- and retrograde perfusion

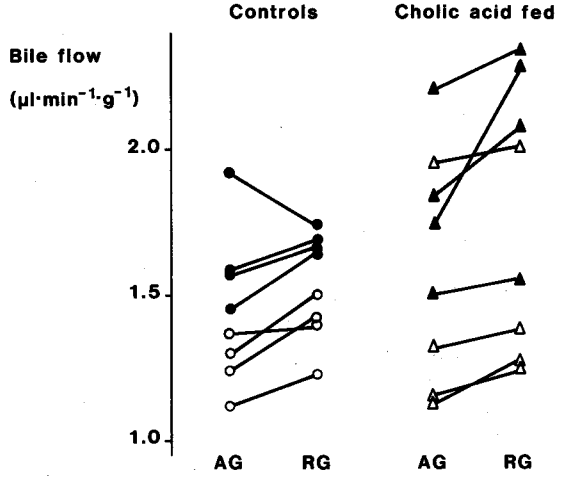
	Pressure ($cm H_2O$)	Flow ($ml \cdot min^{-1} g^{-1}$)	Oxygen consumption ($\mu moles \cdot min^{-1} g^{-1}$)
Antegrade			
Control	10.2 ± 0.2	1.37 ± 0.38	2.06 ± 0.19
Cholic acid fed	10.2 ± 0.4	1.59 ± 0.58	2.31 ± 0.14
Retrograde			
Control	9.9 ± 0.3	1.44 ± 0.39	2.10 ± 0.09
Cholic acid fed	10.2 ± 1.0	1.41 ± 0.56	2.29 ± 0.12 [1]

$\bar{x} \pm SD$ are given. $N = 8$ per group. [1] $p < 0.05$ compared to ante- and retrograde control (by Peritz' F-test).

Table 2. Bile flow ($\mu l \cdot min^{-1}$ g $liver^{-1}$) during ante- and retrograde perfusion in control (CTR) and cholic acid fed (CAF) rats

Taurocholate infusion	0		2 $\mu moles \cdot min^{-1} kg^{-1}$	
	CTR	CAF	CTR	CAF
Antegrade	1.26 ± 0.11	1.38 ± 0.39	1.63 ± 0.20	1.82 ± 0.30
Retrograde	1.38 ± 0.12	1.48 ± 0.36	1.68 ± 0.05	2.07 ± 0.36

$\bar{x} \pm SD$ are given. $n = 4$ per group. Taurocholate infusion significantly ($p < 0.01$) increased bile flow in all experimental groups; there was no effect of cholic acid feeding on bile flow during either perfusion mode.



Bile flow during ante- (AG) and retrograde (RG) perfusion in control (○) and cholic acid fed (△) rats. Bile flow in the absence and presence (0.2 $\mu moles \cdot min^{-1}$ 100 g^{-1}) of exogenous taurocholate are shown by open and solid symbols, respectively. Bile flow during RG perfusion was significantly higher than during AG perfusion ($p < 0.005$ by paired t-test).

Table 3. Effect of ante- and retrograde perfusion on taurocholate extraction (E; %) and excretion rate constant (α_1; min^{-1}) in control (CTR) and cholic acid fed (CAF) rats

Taurocholate infusion	0		2 $\mu moles \cdot min^{-1} kg^{-1}$	
	CTR	CAF	CTR	CAF
Extraction (E)				
Antegrade	98.5 ± 0.8	99.0 ± 0.5	95.8 ± 2.8	95.4 ± 4.5
Retrograde	97.8 ± 1.4	98.0 ± 1.6	94.5 ± 2.3	95.3 ± 1.4
Excretion (α_1)				
Antegrade	0.439 ± 0.123	0.510 ± 0.105	0.377 ± 0.121	0.435 ± 0.090
Retrograde	0.104 ± 0.035	0.038 ± 0.035 [1]	0.113 ± 0.069	0.252 ± 0.026 [2]

[1] $p < 0.05$ compared to control; [2] $p < 0.01$ compared to control.

collaborators were the first to demonstrate in normal liver that centrizonal hepatocytes take up bile acids equally well but they excrete them at a much lower rate than periportal hepatocytes do[4]. We have confirmed these findings in our control animals, extraction being equal during ante- and retrograde perfusion but excretion rate (α_1) being markedly slower during retrograde perfusion.

Oral bile acid feeding has been used by several investigators to expand the bile acid pool[5-8]. Although we did not measure the bile acid pool in the present experiments, the increase in bile flow and basal bile salt secretion was comparable to that in another study using the same feeding schedule[8]. It can be assumed, therefore, that we induced an 8-fold expansion of the bile acid pool similar to that described by previous investigators[8]. Expansion of the bile acid pool has been shown to increase the maximal rate of taurocholate secretion in vivo[5-8]; this has been ascribed to recruitment of hepatocytes[5,6] and to an induction of bile acid receptors[8]. Our results suggest two different phenomena induced by bile acid feeding. In the absence of exogenous taurocholate the excretion rate constant is even slower in bile acid-fed than in control rats. This could be due to induction of bile acid-binding proteins in the hepatocyte[17]. It is not known whether these binders have a preferential acinar localization but the delay in excretion observed during retrograde perfusion is compatible with such a hypothesis. When excretion is studied during a period of moderate taurocholate load, by contrast, a marked acceleration in taurocholate secretion is observed in pool-expanded, but not in control rats. We submit that this phenomenon is due to saturation of the bile acid binders, and excretion thus becomes the rate-limiting step. Under these experimental conditions it becomes obvious that the secretory characteristics of centrizonal hepatocytes have been dramatically changed; they now have the secretory characteristics of periportal hepatocytes. These findings suggest that the previously-described increase in cholic acid receptors induced by bile acid feeding[8] could be occurring mostly in centrizonal hepatocytes.

Two serendipitous observations deserve comment. Livers of cholate-fed animals exhibited significantly higher oxygen consumption than those of control rats (table 1). This is probably due to the induction of Na,K-ATPase as described by Wannagat et al. after cholate feeding[6]. The other one relates to the increase in bile flow observed during retrograde perfusion (fig.). Mention of this phenomenon has also been made by Groothuis et al.[4]. The reason for it is unknown; its occurrence in the absence of exogenous bile acids suggests that it could be related to asymmetric distribution of ionic pumps; improved oxygenation of centrizonal hepatocytes is thought to be mainly responsible for formation of bile salt-independent canalicular bile flow[3].

In conclusion, our study has shown that expansion of the bile acid pool by cholate feeding alters the secretory characteristics of centrizonal hepatocytes, making them behave more like periportal hepatocytes, when challenged with a moderate taurocholate load. In the absence of exogenous bile acids, however, they secrete bile acids more sluggishly, perhaps secondarily to induction of cytosolic bile acid-binding proteins. Taken together, these two mechanisms could protect centrizonal hepatocytes from potentially toxic levels of bile acids.

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Immunohistochemical localization of glutamine synthetase in human liver

R. Gebhardt[a]*, H. Schmid[b] and H. Fitzke[a]

[a] *Physiologisch-chemisches Institut, Hoppe-Seyler-Str. 4, and* [b] *Pathologisches Institut, Liebermeisterstr. 8, Universität Tübingen, D-7400 Tübingen (Federal Republic of Germany)*
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Summary. Glutamine synthetase (GS) of human liver was recognized with a polyclonal antibody to pig brain GS, but failed to stain with an antibody against rat liver GS. Using the latter antibody GS of human liver was shown to be localized within small rings of 1 to 3 hepatocytes surrounding the terminal hepatic venules. This pattern was analogous to that seen in rat and mouse liver.
Key words. Ammonia detoxification; enzyme distribution; glutamine synthetase; human liver; immunohistochemistry.