Biliary lipid secretion and its control

Effect of taurodehydrocholate

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Multiple short pulses of taurocholate (TC) brought about, in isolated perfused rat livers, the secretion of phospholipid and cholesterol into bile; the lipids showed an appreciable lag period behind the bile-salt secretion, and there was considerable variability in response, both between low and high dose pulses of TC and, at the higher dose, even between individual livers. When a background continuous infusion of taurodehydrocholate (a hydrophilic non-micelle-forming bile-salt analogue) was superimposed upon the short TC pulses, the lipid secretion showed much better control, and the lipid peaks were of more uniform size, following more closely, or more coincident with, the bile-salt output peaks. Taurodehydrocholate may provide a signal for the control of the supply and delivery of lipid vesicles to the bile-canalicular membrane, from where the lipid vesicles are then removed by the action of the pulses of TC.

INTRODUCTION

Bile salts play an important role in the regulation of biliary phospholipid and cholesterol secretion. At physiological rates of output, the secretion of phospholipid and cholesterol into bile is dependent on the secretion of bile salts in an approximately linear relationship (Hoffman *et al.*, 1975; Delage *et al.*, 1976; Poupon *et al.*, 1976; Sewell *et al.*, 1979; Casu *et al.*, 1981; Sama *et al.*, 1982); this relationship is not seen, however, at very low or very high bile-salt secretion rates (Hardison & Apter, 1972; Wheeler & King, 1972), indicating that other factors are involved. The characteristics of the bile-salt-associated lipid secretion are dependent on the physical properties of the bile salts. Thus the more hydrophobic bile salts, such as chenodeoxycholate, deoxycholate and their conjugates, stimulate biliary lipid secretion at lower biliary bile-salt concentrations than do hydrophilic bile salts such as cholate and ursodeoxycholate and their conjugates (Montet & Gerolami, 1978; Schersten & Linblad, 1979; Barnwell *et al.*, 1983a,b). More hydrophilic non-micelle-forming bile salts, such as TDHC, do not appear to be able to provoke biliary lipid output (Hardison & Apter, 1972; Barnwell *et al.*, 1984).

In bile, biliary lipids are often associated with bile salts as mixed micelles; increasing the output of bile salts into bile thus results in an increase in its content of mixed micelles. Rapid kinetic studies of the sequence of appearance of materials into rat bile have shown that bile salts are secreted before phospholipid and cholesterol (Lowe *et al.*, 1984). Thus mixed-micelle formation occurs subsequent to the entry of bile salts into the canaliculus. Work from this laboratory suggests that biliary lipid may be removed preferentially from more fluid microdomains within the membrane by the detergent action of bile salts and that this lipid is continuously resupplied *in vivo* from within the cell in order to avoid extensive damage (Coleman *et al.*, 1977; Barnwell *et al.*, 1984; Lowe *et al.*, 1984). Colchicine, a

drug that affects microtubular function and vesicle movement within cells, decreases the biliary output of phospholipid and cholesterol into bile to a much greater extent than it does bile-salt output (Barnwell *et al.*, 1984); this has led to our suggestion that, although bile salts are transported across the hepatocyte as discrete molecules, the lipid is supplied to the membrane in vesicular form, and it is this process that is interfered with by colchicine (Barnwell *et al.*, 1984; Lowe *et al.*, 1984).

Alteration of the rate of a continuous bile-salt secretion within the physiological range brings about a parallel adjustment in biliary lipid secretion and implies that transport of bile salts is somehow sensed by the cell and affects the control of some part(s) of lipid provision, vesicle assembly, vesicle transport and membrane processing (Rahman *et al.*, 1986). However, although multiple short pulses of TC brought about relatively equal biliary bile-salt-output pulse, they did not bring about equal phospholipid outputs and, moreover, the phospholipid peak was delayed (Rahman *et al.*, 1986). This implies that a threshold (intracellular) bile-salt concentration may be required to 'switch on' and deliver the lipids to the canalicular membrane.

The aim of the present study was to investigate whether TDHC, when continuously infused into isolated perfused rat livers, affects (increases?) the rate of delivery of (vesicles transporting) lipids to the canalicular membrane. TDHC, a non-micelle-forming bile-salt analogue, does not itself produce appreciable lipid output (Hardison & Apter, 1972; Barnwell *et al.*, 1984), but may, however, be capable of causing lipid movements within the hepatocyte.

MATERIALS AND METHODS

Materials

The following fine-grade chemicals were obtained: TC and TDHC (Calbiochem–Boehringer Corp., Bishops Cleeve, U.K.), [¹⁴C]TC (Amersham International,

Amersham, Bucks., U.K.), heparin (Weddel Pharmaceuticals, London E.C.1, U.K.), Sagatal (May and Baker, Dagenham, Essex, U.K.). All other reagents were from Fisons Limited., Loughborough, Leics., U.K., and were of the highest grade available. The assay kit for cholesterol was obtained from Boehringer Corp. (London). Lewes, East Sussex, U.K., and the plastic cannula tubing (PP10 and PP30) was obtained from Portex, Hythe, Kent, U.K.

Methods

Male Wistar rats, weighing 250–280 g, were used throughout. Before the experiments, animals were maintained on a standard laboratory diet in a constant-temperature environment (22 °C) and under a constant 12 h-light/12 h-dark cycle. All bile-duct cannulations were performed between 09:00 and 12:00 h with 500 mm of PP10 tubing while the animals were under pentobarbitone (Sagatal) anaesthesia; their livers were then isolated *in situ* (Hems *et al.*, 1966). Liver anoxia was minimized (5–10 s) by commencing recycling perfusion immediately at a constant flow rate of 16 ml·min⁻¹ with 150 ml of Krebs–Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932); this buffer also contained 2 mM-CaCl₂, 5 mM-glucose, 1% (w/v) bovine serum albumin, a physiological amino acid mixture (see Barnwell *et al.*, 1983a) and 40% (v/v) of packed human red blood cells (see Lowe *et al.*, 1984). This solution was recycled, gassed continuously with O₂/CO₂ (19:1, v/v) and maintained at 37 ± 0.5 °C within a thermostatically controlled cabinet similar to that recommended by Collins & Skibba (1980). At the same time, in some experiments, infusion of TDHC (dissolved in red-cell-free and albumin-free perfusion medium) into the perfusion line was initiated at a rate of 12 ml/h (1000 nmol/min per rat) and continued until the completion of the experiment. The livers were perfused for 90 min in order to run down the endogenous biliary bile-salt and lipid levels to basal levels.

After 90 min the liver was converted into a 'single-pass' perfusion of fresh perfusate, and a basal bile sample collected (0–5 min); then either 2500 or 5000 nmol of TC, containing 0.05–0.1 µCi of [¹⁴C]TC,

were infused into the hepatic portal cannula in 60 s. Bile collections were made at 1 min intervals between 5 and 6 min, then at 2 min intervals between 6 and 40 min and at 5 min intervals between 40 and 45 min in preweighed tubes. Subsequently a second and third pulse of TC was injected at 15 min and 25 min, and bile collected on ice and frozen at –20 °C until required for analysis. Bile flow was determined gravimetrically by assuming a density of 1 g·ml⁻¹.

Assays

Bile was assayed for phospholipid after lipid extraction by the method of Bligh & Dyer (1959), with phospholipid-phosphorus determination by the method of Bartlett (1959). The amount of free cholesterol in bile was determined by using an assay kit based on cholesterol oxidase. Bile-salt concentrations in the 0–5 min samples were determined with hydroxysteroid dehydrogenase (EC 1.1.1.150) as described by Coleman *et al.* (1979). The concentration of TC in the remaining samples was determined by comparing the radioactivity in the bile with the specific radioactivity of the TC injected into the liver.

RESULTS

The effect of TC on biliary secretion was determined by the administration of the bile salt into the hepatic portal cannula 5 min after establishing the 'single-pass' liver perfusion; either 2500 nmol or 5000 nmol of TC were introduced in 60 s at 10 min intervals. This was performed in either the absence or the presence of TDHC, which was continuously infused at a rate of 1000 nmol·min⁻¹.

Fig. 1 shows the effect of multiple short pulses of TC (2500 nmol each) on biliary bile-salt, phospholipid and cholesterol output. The peak secretion of bile salt occurred in the 10 min sample (4 min after the TC pulse). The peak secretion of phospholipid and cholesterol output occurred in the 16–18 min samples (10–12 min after the TC pulse). With subsequent second and third pulse of TC the peak bile-salt secretion was maintained

Fig. 1. Effect of TC pulses (2500 nmol) on the biliary output of (i) bile salt (●), (ii) phospholipid (○) and (iii) cholesterol (△) in the isolated liver

Liver isolation, perfusion and bile collection are described in the Materials and methods section. Values at 0–5 min indicate the basal biliary output after 90 min of liver isolation. Three TC pulses of 2500 nmol each were infused at 10 min intervals as indicated by the arrows. Values are means ± S.E.M. (*n* = 5).

and the biliary lipid output lagged behind the respective bile-salt peaks (Fig. 1).

The effects of the three pulses of TC (5000 nmol each) on biliary phospholipid and cholesterol secretion are shown in Fig. 2(a). The peak secretion of bile salt again occurred in the 10 min sample (4 min after the TC pulse). A small initial peak of biliary phospholipid and cholesterol was observed in the 10 min sample, but larger subsequent peaks of phospholipid and cholesterol output were present in the 20 and 30 min samples. Although the lag in biliary lipid output was evident, this was less than that observed in Fig. 1. A large variation in the output of biliary phospholipid and cholesterol in individual

livers was also observed when TC was pulsed at 5000 nmol; these individual results are shown in Figs. 2(b)–2(e).

Fig. 3 represents the effect of three pulses of TC (2500 nmol each) on biliary phospholipid and cholesterol output in the presence of a continuous TDHC infusion ($1000 \text{ nmol} \cdot \text{min}^{-1}$).

The three pulses of TC brought about equal-size biliary bile-salt output peaks at 10, 20 and 30 min; the shape and height of these peaks was the same as in experiments without TDHC infusion (Fig. 1). In contrast with Fig. 1, however, was the response of phospholipid and cholesterol in the presence of TDHC

Fig. 2. Effect of TC pulses (5000 nmol) on the biliary output of (i) bile salt (●), (ii) phospholipid (○) and (iii) cholesterol (△) in the isolated liver

(a) Details are as described in Fig. 1, except that TC was pulsed at 5000 nmol. Values are means $\pm$ S.E.M. ($n = 4$). (b)–(e) are the individual experiments giving rise to the mean values shown in (a).

Fig. 3. Effect of TC pulses (2500 nmol) on the biliary output of (i) bile salt (●), (ii) phospholipid (○) and (iii) cholesterol (△) in the isolated liver in the presence of TDHC infusion

Details are as for Fig. 1. The continuous line in upper part of the Figure represents TDHC, which was continuously infused at a rate of 1000 nmol/min throughout the experiment. Values are means $\pm$ S.E.M. ($n = 5$).

Fig. 4. Effect of TC pulses (5000 nmol) on the biliary output of (i) bile salt (●), (ii) phospholipid (○) and (iii) cholesterol (△) in the isolated liver in the presence of TDHC infusion

Details are as for Fig. 1, except that TC was pulsed at 5000 nmol. The continuous line in upper part of the Figure represents TDHC, which was continuously infused at a rate of 1000 nmol/min throughout the experiment. Values are means $\pm$ S.E.M. ($n = 3$).

infusion, in that three distinct biliary phospholipid and cholesterol peaks were observed. Also, the biliary phospholipid and cholesterol peaks were more nearly coincident with the peaks of TC output, virtually abolishing the lag time of biliary lipid output on pulsing with TC.

The effect of three pulses of larger amounts of TC (5000 nmol each) on biliary phospholipid and cholesterol

output in the presence of a continuous TDHC infusion ($1000 \text{ nmol} \cdot \text{min}^{-1}$) is shown in Fig. 4. The shape and height of bile-salt-output peaks were similar to those in experiments without TDHC infusion (Fig. 2a), but in contrast with Fig. 2(a) were the three distinct phospholipid and cholesterol peaks, which were nearly coincident with the peaks of TC output, virtually abolishing the lag time of biliary lipid output on pulsing with TC.

DISCUSSION

Although bile salts, phospholipids and cholesterol may be associated together ultimately in bile as mixed micelles, Lowe *et al.* (1984) have shown that bile-salt secretion and lipid secretion can be dissociated from one another. The sequence of events therefore seems to be the primary secretion of bile salts into the canaliculus, followed by the biliary lipids, and then the secondary association of the bile salts and lipids to form mixed micelles (Lowe *et al.*, 1984; Coleman *et al.*, 1986). This dissociation of lipid secretion from bile-salt secretion is supported by a number of other observations: (i) colchicine decreases biliary lipid secretion at concentrations having little effect on bile-salt secretion (Barnwell *et al.*, 1984); (ii) several compounds, e.g. ampicillin (Apstein & Robins, 1982) and iodipamide (Apstein & Russo, 1985), decrease biliary lipid secretion, but have little effect on bile-salt secretion.

This dissociation in biliary bile-salt and lipid output is also seen as a lag of phospholipid and cholesterol output behind bile-salt output in experiments represented in Fig. 1 and Fig. 2(a). A low (2500 nmol) and a high (5000 nmol) TC pulse was used in order to study the influence of different amounts of bile salts on the control of biliary lipid output. When TC was pulsed at 2500 nmol, the biliary output of phospholipid and cholesterol lagged behind the bile-salt peaks by several minutes (Fig. 1). This lag was also evident when TC was pulsed at the higher level of 5000 nmol, although it was diminished (Fig. 2a). With the lower pulse (2500 nmol) the phospholipid and cholesterol response was spread generally, whereas, at the higher levels (5000 nmol), peaks of output were evident. An initial small peak and two subsequent larger peaks were present (Fig. 2a).

Though the mean response for the higher pulse (5000 nmol) (Fig. 2a) shows three relatively distinct peaks, these are nevertheless the result of a spectrum of responses (Fig. 2b–2e). It is instructive to consider these individually, since these may indicate different degrees of control operating in different animals. In the experiments of Hardison & Apter (1972) a wide range of biliary phospholipid responses in individual animals was also seen; although the authors have not commented on these, they too may have been studying different degrees of control of biliary lipid output operating in individual animals. In the present experiments, therefore, the following was observed: (i) two peaks of phospholipid and cholesterol lagging behind bile-salt peaks (Fig. 2b); this observation is similar to that reported previously (Rahman *et al.*, 1986); (ii) biliary phospholipid and cholesterol secretion is continuous (Fig. 2c); (iii) three peaks of biliary phospholipid and cholesterol output present and lagging bile-salt peaks; the lag decreases after the first TC pulse (Fig. 2d); (iv) three distinct biliary lipid peaks present, each showing some lag (Fig. 2e).

These four examples may demonstrate increasing degrees of control of lipid delivery to the canalicular membrane in the order Fig. 2(b) < Fig. 2(c) > Fig. 2(d) < Fig. 2(e).

When continuous TDHC infusion (1000 nmol·min⁻¹) was superimposed upon the above experiments, the biliary phospholipid and cholesterol response became more reproducible (Figs. 3 and 4). The biliary lipid-output peaks become more pronounced, regular, and the

lag period became decreased compared with those in Figs. 1 and 2(a). In fact, the biliary phospholipid and cholesterol peaks became virtually coincident with the peaks of TC output, thus abolishing the lag time (Figs. 3 and 4).

The actual point of interaction of the bile salt in controlling the rate of lipid supply is unknown. However, there appears to be an increased occurrence of vesicles in the pericanalicular region during intense bile-salt transport (Boyer *et al.*, 1979; Jones *et al.*, 1979, 1980; Goldsmith *et al.*, 1983). This even occurred during secretion of TDHC, which is secreted in abundance but does not itself involve lipid secretion (Barnwell *et al.*, 1984). Thus this particular abundance of vesicles during TDHC secretion might be explained by a 'queuing' phenomenon whereby, since lipid is not removed from the membrane, the oncoming vesicles approach, but then do not fuse with, the membrane (Barnwell *et al.*, 1984; Lowe *et al.*, 1984; Coleman *et al.*, 1986). These phenomena point to increased vesicle movement during bile-salt secretion. This effect suggests that the cytoskeleton or the cytoskeletal-vesicle interaction may be the key regulatory site which responds to the passage of bile salts through the cell.

Within the physiological range, increasing the bile-salt secretion brings about a parallel adjustment to biliary lipid secretion (see Rahman *et al.*, 1986). This suggests that the transport of bile salts somehow causes the cell to control lipid provision, vesicle assembly and vesicle transport (Rahman *et al.*, 1986). It thus appears that TDHC, whether acting as a messenger on its own or through another molecule, is able to increase the rate of delivery/synthesis of vesicles carrying lipids to the canalicular membrane. The actual site of action of TDHC has yet to be established. The findings of the localization of bile-salt analogues over the Golgi apparatus and other intracellular sites (Suchy *et al.*, 1983) may provide further clues to the site of action of TDHC.

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REFERENCES

- Apstein, M. D. & Robins, S. J. (1982) *Gastroenterology* **83**, 1120–1126
- Apstein, M. D. & Russo, R. (1985) *Dig. Dis. Sci.* **30**, 253–256
- Barnwell, S. G., Godfrey, P. P., Lowe, P. J. & Coleman, R. (1983a) *Biochem. J.* **210**, 549–557
- Barnwell, S. G., Lowe, P. J. & Coleman, R. (1983b) *Biochem. J.* **216**, 107–111
- Barnwell, S. G., Lowe, P. J. & Coleman, R. (1984) *Biochem. J.* **220**, 723–731
- Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–919
- Boyer, J. L., Itabashi, M. & Hruben, Z. (1979) in *The Liver: Quantitative Aspects of Structure and Function* (Preisig, R. & Bircher, J., eds.), pp. 163–178, Editib Cantor Alrendorf, Berne
- Casu, A., Cottalasso, D., Pronzato, M. A. & Bassi, A. M. (1981) *Ital. J. Biochem.* **30**, 389–402
- Coleman, R., Holdsworth, G. & Vyvoda, O. S. (1977) in *Membrane Alterations as a Basis for Liver Injury* (Popper, H., Bionchi, L. & Reutter, W., eds.), pp. 143–156, MTP Press, Lancaster (U.K.)

- Coleman, R., Iqbal, S., Godfrey, P. P. & Billington, D. (1979) *Biochem. J.* **178**, 201–208
- Coleman, R., Rahman, K., Kan, K. S., Lowe, P. J. & Barnwell, S. G. (1986) in *Bile in Health and Disease*. (Proceedings of the Sixth BSG-SK and F International Workshop, 1985) (Elias, E. & Murphy, G., eds.), pp. 23–27, Smith Kline and French Laboratories, Welwyn Garden City
- Collins, F. G. & Skibba, J. L. (1980) *J. Surg. Res.* **28**, 65–70
- Delage, Y., Dumon, M. & Erlinger, S. (1976) *Am. J. Physiol.* **231**, 1875–1878
- Goldsmith, M., Huling, S. & Jones, A. L. (1983) *Gastroenterology* **84**, 978–986
- Hardison, W. G. M. & Apter, J. T. (1972) *Am. J. Physiol.* **222**, 61–67
- Hems, R., Ross, B. D., Berry, M. N. & Krebs, H. A. (1966) *Biochem. J.* **101**, 284–292
- Hoffman, N. E., Donald, D. E. & Hofmann, A. F. (1975) *Am. J. Physiol.* **229**, 714–720
- Jones, A. L., Schmucker, D. L., Monney, J. S. & Ockner, K. (1979) *Lab. Invest.* **40**, 512–517
- Jones, A. L., Schmucker, D. L., Renston, R. H. & Murakami, I. (1980) *Dig. Dis. Sci.* **25**, 609–629
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–36
- Lowe, P. J., Barnwell, S. G. & Coleman, R. (1984) *Biochem. J.* **222**, 631–637
- Montet, J. C. & Gerolami, A. (1978) *Digestion* **17**, 346–364
- Poupon, R., Grosdesuge, M. L., Dumont, M. & Erlinger, S. (1976) *Eur. J. Clin. Invest.* **6**, 279–284
- Rahman, K., Hammond, T. G., Clark, B., Lowe, P. J., Barnwell, S. G. & Coleman, R. (1986) *Biochem. J.* **234**, 421–428
- Sama, C., La Ruso, N. F., Lopez del Pino, V. & Thistle, J. L. (1982) *Gastroenterology* **82**, 515–525
- Sewell, R., Hofmann, N., Cockbrain, S. & Smallwood, R. (1979) in *Biological Effects of Bile Salts* (Paumgartner, G., Steihl, A. & Gerok, W., eds.), pp. 11–17, MTP Press, Lancaster (U.K.)
- Schersten, T. & Linblad, L. (1979) in *Biological Effects of Bile Salts* (Paumgartner, G., Steihl, A. & Gerok, W., eds.), pp. 53–60, MTP Press, Lancaster (U.K.)
- Suchy, R., Balistreri, W., Hung, J., Miller, P. & Garfield, S. A. (1983) *Am. J. Physiol.* **245**, G681–G689
- Wheeler, H. O. & King, K. K. (1972) *J. Clin. Invest.* **51**, 1337–1345

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