Cell swelling increases bile flow and taurocholate excretion into bile in isolated perfused rat liver

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The effects of aniso-osmotically and amino-acid-induced cell-volume changes on bile flow and biliary taurocholate excretion were studied in isolated perfused rat liver. With taurocholate (100 μM) in the influent perfusate, hypo-osmotic exposure (225 mosmol/l) increased taurocholate excretion into bile and bile flow by 42 and 27 %, respectively, whereas inhibition by 32 and 47 %, respectively was observed after hyperosmotic (385 mosmol/l) exposure. The effects of aniso-osmoticity on taurocholate excretion into bile was observed throughout aniso-osmotic exposure, even after completion of volume-regulatory ion fluxes and were fully reversible upon re-exposure to normo-osmotic media. Hypo-osmotic cell swelling (225 mosmol/l) increased the V_{max} of taurocholate translocation from the sinusoidal compartment into bile about 2-fold. Also, cell swelling induced by glutamine and glycine stimulated both bile flow and biliary taurocholate excretion.

There was a close relationship between the aniso-osmotically and amino-acid-induced change of cell volume and taurocholate excretion into bile. The data suggest that liver cell volume plays an important role in regulating bile-acid-dependent bile flow and biliary taurocholate excretion.

INTRODUCTION

Liver cell-volume changes were recently identified as an important modulator of metabolic liver cell function and several long-known, but mechanistically unclear, hormone and amino acid effects on hepatic metabolism were recently explained by hormone- and amino-acid-induced cell-volume changes (for a review, see [1]). For example, cell swelling induced either by insulin, amino acids or hyper-osmotic exposure inhibits hepatic proteolysis, whereas glucagon- and hyperosmolarity-induced cell shrinkage stimulate protein degradation [2]. Nothing is known about the relationship between liver cell volume and bile formation. Studies on hyperosmotic stress in perfused rat liver by increasing the perfusate osmolarity by adding sucrose or mannitol showed a decrease of bile flow; these effects, however, were dependent on the sugar under investigation [3]. Here we report on the effect of aniso-osmotically and amino-acid-induced cell-volume changes on biliary taurocholate excretion and bile flow.

MATERIALS AND METHODS

Liver perfusion

Livers from male Wistar rats (200–300 g body wt.), fed ad libitum on stock diet (Altromin), were perfused as described previously [4] 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. The perfusate was gassed with O_{2}/CO_{2} (19 : 1); the temperature was 37 °C. The perfusate osmolarity was 305 mosmol/l in normo-osmotic perfusions. Aniso-osmotic perfusion conditions were achieved by corresponding changes in the NaCl concentration of the influent.

Determinations

Bile was collected at 1 min intervals into preweighed vessels, and bile flow was determined by gravimetry, using a specific mass of 1 g/ml. Taurocholate excretion into bile was assayed by liquid-scintillation spectrometry of the [14C]urea-accessible space and a [3H]inulin-accessible space. In brief, both radioisotopically labelled compounds were added simultaneously to the influent perfusate for 5 min, i.e. a time period sufficient to achieve equilibration of [14C]urea and [3H]inulin in their respective accessible water spaces [5]. Then radioactivity infusion was stopped and the effluent perfusate collected during the following 5 min and assayed for H and 14C. By using the effluent 3H/14C ratio found during steady-state infusion of radioactivity and that found during the wash-out period, [3H]-inulin and [14C]urea spaces were calculated.

Data from different perfusion experiments are given as means ± S.E.M. (number of experiments).

Materials

[3H]Taurocholate was from New England Nuclear (Dreieich, Germany), L-lactic acid was from Roth (Karlsruhe, Germany) and unlabelled taurocholate was from Sigma (Munich, Germany). All other chemicals were from Merck (Darmstadt, Germany).

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RESULTS AND DISCUSSION

Effect of aniso-osmotic exposure on bile flow and biliary taurocholate excretion

When livers were perfused in the absence of taurocholate in the perfusate, basal bile flow was, if at all, only slightly affected during aniso-osmotic exposure (due to changes of the NaCl concentration in the perfusate); in normo-osmotic (305 mosmol/l), hypo-osmotic (225 mosmol/l) and hyperosmotic (385 mosmol/l) perfusions, basal bile flow was 1.25 ± 0.06 (n = 8), 1.34 ± 0.06 (n = 7) and 1.18 ± 0.07 μl/min per g (n = 4) respectively. Addition of taurocholate (100 μM) to the influent perfusate increased bile flow from 1.25 ± 0.06 μl/min per g (n = 8) to 2.94 ± 0.07 μl/min per g (n = 17) in normo-osmotic perfusions (305 mosmol/l). Sudden exposure to hypo-osmotic perfusion media (225 mosmol/l) by lowering the influent NaCl concentration by 40 mm led to a volume-regulatory K⁺ efflux for about 6 min, as described recently in detail [6], which was accompanied by a stimulation of bile flow and taurocholate excretion (Fig. 1). The initial overshoot in bile flow and taurocholate excretion during the first 6 min of hypo-osmotic exposure may in part be attributed to a mechanical compression of the bile canaliculi and ductules after cell swelling. However, after completion of volume-regulatory K⁺ fluxes, both bile flow and taurocholate excretion into bile remained at an elevated level that persisted throughout hypo-osmotic exposure. This was fully reversible upon re-exposure to normo-osmotic media (Fig. 1). Conversely, hyperosmotic exposure led to a persistent decrease of both bile flow and biliary taurocholate excretion, which was again reversible upon re-exposure to normo-osmotic media (Fig. 1). Taurocholate excretion into bile was saturable, exhibiting a Vₘₐₓ of 387 nmol/min per g (n = 8, linear correlation coefficient, r, 0.933) as determined by double-reciprocal-plot analysis in taurocholate titration (up to 400 μM in influent) studies. During hypo-osmotic (225 mosmol/l) perfusions, the Vₘₐₓ of taurocholate excretion into bile was increased to 716 nmol/min per g (n = 8; r = 0.999).

Taurocholate excretion into bile is a complex process involving Na⁺-dependent taurocholate transport across the sinusoidal plasma membrane [7], intracellular taurocholate transport and canaliculic secretion, probably by an ATP-dependent process [8]; for reviews, see [9,10]). Cell swelling could interfere with these processes. Cell swelling was shown to hyperpolarize the cell membrane potential [11], thereby increasing the driving force for Na⁺-coupled transport across the sinusoidal membrane. In line with this, recent studies showed that cell swelling stimulates Na⁺-coupled amino acid transport into hepatocytes [12], thereby explaining the earlier observation on stimulated amino acid transport via system N by non-system-N substrates, which are transported in a Na⁺-dependent way [13]. Thus it is entirely conceivable that cell swelling also stimulates Na⁺-coupled taurocholate uptake across the sinusoidal membrane. Canaliculic secretion, but not uptake across the sinusoidal membrane, is widely considered to be rate-controlling for biliary taurocholate.

Fig. 1. Effect of aniso-osmotic exposure on bile flow (a) and taurocholate excretion into bile (b) in perfused rat liver

The influent perfusate contained taurocholate at a concentration of 100 μM. Hypo-osmotic (225 mosmol/l) and hyperosmotic (385 mosmol/l) conditions were achieved by lowering or increasing the NaCl concentration in influent perfusate by 40 mm. Results in (a) and (b) are means ± S.E.M. for four different perfusion experiments.

Fig. 2. Effect of glutamine (2 mM) plus glycine (2 mM) on (a) bile flow and (b) taurocholate excretion into bile

The influent perfusate contained taurocholate at a concentration of 100 μM.
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Fig. 3. Relationship between cell-volume changes and taurocholate excretion into bile under the influence of aniso-osmotic exposure, glutamine and glycine

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 to 100% in the individual perfusion experiment, and the effects of aniso-osmoticity or amino acids were determined as the percentage change thereof. Cell-volume changes were determined as changes in the intracellular water space as described recently [5]; for further details, see the text. In normo-osmotic control perfusions the intracellular water space was 548 ± 10 μl/g (n = 44) [5]. Means ± s.e.m. (n = 3–6 different perfusion experiments for each condition). Symbols: ○, aniso-osmoticity (225, 265, 345, 385 mosmol/l); ▪, glutamine (1 and 3 mM); △, glutamine (2 mM) plus glycine (2 mM).

excretion in liver (for reviews, see [9,10]). The finding that hypo-osmotic cell swelling increases the V_{max} for taurocholate excretion into bile therefore suggests that cell swelling also increases canalicular secretion. Canalicular taurocholate transport appears to be ATP-dependent [8]; however, hypo-osmotic cell swelling was shown not to alter the tissue ATP levels [14]. One explanation for the hypo-osmolarity-induced stimulation of taurocholate excretion into bile could be that cell swelling increases the number of active transporters in the canalicular membrane. Alterations in tight-junctional permeability, which could theoretically affect taurocholate regurgitation from the canalicular into the sinusoidal space, probably do not explain our findings, because it was recently shown that vasopressin-induced increases in tight-junctional permeability did not affect taurodehydrocholate excretion into bile or bile flow [15].

Effect of amino acids on bile flow and biliary taurocholate excretion

In the absence of taurocholate in influent perfusate, addition of glutamine (2 mM) plus glycine (2 mM) did not significantly affect bile flow [1.22 ± 0.04 μl/min per g (n = 3)] as against 1.12 ± 0.06 μl/min per g (n = 3). When, however, taurocholate (100 μM) was present in the influent perfusate, the addition of both amino acids stimulated bile flow from 2.62 ± 0.23 to 3.30 ± 0.21 μl/min per g (n = 5), i.e by 0.67 ± 0.10 μl/min per g (n = 5). Simultaneously biliary taurocholate excretion rose by 45 ± 10 nmol/min per g (n = 4) from 174 ± 20 to 219 ± 17 nmol/min per g (n = 4) (see also Fig. 2). These effects were fully reversible upon withdrawal of the amino acids from the influent perfusate (Fig. 3). Similarly, bile flow and biliary taurocholate excretion was stimulated by addition of glutamine (1 mM or 3 mM) or glycine (2 mM) alone (results not shown).

Relationship between taurocholate excretion and cell volume

Aniso-osmotic exposure, glutamine and glycine lead to persistent alterations of liver cell volume; this effect on the intracellular water space, i.e. cell volume in perfused rat liver, were recently quantified by use of a [3H]nitulin/[14C]urea-wash-out technique [5]. When the effects of aniso-osmoticity and amino acids on liver cell volume were related to their effect on biliary taurocholate excretion, a close relationship between both parameters was observed (Fig. 3): cell swelling stimulates taurocholate excretion into bile, whereas cell shrinkage inhibits it, regardless of whether cell volume is modified by aniso-osmoticity or amino acids. It should be mentioned that the cell-volume changes under the influence of amino acids and aniso-osmoticity were determined as a change in the intracellular water space in liver-perfusion experiments without taurocholate in the influent. This is justified, because we could show in separate experiments that the presence of taurocholate (100 μM) had no effect on the degree of cell-volume change exerted by hypo- (225 mosmol/l) or hyperosmotic (385 mosmol/l) exposure.

Taken together, the findings suggest that liver cell-volume changes are an important determinant for bile acid excretion and bile flow (Fig. 3). Thus the data underline the important role of liver cell volume in regulating liver cell function. It remains to be established to what extent cell-volume changes are involved in the regulation of bile formation by hormones.

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REFERENCES


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