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ORGANIC SOLVENTS INCREASE MEMBRANE FLUIDITY AND AFFECT BILE FLOW AND K^+ TRANSPORT IN RAT LIVER*

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Abstract—Following the earlier observation that inhalation of volatile lipid solvents and of narcotic gases causes cholestasis, we studied the effects of various organic solvents on bile flow, plasma membrane fluidity and potassium movement in rat liver. Both *in vivo* and in the isolated perfused liver, applications of CCl_4, $CHCl_3$, dichloromethane, trichloroethylene, halothane, benzene and cyclohexane elicited rapid and sustained but reversible cholestasis. A transient phase of choleresis was observed prior to and after cholestasis, during the increase and fall in liver tissue solvent concentrations, respectively. Tissue concentrations required to produce cholestasis were lower the higher the lipophilicity of the solvent. Membrane fluidity was measured in isolated basolateral liver cell membranes by fluorescence polarization. Fluidity increased with increasing solvent concentration, the increase being associated with either biphasic stimulation and inhibition of membrane enzymes (Na^+,K^+-ATPase, 5′nucleotidase) or with inhibition alone (Mg^{2+}-ATPase). In the isolated perfused liver, application of organic solvents caused hepatic uptake of K^+ that was followed by K^+ release upon withdrawal of the solvent. The magnitude of K^+ uptake elicited by the solvent was comparable with the effect of blocking K^+ channels with 2 mM Ba^{2+}, but Ba^{2+} was ineffective in the presence of the solvent. In contrast, application of ouabain caused K^+ release in equal amounts in the absence and presence of the solvent, indicating that K^+ uptake elicited by the solvent results from inhibition of K^+ efflux through K^+ channels rather than stimulation of the Na^+,K^+ pump. The data show that cholestasis elicited by lipid solvents is associated with an increase in membrane fluidity and with disturbance of liver K^+ homeostasis. The significance of these observations is discussed with respect to other models of experimental cholestasis.

Bile production is accomplished through the composite action of many transport mechanisms that are located at the basolateral and luminal liver cell membranes. These mechanisms include the operation of pumps, carriers and ion channels. Changes in membrane fluidity may either activate or inhibit one or more of the individual transport components, but the significance of perturbations of membrane fluidity in causing changes in bile formation remains controversial.

Analogous to other biological membranes, the fluidity of the liver plasma membrane decreases with low temperature, high Ca^{2+}, high cholesterol content, after application of ethinyl estradiol or chlorpromazine, whereas increase in membrane fluidity occurs *in vitro* with alcohols, or through dietary regimens in animals (for a review see Schachter [1]).

Several observations associate changes in bile flow with changes in membrane fluidity. Thus, ethinyl estradiol induces cholestasis that is associated with a decrease in membrane fluidity [2], but no effect on membrane fluidity was observed in another study [3]. *S*-Adenosyl-L-methionine [4], Triton WR-1339 [5] and a dietary regimen to increase fluidity [6] are able to reverse the cholestatic effect of ethinyl estradiol, but application of *S*-adenosyl-L-methionine alone did not affect bile production [3]. Chlorpromazine decreases bile flow and membrane fluidity concomitantly [7]. On the other hand, the spironolactone derivative cancrenone reduced fluidity but increased bile flow together with increasing the biliary clearance of sucrose and inulin [8]. Local anesthetics increase membrane fluidity in other preparations [9] but inhibit bile formation [10].

Membrane fluidity has also been correlated with membrane enzyme activities and with specific transport functions. An increase in membrane fluidity activates Na^+,K^+-ATPase [2] and adenylate cyclase [11], but reduces the hepatobiliary clearance of cholephilic dyes [12], as well as the carrier-mediated transport of bile acids [13] and of alanine or cysteine [14]. However, a recent study showed no influence of membrane fluidity on basolateral amino acid uptake [15].

No data on the influence of membrane fluidity on ion movements in the liver are available. Various small, lipophilic molecules that are chemically diverse, such as chloroform, diethylether and *n*-butanol, are able to partition into biological and artificial membranes. These solvents alter membrane fluidity [16, 17] and change permeability patterns for different ions [18]. Reversible biological effects such

* Dedicated to Prof. Dr Gustev Paumgartner on the occasion of his 60th birthday.

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as anesthesia caused by volatile organic solvents (e.g. diethylether, chloroform, halothane) have been related to the lipid solubility of these compounds and to interaction with ion channels [19–21].

Previous studies have indicated that lipid solvents may also interact acutely with liver membranes to cause cholestasis [22]. Thus, cholestasis was elicited by short-term administration of CCl_4. This effect was rapid in onset and fully reversible upon withdrawal of the compound and was thus entirely different to liver injury and cirrhosis that are produced by chronic administration of the compound.

This study extends these previous observations to a variety of chemically unrelated lipid solvents and shows that cholestasis is correlated with the lipid solubility of the particular solvents and with their ability to increase membrane fluidity. The data further indicate that the increase in membrane fluidity is associated with closure of cell membrane K^+ channels.

MATERIALS AND METHODS

Materials. Chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), dichloromethane (DCM*), trichloroethylene, benzene and cyclohexane (c-hexane) were of analytical grade and were purchased from Merck (Darmstadt, Germany). Halothane was obtained from Hoechst (Vienna, Austria). 1,6-Diphenyl-1,3,5-hexatriene (DPH) and 1-α-myristoyllecithin were obtained from Sigma Chemie (Munich, Germany).

Animals. Male Louvain rats (Versuchtierzucht und-haltung der Universität Wien, Himberg, Austria), fed *ad lib.* and weighing 200–250 g, were used.

Isolated liver perfusion and in vivo *experiments.* The surgical isolation of the organ was done as described previously [23]. Livers were perfused in a non-recirculating perfusion system at 37° with Krebs–Henseleit buffer containing 1.5% bovine serum albumin and gassed with 95% O_2/5% CO_2. Perfusate flow rate was kept constant in individual experiments (3.1 mL/g liver × min) using a peristaltic pump (Model PA-SF, IKA-Labortechnik, Straufen i. Br., Germany). Lipid solvents were applied by continuous infusion into the perfusion medium through an infusion pump (Perfusar Secura, Braun, Melsungen, Germany). During the experiment perfusion pressure (6 ± 1.5 cm H_2O) was continuously monitored. It remained constant during and after application of organic solvents.

The bile duct was cannulated (Portex cannula, Kent, U.K.), bile was collected drop by drop and bile flow was measured by the frequency and weight of individual bile drops. The weight of single bile drops collected before, during and after applications of solvent was determined. This procedure enabled a good time resolution to observe rapid or transient changes in bile flow. The application of lipid solvents did not influence the weight of individual droplets during one experiment. However, in different experiments depending on the diameter of the bile duct and the corresponding cannula droplet weight varied between 8 and 9 mg.

In studies on liver K^+ balance, 1 mL aliquots of the venous perfusate were collected at 30 sec intervals and net K^+ fluxes between liver and perfusate were calculated from the porto-caval K^+ concentration difference and the perfusate flow rate, and are expressed as nmol/g liver × sec.

For *in vivo* experiments, rats were anesthetized with urethane and the bile duct was cannulated. Urethane was found to be preferable to barbiturates in avoiding respiratory depression during application of solvents that exert narcotic effects. Control experiments were carried out in the isolated perfused liver to show that urethane had no influence on bile flow and potassium movement. For *in vivo* experiments lipid solvents were applied via a tracheal T-cannula, the respiratory air being saturated with the respective solvent. Some perfusion experiments were done in order to approximate the *in vivo* condition with respect to the time course of the rise and decrease in blood solvent concentration. In these experiments, a recirculating system was used where the perfusate passed through an oxygenator prior to entering the liver and a perfusion medium was employed that contained bovine erythrocytes [22]. Gas flowing through the oxygenator was either saturated with or devoid of the lipid solvent. In these experiments the tissue concentrations of organic solvents necessary to produce cholestasis were determined. A N_2 atmosphere was established in the perfusion cabinet when O_2 mixtures that contained inflammable lipid solvents were employed. Control experiments showed that replacing lipid solvents by nitrogen did not influence bile flow.

Plasma membrane preparation and enzyme determinations. Rat liver plasma membranes were isolated as described by Van Amelsvoort *et al.* [24] by differential centrifugation and sucrose density gradient centrifugation. The final membrane pellet was suspended in a solution containing 250 mM sucrose, 10 mM Hepes/KOH, pH 7.4, 0.2 mM $CaCl_2$ to give a protein concentration of 5 mg/mL (stock solution). Na^+,K^+-ATPase, Mg^{2+}-ATPase and 5′nucleotidase were determined as described by Wisher and Evans [25] in both the homogenate and isolated plasma membranes. Na^+, K^+-ATPase and 5′nucleotidase were enriched 10–15-fold above homogenate activity, Mg^{2+}-ATPase only 3–4-fold, thus indicating preferential enrichment of basolateral membranes [25].

Further characterization of the membrane preparation was described in detail in a previous paper [26]. In brief: the enrichment of the glucagon-stimulated adenylate cyclase (19-fold) and alkaline phosphatase (3-fold) as well as a phospholipid/cholesterol molar ratio of 0.43 are also in agreement with a predominantly basolateral origin of the membranes. Cross-contamination with intracellular organelles is low as indicated by the 1.9- and 0.9-fold enrichment of glucose-6-phosphatase (endoplasmic reticulum) and succinate dehydrogenase (mitochondria), respectively.

In order to test the effects of lipid solvents on enzyme activities the solvents were added to the enzyme reaction mixtures at the concentrations given

* Abbreviations: c-hexane, cyclohexane; DCM, dichloromethane; DPH, 1,6-diphenyl-1,3,5-hexatriene.

in the text. Basal enzyme activities in four different preparations were (μmol/mg protein × hr; ±SD): Na^+,K^+-ATPase 11.3 ± 2.2; Mg^{2+}-ATPase 12.7 ± 1.6; 5'nucleotidase 28.9 ± 2.4.

Measurements of fluorescence anisotropy. Membrane fluidity was measured by fluorescence polarization according to Schachter and Shinitzky [27] with the following modifications: the fluorescence probe DPH was dissolved in tetrahydrofurane (2 mM) and diluted to a final concentration of 0.1 mM in a solution containing 50 mM Tris–HCl pH 7.4, 0.5 mM $CaCl_2$ by sonication for 30 min (Labsonic, Braun, Melsungen, Germany). The membrane stock solution was diluted before use with the same buffer to give a protein concentration of 1 mg/mL. Two milliliters of the DPH-containing solution and 100 mL of the membrane vesicle suspension were mixed and incubated for 1 hr in order to incorporate DPH into the membranes. Fluorescence polarization was measured at 25° in aliquots before and immediately after addition of lipid solvents in a covered quartz cuvette on an Aminco 2800 spectrofluorometer (American Instrument Co., U.S.A.) equipped with excitation and emission polarization filters (excitation 360 nm, emission 430 nm). Measurements were repeated with stepwise increasing concentrations of the solvents. Covering of the cuvette was necessary in order to avoid evaporation of the volatile substances. When allowing for evaporation, fluorescence anisotropy rapidly returns to control values. Fluorescence anisotropy (r) was calculated from emission intensities (I) when the excitation polarizer and emission analyser were either parallel (subscript "ii") or crossed at an angle of 90° (subscript "i") using Perrin's equation:

$$r = (I_{ii} - I_i)/(I_{ii} + 2I_i).$$

Background anisotropy with either membranes alone or with the fluorescent dye alone was below 5% of experimental values and was not corrected for. For control experiments, phospholipid vesicles were prepared from 1-α-dimyristoyl-lecithin according to Chen *et al.* [28] and changes in DPH fluorescence polarization by organic solvents were measured as described for the membrane preparation.

Analytical methods and calculations. Tissue concentrations of lipid solvents in the isolated perfused organ were determined 1 min after onset of cholestasis. Liver tissue samples were frozen in liquid N_2, ground and extracted with pyridine. Concentrations of compounds in pyridine were determined by gas chromatography (Varian 2100, column 1.5 m, 3% FE 30, Chromosorb WDMCS 120–140 mesh). Potassium in the perfusion medium was measured electrochemically (Nova 6 Analyzer, Nova Biomedical, Newton, MA, U.S.A.). Octanol/water partition coefficients were calculated according to Fujita and Hansch [29]. Protein concentration was measured according to Bradford [30] with bovine serum albumin as standard. All data are expressed as means ± SD.

RESULTS

Application of organic solvents in the isolated perfused rat liver and the intact rat causes rapid and characteristic changes in bile flow. In general, initial, slight and brief stimulation of bile flow was seen within the first 1–5 min after the animals started to breath air that contained the solvent, or after solvent-saturated O_2/CO_2 was introduced into the oxygenator of the liver perfusion system. In some experiments, this transient choleresis was not seen. It might have escaped detection because of an overlap with the onset of cholestasis during the formation of a single bile drop. A substantial and sustained reduction of bile flow resulted from continuing the application of the solvent for 2–8 min either as vapor or by its infusion into the portal vein. Withdrawing the solvents resulted in slight and transient choleresis in most instances, after which bile flow rate returned to control values. Figure 1 shows an example of this triphasic pattern of bile flow changes obtained with the application (4 min) and withdrawal of DCM in the isolated perfused liver. Figure 2 summarizes the effects of the applications of CCl_4, $CHCl_3$, DCM, trichloroethylene, halothane, benzene and c-hexane.

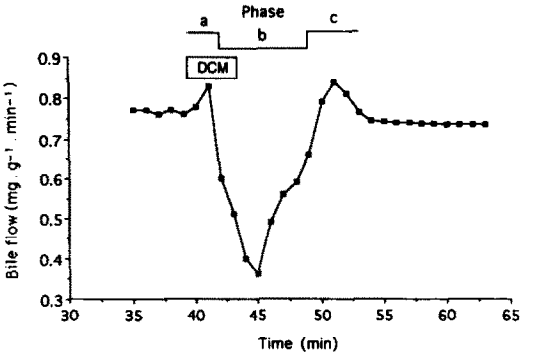

Fig. 1. Representative experiment showing the effect of DCM on bile flow in the isolated perfused rat liver. Dichloromethane was administered through the oxygen supply for 4 min as indicated. An initial rapid stimulation of bile flow (phase a) is followed by cholestasis (phase b) and, upon removal of the solvent, slight choleresis is observed (phase c) before bile flow returns to control secretory rate.

Despite their chemical heterogeneity, all the solvents applied share a high lipophilicity and are able to penetrate lipid bilayers. This ability appeared to be of biological significance in that tissue concentrations required to produce cholestasis were inversely related to the lipophilicity of the compound. This correlation is apparent from Fig. 3 which relates the octanol/water partition coefficient of individual solvents to their liver tissue concentrations measured at the onset of cholestasis. With the exception of c-hexane, in general, lipid solvents with a higher octanol/water partition coefficient required lower total tissue concentrations to inhibit bile salt independent bile flow in the isolated perfused liver.

The correlation of lipophilicity with the ability to inhibit hepatobiliary transport was largely independent of the chemical structure of the individual solvent which indicates that physical interaction of the solvent with membrane lipids could

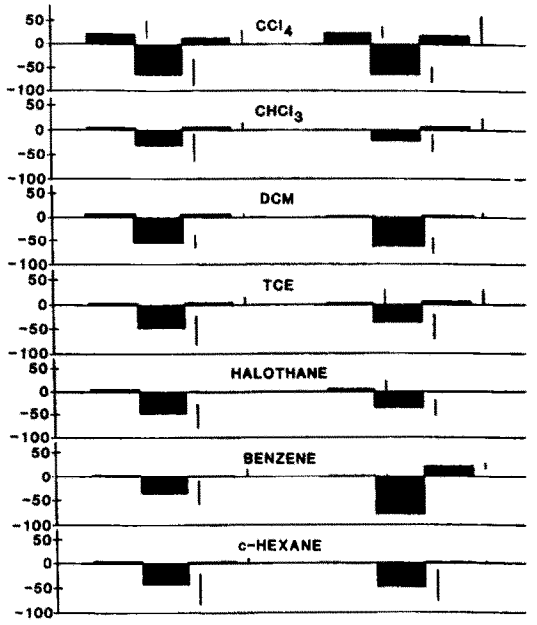

Fig. 2. Changes in bile flow rate obtained through administration and withdrawal of lipid solvents and inhalation anesthetics. Left: *in vivo* experiments, right: liver perfusion experiments. In accord with Fig. 1 the triphasic pattern is represented in % change from control bile flow rate. Column heights represent mean values of maximal choleresis or cholestasis obtained during individual phases. Bars to the right of the columns: ranges of maximal effects. All experiments were repeated at least three times. Substantial cholestasis is produced by all compounds. Cholestasis is preceded and followed by variable choleresis during administration and after withdrawal of the solvents, respectively. Note the lack of initial choleresis after application of $CHCl_3$ and c-hexane (liver perfusion) and of recovery choleresis after halothane (liver perfusion and *in vivo*).

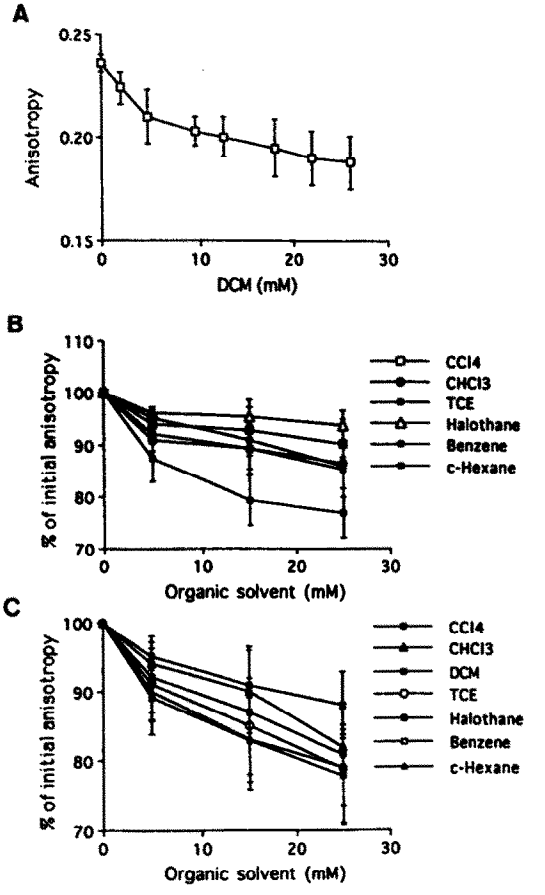

Fig. 4. Effects of lipid solvents on fluidity of lipid membranes determined by measurement of fluorescence anisotropy using DPH. (A) DCM was added to isolated basolateral liver plasma membrane suspensions. Stepwise increase in solvent concentration resulted in increase in membrane fluidity (decrease in anisotropy). (B) Relative changes in anisotropy of basolateral liver plasma membranes produced by different organic solvents. Mean values are represented in % of initial anisotropy obtained from three membrane preparations (±SD). (C) Dependence of membrane fluidity on lipid solvent concentrations in artificial lipid vesicles.

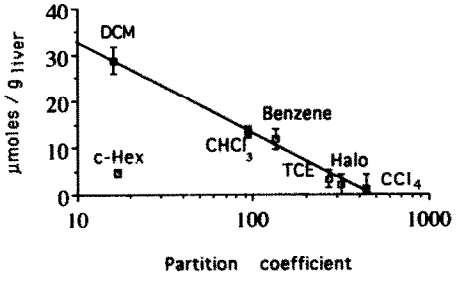

Fig. 3. Tissue concentrations of organic solvents required to produce cholestasis in the isolated perfused liver are plotted against octanol/water partition coefficients. Solvents were administered via the oxygen supply. Tissue samples were taken from the perfused liver after exposure to solvent at the onset of cholestasis and solvent concentrations were determined by gas chromatography as described in Materials and Methods.

be responsible for cholestasis. Therefore, we studied the influence of the solvents on the fluidity of liver cell membrane lipids. Using DPH as sensor and measuring the fluorescence polarization, we determined the anisotropy value of basolateral membranes. In six preparations the *r*-value was 0.234 ± 0.012 (see Materials and Methods). As shown in Fig. 4b, the anisotropy decreased with increasing concentrations of the individual solvent. All solvents effectively increased basolateral liver cell membrane fluidity at concentrations comparable to tissue concentrations obtained during cholestasis, with c-hexane as the most effective. Nearly the same pattern of increasing membrane fluidity with increasing solvent concentrations was observed in artificial lipid vesicles, but with c-hexane being only moderately potent (Fig. 4c).

Preferential partition of the solvent into the lipid membrane phase and simultaneous disturbance of the ordered membrane structure thus appeared necessary for altering membrane transport function so as to cause inhibition of bile secretion. With respect to the complexity of transport mechanisms that cooperate in bile formation, a multitude of individual membrane enzymes, membrane carriers or ion channels could possibly be affected by changes in membrane lipid structure. The following experiments were thus restricted to measure the principal effects of a particular lipid solvent on specific membrane functions. The studies included the effects on selected membrane enzymes activities and on membrane K^+ transport. In particular, we measured the concentration-dependent effect of DCM on the activities of Na^+,K^+-ATPase, 5'nucleotidase and Mg^{2+}-ATPase in isolated plasma membranes, and the effects of the other solvents on these enzymes at selected concentrations. We determined that all solvents induce K^+ uptake by the perfused liver, and, for DCM, we showed that this K^+ uptake is due to inhibition of K^+ efflux through Ba^{2+}-sensitive K^+ channels.

At increasing concentrations DCM exhibited a biphasic effect on Na^+,K^+-ATPase activity in basolateral rat liver plasma membranes. Up to a concentration of 5 mM no influence was detectable, at concentrations around 10 mM the enzyme activity was stimulated, whereas higher concentrations exhibited a slight inhibitory effect. 5'Nucleotidase was also stimulated at concentrations around 10 mM, but was inhibited at higher concentrations. In contrast, Mg^{2+}-ATPase activity was moderately inhibited over the entire concentration range studied (Fig. 5a). Analogous effects on enzyme activities were obtained in single experiments at selected concentrations for each one of the other solvents (Fig. 5b).

Effects on K^+ transport were studied in the isolated perfused organ. K^+ movements were determined from porto-caval K^+ concentration differences and solvents were applied by infusion into the portal vein to reveal perfusate concentrations of 3 mM. This concentration was applied because with all individual solvents bile flow changes and K^+ movements were fully reversible. Application of each solvent resulted in uptake of K^+ that reached near steady state after 5–6 min. Steady state uptake rates ranged from 7.6 (DCM) to 13.2 (CCl_4) nmol K^+/g liver × sec and were negatively correlated to the lipophilicity of the solvent (Fig. 6c). Removal of the solvent led to a commensurate release of the accumulated K^+ (Fig. 6). These effects occurred in parallel with the inhibition and recovery of bile flow rate, respectively (data not shown).

Since liver cells control their K^+ content through the balanced operation of uptake via the Na^+,K^+ pump and efflux through membrane K^+ channels, the observed net uptake could result from either stimulation of the basolateral Na^+,K^+-ATPase or from inhibition of K^+ channels. These two possibilities were investigated by studying the effects of inhibition of the pump by ouabain and of blocking K^+ channels with Ba^{2+}, both in the presence and absence of the lipid solvent. Application of 1 mM

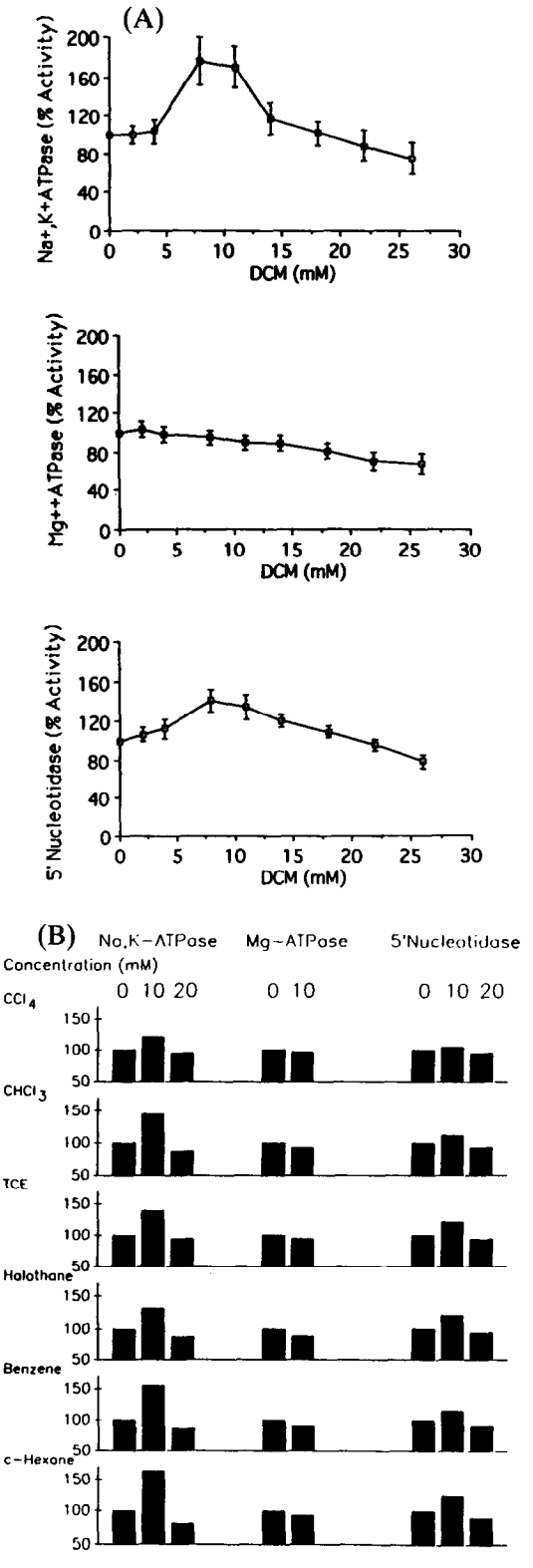

Fig. 5. Effects of lipid solvents on enzyme activities in basolateral liver cell membranes. (A) Effects of increasing concentrations of DCM on enzyme activities (% of control enzyme activity; mean values ± SD; N = 3). (B) Effects of various lipid solvents on enzyme activities at 10 and 20 mM solvent. Single experiments are shown (% of control enzyme activity).

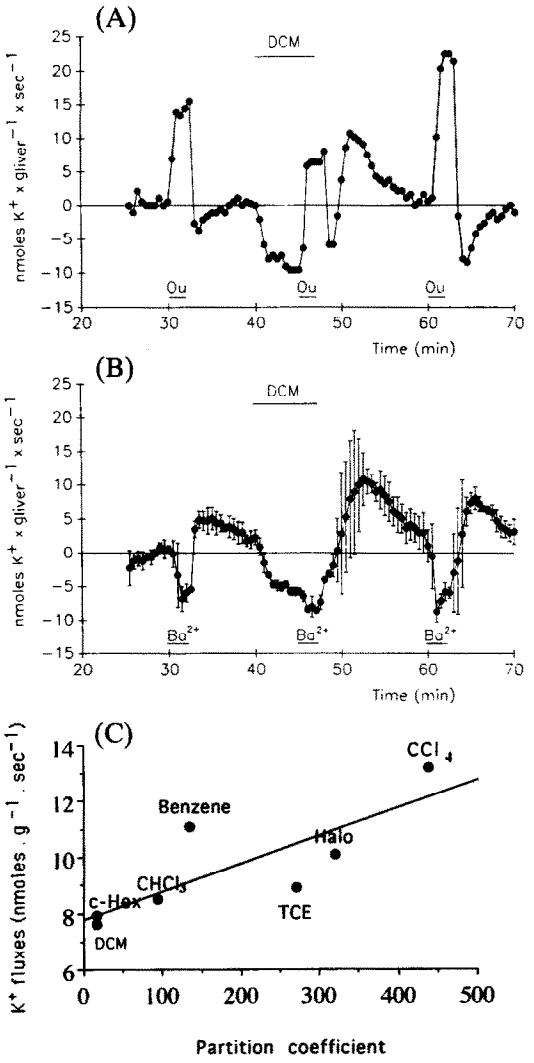

Fig. 6. Analysis of K^+ uptake caused by lipid solvents in the isolated perfused liver. Positive fluxes in (A) and (B) represent release of K^+, negative fluxes hepatic gain of K^+. (A) Effects of short (2 min) application of 1 mM ouabain in the absence and after application of DCM. Note that ouabain causes hepatic K^+ release and that K^+ uptake induced by application of DCM is interrupted by ouabain. The relative amplitude of the ouabain-induced effect is independent of the presence of the solvent. (B) Effect of application of 2 mM Ba^{2+} in the absence and after application of DCM. Note that Ba^{2+} causes hepatic uptake of K^+ and that Ba^{2+} has only a minimal effect after uptake of K^+ has been induced by application of DCM. (C) Maximal rates of hepatic K^+ gain induced by different organic solvents (application time was 10 min in all experiments) plotted against octanol/water partition coefficient.

ouabain in the balanced steady state caused instantaneous release of K^+ with an initial peak efflux of 14.0 ± 2.1 nmol K^+/g liver × sec. The effect was fully reversible by removing ouabain after a 2 min exposure period (data not shown, cf. Ref. 31). Application of ouabain in the presence of DCM (3 mM) shifted the solvent-mediated K^+ uptake towards K^+ release (Fig. 6a). This net K^+ release was smaller than that observed in the absence of the solvent, but, in the presence of the solvent, the difference between K^+ uptake before application of ouabain and efflux after application was not different from the change in K^+ balance observed in the absence of the solvent. Effects of ouabain were thus indistinguishable in their amplitude in the presence and absence of the solvent, and indicate a near equal rate of K^+ pumping by Na^+/K^+-ATPase under control conditions and during solvent-induced cholestasis. The same conclusion was also obtained from two additional experiments where halothane and trichloromethane did not affect the amplitude of ouabain-induced K^+ efflux.

In contrast to the lack of effect on pump activity, DCM exhibited a marked effect on Ba^{2+}-sensitive K^+ flux. As shown in Fig. 6b, a 2 min pulse of 2 mM Ba^{2+} during perfusion under control conditions caused immediate uptake of K^+ which was reversible upon removal of Ba^{2+} and was followed by a phase of compensatory K^+ release. After K^+ uptake had been induced by DCM, the effect of Ba^{2+} on K^+ balance was nearly abolished but it was elicited again at normal amplitude after the solvent had been removed. This inhibition of Ba^{2+}-sensitive K^+ flux indicates that K^+ uptake induced by the solvent results from inhibition of K^+ efflux through K^+ channels and that Ba^{2+} becomes ineffective if applied on top of the inhibition of K^+ efflux already induced by the solvent.

DISCUSSION

Previous studies had shown that CCl_4 [22] and volatile anesthetics [32] induce cholestasis. The rapid onset of cholestasis and its reversibility suggested to us that this effect relates to partition of the compounds into membrane lipids, altering their fluidity and secondarily affecting membrane transport processes, the rapid recovery from cholestasis resulting from the decline in tissue concentration. Chemical modulations, e.g. through radical formation, thus appeared less likely. In order to test this hypothesis we studied the effects of a series of chemically unrelated lipid solvents on bile formation, their ability to partition into lipids and to accumulate in liver tissue, and their effects on membrane fluidity. Our results support this hypothesis in that all lipid solvents tested exhibited a cholestatic effect, all solvents increased membrane fluidity, the effect being concentration dependent, and their potency to induce cholestasis was related to the octanol/water partition coefficient. One particular exception of this correlation is c-hexane, which inhibits bile flow at a considerably lower concentration than expected from its partition coefficient. In isolated plasma membranes c-hexane also increases membrane fluidity more than other solvents but in cholesterol-free liposomes it is only moderately effective (cf. Figs 2 and 4). This suggests that c-hexane might not influence the same microdomains as the other solvents. Its effects on bile flow should be closely related to the cholesterol region within the hepatic plasma membrane.

As outlined in the introduction (cf. also Ref. 1) this observation is in contrast to most other models of experimental cholestasis where a decrease in membrane fluidity rather than an increase was associated with the inhibition of bile flow. Changes of membrane lipid composition appear to be responsible for the decrease in fluidity observed after prolonged administration of the cholestatic agent in these other models (e.g. ethinyl estradiol) [33]. Such changes in lipid composition are unlikely to occur in the experiments described here and this difference from other models could account for the divergent observations on correlations between bile flow and membrane fluidity.

Two observations in this study indicate that a specific range of fluidity of membrane lipids does exist within which operation of membrane transport processes is optimal, and that a deviation from this range to either side could result in decreased bile flow: we observed a transient choleresis that preceded the solvent-induced cholestasis and, likewise, cholestasis was followed by a transient choleresis upon removal of the solvent. In these phases of choleresis, tissue solvent concentration gradually increases or decreases, respectively, and, with respect to the results shown in Fig. 4, lipid fluidity will undergo a period of moderate increase. Analogously, we observed that the activities of membrane Na^+,K^+-ATPase and 5'nucleotidase were increased by the solvents at concentrations that caused a moderate increase in fluidity, whereas higher concentrations and further increase in fluidity resulted in enzyme inhibition (Figs 4 and 5). These latter observations are in agreement with studies on the biphasic effect of organic solvents that showed activation by low and inhibition by high solvent concentrations of protein kinase activities (see Refs 34–36). Furthermore, a number of hepatic membrane enzymes are stimulated by an increase in fluidity upon administration of benzyl alcohol, but inhibition is observed when fluidity is increased beyond a critical level [37]. An optimal range of membrane fluidity thus appears to be necessary for maximal activity of a specific membrane enzyme and the same argument could apply for liver membrane transport proteins. It is conceivable therefore that both an increase and a decrease in membrane fluidity beyond a critical range could result in inhibition of bile formation.

Among the multitude of transport processes that could be affected by changes in membrane lipid fluidity we have chosen to study effects of lipid solvents on K^+ homeostasis. K^+ homeostasis is mtaintained through the balanced operation of K^+ uptake by Na^+,K^+-ATPase and of K^+ efflux through membrane K^+ channels. K^+ homeostasis plays a central role in many cell functions that include control of the internal ionic milieu, cell volume and bile secretion [38, 39]. We observed that administration of lipid solvents resulted in K^+ uptake by the liver. This observation argues against the notion that during short-term solvent application hepatocyte necrosis might be the reason for the observed cholestasis, because necrosis should be accompanied by a marked K^+ loss with a rise in K^+ concentration in the perfusion medium. During solvent-induced cholestasis we could not detect stimulation of ouabain-inhibitable K^+ uptake but efflux through Ba^{2+}-sensitive K^+ channels was dramatically reduced. The method we used is unable, though, to detect transient changes in either active K^+ transport by Na^+,K^+-ATPase or K^+ efflux through K^+ channels that might have occurred during the gradual increase in tissue solvent concentration. This latter fact needs to be emphasized because a transient activation of Na^+,K^+-ATPase would be expected from the data presented in Fig. 5 and, furthermore, an increase in membrane fluidity has been shown to increase membrane K^+ permeability in nervous tissue [18, 19]. It could be speculated however that the alterations in K^+ flux we observe are, at least in part, responsible for the changes in bile flow that are induced by the lipid solvent. This suggestion is based on the fact that: (i) Na^+,K^+-ATPase activity is required to support secondary active transport processes involved in bile formation and (ii) a relative high K^+ permeability is required to maintain the liver cell membrane potential that appears to be also involved in sustaining bile formation by supporting electrogenic transport mechanisms (see Ref. 37). Therefore, it appears possible that an initial transient choleresis is induced by activation of active K^+ transport and by an increase in K^+ permeability, whereas the sustained inhibition of bile flow may result from the observed reduction in K^+ efflux. These sequential changes in K^+ transport could depend on a gradual increase in membrane fluidity, that, as indicated above, could result in a biphasic response of membrane transport functions. The observed K^+ changes might also be accompanied by alterations in the cellular volume. However, the constant perfusion pressure during the experiments rules out microcirculatory changes being the reason for the observed cholestasis.

In conclusion, we have shown that a series of lipid solvents that included inhalation anesthetics induce cholestasis when administered via respiratory air to intact animals or the isolated perfused liver. In addition, transient increases in bile flow were observed during accumulation of the solvent in the liver and after withdrawal. We related these effects to the ability of the solvent to accumulate in the liver, to partition into membrane lipids and to change cell membrane fluidity. The data suggest that the effects on bile flow result mainly from physical interaction with membrane lipids by increasing their fluidity which, in turn, appears to alter membrane transport functions. The data support the concept that maximal enzyme activity and transport function of specific membrane proteins is obtained within a certain range of membrane lipid fluidity. Shifts of membrane fluidity from normal values may thus result in inhibition or activation of protein function.

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