Cell volume and bile acid excretion

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The interaction between cell volume and taurocholate excretion into bile was studied in isolated perfused rat liver. Cell swelling due to hypo-osmotic exposure, addition of amino acids or insulin stimulated taurocholate excretion into bile and bile flow, whereas hyperosmotic cell shrinkage inhibited these. These effects were explained by changes in $V_{max.}$ of taurocholate excretion into bile: $V_{max.}$ increased from about 300 to 700 nmol/min per g after cell swelling by 12–15 % caused by either hypo-osmotic exposure or addition of amino acids under normo-osmotic conditions. Steady-state taurocholate excretion into bile was not affected when the influent K^+ concentration was increased from 6 to 46 mM or decreased to 1 mM with iso-osmoticity being maintained by corresponding changes in the influent Na^+ concentration. Replacement of 40 mM-NaCl by 80 mM-sucrose decreased taurocholate excretion into bile by about 70 %; subsequent hypo-osmotic exposure by omission of sucrose increased taurocholate excretion to 160 %. Only minor, statistically insignficant, effects of aniso-osmotic cell volume changes on the appearance of bolus-injected horseradish peroxidase in bile were observed. Taurocholate (400 μM) exhibited a cholestatic effect during hyperosmotic cell shrinkage, but not during hypo-osmotic cell swelling. Both taurocholate and tauroursodeoxycholate increased liver cell volume. Tauroursodeoxycholate stimulated taurocholate (100 μM) excretion into bile. This stimulatory effect was strongly dependent on the extent of tauroursodeoxycholate-induced cell swelling. During continuous infusion of taurocholate (100 μM) further addition of tauroursodeoxycholate at concentrations of 20, 50 and 100 μM increased cell volume by 10, 8 and 2 % respectively, in parallel with a stimulation of taurocholate excretion into bile by 29, 27 and 9 % respectively. There was a close relationship between the extent of cell volume changes and taurocholate excretion into bile, regardless of whether cell volume was modified by tauroursodeoxycholate, amino acids or aniso-osmotic exposure. The data suggest that: (i) liver cell volume is one important factor determining bile flow and biliary taurocholate excretion; (ii) swelling-induced stimulation of taurocholate excretion into bile is probably not explained by alterations of the membrane potential; (iii) bile acids modulate liver cell volume; (iv) taurocholate-induced cholostasis may depend on cell volume; (v) stimulation of taurocholate excretion into bile by tauroursodeoxycholate can largely be explained by tauroursodeoxycholate-induced cell swelling.

INTRODUCTION

Liver cell volume is an important determinant of metabolic liver cell function. Several long-known, but mechanistically unclear, hormone and amino acid effects on hepatic metabolism found recently an explanation in hormone- and amino acid-induced cell volume changes (for review, see Häussinger & Lang, 1991a). Accordingly, cell volume changes were suggested as a 'second messenger' of hormonal and amino acid control of metabolism (Häussinger et al., 1991; Häussinger & Lang, 1991a,b). Previous studies have indicated that aniso-osmotic cell volume changes also affect bile formation and taurocholate excretion into bile (Chenderovitch et al., 1963; Haddad et al., 1989; Hallbrucker et al., 1992). In rat liver, taurocholate is taken up across the sinusoidal membrane by a Na^+-dependent transporter (Schwarz et al., 1975; Reichen & Paumgartner, 1976; Meier et al., 1984), whereas canalicular excretion is thought to be electrogenic (Meier et al., 1984; Weinmann et al., 1989) and/or to be accomplished by an ATP-dependent transport system (Adachi et al., 1991; Müller et al., 1991; Nishida et al., 1991; Stieger et al., 1992; for reviews see Erlinger, 1988; Sellinger & Boyer, 1990; Nathanson & Boyer, 1991; Boyer et al., 1992). Although the relative contributions of electrogenic and ATP-driven transport for canalicular bile acid secretion are not yet established, the canalicular secretion step is generally assumed to be rate-controlling in the overall process of taurocholate translocation from the sinusoidal into the canalicular compartment

(Erlinger, 1988; Sellinger & Boyer, 1990; Nathanson & Boyer, 1991; Boyer et al., 1992). When infused at higher doses, taurocholate exerts a cholestatic effect (Paumgartner et al., 1974; Herz et al., 1975; Hardison et al., 1981). Taurocholate-induced cholestasis can be prevented by simultaneous addition of tauro-ursodeoxycholate (Kitani & Kanai, 1982). The underlying mechanisms are not understood and are puzzling, because both conjugated bile acids are expected to share common transport mechanisms. Here, we have studied the interaction between liver cell volume and bile acid excretion. The data show that liver cell volume is an important determinant for biliary taurocholate excretion and possibly also for taurocholate-induced cholestasis, and that stimulation of taurocholate excretion by tauroursodeoxycholate can be explained by tauroursodeoxycholate-induced cell swelling.

MATERIALS AND METHODS

Liver perfusion

Livers from male Wistar rats (200–250 g body wt.), fed ad libitum on stock diet (Altromin), were perfused as described previously (Sies, 1978) in a non-recirculating manner with bicarbonate-buffered Krebs–Henseleit saline plus L-lactate (2.1 mM) and pyruvate (0.3 mM). If not indicated otherwise, the influent perfusate contained [³H]taurocholate (37 kBq/l) at a concentration of 100 μM. The influent K^+ concentration was 5.9 mM. In some experiments the K^+ concentration was changed

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to 45.9 mM or 0.9 mM, with iso-osmoticity being maintained by corresponding changes of the influent Na^+ concentration. The perfusate was gassed with O_2/CO_2 (19:1); the temperature was 37 °C. If not stated otherwise, the perfusate osmolarity was 305 mosM and aniso-osmotic perfusion conditions were achieved by corresponding changes of the NaCl concentration in influent. In some experiments, as indicated in the text, 40 mM-NaCl was replaced by 80 mM-sucrose, and hypo-osmotic perfusion conditions without altering the Na^+ concentration were achieved by omission of sucrose from the medium.

Determinations

Bile was sampled at 1 min intervals. Bile flow was determined from the secreted bile mass, assuming a specific mass of 1 g/ml. [^{3}H]Taurocholate excretion into bile was assessed by scintillation spectrometry of the ^{3}H radioactivity present in bile and on the basis of the specific radioactivity of [^{3}H]taurocholate in the influent perfusate.

The effluent K^+ concentration was continuously monitored with a K^+-sensitive electrode (Radiometer, Munich, Germany); net K^+ uptake or release was determined by planimetry of areas under curves and calibration was made by infusion of known amounts of KCl. When present, a baseline drift was taken into account.

The intracellular water space of perfused livers was determined by the [^{3}H]inulin/[^{14}C]urea wash-out technique described in detail by vom Dahl et al. (1991a,b). In brief, both radioisotopes were added to influent perfusate for about 5 min; i.e. a time period sufficient to achieve equilibration of [^{14}C]urea and of [^{3}H]inulin in their respective accessible water spaces. Equilibration was achieved when release of radioactivity into the effluent had reached a steady state. Then radioactivity infusion was stopped, and the effluent perfusate was collected during the following 3–5 min and assayed for ^{3}H and ^{14}C. From the effluent ^{3}H/^{14}C ratio found during steady-state infusion of radioactivity and that found during the wash-out period, [^{3}H]inulin and [^{14}C]urea spaces were calculated. The difference between these two spaces reflects a space which is accessible to added [^{14}C]urea but not to [^{3}H]inulin. This space was considered the intracellular water space ('cell volume'). When [^{3}H]water/[^{14}C]raffinose was used for determination of the intracellular water space instead of [^{14}C]urea/[^{3}H]inulin, almost identical values were obtained (vom Dahl et al., 1991a). From kinetic data on cellular inulin uptake by fluid-phase endocytosis in perfused rat liver as described by Scharschmidt et al. (1986), the error arising from inulin endo-cytosis during the 5 min infusion period is negligible, i.e. less than 0.7% of the intracellular water space. It should be noted that intracellular water-space measurements were only conducted in livers without taurocholate labelling. Accordingly, data on [^{3}H]taurocholate excretion and cell volume were obtained in different experimental series. The approach for measuring cell volume in the intact perfused rat liver allowed repeated determin-ations of the intracellular water space within the individual perfusion experiment. Cell volume changes under the influence of effectors were determined as the difference between two consecutive space measurements (time interval 30 min) in the individual experiment. Control experiments with repeated de-terminations of the intracellular water space in 30 min intervals revealed a spontaneous decrease in the water space of $2.4 \pm 0.3\%$ ($n = 6$) per 30 min of perfusion. The data given on aniso-osmotically and hormone-induced cell volume changes in this paper were corrected for this spontaneous decrease in liver cell volume.

Tight-junctional permeability was assessed as the rapid (within 6 min) appearance of horseradish peroxidase in bile after a bolus injection of horseradish peroxidase into influent perfusate (Lowe

et al., 1985; Hardison & Lowe, 1989; Hayakawa et al., 1990a,b; Llopis et al., 1991). In brief, 0.5 mg of horseradish peroxidase was injected into influent perfusate over a 30s period; bile samples were taken at 1 min intervals and assayed for horseradish peroxidase activity as described by Gallati & Pracht (1985). In these experiments taurocholate was present throughout the experiment at a concentration of 20 μM.

Statistics

Data from different perfusion experiments are given as means $\pm$ S.E.M. (no. of experiments). In the individual perfusion experiments, data on taurocholate excretion were obtained during metabolic and excretory steady states. Steady states of taurocholate excretion after effector addition were achieved within 15 min; steady-state rates of taurocholate excretion were determined between 16 and 20 min after effector institution.

Materials

[^{3}H]Taurocholate was from New England Nuclear (Dreieich, Germany), [^{3}H]inulin and [^{14}C]urea was from Amersham Buchler (Braunschweig, Germany). L-Lactic acid was from Roth (Karlsruhe, Germany). Insulin, horseradish peroxidase (type IV-A), taurocholate and tauroursodeoxycholate were from Sigma (Munich, Germany). All other chemicals were from Merck (Darmstadt, Germany).

RESULTS

Taurocholate excretion into bile during aniso-osmotic perfusion

As shown in Fig. 1, steady-state taurocholate excretion into bile as well as taurocholate-induced stimulation of bile flow were saturable processes under normo-osmotic conditions (305 mosM); maximal effects were observed at taurocholate concentrations above 100 μM in the influent perfusate. When similar titration studies were performed with livers perfused with a hypo-osmotic medium (225 mosM, obtained by lowering the perfusate NaCl concentration by 40 mM), taurocholate excretion into bile as well as taurocholate-induced stimulation of bile flow during steady states were markedly stimulated (Fig. 1). Double-reciprocal-plot analysis of the data in Fig. 1(a) yielded maximal rates of taurocholate excretion into bile for normo- and hypo-osmotic perfusions of 287 nmol/min per g ($n = 8$, linear re-gression coefficient $r = 0.994$) and 727 nmol/min per g ($n = 8$, $r = 0.999$) respectively. Corresponding $k_{0.5}$ values for portal taurocholate additions were 252 and 712 nmol/min per g, equivalent to taurocholate concentrations in influent perfusate of about 60 and 180 μM. The relationship between bile salt excretion and bile flow was linear for hypo-osmotic and normo-osmotic perfusions (Fig. 2); however, the amount of fluid secreted per μmol of taurocholate excreted was 9.26 μl and 7.0 μl for hypo-(225 mosM) and normo- (305 mosM) osmotic perfusions re-spectively. This is an expected finding, because taurocholate-induced choleresis is thought to be driven by the osmotic gradient (for reviews see Erlinger, 1988; Sellinger & Boyer, 1990), and lowering of the perfusate osmolarity from 305 to 225 mosM should therefore increase taurocholate-induced choleresis by 35%. This theoretical value closely agrees with the measured increase of 32%. Extrapolation of the data in Fig. 2 to zero taurocholate excretion yielded a so-called 'bile-acid-independent bile flow' of 1.43 and 1.40 μl/min per g for the hypo- and normo-osmotic conditions respectively, i.e. values closely matching bile flow rates determined in the absence of taurocholate infusion of 1.34 ± 0.6 ($n = 7$) and 1.25 ± 0.6 ($n = 8$) μl/min per g respectively. No cholestatic effect of taurocholate was observed in the tested concentration range (0–400 μM) during normo- or hypo-osmotic
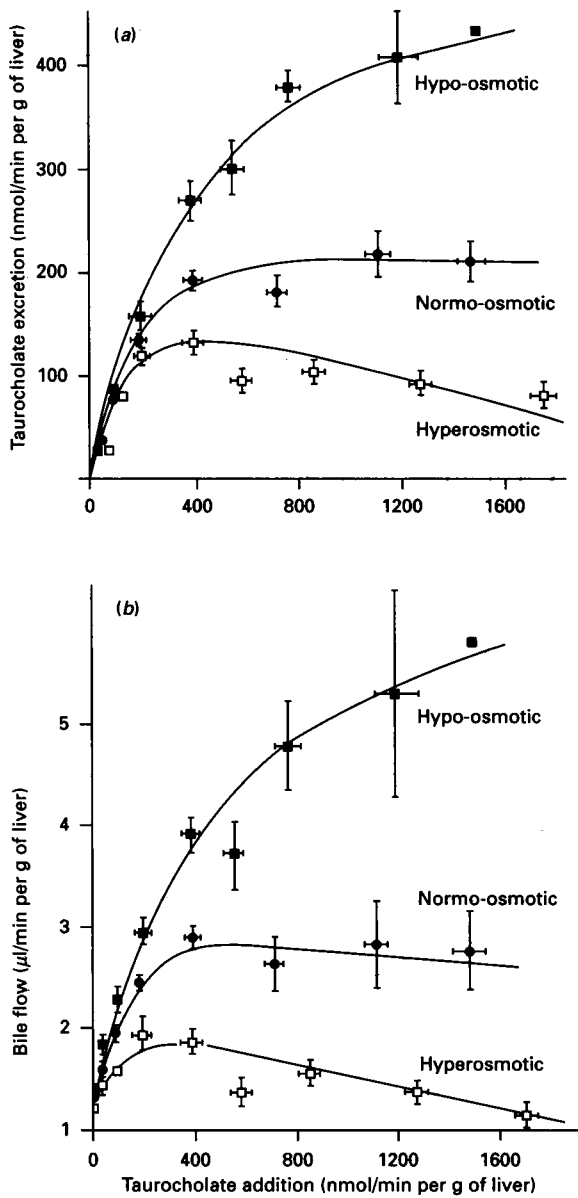
Fig. 1. Taurcholate excretion (a) and bile flow (b) in normo-osmotic (305 mosM), hypo-osmotic (225 mosM) and hyperosmotic (385 mosM) liver perfusions

Hypo-osmotic (225 mosM) and hyperosmotic (385 mosM) conditions were obtained by lowering or increasing the NaCl concentration in influent perfusate by 40 mM. Data were obtained during metabolic steady states and are given as means±S.E.M. (n = 3–10 different perfusion experiments). A taurocholate addition of about 400 nmol/min per g corresponds to a taurocholate concentration of 100 μM in the influent perfusate. In the absence of taurocholate, the bile flow was 1.26±0.04 (n = 12), 1.39±0.05 (n = 10) and 1.18±0.05 (n = 5) μl/min per g of liver in normo-, hypo- and hyper-osmotic perfusions respectively. A cholestatic effect of taurocholate is only seen during hyperosmotic exposure.
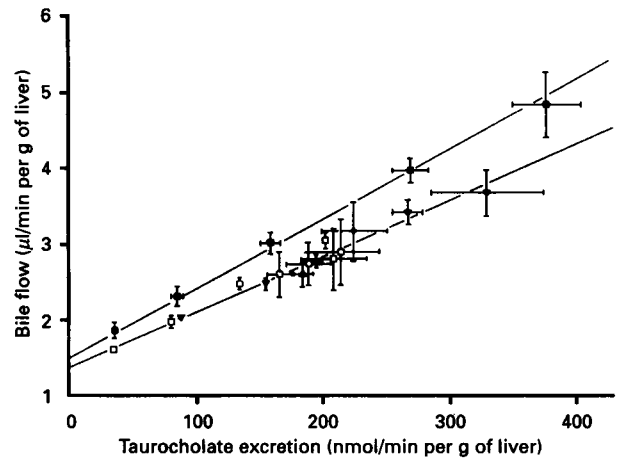

Fig. 2. Relationship between bile flow and taurocholate excretion into bile during normo-osmotic (305 mosM) and hypo-osmotic (225 mosM) perfusions

Taurocholate excretion into bile was varied by altering the taurocholate concentration in the influent perfusate between 10 and 400 μM during normo-osmotic (305 mosM) (□) and hypo-osmotic (225 mosM) perfusions (●). In addition, for the normo-osmotic condition, data are included from experiments in which taurocholate excretion into bile was modified by further addition of amino acids, such as glutamine (1 or 3 mM) (▲), alanine (2 mM) (■) or glutamine plus glycine (2 mM each) (▼). Linear-regression analysis of the data presented revealed the following relationships for the normo- and hypo-osmotic conditions respectively: $y = 0.007x + 1.39$ (n = 15; r = 0.983) and $y = 0.0093x + 1.43$ (n = 6; r = 0.985).

because at concentrations above 100 μM a cholestatic effect of taurocholate was observed (Fig. 1), as it was reported to occur at much higher taurocholate concentrations under normo-osmotic conditions (Herz et al., 1975).

As shown recently (Fig. 1 of Hallbrucker et al., 1992), after hypo-osmotic exposure produced by lowering the influent NaCl concentration by 40 mM, the stimulation of taurocholate excretion into bile was biphasic: during the first 5 min of hypo-osmotic exposure there was a transient increase in taurocholate excretion, followed by a second sustained stimulation of taurocholate excretion, which reached a steady state after about 15 min and which was maintained for the duration of hypo-osmotic exposure. Interestingly, taurocholate uptake from the perfusate (determined from the influent/effluent taurocholate concentration difference) started to increase to a new steady state after about 5 min of hypo-osmotic exposure and was not significantly affected during the first 5 min (results not shown), although taurocholate excretion into bile transiently increased. This would be compatible with the idea that canalicular taurocholate secretion is stimulated primarily by hypo-osmotic cell swelling and that net taurocholate influx across the sinusoidal plasma membrane increases secondarily. This interpretation is also in line with the general assumption that canalicular secretion, but not transport across the sinusoidal membrane, is rate-controlling for transcellular bile acid transport.

conditions (Fig. 1). On the other hand, after hyperosmotic cell shrinkage by increasing the perfusate osmolarity from 305 to 385 mosM by addition of 40 mM-NaCl, taurocholate excretion into bile as well as taurocholate-induced stimulation of bile flow were markedly diminished compared with the normo-osmotic condition (Fig. 1). Clearly, hyperosmotic exposure decreased the $V_{max.}$ of taurocholate excretion into bile. However, double-reciprocal analysis of the data in Fig. 1 was not performed,

Effect of ionic substitutions on taurocholate excretion into bile

As shown in Fig. 3, replacement of 40 mM-Na$^+$ by 40 mM-K$^+$ in the influent perfusate transiently lowered both bile flow and taurocholate excretion into bile; however, taurocholate excretion fully recovered to the baseline level within 7 min. Likewise, lowering the influent K$^+$ concentration from 5.9 mM to 0.9 mM (and increasing the Na$^+$ concentration by 5 mM) had no effect on taurocholate excretion into bile.
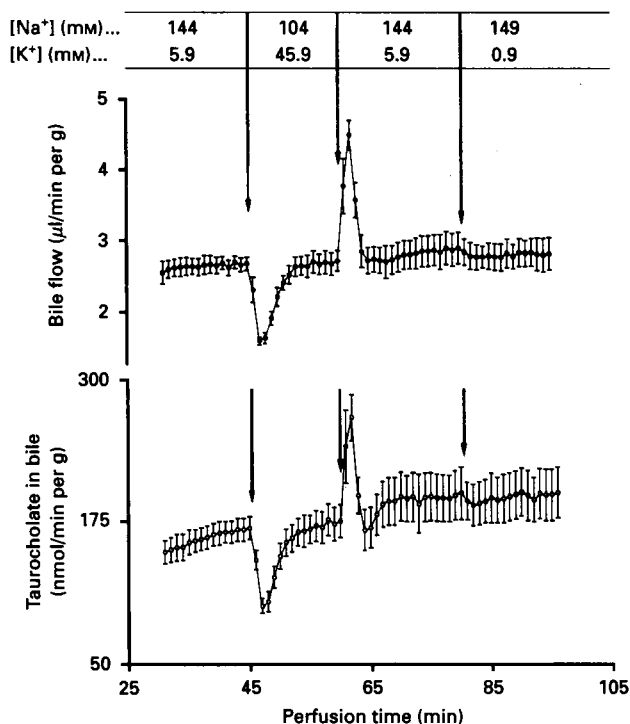
| [Na$^+$] (mM)... | 144 | 104 | 144 | 149 |
| [K$^+$] (mM)... | 5.9 | 45.9 | 5.9 | 0.9 |

Fig. 3. Effect of extracellular K$^+$ on bile flow and taurocholate excretion into bile

Livers were perfused with a medium containing [^{3}H]taurocholate (100 μM). At the time points indicated, the K$^+$ concentration in the influent perfusate was changed from 5.9 mM to 45.9 mM or 0.9 mM respectively. Normo-osmoticity (305 mosM) was preserved by corresponding alterations of the Na$^+$ concentration in influent. Data are given as means $\pm$ S.E.M. and are from four different perfusion experiments.

	Normo-osmotic		Hypo-osmotic	Normo-osmotic	
[Na$^+$] (mM)...	144		104	144	
[Sucrose] (mM)...	0	80	0	80	0

Fig. 4. Effect of replacing 40 mM-NaCl by 80 mM-sucrose on taurocholate excretion into bile

Livers were perfused with a medium containing [^{3}H]taurocholate. Replacement of 40 mM-NaCl by 80 mM-sucrose markedly inhibits taurocholate excretion into bile, whereas subsequent omission of sucrose (hypo-osmotic condition) markedly stimulates taurocholate excretion into bile. Data are given as means $\pm$ S.E.M. and are from three different perfusion experiments.

As shown in Fig. 4, replacement of 40 mM-NaCl by 80 mM-sucrose in the perfusate markedly inhibited taurocholate excretion into bile, whereby hypo-osmotic exposure caused by removal of sucrose stimulated taurocholate excretion into bile to about 60% above the starting level. These data show that even when the extracellular osmolarity is maintained, addition of a non-permeant sugar such as sucrose (Tavoloni, 1984) markedly inhibits bile acid secretion, as was also shown when hyperosmotic stress was induced by addition of sucrose (Haddad et al., 1989).

Tight-junctional permeability during aniso-osmotic exposure

The possibility that alterations of tight-junctional permeability could account for the marked effects of aniso-osmotic exposure on $V_{max.}$ of biliary taurocholate excretion (Fig. 1a) was tested in perfusion experiments monitoring the appearance of bolus-injected horseradish peroxidase in bile. This technique has frequently been used to assess tight-junctional permeability in perfused rat liver (Lowe et al., 1985, 1988; Hardison & Lowe, 1989; Hayakawa et al., 1990a,b; Llopis et al., 1991). In line with these previous reports, there was a biphasic appearance of horseradish peroxidase in bile (Fig. 5). A first peak occurred almost immediately (but is detected after about 5 min, owing to the dead-space of the bile-draining system), and is considered to reflect horseradish peroxidase entry via the paracellular pathway (Lowe et al., 1985, 1988; Hardison & Lowe, 1989), but may also include a rapid vesicular transport component (Hayakawa et al., 1990a). A second, larger, peak appeared after about 15 min (detected after 20 min), reflecting a transcytotic vesicle pathway
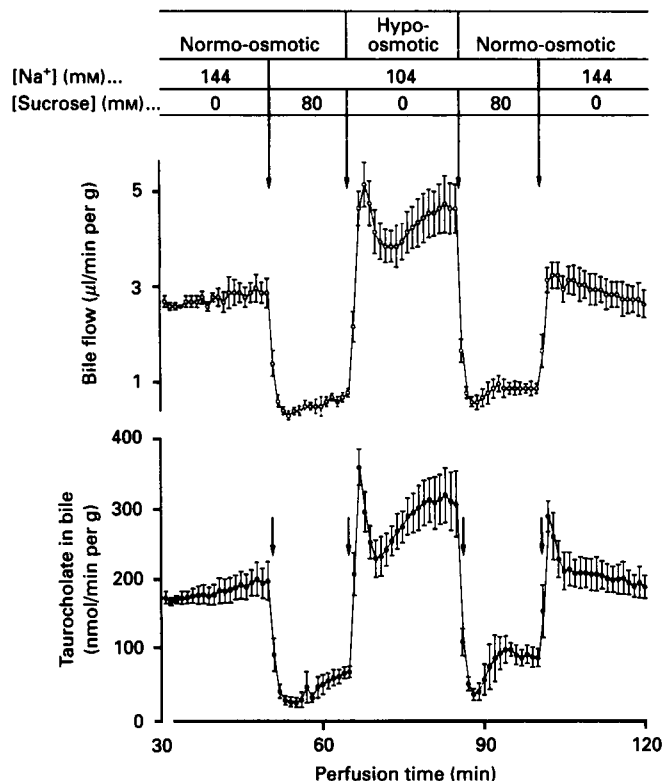
(Lowe et al., 1985; Hardison & Lowe, 1989; Hayakawa et al., 1990a,b; Llopis et al., 1991). As shown in Fig. 5, the pattern of horseradish peroxidase appearance in bile during normo-osmotic (305 mosM) perfusions was almost identical with that found in livers exposed to hyperosmotic (385 mosM) perfusion media. Hypo-osmotic conditions (225 mosM), however, slightly diminished the first peak of horseradish peroxidase appearance, but this effect was statistically not significant. Also the amounts of horseradish peroxidase recovered in bile were not significantly different during normo-, hypo- and hyper-osmotic perfusions: within 60 min after horseradish peroxidase bolus injection 0.000347 $\pm$ 0.000055% ($n = 3$), 0.000326 $\pm$ 0.000021% ($n = 3$) and 0.000340 $\pm$ 0.000089% ($n = 3$) of the injected dose respectively were recovered in bile. These data suggest that the steady-state degree of cell swelling/shrinkage does not affect paracellular or transcytotic horseradish peroxidase transport in perfused liver. On the other hand, in normo-osmotic perfusions vasopressin, which is known to increase tight-junctional permeability (Lowe et al., 1988; Hardison & Lowe, 1989), significantly increased the first peak of horseradish peroxidase appearance about 7-fold (results not shown). As found in three separate experiments with 100 μM-taurocholate in the influent, a vasopressin (35 nM)-induced increase in tight-junctional permeability was, however, not accompanied by detectable alterations of bile flow and taurocholate excretion during metabolic steady states (results not shown). Similar findings have been reported by others for taurodehydrocholate excretion into bile (Hardison & Lowe, 1989). Accordingly, alterations of the paracellular pathway
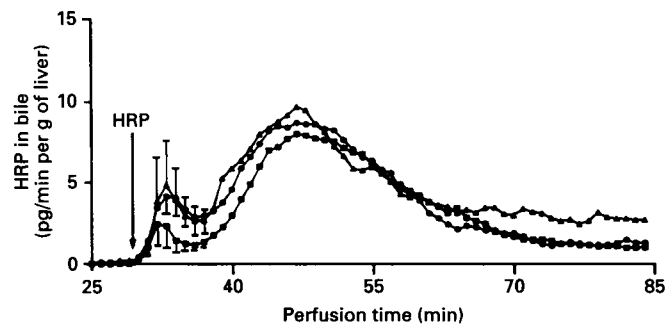
Fig. 5. Horeradish peroxidase (HRP) appearance in bile after bolus injection during normo-osmotic (●; 305 mosM), hypo-osmotic (■; 225 mosM) and hyperosmotic (▲; 385 mosM) perfusions

For experimental details see the Materials and methods section. Hypo-osmotic (225 mosM) and hyperosmotic (385 mosM) conditions were achieved by lowering or increasing the NaCl concentration in the influent perfusate by 40 mM. Horseradish peroxidase was bolus-injected 20 min after onset of the aniso-osmotic perfusion medium, i.e. after completion of volume-regulatory ion fluxes. Data are given as means and are from three different perfusion experiments for each condition. For clarity, respective s.e.m. values were only included for the first peak of horseradish peroxidase appearance; for other time points the respective s.e.m. values were of similar size to that shown during the first peak.

do not explain the effects of aniso-osmoticity on taurocholate excretion into bile. It should be emphasized that horseradish peroxidase bolus injection in the experiments depicted in Fig. 5 was performed about 20 min after the onset of aniso-osmotic exposure, i.e. after the time period required for completion of volume-regulatory K$^+$ fluxes and adjustment of a new steady-state cell volume (Lang et al., 1989; Häussinger et al., 1990a). Thus the findings in Fig. 5 do not rule out the possibility that alterations of tight-junctional permeability or of transcytotic horseradish peroxidase transport may occur during volume-regulatory responses, i.e. within the first 10 min of aniso-osmotic exposure.

Effect of amino acids and insulin on taurocholate excretion into bile

Glutamine and glycine induce liver cell swelling in the isolated perfused rat liver, owing to concentrative uptake of these amino acids into the cells (Häussinger et al., 1990b, 1991; vom Dahl et al., 1991a; Hallbrucker et al., 1991a). As shown in Fig. 6, with these amino acids present in the influent perfusate, taurocholate excretion into bile and bile flow were markedly stimulated: V_{max} for taurocholate excretion rose from 287 nmol/min per g ($n = 8$; linear correlation coefficient $r = 0.994$) to 699 nmol/min per g ($n = 4$; $r = 0.998$). Stimulation of taurocholate excretion by the amino acids tested appeared to parallel their ability to swell the cells (Table 1). Although amino acids led to cell swelling and stimulation of taurocholate excretion into bile, they did not affect the relationship between taurocholate excretion and bile flow (Fig. 2), in line with the previous finding that addition of these amino acids did not significantly affect bile flow in the absence of taurocholate (Hallbrucker et al., 1992).

Insulin causes cell swelling owing to intracellular accumulation of K$^+$ via amiloride- and bumetanide-sensitive mechanisms (Hallbrucker et al., 1991b). As shown in Fig. 6, during the continuous presence of insulin (35 nM) bile flow and taurocholate excretion into bile were significantly stimulated. Insulin (35 nM) increased V_{max} of taurocholate excretion from 287 (control; see Fig. 1a) to 429 nmol/min per g ($n = 4$; $r = 0.989$).
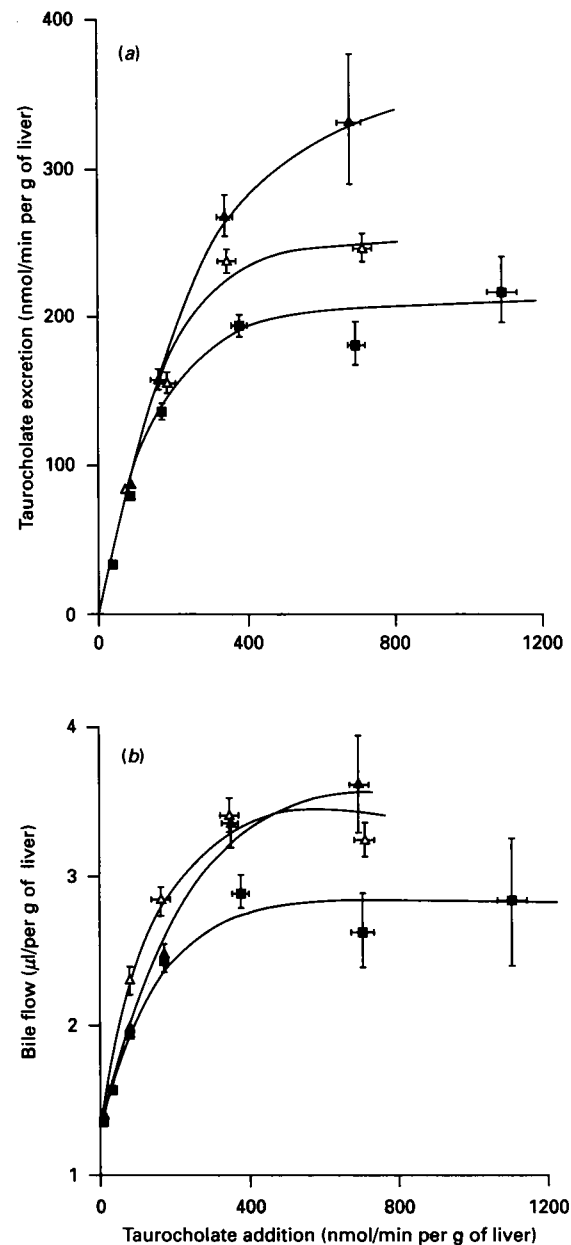

Fig. 6. Taurocholate excretion (a) and bile flow (b) in the absence or presence of glutamine/glycine or insulin

Livers were perfused with normo-osmotic perfusion medium (305 mosM) and [³H]taurocholate was added at different concentrations to the influent. A taurocholate addition of about 400 nmol/min per g corresponds to a taurocholate concentration of 100 μM in the influent perfusate. Insulin (35 nM) and glutamine/glycine (2 mM each) were added 20 min before infusion of taurocholate, i.e. taurocholate was added after completion of insulin- and amino acid-induced K$^+$ movements. Data were obtained during metabolic steady states and are given as means ± s.e.m. ($n = 4$–5). In the absence of taurocholate, the bile flow was 1.26 ± 0.04 ($n = 12$), 1.20 ± 0.06 ($n = 5$) and 1.34 ± 0.06 ($n = 5$) μl/min per g of liver in normo-osmotic control experiments and in the presence of glutamine/glycine or insulin respectively. ■, Control; ▲, glutamine plus glycine; △, insulin.
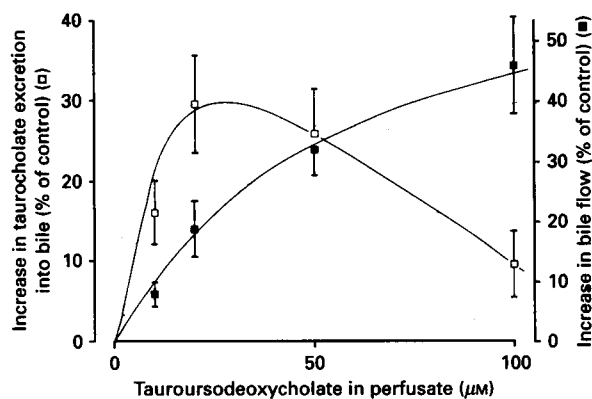
Effect of tauroursodeoxycholate on taurocholate excretion into bile and on cell volume

During constant infusion of [³H]taurocholate, further addition of tauroursodeoxycholate (10–100 μM) stimulated both bile flow and taurocholate excretion into bile. This effect of

Table 1. Effect of amino acids on taurocholate excretion into bile in isolated perfused rat liver

Livers were perfused in an open non-recirculating system. The influent perfusate contained [^{3}H]taurocholate (100 μM). Tauro-cholate excretion into bile in the absence of added amino acids was set at 100% in the individual perfusion experiment and the effect of amino acids is expressed as a percentage hereof. Taurocholate excretion into bile was 201 ± 6 nmol/min per g ($n = 12$) in the absence of added amino acids. Under these conditions bile flow was 3.0 ± 0.1 μl/min per g (100%). The effect of amino acids on cell volume was determined in separate experiments without taurocholate in influent as intracellular water space as described (vom Dahl et al., 1991a). Cell volume in the absence of amino acids was set at 100%, and their effect on cell volume is expressed as a percentage thereof.

Condition	Increase in taurocholate excretion (%)	Increase in bile flow (%)	Increase in cell volume (%)
Glutamine (1 mM)	14 ± 1	13 ± 2	5.7 ± 1.0
Glutamine (3 mM)	23 ± 1	18 ± 3	9.6 ± 1.6
Glutamine (2 mM) + glycine (2 mM)	32 ± 8	27 ± 5	12.1 ± 1.4
Alanine (2 mM)	25 ± 5	15 ± 2	6.0 ± 0.4

Fig. 7. Effect of tauroursodeoxycholate on taurocholate (100 μM) excretion into bile and bile flow in perfused rat liver

Livers were perfused with normo-osmotic (305 mosM) medium containing taurocholate at a concentration of 100 μM, and tauroursodeoxycholate was added at concentrations of 10, 20, 50 and 100 μM. Data are given as means $\pm$ s.e.m. ($n = 3$–7). Bile flow and taurocholate excretion in the absence of tauroursodeoxycholate were set at 100% in the individual perfusion experiments, and the effect of tauroursodeoxycholate on these parameters in the individual perfusion experiments is expressed as a percentage thereof. In these experiments, bile flow and taurocholate excretion in the absence of tauroursodeoxycholate (i.e. the 'control values') were 2.98 ± 0.16 μl/g ($n = 8$) and 180 ± 7 nmol/min per g ($n = 8$) respectively.

tauroursodeoxycholate on taurocholate excretion was strongly dependent on the tauroursodeoxycholate concentration infused (Fig. 7). It was maximal at tauroursodeoxycholate concentrations of 20–50 μM; at higher concentrations of tauroursodeoxycholate bile flow continued to increase, whereas taurocholate excretion into bile decreased again (Fig. 7). As shown in Fig. 8, tauroursodeoxycholate (50 μM) stimulated $V_{max.}$ of taurocholate excretion into bile.
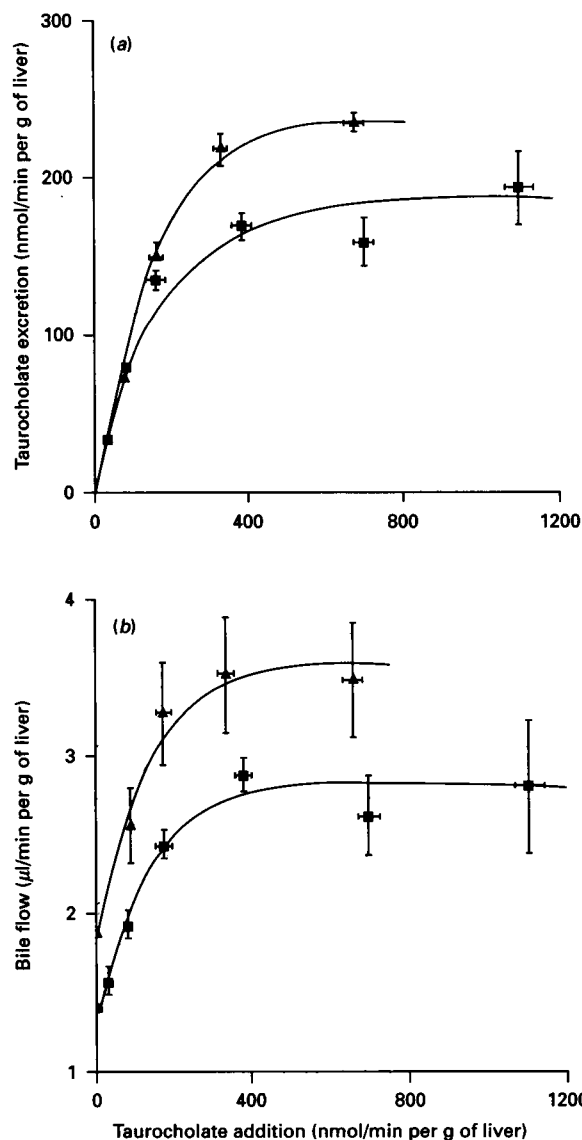
Fig. 8. Stimulation of taurocholate excretion (a) and bile flow (b) by tauroursodeoxycholate (50 μM)

Livers were perfused continuously with ($\blacktriangle$) or without ($\blacksquare$) tauro-ursodeoxycholate (50 μM) and the excretion of [^{3}H]taurocholate into bile was monitored as a function of the portal taurocholate addition. A taurocholate addition of about 400 nmol/min per g corresponds to a taurocholate concentration of 100 μM in the influent perfusate. Data are given as means $\pm$ s.e.m. from 3–7 different experiments.

The effect of tauroursodeoxycholate on liver cell volume was assessed by measurements of the intracellular water space. During continuous infusion of taurocholate (100 μM), tauroursodeoxycholate induced dose-dependent cell swelling (Table 2). Tauroursodeoxycholate-induced cell swelling roughly paralleled its effect on taurocholate excretion into bile (Table 2).

Relation to cell volume

Addition of taurocholate (100 μM) to influent perfusate of perfused rat liver led to an increase in the intracellular water space by 10.5 ± 1.4% ($n = 7$). Although taurocholate itself induced liver cell swelling, liver cell volume changes after aniso-osmotic exposure or addition of insulin or glutamine plus glycine were not influenced by the presence of taurocholate (Table 3). Accordingly, cell swelling induced by taurocholate and amino

Table 2. Effect of tauroursodeoxycholate on liver cell volume and taurocholate excretion into bile

Livers were perfused with a medium containing taurocholate (100 μM). Taurocholate excretion and cell volume under these conditions were set to 100% in the individual perfusion experiment. Then tauroursodeoxycholate was added at the concentrations listed and the resulting change of cell volume and taurocholate excretion is given as a percentage thereof. In the absence of tauroursodeoxycholate, but with taurocholate (100 μM) present, the intracellular water space was 518 ± 11 μl/g of liver ($n = 15$). Data are given as means $\pm$ s.e.m. from three different experiments for each condition.

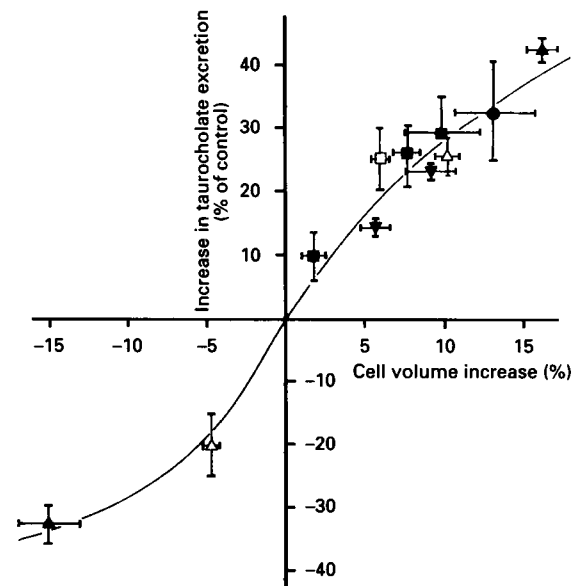| Tauroursodeoxycholate in influent (μM)	Cell volume increase		Stimulation of taurocholate excretion (%)
	(μl/g)	(%)	
20	$+44 \pm 10$	$+9.9 \pm 2.4$	$+29 \pm 6$
50	$+40 \pm 4$	$+7.6 \pm 0.9$	$+26 \pm 5$
100	$+9 \pm 4$	$+1.7 \pm 0.8$	$+9 \pm 4$

Table 3. Cell volume changes after aniso-osmotic exposure, addition of glutamine plus glycine (2 mM each) or insulin (35 nM) in the presence and absence of taurocholate (100 μM)

Data are means $\pm$ s.e.m. from 3–15 experiments for each condition. Cell volume was determined as intracellular water space as described in the Materials and methods section and was set to 100% in the individual perfusion experiment, and the volume effects of aniso-osmotic exposure, insulin and glutamine/glycine (2 mM each) are expressed as percentages thereof. As shown in a separate series of experiments, taurocholate (100 μM) itself increased the intracellular water space by 51 ± 6 μl/g of liver ($n = 7$), corresponding to a cell volume increase of 10.5 ± 1.4%.

Condition	Taurocholate	Cell volume increase	
		(μl/g)	(%)
Hypo-osmotic (225 mosM)	Absent	$+94 \pm 15$	$+18.9 \pm 2.6$
	Present	$+86 \pm 3$	$+16.1 \pm 0.9$
Hyperosmotic (385 mosM)	Absent	-74 ± 1	-12.1 ± 0.2
	Present	-76 ± 8	-15.1 ± 2.0
Insulin (35 nM)	Absent	$+59 \pm 6$	$+12.2 \pm 1.4$
	Present	$+54 \pm 6$	$+10.8 \pm 1.1$
Glutamine (2 mM) + glycine (2 mM)	Absent	$+68 \pm 8$	$+12.1 \pm 1.4$
	Present	$+71 \pm 13$	$+13.3 \pm 2.6$

acids was additive. This was probably also true for the insulin-induced net K^+ accumulation inside the cells: in the absence and presence of taurocholate (100 μM) insulin-induced net K^+ uptake was 4.5 ± 0.2 ($n = 15$), and 4.3 ± 0.4 ($n = 3$) μmol/g respectively.

As shown in Fig. 9, there was a close relationship between cell volume and taurocholate excretion into bile. Cell swelling stimulates, whereas cell shrinkage inhibits, taurocholate excretion into bile. This relationship is maintained regardless of whether cell volume changes were induced by aniso-osmotic exposure, amino acids or tauroursodeoxycholate. The effect of insulin on taurocholate excretion into bile was somewhat lower than that predicted from its swelling effect. Although both hypo-osmotic exposure (265 mosM) and insulin led to a comparable increase in cell volume (i.e. by 10.1 ± 0.8% and 10.8 ± 1.1% respectively), their stimulatory effects on taurocholate excretion into bile were 25 ± 3% ($n = 3$) and 18 ± 3% ($n = 4$) respectively. Apparently,

Fig. 9. Relationship between cell volume changes and taurocholate excretion into bile under the influence of aniso-osmotic exposure, tauroursodeoxycholate, alanine, glutamine, glycine and insulin

In all experiments the influent perfusate contained taurocholate at a concentration of 100 μM. Taurocholate excretion into bile in the absence of amino acids and during normo-osmotic perfusion was set at 100% in the individual perfusion experiment, and the effects of aniso-osmoticity, tauroursodeoxycholate or amino acids were determined as the percentage change thereof. Cell volume changes were determined as change in the intracellular water space during continuous perfusion with taurocholate (100 μM) ($\blacktriangle$, $\blacksquare$, $\bullet$). In some experiments ($\blacktriangledown$, $\square$, $\triangle$), cell volume changes under the influence of effectors on cell volume were determined in the absence of taurocholate. This appears justified, because the volume effects of aniso-osmoticity and amino acids were not affected by the presence of taurocholate (see Table 3). Data are given as means $\pm$ s.e.m. ($n = 3$–6 different perfusion experiments for each condition). Symbols: $\blacktriangle$, aniso-osmoticity (225 and 385 mosM); $\bullet$, glutamine (2 mM) plus glycine (2 mM); $\blacksquare$, tauroursodeoxycholate (20, 50 and 100 μM); $\triangle$, aniso-osmoticity (265 and 345 mosM), $\blacktriangledown$, glutamine (1 and 3 mM); $\square$, alanine (2 mM).

mechanisms distinct from cell swelling come into play during insulin-induced stimulation of taurocholate excretion.

DISCUSSION

Cell volume and bile formation

The data in this paper suggest that liver cell volume is a major determinant for taurocholate excretion into bile (Fig. 9), in line with our previous report (Hallbrucker et al., 1992). Cell swelling, whether induced by hypo-osmotic perfusion, amino acids, tauroursodeoxycholate or insulin, apparently increases $V_{max.}$ of taurocholate excretion into bile (Figs. 1a, 6a, 8a). Conversely, hyperosmotic cell shrinkage decreases the capacity of the liver to excrete taurocholate, and the known cholestatic effect of taurocholate becomes apparent at concentrations below 400 μM (Fig. 1a). The effects of aniso-osmoticity on taurocholate excretion (Fig. 1) are probably not explained by alterations of tight-junctional permeability. Opening of tight junctions by vasopressin had no effect on the steady-state taurocholate (the present paper) and taurodehydrocholate excretion (Lowe et al., 1985). Further, as suggested by the data in Fig. 5, aniso-osmotic exposure did not significantly alter tight-junctional permeability,

despite a marked effect on taurocholate excretion into bile (Fig. 1a).

Theoretically, the stimulation of taurocholate excretion and bile flow after decreasing the perfusate NaCl concentration (Fig. 1) could have been due to decreased osmolarity with subsequent cell swelling, or due to the decrease in NaCl concentration as such. This latter possibility is rather unlikely, since decreasing perfusate Na$^+$ activity is expected to compromise, not to stimulate, transcellular taurocholate transport. Furthermore, fluid transport into the canaliculi depends in part on the movement of Na$^+$ through the paracellular shunt (Boyer et al., 1992), which should be impaired after decreased perfusate Na$^+$ activity. In fact, the iso-osmotic replacement of NaCl by sucrose leads to a profound decrease in taurocholate excretion (Fig. 4). Subsequent decrease in perfusate osmolarity by omission of sucrose at constant NaCl concentration leads to a rapid stimulation of taurocholate excretion (Fig. 4). Thus, the decrease in osmolarity rather than the decrease in the NaCl concentration stimulates taurocholate excretion. This view is also augmented by the finding that cell swelling owing to amino acid accumulation inside the cells stimulates taurocholate excretion, and the close correlation between cell volume and taurocholate excretion depicted in Fig. 9.

It is likely that the swelling-induced stimulation of taurocholate excretion into bile reflects a stimulation of the canalicular taurocholate secretion process, which is known to be the rate-limiting step in the overall process of taurocholate translocation from the sinusoidal into the biliary compartment (Erlinger, 1988; Sellinger & Boyer, 1990; Nathanson & Boyer, 1991; Boyer et al., 1992). This view is also augmented by the finding that the transient stimulation of taurocholate excretion into bile during the first 5 min of hypo-osmotic exposure was not accompanied by an increased taurocholate uptake from the perfusate; however, washout effects owing to hypo-osmoticity-stimulated bile flow may contribute to the transient stimulation of taurocholate excretion.

The mechanisms underlying the swelling-induced stimulation of taurocholate excretion remain speculative. Evidence has been given for a membrane-potential-driven canalicular taurocholate secretion (Meier et al., 1984; Weinmann et al., 1989). Although hypo-osmotic exposure (225 mosM) of isolated mouse liver cells was reported to hyperpolarize the membrane-potential from about −40 to −47 mV (Graf et al., 1988), the membrane-potential effects on canalicular taurocholate secretion appear to be too small in order to account quantitatively for the marked stimulation of taurocholate excretion after hypo-osmotic cell swelling (Fig. 1). In addition, our own electrophysiological studies revealed that hypo-osmotic exposure of rat hepatocytes led only to a transient hyperpolarization (for about 1 min), followed by a sustained depolarization (F. Lang, M. Ritter & D. Häussinger, unpublished work), contrasting with the persistent stimulation of taurocholate excretion into bile. In line with our conclusion that swelling-induced changes of the membrane potential do not explain the effect on taurocholate excretion are also the findings in Fig. 3, demonstrating that marked alterations of the extracellular K$^+$ concentration do not affect steady-state taurocholate excretion into bile. Recent studies revealed that canalicular taurocholate secretion is accomplished by an ATP-driven transport system (Adachi et al., 1991; Müller et al., 1991; Nishida et al., 1991; Stieger et al., 1992), yet hypo-osmotic cell swelling was without effect on tissue ATP levels in perfused rat liver (Häussinger et al., 1990c). Thus the possibility must be envisaged that the swelling-induced increase in $V_{max.}$ of taurocholate excretion into bile may involve the recruitment of previously inactive or latent transporter molecules, as suggested previously (Hallbrucker et al., 1992; Boyer et al., 1992).

Cell volume and the effect of tauroursodeoxycholate on taurocholate excretion

Both taurocholate and tauroursodeoxycholate induce cell swelling (Table 2). In the concentration range studied, no alterations of cellular K$^+$ balance could be detected under the influence of these bile acids. It is likely that bile-acid-induced cell swelling is, at least in part, explained by an osmotically active intra-/extra-cellular bile acid concentration gradient which is built up by the Na$^+$-dependent bile acid carrier in the sinusoidal membrane. In line with this, a 200-fold intracellular accumulation of taurocholate has been observed in the presence of physiological taurocholate concentrations (Schwartz et al., 1975). Apart from this, the mechanisms underlying taurocholate- or tauroursodeoxycholate-induced cell swelling remain obscure. Na$^+$/H$^+$ exchange is turned on for cell volume increase under a variety of conditions (for review see Häussinger & Lang, 1991a), and ursodeoxycholate, but not its taurine conjugate studied here, was shown to stimulate Na$^+$/H$^+$ exchange in rat liver basolateral plasma-membrane vesicles (Moseley et al., 1987).

The findings that cell swelling stimulates taurocholate excretion and that taurocholate itself induces cell swelling may be seen as a feed-forward mechanism augmenting bile acid excretion, at least at bile acid concentrations below 100 μM. Although one might expect competition between taurocholate and tauroursodeoxycholate for transport across the sinusoidal membrane, addition of tauroursodeoxycholate at low concentrations to perfused rat liver increased taurocholate excretion into bile (Fig. 7). The most likely explanation for this effect is tauroursodeoxycholate-induced cell swelling. This view is supported by the findings (i) that tauroursodeoxycholate-induced stimulation of taurocholate excretion can roughly be mimicked by equipotent hypo-osmotic or amino acid-induced cell swelling (Fig. 9) and (ii) that the effect of tauroursodeoxycholate on taurocholate excretion is paralleled by the extent of tauroursodeoxycholate-induced cell swelling, but not by its infused concentration (Table 2, Fig. 9). Stimulation of bile acid excretion by tauroursodeoxycholate-induced cell swelling may well contribute to the beneficial effect of ursodeoxycholate in several cholestatic diseases, such as primary biliary cirrhosis or primary sclerosing cholangitis (Poupon et al., 1991; Podda et al., 1991), and evidence has been presented that ursodeoxycholate is conjugated in vivo with taurine or glycine (Heumann et al., 1991).

The finding that raising the tauroursodeoxycholate concentration from 20 to 100 μM almost abolishes both tauroursodeoxycholate-induced cell swelling and stimulation of taurocholate excretion is difficult to explain. Possible explanations at present remain speculative, because both the kinetics of sinusoidal taurocholate/tauroursodeoxycholate uptake and canalicular secretion and the steady-state intracellular concentrations of both bile acids are not known. In the presence of 100 μM-taurocholate (which according to Fig. 1a leads to an already maximal biliary taurocholate excretion), further addition to tauroursodeoxycholate (100 μM) has almost no effect on both cell volume and taurocholate excretion into bile (Fig. 7, Table 2). Clearly, under these conditions total bile acid (taurocholate plus tauroursodeoxycholate) excretion increases, as suggested by the increase in bile flow (Fig. 7). This finding would be compatible with the suggestion by Kitani & Kanai (1982) that different mechanisms could be involved in the biliary secretion of taurocholate and tauroursodeoxycholate.

Physiological relevance

Under physiological conditions the portal bile acid load will rarely exceed 100 nmol/min per g. Such a small amount is almost completely cleared by the liver regardless of the perfusate

osmolarity (see Fig. 1). Accordingly, one might argue that cell volume changes may not play a role in bile acid excretion under physiological conditions. Here, however, the acinar organization of the liver has to be considered. As shown by histo-autoradiography, only the first upstream hepatocytes are normally engaged in bile acid excretion, but all other, more downstream, hepatocytes are recruited when the bile acid load increases or when cholestasis is present (Buscher et al., 1989, 1991). Although it is not known whether cell swelling increases the $V_{max.}$ of taurocholate excretion in all hepatocytes uniformly, our data may suggest that, in the presence of physiologically low bile acid load, the number of hepatocytes engaged in bile acid extraction and secretion will increase and decrease after cell shrinkage and cell swelling, respectively. Thus under physiological conditions, cell volume will determine the number of hepatocytes transporting bile acids, rather than the amount of bile acids excreted into bile.

Amino acids were shown to inhibit taurocholate uptake into isolated plasma-membrane vesicles owing to competition for the Na^+ gradient (Blitzer & Bueler, 1985), and inhibition of taurocholate uptake by amino acids was suggested to play a role in the pathogenesis of cholestasis during total parenteral nutrition (Sellinger & Boyer, 1990). Such a mechanism, however, appears unlikely, because amino acids which are transported in a Na^+-dependent way, such as glutamine, glycine or alanine, actually stimulate taurocholate excretion into bile in the intact liver (Table 1, Figs. 6a and 9). The most likely explanation is an amino acid-induced cell swelling which increases the $V_{max.}$ for canalicular taurocholate secretion. Because the latter is the rate-controlling step for transcellular taurocholate transport, amino acids will stimulate overall trans-epithelial taurocholate transport, although some inhibition of sinusoidal taurocholate uptake by the amino acids may occur.

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