Albumin stimulates the release of lysophosphatidylcholine from cultured rat hepatocytes

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The effect of albumin on the release of [^3H]lysophosphatidylcholine from cultured rat hepatocytes prelabelled with [$Me\text{-}^3\text{H}$]choline was studied. In the absence of serum and albumin from the medium, the cells released essentially no [^3H]lysophosphatidylcholine. Albumin stimulated this process dramatically, and it reached a plateau at 2 mg/ml. After an initial lag of 30 min, the release of [^3H]lysophosphatidylcholine was linear for at least 4 h. At low concentrations, albumin slightly stimulated [^3H]phosphatidylcholine release. The albumin had no measurable effect on the metabolism of cellular [^3H]phosphatidylcholine, [^3H]lysophosphatidylcholine or [^3H]glycerophosphocholine. In addition, albumin did not alter the release of ^3H -labelled water-soluble compounds, including [^3H]glycerophosphocholine, into the medium. The possibility that the [^3H]lysophosphatidylcholine was arising from catabolism of [^3H]phosphatidylcholine in the medium by secreted enzymes was excluded. The effect on [^3H]lysophosphatidylcholine secretion was also observed when the cells were incubated with α -cyclodextrin, a cyclic polysaccharide that has the ability to bind lysophosphatidylcholine. The albumin-released lysophosphatidylcholine was enriched in unsaturated fatty acids. Alteration of the fatty acid composition of cellular phosphatidylcholine gave rise to parallel changes in phosphatidylcholine and lysophosphatidylcholine in the medium. It is concluded that phosphatidylcholine is constantly being degraded in the rat hepatocyte to lysophosphatidylcholine which is released into the medium only when a suitable acceptor is present.

INTRODUCTION

In rat liver, lysophosphatidylcholine is formed by the deacylation of phosphatidylcholine by the action of phospholipases A_1 and A_2 (Thompson, 1980; Van den Bosch, 1980, 1982). Lysophosphatidylcholine is a cytotoxic agent (Weltzien, 1979) and does not accumulate, but is mainly metabolized by reacylation to phosphatidylcholine by acyltransferases (Lands, 1965; Thompson, 1980) or further deacylation to glycerophosphocholine by lysophospholipases (Thompson, 1980; Van den Bosch, 1982). Glycerophosphocholine is hydrolysed to unesterified choline and glycerophosphate by glycerophosphocholine phosphodiesterase (Dawson, 1956; Lloyd-Davies *et al.*, 1972). The liberated choline can presumably be used for phosphatidylcholine synthesis via the CDP-choline pathway (Pelech & Vance, 1984), thereby completing a cycle for phosphatidylcholine synthesis and turnover.

Lysophosphatidylcholine constitutes the second most prevalent phospholipid in the plasma of rats and several other species (Nelson, 1967), where lysophosphatidylcholine is bound to albumin (Switzer & Eder, 1965). Lysophosphatidylcholine can be produced in the plasma by the action of lecithin-cholesterol acyltransferase which catalyses the transfer of a fatty acid from the 2-position of phosphatidylcholine to cholesterol, forming cholesterol ester (Glomset, 1962). However, human patients with an inherited familial absence of lecithin-cholesterol acyltransferase and a corresponding virtual absence of plasma cholesterol ester have only marginal decreases in plasma lysophosphatidylcholine (Norum &

Gjone, 1967). Sekas *et al.* (1985) reported that, when isolated rat liver is perfused with lipid-free medium containing albumin, the accumulation of lysophosphatidylcholine exceeds that of phosphatidylcholine in the perfusate. The lysophosphatidylcholine is not formed by the hydrolysis of phosphatidylcholine in the perfusate, and must therefore originate from the phosphatidylcholine of liver. In addition, the perfusate lysophosphatidylcholine is predominantly unsaturated, unlike the highly saturated lysophosphatidylcholine resulting from lecithin-cholesterol acyltransferase activity (Glomset, 1962).

The secretion of lysophosphatidylcholine by cultured rat hepatocytes has also been observed (Mangiapan & Brindley, 1986; Graham *et al.*, 1988a). It is evident that its appearance in the medium occurs by a mechanism different from that of lipoprotein secretion because, unlike the latter, it is not stimulated by dexamethasone or inhibited by colchicine (Mangiapan & Brindley, 1986). The aim of the present work was to investigate in detail the albumin-mediated release of lysophosphatidylcholine from cultured rat hepatocytes.

EXPERIMENTAL

Animals

Male Sprague-Dawley rats (*Rattus norvegicus*) weighing 75–100 g were used. They were housed in wire-mesh cages and given water and pelleted rat diet (Wayne Lab-Blox; Continental Grain Co., Chicago, IL, U.S.A.) *ad libitum*.

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Preparation and incubation of hepatocytes

Hepatocytes were isolated by a collagenase-perfusion technique similar to that previously described (Davis *et al.*, 1979). The isolated hepatocytes were washed and resuspended in choline-, methionine- and arginine-free Dulbecco's modified Eagle's medium containing 17% (v/v) delipidated fetal-calf serum, 0.4 mM-L-ornithine, 1.7 μ M-insulin, 100 units of penicillin G/ml, 100 μ g of streptomycin sulphate/ml and 10 mM-Hepes, pH 7.4 (1×10^6 cells/ml). These cells were dispersed into plastic culture dishes (Falcon 3802 Primaria; 60 mm $\times$ 15 mm; Becton Dickinson and Co., Oxnard CA, U.S.A.) (3 ml/dish) and incubated at 37 °C under an atmosphere of air/CO₂ (19:1). Hepatocytes were maintained in monolayer culture for approx. 18 h before all experiments. This caused the cellular choline and phosphocholine pools to be partially depleted which allowed efficient incorporation of radiolabelled choline into the choline-containing phospholipids via the CDP-choline pathway.

The dishes of cultured hepatocytes were washed with 2 $\times$ 3 ml of serum-free medium (choline-, methionine- and arginine-free modified Eagle's medium described above) to remove the delipidated serum and non-viable cells. The dishes of cells were incubated for 30 min with 2 ml of serum-free medium that contained 10 μ Ci of [*Me*-³H]choline chloride (15 Ci/mmol) and then washed twice with unlabelled medium. The prelabelled cells were subsequently incubated for up to 4 h with 2 ml of serum-free medium containing 0–20 mg of fatty acid-poor bovine serum albumin/ml and 28 μ M-choline chloride (two dishes at each albumin concentration per time point).

At the end of the incubation period, the medium was removed and the monolayer of cells washed with 2 $\times$ 0.5 ml of serum-free medium. The medium and cell washings were combined, and centrifuged at 12000 *g* for 15 min to remove detached cells and cell debris. The supernatant was used for extraction and analysis of lipids and water-soluble compounds. The cells were scraped from the dishes in 2 ml of distilled water with a rubber policeman. The cell suspension was sonicated for 30 s with an ultrasonic processor W-385 (Heat Systems-Ultrasonics Inc., Farmingdale, NY, U.S.A.) with 20% duty cycle and output setting 2. A portion of the sonicated cells (0.1 ml) was taken for protein determination by the Bio-Rad assay, based on the method of Bradford (1976), and the remainder used for extraction and analysis of lipids and water-soluble compounds. Trypan Blue stain exclusion and constant cellular protein content indicated that hepatocytes were viable throughout the incubation. In support of the view that the hepatocytes were completely viable in the absence of choline, it should be noted that hepatocytes prepared from 3-day-choline-deficient rats were equally viable whether they were supplemented with choline or not (Yao & Vance, 1988). Some modifications to this standard procedure were used and are described in the legends to the Figures and Tables.

Extraction and analysis of lipids and water-soluble compounds

Lipids and water-soluble compounds were extracted from sonicated cell preparations (2 ml) and centrifuged media (3 ml) by adding 4 vol. of chloroform/methanol (2:1, v/v) containing the antioxidant 2,6-di-*t*-butyl-4-

methylphenol (50 mg/l), by the method of Folch *et al.* (1957). After centrifugation of the extract at 1000 *g* for 15 min, the upper aqueous phase was removed and the lower chloroform phase washed once with the appropriate volume of upper phase prepared from methanol/water (1:1, v/v).

A portion of the lower chloroform phase (0.2 ml) containing lipids was evaporated to dryness under N₂ in a scintillation vial. The residue was redissolved in 5 ml of scintillation fluid, and radioactivity was measured with a Beckman LS 3801 liquid-scintillation counter, which automatically corrected for quenching by the sample-channels-ratio technique.

A portion of the chloroform phase (1–4 ml, evaporated to a small volume under N₂) along with 10 μ g of lysophosphatidylcholine, sphingomyelin and phosphatidylcholine standards were applied to a silica-gel 60 t.l.c. plate (E. Merck, Darmstadt, West Germany), which was developed in the solvent system chloroform/methanol/acetic acid/water (50:30:8:3, by vol.) containing the antioxidant 2,6-di-*t*-butyl-4-methylphenol (50 mg/l). After the plate was air-dried, the lysophosphatidylcholine, sphingomyelin and phosphatidylcholine areas were made visible with I₂ vapour and scraped into scintillation vials. Scintillation fluid (5 ml) was added and radioactivity measured after 24 h.

The fatty acid composition and chemical amounts of lysophosphatidylcholine and phosphatidylcholine in the chloroform phase were determined as follows: a concentrated portion of the chloroform layer was subjected to t.l.c. as described above. The lysophosphatidylcholine and phosphatidylcholine areas on the developed plate were detected with the spray reagent Primulin (Wright, 1971) and scraped into 10 ml screw-capped tubes for immediate methanolysis. A known amount of heptadecanoic acid (usually 50 nmol) was added as an internal standard. To this sample was added 1 ml of 14% (v/v) BF₃ in methanol, and the tightly closed tube was heated in a boiling-water bath for 10 min. The methanolysis reaction was terminated by the addition of 1 ml of distilled water, and the fatty acid methyl esters were extracted into 2 ml of hexane, which was subsequently concentrated to a small volume under N₂. The fatty acid methyl esters were assayed with a Perkin-Elmer 8420 gas-liquid chromatograph coupled to a GP-100 graphics printer, by using a 1.83 m (6 ft) $\times$ 3.2 mm ($\frac{1}{8}$ in) stainless-steel column packed with 5% DEGS-PS on 100–120-mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The helium carrier-gas pressure was 170 kPa, and the injector and detector temperatures were 250 °C. The column was initially maintained at 145 °C for 2 min, after which the temperature was increased by 2 °C/min to 190 °C, and maintained for 5.5 min. The temperature was then increased by 5 °C/min to 195 °C and maintained for 17 min. The amount of each fatty acid methyl ester was determined by comparison of the area of each peak to that of the known amount of internal standard.

A portion of the upper aqueous phase (0.5 ml), containing choline, phospholine, betaine and glycerophosphocholine, was placed in a scintillation vial along with 5 ml of scintillation fluid, and radioactivity was determined.

Radioactivity associated with glycerophosphocholine was measured as follows: a portion of the aqueous phase (1–2 ml) was applied to a column (0.8 cm $\times$ 3 cm) of Dowex AG 50W (X8; 200–400 mesh; H⁺ form) cation-

exchange resin and washed with 25 ml of distilled water. The eluent exclusively contained glycerophosphocholine (Illingworth & Portman, 1972; Robinson, 1985) and a sample was taken for radioactivity determination. The specificity of this technique was checked by applying a concentrated portion of the aqueous phase along with 20 μ g of choline, phosphocholine, betaine and glycerophosphocholine standards on a silica-gel 60 t.l.c. plate, which was developed in the solvent system methanol/1.2% NaCl/NH₃ (10:10:1, by vol.). The glycerophosphocholine area was located with I₂ vapour and scraped into a scintillation vial. Radioactivity was determined after adding 5 ml of scintillation fluid and 0.5 ml of 1 M-HCl.

Chemicals

These were obtained as follows: [*Me*-³H]choline chloride (sp. radioactivity 15 Ci/mmol; radiochemical purity 98%) and liquid-scintillation fluid (ACS) from Amersham Canada Ltd., Oakville, Ontario, Canada; choline-, methionine- and arginine-free Dulbecco's modified Eagle's medium, minimum essential medium, penicillin G and streptomycin sulphate from Gibco Laboratories, Life Technologies Inc., Grand Island, NY, U.S.A.; Dowex AG 50W (X8; 200–400 mesh; H⁺ form) cation-exchange resin from Bio-Rad Laboratories, Richmond, CA, U.S.A.; 14% BF₃ in methanol from Pierce Chemical Co., Rockford, IL, U.S.A.; choline chloride, phosphorylcholine chloride (calcium salt), L- α -glycerophosphorylcholine (free base, grade V, from egg yolk), betaine hydrochloride, L- α -lysophosphatidylcholine (type I, from egg yolk), sphingomyelin (from bovine brain), L- α -phosphatidylcholine (type V-E, from egg yolk), L-ornithine hydrochloride, Hepes, insulin (from bovine pancreas), α -cyclodextrin, Trypan Blue stain (0.4%), primulin, heptadecanoic acid, 2,6-di-*t*-butyl-4-methylphenol and bovine serum albumin (essentially fatty acid-free) from Sigma Chemical Co., St. Louis, MO, U.S.A.; and fetal-calf serum from BDH Chemicals Canada Ltd., Edmonton, Alberta, Canada. The fetal-calf serum was delipidated by the method of Cham & Knowles (1976). All other chemicals were reagent grade.

RESULTS AND DISCUSSION

Release of lysophosphatidylcholine from cultured rat hepatocytes as a function of albumin concentration

The concentration of albumin in rat plasma is about 40 mg/ml, and it binds fatty acids, lysophosphatidylcholine and other metabolites (Dimopoulos, 1970; Andersson *et al.*, 1979). Lipid-poor bovine serum albumin was used in the present study to obtain maximal effects. SDS/polyacrylamide-gel electrophoresis and t.l.c. revealed that this albumin was free of contaminating proteins and lipids. Fig. 1 shows the effect of albumin concentration on the release of [³H]lipids from cultured rat hepatocytes prelabelled with [*Me*-³H]choline. The release of total [³H]lipids into the medium was enhanced with an albumin concentration as low as 0.1 mg/ml, and approached a plateau when the concentration was greater than 2 mg/ml. Since the effect on lysophosphatidylcholine release was observed at such a low concentration of protein, a non-specific osmolality effect is unlikely. [³H]Lysophosphatidylcholine and [³H]phosphatidylcholine accounted for about 80% of the labelled lipids in the medium. The enhanced release

Fig. 1. Effect of albumin concentration on the release of [³H]-lipids from cultured rat hepatocytes prelabelled with [*Me*-³H]choline

Rat hepatocytes, cultured for approx. 18 h in choline-, methionine- and arginine-free Dulbecco's modified Eagle's medium containing delipidated fetal-calf serum, were incubated for 30 min in 2 ml of serum-free medium containing 10 μ Ci of [*Me*-³H]choline. The prelabelled hepatocytes were subsequently incubated for 1 h in 2 ml of serum-free medium containing 0–20 mg of fatty acid-poor bovine serum albumin/ml and 28 μ M-choline. Media were collected, and the ³H radioactivity associated with total lipids (■), lysophosphatidylcholine (▲) and phosphatidylcholine (□) was determined as described in the Experimental section. Each point represents the mean of two dishes, and the ranges were less than 5% of the mean. This experiment was performed three times with similar results.

of [³H]lipids from cultured hepatocytes by albumin was mainly associated with [³H]lysophosphatidylcholine (Fig. 1). Albumin binds lysophosphatidylcholine in a molar ratio of about 1:1 (Switzer & Eder, 1965; Klopfenstein, 1969), and non-esterified fatty acids in a molar ratio of approx. 1:3–8 (Spector, 1986). To ensure that the binding capacity of albumin for lysophosphatidylcholine and non-esterified fatty acids was not exceeded in subsequent experiments, an albumin concentration of 5 mg/ml (equivalent to about 150 nmol/dish) was used.

Release of lysophosphatidylcholine from cultured rat hepatocytes by albumin as a function of time

A time course of the albumin-mediated release of [³H]-lipids from cultured rat hepatocytes prelabelled with [*Me*-³H]choline is presented in Fig. 2. The release of total [³H]lipids into the medium approximately doubled in the presence of albumin through the first 1 h and continued to increase between 1 and 4 h (Fig. 2a). The enhanced release of total [³H]lipids during the 1–4 h time period was almost entirely due to the presence of [³H]-lysophosphatidylcholine (Figs. 2a and 2b). It is striking

Fig. 2. Time course of albumin-mediated release of [^3H]lipids from cultured rat hepatocytes prelabelled with [$\text{Me-}^3\text{H}$]choline

Rat hepatocytes, cultured for approx. 18 h in choline-, methionine- and arginine-free Dulbecco's modified Eagle's medium containing delipidated fetal-calf serum, were incubated for 30 min in 2 ml of serum-free medium containing 10 μCi of [$\text{Me-}^3\text{H}$]choline. The prelabelled hepatocytes were subsequently incubated for up to 4 h in 2 ml of serum-free medium containing 28 μM -choline with and without 5 mg of fatty acid-poor bovine serum albumin/ml. Media were collected at the times indicated, and ^3H radioactivity associated with (a) total lipids (with albumin, $\blacksquare$; without albumin, $\square$), (b) lysophosphatidylcholine (with albumin, $\blacktriangle$; without albumin, $\triangle$) and (c) phosphatidylcholine (with albumin, $\blacksquare$; without albumin, $\square$) was determined as described in the Experimental section. Each point represents the mean of two dishes, and the ranges were less than 5% of the mean. This experiment was repeated twice with similar results.

Fig. 3. Effect of albumin on the metabolism of [^3H]lipids in cultured rat hepatocytes prelabelled with [$\text{Me-}^3\text{H}$]choline

Rat hepatocytes were treated as described for Fig. 2. Cells were collected at the times indicated, and the ^3H radioactivity associated with (a) total lipids (with albumin, $\blacksquare$; without albumin, $\square$), (b) phosphatidylcholine (with albumin, $\blacksquare$; without albumin, $\square$), (c) lysophosphatidylcholine (with albumin, $\blacktriangle$; without albumin, $\triangle$) was determined as described in the Experimental section. Each point represents the mean of two dishes, and the ranges were less than 5% of the mean.

that in the absence of albumin virtually no [^3H]lysophosphatidylcholine was released into the medium. It is noteworthy that during the first 1 h [^3H]phosphatidylcholine release was stimulated by albumin (Fig. 2c). The release of [^3H]phosphatidylcholine exceeded that of [^3H]lysophosphatidylcholine during the first 30 min in the presence of albumin (Figs. 2b and 2c). This phosphatidylcholine was probably albumin-bound rather than associated with lipoproteins, as secretion of very-low-density lipoproteins from cultured rat hepatocytes has been reported to be inhibited by albumin in the medium (Davis *et al.*, 1980).

Figs. 3(a) and 3(b) show that albumin caused no detectable change in the total [^3H]lipids or [^3H]phosphatidylcholine of cultured rat hepatocytes

during the 4 h incubation. There was a small decrease in cellular [^3H]lysophosphatidylcholine in the presence of albumin, but this was insufficient to account for the observed increase in [^3H]lysophosphatidylcholine in the medium (Fig. 3c). The treatment of hepatocytes with albumin for 4 h did not appear to modulate the total ^3H -labelled water-soluble compounds (betaine, choline, phosphocholine and glycerophosphocholine) or [^3H]glycerophosphocholine in the cells, or the production of total ^3H -labelled water-soluble compounds (betaine, choline, phosphocholine and glycerophosphocholine) or [^3H]glycerophosphocholine in the medium. Although there was clearly an increase in [^3H]lipids in the medium (mainly as [^3H]lysophosphatidylcholine) when [$\text{Me-}^3\text{H}$]choline-labelled cultured hepatocytes were incubated

Fig. 4. [³H]Lysophosphatidylcholine in the medium is not formed by the hydrolysis of [³H]phosphatidylcholine in the medium

Rat hepatocytes, cultured for approx. 18 h in choline-, methionine- and arginine-free Dulbecco's modified Eagle's medium containing delipidated fetal-calf serum, were incubated for 30 min in 2 ml of serum-free medium containing 10 μ Ci of [*Me*-³H]choline. The prelabelled hepatocytes were subsequently incubated for 4 h in 2 ml of serum-free medium containing 28 μ M-choline and 5 mg of fatty acid-poor bovine serum albumin/ml. Medium (combined from ten dishes) was centrifuged at 12000 *g* for 15 min and further incubated for up to 5 h in the absence of cells. Samples of this medium (2 ml) were collected at the times indicated, and the ³H radioactivity associated with lysophosphatidylcholine (▲), phosphatidylcholine (■) and glycerophosphocholine (◇) was determined as described in the Experimental section. Each point represents the mean of duplicate analyses, and the ranges were less than 5% of the mean. This experiment was performed twice with similar results.

for 4 h in the presence of albumin (Fig. 2a), it could not be accounted for by changes in the cellular [³H]lipids and ³H-labelled water-soluble compounds and in ³H-labelled water-soluble compounds in the medium. It is evident that the magnitude of the radioactivity in these compounds in the cells and medium was sufficiently large to mask the changes caused by albumin.

Fig. 4 shows that further incubation of medium removed from [*Me*-³H]choline-labelled cultured rat hepatocytes previously incubated with albumin for 4 h resulted in no appreciable change in [³H]-lysophosphatidylcholine, [³H]phosphatidylcholine or [³H]glycerophosphocholine. This demonstrates that the [³H]lysophosphatidylcholine in the medium did not originate from the hydrolysis of [³H]phosphatidylcholine present in the medium by secreted enzymes (e.g. lecithin-cholesterol acyltransferase, hepatic lipase), and must have arisen from the degradation of hepatocyte [³H]phosphatidylcholine and was trapped in

Fig. 5. Time course of α -cyclodextrin- and albumin-mediated release of [³H]lysophosphatidylcholine from cultured rat hepatocytes prelabelled with [*Me*-³H]choline

Rat hepatocytes, cultured for approx. 18 h in choline-, methionine- and arginine-free Dulbecco's modified Eagle's medium containing delipidated fetal-calf serum, were incubated for 30 min in 2 ml of serum-free medium containing 10 μ Ci of [*Me*-³H]choline. The prelabelled hepatocytes were subsequently incubated for up to 4 h in 2 ml of serum-free medium containing 28 μ M-choline with and without 5 mg of α -cyclodextrin/ml or 5 mg of fatty acid-poor bovine serum albumin/ml. Media were collected at the times indicated, and the ³H radioactivity associated with lysophosphatidylcholine (with α -cyclodextrin, ■; with albumin, ▲; and without α -cyclodextrin and albumin, △) was determined as described in the Experimental section. Each point represents the mean of two dishes, and the ranges were less than 5% of the mean. This experiment was repeated twice with similar results.

the medium by albumin. In addition, the [³H]-lysophosphatidylcholine in the medium underwent only marginal degradation to [³H]glycerophosphocholine, suggesting that the [³H]glycerophosphocholine in the medium was also released from the hepatocytes. These results are consistent with those of Sekas *et al.* (1985), which indicated that the isolated rat liver perfused with lipid-free medium containing albumin directly secretes lysophosphatidylcholine generated from the hydrolysis of hepatic phosphatidylcholine.

Hepatic lipase, synthesized and secreted by parenchymal cells, becomes loosely attached to non-parenchymal cell types (e.g. endothelial cells) in the liver, and the lipase is heparin-releasable (Jansen *et al.*, 1978; Nilsson-Ehle *et al.*, 1980). Pretreatment of cultured rat hepatocytes with heparin did not affect the production of [³H]lysophosphatidylcholine in the medium due to the presence of albumin. This suggests that any hepatic lipase bound to the isolated parenchymal cells used in this study was not involved in the production of albumin-released [³H]lysophosphatidylcholine.

Table 1. Fatty acid compositions of cellular phosphatidylcholine and of lysophosphatidylcholine in the medium after rat hepatocytes (previously cultured with fetal-calf serum in the presence of choline and methionine) had been incubated with albumin

Rat hepatocytes were cultured for approx. 18 h in minimum essential medium containing 17% fetal-calf serum, 28 μ M-choline, 200 μ M-L-methionine, 1.7 μ M-insulin, 100 units of penicillin G/ml, 100 μ g of streptomycin sulphate/ml and 26.2 mM- NaHCO_3 , pH 7.4 (1×10^6 cells/ml, 3 ml/dish). The hepatocytes were subsequently incubated for up to 10 h in 2 ml of serum-free medium containing 5 mg of fatty acid-poor bovine serum albumin/ml. Cells and medium (combined from ten dishes) were collected at the times indicated, and the fatty acid compositions of the cellular phosphatidylcholine and of lysophosphatidylcholine in the medium were determined as described in the Experimental section. Each value is the mean $\pm$ range of duplicate analyses. This experiment was repeated once with similar results. Abbreviations used: PC, phosphatidylcholine; LPC, lysophosphatidylcholine; UFA:SFA, ratio of unsaturated fatty acids to saturated fatty acids.

Phospholipid	Incubation time (h)	Fatty acid composition (mol%)								UFA:SFA	Amount of phospholipid (nmol/dish)
		C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:3}	C _{20:4}	C _{22:6}		
Cellular PC	0	25.6±0.6	2.8±0.6	21.2±0.1	7.3±0.2	13.3±0.2	1.1±0.1	19.9±0.7	8.8±0.6	1.14	175.6±4.5
	1	25.2±0.7	3.6±0.2	20.5±0.2	7.7±0.1	13.5±0.1	1.2±0.1	19.7±0.3	8.6±0.3	1.19	176.5±3.0
	2	24.8±0.6	3.6±0.1	20.1±0.2	7.8±0.1	13.4±0.0	1.2±0.2	20.2±0.2	8.9±0.3	1.23	173.9±0.6
	5	25.2±0.7	3.5±0.1	20.2±0.1	7.9±0.1	13.2±0.1	1.1±0.1	20.5±0.6	8.4±0.6	1.20	178.4±3.8
	10	24.8±0.3	2.9±0.4	20.6±0.1	8.4±0.2	12.4±0.1	1.0±0.1	21.6±0.3	8.3±0.2	1.20	182.9±5.6
Medium LPC	1	20.7±0.2	6.5±0.3	21.4±0.9	13.8±0.5	15.1±0.6	2.6±0.4	14.4±0.2	5.5±0.2	1.38	2.8±0.1
	2	18.8±0.3	5.6±0.3	18.2±0.2	13.5±0.4	15.8±0.2	2.5±0.1	18.7±0.4	6.9±0.3	1.70	3.6±0.2
	5	18.4±0.2	4.8±0.2	15.4±0.2	11.6±0.1	16.4±0.2	1.9±0.0	22.9±0.0	8.6±0.1	1.96	11.7±0.1
	10	19.8±1.1	4.3±0.2	15.1±0.2	12.1±0.9	15.3±0.9	1.5±0.4	23.6±0.7	8.3±0.4	1.87	18.6±0.6
	1	28.2±1.5	5.2±0.1	18.1±0.2	11.5±1.5	13.5±1.1	1.7±0.2	15.2±0.6	6.6±0.7	1.16	2.2±0.1
Medium PC	2	27.4±0.8	5.7±1.5	18.5±0.9	12.5±0.4	14.5±0.4	1.5±0.3	13.9±1.4	6.0±0.5	1.18	2.5±0.0
	5	26.2±0.7	3.9±0.2	20.1±0.4	9.9±0.4	13.5±0.6	1.5±0.4	17.0±0.2	7.9±0.3	1.16	5.9±0.1
	10	26.0±0.8	5.1±0.7	19.2±0.2	11.0±0.3	14.1±0.1	1.6±0.2	16.7±0.8	6.3±0.5	1.21	9.6±0.3

Release of lysophosphatidylcholine from cultured rat hepatocytes can also be stimulated by α -cyclodextrin

It was important to provide evidence that albumin released lysophosphatidylcholine from hepatocytes in the previous experiments because of its ability to bind lysophosphatidylcholine. Hence, the molecular encapsulating agent α -cyclodextrin was incubated with hepatocytes to determine if it could also release lysophosphatidylcholine. α -Cyclodextrin consists of six glucose residues arranged in a rigid conical molecular structure with a hollow interior that is capable of forming inclusion complexes with polar hydrophobic molecules (Szejtli, 1982). A time course of the release of [3 H]lysophosphatidylcholine from cultured rat hepatocytes prelabelled with [Me - 3 H] choline in the presence of α -cyclodextrin and albumin is presented in Fig. 5. It appears that α -cyclodextrin was more effective than albumin, on a mass basis, at releasing [3 H]-lysophosphatidylcholine into the medium during the 4 h incubation period. Since the M_r of α -cyclodextrin is 973 and that of albumin is 66000, on a molar basis albumin was considerably more effective as an acceptor. The dimensions of the internal cavity of α -cyclodextrin were apparently large enough to accommodate the acyl chain of the [3 H]lysophosphatidylcholine. One explanation for the differences in lysophosphatidylcholine released during the 4 h incubation (Fig. 5) is that lysophosphatidylcholine is more readily taken up by the hepatocytes from albumin than from α -cyclodextrin. All cellular membranes, including plasma membranes, have a high affinity for lysophosphatidylcholine (Ansell & Spanner, 1982).

It is evident from these results that albumin, like α -cyclodextrin, releases lysophosphatidylcholine from hepatocytes owing to its lipid-complexing properties, and acts as a reservoir for this lipid in the medium. Albumin and α -cyclodextrin presumably stimulate release of lysophosphatidylcholine (from the plasma membrane) that is present transiently during phosphatidylcholine degradation. In the absence of albumin or α -cyclodextrin, lysophosphatidylcholine undergoes reacylation with acyl-CoAs (Lands, 1965) or deacylation by lysophospholipase activity (Van den Bosch, 1982). Consequently, with albumin or α -cyclodextrin present there is a competition for the product of phospholipase A activity between the acylating enzyme and the lipid-complexing compounds. The release of lysophosphatidylcholine is likely to be favoured because of the additional depletion of cellular non-esterified fatty acids and acyl-CoAs by albumin and cyclodextrin (Dimopoulos, 1970; Andersson *et al.*, 1979). Plasma lipoproteins also have the ability to bind lysophosphatidylcholine (Portman & Illingworth, 1973), and at high concentrations might also stimulate release of lysophosphatidylcholine from the liver.

Fatty acid composition of lysophosphatidylcholine released from cultured rat hepatocytes by albumin

An investigation was made on the fatty acid composition of albumin-released lysophosphatidylcholine from rat hepatocytes cultured under different conditions. Table 1 shows the fatty acid compositions of cellular phosphatidylcholine and of lysophosphatidylcholine and phosphatidylcholine in the medium after rat hepatocytes [previously cultured with fetal-calf serum (non-delipid-

Table 2. Fatty acid compositions of cellular phosphatidylcholine and lysophosphatidylcholine and phosphatidylcholine in the medium after rat hepatocytes (previously cultured with delipidated fetal-calf serum in the absence of choline and methionine) had been incubated with albumin

Rat hepatocytes were cultured for approx. 18 h in choline-, methionine- and arginine-free Dulbecco's modified Eagle's medium containing delipidated fetal-calf serum. The hepatocytes were subsequently incubated for up to 5 h in 2 ml of serum-free medium containing 5 mg of fatty acid-poor bovine serum albumin/ml and 28 μ M-choline. Cells and medium (combined from ten dishes) were collected at the times indicated, and the fatty acid compositions of the cellular phosphatidylcholine and of lysophosphatidylcholine and phosphatidylcholine in the medium were determined as described in the Experimental section. Each value is the mean $\pm$ range of duplicate analyses. This experiment was repeated once with similar results. Abbreviations used: PC, phosphatidylcholine; LPC, lysophosphatidylcholine; UFA: SFA, ratio of unsaturated fatty acids to saturated fatty acids.

Phospholipid	Incubation time (h)	Fatty acid composition (mol %)								UFA :SFA	Amount of phospholipid (nmol/dish)
		C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:3}	C _{20:4}	C _{22:6}		
Cellular PC	0	37.0±0.2	2.7±0.1	19.7±0.1	8.7±0.2	11.5±0.2	1.8±0.3	14.1±0.3	4.5±0.1	0.76	195.6±1.5
	1	39.6±0.1	2.6±0.0	23.6±0.1	8.3±0.1	8.9±0.1	0.0±0.0	13.3±0.3	3.7±0.2	0.58	174.8±2.4
	2	35.6±0.1	2.9±0.0	19.2±0.1	9.5±0.0	11.4±0.0	1.5±0.0	15.3±0.1	4.6±0.1	0.83	192.3±1.8
	5	37.0±0.2	3.2±0.1	17.4±0.1	10.9±0.1	11.5±0.1	1.2±0.1	14.4±0.1	4.4±0.0	0.84	183.3±2.2
Medium LPC	1	38.8±0.3	2.6±0.0	25.7±0.2	13.3±0.1	10.3±0.1	0.0±0.0	7.6±0.4	1.7±0.4	0.55	7.0±0.1
	2	37.7±0.2	3.1±0.0	22.3±0.3	13.6±0.1	11.1±0.4	0.0±0.0	9.7±0.4	2.5±0.0	0.67	9.7±0.0
	5	34.8±0.4	4.3±0.3	16.5±0.2	15.2±0.2	14.2±0.2	0.0±0.0	12.2±0.1	2.8±0.2	0.95	17.4±0.2
	1	43.3±0.4	0.0±0.0	21.2±0.7	15.4±0.1	12.2±0.1	0.0±0.0	7.9±0.1	0.0±0.0	0.55	1.6±0.0
Medium PC	2	43.3±0.6	5.6±0.1	18.2±0.2	13.3±0.1	11.9±0.1	0.0±0.0	7.7±0.1	0.0±0.0	0.63	2.1±0.0
	5	36.8±0.6	2.8±0.0	19.2±0.1	11.5±0.1	12.7±0.4	0.0±0.0	12.0±0.1	5.0±0.0	0.79	8.0±0.1

ated) in the presence of choline and methionine] had been incubated with albumin for up to 10 h. The fatty acid composition of the cellular phosphatidylcholine was similar to that previously reported for rat hepatocytes (Vance & Vance, 1985, 1986). The ratio of unsaturated to saturated fatty acids of the cellular phosphatidylcholine remained constant throughout the 10 h incubation period with albumin. The lysophosphatidylcholine released into the medium owing to the presence of albumin was mainly unsaturated, which is consistent with the finding of Sekas *et al.* (1985) using isolated perfused rat livers. The ratio of unsaturated to saturated fatty acids of the lysophosphatidylcholine in the medium increased during the incubation, which was predominantly due to an increase in arachidonic acid ($C_{20:4}$) and docosahexaenoic acid ($C_{22:6}$) and a decrease in stearic acid ($C_{18:0}$). The unsaturated to saturated fatty acid ratio of the phosphatidylcholine in the medium remained constant throughout the albumin treatment and reflected that of the cellular phosphatidylcholine. Although the unsaturated to saturated fatty acid ratios of phosphatidylcholine in the medium and in cells were similar, the distribution of the various unsaturated fatty acids differed. For instance, there is a greater relative content of oleic acid ($C_{18:1}$) and a lower content of arachidonic acid ($C_{20:4}$) and docosahexaenoic acid ($C_{22:6}$) in phosphatidylcholine in the medium compared with the cellular phosphatidylcholine. This implies that there is some selectivity in the types of phosphatidylcholine that are secreted into the medium. The fatty acid composition of the medium phosphatidylcholine was consistent with previous reports (Vance *et al.*, 1986). Throughout the incubation with albumin, the absolute amounts of lysophosphatidylcholine and phosphatidylcholine in the medium increased. The amount of lysophosphatidylcholine released into the medium was always higher than that of phosphatidylcholine. There was no appreciable change in the amount of cellular phosphatidylcholine with time (Table 1).

The highly unsaturated lysophosphatidylcholine released from the cultured rat hepatocytes by albumin (Table 1) is markedly dissimilar to the highly saturated lysophosphatidylcholine formed by lecithin-cholesterol acyltransferase in plasma (Glomset, 1962). The lysophosphatidylcholine in rat plasma is mainly unsaturated (Sekas *et al.*, 1985). The predominantly unsaturated lysophosphatidylcholine released from hepatocytes by albumin most likely originates by the action of phospholipases A_1 and A_2 (phospholipase A_1 being more active) on phosphatidylcholine in the plasma membrane. This is assuming that the phosphatidylcholine has a normal fatty acid distribution, i.e. saturated and unsaturated fatty acids mainly present at the 1 and 2 positions respectively. The results in Table 1 imply that arachidonic acid ($C_{20:4}$) and docosahexaenoic acid ($C_{22:6}$) are preferentially incorporated into the phosphatidylcholine that is the precursor of lysophosphatidylcholine released from hepatocytes by albumin. Graham *et al.* (1988a) demonstrated that the secretion of lysophosphatidylcholine from rat hepatocytes is preferentially stimulated by exogenously supplied unsaturated fatty acids and that these fatty acids are incorporated into the lysophosphatidylcholine. The increasing proportion of arachidonic acid ($C_{20:4}$) and docosahexaenoic acid ($C_{22:6}$) in the released lysophosphatidylcholine with time (Table 1) might then arise from their early release by a phospho-

lipase A_2 and their preferential incorporation into lysophosphatidylcholine. Graham *et al.* (1988a) have suggested that this transport of lysophosphatidylcholine may be physiologically important in supplying extrahepatic tissues with essential fatty acids and choline.

Because the labelling experiments were done in hepatocytes cultured in the absence of choline, methionine and serum lipids, it was pertinent to analyse the fatty acid composition of choline phospholipids under these conditions. The fatty acid compositions of cellular phosphatidylcholine and of lysophosphatidylcholine and phosphatidylcholine in the medium after rat hepatocytes (previously cultured with delipidated fetal-calf serum in the absence of choline and methionine) had been incubated with albumin for up to 5 h is depicted in Table 2. Depletion of choline, methionine and serum lipids resulted in the cellular phosphatidylcholine and lysophosphatidylcholine and phosphatidylcholine in the medium being predominantly saturated, which was in direct contrast with previous observations (Table 1). The phospholipids contained an unusually high amount of palmitic acid ($C_{16:0}$) and low amounts of arachidonic acid ($C_{20:4}$) and docosahexaenoic acid ($C_{22:6}$). The unsaturated to saturated fatty acid ratio of the lysophosphatidylcholine and phosphatidylcholine in the medium increased during the incubation with albumin. This was mainly due to an increase in linoleic acid ($C_{18:2}$) and arachidonic acid ($C_{20:4}$) and a decrease in palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$). The results in Tables 1 and 2 show that the fatty acid composition of the lysophosphatidylcholine released into the medium by albumin is dependent on that of the cellular phosphatidylcholine.

The results of the present work and the data of Sekas *et al.* (1985) and Graham *et al.* (1988a,b) suggest that the albumin-mediated release of lysophosphatidylcholine from the liver makes a substantial contribution to the plasma lysophosphatidylcholine of rats. The hepatic enzymes involved in this process need to be characterized. In addition, it remains to be determined whether lysophosphatidylcholine is also released from extrahepatic tissues by albumin.

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REFERENCES

- Andersson, L. O., Birke, G., Liljedahl, S.-O. & Rothschild, M. (1979) in *Plasma Proteins* (Blomback, B. & Hanson, L. A., eds.), pp. 43–71, John Wiley and Sons, Chichester and New York
- Ansell, G. B. & Spanner, S. (1982) *New Compr. Biochem.* **4**, 29
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Cham, B. E. & Knowles, B. R. (1976) *J. Lipid Res.* **17**, 176–181
- Davis, R. A., Engelhorn, S. C., Pangburn, S. H., Weinstein, D. B. & Steinberg, D. (1979) *J. Biol. Chem.* **254**, 2010–2016
- Davis, R. A., Engelhorn, S. C., Weinstein, D. B. & Steinberg, D. (1980) *J. Biol. Chem.* **255**, 2039–2045
- Dawson, R. M. C. (1956) *Biochem. J.* **62**, 689–693

- Dimopoulos, G. T. (1970) in *Clinical Biochemistry Of Domestic Animals* (Kaneko, J. J. & Cornelius, C. E., eds.), vol. 1, 2nd edn., pp. 97–129, Academic Press, New York and London
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
- Glomset, J. A. (1962) *Biochim. Biophys. Acta* **65**, 128–135
- Graham, A., Zammit, V. A. & Brindley, D. N. (1988a) *Biochem. J.* **249**, 727–733
- Graham, A., Bennett, A. J., McLean, A. M., Zammit, V. A. & Brindley, D. N. (1988b) *Biochem. J.* **253**, 687–692
- Illingworth, D. R. & Portman, O. W. (1972) *J. Chromatogr.* **73**, 262–264
- Jansen, H., Van Berkel, T. J. C. & Hulsmann, W. C. (1978) *Biochem. Biophys. Res. Commun.* **85**, 148–152
- Klopfenstein, W. E. (1969) *Biochim. Biophys. Acta* **181**, 323–325
- Lands, W. E. M. (1965) *Annu. Rev. Biochem.* **34**, 313–346
- Lloyd-Davies, K. A., Michell, R. H. & Coleman, R. (1972) *Biochem. J.* **127**, 357–368
- Mangiapane, E. H. & Brindley, D. N. (1986) *Biochem. J.* **233**, 151–160
- Nelson, G. J. (1967) *Lipids* **2**, 323–328
- Nilsson-Ehle, P., Garfinkel, A. S. & Schotz, M. C. (1980) *Annu. Rev. Biochem.* **49**, 667–693
- Norum, K. R. & Gjone, E. (1967) *Scand. J. Clin. Lab. Invest.* **20**, 231–243
- Pelech, S. L. & Vance, D. E. (1984) *Biochim. Biophys. Acta* **779**, 217–251
- Portman, O. W. & Illingworth, D. R. (1973) *Biochim. Biophys. Acta* **326**, 34–42
- Robinson, B. S. (1985) Ph.D. Thesis, University of Adelaide
- Sekas, G., Patton, G. M., Lincoln, E. C. & Robins, S. J. (1985) *J. Lab. Clin. Med.* **105**, 190–194
- Spector, A. A. (1986) *Methods Enzymol.* **128**, 320–339
- Switzer, S. & Eder, H. A. (1965) *J. Lipid Res* **6**, 506–511
- Szejtli, J. (1982) *Cyclodextrins And Their Inclusion Complexes*, Akademiai Kiado, Budapest
- Thompson, G. A., Jr. (1980) *The Regulation of Membrane Lipid Metabolism*, pp. 75–103, CRC Press, Boca Raton, FL
- Vance, J. E. & Vance, D. E. (1985) *Can. J. Biochem. Cell Biol.* **63**, 870–881
- Vance, J. E. & Vance, D. E. (1986) *J. Biol. Chem.* **261**, 4486–4491
- Vance, J. E., Nguyen, T. M. & Vance, D. E. (1986) *Biochim. Biophys. Acta* **875**, 501–509
- Van den Bosch, H. (1980) *Biochim. Biophys. Acta* **604**, 191–246
- Van den Bosch, H. (1982) *New Compr. Biochem.* **4**, 313–357
- Weltzien, H. U. (1979) *Biochim. Biophys. Acta* **559**, 259–287
- Wright, R. S. (1971) *J. Chromatogr.* **59**, 220–221
- Yao, Z. & Vance, D. E. (1988) *J. Biol. Chem.* **263**, 2998–3004

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