Oestradiol 17 β -glucuronide increases tight-junctional permeability in rat liver

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By using rat liver perfusion under one-pass conditions with a single pulse of horseradish peroxidase (HRP), the biliary output of HRP was used as an indicator of paracellular permeability change caused by the cholestatic compound oestradiol 17 β -glucuronide (E₁₇G). Since E₁₇G reduced bile flow, we have also used, during the assessment of junctional permeability after E₁₇G treatment, the choleric compound taurodehydrocholate to enhance bile flow back to control levels. At both low and restored bile flow rates, the acute administration of E₁₇G (3.4 μ mol) increased the HRP peak height, thereby indicating that one of the hepatotoxic actions of E₁₇G is to increase the permeability of hepatic tight junctions. The action of E₁₇G in affecting bile acid secretion and biliary volume are also explored.

INTRODUCTION

Intrahepatic cholestasis is observed in some women during the last trimester of pregnancy, when plasma oestrogen concentrations are high, sometimes in women using oral steroidal contraceptives and also in animals treated with high doses of oestrogens. It is thought to be due to the effects of increased amounts of minor oestrogen metabolites being present under such circumstances, particularly D-ring glucuronide conjugates of oestrogens, which show potent dose-dependent reversible cholestatic properties (Meyers *et al.*, 1980, 1981; Vore, 1981; Vore *et al.*, 1983; Vore & Slikker, 1985).

The mechanism(s) of the toxic action of these compounds are still little understood. A number of possibilities have been advanced including: (i) reduction in (Na⁺ + K⁺)-dependent ATPase activity, (ii) decrease in bile acid synthesis, (iii) decrease in bile acid transport, (iv) change in the plasma-membrane lipid composition, and (v) an alteration in the junctional permeability (Forker, 1969; Cooper, 1977; Davis *et al.*, 1978; Meyers *et al.*, 1981; Elias *et al.*, 1983).

Although an involvement of hepatic tight junctions has been suggested as a contributor to cholestasis caused by oestrogen metabolites (Forker, 1969; Peterson & Fujimoto, 1977; Vore, 1981, 1987; Bradley, 1982; Elias *et al.*, 1983; Aldinolfi *et al.*, 1984; Iqbal *et al.*, 1984, 1985; Jaeschke *et al.*, 1987a), contradictory results have been obtained by others (Meyers *et al.*, 1980; Jaeschke *et al.*, 1983, 1987b). Such discrepancies may arise from problems in the techniques used to assess junctional permeability.

Measurement of the bile/plasma (or perfusate) ratio for an inert carbohydrate is probably the most widely used technique in experimental animals, but its reliability is limited by certain considerations: (i) some commonly used compounds may be transferred to bile not only by the paracellular route, but also by a transcytotic route (Lorenzini *et al.*, 1986); only recently has an attempt been made to take an account of the transcytotic

contribution (Jaeschke *et al.*, 1987a); (ii) the bile/plasma (B/P) ratio is also influenced by the bile flow rate (see Forker, 1969; Elias *et al.*, 1983), i.e. a depression in this flow rate may enhance the diffusion rate of the inert compound, artificially increasing the B/P ratio.

When isolated perfused liver was pulsed under single-pass conditions with the marker compound horseradish peroxidase (HRP), the height of the first peak of the biliary HRP output appeared to be a good indicator of junctional permeability (Lowe *et al.*, 1985) and has been used in a number of recent studies involving changes in junctional permeability (Kan & Coleman, 1986, 1988; Lowe *et al.*, 1988).

In the present work we have studied the effect of the cholestatic compound, oestradiol 17 β -glucuronide (E₁₇G), upon hepatic tight-junctional permeability using this new HRP-pulsing technique, in an attempt to clarify the situation on junctional-permeability change, and to eliminate any contribution from transcytotic mechanisms. We have studied the effects of E₁₇G upon livers largely depleted of bile acids and also continuously perfused with taurodehydrocholate (TDHC), a bile acid analogue which is a hydrophilic non-toxic hydrocholeric compound. This has been used to restore cholestatic bile flow to normal levels and thereby to circumvent the problem of reduced bile flow during permeability assessment.

MATERIALS AND METHODS

Materials

E₁₇G, dimethyl sulphoxide (DMSO), tetramethylbenzidine, bovine serum albumin (BSA), HRP and other fine-grade chemicals were purchased from Sigma Chemical Co., Poole, Dorset, U.K. H₂O₂ solution (100 vol.) was obtained from Fisons Scientific Apparatus, Loughborough, Leics., U.K. The sodium salt of TDHC was purchased from Calbiochem-Behring Corp., Bishops Stortford, Herts., U.K. Cyclobutylol (CB) was obtained

Abbreviations used: E₁₇G, oestradiol 17 β -glucuronide; B/P, bile/plasma (ratio); HRP, horseradish peroxidase; TDHC, taurodehydrocholate; DMSO, dimethyl sulphoxide; BSA, bovine serum albumin; CB, cyclobutylol.

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from Dr. R. Jimenez, Department of Physiology and Pharmacology, University of Salamanca, Salamanca, Spain. Norursodeoxycholate was kindly provided by Professor A. F. Hoffmann, Division of Gastroenterology, University of California, San Diego, CA, U.S.A. The plastic cannula tubing (PP10) was supplied by Portex, Hythe, Kent, U.K.

Methods

Male Wistar rats, approx. 250 g body wt. each, were used throughout the study. The bile duct was cannulated using PP10 tubing during pentobarbital anaesthesia. The liver was isolated *in situ* as described by Hems *et al.* (1966) and perfused with 150 ml of Krebs–Ringer bicarbonate solution (Krebs & Henseleit, 1932) containing 1% (w/v) BSA, 5 mM-glucose, 1.2 mM- CaCl_2 , a physiological amino acid mixture (see Barnwell & Coleman, 1983) and 20% (v/v) packed ox red cells. The solution was continuously gassed with O_2/CO_2 (19:1) and was maintained at 37 °C in a thermostatically controlled cabinet.

At 15 min after the start of the perfusion, a continuous portal infusion into the liver of either sodium TDHC

(15 mM) at a rate of 1 $\mu\text{mol}/\text{min}$ (in 0.9% NaCl solution), or 0.9% NaCl solution, was initiated. At 15 min after the infusion of the TDHC or saline alone, 200 μl of DMSO containing either 3.4 μmol of E_{17}G or nothing (in control experiments) was injected, within 1 min, via the portal cannula into the liver.

At 15 min after the injection of E_{17}G , the liver perfusion was converted to single-pass conditions, and 3 mg of HRP, dissolved in 1 ml of Krebs–Ringer bicarbonate solution, was then infused, within 1 min, into the liver at the same entry point as the drug injection (Lowe *et al.*, 1985; Kan & Coleman, 1988).

Bile was collected into preweighed tubes, and the flow rates were determined gravimetrically (assuming a density of 1 g/ml) throughout each experiment. The HRP in the collected bile was assayed spectrophotometrically as described by Gallati & Pracht (1975), and the location and height of the first peak of biliary HRP was identified. Further details are given in the legend to Fig. 3 (below).

Bile acids in bile were assayed by using hydroxysteroid dehydrogenase (EC 1.1.1.50) as described by Coleman *et al.* (1979). In the case of experiments with cyclobutylol, details are given in the legend to Fig. 2 (below).

Fig. 1. Effects of E_{17}G on bile flow and bile acid secretion in control and TDHC-infused isolated perfused liver preparations

Isolated livers were perfused with medium to which was added continuously into the portal flow line: (a) and (c) 0.9% NaCl alone (16 $\mu\text{l}/\text{min}$); (b) and (d) TDHC (1 $\mu\text{mol}/\text{min}$) in 0.9% NaCl (16 $\mu\text{l}/\text{min}$). At 15 min after the start of these infusions, 200 μl of DMSO alone (control, C, ○) or 200 μl of DMSO containing 3.4 μmol of E_{17}G (●) was injected into the liver over 1 min via the portal flow line. For DMSO controls, $n = 3$; for E_{17}G , $n = 4$. Bars represent the S.E.M.

RESULTS AND DISCUSSION

Effects of E₁₇G on bile volume

The effect of E₁₇G upon the perfused liver under standard conditions (Fig. 1a) is to cause a substantial reduction in flow. Since most of the bile acid pool in the liver had been depleted by the prior perfusion conditions, the flow reduction caused by E₁₇G is therefore largely bile-acid-independent. Reduction of bile-acid-independent flow in other experimental designs has been observed by other groups of workers (Meyers *et al.*, 1980; Iqbal *et al.*, 1984).

Infusion of TDHC increased the control bile flow by 12 μ l/min (Fig. 1b), but the subsequent administration of E₁₇G then substantially reduced the flow back to the untreated control level. The fall in this case was greater than under saline-infusion conditions, indicating that the portion of flow stimulated by TDHC was reduced (i.e. a bile-acid-dependent flow), in addition to reducing the flow independent of bile acids. This is supported by the concomitant fall in secretion of bile acids (Figs. 1c and 1d); in the case of TDHC, this is largely the hydroxy metabolites of TDHC, since hydroxysteroid dehydrogenase does not measure TDHC directly.

The fall in bile flow is partially reversed 15 min after E₁₇G administration (see also Aldinolfi *et al.*, 1984). This reversal coincides with the restoration of bile-acid secretion (TDHC metabolites) (Fig. 1d). Appreciable flow reduction, however, still remains after this point (Fig. 1b) and is thus likely to be due to the continuing inhibition of the flow independent of bile acids. Administration of E₁₇G at a high dosage caused complete biliary reduction in perfused livers and in bile-fistula rats (results not shown), partly indicating that both the bile-acid-dependent flow and the bile-acid-independent flow could be affected.

Meyers *et al.* (1981) have noted a structural similarity between D-ring steroidal glucuronides and bile acids, which led to the suggestion that cholestatic steroids may specifically inhibit the bile-acid carrier. In the present experiments with the choleretic anion CB [see Fig. 2 and also Roquet & Jousse (1960)], and in which most of the endogenous bile-salt pool has been depleted before CB infusion, the portion of bile flow stimulated by this non-bile acid choleretic was severely reduced. Thus E₁₇G, in addition to its inhibitory effects on the bile-acid-transport system, may also interfere with other anion transports which may play a part in generating the total flow.

Effect on junctional permeability

In Vore's (1987) review, a word of caution on the possibility of junctional permeability change caused by oestrogens was sounded in relation to B/P equilibrium measurement with high-molecular-mass markers, since changes in the relative contributions of paracellular movement and transcellular vesicular transport to the B/P ratio may be subject to differential change. By using the present method of HRP peak height, however, the contribution of vesicular transcytosis has been thereby eliminated, and thus the permeability change of the junction is brought into clearer focus. The use of a choleretic compound, e.g. TDHC, to circumvent the problem of the influence of low flow rate on permeability studies has been outlined in the Introduction.

Thus at both low and restored bile flow rates (Figs. 3a and 3b), an increase in HRP peak height was observed

Fig. 2. Effect of E₁₇G on bile flow and bile-acid secretion on isolated perfused liver preparations perfused with CB

Isolated livers were perfused with medium. At 60 min, CB (1 mg/min in 0.9% NaCl; 83 μ l/min) was continuously added to the portal flow line. At 80 min, 3.4 μ mol of E₁₇G in 200 μ l of DMSO was injected into the portal flow line over the course of 1 min. Bile flow: ○, before, and ●, after, E₁₇G. Bile acids: □, before, and ■, after, E₁₇G ($n = 3$; bars represent S.E.M.).

subsequent to E₁₇G administration (Figs. 3c and 3d), strongly indicating that an increase in junctional permeability has been caused by exposure to E₁₇G. Increase in junctional permeability therefore appears to be a feature of cholestasis caused by E₁₇G.

Although increase in permeability of hepatic tight junctions is evident after E₁₇G, it is not yet clear, however, whether bile flow reduction is a direct consequence, or independent, of an increase in junctional permeability. A regurgitation of osmotically active anions through 'leaky' junctions, resulting in reduction of osmotic water, may be a possible mechanism of flow reduction (Elias & Boyer, 1978). In contrast, however, other studies also have indicated that increase in junctional permeability may not directly lead to flow reduction: (i) increase in junctional permeability during early 1-naphthyl isothiocyanate pretreatment occurs without flow reduction (Kan & Coleman, 1986); (ii) low bile flow rates precede junctional-permeability change in rats treated with oestradiol valerate (Jaeschke *et al.*, 1987b). It thus seems that permeability increase is not concomitant with volume change and, therefore, junctional-permeability change and bile-volume change may be unrelated. In another recent report, other potential causes of E₁₇G-induced cholestasis, e.g. (Na⁺ + K⁺)-dependent ATPase activity and membrane fluidity, have also been shown to be independent of the volume reduction (Smith & Gordon, 1988).

The higher junctional permeability seen in E₁₇G-treated livers infused with TDHC, as compared with the saline control, is of some interest. This choleretic compound did not appear to affect the junctional permeability alone, as evidenced by the control (Fig. 1), and thus the effect is to enhance the permeability effect after E₁₇G treatment. In a study by Utili *et al.* (1985), it was observed that TDHC did not affect the excretion of E₁₇G into bile, and thus it is unlikely that the enhancement of HRP peak height is due to a reduction in the excretion rate of E₁₇G from the liver, thereby prolonging its presence. Moreover, in preliminary experiments with

Fig. 3. Effect of E₁₇G on bile flow and paracellular permeability in control and THDC-infused livers

Livers were isolated, infused with 0.9% NaCl or TDHC in 0.9% NaCl and treated with DMSO or E₁₇G/DMSO as described in the legend to Fig. 1. (a) and (c) Saline infusion; (b) and (d) TDHC infusion. At 15 min after the injection of DMSO or E₁₇G/DMSO the livers were then pulsed under single-pass conditions with 3 mg of HRP for the assessment of junctional permeability (see the Materials and methods section). Bile flow and HRP in the bile were measured, and the location of the first peak was identified. The shapes of the first peaks were similar, but the time at which the peak height occurred varied slightly, by one collection tube, i.e. 5 ± 2 min. At higher flow rates the peak was reached in less than 5 min and at lower flow rates at more than 5 min. These small differences in peak time are thought to be a consequence of the difference in bile flow rates. In spite of these small differences in location, the peak height is still a good reflection of the area under the peak. Open columns represent DMSO controls ($n = 3$); hatched columns represent E₁₇G treatment ($n = 4$) and bars represent S.E.M.

another choleretic compound, namely norursodeoxycholate, junctional permeability was increased to the same level as that observed with TDHC.

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