RESEARCH COMMUNICATION

Involvement of microtubules in the swelling-induced stimulation of transcellular taurocholate transport in perfused rat liver

Dieter HÄUSSINGER,† Nirmalendu SAHA, Christian HALLBRUCKER, Florian LANG,* and Wolfgang GEROK

Medizinische Universitätsklinik, Hugstetterstrasse, D-7800 Freiburg, Germany and *Physiologisches Institut der Universität Tübingen, Tübingen, Germany

An increase of the hepatocellular hydratation state, induced by hypotonic exposure, amino acids or tauroursodeoxycholate, was shown to increase within minutes the $V_{max.}$ of transcellular taurocholate transport and excretion into bile [Häussinger, Hallbrucker, Saha, Lang and Gerok (1992) Biochem. J. **288**, 681–689]. This stimulatory effect of cell swelling on taurocholate excretion into bile is abolished in the presence of colchicine (5 μM). On the other hand, colchicine did not affect the stimulatory action of hypotonic cell swelling on $^{14}CO_2$ production from [1-^{14}C]glycine or [1-^{14}C]glucose. Likewise, volume regulatory K^+ fluxes following anisotonic exposure were not influenced in the presence of colchicine. Lumicolchicine (5 μM), a stereoisomer of colchicine without an inhibitory effect on microtubules, did not abolish the stimulation of taurocholate excretion into bile following hypo-osmotic exposure. Hypertonic cell shrinkage decreased taurocholate excretion into bile by about 35%; this effect was fully reversible upon normotonic re-exposure. With colchicine pretreatment, however, the hypertonicity-induced inhibition of taurocholate excretion was blunted and was no longer reversible upon normotonic re-exposure. The results suggest that stimulation of taurocholate excretion into bile in response to cell swelling involves a colchicine-sensitive, probably microtubule-dependent, mechanism, but not the stimulation of other cell-volume-sensitive pathways such as glycine oxidation or the pentose-phosphate shunt. It is hypothesized that the swelling-induced stimulation of taurocholate excretion into bile is due to a microtubule-dependent insertion of bile acid transporter molecules into the canalicular membrane.

INTRODUCTION

Alterations of the hepatocellular hydratation state were recently recognized as an important modulator of hepatocellular function (for reviews see Häussinger and Lang, 1991; Lang and Häussinger, 1993). Cell swelling, induced by either hypotonic exposure or addition of amino acids, insulin or tauroursodeoxycholate, increases the $V_{max.}$ of transcellular taurocholate transport in perfused rat liver within minutes, whereas hypertonic cell shrinkage decreases transport capacity (Hallbrucker et al., 1992; Häussinger et al., 1992a). However, the underlying mechanism remained unclear. Cell swelling leads to marked cytoskeletal alterations, such as a rapid polymerization of actin (Theodoropoulos et al., 1992) and an increase of cellular mRNA levels for β-actin (Schulz et al., 1991; Theodoropoulos et al., 1992) and tubulin (B. Stoll, C. Stournaras and D. Häussinger, unpublished work). Also in other cell types, evidence has been given for a rearrangement of cytoskeletal structures after anisotonic exposure (Cornet et al., 1987; Linshaw et al., 1992). Bile formation was repeatedly shown to be sensitive to inhibitors of cytoskeletal structures (for reviews see Erlinger, 1988; Sellinger and Boyer, 1990; Nathanson and Boyer, 1991; Boyer et al., 1992) and the existence of a vesicular bile acid transport mechanism in liver has been discussed repeatedly (Dubin et al., 1980; Kacich et al., 1983; Goldsmith et al., 1983; Suchy et al., 1983; Lamri et al., 1988; Crawford and Gollan, 1988; Crawford et al., 1988; Stolz et al., 1989; Aoyama et al., 1991; Dumont et al., 1991). Thus, we addressed the question whether the cell-volume-dependence of transcellular taurocholate transport in liver is sensitive to inhibitors of microtubule formation.

MATERIALS AND METHODS

Haemoglobin-free liver perfusion

Livers of male Wistar rats of 140–240 g body weight, fed *ad libitum* on stock diet (Altromin), were perfused *in situ* from the portal to the hepatic vein as described previously (Sies, 1978) without recirculation of the perfusate using the bicarbonate-buffered Krebs–Henseleit saline plus L-lactate (2.1 mM) and pyruvate (0.3 mM). The K^+ concentration in the influent was 5.9 mM; in normotonic perfusion experiments the osmolarity was 305 mosmol/l. Hypotonic (225 mosmol/l) and hypertonic (385 mosmol/l) conditions were achieved by removing or adding 40 mM NaCl from the perfusion fluid. The perfusion fluid was equilibrated with O_2/CO_2 (95:5, v/v). The temperature was 37 °C. The perfusion flow rate was 3.2–4.5 ml/min per g of liver and was constant to within 5% throughout each individual experiment.

Assays

Bile was sampled at 1 min intervals and bile flow was determined gravimetrically assuming a specific mass for secreted bile of 1 g/ml.

Taurocholate excretion into bile was calculated on the basis of the specific radioactivity of added [^{3}H]taurocholate (100 μM; 75 kBq/l) from the ^{3}H radioactivity recovered in bile by scintillation spectrometry. A similar approach was used to determine biliary excretion of added [^{3}H]colchicine (5 μM; approx. 100 kBq/l).

K^+ in the effluent perfusate was monitored continuously with

Abbreviation used: LDH, lactate dehydrogenase.

† To whom correspondence should be addressed.

a K^+-sensitive electrode (Radiometer, Munich, Germany). The electrode was calibrated by infusion of known amounts of KCl. Data on K^+ release were obtained by planimetric calculations of areas under curves. When present, a baseline drift was taken into account.

Portal perfusion pressure was routinely monitored with a pressure transducer (Hugo Sachs Electronics, Hugstetten).

$^{14}CO_2$ from [1-^{14}C]glucose and [1-^{14}C]glycine was determined as described previously (Häussinger et al., 1992b; Saha et al., 1992). In brief, perfusate containing [1-^{14}C]glucose (5 mM, 120 kBq/ml) or [1-^{14}C]glycine (0.3 mM, 75 kBq/ml) in influent, was pooled over 1 min periods and collected in conical stoppered flasks. $^{14}CO_2$ was liberated by acidification and trapped in phenylethylamine. Rates of $^{14}CO_2$ production were calculated from the specific radioactivity of influent glucose or glycine respectively. Results are given as means ± S.E.M. (n = number of perfusion experiments).

Materials

[1-^{14}C]Glycine, [1-^{14}C]glucose, [^{3}H]colchicine and [^{3}H]taurocholate were from Amersham (Frankfurt, Germany). Unlabelled taurocholate, tauroursodeoxycholate, lumicolchicine and colchicine were from Sigma (Munich, Germany). L-Lactic acid was from Roth (Karlsruhe, Germany). All other chemicals were from Merck (Darmstadt, Germany).

RESULTS

Effect of anisotonicity on colchicine handling by perfused rat liver

When [^{3}H]colchicine (5 μM) was infused to hypotonically (225 mosmol/l) perfused rat liver, corresponding to an influent dose of 19.7 ± 0.5 nmol/g per min, it took about 20 min until a steady-state release of radioactivity into bile and effluent perfusate was reached, indicating that colchicine was trapped inside the liver only during this period. After 20 min of [^{3}H]colchicine infusion, steady-state conditions were reached: 21 ± 3 % (n = 3) and 79 ± 3 % (n = 3) of the infused radiolabel were recovered in bile and effluent perfusate, respectively (Figure 1a). Thus, intracellular colchicine-binding sites became saturated apparently within 20 min under these conditions. When [^{3}H]colchicine (5 μM) was infused into hyperosmotically (385 mosmol/l) preshrunken livers, corresponding to an influent dose of 20.7 ± 0.5 nmol/g per min (Figure 1b), the kinetics of radiolabel release into effluent perfusate and bile were largely identical with that found during hypotonic perfusions; steady-state release rates were found after about 20 min, with 29 ± 1 % and 71 ± 1 % (n = 3) of the infused radiolabel being recovered in bile and effluent perfusate respectively. These results suggest that the perfusate osmolarity has no effect on the extent and time course of colchicine trapping inside the liver.

Effect of colchicine on taurocholate excretion into bile

As shown in Figure 2, and in line with previous data (Hallbrucker et al., 1992; Häussinger et al., 1992a), taurocholate (100 μM) excretion into bile was significantly higher in hypotonic (225 mosmol/l) than in normotonic (305 mosmol/l) perfusions. This effect was observed throughout an 80 min perfusion period (not shown, but for the constancy of taurocholate excretion over a 100 min normotonic perfusion period in the absence of colchicine; see Figure 3 below). When, however, colchicine (5 μM) was added to influent perfusate both bile flow (not shown) and taurocholate excretion into bile became progressively inhibited and, after a 45 min period of colchicine (5 μM) infusion, taurocholate excretion into bile was no longer different in normotonic and hypotonic perfusions (Figure 2). Interestingly, taurocholate excretion into bile dropped only slightly during the first 10–20 min of colchicine addition. In the light of the results in Figure 1, this observation may suggest that saturation of colchicine-binding sites in the liver is required for the cholestatic effect of this microtubule inhibitor. Hepatocellular integrity, as assessed by lactate dehydrogenase (LDH) release into effluent perfusate was only slightly affected by colchicine (5 μM). After a 50 min normotonic perfusion with taurocholate (100 μM), LDH activity in effluent perfusate was 6 ± 1 units/l (n = 6) and was 11 ± 1 units/l (n = 3) when colchicine (5 μM) was present between 20 and 50 min of taurocholate infusion. Corresponding

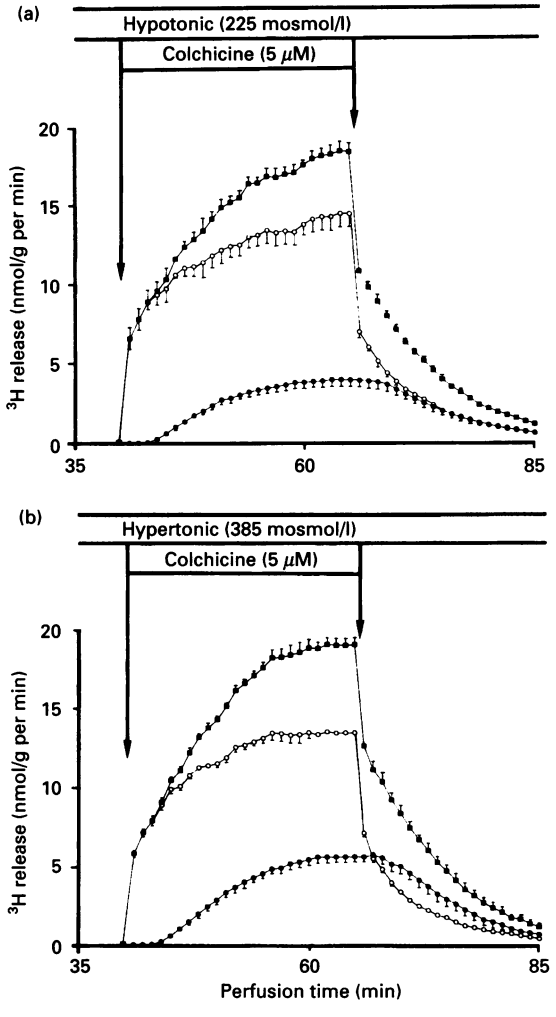

Figure 1 [^{3}H]Colchicine handling by hypotonically (225 mosmol/l) (a) and hypertonically (385 mosmol/l) (b) perfused rat liver

Livers were perfused with a hypotonic (225 mosmol/l) or hypertonic (385 mosmol/l) medium and [^{3}H]colchicine (5 μM) was added during the time period indicated. ^{3}H release was measured in effluent perfusate (○) and in bile (●); (■) refers to the sum of ^{3}H release into bile plus perfusate. [^{3}H]Colchicine addition to influent was approx. 20 nmol/g per min. Steady states are reached only after an infusion period of about 15–20 min with radioactivity in influent equalling that in bile plus effluent perfusate. No significant difference is seen between hypotonically and hypertonically perfused livers, suggesting that the perfusate tonicity is without effect on ^{3}H trapping in the liver. Results are given as means ± S.E.M. (n = 3 for each condition).

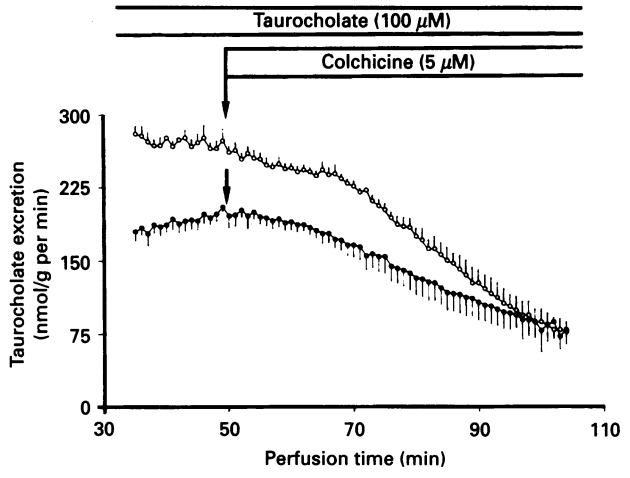

Figure 2 Effect of colchicine (5 μM) on taurocholate excretion into bile in normotonically (●) and hypotonically perfused (○) rat livers

Livers were perfused with a medium containing 100 μM [^{3}H]taurocholate and [^{3}H]taurocholate excretion into bile was determined. In line with previous results (Hallbrucker et al., 1992; Häussinger et al., 1992a) taurocholate excretion into bile was significantly higher in hypotonically (○) than in hypertonically (●) perfused livers. In control experiments, i.e. without colchicine addition, taurocholate excretion into bile was constant over a 100 min period (not shown, but see Figures 3–5). Addition of colchicine had little effect on taurocholate excretion during the first 15–20 min, but inhibits taurocholate excretion progressively thereafter. After a 40 min infusion period of colchicine, taurocholate excretion into bile is no longer different between normotonic and hypotonic perfusions. Results are given as means ± S.E.M. ($n = 3$ for each condition).

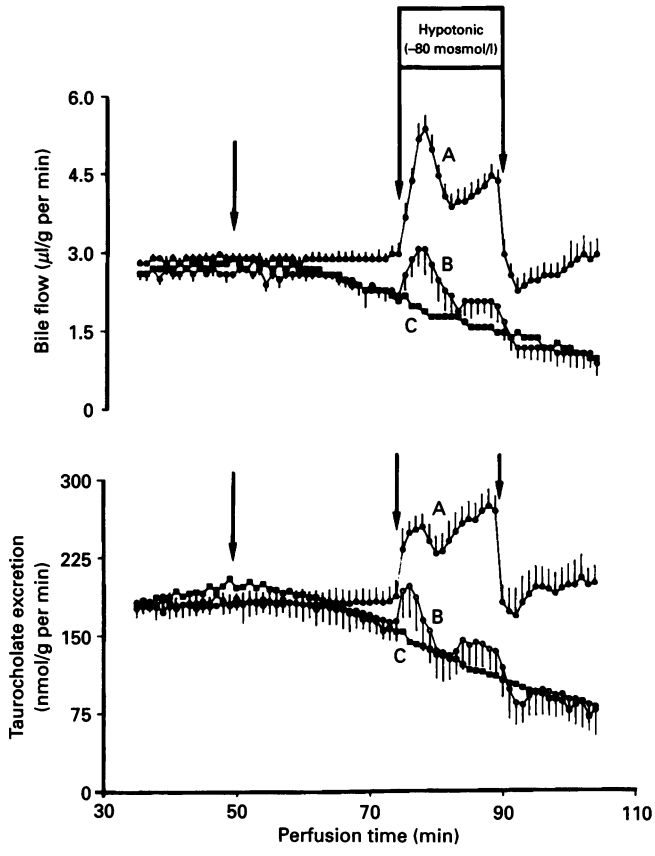

Figure 3 Effect of hypotonic (225 mosmol/l) exposure on bile flow and taurocholate excretion into bile in the absence and presence of colchicine (5 μM)

Livers were perfused with a medium containing taurocholate (100 μM) and hypotonic perfusion conditions were instituted from 76–90 min of perfusion. With colchicine absent, hypotonic exposure increases taurocholate excretion into bile (A). With colchicine (5 μM) present (onset of colchicine infusion is indicated by the first arrow), the stimulatory effect of hypotonic exposure on taurocholate excretion is largely abolished (B). As for controls, results are given for taurocholate excretion during colchicine addition in experiments without hypotonic exposure (C); these results are taken from Figure 2 and are given without S.E.M. values for clarity (for statistics see results depicted in Figure 2). Results are given as means ± S.E.M. ($n = 3$ or 4 for each condition).

values for hypotonic (225 mosmol/l) perfusion were 7 ± 1 units/l ($n = 4$) and 10 ± 1 units/l ($n = 3$) respectively.

Colchicine had no effect on the portal perfusion pressure in both normotonic and hypotonic perfusions. In normotonic (and hypotonic) perfusions, the portal perfusion pressure was 3.0 ± 0.2 cmH_2O (2.6 ± 0.5 cmH_2O) ($n = 3$) and was 3.4 ± 0.3 cmH_2O (3.0 ± 0.6 cmH_2O) after a 60 min perfusion period with colchicine (5 μM). This suggests that the colchicine-induced cholestasis is not explained by an inhomogeneous perfusion of the liver parenchyma.

Colchicine abolishes the cell-swelling-induced stimulation of taurocholate excretion into bile

As shown in Figure 3, cell swelling due to hypotonic (225 mosmol/l) exposure stimulated taurocholate excretion into bile and bile flow, in line with previous results (Hallbrucker et al., 1992; Häussinger et al., 1992a). When, however, hypotonic exposure was instituted 20 min after onset of colchicine infusion, i.e. at a time point when taurocholate excretion started to decrease under the influence of colchicine in control experiments (Figures 2 and 3), the stimulatory effect of hypo-osmotic cell swelling on taurocholate excretion into bile was largely abolished. Here, hypo-osmotic exposure produced only a slight and transient stimulation of taurocholate excretion, which can be explained by an accelerated washout of taurocholate already present in the biliary tree due to an osmotic water shift into the biliary compartment. Also when cell swelling was induced in normotonic perfusions by addition of amino acids such as glutamine and glycine (2 mM each; Figure 4), taurocholate excretion into bile was increased in the absence of colchicine, but not when colchicine was added 20 min before. Interestingly, colchicine also markedly diminished the initial overshooting stimulation of taurocholate excretion into bile which is observed during the first 6 min of amino-acid addition. As shown recently, tauroursodeoxycholate at concentrations below 100 μM induces cell swelling when added in addition to taurocholate (100 μM) (Häussinger et al., 1992a), but not when added in the absence of taurocholate (C. Hallbrucker, S. vom Dahl and D. Häussinger, unpublished work). This tauroursodeoxycholate-induced cell swelling quantitatively explains the tauroursodeoxycholate-induced stimulation of taurocholate excretion into bile (Häussinger et al., 1992a). As shown in Figure 5, in the presence of colchicine (5 μM) the stimulatory action of tauroursodeoxycholate on taurocholate excretion was abolished.

The lack of effect of hypotonic exposure (Figure 3), amino acids (Figure 4) and tauroursodeoxycholate (Figure 5) on taurocholate excretion in the presence of colchicine is probably not explained by a colchicine-induced inhibition of cell swelling in response to these manoeuvres. This is suggested by the finding that neither the time courses (not shown) nor the extent (Table 1) of volume-regulatory K^+ fluxes, induced by hypotonic exposure

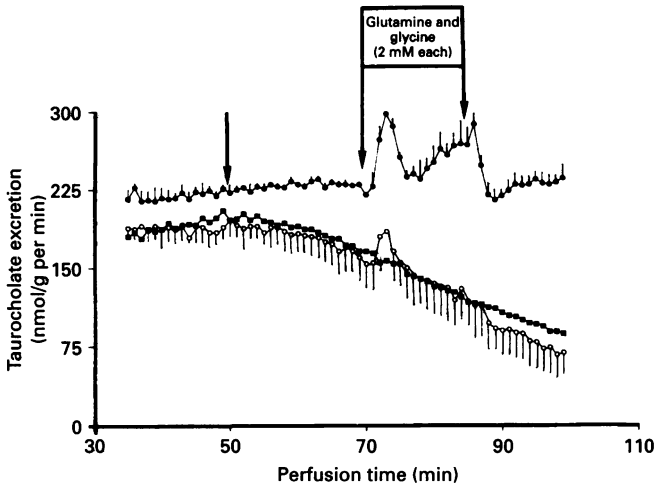

Figure 4 Effect of glutamine plus glycine (2 mM each)-induced cell swelling on taurocholate excretion into bile in the absence and presence of colchicine (5 μM)

Livers were perfused with a medium containing taurocholate (100 μM) and glutamine plus glycine (2 mM each) was added from 71–85 min of perfusion, in order to achieve isotonic cell swelling. With colchicine absent, addition of the amino acids increased taurocholate excretion into bile in a biphasic way (●). With colchicine (5 μM) present (onset of colchicine infusion is marked by first arrow), the stimulatory effect of glutamine plus glycine on taurocholate excretion was largely abolished (○). As for controls, results are given for taurocholate excretion during colchicine addition in experiments without amino-acid addition (■) (S.E.M. values have been omitted for clarity, but see results in Figure 2). Results are given as means ± S.E.M. (n = 3 or 4 for each condition).

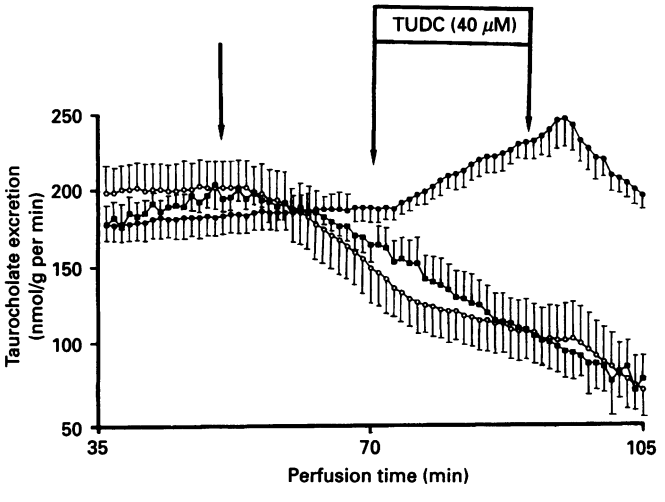

Figure 5 Effect of tauroursodeoxycholate (TUDC, 40 μM) on taurocholate excretion into bile in the absence and presence of colchicine (5 μM)

Livers were perfused with a medium containing taurocholate (100 μM) and tauroursodeoxycholate (TUDC, 40 μM) was added from 70–89 min of perfusion. With colchicine absent, tauroursodeoxycholate increases taurocholate excretion into bile (●). With colchicine (5 μM) present (onset of colchicine infusion is marked by first arrow), the stimulatory effect of tauroursodeoxycholate on taurocholate excretion is largely abolished (○). As for controls, results are given for taurocholate excretion during colchicine addition in experiments without tauroursodeoxycholate addition (■). Results are given as means ± S.E.M. (n = 3 or 4 for each condition).

Table 1 Effect of colchicine (5 μM) on the hypo-osmolarity-induced effects on $^{14}CO_2$ production from [1-^{14}C]glycine (0.3 mM) and [1-^{14}C]glucose (5 mM) and volume-regulatory K^+ efflux

Livers were perfused with a medium containing lactate (2.1 mM), pyruvate (0.3 mM) plus either [1-^{14}C]glycine (0.3 mM) or [1-^{14}C]glucose (5 mM). Hypotonic conditions (225 mosmol/l) were achieved by lowering the influent NaCl concentration by 40 mM. Colchicine (5 μM) was added to the perfusion medium 20–25 min before the onset of hypotonic conditions. Results are given as means ± S.E.M. (n = 3–10). Results on volume-regulatory K^+ fluxes were obtained in experiments without glucose or glycine in the medium. Results are given as means ± S.E.M. and are from 3–6 experiments for each condition.

Parameter	Control	Colchicine-treated (5 μM)
$^{14}CO_2$ production from [1-^{14}C]glycine (nmol/g per min)		
Normotonic	14.2 ± 0.7	18.9 ± 1.2
Hypotonic	26.5 ± 0.7	36.0 ± 1.3
Change after swelling (%)	+87 ± 11	+94 ± 7
$^{14}CO_2$ production from [1-^{14}C]glucose (nmol/g per min)		
Normotonic	15.7 ± 2.8	13.1 ± 0.4
Hypotonic	29.8 ± 5.9	22.8 ± 2.3
Change after swelling (%)	+88 ± 9	+73 ± 13
Volume-regulatory K^+ fluxes (μmol/g)		
Efflux after hypotonic exposure	12.7 ± 0.8	13.9 ± 0.5
Reuptake after normotonic re-exposure	11.5 ± 0.8	13.1 ± 1.0
Net efflux after glutamine/glycine addition (2 mM each)	2.1 ± 0.2	2.3 ± 0.2

or glutamine plus glycine, were affected by the presence of colchicine.

In order to rule out unspecific effects of colchicine on bile-acid secretion, the effect of lumicolchicine (5 μM), a stereoisomer of colchicine which has no effect on taurocholate and horseradish peroxidase excretion into bile and on microtubules (Wilson and Friedkin, 1966; Dubin et al., 1980; Bruck et al., 1992), was tested. Lumicolchicine had no effect on taurocholate excretion into bile and the stimulatory effect of hypotonic exposure on taurocholate excretion into bile was largely preserved. In normotonic control perfusions, taurocholate excretion into bile was 206 ± 8 nmol/g per min (n = 4) and was 213 ± 9 nmol/g per min (n = 4) after a 25 min period of lumicolchicine infusion. Hypotonic (225 mosmol/l) exposure stimulated taurocholate excretion into bile by 41 ± 3 % (n = 3) and 30 ± 8 % (n = 4) in the absence and presence of lumicolchicine (5 μM) respectively. These results suggest that the inhibition of the swelling-induced stimulation of taurocholate excretion into bile by colchicine (Figures 2–5) is due to its effect on microtubules.

Effect of colchicine on the inhibition of taurocholate excretion into bile after cell shrinkage

As shown recently, hypertonic (385 mosmol/l) cell shrinkage inhibits taurocholate excretion into bile (Hallbrucker et al., 1992; Häussinger et al., 1992a). A persistent (about 35 %) inhibition was also observed when lumicolchicine (5 μM) was added 20–25 min before hypertonic exposure; this effect was fully reversible upon normotonic re-exposure (Figure 6). When, however, colchicine (5 μM) was infused instead of lumicolchicine, the inhibitory effect of hypertonic cell shrinkage on taurocholate excretion into bile was smaller and was no longer reversible upon normotonic re-exposure.

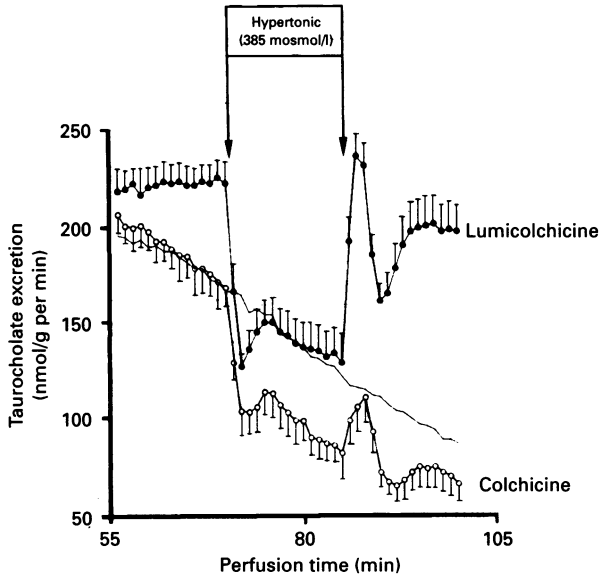

Figure 6 Effect of hypertonic exposure (385 mosmol/l) on taurocholate excretion into bile in the presence of lumicolchicine (5 μM; ●) or colchicine (5 μM; ○)

Livers were perfused with a medium containing taurocholate (100 μM) and hypertonic exposure was instituted from 71–85 min of perfusion. Colchicine (5 μM) or lumicolchicine (5 μM) was added to influent perfusate at 50 min of perfusion. In the presence of lumicolchicine, hypertonic exposure inhibited taurocholate excretion into bile (●), an effect which was fully reversible upon normotonic re-exposure. When, however, colchicine (5 μM) was added instead of lumicolchicine (○), the inhibitory effect of hypertonic exposure was diminished and was no longer reversible after normotonic re-exposure. The line (without symbols) refers to the effect of colchicine (5 μM) on taurocholate excretion with normotonic perfusion conditions throughout the experiment. This control curve (S.E.M. values omitted for clarity) is taken from Figure 2. Results are given as means ± S.E.M. ($n = 3$ or 4 for each condition).

Effect of colchicine on the swelling-induced stimulation of $^{14}CO_2$ production from [1-^{14}C]glycine and [1-^{14}C]glucose metabolism

Hypotonic cell swelling is known to stimulate $^{14}CO_2$ production from [1-^{14}C]glycine (Häussinger et al., 1992b) and [1-^{14}C]glucose (Saha et al., 1992). As shown in Table 1, addition of colchicine (5 μM) 20 min before hypotonic exposure did not significantly affect $^{14}CO_2$ production from [1-^{14}C]glycine and [1-^{14}C]glucose and its stimulation by hypotonic cell swelling. This suggests that microtubules are probably not involved in the swelling-induced stimulation of glycine oxidation (Häussinger et al., 1992b) or the swelling-induced stimulation of pentose-phosphate-cycle flux (Saha et al., 1992).

DISCUSSION

The results in this paper show that the recently reported cell volume effects on transcellular taurocholate transport (Hallbrucker et al., 1992; Häussinger et al., 1992a) are sensitive to colchicine, but not to lumicolchicine. This suggests a role for microtubules in bringing about the rapid increase of transcellular bile-acid transport capacity following increases in the cellular hydratation state. There is general agreement that the canalicular bile-acid secretion step, but not the sinusoidal bile-acid uptake process, is rate-controlling for transcellular bile-acid transport in liver (Erlinger, 1988; Sellinger and Boyer, 1990; Nathanson and Boyer, 1991; Boyer et al., 1992). Thus, alterations of taurocholate excretion into bile as reported here and elsewhere recently (Hallbrucker et al., 1992; Häussinger et al., 1992a) in response to cell swelling/shrinkage may reflect cell-volume effects on canalicular bile-acid secretion. The present findings support the previous speculation that cell swelling leads to a rapid insertion into the canalicular membrane of intracellularly stored bile-acid transporter molecules (Boyer et al., 1992; Häussinger et al., 1992a), which have been identified recently as primary active, i.e. ATP-dependent, transporters (Adachi et al., 1991; Müller et al., 1991; Nishida et al., 1991; Stieger et al., 1992). The currently available evidence for such a cell-volume control of short-term bile-acid-transporter insertion/retrieval in the canalicular membrane is as follows. (i) The $V_{max.}$ of taurocholate excretion into bile doubles within minutes following a 10–15% increase of the cellular hydratation state (Häussinger et al., 1992a), whereby effects on the known driving forces for canalicular bile-acid secretion can not provide a satisfactory explanation. Here, no significant effects of cell swelling on tissue levels of cellular ATP are detectable (Häussinger et al., 1990a) and cell swelling results after a transient hyperpolarization of the membrane potential in a persistent depolarization (F. Lang, M. Ritter and D. Häussinger, unpublished work obtained with micropuncture studies in isolated hepatocytes), which would even tend to diminish potential-driven taurocholate excretion. (ii) The swelling-induced stimulation of taurocholate excretion into bile is abolished by colchicine, an inhibitor of the microtubular system (this paper), and microtubules are known to be involved in vesicular transport. (iii) Cell swelling leads to a transient membrane flow from the cellular interior to the plasma membrane, suggestive of a stimulation of exocytosis during the first few minutes of hypotonic exposure (Pfaller et al., 1993). (iv) Sudden hypo-osmotic exposure stimulates transcellular vesicular transport of horseradish peroxidase in a colchicine-sensitive way (Bruck et al., 1992). The idea that membrane transport is regulated by insertion/retrieval of transporter molecules, as suggested here for hepatic bile-acid secretion, is not new. Evidence for such a mechanism has been given previously for glucose transport in adipocytes (for review see Pessin and Bell, 1992) and for H^+ transport in the kidney (for review see Burckhardt and Burckhardt, 1988).

In the past, evidence has been given repeatedly for a vesicular bile-acid transport facility (Dubin et al., 1980; Goldsmith et al., 1983; Kacich et al., 1983; Suchy et al., 1983; Crawford and Gollan, 1988; Crawford et al., 1988; Lamri et al., 1988; Stolz et al., 1989; Dumont et al., 1991; Aoyama et al., 1991), in part based on the electron-microscopic demonstration of bile-acid-containing vesicles inside hepatocytes (Suchy et al., 1983; Lamri et al., 1988). Here re-interpretation of established facts must be considered. The ATP-dependent bile-acid transporter in the canalicular membrane (Adachi et al., 1991; Müller et al., 1991; Nishida et al., 1991; Stieger et al., 1992) is probably, like other plasma membrane transport proteins, processed and synthesized in the Golgi apparatus and the endoplasmic reticulum before its targeting to the canalicular membrane. Thus, a considerable fraction of these transporter molecules may be present in intracellular vesicles. Assuming the carrier to be active in these vesicles, it will not be surprising that bile acids will accumulate in their lumens. In view of our hypothesis, the primary function of these vesicles, however, is to transport the canalicular transporter rather than to transport bile acids. Here, cell swelling apparently acts as a signal to the target and inserts these transporter-containing vesicles within minutes into the canalicular membrane. Such a hypothesis would predict that during the swelling-induced fusion process the bile acids concentrated inside the vesicles will be poured out into the canalicular lumen in a colchicine-sensitive

way. Indeed, a biphasic stimulation of taurocholate excretion is observed after isotonic cell swelling by glutamine plus glycine (Figure 4). There is an initial overshooting stimulation of taurocholate excretion during the first 6 min of glutamine plus glycine addition; i.e. the time period required for maximal amino-acid-induced swelling (Häussinger et al., 1990b) (Figure 4). This transient overshoot is followed by a sustained phase of stimulated taurocholate excretion (Hallbrucker et al., 1992), which probably reflects the increased canalicular transport capacity resulting from the insertion of transporter molecules. Both phases are sensitive to colchicine inhibition. It should be emphasized that addition of glutamine and glycine *per se* (i.e. in the absence of taurocholate) does not affect bile flow (Hallbrucker et al., 1992), ruling out the possibility that a choleretic effect of the amino acids themselves creates the first peak of taurocholate excretion due to a washout of the biliary tree (as it occurs after hypotonic exposure). That such an initially overshooting taurocholate excretion does not occur under the influence of tauroursodeoxycholate (Figure 5) could be explained by a slower time course of tauroursodeoxycholate-induced cell swelling.

Theoretically one might predict from the above hypothesis that inhibition of taurocholate excretion after cell shrinkage should also be sensitive to colchicine. However, hypertonic exposure in the presence of colchicine was effective, albeit to a smaller extent than in the presence of lumicolchicine, and without reversibility (Figure 6). This could reflect differences in colchicine sensitivity for endo- and exo-cytosis respectively. Interestingly, the stimulatory effect of cell swelling on mitochondrial glycine oxidation and the pentose-phosphate-cycle flux was not affected by colchicine. This suggests that mechanisms unrelated to microtubule alterations bring about these responses. On the other hand, the cytoskeleton was suggested to be involved in the regulation of protein turnover (Hesketh and Pryme, 1991). Here, the interesting question arises as to what extent cytoskeletal alterations are involved in mediating the cell-volume effects on proteolysis (Häussinger et al., 1991) and protein synthesis (Stoll et al., 1992).

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