Effects of selective zonal injury on bile acid-induced bile flow in the isolated rat liver

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Monte, Maria J., Maria D. Badia, Felisa Palomero, Mohamad Y. El-Mir, Jose R. Alonso, and Jose J. G. Marin. Effects of selective zonal injury on bile acid-induced bile flow in the isolated rat liver. Am. J. Physiol. 264 (Gastrointest. Liver Physiol. 27): G1103-G1111, 1993.—The importance of acinar heterogeneity in ursodeoxycholic acid (UDCA)-induced bile flow was assessed in isolated rat livers that underwent restricted acinar damage by antegrade (A; 50 nmol) or retrograde (R; 500 nmol) digitonin infusion, as confirmed by histological evaluation. Stability of reduced (−40%) bile flow and perfusion flow (−25%) at constant pressure and potassium lactate dehydrogenase release indicated similar viability of A and R preparations. They also showed similar abilities to secrete increasing doses of taurocholate (TC; maximal secretion rate ∼105 nmol·min⁻¹·g liver⁻¹). TC-induced bile flow was not reduced by digitonin. In contrast, UDCA-induced cholinolysis was sensitive to zonal injury. Moreover, increases in bile flow and bicarbonate secretion observed under UDCA infusion (1.5 μmol/min) were lower in R than in A (−33 and −51%, respectively). No significant difference was observed in UDCA amiation or glucuronation between A and R preparations. With the use of single-pass perfusion on intact isolated livers that received 1 or 10 μmol UDCA, an early peak in bile acid output was observed to occur before the appearance of the major secretary peak. This was not found when 1 μmol of chenodeoxycholic acid bolus or trace amounts of [14C]TC were given. High-performance liquid chromatographic analysis of the early peak revealed it to be mainly due to unconjugated UDCA. This suggests the existence of a diffusion pathway for protonated bile acids and hence that the exit of lipophilic UDCA from bile during its way through the intra-acinar canaliculi across this pathway is also possible. Taken together, these results indicate that liver parenchyma microanatomy plays an important role in UDCA-induced hypercholeresis, which may be due in part to the existence of a diffusion flux of protonated UDCA molecules flowing a yet unknown intra-acinar circuit.

ursodeoxycholic acid; liver parenchyma

THE ABILITY of ursodeoxycholic acid (UDCA) to induce hypercholeresis has been studied by several groups during the last decade (11, 24, 33, 39, 40). Despite many important data reported in a large number of papers published on the mechanism by which UDCA induces hypercholeresis, the overall process is poorly understood. Nevertheless, some interesting hypotheses, recently reviewed (12), have been proposed to explain the hypercholeresis phenomenon. In brief, two major lines of thought can be considered. Dumont et al. (11) first proposed bicarbonate secretion by the hepatocytes in response to UDCA stimulation as the mechanism responsible for the very high choleresis found in experimental animals under UDCA infusion. The existence of Cl⁻·HCO₃⁻ (30) and Na⁺·H⁺ (2) exchangers in the plasma membrane of the hepatocyte and the sensitivity of Na⁺·H⁺ exchange to UDCA in plasma membrane preparations (32) are consistent with the fact that UDCA-induced bile flow and bicarbonate secretion are inhibited by amiloride and its analogues (24, 35); this seems to support the hypothesis of a process taking place at the cellular level (38). However, some results on UDCA-induced modifications in intracellular pH and Na⁺·H⁺ exchange activation are controversial when studied in isolated hepatocytes (1, 39). An alternative hypothesis suggests the existence of a cholehepatic shunting (33), according to which the unconjugated form of UDCA would be secreted into bile as an anion but, once there, would undergo protonation due to its relatively high pKₐ value. The sequestration of H⁺ by UDCA induces the hydration of CO₂ to H₂CO₃, which is rapidly dissociated into H⁺ and HCO₃⁻. This reaction, if occurring within the biliary lumen, may be catalyzed by carbonic anhydrase, which has been reported to play a role in UDCA-induced bicarbonate secretion into bile (28) and which is located on the plasma membrane of rat hepatocytes (26). Protonation of the bile acid molecule markedly changes its physicochemical properties, making the UDCA more lipophilic and enabling it to be passively reabsorbed from the biliary lumen (13). Recycling of UDCA from the peribiliary capillary plexus to the sinusoids and subsequent resecretion into the canaliculus would generate an additional bile flow, resulting in a bicarbonate-rich hypercholeresis. In agreement with this hypothesis, it has been reported that UDCA-induced biliary bicarbonate secretion is due to H⁺ extrusion from bile rather than to the transport of bicarbonate itself (27). Recently, this point of view has been supported by other authors using different experimental approaches (39). However, even if the cholehepatic shunt pathway does exist, the recirculation of unconjugated UDCA back to the sinusoids does not seem to be mandatory for UDCA-induced bicarbonate-rich hypercholeresis (34), and hence additional mechanisms may be operating. Among these, the formation of UDCA-glucuronide has been reported (40). This compound behaves as a strong choleric bile acid in part because of its ability to stimulate bicarbonate secretion (40). This could partly be due to the presence of UDCA-glucuronide in bile as a valent anion that is presumably secreted together with two counterions (12). However, the quantitative contribution of UDCA-glucuronide secretion to the choleric effect of UDCA is unclear, because other authors have found <1% of the total bile acid infused present in bile as the 3-ether glucuronide derivative of UDCA (23).

Acinar heterogeneity in hepatic functions has been reported to affect processes involved in bile acid transport (18), metabolism (4, 42), and the effect of bile acids
on bile formation (3, 19). However, the role of parenchymal microanatomy on UDCA-induced choleresis has only recently been explored (34, 39). Digitonin-collagenase perfusion is a useful tool for separating periporal and perivenous hepatocytes from rat livers (25). This technique is based on initial selective destruction of one acinar region by the cholesterol-complexing properties of digitonin, administered through the portal or the hepatic vein, followed by isolation of the cells from the opposite intact acinar zone. The dose of digitonin reported in most papers on rats is ~35 μmol. This is a convenient dose when destruction, as complete as possible, of one of the acinar zones is desired. Nevertheless, this procedure causes considerable damage that renders the liver useless for steady-state perfusions. In this work, we first selected an experimental model of isolated "in situ" rat liver perfusion with restricted damage to the periportal or perivenous area of the acinus but able to maintain a functional steady state for performing perfusion experiments. We then infused UDCA into the portal vein to gain information on the relative contributions of hepatocytes from acinar 1 zone and perivenous 6 zone. This procedure causes considerable damage that renders the liver useless for steady-state perfusions. In this work, we first selected an experimental model of isolated "in situ" rat liver perfusion with restricted damage to the periportal or perivenous area of the acinus but able to maintain a functional steady state for performing perfusion experiments. We then infused UDCA into the portal vein to gain information on the relative contributions of hepatocytes from acinar zone 1 or 3 to the hypercholeretic response to UDCA. A similar attempt to perform experiments on isolated rat livers after a digitonin pulse through the hepatic vein has recently been reported (39).

**MATERIALS AND METHODS**

**Materials.** Sodium taurocholate (TC), UDCA, chenodeoxycholic acid (CDCA), 3α-hydroxy steroid dehydrogenase, β-gluconidase, dimethyl sulfoxide (DMSO), trypan blue, and digitonin (50% pure) were purchased from Sigma (St. Louis, MO). [14C]TC (sp act 46.7 mCi/mmol) was obtained from New England Nuclear (Itisa, Madrid, Spain). Bile acids were >95% pure by thin-layer chromatography. TC, CDCA, and UDCA were dissolved in 0.15 M NaCl and 0.1 M Na2CO3 (1:1, vol/vol). The pH of the solution containing bile acid was adjusted to 8.3 with 5 M HCl. The enzymes used in bicarbonate measurements were supplied by Beckman (Dri-STAT Reagent, Beckman, Madrid, Spain). NADH was from Boehringer (Mannheim, Germany).

**Experimental protocols.** Nonfasting male Wistar rats (Faculty of Pharmacy, Salamanca, Spain) weighing 240–270 g were used as donors. They were fed ad libitum on commercial rat food (Panlab, Madrid). The animals were anesthetized intraperitoneally with 5 mg/100 g body wt pentobarbital sodium (Nembutal, Abbot, Madrid). Cannulation of the common bile duct, portal vein, and inferior vena cava was carried out as described previously (28). The hepatic artery was ligated in all preparations. The isolated in situ liver was then moved to a prewarmed (37°C) in a 50% water-50% methanol-KH2PO4 (0.01 M) buffer. The elution protocol was the following: 0–20 min, from 50% water-50% solvent A [methanol-KH2PO4 (0.01 M), 76:24 vol/vol, pH 5.45] to 100% solvent A; 20-40 min, from 100% solvent A; 40–90 min, from 100% solvent A to 100% methanol. The solvent rate was 1 ml/min. Integration was carried out with an IBM computer (model 30286, IBM, Portsmouth, UK) using System Gold software from Beckman. The elution protocol was the following: 0–20 min, from 50% water-50% solvent A [methanol-KH2PO4 (0.01 M), 76:24 vol/vol, pH 5.45] to 100% solvent A; 20-40 min, isocratic solvent A; 40–90 min, from 100% solvent A to 100% methanol. The solvent rate was 1 ml/min.

**Histology.** In some preparations, once the experimental period had ended, the livers were perfused with trypan blue (0.2 mM in 150 mM NaCl) for 10 min at 2.5 ml/min. After being washed with perfusion medium for 10 min, the livers were perfused with Boun fixative (picric acid-formaldehyde-glacial acetic acid; 75:30:2, vol/vol/vol) for 10 min. Several 5- to 7-mm-thick blocks were cut from both superficial and deep parts of all lobes and postfixed in the same fixative for an additional 4 h.
The blocks were then dehydrated in ethanol series and embedded in Paraplast (Merck, Darmstadt, Germany). Sections were cut (10-μm thick) and stained with eosin or with hematoxylin-eosin and were photographed with the use of a Zeiss III photomicroscope (Oberkochen, Germany). Additionally, in a second set of preparations the livers were perfused, after washing, with 1% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.25). After postfixation in the same mixture for 4 h, 0.1-mm-thick sections were dehydrated in ethanol (block stained with 1% uranyl acetate in 70% ethanol) and embedded in Araldite (Emscope, Ashford, UK). Semithin (0.8-1 μm) and ultrathin (40-60 nm) sections were cut in a Reichert Jung Ultracut E ultramicrotome. The semithin sections were mounted on slides and stained with 1% toluidine blue. The thin sections were mounted on Formvar-coated slot grids, stained with lead citrate, and studied in a Zeiss electron microscope. A Quantimet-500 Image Analysis System (Leica, Cambridge, UK) was used to measure damaged zones of livers treated with digitonin and observed with the photomicroscope.

**Digitonin-induced restricted damage.** To select an experimental model, preliminary studies on dose dependence of the reduction in bile formation vs. the amount of digitonin infused through the portal or the hepatic vein were carried out (Fig. 1). We found that a dose fourfold lower than that used by Strazzabosco et al. (39) caused a reduction in bile flow below 10% of the control values, together with a continuous loss of viability during the perfusion period. In our laboratory, viable preparations, with a steady-state bile flow of ~60% of pre-digitonin-pulse values (Fig. 2), were obtained with far lower doses of digitonin (0.50 and 0.05 mmol, in R and A experiments, respectively). The different sensitivity of the acinar zones to digitonin treatment, despite similar ultrastructural changes (36), is probably due to a larger dead space when perfusion was carried out through the hepatic vein, as suggested by the longer time required for hepatocyte destruction observed by others in retrograde experiments involving digitonin infusion (25). The combination of zone-specific damage and trypan blue exclusion was used to evaluate liver integrity (3). With the use of doses of digitonin >20-fold higher than those used in the present work, a net coloring of the permeabilized cells up to acinar zone 2, regardless of the direction of digitonin administration, has been reported (15), and it has also been shown that the percentage of cells stained with dye correlates with the release of LDH into the perfusate (6). Therefore, this procedure was selected by us and others (39, 43) to confirm whether portal or perivenous hepatocytes are spared after damage to the contralateral acinar zone. However, our results indicate that the trypan blue staining test underestimates the boundaries of the functionally impaired zone. This test revealed that histological damage is indeed restricted to zone 1 or zone 3 hepatocytes, depending on the direction of the digitonin-pulse perfusion. In both preparations, the opposite zone of the acinus was essentially spared. Likewise, in both cases the proportion of the acinar area unable to exclude the trypan blue was <5% of the acinus length, which was not proportional to the more important functional impairment observed. In fact, larger injured areas were observed by studying semithin sections of liver that were stained with toluidine blue. These observations revealed the presence of relative homogeneity in shape and size of injured zones both in A and R preparations whose mean area was 42.3 ± 3.2 μm² (×10^3) when digitonin was administered through the hepatic vein and 39.2 ± 2.7 μm² (×10^3) when the injury was located in acinar zone 1 (P > 0.05; in both cases 16 measurements were carried out on 8 different slides). Electron microscopy confirmed the observations in toluidine blue-stained preparations, showing a sharp limit between injured and apparently unaffected hepatocytes. Similar ultrastructural changes were confirmed in both the A and R experiments.

**Viability of preparations.** Perfusate concentrations of potassium and LDH activity in the perfusate were measured at the end of the experiments, i.e., 60 min after the digitonin pulse in both the A and R experiments. The results were as follows. The potassium concentration (in mM) for A experiments (n = 4) was 6.4 ± 0.2 and for R experiments (n = 4) was 6.4 ± 0.4. LDH activity (in μU/ml) in A experiments (n = 10) was 0.9±0.17 and in R experiments (n = 10) was 0.98 ± 0.15. The absence of significant differences in the release of potassium and LDH between both types of preparations suggests a restricted damage of similar magnitude. The stability in bile flow (Fig. 2A) and in perfusion flow (Fig. 2B) at constant perfusion pressure (12 cmH₂O) together with the oxygen consumption during this period (~1.6 mmol · min⁻¹ · g liver⁻¹) provide evidence of steady state in the viability of the preparations despite the initial loss of hepatic functional mass. Similar biliary response to the TC in A and R preparations, which will be discussed in RESULTS, also supports this concept.

**Statistical analysis.** Unless otherwise indicated, values are given as means ± SE. The Bonferroni method of multiple-range testing was used for calculating the statistical significance of
differences. Single comparisons of two means were performed with the use of a paired or unpaired Student's t test. Regression lines were calculated by the least-squares method. Statistical analyses were performed using a Macintosh SE Computer (Apple Computer, Cupertino, CA).

RESULTS

Choleretic effect of TC or UDCA infusion. Infusion of the A or R preparations with increasing doses of TC (from 0 to 600 nmol/min) induced increases in the bile acid secretory rate of similar magnitude to that of the infusion rate (Fig. 3). When the relationship between bile acid output and bile flow was plotted (Fig. 4), similar slopes in the regression lines were obtained for the control and A and R livers, which suggests that the choleretic capability of TC was not significantly modified by digitonin. Nevertheless, compared with the controls, both A and R preparations showed similar decreases in the value of the y-intercept, which is usually considered to represent the bile acid-independent fraction of bile flow. In separate experiments in which the TC infusion rate was increased to attain a toxic effect, the SRmax was calculated to be similar in the A and R preparations (104 ± 5 nmol·min⁻¹·g liver⁻¹, n = 6), which was significantly lower than that found in intact livers (125 ± 7 nmol·min⁻¹·g liver⁻¹, n = 3). Despite the similarity of the biliary response of A and R preparations to TC infusion, a statistically significant difference in the response to UDCA was found (Fig. 5). After digitonin treatment, the UDCA-induced bile flow was dramatically reduced in both A and R. Figure 5 shows that the accumulated increase in bile flow, measured over 50 min under UDCA infusion, was markedly reduced in both A and R compared with control livers. This reduction was significantly higher in R (−70%) than in A (−55%). As expected based on previous studies (34), the more marked effect on UDCA-induced bile formation found when the damaged area was acinar zone 3 was consistent with changes in bile electrolyte composition (Table 1). Thus UDCA-induced biliary bicarbonate secretion in R preparations was only 49% of that found in A livers.

Biotransformation of infused UDCA. In contrast to the difference in UDCA-induced bile flow, bile acid output determined by 3α-hydroxysteroid dehydrogenase analysis was not significantly different in A and R (Table 2). This value was ~35% of the infused dose. HPLC analysis of bile samples revealed that almost all of the bile acids detected in fresh bile as 3α-hydroxy bile acids by that method were amidated forms of UDCA. Negligible amounts of unconjugated UDCA were secreted into bile both in A and R. Addition of tauro-UDCA and glyco-UDCA as detected by HPLC accounted for almost the entire amount of bile acids as measured by 3α-hydroxysteroid dehydrogenase. Moreover, unconjugated-UDCA was usually below the sensitivity of the HPLC detector. The digitonin pulse did not induce significant changes in the conjugation pattern of UDCA (Table 3). Treatment of the bile samples with β-glucuronidase followed by 3α-hydroxysteroid dehydrogenase analysis indicated that an additional 15% of the UDCA dose infused was secreted as glucuronide derivatives (Table 2). The proportion of bile acids found to be biotransformed in this way was not significantly different in A (29.8 ± 2.9% of total bile acid output) compared with R (25.9 ± 2.2%).

Effect of UDCA, CDCA, or labeled TC bolus in single-pass experiments in intact isolated livers. Results obtained in these experiments are shown in Figs. 7 and 8. After 1 μmol UDCA but not CDCA bolus, a rapid increase in bile acid output was observed to occur before the appearance of the major secretory peak. The size of this early peak increased when the liver received 10 μmol UDCA bolus. This amount was also assayed for a CDCA bolus, but this was found to be cholestatic. We interpret this first peak as being due to a rapid flux of lipophilic molecules of protonated unconjugated UDCA into bile. To assess this possibility, HPLC analysis was carried out on samples collected before 10 μmol UDCA bolus (basal bile) and at the maximum of the first and second secretory peaks (Fig. 9). Taurocholate was the major bile acid secreted in basal bile. Conjugated UDCA was ~27% of total basal bile acid output, but unconjugated UDCA was not detected. At the maximum of the first peak a net increase in unconjugated UDCA was found. At this time, bile acid output was increased by 85% with no marked changes in bile acid output.
jugation with glycine or taurine, whereas the secretion of UDCA in unconjugated form indicates that a small fraction of UDCA molecules does not enter the conjugation pathway, appearing earlier in bile. If this effect is due only to metabolic mechanisms, conjugated bile acids should reach bile more or less at the same time as that found for the early peak of UDCA. Therefore, the time course of bile output was investigated after infusing together both 10 μmol/min of unlabeled UDCA and a tracer dose of labeled TC. Figure 8 shows that labeled TC was collected earlier than most UDCA molecules which may be explained by the time required for UDCA conjugation. However, an early peak in UDCA secretion appeared even before TC output, which suggest the existence of a faster pathway for unconjugated UDCA than for TC to cross from the perfusate to bile.

Table 1. Basal values and UDCA-induced increase in bile electrolyte concentration and output

<table>
<thead>
<tr>
<th>Concentration, mM</th>
<th>Output, nmol·min⁻¹·g liver⁻¹</th>
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<tr>
<td>Na⁺ Basal</td>
<td>138.9±2.3</td>
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<tr>
<td>Increase A</td>
<td>20.3±2.8</td>
</tr>
<tr>
<td>Increase R</td>
<td>25.3±4.4</td>
</tr>
<tr>
<td>K⁺ Basal</td>
<td>7.7±0.15</td>
</tr>
<tr>
<td>Increase A</td>
<td>1.19±0.26</td>
</tr>
<tr>
<td>Increase R</td>
<td>1.20±0.23</td>
</tr>
<tr>
<td>Cl⁻ Basal</td>
<td>100.1±2.6</td>
</tr>
<tr>
<td>Increase A</td>
<td>-8.8±2.2</td>
</tr>
<tr>
<td>Increase R</td>
<td>-3.4±6.4</td>
</tr>
<tr>
<td>HCO₃⁻ Basal</td>
<td>22.0±0.6</td>
</tr>
<tr>
<td>Increase A</td>
<td>14.7±3.3</td>
</tr>
<tr>
<td>Increase R</td>
<td>5.7±1.3 (NS)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Basal values are average values obtained from minute 0 to minute 20. Increase values were calculated by subtracting the basal value from the average value obtained from minute 30 to minute 80 in each single experiment. UDCA, ursodeoxycholic acid. A (n = 5) and R (n = 5) were experiments with antegrade and retrograde digitonin-pulse perfusion, respectively; they were compared by an unpaired t test; * P < 0.01.

Table 2. Bile concentration and output of glucuronated and nonglucuronated bile acids under UDCA infusion

<table>
<thead>
<tr>
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<th>Concentration, mM</th>
<th>Output, nmol·min⁻¹·g liver⁻¹</th>
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<tbody>
<tr>
<td>A, mM</td>
<td>38.4±4.4</td>
<td>26.9±3.3</td>
</tr>
<tr>
<td>R, mM</td>
<td>44.0±7.7</td>
<td>29.3±6.4</td>
</tr>
<tr>
<td>A, nmol·min⁻¹·g liver⁻¹</td>
<td>61.4±10.1</td>
<td>46.6±7.4</td>
</tr>
<tr>
<td>R, nmol·min⁻¹·g liver⁻¹</td>
<td>56.1±8.2</td>
<td>42.2±6.7</td>
</tr>
</tbody>
</table>

Values are means ± SE from analysis of pooled samples collected from minute 30 to minute 80. Glucuronated bile acid concentrations were calculated from the subtraction between 3α-hydroxy bile acid measurement before and after incubation with β-glucuronidase. UDCA, ursodeoxycholic acid. A (n = 5) and R (n = 6) were livers with antegrade and retrograde digitonin-pulse perfusion, respectively; they were compared by an unpaired t test. No significant difference was found.

DISCUSSION

Three major findings are outstanding in this paper. One shows the importance of the integrity of the hepatic parenchyma microanatomy for UDCA-induced bicroarbonate-rich hypercholeresis; another suggests that perivenous hepatocytes have a higher contribution to this process; and finally evidence is provided for the existence of a faster pathway for unconjugated UDCA molecules between the perfusate and bile. The flux of these molecules toward bile was faster than that of the less lipopholic unconjugated CDCA or even of bile acid molecules that do not have to undergo conjugation, such as TC. Possible explanations of these findings are discussed below.

At physiological doses of bile acid load, the main agents responsible for portal clearance of bile acids are the hepatocytes located at acinar zone 1 (19). However, all acinar regions are able to efficiently carry out bile acid uptake and secretion. Thus the relative quantitative contribution of each zone to bile formation is determined by the gradient of bile acid concentration within the sinusoids (7). This does not exclude the possibility that peculiarities in the transfer of bile acids from blood to bile might exist when comparing hepatocytes from acinar zone 1 or 3 (7, 19). An interesting question arises as to whether, once secreted into the canaliculi, bile acid molecules are able to induce a similar bile flow at zone 1 or 3 of the acinus, i.e., whether their osmotic activity is similar, regardless of the differences in the architecture of the canicular network in the periportal and perivenous regions (22). In agreement with the results obtained by Gonzalez et al. (17) in anesthetized rabbits, the findings reported in this article show the absence of a significant difference in the choleretic capability of secreted bile acid molecules,
**Table 3. Bile concentration and output of amidated UDCA**

<table>
<thead>
<tr>
<th></th>
<th>Concentration, mM</th>
<th>Output, nmol·min⁻¹·g liver⁻¹</th>
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<tbody>
<tr>
<td></td>
<td>GUDCA</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>16.5±4.5</td>
<td>26.3±6.6</td>
</tr>
<tr>
<td>R</td>
<td>15.5±5.7</td>
<td>22.0±8.9</td>
</tr>
<tr>
<td></td>
<td>TUDCA</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>15.4±3.2</td>
<td>27.1±7.3</td>
</tr>
<tr>
<td>R</td>
<td>17.4±5.3</td>
<td>26.3±10.9</td>
</tr>
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</table>

Values are means ± SE from high-performance liquid chromatographic analysis of pooled samples collected from minute 30 to minute 80. TUDCA, tauroursodeoxycholic acid; GUDCA, glycoursodeoxycholic acid; A (n = 4) and R (n = 4) were livers with antegrade and retrograde digitonin-pulse perfusion, respectively; they were compared by an unpaired t test. No significant difference was found.

Fig. 6. Time course (A) of bile acid output in control isolated rat livers (C, n = 5) and after digitonin-pulse perfusion, administered when indicated in the figure, either through the portal (antegrade, A, n = 5) or the hepatic (retrograde, R, n = 5) vein, and comparison of accumulated UDCA-induced increase in bile acid output from minute 30 to minute 80 (B). UDCA was infused into portal vein at 1.5 μmol/min from minute 30. Values are means ± SE. *P < 0.05 compared with A by a multiple-range test.

regardless of the acinar zone considered. Therefore, differences in both the ability to secrete an exogenous load of bile acid or in the choleretic effect of secreted molecules can be reasonably ruled out as explanations for the lower UDCA-induced choleresis observed in R compared with A preparations. The existence of an intra-acinar gradient of UDCA concentration within the sinusoids was not expected, because, in this study, the livers were perfused in the prograde mode under large doses of UDCA infusion to avoid such gradients.

Biotransformation of UDCA has been proposed as one of the causes of hypercholeresis (40). Glucuronide derivatives escape the 3α-hydroxysteroid dehydrogenase method traditionally used to measure bile acid concentrations, and hence the choleretic ability of the detected molecules would be overestimated if the amount of glucuronated bile acids was large enough. Indeed, we found that a considerable amount of UDCA was secreted in glucuronated form, but no marked differences were found between the proportions of this compound, independently of whether the damage was induced in acinar zone 1 or 3. Including glucuronated bile acids, the relationship between UDCA-induced bile flow and bile acid output was ~4 and 7 μl/μmol in R and A, respectively. These values are not higher than those found for TC. We have previously reported that a selective increase in UDCA amidation with glycine vs. taurine may be one of the factors involved in the modification of UDCA-induced
Fig. 9. High-performance liquid chromatography of bile samples collected before (basal bile) and after portal administration of 10 pmol UDCA as a bolus to an intact isolated perfused rat liver. Results obtained after injection of 3 samples are shown. A: 8 μl basal bile containing 17 nmol total bile acid (measured by 3α-hydroxysteroid dehydrogenase in a separate aliquot); B: 10 μl bile sample collected 4 min after bolus, containing 26.7 nmol total bile acid; C: 5-μl bile sample collected 15 min after bolus, containing 81.2 nmol total bile acid. Elution times for tauroursodeoxycholate (1), glycoursoodeoxycholate (2), taurocholate (3), and UDCA (4) are indicated in each chromatogram.

After considering several of the possibilities discussed above, an additional explanation can be proposed. Prior to doing so, however, certain aspects should be remembered. 1) Both the lack of UDCA hypercholeretic ability in hepatocyte couplets (16) and the importance of the direction of perfusion in UDCA-induced hypercholeresis (34) suggested that the microanatomy of the liver parenchyma is important for the effect of UDCA on bile flow. The results on restricted acinar damage reported here support this point of view. 2) There exists some experimental evidence that the escape of protonated UDCA from the canalicus and/or ductular lumen rather than the recycling of these molecules to the sinusoidal pole of the hepatocyte is a determinant process in bicarbonate generation under UDCA infusion (27, 39). 3) The need of additional exposure of hepatocytes to recycling UDCA seems unlikely because UDCA hypercholeresis is only observed at large doses of UDCA infusion (11, 23). 4) UDCA-induced hypercholeresis correlates with the amount of unconjugated UDCA measured in bile (20). 5) Using a similar experimental protocol to that described in this article and a 10-μmol dose of bile acid bolus, Gurantz et al. (20) have shown that UDCA is almost completely amidated (~99%) by the isolated perfused rat liver. These authors reported that during the first few minutes after its injection amidated forms of UDCA were only 80% of secreted UDCA, which suggests a more rapid transfer into bile of the protonated form of UDCA. In the present work, a shorter collection time (1 min) allowed us to better observe this effect. The existence of an early appearance of UDCA but not of CDCA in bile after bile acid bolus before the major secretory peak occurred indicates a faster flux between the sinusoids and the canaliculus for UDCA than for CDCA. Therefore, a possible explanation for this effect may be the existence of a non-ionic diffusional pathway between the sinusoid and the canaliculus for relatively lipophilic protonated UDCA, i.e., by a process similar to that reported for cholic acid incorporation into liposomes (8). The anatomic location of this pathway within the liver parenchyma is unclear, but if it does exist, the quantitative role of this pathway may be much more important in the bile-sinusoid direction, because UDCA is concentrated in the canaliculus.
Nevertheless, it should be kept in mind that alternative explanations for this finding cannot be ruled out, such as diffusion across the peribiliary vascular plexus into bile if UDCA reaches the arterial vessel via arterioporal anastomoses (31). Bearing all of the aforementioned in mind, the results obtained in this work can be interpreted according to a new hypothesis, which covers most of the concepts already included in the cholehepatic shunt hypothesis but in which UDCA recycling also includes the hepatic acini. The model assumes that secretion of UDCA may partly occur in unconjugated (lipophile) form. After accumulation downstream by the continuous supply of hepatocytes located in the pathway of bile from zone 3 to zone 1 and protonation within the biliary lumen, protonated UDCA may then undergo subsequent passive backdiffusion. The proposed hypothesis suggests that the escape of protonated UDCA would generate a bicarbonate ion that would appear in bile instead of the missing bile acid molecule. Moreover, if UDCA diffusion occurs at zones 2 and 1 reaching the sinusoid, reuptake and resecretion of these molecules by zone 3 would generate an additional bile flow. The fact that UDCA infusion causes hypercholeresis only if it is given above a certain dose may be due in part to the necessity of overloading the conjugation machinery but also of recruiting hepatocytes from locations farther away than acinar zone 1, which would enlarge the diffusional surface. It is important to note that the existence of an intra-acinar recycling of UDCA does not exclude, but rather supports, the existence of an additional cholehepatic recycling, which would occur, because of similar driving forces, at different anatomic locations. Both hypotheses are fairly complementary when one attempts to understand most of the available experimental data.

To what extent is this hypothesis consistent with the results obtained on restricted damage preparations? In experiments where periporal hepatocytes were damaged, UDCA secretion processes can occur at the spared zone 3. UDCA secreted upward into the canalicul tree may undergo subsequent passive diffusion. Thus, although impaired, intra-acinar recycling would be possible even though the functional integrity of periporal hepatocytes was not preserved. By contrast, when zone 3 is damaged, a UDCA-insensitive dead space is created, because actively secreting hepatocytes are only those located in zone 1 and 2; hence, the effective path length along the acinar canaliculi is presumably shortened. Extension of the possible area for diffusion of protonated UDCA is reduced to the periportal branches of the canalicul tree. Contribution of the biliary epithelium via the cholehepatic shunt is expected to be similar in both preparations.

In summary, these results indicate that digitonin-pulse perfusion is a useful model to investigate the selective role of acinar zone 1 or zone 3 hepatocytes in the metabolic and biliary response to bile acid infusion. These studies provide evidence indicating that liver parenchyma microanatomy plays an important role in UDCA-induced hypercholeresis. They also suggest the existence of a different contribution of periportal and perivenous hepatocytes to UDCA-induced bicarbonate-rich hypercholeresis.

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