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Acute effects of cholestatic and choleretic bile salts on vasopressin- and glucagon-induced hepato-biliary calcium fluxes in the perfused rat liver

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The effects were investigated of the choleretic bile salt glycoursodeoxycholate (G-UDCA) and of the cholestatic bile salt taurochenodeoxycholate (T-CDCA) on changes in perfusate Ca^{2+}, glucose and oxygen and in bile calcium and bile flow induced by the administration of (*a*) vasopressin, (*b*) glucagon and (*c*) glucagon plus vasopressin together to the perfused rat liver [Hamada, Karjalainen, Setchell, Millard & Bygrave (1992) Biochem. J. **281**, 387–392]. G-UDCA itself increased the secretion of calcium in the bile several-fold, but its principal effect was to augment each of the above-mentioned metabolic events except glucose and oxygen output; particularly noteworthy was its ability to augment the 'transients' in bile calcium and bile flow seen immediately after the administration of vasopressin with or without glucagon. T-CDCA, by contrast, produced opposite effects and attenuated all of the parameters measured, and in particular the transients in bile calcium and bile flow. The data provide evidence of a strong correlation between calcium fluxes occurring on both the sinusoidal and the bile-canalicular membranes and that all are modifiable by glucagon, Ca^{2+}-mobilizing hormones and bile salts.

INTRODUCTION

Recently we described experiments using the perfused rat liver in which changes in Ca^{2+} fluxes were measured in both the perfusate and in the bile after the administration of vasopressin in the absence and presence of glucagon (Hamada *et al.*, 1992). The information gained from this experimental approach, which simultaneously monitors Ca^{2+} mobilization across both the sinusoidal and canalicular plasma membranes, has provided a basis for allowing one to gain a more complete understanding of Ca^{2+} homoeostasis in the intact rat liver. One of the more important discoveries in that study was the detection of a 'transient' in biliary calcium and biliary secretion that appears within seconds of the co-administration of vasopressin and glucagon to the portal vein.

In recent years, it has become apparent that bile salts are able to mobilize Ca^{2+} in hepatocytes (Combettes *et al.*, 1988, 1989, 1990; Anwer *et al.*, 1989) and in perfused rat liver (Anwer *et al.*, 1989), though there appears to be some disagreement as to the mechanism by which these salts mobilize the ion (see, e.g., Combettes *et al.*, 1990). Bile salts are continuously flowing through the liver as part of the enterohepatic circulation, either as a result of their synthesis therein or in the course of their passage from the blood plasma to the bile (for a review see Carey & Cahalane, 1988). We therefore considered it important to analyse in our experimental system with the perfused rat liver (Hamada *et al.*, 1992), the influence of various bile salts on their ability both to mobilize biliary Ca^{2+} and bile flow and to alter hormone-induced perfusate Ca^{2+} fluxes.

In the present study we have examined and compared the effects of a choleretic bile salt, glycoursodeoxycholate (G-UDCA; see, e.g., Kitani & Kanai, 1982), with those of a cholestatic bile salt, taurochenodeoxycholate (T-CDCA; see, e.g., Heuman *et al.*, 1991). The results of these experiments show that G-UDCA augments the biliary calcium transient induced by the action of vasopressin, an effect greatly enhanced by the co-administration of glucagon (Hamada *et al.*, 1992). By contrast, T-CDCA has an attenuating effect on the biliary calcium transients and on biliary flow as well as on perfusate Ca^{2+} fluxes.

EXPERIMENTAL

Animals and perfusions

Male Wistar-strain albino rats, initially weighing approx. 280 g and having free access to food, were used in all experiments. Rats were anaesthetized with sodium pentobarbitone (50 mg/kg body wt.). Considerable care was taken to ensure the animal did not suffer in the course of isolating the liver, which was perfused with Krebs–Henseleit (1932) bicarbonate buffer equilibrated with O_2/CO_2 (19:1) and containing 1.3 mM-$CaCl_2$. Perfusions were conducted in a non-recirculating mode, and the perfusate was delivered at a constant volume of 3.5 ml/min per g wet wt. of liver by means of an LKB 2115 peristaltic pump. For each experiment the liver was first pre-perfused for at least 15 min before the infusion of any agent, each of which was administered by a pump-driven infusion syringe. In these experiments the strategy generally adopted was to infuse the appropriate bile salt from 20 min; the first hormone pulse was delivered 10 min later. Details are contained in the legends to the Figures. Additionally, a fine plastic cannula was inserted into the bile duct to enable collection of samples after administration of agents to the inflow cannula in the portal vein.

Perfusate Ca^{2+} measurements

The perfusate Ca^{2+} concentration was monitored continuously with a Radiometer F2112 Ca^{2+}-selective electrode in a flow-

Abbreviations used: G-UDCA, sodium glycoursodeoxycholate [sodium (3α,7β-dihydroxy-24-oxo-5β-cholan-24-yl)aminoacetate]; T-CDCA, sodium taurochenodeoxycholate {sodium 2-[(3α,7α-dihydroxy-24-oxo-5β-cholan-24-yl)amino]ethanesulphonate}.

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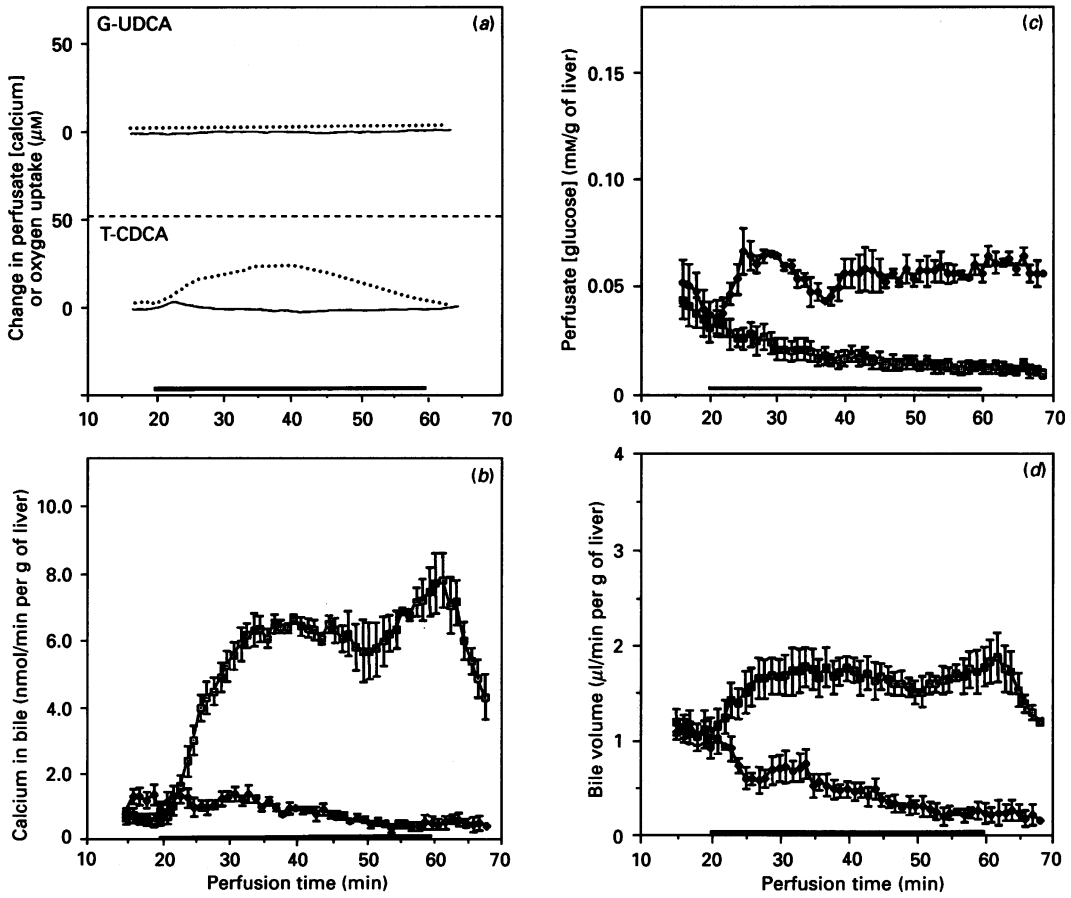

Fig. 1. Effects of G-UDCA and T-CDCA on perfusate Ca^{2+}, glucose output and oxygen uptake and on bile calcium and bile flow in the perfused rat liver

Livers were perfused with Krebs–Henseleit bicarbonate medium containing 1.3 mM-Ca^{2+}. After a pre-perfusion period of 20 min, either G-UDCA or T-CDCA (100 μM) was infused for 40 min, as indicated by the horizontal line. The Ca^{2+}-selective-electrode trace (——) and oxygen-electrode trace (· · · · · ·) are shown in (*a*); the concentration of calcium in the bile determined by atomic-absorption spectroscopy is shown in (*b*); perfusate glucose output is shown in (*c*); bile flow is shown in (*d*). For further details see the Experimental section. Each trace is a representative of at least four experiments performed independently. The data in (*b*), (*c*) and (*d*) are means ± S.E.M. of four independent experiments: ⊡, G-UDCA; ◆, T-CDCA.

through chamber placed on the outflow side of the liver; this is described in detail elsewhere (Reinhart *et al.*, 1982*a*). An upward deflection in the Ca^{2+} traces shown in the Figures presented reflects net Ca^{2+} efflux from the liver, whereas a downward deflection reflects net Ca^{2+} uptake by the liver. The electrode was coupled to a Radiometer K801 reference electrode via an agarose/KCl salt-bridge, and the combined signals were fed via an Orion microprocessor ion-analyser to a SP4100 computing integrator for recording and analysis. For other details see Altin & Bygrave (1985).

Other measurements

Oxygen consumption and glucose release by the liver were determined as previously described (Reinhart *et al.*, 1982*b*). Total atomic calcium in the bile samples was measured with a Varian AA20 atomic-absorption spectrophotometer. Samples were extracted with 1 M-$HClO_4$ (final concn.) and, after centrifugation in an Eppendorf microfuge, the resulting supernatant was analysed for calcium in the presence of $SrCl_2$ (0.2% final concn.) and KCl (0.1% final concn.). Bile flow was measured by weighing the bile fluid collected for each 1 min, assuming that 1 ml is equivalent to 1 mg wet wt. Calculations carried out to determine the relative lag time in each of the perfusate and bile-flow cannulae showed that these generally were within 5–10 s of each other, but that with cholestatic agents bile flow could lag by up to 40 s.

Chemicals and materials

Hormones were obtained from Sigma Chemical Co., St Louis, MO, U.S.A. G-UDCA and T-CDCA were gifts from Tokyo Tanabe Pharmaceutical Co., Tokyo, Japan. These agents were dissolved in Krebs–Henseleit bicarbonate buffer before infusion into the liver. For T-CDCA, a stock solution was first made up in 100% dimethyl sulphoxide. The final concentration of dimethyl sulphoxide in the perfusate did not exceed 0.03%; this concentration of dimethyl sulphoxide alone did not have any effect on any of the metabolic parameters studied in this paper (results not shown). The infusion rate of the bile salts was of the order of 10 μmol/min per kg body wt. (see Drew & Priestly, 1979). Ca^{2+}-selective electrode membranes (F2112) and filling solutions (S43316) were obtained from Radiometer, Copenhagen, Denmark. Other chemicals used were of analytical-reagent grade.

Expression of data

All experiments were performed at least three times. Where indicated, data are expressed as means ± S.E.M. for the numbers of independent experiments described.

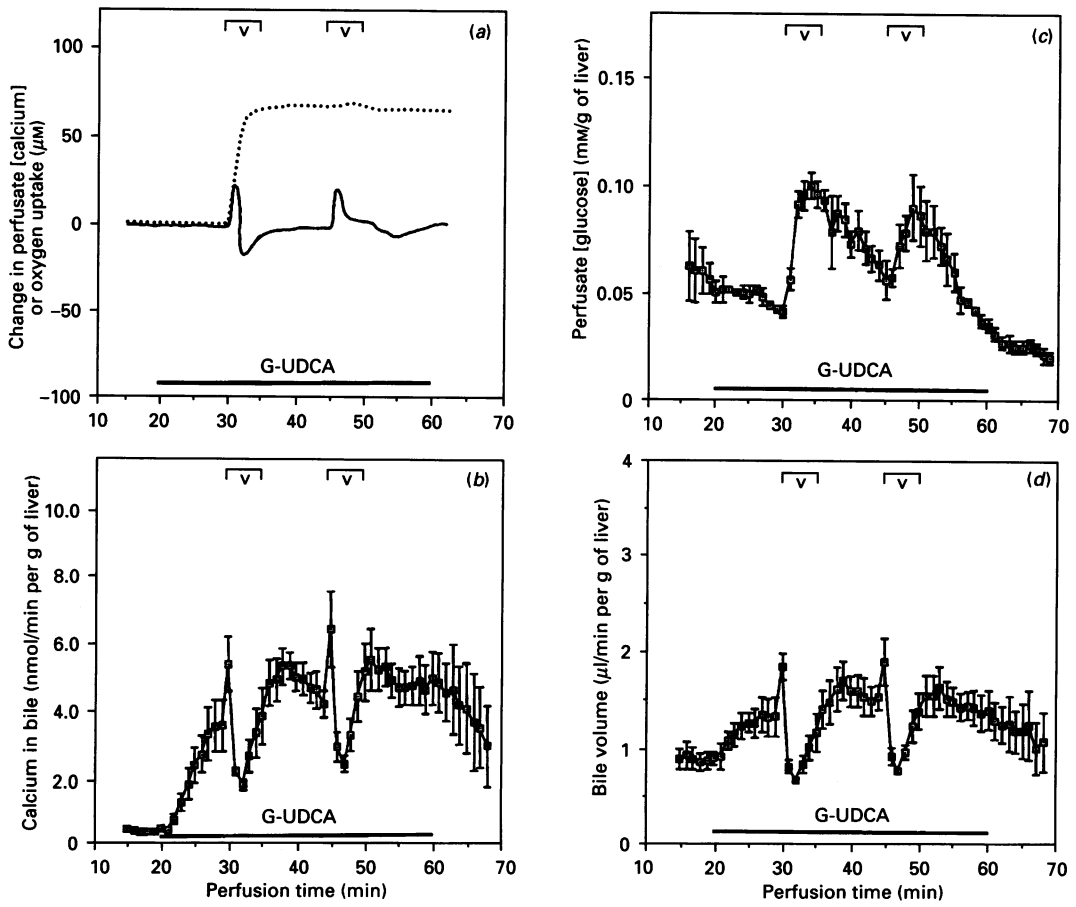

Fig. 2. Augmentation by G-UDCA of vasopressin-induced changes in perfusate Ca^{2+}, glucose output and oxygen uptake and on bile calcium and bile flow in the perfused rat liver

Livers were perfused with Krebs–Henseleit bicarbonate medium containing 1.3 mM-Ca^{2+}. After a pre-perfusion period of 20 min, G-UDCA (100 μM) was infused for 40 min, as indicated by the horizontal bar. Vasopressin (V; 10 nM) was infused twice each for 5 min beginning at times 30 min and 45 min. The Ca^{2+}-selective-electrode trace (——) and oxygen-electrode trace (· · · · · ·) are shown in (*a*); the concentration of calcium in the bile determined by atomic-absorption spectroscopy is shown in (*b*); perfusate glucose output is shown in (*c*); bile flow is shown in (*d*). For further details see the Experimental section. Each trace is a representative of at least four experiments performed independently. The data in (*b*), (*c*) and (*d*) are means ± S.E.M. of four independent experiments.

RESULTS

Effect of bile salts on basal perfusate Ca^{2+}, glucose output and oxygen uptake and on biliary flow and calcium content

An initial set of experiments was undertaken to determine whether the bile salts used in this work influenced the basal metabolic parameters under investigation. Data in Fig. 1 show that the principal effect of G-UDCA is to promote the secretion of calcium in the bile (Fig. 1*b*). The rate of secretion increases markedly, by some 7-fold, especially over the initial 10 min of bile-salt infusion; thereafter the rate levels off, except for a relatively minor fluctuation at 50–60 min. The concentration of calcium in the bile increased from the basal of approx. 0.6 mM (see Hamada *et al.*, 1992) to a maximum of the order of 3.5 mM (results not shown). Cessation of bile-salt infusion results in a rapid diminution in the rate of biliary calcium secretion. Fig. 1(*d*) shows that, at the concentration of G-UDCA used in this work, bile flow is increased by little more than 50%. Perfusate Ca^{2+} fluxes and oxygen uptake (Fig. 1*a*) are unaffected by G-UDCA infusion, as is glucose output (Fig. 1*c*).

The effect of T-CDCA on these same parameters is also shown in Fig. 1. This bile salt produces actions that are different from those of G-UDCA. First, T-CDCA produces a small but discernible increase in perfusate Ca^{2+} efflux and induces a gradual increase in oxygen output by the liver during the initial 10–15 min of its infusion. Second, T-CDCA induces a significant increase in perfusate glucose over the initial 10 min. Third, there is little significant change in bile calcium; if anything, only a slight fall occurs after approx. 15–20 min of T-CDCA infusion. Finally, and consistent with this last point, there appears to be a gradual attenuation of bile flow that becomes more evident as the infusion of the bile salt continues. The concentration of calcium in the bile increased by approx. 0.7 mM during the first 10 min of T-CDCA infusion and thereafter remained at a constant value (results not shown).

Effect of G-UDCA on vasopressin-induced perfusate Ca^{2+}, glucose output and oxygen uptake and on biliary flow and calcium content

Data in Fig. 2 show the effect of G-UDCA on the metabolic parameters examined in Fig. 1 after the administration of two 5 min pulses of vasopressin to the perfused rat liver. The oxygen response (Fig. 2*a*) is not very different from that seen in the absence of the bile salt (e.g. Altin & Bygrave, 1985; Hamada *et al.*, 1992), but the pattern of perfusate Ca^{2+} fluxes differs in two major respects. The first is seen after the initial pulse of the hormone, where Ca^{2+} efflux is quickly followed by a spontaneous influx of the ion, and then a second, more gradual, efflux. The

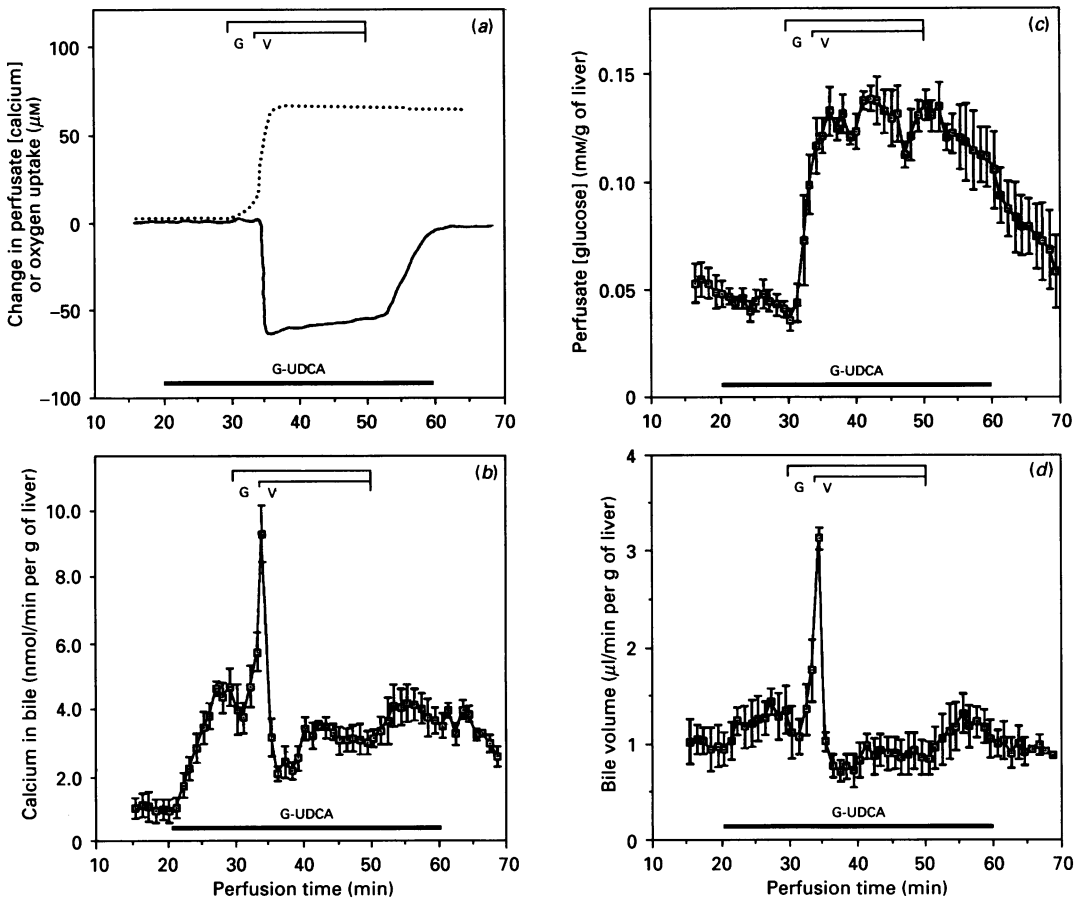

Fig. 3. Augmentation by G-UDCA of the synergistic action of glucagon and vasopressin on perfusate Ca^{2+}, glucose output and oxygen uptake and on bile calcium and bile flow in the perfused rat liver

The experimental procedure was as described in Fig. 2, except that glucagon (G; 10 nM) infusion commenced at time 30 min, and 4 min later vasopressin (V; 10 nM) infusion commenced. The infusion of the hormones was terminated at time 50 min as indicated. The Ca^{2+}-selective-electrode trace (——) and oxygen-electrode trace (· · · · · ·) are shown in (*a*). Each trace is a representative of at least four experiments performed independently. The data in (*b*), (*c*) and (*d*) are means ± S.E.M. of four independent experiments.

extent to which this latter efflux occurs here is considerably attenuated as compared with the case without added bile salt (cf. Hamada *et al.*, 1992). The second feature of perfusate Ca^{2+} fluxes altered by the presence of G-UDCA is that vasopressin, in the second pulse, induces a phenylephrine-like response (see Altin & Bygrave, 1985), in that the cessation of vasopressin infusion (at 50 min) induces a slight but reproducible influx of the ion. The response of glucose output to vasopressin (Fig. 2*c*) is not changed by the prior administration of G-UDCA.

Figs. 2(*b*) and 2(*d*) show the changes in biliary calcium and in bile flow after vasopressin administration while G-UDCA is concomitantly infused. Although the pattern of response is qualitatively similar to that seen in the absence of G-UDCA (Hamada *et al.*, 1992), each of the vasopressin-induced transients is considerably augmented and 'sharper' in the present experiments. This is reflected first in the magnitude of the transients, second in the shorter refractory period at the trough of the transients and third in the fact that the second vasopressin pulse also induces a transient. Since, during each of the two transients, both the flow of bile and the calcium secreted into the bile increased virtually simultaneously, the calcium concentration in the bile did not change significantly (results not shown). The transient rise in bile flow reported here was observed also by Hardison *et al.* (1991), but in the presence of either taurocholate or taurodehydrocholate. Their data did not indicate whether the transient occurred in the absence of these bile salts.

Effect of G-UDCA on glucagon- plus vasopressin-induced perfusate Ca^{2+}, glucose output and oxygen uptake and on biliary flow and calcium content

The experiments undertaken in Fig. 2 were repeated, but under conditions where glucagon was first administered to the liver, followed soon after by vasopressin infusion (Altin & Bygrave, 1986). Figs. 3(*a*) and 3(*c*) show the changes occurring in perfusate Ca^{2+}, oxygen and glucose. Glucagon administration induces a slight Ca^{2+} efflux and little oxygen uptake, but a large glucose output (Fig. 3*c*). Immediately after the co-administration of vasopressin, the typical very slight Ca^{2+} efflux followed by the pronounced and prolonged Ca^{2+} influx is observed. The normal Ca^{2+} flux pattern thus is modified by the presence of G-UDCA (Fig. 3*a*) in two main respects. First, the maximal rate of influx is maintained for as long as the hormones are administered; in the absence of the bile salt, the rate of Ca^{2+} influx is considerably more attenuated (Altin & Bygrave, 1986; Hamada *et al.*, 1992). The second point is that the rate at which net Ca^{2+} influx returns to zero is slower here and no efflux of the ion occurs, as is always observed in the absence of the bile salt (cf. Altin & Bygrave, 1986).

The concomitant changes in biliary calcium content and secretion are shown in Figs. 3(*b*) and 3(*d*). Immediately after the administration of glucagon there occurs a small but discernible decline in both parameters. However, the most striking finding is

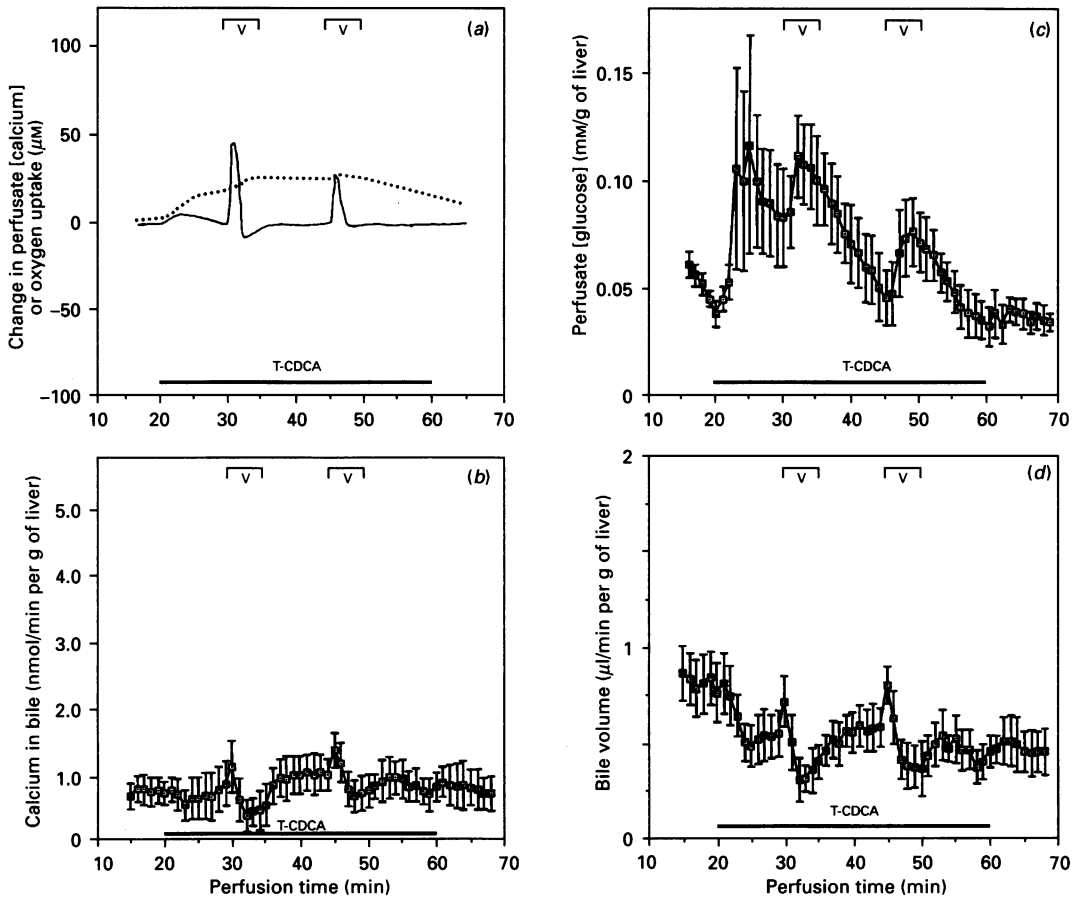

Fig. 4. Attenuation by T-CDCA of vasopressin-induced changes in perfusate Ca^{2+}, glucose output and oxygen uptake and on bile calcium and bile flow in the perfused rat liver

The experimental procedure was as described in Fig. 2, except that T-CDCA (100 μM) was infused in place of G-UDCA. The Ca^{2+}-selective-electrode trace (——) and oxygen-electrode trace (· · · · · ·) are shown in (*a*). Each trace is a representative of at least four experiments performed independently. The data in (*b*), (*c*) and (*d*) are means ± S.E.M. of four independent experiments.

that, immediately after vasopressin co-administration, there occurs an even sharper transient and shorter refractory period in both calcium secretion (Fig. 3*b*) and bile flow (Fig. 3*d*) than that seen when G-UDCA is absent (cf. Hamada *et al.*, 1992). Under the present conditions, the rate of calcium secretion was augmented from approx. 5 to 9 nmol/min per g of liver, as compared with an augmentation from approx. 0.5 to 1.5 nmol/min per g of liver in the absence of the bile salt. The concentration of calcium in the bile was not significantly altered by the action of the hormones in these experiments (results not shown). The flow of bile stimulated by the combined actions of vasopressin and glucagon in these experiments was approx. 50% greater than that occurring in the absence of G-UDCA (cf. Hamada *et al.*, 1992). It should be noted that the stimulatory action of glucagon contrasts with the attenuating action of this hormone on the initial phase of vasopressin-induced Ca^{2+} efflux in the perfusate. A second important action of the glucagon is to offset partially, after this sharp transient, the vasopressin-induced decrease in bile secretion and biliary calcium (Figs. 3*b* and 3*d*).

Effect of T-CDCA on vasopressin-induced perfusate Ca^{2+}, glucose output and oxygen uptake and on biliary flow and calcium content

Data in Fig. 4 show the effect of T-CDCA on the metabolic parameters examined in Fig. 1 after the administration of two 5 min pulses of vasopressin to the perfused rat liver. Oxygen uptake (Fig. 4*a*) induced by vasopressin is markedly attenuated, and the pattern of perfusate Ca^{2+} fluxes is quite different from that seen in the presence of G-UDCA in the following respects. First, a greater Ca^{2+} efflux is observed, especially after the initial pulse of the hormone; second, little discenible Ca^{2+} influx follows; third, the second pulse of the hormone induces only Ca^{2+} efflux. Outside of the effects of T-CDCA seen in the control experiments (Fig. 1*c*), the response of glucose output to the initial pulse of vasopressin is not changed by the prior administration of the bile salt (Fig. 4*c*); however, the response to the second pulse of vasopressin appears to be slightly attenuated compared with the effects of G-UDCA (cf. Fig. 2*c*).

Figs. 4(*b*) and 4(*d*) clearly indicate the attenuation of the vasopressin-induced transients in biliary calcium and bile flow that occur after the prior infusion of T-CDCA to the perfused liver. These attenuated responses contrast with the augmented responses induced by G-UDCA infusion (cf. Figs. 2*b* and 2*d*).

Effect of T-CDCA on glucagon- plus vasopressin-induced perfusate Ca^{2+}, glucose output and oxygen uptake and on biliary flow and calcium content

Fig. 5 shows the results of experiments carried out identical with those in Fig. 3 except that T-CDCA instead of G-UDCA was infused for 40 min. In respect of changes occurring in perfusate Ca^{2+}, glucose output and oxygen uptake (Figs. 5*a* and 5*c*), three things are evident. First is the marked attenuation of

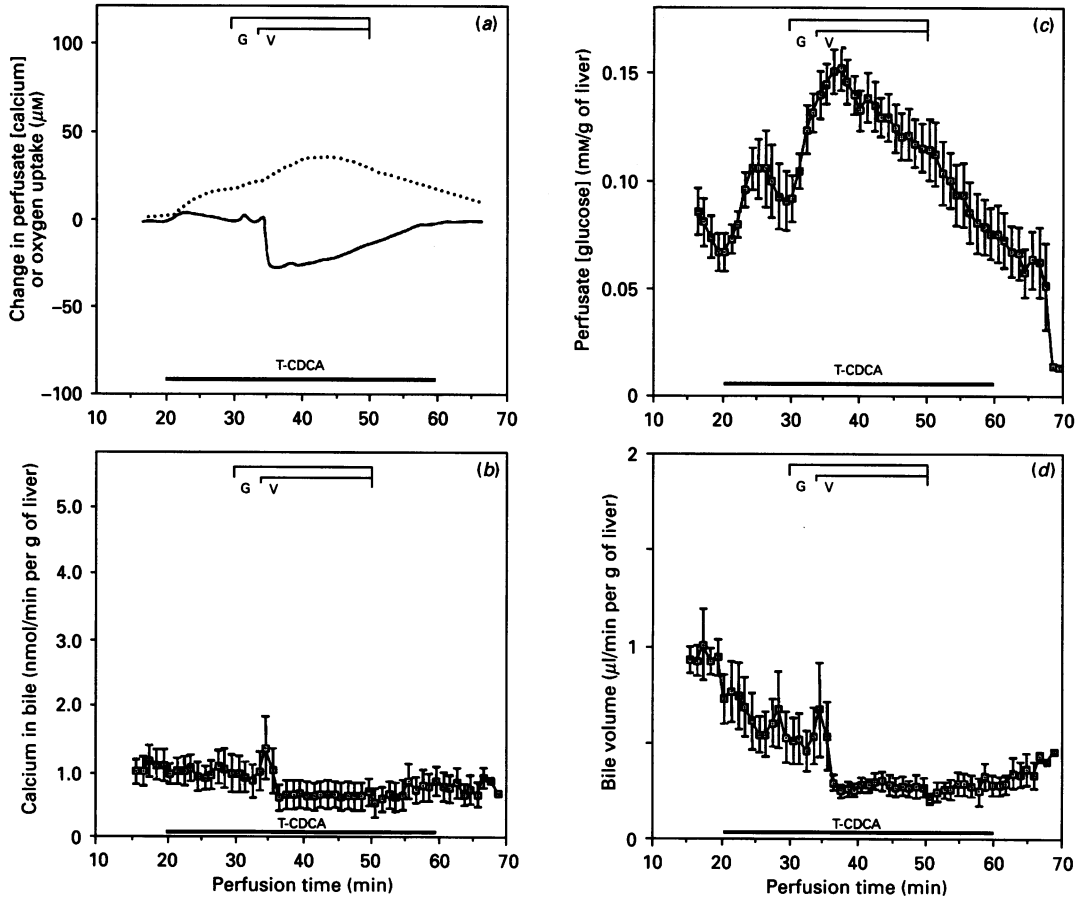

Fig. 5. Attenuation by T-CDCA of the synergistic action of glucagon and vasopressin on perfusate Ca^{2+}, glucose output and oxygen uptake and on bile calcium and bile flow in the perfused rat liver

The experimental procedure was as described in Fig. 3, except that T-CDCA (100 μM) was infused instead of G-UDCA. The Ca^{2+}-selective-electrode trace (——) and oxygen-electrode trace (· · · · · ·) are shown in (*a*). Each trace is a representative of at least four experiments performed independently. The data in (*b*), (*c*) and (*d*) are means ± S.E.M. of four independent experiments.

Ca^{2+} influx that would have occurred immediately after the infusion of vasopressin; second, there is little vasopressin-induced increase in oxygen uptake; third, glucose output begins to decline from a peak at around 35–40 min, or only 2–3 min after vasopressin infusion (cf. Fig. 5*c* with Fig. 3*c*).

The concomitant changes in biliary calcium content and secretion are shown in Figs. 5(*b*) and 5(*d*). It is apparent that, as was the case in the previous set of experiments when vasopressin alone was administered, the prior administration of T-CDCA prevents the manifestation of the synergistic effects of glucagon plus vasopressin in inducing the very sharp transients. If anything, the effects of these hormones under these conditions are to attenuate even further biliary calcium secretion and bile flow.

In other experiments (results not shown) we observed that, taking account of the effects of the bile salts alone, the effects of 5 min pulses of glucagon on the parameters measured here were not significantly different from those seen when the hormone was administered in the absence of the bile salts (Hamada *et al.*, 1992).

DISCUSSION

In an earlier paper (Hamada *et al.*, 1992), we reported that the co-administration of glucagon and vasopressin to the perfused rat liver rapidly induces (i.e. in less than 1 min) a 'transient' or pulse in biliary calcium and biliary flow. In the present paper we have shown that this transient can be modified markedly by the acute administration of either choleretic or cholestatic bile salts. The data clearly indicate that the choleretic bile salt G-UDCA both augments the peak height and shortens the duration of this transient. The cholestatic bile salt T-CDCA, by contrast, practically obliterates the peak, but does not impede the subsequent vasopressin-induced attenuation of bile flow.

Comparison of data in Fig. 1(*b*) with 1(*d*) and Fig. 3(*b*) with 3(*d*) reveals that, before and during the hormone-induced transient, G-UDCA increases bile calcium to a much greater extent than it increases bile flow. Also, the concentration of calcium in the bile increases several-fold over this time. Thus in addition to inducing choleresis, G-UDCA also appears to have a specific effect on stimulating output of bile calcium. We are led to conclude from this that, although the increase in hormone-induced biliary flow can be attributable to the choleretic effect of G-UDCA, at the same time some additional mechanism(s) must contribute to the increase in hormone-induced transient in biliary calcium that follows administration of G-UDCA.

Our data reveal that, besides their effects on biliary output, these bile salts at the same time induce quite large changes in the pattern of hormone-induced perfusate Ca^{2+} fluxes. The changes are particularly evident after the co-administration of glucagon and vasopressin, conditions wherein a synergistic action on Ca^{2+} influx is known to occur (e.g. Morgan *et al.*, 1983; Mauger &

Claret, 1988; Altin & Bygrave, 1988). Thus, and concomitant with the biliary changes mentioned above, we see a marked increase in hormone-induced Ca^{2+} influx into the liver after G-UDCA administration (Fig. 3) and marked attenuation of these same fluxes after T-CDCA administration (Fig. 5).

By contrast with their different actions on perfusate Ca^{2+} fluxes, biliary flow and biliary calcium, these two bile salts have little effect on glucose output apart from the slight enhancing effect of T-CDCA alone (Fig. 1*c*); this enhancement can be correlated with a slight mobilization of perfusate Ca^{2+} and enhanced oxygen uptake (Fig. 1*a*). Thus, on the one hand G-UDCA and T-CDCA have different modes of action on perfusate and biliary calcium fluxes and on bile flow, yet on the other, little effect on glucose output.

As mentioned in the Introduction, the ability of bile salts to influence hormone-induced Ca^{2+} mobilization in hepatocytes and the perfused rat liver has been investigated by several groups (Anwer *et al.*, 1989; Combettes *et al.*, 1990). A direct comparison of the present study with those reports unfortunately is not possible, because none of the bile salts used were common to both sets of research. In light of the rapidity of bile-salt action in our study (sensitization to either G-UDCA or T-CDCA occurs within 10 min), it is noteworthy, however, that taurolithocholate and taurolithocholate sulphate released 85% of the hepatocyte Ca^{2+} pool within 60 s (Combettes *et al.*, 1989) and that this pool was identical with that released by Ins(1,4,5)P_3. Of especial relevance to the present work was the finding by Combettes *et al.* (1989) that neither of these bile salts altered Ca^{2+} influx stimulated by the combined effects of glucagon plus vasopressin.

Lowe *et al.* (1988) have reported that Ca^{2+}-mobilizing hormones are able to increase rapidly the permeability of tight junctions, and they suggested that this represents a mechanism by which bile flow can be physiologically controlled. These authors also provided evidence that this mechanism involved a route of paracellular transport. This was based on the rapidity with which the hormone-induced events took place. Our results are not inconsistent with this concept; in particular, the events measured in the present work took place equally rapidly. The recent review by Nathanson & Boyer (1991) has summarized current views on the hormonal control of bile secretion. Central to those views are the relationships between the actions of Ca^{2+}-mobilizing hormones and adenylate cyclase and the second messengers produced therefrom. The proposed schema is that increased cytoplasmic Ca^{2+} concentration arising from the mobilization of intracellular and/or extracellular stores induces the contraction of pericanalicular microfilaments and increases the permeability of tight junctions. Increased cytoplasmic cyclic AMP is visualized as promoting bile-salt uptake across the sinusoidal membrane. What is not clear, however, from either our work or from any information in their schema is (i) how G-UDCA might promote an increase in biliary calcium independent of any choleretic effect (the concentration ratio G-UDCA/biliary calcium appears to change significantly along the time course, commencing at approx. 1:6 and presumably reaching approx. 1:20 after 10 min), and (ii) how glucagon and vasopressin act synergistically in promoting both the transient in bile function and the massive influx of Ca^{2+} into the perfused liver; for the latter, however, we have attempted to consider several possibilities (see, e.g., Altin & Bygrave, 1988).

The physicochemical properties of bile salts have been raised by others elsewhere in attempting to offer explanations for the action of these agents in hepatobiliary function (see, e.g., Anwer *et al.*, 1989; Heuman *et al.*, 1991; but see also Combettes *et al.*, 1989). Clearly the issue of the chemical structure of various bile salts and their ability to augment or attenuate the transients observed in this work will be worth further study.

We also could begin to think in terms of a mechanism(s) involving, for instance, a cyclic AMP-dependent phosphorylation that is able to promote the subsequent action of vasopressin, which in turn is known to promote secretory events occurring at the tight junctions (Hill *et al.*, 1985; Lowe *et al.*, 1988; Llopis *et al.*, 1991) and which also are now thought to involve Ca^{2+} (Kan & Coleman, 1988). It would be of interest to determine, therefore, if the potentiating action of glucagon on the Ca^{2+}-mobilizing hormone seen here has a similar action on the permeability of tight junctions.

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