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Influence of common bile duct cannula size on maximal secretory rate of taurocholate in the rat

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Summary

The common bile duct of male Sprague-Dawley rats was cannulated with either PE 10 or PE 50 tubing. Maximal secretory rate of taurocholate averaged 389 ± 67 (SD) and 657 ± 115 nmoles·min^{-1}·g liver^{-1} in the PE 10 and PE 50 group, respectively ($p < 0.005$). Maximal bile secretory pressure was significantly higher in the PE 10 group (240 ± 28 vs 174 ± 8 mm H20; $p < 0.005$). When the maximal secretory rate was exceeded, bile flow decreased in both groups but this was accompanied with a decrease in maximal bile secretory pressure in the PE 10 group only. Maximal secretory rate of bile salts is markedly influenced by experimental technique. Use of small caliber common bile duct cannulae leads to partial obstruction and decreases the apparent maximal secretory rate for taurocholate.

Secretion of bile salts into bile is one of the major determinants of bile formation[4,16]. Secretion depends upon at least 2 steps, namely uptake into the hepatocyte and secretion across the canalicular membrane into bile. While the first step has been well characterized as a carrier-mediated, sodium dependent process[13] the excretory step is much less well undestood. It has classically been defined by the secretory transport maximum[12]. This concept has been criticized recently, since the maximal rate by which bile salts are secreted depends on a balance between load and toxicity of the bile salt studied[7]. It has therefore been proposed to use the term maximal secretory rate (SRm) rather than transport maximum for this step[7]. When the maximal secretory rate of taurocholate is exceeded, cholestasis ensues[8] lending further support to the concept that the maximal secretory rate is a balance between load, true transport maximum and toxicity[7].

PE 10 cannulae are customarily employed for common bile duct cannulation in the rat[4,8,11,12,14], but in a recent study a larger PE 50 cannula was employed and the authors found a higher maximal secretory rate than is usually reported[7]. We therefore studied whether PE 10 catheters induce partial biliary obstruction which might influence the maximal secretory rate of taurocholate.

Materials and methods

Male Sprague-Dawley rats (Charles-River Breeding Laboratories; Wilmington, MA) were maintained in temperature and humidity controlled animal quarters on a 12-h light-dark cycle and allowed free access to standard rat food and tap water. Body and liver weights at the time of study averaged 264 ± 34 and 10.0 ± 1.3 g, respectively.

Taurocholate sodium was grade A from Calbiochem, La Jolla, CA. It was > 96% pure by thin-layer chromathography[9]. After hydrolysis only 1 peak corresponding to cholic acid was seen by gas-liquid chromatography[10]. NAD was from Boehringer-Mannheim Biochemicals, Indianapolis, IN and 3-hydroxy steroid dehydrogenase from U.S. Biochemical Corporation, Cleveland, OH.

All animals were allowed access to food until the time of study which was always between 08.00 and 09.00 h. The animals were anesthetized with pentobarbital sodium (50 mg/kg) i.p. The common bile duct was cannulated with either PE 10 or PE 50 tubing (Clay-Adams, Parsippany, NJ). The inner diameter as stated by the manufacturer was 0.28 and 0.58 mm for PE 10 and PE 50 tubing, respectively. In addition to the common bile duct, a jugular vein was cannulated with PE 50 tubing. Body temperature was monitored with a rectal thermometer and kept between 37.5 and 38.50 °C by means of a heating lamp. Bile was collected in 10-min intervals into tared tubes and weighed. During a control period of 20 min, normal saline was infused through the jugular vein catheter at a rate of 2 ml/h. Thereafter, taurocholate dissolved in normal saline was infused at rates of 0.8–2.0 μmoles·min^{-1}·100 g^{-1}. Each infusion rate was maintained for 30 min.

Maximal bile secretory pressure was measured in 2 groups of animals. In the method 'from below' the cannula was elevated above the hilus of the liver until a stable pressure was achieved (usually within 5 min). In the method 'from above' the cannula was connected to a manometer which had previously been filled with normal saline. Biliary bile salt concentrations were measured photometrically using the hydroxy steroid dehydrogenase method[11].

All results are expressed as mean ± 1 SD. Means of 2 groups were compared with Student's t-test after testing the equality of variances with an F-test[15]. If the variances were unequal, a modified t-test was employed[17]. Linear regression analysis was performed by the method of least squares[15]. $p < 0.05$ was considered statistically significant.

Results

Bile flow and bile salt secretion rate did not differ during the control period between the groups fitted with a PE 10 and a PE 50 common bile duct cannula (table 1). By contrast, the maximal secretory rate for taurocholate was 69% higher in the PE 50 than in the PE 10

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Table 1. Effect of catheter size on bile flow and bile salt secretion rate in the bile fistula rat

	PE 10 Bile flow	Bile salt secretion	PE 50 Bile flow	Bile salt secretion
Control period	1.96 ± 0.13	90 ± 25	1.95 ± 0.14	76 ± 17
Taurocholate SRm	3.76 ± 0.11	389 ± 67	5.41 ± 0.27[a]	657 ± 115[b]
Cholestasis[d]	2.67 ± 0.42	213 ± 24	4.12 ± 0.85[c]	394 ± 80[b]

Bile flow is expressed as $\mu l \cdot min^{-1} \cdot g\ liver^{-1}$ and bile salt secretion as $nmoles \cdot min^{-1} \cdot liver^{-1}$; $\bar{X} \pm 1$ SD are given (N = 4); [a] $p < 0.001$ compared to PE 10; [b] $p < 0.005$ compared to PE 10; [c] $p < 0.01$ compared to PE 10; [d] Cholestasis relates to the period immediately following SRm.

group ($p < 0.005$; table 1). In the period immediately following taurocholate maximum secretory rate, bile flow fell by 41 and 24% in the PE 10 and PE 50 group, respectively (table 1). There was no difference in the relationship between bile flow and bile salt secretion rate between the 2 groups (fig.). In both cases, this relationship could be described by a straight line, the intercept being 1.70 and 1.87 $\mu l \cdot min^{-1} \cdot g\ liver^{-1}$ in the PE 10 and the PE 50 group, respectively. The corresponding slopes were 5.37 and 5.05 µl/µmole. Neither difference was statistically significant. Maximal bile secretory pressure was measured in 2 different groups of animals 'from below' and 'from above'. The 2 methods gave identical results under basal conditions (table 2). The maximal bile secretory pressures in response to taurocholate are given in table 3. The method 'from below' was used in these experiments. Maximal bile secretory pressure was significantly higher in the PE 10 group during both the control period and once taurocholate SRm had been reached. After exceeding SRm, bile flow decreased (table 1) and there was a sudden and significant drop in the maximal bile secretory pressure in animals fitted with a PE 10, but not with a PE 50 cannula (table 3).

Table 2. Bile secretory pressure assessed by two different methods in the fistula rat

	PE 10	PE 50
From above (N = 3)[a]	228 ± 19	178 ± 16[c]
From below (N = 4)[b]	240 ± 28	174 ± 8[c]

Bile secretory pressure is expressed in mm H_2O; $\bar{X} \pm$ SD are given. The results of the 2 methods did not differ statistically within the PE 10 and PE 50 group; [a] from above: bile secretory pressure was measured by connecting a saline filled manometer to the common bile duct cannula; [b] from below: bile secretory pressure was measured by elevating the common bile duct cannula above the liver hilus; [c] $p < 0.005$ compared to PE 10.

Table 3. Effect of catheter size on bile secretory pressure in the bile fistula rat

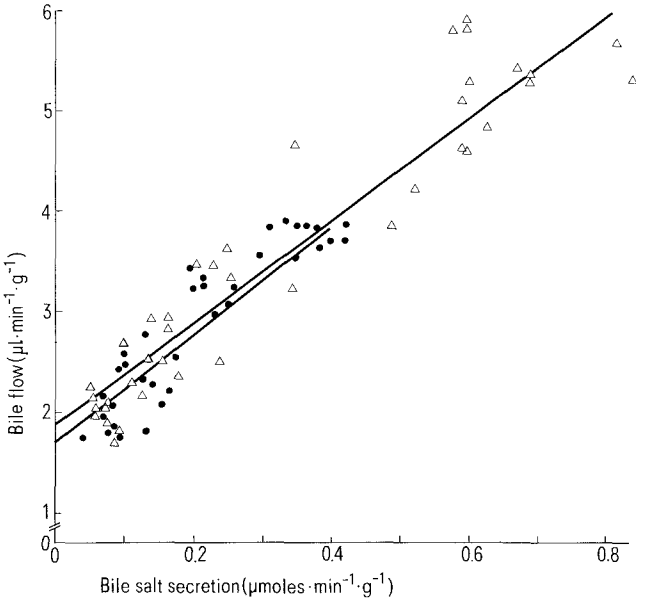
	PE 10	PE 50
Control period	240 ± 28	174 ± 8[a]
Taurocholate SRm	205 ± 27	156 ± 9[b]
Cholestasis[e]	173 ± 18[c]	150 ± 17[d]

Bile secretory pressure measured from below is expressed in mm H_2O. $\bar{X} \pm$ SD are given (N = 4). Differences between the groups were calculated with Student's t-test, within the group with a paired t-test; [a] $p < 0.005$ compared to PE 10; [b] $p < 0.01$ compared to PE 10; [c] $p < 0.005$ compared to control period; [d] $p < 0.025$ compared to control period; [e] cholestasis relates to the period immediately following SRm.

Discussion

The main finding of the present study is that catheter size influences the magnitude of the maximal secretory rate for taurocholate (SRm). This finding lends further support to the notion that SRm does not reflect the maximal capacity of the bile salt secretory transport system as implied by the term transport maximum (Tm)[7]. The values found for SRm in the present study are similar to comparable values in the literature. Thus, Tm in the rat has been described to be 348 $nmoles \cdot min^{-1} \cdot g\ liver^{-1}$ when a PE 10 common bile duct cannula had been used[12,14]. This compares with our value of 389 $nmoles \cdot min^{-1} \cdot g\ liver^{-1}$ in the PE 10 group. When a PE 50 cannula was employed taurocholate SRm was 68% higher; this value is similar to the SRm reported by Hardison and coworkers who also employed a PE 50 cannula[7].

The most likely reason for this difference is partial biliary obstruction. PE 10 catheters of varying lengths have been successfully used to induce partial biliary obstruction in the rat[2]. Under the present experimental conditions, this was not manifest under basal conditions as the control bile flow and bile salt secretion rates did not differ between the 2 groups (table 1). The additional demand put on the liver by bile salt infusion, however, could not be accommodated, resulting in a lower taurocholate SRm in the partially obstructed PE 10 animals. Biliary secretory pressure is independent of bile flow under physiologic conditions[4,5]. This was also seen in the present study, where maximal bile secretory pressure, after an initial drop, remained constant despite increasing bile flow until taurocholate SRm was reached



Relationship between bile flow and bile salt secretion rate in animals fitted with PE 10 (○) or PE 50 (△) cannulae. Only values until and including maximal secretory rate of taurocholate are shown. In both groups, there was a significant linear correlation between bile flow and bile salt secretion rate, the equations being $y = 1.70 + 5.37 \times$ ($r = 0.885$; $p < 0.001$) and $y = 1.87 + 5.05 \times$ ($r = 0.938$; $p < 0.001$) in the PE 10 and PE 50 groups, respectively. Neither slope nor y-intercept differed significantly between the 2 groups.

(table 3). Maximal bile secretory pressure was significantly higher in animals with a PE 10 cannula. It is unlikely that this difference is due to intrinsic properties of the catheter. Similar pressure measurements were obtained whether the limiting pressure was obtained from above or from below (table 2). The difference cannot be ascribed to capillary forces, since no such effect was detectable using bile in vitro. Since the liver performs greater work during partial obstruction[2], we speculate that the increased work required of the liver results in higher secretory pressure. The sudden drop in pressure observed when taurocholate SRm is exceeded suggests the loss of some driving force of bile or regurgitation of bile. Our data do not allow differentiation between these two possibilities.

Analysis of the relationship beween bile flow and bile salt secretion rate shows that it could adequately be described by a straight line. In this classical analysis, the slope is taken as an indicator of the osmotic property of the bile salt studied, while the intercept with the ordinate reflects the so-called bile salt-independent fraction of bile flow[6]. Although this concept is an oversimplification[3] and other analyses could be applied to our data, this simple analysis proposed by Erlinger[6] still offers a valuable first approach. In our study, there was no difference in slope or intercept (fig. 1). Thus, neither bile salt-independent bile formation nor the osmotic property of taurocholate seem to be affected by the partial obstruction induced by the PE 10 cannula. The latter is in contrast to a recent report demonstrating increased osmotic activity of taurocholate in the presence of partial biliary obstruction[2]. This coud be due to the greater degree of obstruction in that investigation[2].

Similar to other organic anions, taurocholate leads to cholestasis when administered in excess of its secretory maximum in the perfused rat liver[8]. The present investigation confirms that finding in vivo. In conclusion, this study demonstrates that the maximal secretory rate for taurocholate is affected by the caliber of catheter used. It is proposed that the lower SRm observed when the customary PE 10 cannula is used for common bile duct cannulation is due to partial obstruction. Therefore, attention should be paid to the size of catheter used in future studies of biliary physiology.

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