Lysophosphatidylcholine metabolism and lipoprotein secretion by cultured rat hepatocytes deficient in choline

Brenton S. ROBINSON,* Zemin YAO, Derek J. BAISTED† and Dennis E. VANCE‡

Lipid and Lipoprotein Research Group and Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

The metabolism of lysophosphatidylcholine was studied in cultured rat hepatocytes deficient in choline and methionine. Even though the cells were defective in phosphatidylcholine biosynthesis, the albumin-stimulated release of lysophosphatidylcholine (1.9 nmol/h per mg of cellular protein) was similar to that in hepatocytes supplemented with choline. Albumin also stimulated (1.4-fold) the release of phosphatidylcholine from the deficient cells. The extra phosphatidylcholine and lysophosphatidylcholine in the medium were largely recovered in the albumin fraction (density > 1.18 g/ml), suggesting that albumin released these lipids from hepatocytes because of binding to this protein. The secretion of glycerophosphocholine was decreased by about 40 % by the addition of albumin. When choline-deficient hepatocytes were supplemented with lysophosphatidylcholine, it was transported into the cells and mainly acylated to form phosphatidylcholine, which increased in mass by 30–35 % in the first 4 h of incubation. Lysophosphatidylcholine was shown to be as effective as choline in restoring the secretion of very-low-density lipoproteins to normal amounts, as judged by the secretion of triacylglycerol, phosphatidylcholine and the apolipoproteins associated with very-low-density lipoproteins. Thus phosphatidylcholine synthesis via reacylation of lysophosphatidylcholine, via the CDP-choline pathway or via methylation of phosphatidylethanolamine, will satisfy the requirements for secretion of very-low-density lipoprotein from hepatocytes.

INTRODUCTION

Lysophosphatidylcholine constitutes less than 2 % of the total phospholipids in the membranes of rat liver and most other tissues (White, 1973; Ansell & Spanner, 1982a). The level of this lysophosphatide must be strictly controlled, since it has cytolytic and membrane-perturbing properties (Weltzien, 1979). In rat liver, lysophosphatidylcholine is generated by the deacylation of phosphatidylcholine by the action of phospholipases A₁ and A₂ (Thompson, 1980; Van den Bosch, 1980, 1982). Lysophosphatidylcholine 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). This lyso lipid is an important intermediate in the remodelling of the fatty acyl moieties of phosphatidylcholine (Lands, 1965; Holub & Kuksis, 1978), and has been suggested to play a role in the regulation of a number of membrane-associated enzymes (Weltzien, 1979).

Lysophosphatidylcholine is the second most prevalent phospholipid in the plasma of rats and several other species (Nelson, 1967), where it is associated with albumin (Switzer & Eder, 1965). Lysophosphatidylcholine is readily taken up by tissues and can be used for the synthesis of phosphatidylcholine, or for the production of acetylcholine in nervous tissue (Stein & Stein, 1966; Illingworth & Portman, 1972; Portman & Illingworth, 1974; Savard & Choy, 1982; Ansell & Spanner, 1982b; Esko & Matsuoka, 1983). It has subsequently been

reported that albumin enhances the release of lysophosphatidylcholine from isolated perfused rat liver (Sekas *et al.*, 1985) and cultured rat hepatocytes (Mangiapane & Brindley, 1986; Graham *et al.*, 1988a,b; Baisted *et al.*, 1988), which is most probably generated by hydrolysis of cellular phosphatidylcholine and is independent of lipoprotein secretion. This hepatic lysophosphatidylcholine is mainly unsaturated, unlike the highly saturated lysophosphatidylcholine formed by lecithin-cholesterol acyltransferase in plasma (Glomset, 1962; Sekas *et al.*, 1985; Baisted *et al.*, 1988). The liver could be the major source of plasma lysophosphatidylcholine, since its concentration is relatively high in human patients with plasma lecithin-cholesterol acyltransferase deficiency (Norum & Gjone, 1967). Graham *et al.* (1988a) have suggested that transport of lysophosphatidylcholine from the liver may be physiologically important in supplying extrahepatic tissues with essential fatty acids and choline.

In studies on choline-deficient hepatocytes, Yao & Vance (1988) reported that decreased secretion of triacylglycerol and phosphatidylcholine was due to impaired secretion of very-low-density lipoproteins, but not high-density lipoproteins. Addition of either choline or methionine rapidly corrected the defective very-low-density lipoprotein secretion from the choline-deficient cells. Synthesis of phosphatidylcholine is stimulated and the cellular phosphatidylcholine concentration is increased by the addition of choline or methionine, indicating that active synthesis of phosphatidylcholine via the CDP-choline pathway or by the methylation of

* Present address: Department of Chemical Pathology, Adelaide Children's Hospital, North Adelaide, South Australia 5006, Australia.

† Present address: Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, U.S.A.

‡ To whom correspondence should be addressed.

phosphatidylethanolamine was required for very-low-density lipoprotein secretion.

We were curious whether or not the secretion of lysophosphatidylcholine would be perturbed in the choline-deficient cells, and whether or not phosphatidylcholine synthesis from exogenous lysophosphatidylcholine could overcome the impaired very-low-density lipoprotein secretion from these choline-deficient cells. Lysophosphatidylcholine metabolism in the choline-deficient liver has not been previously studied.

EXPERIMENTAL

Animals and diet

Male Sprague-Dawley rats (*Rattus norvegicus*), weighing 60–80 g, were housed in wire-mesh cages and given water and a choline-deficient diet (ICN Nutritional Biochemicals, Cleveland, OH, U.S.A.) *ad libitum* for 3 days before preparation of hepatocytes. The diet composition was (by wt.) 10 % vitamin-free casein, 10 % α -protein, 20 % lard, 56 % sucrose, 4 % salt mixture (Wesson), and ICN vitamin fortification mixture except choline chloride.

Preparation and incubation of hepatocytes

Hepatocytes were isolated by a collagenase-perfusion technique essentially as described previously (Davis *et al.*, 1979; Yao & Vance, 1988). The isolated hepatocytes were resuspended in Dulbecco's modified Eagle's medium (choline-, methionine- and arginine-free) 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). The cells were dispensed into Primaria culture dishes (Falcon 3802, 60 mm $\times$ 15 mm; Becton Dickinson and Co., Oxnard, CA, U.S.A.) (3 ml/dish) and maintained in monolayer culture at 37 °C under an atmosphere of air/CO₂ (19:1) for at least 6 h before all experiments.

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 monolayer of hepatocytes was subsequently incubated with 2 ml of serum-free medium that contained radiolabelled and/or unlabelled metabolites as described in the legends to Figures and Tables. In radiolabelling experiments, 10–20 μ Ci of [*Me*-³H]choline chloride (15 Ci/mmol), 15 μ Ci of L-[4,5-³H]leucine (45 Ci/mmol) or 23 nCi of [*Me*-³H]lysophosphatidylcholine (114 μ Ci/mmol) was added to each dish of cells. At the end of the incubation period, the medium was removed and the cells were 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 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 sonicate cells (0.1 ml) was taken for protein determination by the Bio-Rad assay based on the method of Bradford (1976), and the remainder was used for the analysis of lipids and water-soluble compounds. Trypan

Blue stain exclusion and constant cellular protein content indicated that hepatocytes were viable throughout the incubation, as observed previously (Yao & Vance, 1988). The viability of the hepatocytes was also unaffected by exposure to 100 μ M-lysophosphatidylcholine for up to 24 h.

Extraction and analysis of lipids and water-soluble compounds from hepatocytes and culture medium

The lipids and water-soluble compounds were extracted and radioactivity was determined as previously described (Baisted *et al.*, 1988). The chemical amounts of lysophosphatidylcholine, phosphatidylcholine and triacylglycerol in the chloroform phase were determined as follows: a concentrated portion of the chloroform layer was applied to a silica-gel 60 t.l.c. plate along with 10 μ g of each of lysophosphatidylcholine, phosphatidylcholine and triolein standards. The plate was developed half-way in the solvent system chloroform/methanol/acetic acid/formic acid/water (35:15:6:2:1, by vol.) to separate phospholipids. The plate was dried and developed to the top in the solvent hexane/di-isopropyl ether/acetic acid (65:35:2, by vol.) to separate neutral lipids. The solvents contained the antioxidant 2,6-di-*t*-butyl-4-methylphenol (50 mg/l). The lysophosphatidylcholine, phosphatidylcholine and triacylglycerol areas were made visible with I₂ vapour and scraped into 15 ml test tubes. Phospholipids were digested with HClO₄ and the phosphorus content was measured (Rouser *et al.*, 1966). Triacylglycerol was treated with alkaline hydroxylamine and the content of fatty acyl groups determined (Snyder & Stephens, 1959).

Fractionation of lipoproteins in culture medium and analysis of lipids and apolipoproteins

The techniques for these procedures have been described by Yao & Vance (1988).

Chemicals

The source of most chemicals has been described (Baisted *et al.*, 1988; Yao & Vance, 1988). Phospholipase A₂ (from *Crotalus adamanteus* venom) was from Sigma Chemical Co., St. Louis, MO, U.S.A. Lysophosphatidyl[*Me*-³H]choline was prepared as follows: choline-deficient rat hepatocytes, cultured for approx. 6 h in choline-, methionine- and arginine-free Dulbecco's modified Eagle's medium containing delipidated fetal-calf serum, were incubated for 2 h in 2 ml of serum-free medium containing 12 μ Ci of [*Me*-³H]choline. Lipids were extracted from the cells, and phosphatidyl[*Me*-³H]choline was isolated by t.l.c. The phosphatidyl[*Me*-³H]choline (6 mCi/mmol) was treated with phospholipase A₂ as described by Tam *et al.* (1984), and lysophosphatidyl[*Me*-³H]choline was purified by t.l.c. The lysophosphatidyl[*Me*-³H]choline was dissolved in water, and lysophosphatidylcholine (from egg yolk) was added to give a specific radioactivity of 250 d.p.m./nmol. All other chemicals were reagent grade.

RESULTS AND DISCUSSION

Release of lysophosphatidylcholine and choline-deficient hepatocytes by albumin

Lipid-poor bovine serum albumin, essentially free of contaminating proteins and lipids (determined by SDS/polyacrylamide-gel electrophoresis and t.l.c.), was

Fig. 1. Effect of albumin on the release of [^3H]lipids from choline-deficient cultured rat hepatocytes prelabelled with [$Me\text{-}^3\text{H}$]choline

Choline-deficient 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 2 h in 2 ml of serum-free medium containing 10 μCi of [$Me\text{-}^3\text{H}$]choline. The prelabelled hepatocytes were subsequently incubated for up to 8 h in 2 ml of serum-free medium containing 28 μM -choline without (□) or with (■) 5 mg of fatty acid-poor bovine serum albumin/ml. Media were collected at the times indicated, and the ^3H associated with (a) total lipids, (b) lysophosphatidylcholine and (c) 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 twice with similar results.

Fig. 2. Effect of albumin on the metabolism of [^3H]lipids in choline-deficient cultured rat hepatocytes prelabelled with [$Me\text{-}^3\text{H}$]choline

Choline-deficient rat hepatocytes were treated as described for Fig. 1. Cells were collected at the times indicated and the ^3H associated with (a) total lipids, (b) phosphatidylcholine and (c) lysophosphatidylcholine [without (□) or with (■) 5 mg of fatty acid-poor bovine serum albumin/ml] 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 twice with similar results.

used in this study. Fig. 1 shows the effect of albumin on the release of [^3H]lipids from choline-deficient hepatocytes prelabelled with [$Me\text{-}^3\text{H}$]choline. The release of total [^3H]lipids into the medium increased approx. 4-fold in the presence of albumin after the first 1 h, and this

was maintained between 1 and 8 h (Fig. 1a). The presence of [^3H]lysophosphatidylcholine was mainly responsible for the stimulated release of total [^3H]lipids during the 8 h time period (Figs. 1a and 1b). Negligible [^3H]lysophosphatidylcholine was released into the medium in

the absence of albumin. It is intriguing that albumin enhanced [^3H]phosphatidylcholine release throughout the 8 h incubation (Fig. 1c).

Treatment of the choline-deficient hepatocytes with albumin did not have a significant effect on cellular total [^3H]lipids and [^3H]phosphatidylcholine after 8 h, as depicted in Figs. 2(a) and 2(b). Fig. 2(c) shows there was a small decrease in cellular [^3H]lysophosphatidylcholine in the presence of albumin, which may be responsible for some of the observed increase in [^3H]lysophosphatidylcholine in the medium. The treatment of hepatocytes with albumin inhibited the production of [^3H]glycerophosphocholine in the medium (Fig. 3b), which is possibly due to lower amounts of [^3H]lysophosphatidylcholine in the cells. In contrast, albumin did not modulate the total ^3H -labelled (0.75×10^6 d.p.m./dish) water-soluble compounds (betaine, choline, phosphocholine and glycerophosphocholine) or [^3H]glycerophosphocholine (0.3×10^6 d.p.m./dish) in the cells for up to 8 h. Changes in the cellular [^3H]lipids and ^3H -labelled water-soluble compounds in the medium could not clearly account for the increase in [^3H]lipids in the medium (predominantly as [^3H]lysophosphatidylcholine) when the [$\text{Me-}^3\text{H}$]choline-labelled hepatocytes were incubated with albumin. Previous work has provided evidence that cellular phosphatidylcholine is the likely precursor of the increased lysophosphatidylcholine in the medium (Sekas *et al.*,

Fig. 3. Effect of albumin on the release of ^3H -labelled water-soluble compounds from choline-deficient cultured rat hepatocytes prelabelled with [$\text{Me-}^3\text{H}$]choline

Choline-deficient rat hepatocytes were treated as described for Fig. 1. Media were collected at the times indicated and the ^3H associated with (a) total water-soluble compounds and (b) glycerophosphocholine [without (□) or with (■) 5 mg of fatty acid-poor bovine serum albumin/ml] 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 twice with similar results.

Table 1. Effect of albumin and choline on the production of phosphatidylcholine and lysophosphatidylcholine by choline-deficient cultured rat hepatocytes

Choline-deficient rat hepatocytes were cultured for approx. 5 h in choline-, methionine- and arginine-free Dulbecco's modified Eagle's medium containing delipidated fetal-calf serum. The hepatocytes were subsequently incubated for 4 h in 2 ml of serum-free medium with no addition, with 5 mg of fatty acid-poor bovine serum albumin/ml, with 100 μM -choline or with both fatty acid-poor bovine serum albumin and choline. Cells and media (combined from ten dishes) were collected and the amounts of phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) 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.

Treatment	Amount of phospholipid (nmol/mg of cellular protein)			
	Cells		Medium	
	PC	LPC	PC	LPC
No addition	108.4 $\pm$ 1.3	0.9 $\pm$ 0.1	2.8 $\pm$ 0.3	0.1 $\pm$ 0.1
+ Albumin	97.7 $\pm$ 1.7	1.0 $\pm$ 0.1	4.0 $\pm$ 0.2	7.4 $\pm$ 0.3
+ Choline	146.6 $\pm$ 2.4	0.7 $\pm$ 0.2	3.8 $\pm$ 0.1	0.1 $\pm$ 0.0
+ Albumin, + choline	134.1 $\pm$ 5.0	0.8 $\pm$ 0.1	4.4 $\pm$ 0.3	8.3 $\pm$ 0.4

1985; Mangiapane & Brindley, 1986; Graham *et al.*, 1988a; Baisted *et al.*, 1988). The relatively high radioactivity associated with cellular phosphatidylcholine in the present study made it difficult to detect changes caused by albumin.

Table 1 shows the effects of albumin and choline on the amounts of phosphatidylcholine and lysophosphatidylcholine produced by choline-deficient hepatocytes. When the hepatocytes were incubated with albumin for 4 h there was a small decrease in the amount of cellular phosphatidylcholine. There was no appreciable change in lysophosphatidylcholine mass in the cells. Albumin caused the amounts of phosphatidylcholine and lysophosphatidylcholine that were released into the medium to increase 1.4- and 74-fold respectively. The mass of lysophosphatidylcholine in the medium was higher than that of phosphatidylcholine after the incubation with albumin. As shown in Table 1, there was a good correlation between loss of cellular phosphatidylcholine in the presence of albumin and an increase in the medium of phosphatidylcholine and lysophosphatidylcholine. Incubation of the choline-deficient hepatocytes with choline for 4 h increased the amount of phosphatidylcholine in the cells and medium (1.4-fold). Addition of choline did not influence the amounts of lysophosphatidylcholine in cells and medium. When the choline-deficient hepatocytes were incubated with both choline and albumin there was less phosphatidylcholine in the cells and more phosphatidylcholine and lysophosphatidylcholine released into the medium than with choline alone. Again the presence of albumin caused the mass of lysophosphatidylcholine to be higher than that of phosphatidylcholine in the medium. The effects of albumin shown in Table 1 are consistent with those obtained in the radiolabelling experiments.

Table 2. Effect of albumin on the distribution of [³H]choline-labelled lysophosphatidylcholine and phosphatidylcholine in lipoprotein fractions released from choline-deficient cultured rat hepatocytes

Choline-deficient 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 2 h in 2 ml of serum-free medium containing 20 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 with or without 5 mg of fatty acid-poor bovine serum albumin/ml. Media (combined from eight dishes) were collected and subjected to ultracentrifugation to fractionate lipoproteins. ³H radioactivity associated with lysophosphatidylcholine and phosphatidylcholine in the dense-protein (BF), high-density-lipoprotein (HDL), low-density-lipoprotein (LDL) and very-low-density-lipoprotein (VLDL) fractions was determined as described in the Experimental section. Each value is the mean $\pm$ range of duplicate determinations. A repeat of this experiment gave essentially identical results.

Lipoprotein fraction	Radioactivity (d.p.m./dish)			
	Lysophosphatidylcholine		Phosphatidylcholine	
	+ Albumin	No albumin	+ Albumin	No albumin
BF	722 390 $\pm$ 45 100	2230 $\pm$ 230	114 120 $\pm$ 10 550	25 730 $\pm$ 1000
HDL	98 360 $\pm$ 9950	890 $\pm$ 20	71 520 $\pm$ 1030	50 270 $\pm$ 3310
LDL	260 $\pm$ 40	400 $\pm$ 80	30 920 $\pm$ 2700	19 760 $\pm$ 990
VLDL	490 $\pm$ 90	1270 $\pm$ 40	77 550 $\pm$ 9260	83 800 $\pm$ 4760

Fig. 4. Effect of lysophosphatidylcholine and choline on the amount of phosphatidylcholine in choline-deficient cultured rat hepatocytes

Choline-deficient rat hepatocytes were plated for approx. 5 h in choline- and methionine-free medium containing delipidated fetal-calf serum. The hepatocytes were subsequently incubated for up to 24 h in 2 ml of serum-free medium with no addition ($\square$), 100 μ M-lysophosphatidylcholine ($\blacktriangle$) or 100 μ M-choline ($\blacksquare$). Cells were collected at the times indicated and the amount of phosphatidylcholine 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. Inset: hepatocytes were incubated for up to 24 h in 2 ml of serum-free medium containing [*Me*-³H]lysophosphatidylcholine (250 d.p.m./

The influence of albumin on the distribution of [³H]lysophosphatidylcholine and [³H]phosphatidylcholine in lipoprotein fractions released from choline-deficient cultured rat hepatocytes is shown in Table 2. [³H]Lysophosphatidylcholine was mainly present in the dense-protein fraction of the medium when the hepatocytes were incubated with or without albumin. There was a large increase in the amount of [³H]lysophosphatidylcholine in the dense-protein and high-density-lipoprotein fractions (324- and 111-fold respectively) with albumin treatment. Albumin caused a 1.5- and 2.6-fold decrease in [³H]lysophosphatidylcholine in the low-density-lipoprotein and very-low-density-lipoprotein fractions. [³H]Phosphatidylcholine was predominantly associated with the very-low-density-lipoprotein fraction when the hepatocytes were incubated in the absence of albumin. In contrast, when the cells were incubated with albumin, [³H]phosphatidylcholine was mainly present in the dense-protein fraction. The amount of [³H]phosphatidylcholine increased in the dense-protein, high-density-lipoprotein and low-density-lipoprotein fractions (4.4-, 1.4- and 1.6-fold respectively) and slightly decreased in the very-low-density-lipoprotein fraction with the addition of albumin. Table 2 indicates that albumin released lysophosphatidylcholine and phosphatidylcholine from the hepatocytes because of its ability to bind these phospholipids.

Previous work has provided evidence that the lysophosphatidylcholine released from cultured rat hepatocytes by albumin originates from the degradation of cellular phosphatidylcholine by phospholipases A₁ and A₂ and is not produced by the hydrolysis of phosphatidylcholine previously secreted (Sekas *et al.*, 1985; Baisted *et al.*, 1988; Graham *et al.*, 1988b). The

nmol; 100 μ M). Cells were collected at the times indicated and ³H associated with phosphatidylcholine was determined. Each point represents the mean of two dishes, and the ranges were less than 5% of the mean. These experiments were repeated once with similar results.

present results demonstrate that phosphatidylcholine is constantly being degraded in choline-deficient cultured rat hepatocytes to lysophosphatidylcholine which is released into the medium if albumin is present. In addition, the results show that albumin can directly release phosphatidylcholine from these cells. The synthesis of phosphatidylcholine via the CDP-choline pathway and methylation of phosphatidylethanolamine is restricted in choline-deficient hepatocytes (Yao & Vance, 1988), and most of the albumin-released lysophosphatidylcholine and phosphatidylcholine is probably derived from a pre-existing pool of cellular phosphatidylcholine. This hepatic phosphatidylcholine pool could be decreased if some of the lysophosphatidylcholine and phosphatidylcholine released into the medium in the presence of albumin were not transported back into the cells.

Baisted *et al.* (1988) reported that glycerophosphocholine is directly secreted by cultured rat hepatocytes. It has been shown here that in the presence of albumin the secretion of glycerophosphocholine from choline-deficient hepatocytes is decreased (Fig. 3). A likely explanation for this phenomenon is that albumin removes lysophosphatidylcholine from the cells, leaving less precursors available for the formation of glycerophosphocholine to be secreted. Since the levels of lysophosphatidylcholine were unchanged in albumin-treated cells (Table 1), the steady-state level of lysophosphatidylcholine is apparently unaffected.

The lysophosphatidylcholine and phosphatidylcholine released from choline-deficient hepatocytes by albumin is albumin-bound rather than associated with secreted

lipoproteins. However, some of the lysophosphatidylcholine and phosphatidylcholine initially secreted with lipoproteins may later become associated with albumin in the medium. Davis *et al.* (1980) found that secretion of very-low-density lipoprotein by cultured rat hepatocytes is inhibited by high concentrations of albumin, owing to a viscosity effect. It is unlikely that this occurred in the present work, as a relatively low albumin concentration was used.

The release of lysophosphatidylcholine and phosphatidylcholine from the liver by albumin may provide a vital source of choline and fatty acids to the brain and other extrahepatic tissues during choline deficiency, when very-low-density lipoprotein secretion is suppressed. The intracellular location of the hepatic phosphatidylcholine pool from which the released lysophosphatidylcholine and phosphatidylcholine are derived and the phospholipases involved need to be determined.

Effect of lysophosphatidylcholine on the synthesis of phosphatidylcholine and secretion of lipoproteins by choline-deficient cultured rat hepatocytes

The choline-deficient rat hepatocytes effectively utilized exogenous lysophosphatidylcholine to synthesize phosphatidylcholine. Fig. 4 indicates that the amount of cellular phosphatidylcholine increased approx. 1.4-fold after 4 h of lysophosphatidylcholine supplementation, and this concentration was maintained for up to 24 h. The stimulatory effect of lysophosphatidylcholine on the level of cellular phosphatidylcholine was identical with that of choline (Fig. 4). Lysophosphatidyl[Me-³H]choline was

Fig. 5. Effect of lysophosphatidylcholine and choline on the secretion of triacylglycerol and phosphatidylcholine from choline-deficient cultured rat hepatocytes

Choline-deficient rat hepatocytes were treated as described for Fig. 4. Media were collected at the times indicated and the amounts of (a) triacylglycerol and (b) phosphatidylcholine (□, no addition; ▲, 100 μM-lysophosphatidylcholine; ■, 100 μM-choline) 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. Inset: hepatocytes were incubated with lysophosphatidyl[Me-³H]choline as for Fig. 4 inset. Media were collected at the times indicated and ³H associated with phosphatidylcholine was determined. Each point represents the mean of two dishes, and the ranges were less than 5% of the mean. The experiment was repeated twice with similar results.

used as a tracer to determine the fate of the exogenous lysophosphatidylcholine. During the 24 h incubation period, less than 5 % of the total radioactivity added was found in cellular and medium water-soluble compounds, indicating minimal degradation of the lysophosphatidylcholine. At least 95 % of the total radioactivity incorporated in the hepatocytes was present in lipid compounds, of which about 90 %, 4 % and 4 % was associated with phosphatidylcholine, lysophosphatidylcholine and sphingomyelin respectively. These results suggest that most of the supplemented lysophosphatidylcholine was transported as an intact molecule into the cells and rapidly acylated to form phosphatidylcholine. The synthesis of cellular [^3H]phosphatidylcholine was almost constant with time during the first 12 h of incubation with [^3H]lysophosphatidylcholine (Fig. 4, inset).

When the choline-deficient hepatocytes were supplemented with lysophosphatidylcholine or choline, the mass secretion of triacylglycerol and phosphatidylcholine into the medium was stimulated 3- and 2-fold respectively (Figs. 5a and 5b). An almost linear secretion of [^3H]phosphatidylcholine was observed when the hepatocytes received [$\text{Me-}^3\text{H}$]lysophosphatidylcholine as a tracer (Fig. 5b, inset), indicating secretion of newly synthesized phosphatidylcholine made with the supplemented lysophosphatidylcholine.

To determine whether or not the stimulated secretion of triacylglycerol and phosphatidylcholine was a consequence of increased lipoprotein secretion, the culture medium was fractionated into very-low-density-lipoprotein, low-density-lipoprotein, high-density-lipoprotein and dense protein fractions and analysed. High-performance t.l.c. of lipids in each of the four fractions showed that the increased secretion of triacylglycerol and phosphatidylcholine by the hepatocytes in the presence of lysophosphatidylcholine or choline was mainly due to stimulated very-low-density lipoprotein secretion. The stimulatory effect of lysophosphatidylcholine and choline on very-low-density-lipoprotein lipid secretion was almost identical. The secretion of lipids associated with other fractions was only slightly affected by the addition of lysophosphatidylcholine or choline. The secretion of [^3H]leucine-labelled apolipoproteins in each of the four fractions was examined by SDS/polyacrylamide-gel electrophoresis followed by fluorography. The secretion of very-low-density-lipoprotein apolipoproteins (mainly apo B_H, apo B_L and apoE) was markedly stimulated by the addition of lysophosphatidylcholine or choline (Fig. 6), further indicating enhanced release of apo B/triacylglycerol-rich lipoproteins. The secretion of high-density-lipoprotein apolipoproteins (mainly apo AI and apo E), albumin and other dense proteins by the hepatocytes was not changed by lysophosphatidylcholine or choline supplementation (Fig. 6).

Yao & Vance (1988) demonstrated that choline deficiency causes impaired secretion of very-low-density lipoproteins, but not high-density lipoproteins, from cultured rat hepatocytes, and this can be reversed by phosphatidylcholine synthesis via the CDP-choline pathway or phosphatidylethanolamine methylation. The present results confirm these findings (Yao & Vance, 1988) and show that phosphatidylcholine synthesis by the acylation of exogenous lysophosphatidylcholine can also stimulate very-low-density lipoprotein secretion. The experiments were conducted in the absence of albumin to maximize

Fig. 6. [^3H]Leucine-labelled apolipoproteins of fractionated lipoproteins secreted by choline-deficient cultured rat hepatocytes supplemented with lysophosphatidylcholine or choline

Choline-deficient rat hepatocytes were cultured for approx. 5 h in choline- and methionine-free medium containing delipidated fetal-calf serum, and subsequently incubated for 12 h in 2 ml of serum-free medium containing 15 μCi of L-[4,5- ^3H]leucine with no addition (—), 100 μM -choline (C) or 100 μM -lysophosphatidylcholine (L). Media (combined from eight dishes) were collected and subjected to ultracentrifugation to fractionate lipoproteins. Lipoproteins in the dense-protein (BF), high-density-lipoprotein (HDL), low-density-lipoprotein (LDL) and very-low-density-lipoprotein (VLDL) fractions were concentrated by absorption on to Cab-O-Sil. [^3H]Apolipoproteins absorbed to the Cab-O-Sil were solubilized, and equal portions of the samples were subjected to SDS/polyacrylamide-gel electrophoresis and fluorography as described in the Experimental section. The X-ray film was exposed for 72 h for VLDL and 18 h for HDL and BF. There were no visible bands for LDL samples after exposure for 3 days. The positions of apolipoprotein standards are indicated on the left: Alb, albumin. This experiment was repeated twice with similar results.

cellular uptake of lysophosphatidylcholine. The acylation of lysophosphatidylcholine to form phosphatidylcholine for lipoprotein secretion presumably occurs in the endoplasmic reticulum or Golgi membranes. It has recently been found that phosphatidylcholine synthesis is specifically required for normal hepatic very-low-density lipoprotein secretion, and cannot be replaced by the synthesis of phosphatidylethanolamine, phosphatidylmonomethylethanolamine or phosphatidyltrimethylethanolamine (Z. Yao & D. E. Vance, unpublished work). The reason for this specific phospholipid requirement is unknown and warrants further investigation.

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