Comparison of albumin-mediated release of lysophosphatidylcholine and lysophosphatidylethanolamine from cultured rat hepatocytes

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We have investigated the albumin-stimulated release from cultured rat hepatocytes of lysophosphatidylcholine derived from methylation of phosphatidylethanolamine and of lysophosphatidylethanolamine. In the presence of albumin, neither lysophosphatidylethanolamine nor lysophosphatidylcholine was released into the culture medium. Albumin stimulated the accumulation of both phospholipids in the medium. After 2 h, 14.1 nmol of lysophosphatidylcholine and 2.0 nmol of lysophosphatidylethanolamine per 3×10^6 cells had accumulated in the medium. The rate of release of [^3H]ethanolamine-labelled lysophosphatidylethanolamine was rapid in the first 2 h and then was decreased, whereas there was a 1 h lag in the release of [^3H]ethanolamine-labelled lysophosphatidylcholine. This apparent lag probably reflected the time necessary for the synthesis of phosphatidylcholine from phosphatidylethanolamine in the cells. Albumin caused a decrease in labelled cellular lysophosphatidylethanolamine and lysophosphatidylcholine which only partially accounted for the accumulation of the labelled phospholipids in the medium. Albumin also stimulated the release of labelled phosphatidylethanolamine (almost 3-fold) and phosphatidylcholine (2-fold) into the medium. There was no detectable change in the labelling of the cellular pools of these phospholipids, most likely owing to the large amounts in the cells compared with the medium. The labelled lysophospholipids did not arise from catabolism of the parent phospholipid in the medium. Analysis of the fatty acids of the secreted lysophospholipids showed a preferential release of unsaturated fatty acyl species of lysophosphatidylcholine, whereas lysophosphatidylethanolamine contained similar amounts of saturated and unsaturated fatty acids.

INTRODUCTION

Lysophosphatidylcholine is the second most prevalent phospholipid in the plasma of rats and several other species (Nelson, 1967), where it is bound to albumin (Switzer & Eder, 1965). It has been shown that albumin stimulates the release of lysophosphatidylcholine from isolated perfused rat liver (Sekas *et al.*, 1985) and cultured rat hepatocytes (Mangiapan & Brindley, 1986; Graham *et al.*, 1988a,b; Baisted *et al.*, 1988; Robinson *et al.*, 1989). The released lysophosphatidylcholine appears to be formed by the action of phospholipases A_1 and A_2 on cellular phosphatidylcholine and not by the degradation of secreted lipoprotein phosphatidylcholine. This hepatic lysophosphatidylcholine is predominantly unsaturated, which is markedly dissimilar from the highly saturated lysophosphatidylcholine synthesized by lecithin-cholesterol acyltransferase in plasma (Glomset, 1962; Sekas *et al.*, 1985; Baisted *et al.*, 1988). The liver could be a major source of plasma lysophosphatidylcholine, since its concentration remains relatively high even in patients suffering from a deficiency of lecithin-cholesterol acyltransferase (Norum & Gjone, 1967). The physiological function of the albumin-mediated release of lysophosphatidylcholine from the liver may be to supply extrahepatic tissues with choline and essential fatty acids (Graham *et al.*, 1988a).

Biosynthesis of phosphatidylcholine in rat liver occurs mainly by two pathways: from unesterified choline via the CDP-choline pathway, and by the methylation of phosphatidylethanolamine (Pelech & Vance, 1984). Previous work has indicated that lysophosphatidylcholine released from cultured rat hepatocytes by albumin can be derived from cellular phosphatidylcholine formed via the CDP-choline pathway (Graham *et al.*, 1988a,b; Baisted *et al.*, 1988; Robinson *et al.*, 1989). In the present paper we investigated whether cellular phosphatidylcholine formed by the methylation of phosphatidylethanolamine can also be a source of lysophosphatidylcholine released from rat hepatocytes by albumin. We were also interested whether albumin can enhance the release of lysophosphatidylethanolamine in addition to lysophosphatidylcholine from rat liver cells. Lysophosphatidylethanolamine transport from liver has not been previously studied.

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 a 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 described previously (Davis *et al.*, 1979; Yao & Vance, 1988). The isolated hepatocytes were resuspended in ethanolamine- and serine-free minimum essential medium containing 17% (v/v) 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-NaHCO₃, pH 7.4 (1×10^6 cells/ml). The cells were dispersed in 3 ml into plastic culture dishes (Falcon 3802 Primaria; 60 mm $\times$ 15 mm; Becton Dickinson and Co., Oxnard, CA, U.S.A.) and maintained in monolayer culture at 37 °C under an atmosphere of air/CO₂ (19:1) for approx. 18 h before all experiments.

The dishes of cultured hepatocytes were washed with 2 $\times$ 3 ml of serum-free medium (ethanolamine- and serine-free minimum essential medium described above) to remove the serum and non-viable cells. The monolayers of hepatocytes were incubated for 1 h in 2 ml of serum-free medium that contained 20 μ Ci of [1-³H]-ethanolamine (30 Ci/mmol), and then were washed twice with unlabelled medium. The prelabelled cells were subsequently incubated for up to 4 h in 2 ml of serum-free medium with or without 5 mg of fatty acid-poor bovine serum albumin/ml. SDS/polyacrylamide-gel electrophoresis and t.l.c. showed that the albumin was essentially free of contaminating proteins and lipids.

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 sonicated cell preparation (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 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 (Baisted *et al.*, 1988; Yao & Vance, 1988). Some modifications to this standard procedure were used and are described in the legends to the Figures and Table.

Extraction and analysis of lipids and water-soluble compounds

Lipids and water-soluble compounds were extracted from sonicated cell preparations and centrifuged media, and total radioactivity was determined as previously described (Baisted *et al.*, 1988). Radioactivity associated with lysophosphatidylcholine, phosphatidylcholine, lysophosphatidylethanolamine and phosphatidylethanolamine was determined as follows: a concentrated portion of the chloroform phase was applied to a silica-gel 60 t.l.c. plate (E. Merck, Darmstadt, Germany) along with 10 μ g each of lysophosphatidylcholine, phosphatidylcholine, lysophosphatidylethanolamine and phosphatidylethanolamine standards. The plate was developed in the

first direction in the solvent system chloroform/methanol/acetic acid/water (50:30:8:3, by vol.). After air-drying, the plate was developed in the second direction in the solvent system chloroform/methanol/ammonia (65:30:4, by vol.) The solvents contained the antioxidant 2,6-di-*t*-butyl-4-methylphenol (50 mg/l). The phospholipid areas were made visible with I₂ vapour and scraped into scintillation vials. Water (0.5 ml) and scintillation fluid (5 ml) were added, and radioactivity was measured after 24 h.

The fatty acid compositions and chemical amounts of lysophosphatidylcholine, phosphatidylcholine, lysophosphatidylethanolamine and phosphatidylethanolamine in the chloroform phase were determined by the procedure of Baisted *et al.* (1988).

Chemicals

[1-³H]Ethan-1-ol-2-amine hydrochloride (sp. radioactivity 30 Ci/mmol; radiochemical purity 99%) was obtained from Amersham Canada Ltd., Oakville, Ontario, Canada. Ethanolamine- and serine-free minimum essential medium was from Gibco Laboratories, Life Technologies Inc., Grand Island, NY, U.S.A. The sources of other chemicals have been described (Baisted *et al.*, 1988).

RESULTS AND DISCUSSION

The effect of albumin on the release of [³H]lipids from cultured rat hepatocytes prelabelled with [1-³H]-ethanolamine is depicted in Fig. 1. The release of total [³H]lipids into the medium increased approx. 4-fold after the 1 h in the presence of albumin, and this was maintained between 1 and 4 h (Fig. 1a). The albumin-stimulated release of total [³H]lipids during the 4 h time period was mainly due to the presence of [³H]lysophosphatidylcholine and [³H]lysophosphatidylethanolamine (Figs. 1b and 1d). In the absence of albumin, negligible [³H]lysophosphatidylcholine and [³H]lysophosphatidylethanolamine were released into the medium. Interestingly, albumin also enhanced [³H]-phosphatidylethanolamine and [³H]phosphatidylcholine release throughout the 4 h incubation (Figs. 1e and 1c). Robinson *et al.* (1989) demonstrated that phosphatidylcholine released from hepatocytes by albumin is albumin-bound rather than associated with secreted lipoproteins. It is noteworthy that the rate of release of [³H]-lysophosphatidylethanolamine and [³H]phosphatidylethanolamine was initially rapid in the medium, and the rate of release decreased after 1 h. In contrast, in repeated experiments [³H]lysophosphatidylcholine and [³H]-phosphatidylcholine appeared in the medium after an initial lag time after which there was a steady increase (Figs. 1b, 1c and 1d). This labelling pattern in the medium is most likely due to cellular phosphatidylethanolamine being initially labelled with [1-³H]ethanolamine and subsequently converted into [³H]phosphatidylcholine. Incubation of hepatocytes with albumin for 4 h did not appear to modulate the production of ³H-labelled water-soluble compounds in the medium (results not shown).

Treatment of the cultured rat hepatocytes with albumin caused no detectable change in cellular ³H-labelled total lipids, [³H]phosphatidylcholine and [³H]phosphatidylethanolamine through 4 h (Figs. 2a, 2b and 2d). [³H]Phosphatidylethanolamine decreased and

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

Rat hepatocytes, cultured for approx. 18 h in ethanolamine- and serine-free minimum essential medium containing fetal-calf serum, were incubated for 1 h in 2 ml of serum-free medium containing 20 μ Ci of [1-3H]ethanolamine. The prelabelled hepatocytes were subsequently incubated for up to 4 h in 2 ml of serum-free medium with (■) or without (□) 5 mg of fatty acid-poor bovine serum albumin/ml. Media were collected at the times indicated, and the ³H radioactivity associated with (a) total lipids, (b) phosphatidylcholine, (c) lysophosphatidylethanolamine and (d) lysophosphatidylethanolamine and (e) phosphatidylethanolamine 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 cultured rat hepatocytes prelabelled with [1-3H]ethanolamine

Rat hepatocytes were treated as described for Fig. 1. Cells were collected at the times indicated, and the ³H radioactivity associated with (a) total lipids, (b) phosphatidylcholine, (c) lysophosphatidylethanolamine and (d) lysophosphatidylethanolamine [with (■) or without, (□) 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.

[^3H]phosphatidylcholine increased in the cells which reflects the methylation of phosphatidylethanolamine to phosphatidylcholine. Figs. 2(c) and 2(e) indicate there was less radioactivity in cellular [^3H]lysophosphatidylcholine (~ 4000 d.p.m.) and [^3H]lysophosphatidylethanolamine (~ 20000 d.p.m.) in the presence of albumin, but was inadequate to account for the observed increase in [^3H]lysophosphatidylcholine (~ 270000 d.p.m.) and [^3H]lysophosphatidylethanolamine (~ 80000 d.p.m.) in the medium (Figs. 1b and 1d). Albumin did not have an appreciable effect on ^3H -labelled water-soluble compounds in the cells for up to 4 h. The increase in ^3H -labelled lipids in the medium (predominantly as [^3H]lysophosphatidylcholine and [^3H]lysophosphatidylethanolamine) when [$1\text{-}^3\text{H}$]ethanolamine-labelled cultured hepatocytes were incubated with albumin could not be readily accounted for by changes in the cellular ^3H -labelled lipids and ^3H -labelled water-soluble compounds and in ^3H -labelled water-soluble compounds in the medium. Changes in cellular phosphatidylcholine and phosphatidylethanolamine induced by albumin were difficult to monitor, owing to the relatively high radioactivity associated with these phospholipids. The ratio of [^3H]lysophosphatidylcholine in the medium to cellular [^3H]phosphatidylcholine (~ 0.04) was higher than that of [^3H]lysophosphatidylethanolamine in the medium to cellular [^3H]phosphatidylethanolamine (~ 0.01) after the 4 h albumin treatment. This suggests that the albumin-mediated release of [^3H]lysophosphatidylcholine was greater than that of [^3H]lysophosphatidylethanolamine from hepatocytes.

Fig. 3 shows that subsequent incubation of medium removed from [$1\text{-}^3\text{H}$]ethanolamine-labelled cultured rat hepatocytes previously incubated with albumin for 3 h resulted in no significant alteration in [^3H]lysophosphatidylcholine, [^3H]phosphatidylcholine, [^3H]lysophosphatidylethanolamine or [^3H]phosphatidylethanolamine. This indicates that the elevated [^3H]lysophosphatidylcholine and [^3H]lysophosphatidylethanolamine in the medium did not originate from the breakdown of [^3H]phosphatidylcholine and [^3H]phosphatidylethanolamine in the medium by secreted enzymes. These lysophospholipids most likely arose from the degradation of cellular [^3H]phosphatidylcholine and [^3H]phosphatidylethanolamine respectively and subsequent sequestration by albumin. Previous work has suggested that lysophosphatidylcholine released from cultured rat hepatocytes by albumin is generated by the hydrolysis of cellular phosphatidylcholine by phospholipases A_1 and A_2 and is not produced by the degradation of secreted phosphatidylcholine (Sekas *et al.*, 1985; Baisted *et al.*, 1988; Graham *et al.*, 1988b). The present results indicate that lysophosphatidylcholine released from cultured rat hepatocytes by albumin can be derived from cellular phosphatidylcholine synthesized by the methylation of phosphatidylethanolamine in addition to phosphatidylcholine formed via the CDP-choline pathway (Graham *et al.*, 1988a,b; Baisted *et al.*, 1988; Robinson *et al.*, 1989). The labelling experiments show that albumin-released lysophosphatidylethanolamine can originate from hepatocyte phosphatidylethanolamine synthesized via the CDP-ethanolamine pathway. It remains to be determined whether cellular phosphatidylethanolamine formed by the decarboxylation of phosphatidylserine can also be the precursor of lysophosphatidylethanolamine released from hepatocytes by albumin.

Fig. 3. [^3H]Lysophosphatidylcholine and [^3H]lysophosphatidylethanolamine in the medium are not formed by the hydrolysis of [^3H]phosphatidylcholine and [^3H]phosphatidylethanolamine in the medium

Rat hepatocytes, cultured for approx. 18 h in ethanolamine- and serine-free minimum essential medium containing fetal-calf serum, were incubated for 1 h in 2 ml of serum-free medium containing $20\ \mu\text{Ci}$ of [$1\text{-}^3\text{H}$]ethanolamine. The prelabelled hepatocytes were subsequently incubated for 3 h in 2 ml of serum-free medium containing 5 mg of fatty-acid-poor bovine serum albumin/ml. Medium (combined from 11 dishes) was centrifuged at $12000\ g$ for 15 min and further incubated for up to 4 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 (■), lysophosphatidylethanolamine (△) and phosphatidylethanolamine (□) 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.

The fatty acid compositions and chemical amounts of cellular phosphatidylcholine and phosphatidylethanolamine and of lysophosphatidylcholine, lysophosphatidylethanolamine, phosphatidylcholine and phosphatidylethanolamine in the medium after cultured rat hepatocytes had been incubated with albumin for up to 5 h are presented in Table 1. The fatty acid compositions of the cellular phosphatidylcholine and phosphatidylethanolamine were consistent with those previously reported for rat hepatocytes maintained in culture (Vance & Vance, 1985, 1986). Phosphatidylethanolamine was slightly more unsaturated than phosphatidylcholine in the cells. The ratio of unsaturated to saturated fatty acids of the cellular phosphatidylcholine and phosphatidylethanolamine remained constant throughout the 5 h treatment with albumin. The lysophosphatidylcholine released into the medium by albumin was predominantly unsaturated. The ratio of unsaturated to saturated fatty acids of the medium lysophosphatidylcholine increased during the incubation. These findings are similar to those of Baisted *et al.* (1988). It is intriguing that in repeated experiments lysophosphatidylethanolamine was less unsaturated than lysophosphatidylcholine in the medium [mainly owing to less arachidonic acid ($\text{C}_{20:4}$) and

Table 1. Fatty acid compositions and chemical amounts of cellular and medium phospholipids after cultured rat hepatocytes had been incubated with albumin

Rat hepatocytes were cultured for approx. 18 h in ethanolamine- and serine-free minimum essential medium containing 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. Cells and medium (combined from 16 dishes) were collected at the times indicated, and the fatty acid compositions and chemical amounts of phospholipids 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; PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; 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	20.7±0.4	0.5±0.1	25.6±0.3	5.4±0.2	14.8±0.0	1.1±0.1	26.0±0.3	5.9±0.1	1.16	295.4±14.0
	2	20.4±0.6	0.6±0.0	25.6±0.4	5.2±0.1	14.4±0.1	1.3±0.1	26.4±0.5	6.0±0.4	1.17	315.8±2.1
	5	20.7±0.1	0.6±0.1	26.7±0.7	4.6±0.2	13.4±0.1	0.8±0.3	27.5±0.2	5.7±0.5	1.11	299.9±10.4
Cellular PE	0	14.8±0.2	2.0±0.1	30.4±0.4	4.9±0.2	8.8±0.7	0.6±0.0	30.5±0.4	8.0±0.1	1.21	127.5±0.9
	2	14.3±0.4	1.2±0.3	30.7±0.5	4.5±0.2	8.1±0.2	0.8±0.1	31.4±0.5	9.0±0.2	1.22	143.4±4.1
	5	13.8±0.3	1.0±0.4	32.0±0.5	4.1±0.1	7.5±0.2	0.7±0.1	32.0±0.2	8.9±0.1	1.18	132.8±0.1
Medium LPC	2	19.5±0.5	1.8±0.4	21.1±0.9	5.2±0.6	17.1±0.1	0.3±0.3	32.3±0.9	2.7±0.8	1.46	14.1±0.1
	5	18.5±0.7	0.8±0.2	17.9±0.2	5.0±0.3	17.2±0.3	0.8±0.2	34.8±1.2	5.0±0.3	1.75	21.7±0.9
	2	15.0±0.4	6.7±0.3	32.2±0.4	6.0±1.0	15.0±0.4	0.0±0.0	23.8±0.2	1.3±0.1	1.12	2.0±0.2
Medium LPE	5	17.7±0.5	1.5±0.1	29.6±0.6	8.1±0.3	17.6±0.5	0.0±0.0	24.3±0.2	1.2±0.1	1.11	2.2±0.1
	2	23.8±0.1	3.1±0.1	23.6±0.3	10.0±0.1	18.2±0.6	0.5±0.0	18.6±0.3	2.2±0.2	1.11	5.9±0.0
	5	23.4±0.1	2.2±0.0	23.5±0.3	7.3±0.1	15.9±0.1	0.6±0.1	23.2±0.1	3.9±0.3	1.13	8.5±0.1
Medium PC	2	20.5±0.4	1.6±0.3	24.4±0.2	6.1±0.3	22.6±0.2	0.0±0.0	23.5±0.2	1.3±0.3	1.23	1.0±0.2
	5	20.5±0.2	1.2±0.4	25.1±0.5	6.6±0.2	21.5±0.3	0.0±0.0	22.8±0.4	2.3±0.1	1.19	1.6±0.2

docosahexaenoic acid ($C_{22:6}$) and more stearic acid ($C_{18:0}$) being present], even though phosphatidylethanolamine was possibly more unsaturated than phosphatidylcholine in the cells. The unsaturated to saturated fatty acid ratio of the lysophosphatidylethanolamine in the medium remained unchanged with time. The ratios of unsaturated to saturated fatty acids of phosphatidylcholine and phosphatidylethanolamine in the medium reflected those of cellular phosphatidylcholine and phosphatidylethanolamine, and remained static throughout the albumin incubation. Although the unsaturated-to-saturated fatty acid ratios of phosphatidylcholine and phosphatidylethanolamine in the medium and in the cells were similar, the composition of the various unsaturated fatty acids differed [e.g. there was a greater content of oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) and a lower content of arachidonic acid ($C_{20:4}$) and docosahexaenoic acid ($C_{22:6}$) in phosphatidylcholine and phosphatidylethanolamine of the medium compared with that of the cells]. There appears to be a degree of selectivity in the molecular species of phosphatidylcholine and phosphatidylethanolamine transported into the medium, as previously shown (Vance & Vance, 1986; Vance *et al.*, 1986). In the hepatocytes the absolute amount of phosphatidylcholine was approximately double that of phosphatidylethanolamine. There was no significant change in the amount of cellular phosphatidylcholine and phosphatidylethanolamine with time. The amount of lysophosphatidylcholine was severalfold higher than that of lysophosphatidylethanolamine in the medium throughout the albumin incubation, taking into consideration the proportions of cellular phosphatidylcholine and phosphatidylethanolamine. Throughout the treatment with albumin the amount of phosphatidylcholine was higher than that of phosphatidylethanolamine in the medium. The mass of lysophosphatidylcholine and lysophosphatidylethanolamine released into the medium was always higher than that of phosphatidylcholine and phosphatidylethanolamine respectively.

The present work suggests that phosphatidylcholine and phosphatidylethanolamine are constantly being degraded by phospholipase A_1 and A_2 in cultured rat hepatocytes to lysophosphatidylcholine and lysophosphatidylethanolamine, which are released into the medium if albumin is present. The absolute amount and degree of unsaturation of lysophosphatidylcholine was higher than for lysophosphatidylethanolamine in the medium, which probably reflects the cellular phosphatidylcholine and phosphatidylethanolamine precursors being hydrolysed by phospholipases at different rates. The highly unsaturated lysophosphatidylcholine released from hepatocytes by albumin is very dissimilar to the highly saturated lysophosphatidylcholine formed by plasma lecithin-cholesterol acyltransferase (Glomset, 1962). Since lysophosphatidylcholine in rat plasma is predominantly unsaturated (Sekas *et al.*, 1985), the albumin-mediated release of lysophosphatidylcholine from liver appears to be a quantitatively important source of plasma lysophosphatidylcholine. To our knowledge there are no reports in the literature on the fatty acid composition of lysophosphatidylethanolamine in rat plasma. Graham *et al.* (1988a) speculated that the release of lysophosphatidylcholine from the liver by albumin may be important in supplying extrahepatic tissues with choline and essential fatty acids. Saito *et al.* (1988) demonstrated that lysophosphatidylcholine is an

endothelium-dependent vascular-smooth-muscle relaxant, and it is therefore conceivable that hepatic release of lysophosphatidylcholine by albumin plays a significant role in the regulation of regional blood flow. The albumin-stimulated release of lysophosphatidylethanolamine from the liver may have similar physiological functions. It is also noteworthy that lysophosphatidylcholine has been shown to be a chemotactic factor for human monocytes (Quinn *et al.*, 1988). However, lysophosphatidylethanolamine did not have this function.

Lysophosphatidylcholine and lysophosphatidylethanolamine released from rat hepatocytes by albumin might originate by the action of phospholipases A_1 and A_2 on phosphatidylcholine and phosphatidylethanolamine in the plasma membrane. It has been found that phospholipids are asymmetrically distributed across the lipid bilayer of rat liver plasma membrane, with most of the phosphatidylcholine in the outer leaflet and phosphatidylethanolamine in the inner leaflet (Higgins & Evans, 1978; Cullis & Hope, 1985). It is feasible that phosphatidylcholine is predominantly hydrolysed by phospholipase A_1 in the outer leaflet of the bilayer, resulting in highly unsaturated lysophosphatidylcholine which is in close proximity to albumin and readily removed. Conversely, phosphatidylethanolamine may be almost equally hydrolysed by phospholipase A_1 and A_2 in the inner leaflet of the bilayer, resulting in moderately unsaturated lysophosphatidylethanolamine, which is less readily removed by albumin because it has to 'flip-flop' across the membrane. This is assuming that the phosphatidylcholine and phosphatidylethanolamine precursors have a normal fatty acid distribution, i.e. saturated and unsaturated fatty acids mainly present at the 1 and 2 positions of the molecule respectively. There is evidence for both phospholipases A_1 and A_2 in plasma membrane of liver, with A_1 predominating (Nachbaur *et al.*, 1972; Newkirk & Waite, 1971).

Both lysophosphatidylethanolamine and lysophosphatidylcholine in human serum have more saturated fatty acids ($\sim 60\%$) than unsaturated fatty acids ($\sim 40\%$), and the concentration of lysophosphatidylethanolamine is much lower than that of lysophosphatidylcholine (Williams *et al.*, 1966). This appears somewhat contradictory to our observed preferential secretion of unsaturated lysophosphatidylcholine. However, the plasma lipids will reflect both secretion and metabolism. Åkesson *et al.* (1976) found that, when different molecular species of lysophosphatidylcholine and lysophosphatidylethanolamine were injected intraperitoneally into rats, the uptake into the liver within 15 min was higher for lysophosphatidylethanolamine than for lysophosphatidylcholine and the unsaturated species of each was taken up and acylated more rapidly than the saturated species. Thus, because the circulating level of lysophosphatidylcholine in serum is a result of its formation and uptake, the preponderance of the saturated species might reflect the greater rate of uptake of the unsaturated species. Figs. 1(b) and 1(d) also reflect the difference in the release versus uptake of lysophosphatidylcholine and lysophosphatidylethanolamine during the time course. The accumulation of radioactivity in lysophosphatidylcholine is linear, whereas that in lysophosphatidylethanolamine is approaching a plateau. Finally, the relatively more saturated lysophosphatidylethanolamine than lysophosphatidylcholine in the medium may be a

consequence of the unsaturated lysophosphatidylethanolamine being the most efficiently removed by the hepatocyte.

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REFERENCES

- Åkesson, B., Arner, A. & Sundler, R. (1976) *Biochim. Biophys. Acta* **441**, 453–464
- Baisted, D. J., Robinson, B. S. & Vance, D. E. (1988) *Biochem. J.* **253**, 693–701
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Cullis, P. R. & Hope, M. J. (1985) in *Biochemistry of Lipids and Membranes* (Vance, D. E. & Vance, J. E., eds.), pp. 25–72, Benjamin/Cummings Publishing Co., Menlo Park, CA
- Davis, R. A., Engelhorn, S. C., Pangburn, S. H., Weinstein, D. B. & Steinberg, D. (1979) *J. Biol. Chem.* **254**, 2010–2016
- 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. A. M., Zammit, V. A. & Brindley, D. N. (1988b) *Biochem. J.* **253**, 687–692
- Higgins, J. A. & Evans, W. H. (1978) *Biochem. J.* **174**, 563–567
- Mangiapane, E. H. & Brindley, D. N. (1986) *Biochem. J.* **233**, 151–160
- Nachbaur, J., Colbeau, A. & Vignais, P. M. (1972) *Biochim. Biophys. Acta* **247**, 426–440
- Nelson, G. J. (1967) *Lipids* **2**, 323–328
- Newkirk, J. D. & Waite, B. M. (1971) *Biochim. Biophys. Acta* **225**, 224–230
- 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
- Quinn, M. T., Parthasarathy, S. & Steinberg, D. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2805–2809
- Robinson, B. S., Yao, Z., Baisted, D. J. & Vance, D. E. (1989) *Biochem. J.* **260**, 207–214
- Saito, T., Wolf, A., Menon, N. K., Saeed, M. & Bing, R. J. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8246–8250
- Sekas, G., Patton, G. M., Lincoln, E. C. & Robins, S. J. (1985) *J. Lab. Clin. Med.* **105**, 190–194
- Switzer, S. & Eder, H. A. (1965) *J. Lipid Res.* **6**, 506–511
- 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
- Williams, J. H., Kuchmak, M. & Witter, R. F. (1966) *Lipids* **1**, 89–97
- Yao, Z. & Vance, D. E. (1988) *J. Biol. Chem.* **263**, 2998–3004

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