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HEPATIC CLEARANCE AND BILIARY SECRETORY RATE MAXIMUM OF TAUROCHOLATE IN THE RECIRCULATING AND SINGLE PASS ISOLATED PERFUSED RAT LIVER

EFFECTS OF THE CHOLESTATIC AGENT, ESTRADIOL-17β-(β-D-GLUCURONIDE)

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Abstract—The ability of the cholestatic steroid glucuronide, estradiol-17β-(β-D-glucuronide) ($E_2$17G), to inhibit the hepatic clearance (Cl_H) and biliary secretory rate maximum (SR_m) of taurocholate was investigated in the recirculating and single pass isolated perfused male rat liver. In the recirculating perfused liver, $E_2$17G (0, 2, 4, or 6 μmol) was added as a bolus dose to the reservoir at zero time while taurocholate was infused into the portal vein in increasing amounts (15, 30, 45, or 60 μmol/mL; 1 mL/hr for 15 min each). $E_2$17G (4 μmol) caused a significant ($P < 0.05$) inhibition of bile flow and bile acid secretion at 10–15 min during infusion of 15 μmol/hr taurocholate but did not inhibit the SR_m which occurred at 42 min, indicating that $E_2$17G had not caused an irreversible inhibition of taurocholate transport. $E_2$17G (6 μmol) caused a profound and irreversible inhibition of bile flow attributable to retention of $E_2$17G in the liver. The noncholestatic estradiol-3-(β-D-glucuronide) ($E_2$3G; 6 μmol) had no significant effect on bile flow or the SR_m. In the single pass perfused liver (10 mL/min flow rate), $E_2$17G (0, 1, 2, 5, or 10 nmol/mL) or $E_2$3G (2 nmol/mL) was added to the perfusate resulting in a stable infusion to the liver. [^{3}H]Taurocholate was infused into the portal vein in increasing amounts to give inflow concentrations (C_{in}) of 25, 50, 75 or 100 nmol/mL. In the absence of $E_2$17G, taurocholate Cl_H decreased from 0.92 to 0.70 mL/min/g liver with increasing taurocholate concentrations. Neither $E_2$17G nor $E_2$3G altered the Cl_H of 25 nmol/mL taurocholate. $E_2$17G (10 nmol/mL) inhibited bile flow and bile acid secretion first at 20–25 min, followed by inhibition of Cl_H of 75 and 100 nmol/mL taurocholate (35–60 min). In contrast, $E_2$3G stimulated bile acid secretion and increased the SR_m by 80%. Thus, at doses that did not block its own elimination, $E_2$17G did not cause an irreversible inhibition of taurocholate transport into bile. $E_2$17G did not directly inhibit the uptake of taurocholate into the liver but first inhibited the biliary excretion of taurocholate, resulting in its intrahepatic accumulation and decreased clearance from the perfusate.

In the rat, bile secretion is attributed to osmotic water flow in response to the active transport of solutes into the canalicular space [1]. Bile acids are considered to be one of the major solutes generating bile flow because of the excellent correlation observed between bile flow and bile acid secretory rate. Taurocholate (TC) is the single most important bile acid in the rat. Its uptake into the hepatocyte has been shown to be carrier-mediated and dependent on the sodium gradient generated by Na^+,K^+-ATPase [2, 3]. The secretion of TC across the canalicular membrane is also carrier-mediated and is driven by the intracellular negative membrane potential [4]. Because the capacity for uptake of bile acids into the hepatocyte exceeds that of biliary secretion by 6- to 10-fold [5], and because of the toxicity of the bile acids, a true stable transport maximum (T_m) for bile acid secretion is not observed. Rather, a secretory rate maximum (SR_m) is reached, followed by a decrease in bile flow and bile acid secretion [6].

Estradiol-17β-(β-D-glucuronide) ($E_2$17G) is a naturally occurring metabolite of estradiol which induces a dose-dependent, reversible cholestasis *in vivo* in the rat and in the isolated perfused rat liver [7–9]. The cholestatic properties of $E_2$17G are shared by other glucuronide conjugates of the steroid D-ring, but not by the glucuronide conjugates of the steroid A-ring, such as estradiol-3-(β-D-glucuronide) ($E_2$3G) [7, 8]. $E_2$17G decreases the bile acid secretory rate, suggesting inhibition of bile salt secretion as a mechanism of cholestasis. We [9] and others [10] have shown that infusion of TC into the recirculating isolated perfused rat liver protects against cholestasis induced by a single bolus dose of $E_2$17G. The log dose–response curve for $E_2$17G-induced cholestasis is shifted to the right but with an increased slope in the presence of TC [9].

The present studies were designed to determine if the inhibition of bile acid secretion induced by $E_2$17g is reversible or irreversible. We have shown [7–9] that bile flow and bile acid secretory rate return to control levels after a single bolus dose of $E_2$17G. However, in these studies we had not determined if the maximal bile acid secretory rate (SR_m) was

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compromised. Thus, it is possible that $E_2$17G could irreversibly inhibit bile acid secretion at a limited number of sites/carriers, but that "spare" carriers, perhaps located along the liver lobule, could compensate and restore bile flow and bile acid secretory rate to initial levels.

We therefore determined whether addition of a single cholestatic dose of $E_2$17G to the recirculating isolated perfused liver caused a permanent inhibition of the SR_m. In a second series of experiments, we used the single pass perfused liver system to determine the effects of a stable infusion of $E_2$17G on the ability of the liver to extract TC from the perfusate and secrete it in bile.

MATERIALS AND METHODS

Materials. [^{3}H]TC (8.1 Ci/mmol; 98.2% purity) was purchased from New England Nuclear (Boston, MA). 3α-Hydroxy steroid dehydrogenase was purchased from the Worthington Biochemical Corp. (Freehold, NJ). $E_2$17G sodium salt, sodium taurocholic acid, $E_2$3G sodium salt and the GOT assay kit were purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade.

Animals. Male Sprague–Dawley rats (254 ± 15 g; mean ± SEM) (Harlan Industries, Indianapolis, IN) were housed in cages with Sani-chips bedding (PJ Murphy Forest Products, Montville, NJ), had free access to Purina rat chow and water, and were maintained on a 12-hr automatically timed light and dark cycle. Male retired breeder Sprague–Dawley rats were used as blood donors for the recirculating perfused liver and were housed similarly.

Recirculating isolated perfused liver. Untreated male rats anesthetized with urethane (1 g/kg, i.p.) were used as liver donors. The bile duct was cannulated with PE-10 tubing and the livers were perfused via the portal vein as described earlier [11] with 20% rat donor blood in Kreb's Ringer Bicarbonate buffer at a constant flow rate of 20 mL/min in a recirculating design. Perfusate was oxygenated with 95% O_2–5% CO_2 using the lung described by Hamilton *et al.* [12]. Total volume in the reservoir was 80 mL. Liver and perfusate were allowed to equilibrate for 20 min in a humidified plexiglass box at 37° while saline (1 mL/hr) was infused into the tubing leading to the portal vein. Two 5-min initial bile samples were collected at the end of the equilibration period. At zero time, the saline infusion was stopped and TC was infused similarly at increasing concentrations (30, 40, 50 and 60 μmol TC/mL saline; 1 mL/hr) for 15 min each. A 1-mL bolus dose of either vehicle (10:4:1; saline:propylene glycol:ethanol), $E_2$3G (6 μmol in vehicle) or $E_2$17G (2, 4 or 6 μmol in vehicle) was added to the reservoir at zero time. Bile was collected in tared tubes for 5-min intervals, and the amount of bile was determined gravimetrically, assuming a density of 1 g/mL. Bile acids were measured using the method of Talalay [13]. The liver was used only if initial bile flow was greater than 15 μL/min/kg (0.6 μL/min/g liver).

Single pass isolated perfused liver. Outdated (less than 1 week) packed human red blood cells (250 mL; Central Kentucky Blood Center, Lexington, KY) were washed three times with 2–3 vol. of 0.9% NaCl at 4° followed by three washes with 4° lactated Ringer's solution (Travenol Laboratories, Inc., Deerfield, IL). Cells were centrifuged in a GSA rotor at 4500 rpm for 6 min, and the supernatant and surface buffy coat were removed after each wash. Packed cells were then diluted with 800–1000 mL of 4° Kreb's Ringer Bicarbonate to give a hematocrit of 10–15%. Blood and buffer were then warmed to 37° in a circulating water bath. The portal vein and bile duct were cannulated and the liver was surgically isolated as described for the recirculating system. Perfusate was oxygenated as described above and the liver perfused at a flow rate of 10 mL/min in a single pass design in a humidified plexiglass box and maintained at 37°.

The liver was allowed to equilibrate for 30 min during which time TC (15 μmol/mL; 1 mL/hr) was infused into the tubing leading to the portal vein to give a theoretical concentration of 25 nmol/mL perfusate. Bile was collected for two 5-min periods to determine initial bile flow and livers were used only if bile flow was greater than 15 μL/min/kg (0.6 μL/min/g liver).

At zero time, the initial perfusate was replaced with perfusate containing either the vehicle (1 mL added to 650 mL perfusate) or $E_2$17G (final concentration 1, 2, 5 or 10 nmol/mL) in vehicle, or $E_2$3G (final concentration 2 nmol/mL) in vehicle. Also at zero time, [^{3}H]TC (15 μmol/mL; 1 mL/hr) was infused into the tubing, replacing the initial unlabeled TC infusion. The concentration of [^{3}H]TC infused was increased every 15 min from 15 μmol/mL (0–15 min) to 30 μmol/mL (15–30 min) to 45 μmol/mL (30–45 min) to 60 μmol/mL (45–60 min) giving estimated inflow perfusate concentrations (C_{in}) of 25, 50, 75 and 100 nmol/mL respectively. Inflow perfusate samples (1 mL) were collected with a syringe at 9, 14, 24, 29, 39, 44, 54 and 59 min and showed stable concentrations for each C_{in} of [^{3}H]TC. The actual measured C_{in} of [^{3}H]TC are shown in Figs. 2 and 3. Outflow perfusate samples (1 mL) were collected at the midpoint of each 5-min bile collection period. The last two outflow samples in each 15-min period were essentially identical indicating that steady state had been achieved. The mean of these two values was used as the outflow concentration (C_{out}) of [^{3}H]TC.

Inflow and outflow perfusate samples were centrifuged to separate blood from plasma, and 200 μL of the supernatant was assayed for radioactivity using 5 mL of Safety Solve (Research Products International Corp., Mt. Prospect, IL) in a 1500 series Tricarb liquid scintillation analyzer (Packard Instrument Co., Downers Grove, IL).

Glutamic-oxalacetic transaminase (GOT) activity was monitored in outflow perfusate samples, using a Gilford Response Series UV-Vis spectrophotometer (Ciba Corning Diagnostics Corp., Oberlin, OH). An open manometer in the segment between the pump and the liver was used to monitor the inflow perfusion pressure throughout the experiment.

Data analyses. In the recirculating isolated perfused liver, a one-way ANOVA followed by a Dunnett's test was used to compare the effects of

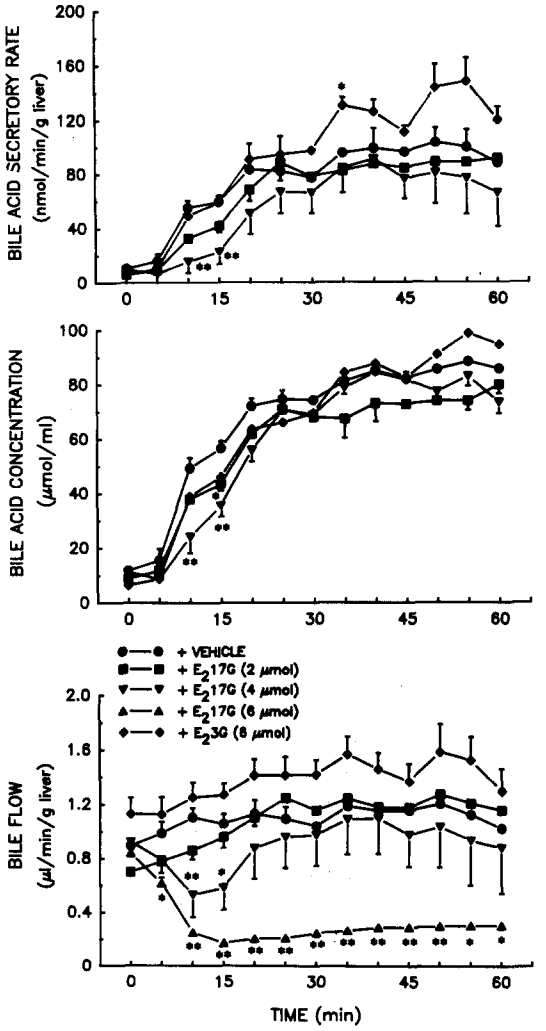

Fig. 1. Effect of a single bolus dose of vehicle or $E_2$17G (2, 4, or 6 μmol) or 6 μmol $E_2$3G on bile flow, bile acid concentration and bile acid secretory rate in the recirculating perfused liver. Taurocholate was infused (1 mL/hr) at increasing concentrations (15, 30, 45 and 60 μmol/mL, for 15 min each) as described in Materials and Methods. Values are means ± SEM, N = 4 per group. Statistics: one-way ANOVA followed by Dunnett's multiple range test: (*) $P < 0.05$, and (**) $P < 0.01$, significantly different from vehicle control. $E_2$17G at 6 μmol reduced bile flow to levels inadequate for bile acid analysis

saline, $E_2$3G (6 μmol) and $E_2$17G (2, 4 or 6 μmol) on bile flow, bile acid concentration and bile acid secretory rate.

For the single pass perfusion, hepatic clearance (Cl_H) of TC was calculated according to the equation $Cl_H = Q \cdot E$, where Q = flow rate and E = extraction ratio. The extraction ratio was calculated as $(C_{in} - C_{out})/C_{in}$. One-way ANOVA followed by a Student–Neuman–Keuls multiple range test was used to compare the effects of vehicle and $E_2$17G(1, 2, 5 or 10 nmol/mL) in the presence of [^{3}H]TC on bile flow, bile acid concentration, bile acid secretory rate, and Cl_H of [^{3}H]TC. In all cases the criteria for statistical significance was $P < 0.05$.

Table 1. Effects of $E_2$17G and $E_2$3G on the bile acid secretory rate maximum (SR_m) in the recirculating isolated perfused liver

Additions (μmol)	SR_m (nmol/min/g liver)	Time (min)
$E_2$17G		
0	120 ± 15	47.5 ± 4.8
2	107 ± 7	43.8 ± 7.5
4	96 ± 23	42.5 ± 4.3
$E_2$3G		
6	154 ± 14	48.8 ± 4.7

Values are means ± SEM, N = 4. SR_m and time data were determined as the maximal bile acid secretory rate and the time at which it occurred for each liver perfusion experiment.

RESULTS

Recirculating isolated perfused liver. Infusion of increasing concentrations of TC in the control group caused a modest increase in bile flow and large increases in bile acid concentration and bile acid secretory rate (Fig. 1). These data also show that the bile acid secretory rate had reached a maximum within each 15-min period for the 30, 45 and 60 μmol/hr TC infusions. Addition of 2 μmol $E_2$17G had no significant effect on bile flow or bile acid secretory rate although the bile acid concentration was decreased at 15 min. Four micromoles of $E_2$17G significantly inhibited bile flow at 10 and 15 min; bile flow recovered to control levels although with a good deal of variability. The bile acid concentration and bile acid secretory rate were also significantly inhibited at 10 and 15 min, but again recovered to control levels. As shown in Table 1, neither 2 nor 4 μmol $E_2$17G had a significant effect on SR_m. These data indicate that the inhibition of bile flow and bile acid secretion by $E_2$17G is reversible and that the liver can attain the same SR_m after exposure to $E_2$17G. Six micromoles $E_2$17G decreased bile flow markedly and irreversibly, however, and bile flow was inadequate for quantitation of bile acids. The lack of recovery in this case was due to the inability of the liver to eliminate $E_2$17G in bile. We [9] have shown that addition of 2.1 μmol $E_2$17G to the recirculating isolated perfused liver in the absence of bile acids decreases bile flow to about 0.4 μL/min/g liver; [^{3}H]$E_2$17G is excreted in bile at a stable, maximal rate of 1.5 nmol/min/g liver. Assuming a similar excretion rate in the present studies, only 900 nmol or 15% of the dose of 6 μmol would be excreted during the 60-min perfusion period. In contrast, 6 μmol $E_2$3G had no effect on bile flow or the bile acid concentration, but increased the bile acid secretory rate at 35 min. The SR_m was increased by 28% but this did not reach statistical significance.

Single pass isolated perfused liver. The concentrations of GOT in effluent perfusate ranged from 2 to 10 units/L and were within the normal range for the liver [14]. Perfusion pressures were normal [14] and ranged from 12 to 14 cm of H_2O; neither $E_2$17G nor $E_2$3G influenced perfusion pressure. Liver to body weight ratios averaged 4.3%;

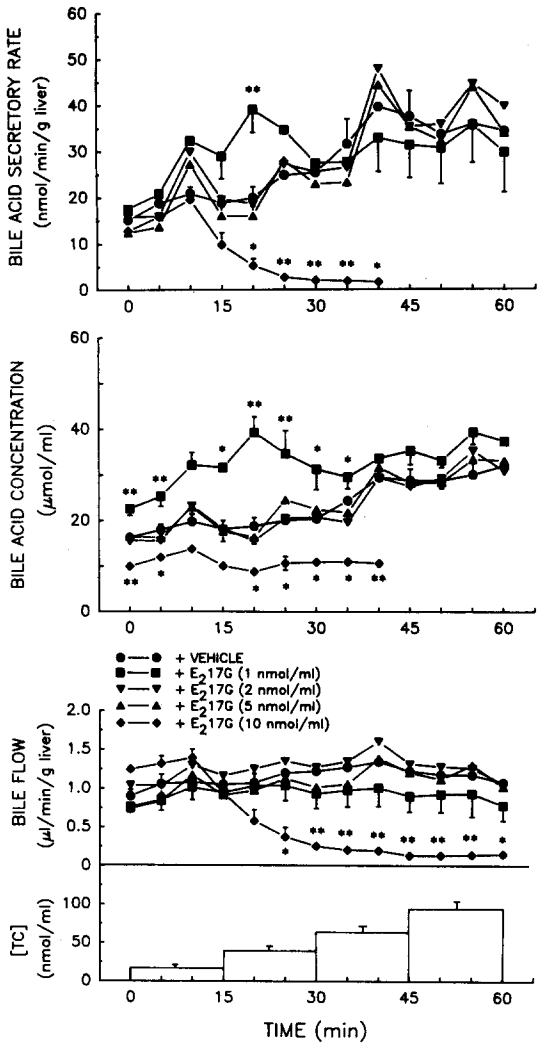

Fig. 2. Effect of a continuous infusion of vehicle or $E_2$17G (1, 2, 5 or 10 nmol/mL) on bile flow, bile acid concentration and bile acid secretory rate in the single pass perfused liver. Taurocholate was co-infused (1 mL/hr) to yield concentrations shown in the bottom panel. Values are means ± SEM, N = 4 per group. Statistics: one-way ANOVA followed by the Student–Neuman–Keuls test: (*) $P < 0.05$, and (**) $P < 0.01$, significantly different from vehicle control.

analysis of variance of these data showed no differences among the groups.

As shown in Fig. 2, bile acid secretion reached a maximum and remained stable or declined within each 15-min period of TC infusion in the vehicle control group. Bile flow increased modestly, whereas bile acid concentration and bile acid secretion increased markedly as the TC infusion increased. The presence of $E_2$17G in the perfusate at 1, 2 or 5 nmol/mL had no consistent significant effect on bile flow or bile acid secretory rate. The effect on bile acid concentration was complicated by the significant differences in initial bile acid concentration in two of the groups ($E_2$17G; 1 and 10 nmol/mL). The basis for these differences is not known but

Table 2. Effects of $E_2$17G and $E_2$3G on the bile acid secretory rate maximum (SR_m) in the single pass perfused liver

Additions (nmol/mL)	SR_m (nmol/min/g liver)	C_{in} of [^{3}H]TC (nmol/mL)
$E_2$17G		
0	40.6 ± 6.0	83.1 ± 2.5
1	42.3 ± 5.8	54.4 ± 16.8
2	56.4 ± 8.1	66.7 ± 18.3
5	46.8 ± 8.1	52.5 ± 8.6
10	19.5 ± 3.2*	12.8 ± 1.4*
$E_2$3G		
2	73.4 ± 6.7*	85.9 ± 8.1

Values are means ± SEM, N = 4. The SR_m and TC concentration were determined as the maximal bile acid secretory rate and the infused TC concentration at which it occurred for each liver perfusion experiment. Statistics: one-way ANOVA followed by Student–Newman–Keuls multiple range test.

* $P < 0.05$ vs vehicle control.

apparently reflects a difference in the bile acid independent flow since bile acid secretory rates were very similar in the five groups. When bile acid concentrations were normalized based on the percent of initial bile acid concentration, the only significant difference from the vehicle control occurred at 20 min in the presence of 1 nmol/mL $E_2$17G. This increase was reflected in an increased bile acid secretory rate at 20 min (Fig. 2). As shown in Table 2, $E_2$17G at 1, 2 and 5 nmol/mL had no effect on the bile acid SR_m. At 10 nmol/mL however, $E_2$17G significantly decreased bile flow beginning at 25-min and bile acid secretory rate beginning at 20 min. Bile flow was inadequate for quantitation of bile acids after 40 min. The bile acid SR_m was decreased significantly by 52% and occurred at a significantly lower concentration of infused TC relative to controls (Table 2).

In contrast, 2 nmol/mL $E_2$3G had no effect on bile flow, but markedly increased the bile acid concentration and the bile acid secretory rate (Fig. 3). $E_2$3G significantly increased the SR_m to 180% of that in the vehicle control (Table 2).

Table 3 shows the effects of $E_2$17G and $E_2$3G on the clearance of TC. The extraction ratio for TC in control livers ranged from 0.89 to 0.67 and is in excellent agreement with the literature values of 0.78 obtained *in vivo* in the rat [15] and 0.75 in the isolated perfused rat liver [16]. The decrease in clearance with increasing substrate concentration confirms the well-established saturable uptake of TC by the liver [1]. $E_2$17G at concentrations of 1 and 2 nmol/mL perfusate decreased TC clearance slightly at the higher concentrations of TC, but these changes were not statistically different, in part because of the increased variability. $E_2$17G at concentrations of 5 and 10 nmol/mL significantly decreased the clearance of 75 nmol/mL TC. In three of the livers in which $E_2$17G was infused at 10 nmol/mL, the C_{out} exceeded C_{in} at the 100 nmol/mL TC concentration. It is important to note that $E_2$17G had no effect on the clearance of 25 nmol/mL TC at early time

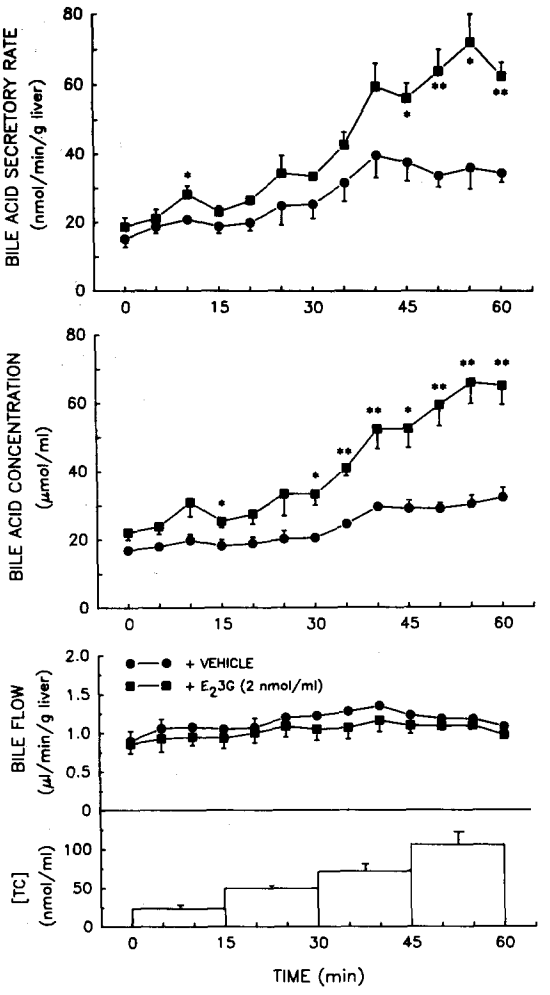

Fig. 3. Effect of a continuous infusion of vehicle or 2 nmol/mL $E_2$3G on bile flow, bile acid concentration and bile acid secretory rate in the single pass perfused liver. Taurocholate was infused (1 mL/hr) to yield concentrations shown in the bottom panel. Values are means ± SEM, N = 4 per group. Statistics: (*) $P < 0.05$, and (**) $P < 0.01$, significantly different from vehicle control.

periods, but did inhibit TC clearance at later times, after bile acid secretory rate and bile flow were inhibited. In contrast, $E_2$3G had no effect on the clearance of TC.

DISCUSSION

Studies in the recirculating perfused liver system showed clearly that at doses that caused a significant inhibition of bile flow and bile acid secretory rate, $E_2$17G did not diminish the maximal capacity of the liver to secrete bile acids. The SR_m in livers exposed to 2 or 4 μmol $E_2$17G was not decreased relative to that in the controls. These data indicate that $E_2$17G does not induce any permanent, irreversible inhibition of the ability of the liver to secrete bile acids. The prolonged cholestasis seen after 6 μmol $E_2$17G is apparently due to the high levels retained in the liver/perfusate which cannot be eliminated. At this dose of $E_2$17G, increasing taurocholate concentrations could not overcome the cholestasis. In our previous studies characterizing $E_2$17G cholestasis in the recirculating perfused liver, a bolus dose of 2.1 μmol $E_2$17G in the absence of any infused bile acids caused a 75% inhibition of bile flow which recovered only slightly over a 2-hr period [9]. Infusion of taurocholate in increasing concentrations in the present studies thus increased the dose of $E_2$17G necessary to cause a profound and prolonged cholestasis about 3-fold.

Experiments in the single pass isolated perfused liver were designed to determine the effects of a stable infusion of $E_2$17G on SR_m and Cl_H of taurocholate. $E_2$17G at 1, 2, 5 or 10 nmol/mL did not inhibit the clearance of 25 nmol/mL taurocholate from the perfusate. These data indicate that $E_2$17G does not inhibit directly the uptake of taurocholate across the basolateral domain of the hepatocyte. These data are consistent with data in isolated hepatocytes showing that $E_2$17G and TC do not compete at the major Na^+-dependent TC uptake site [17, 18]. Similarly, Zimmerli *et al.* [19] have shown that $E_2$17G does not inhibit the Na^+-dependent uptake of TC into hepatic basolateral membrane vesicles. The decreased TC clearance

Table 3. Effects of $E_2$17G and $E_2$3G on clearance of [^{3}H]TC in the single pass perfused liver

Additions (nmol/mL)	Cl_H (mL/min/g liver)			
	C_{in} of [^{3}H]TC (nmol/mL)			
	25	50	75	100
$E_2$17G				
0	0.92 ± 0.11	0.86 ± 0.09	0.77 ± 0.10	0.70 ± 0.09
1	0.82 ± 0.10	0.75 ± 0.10	0.63 ± 0.14	0.48 ± 0.12
2	0.74 ± 0.09	0.79 ± 0.06	0.72 ± 0.07	0.53 ± 0.11
5	0.69 ± 0.09	0.51 ± 0.16	0.44 ± 0.07*	0.34 ± 0.10
10	0.86 ± 0.09	0.60 ± 0.14	0.20 ± 0.09†	0.14
$E_2$3G				
2	0.82 ± 0.04	0.77 ± 0.05	0.71 ± 0.05	0.67 ± 0.03

Values are means ± SEM, N = 4. Statistics: one-way ANOVA followed by Student–Newman–Keuls multiple range test.

*† Significantly different from controls: *$P < 0.05$, and †$P < 0.01$.

seen at the highest doses of $E_2$17G and in the presence of the higher inflow concentrations of TC appears to be due to inhibition of the biliary excretion of TC. Thus, at 10 nmol/mL $E_2$17G, inhibition of the bile acid secretory rate at 20 min preceded a decrease in TC clearance. These data indicate that $E_2$17G acts at some site distal to the uptake site, either an intracellular site or at the canalicular membrane, to inhibit the biliary excretion of taurocholate. Since biliary excretion of taurocholate is the rate-limiting step in its transhepatic transport, inhibition of its excretion into bile results in its intracellular accumulation and a subsequent decrease in its uptake from the perfusate. Thus, at intracellular concentrations that markedly inhibited further uptake, TC could not overcome the cholestasis achieved by a constant infusion of 10 nmol/mL $E_2$17G. It may simply not be possible to achieve intracellular levels of TC adequate for overcoming a competitive inhibition at this dose of $E_2$17G. Alternatively, TC and $E_2$17G may not act at the same site. Thus, $E_2$17G may act indirectly to inhibit the canalicular secretion of TC and is not a direct competitive inhibitor of TC transport. Resolution of this issue is not possible in a complex physiological system like the perfused liver but will require a kinetic analysis of TC transport in canalicular membrane vesicles.

The present studies also show that $E_2$3G stimulated the bile acid secretory rate and markedly increased the SR_m for taurocholate. These data confirm our previous observations that $E_2$3G increases the bile acid secretory rate and protects against an otherwise cholestatic infusion of taurocholate [9]. $E_2$17G showed similar effects in our previous study and there was some suggestion of this ability in the present studies. Thus, 1 nmol/mL $E_2$17G significantly increased the bile acid secretory rate at 20 min in the presence of a 50 nmol/mL TC infusion. The erratic effects of $E_2$17G appear to reflect a compromise between its cholestatic and stimulatory properties. Bile acids and non-bile acid organic anions are thought to be secreted by separate carriers in the canalicular membrane since they do not compete for transport [20]. Rather, numerous studies have shown that taurocholate facilitates the biliary excretion of a variety of non-bile acid organic anions such as bromosulfophthalein [21, 22], indocyanine green [23], iopanoic acid [24] and ampicillin [25]. We do not yet understand the mechanism by which $E_2$3G can stimulate the SR_m of taurocholate, but the data implicate an interaction between the carriers.

In summary, the present studies have shown that $E_2$17G is not an irreversible inhibitor of TC secretion into the bile in that the same SR_m can be achieved after exposure of the liver to a cholestatic dose of $E_2$17G. The present studies also show that under physiological conditions, $E_2$17G does not inhibit the uptake of TC by the liver. $E_2$17G inhibited the biliary secretion of TC into the bile at a site distal to the uptake site, but it was not possible to determine whether TC and $E_2$17G competed for transport at a single site or whether $E_2$17G acted indirectly to inhibit TC secretion into bile. Finally, $E_2$3G stimulated the secretion of TC into bile and increased SR_m for TC by 80%.

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REFERENCES

1. Blitzer BL and Boyer JL, Cellular mechanisms of bile formation. *Gastroenterology* **82**: 346–357, 1982.
2. Schwarz LR, Burr R, Schwenk M, Pfaff E and Greim H, Uptake of taurocholate acid into isolated rat liver cells. *Eur J Biochem* **55**: 617–623, 1975.
3. Scharschmidt BF and Stephens JE, Transport of sodium, chloride, and taurocholate by cultured rat hepatocytes. *Proc Natl Acad Sci USA* **78**: 986–990, 1981.
4. Meier PJ, Meier-Abt AS and Boyer JL, Properties of the canalicular bile acid transport system in rat liver. *Biochem J* **242**: 465–469, 1987.
5. Reichen J and Paumgartner G, Kinetics of taurocholate by the perfused rat liver. *Gastroenterology* **68**: 132–136, 1975.
6. Hardison WGM, Hatoff DE, Miyai K and Weiner RG, Nature of bile acid maximum secretory rate in the rat. *Am J Physiol* **241**: G337–G343, 1981.
7. Meyers M, Slikker W, Pascoe G and Vore M, Characterization of cholestasis induced by estradiol-17β-D-glucuronide in the rat. *J Pharmacol Exp Ther* **214**: 87–93, 1980.
8. Meyers MB, Slikker W and Vore M, Steroid D-ring glucuronides: Characterization of a new class of cholestatic agents in the rat. *J Pharmacol Exp Ther* **218**: 63–73, 1981.
9. Durham S and Vore M, Taurocholate and steroid glucuronides: Mutual protection against cholestasis in the isolated perfused rat liver. *J Pharmacol Exp Ther* **237**: 490–495, 1986.
10. Adinolfi LE, Utili R, Garta GB, Abernathy CD and Zimmerman HJ, Cholestasis induced by estradiol 17β-D-glucuronide: Mechanisms and prevention by sodium taurocholate. *Hepatology* **4**: 30–37, 1984.
11. Vore M, Bauer J and Pascucci V, The effect of pregnancy on the metabolism of [^{14}C]phenytoin in the isolated perfused rat liver. *J Pharmacol Exp Ther* **206**: 439–447, 1978.
12. Hamilton RL, Berry MN, Williams MC and Severinghause EM, A simple and inexpensive membrane "lung" for small organ perfusion. *J Lipid Res* **15**: 182–186, 1974.
13. Talalay P, Enzymatic analysis of steroid hormones. *Methods Biochem Anal* **8**: 119–143, 1960.
14. St. Pierre M, Schwab AJ, Goresky CA, Lee W-F and Pang KS, The multiple-indicator dilution technique for characterization of normal and retrograde flow in once-through rat liver perfusions. *Hepatology* **9**: 285–296, 1989.
15. Iga T and Klassen CD, Hepatic extraction of bile acids in rats. *Biochem Pharmacol* **31**: 205–209, 1982.
16. Hoffman AF, Bile acids. In: *The Liver: Biology and Pathobiology* (Eds. Arias IM, Popper H, Jakoby WB, Schachter D and Shafritz DA), 2nd Edn, pp. 553–572. Raven Press, New York, 1988.
17. Brock WJ, Durham S and Vore M, Characterization of the interaction between estrogen metabolites and taurocholate for uptake into isolated hepatocytes. Lack of correlation between cholestasis and inhibition of taurocholate uptake. *J Steroid Biochem* **20**: 1181–1185, 1984.
18. Brouwer KLR, Durham S and Vore M, Multiple carriers

for uptake of [^{3}H]estradiol-17-β(β-D-glucuronide) in isolated rat hepatocytes. *Mol Pharmacol* **32**: 519–523, 1987.
19. Zimmerli B, Valentinas J and Meier PJ, Multispecificity of Na^+-dependent taurocholate uptake in basolateral (sinusoidal) rat liver plasma membrane vesicle. *J Pharmacol Exp Ther* **250**: 301–308, 1989.
20. Erlinger S, Secretion of bile. In: *Diseases of the Liver* (Eds. Schiff L and Schiff ER), 5th Edn, pp. 93–118. J. P. Lippincott Co., Philadelphia, 1982.
21. O'Maille ERL, Richards TG and Short AH, Factors determining the maximal rate of organic anion secretion by the liver and further evidence on the hepatic site of action of the hormone secretin. *J Physiol (Lond)* **186**: 424–438, 1966.
22. Boyer JL, Scheig RL and Klatskin G, The effect of sodium taurocholate on the hepatic metabolism of sulfobromophthalein sodium (BSP): The role of bile flow. *J Clin Invest* **49**: 206–215, 1970.
23. Vonk RJ, Veen H, Prop G and Meijer DKF, The influence of taurocholate and dehydrocholate choleresis on plasma disappearance and biliary excretion of indocyanine green in the rat. *Naunyn Schmiedebergs Arch Pharmacol* **282**: 401–410, 1974.
24. Berk BN, Goldberger LE and Loeb PM, The role of bile salts in the hepatic excretion of iopanoic acid. *Radiology* **9**: 7–15, 1974.
25. Mandiola S, Johnson BL, Winters RE and Longmire WP, Biliary excretion of ampicillin in the anesthetized dog. I. Effect of serum ampicillin concentration, taurocholate infusion rate, biliary secretion pressure and secretion infusion. *Surgery* **71**: 664–674, 1972.