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SOME STUDIES ON THE BIOSYNTHESIS OF THE MOLECULAR SPECIES OF PHOSPHATIDYLCHOLINE FROM RAT LUNG AND PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE FROM RAT LIVER

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SUMMARY

1. At different time intervals after injection of [1(3)-^{3}H]glycerol, the incorporation of glycerol into the various molecular species of phosphatidylcholine and phosphatidylethanolamine from rat liver, and phosphatidylcholine from rat lung was determined.

2. The results indicate that, in liver, a *de novo* synthesis is primarily operating in the biosynthesis of linoleic acid-containing molecules of lecithin and of the hexaenoic molecular species of phosphatidylethanolamine. An acylation of monoacyl derivatives of these phospholipids is suggested to play an important role particularly in the formation of arachidonic acid containing molecular species of these phospholipids.

3. In lung, the *de novo* synthesis was found to contribute also primarily to the linoleic acid-containing lecithins, though it also represents an important pathway for the synthesis of tetraenoic, monoenoic and, perhaps to a lesser extent, disaturated lecithins. A deacylation–reacylation mechanism may contribute significantly to the formation of dipalmitoyl lecithin, a major constituent of lung pulmonary surfactant.

4. Acylation of 1-palmitoyl-*sn*-glycero-3-phosphorylcholine with various labeled fatty acids or acyl-CoA esters was studied in the presence of microsomes from rat lung and liver. In the presence of microsomes from lung a significant uptake of palmitic acid was observed into the 2-position of lecithin, this in strong contrast to the findings with liver microsomes where only a very limited uptake of palmitic acid was observed. The results endorse the findings from the *in vivo* studies that the acylation of monoacyl-*sn*-glycero-3-phosphorylcholine may play an important additional role in maintaining the high level of dipalmitoyl lecithin in lung.

5. Comparison of the composition of phosphatidylethanolamine and lecithin from lung suggested, in support of previous observations by other investigators, that the methylation of phosphatidylethanolamine does not represent an important pathway for the formation of dipalmitoyl lecithin.

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INTRODUCTION

Naturally occurring phospholipids such as phosphatidylcholine and phosphatidylethanolamine comprise a heterogeneous collection of molecules differing with respect to their fatty acyl constituents[1,2]. Analyses on the detailed molecular make-up of lecithins from a variety of tissues and mammalian species have shown that in most tissues lecithins containing a saturated fatty acid at the 1- position and an unsaturated at the 2-position are predominant[3,4]. However, in contrast to other tissues, lung tissues from all mammals investigated contained dipalmitoyl lecithin as a major component[5]. The presence of high amounts of this particular lecithin species in beef lung has been reported already in 1946 by THANNHAUSER *et al.*[5]. Dipalmitoyl lecithin is considered to play an important role in determining the surface properties of pulmonary surfactant[6–10]. Because alveolar surface tension would become very high, especially at low lung volumes during respiration, the surface force at the air–water interface must be reduced, probably by the surfactant, in order to prevent collapse of the alveoli[11–14]. Recent studies by THOMAS AND RHODES[15] provided evidence that alveolar lecithin is probably derived from lecithin from pulmonary parenchyma cells and subsequently discharged with time onto the alveolar surface. It was thought of interest to investigate in the present study which of the known pathways for the biosynthesis of lecithin is mainly responsible for the formation of dipalmitoyl lecithin in lung tissues.

In liver, it has been demonstrated that the various pathways leading to the synthesis of lecithin, *viz. de novo* synthesis[16], methylation of phosphatidylethanolamine[17] and acylation of monoacyl-*sn*-glycero-3-phosphorylcholines[18–20] contribute in a different way to the synthesis of the various molecular species of lecithin: whereas *de novo* synthesis *via* CDP-choline is considered to be involved mainly in the formation of monoenoic and, especially, dienoic molecular species, an acylation of 1-acyl-*sn*-glycero-3-phosphorylcholine is probably mainly operative in the biosynthesis of arachidonic acid containing molecules[21–26].

The methylation of phosphatidylethanolamine is suggested to play an important role in the formation of hexaenoic phosphatidylcholines[23,27,28]. Several authors have attempted to study the relative contributions of these various pathways in the formation of dipalmitoyl lecithin in lung tissue. MORGAN[29] suggested that the methylation of phosphatidylethanolamine would be primarily responsible for the synthesis of this compound in dog lung. This suggestion is not supported by the observations of other investigators[30–33].

Preliminary experiments in this laboratory by MONTFOORT[34] suggested a possible participation of the acylation of monoacyl-*sn*-glycero-3-phosphorylcholine in the biosynthesis or maintaining of dipalmitoyl lecithin in rat lung. Similar findings in dog lung were recently reported by FROSOLONO *et al.*[35]. In order to extend the observations of MONTFOORT[34] and FROSOLONO *et al.*[35] it was attempted in the present study to evaluate the contribution of *de novo* synthesis and the deacylation–reacylation mechanism in the synthesis of the various molecular components of lecithin in rat lung and, for comparison, of lecithin and phosphatidylethanolamine in rat liver.

EXPERIMENTAL

In vivo experiments: injection procedure and extraction of lipids

0.5 mC [1(3)-^{3}H]glycerol in 1 ml of 0.9% NaCl was injected into the tail vein of male white rats (Wistar strain) which had been fasted overnight. After the indicated time intervals, the animals were killed by decapitation. The livers and lungs were quickly removed and immediately pressed between 2 blocks of dry ice. As quickly as possible the tissues were homogenized in methanol–chloroform–water (2:1:1, v/v/v) using a cooled Sorvall mixer. Lipids were then extracted following the procedure of BLIGH AND DYER[36].

Incorporation of [1(3)-^{3}H]glycerol into the molecular species of phosphatidylcholine of lung and phosphatidylcholine and phosphatidylethanolamine of liver

Phosphatidylcholine and phosphatidylethanolamine were isolated from the total lipids of lung and liver by means of thin-layer chromatography on silica H plates impregnated with 1 mM Na_2CO_3 using chloroform–methanol–acetic acid–water (50 28:10:5, by vol.) as a developer[37]. After development of the plates, the spots were visualized by slight exposure to iodine vapor. The lecithin and phosphatidylethanolamine fractions were exhaustively eluted from the silica gel with methanol–chloroform (80: 20, v/v). Both phospholipids were resolved into their various molecular species after conversion into 1,2-diacyl-*sn*-glycerols with phospholipase C from *Bacillus cereus*, followed by fractionation on $AgNO_3$-impregnated plates. The details of this procedure have been described previously[38]. The fractionated diacylglycerols were quantitatively eluted from the silica with chloroform–methanol (80:20, v/v). After washing according to the procedure of FOLCH *et al.* [39] the solvents were evaporated *in vacuo* and the residues dissolved in 1 ml of methanol. 0.7-ml aliquots were then transferred into scintillation vials containing 16 ml of a scintillation solution containing 0.5% PPO (w/v) and 0.03% dimethyl POPOP (w/v) in toluene. The samples were counted in a Packard TriCarb liquid scintillation spectrometer using an external standard for quenching corrections. The absolute amounts of the various molecular species of phosphatidylcholine and phosphatidylethanolamine were determined by means of gas–liquid chromatography as described previously[38].

In vitro experiments: isolation of cell fractions

A 10% homogenate of rat lung and liver in 0.25 M sucrose (adjusted to pH 7.4 with 1 M Tris) was prepared by homogenization with the aid of a Potter–Elvehjem tube. Nuclei and cell debris were removed by centrifugation for 5 min at $1000 \times g$ in a IEC-PR-6 centrifuge. Mitochondria and lysosomes were sedimented by centrifugation at $20000 \times g$ for 10 min in a SS34 rotor of the Sorvall RC-2B centrifuge. This pellet was discarded and a microsomal fraction was obtained by spinning the $20000 \times g$ supernatant in the 30 rotor of the Beckman L2-65B ultracentifruge at $100000 \times g$ for 60 min.

The microsomal pellet was rinsed several times with 0.125 M KCl–0.1 M Tris (pH 7.4) and finally suspended in this medium in a concentration of 2 mg protein per ml. All manipulations were carried out between 0 and 3°. Protein was determined following the method of LOWRY *et al.*[40].

Acylation of 1-palmitoyl-sn-glycero-3-phosphorylcholine in the prescence of microsomes from lung and liver

The basic incubation mixture contained, unless otherwise stated, the following components: 25 μl of a synthetic 1-palmitoyl-*sn*-glycero-3-phosphorylcholine (pipetted from a clear emulsion of 2 mM in 0.125 M KCl–0.1 M Tris (pH 7.4) obtained by ultrasonication with a MSE sonifier); 25 μl 0.1 M $MgCl_2$, 10 μl of the indicated acyl-CoA ester (1.6 mM), 25 μl of protein (2 mg/ml) and 0.125 M KCl–0.1 M Tris buffer (pH 7.4 to a total volume of 250 μl.

In experiments where free fatty acids were used, 50 μl of ATP-CoA solution (50 mM ATP, 1 mM CoA; pH 7.4) were added. 25 μl of the indicated free fatty acids were added from an emulsion in 0.125 M KCl–0.1 M Tris (pH 7.4) which was obtained by ultrasonication for 2×0.5 min with a MSE sonifier. After the indicated periods of incubation at 37° in a shaking waterbath, the reaction was stopped by the addition of 1 ml methanol–chloroform (2:1, v/v). After extraction of the lipids[86] lecithin was isolated by means of thin-layer chromatography[88]. After staining with iodine vapor, the lecithin fractions were scraped directly into vials containing 15 ml of scintillation fluid. For ^{14}C-labeled compounds the scintillation medium consisted of 0.7% (w/v) PPO, 0.03% (w/v) dimethyl-POPOP and 10% naphtalene in dioxane, diluted 5 parts to 1 of water (v/v).

For tritiated samples a 0.5% (w/v) PPO and 0.03% (w/v) dimethyl-POPOP solution in toluene was diluted with Triton X-100 and water in a ratio of 2:1:0.2 (v/v/v). In experiments where both ^3H- and ^{14}C-labeled fatty acids were used, the lecithins were eluted from the silica with methanol–chloroform (80:20, v/v). After evaporation of the solvent, the residue was dissolved in 1 ml of methanol. 0.7-ml aliquots were then counted in 15 ml 0.5% PPO (w/v) and 0.03% dimethyl POPOP (w/v) in toluene.

Chemicals

[1(3)-^3H]glycerol, [1-^{14}C]palmitate, [9,10-$^3H_2$]palmitate, [9,10-$^3H_2$]oleate and

TABLE I

FATTY ACID COMPOSITION AND WEIGHT PERCENTAGE OF THE VARIOUS MOLECULAR SPECIES OF LECITHIN FROM RAT LUNG AND LECITHIN AND PHOSPHATIDYLETHANOLAMINE FROM RAT LIVER

	Molecular species	*Fatty acid composition*							*Weight %*
		16:0	*16:1*	*18:0*	*18:1*	*18:2*	*20:4*	*22:6*	
Lung phosphatidylcholine	Δ 0	91.2	–	6.6	2.2	–	–	–	32.2
	Δ 1	51.9	13.8	4.3	30.0	–	–	–	27.9
	Δ 2	36.6	1.4	10.1	6.4	45.5	–	–	24.1
	Δ 3	41.1	1.8	15.5	18.8	19.0	–	–	4.3
	Δ 4	28.5	1.2	18.6	8.2	3.1	39.4	–	11.5
Liver phosphatidylcholine	Δ 1*	50.7	+	17.3	32.0	–	–	–	7.8
	Δ 2	32.0	–	22.8	–	45.2	–	–	26.0
	Δ 3	35.0	–	28.2	17.0	16.8	–	–	2.9
	Δ 4	26.2	–	30.6	–	–	43.2	–	51.6
	Δ 6	30.6	–	24.8	7.3	11.1	6.4	19.8	11.7
Liver phosphatidylethanolamine	Δ 1	36.3	+	22.4	35.3	6.0	–	–	3.1
	Δ 2	23.4	–	22.7	6.1	47.8	–	–	21.2
	Δ 4	13.0	–	36.6	4.3	0.6	45.5	–	48.9
	Δ 6	27.6	–	19.4	5.2	3.8	8.9	35.1	26.8

* This fraction contains minor amounts of Δ 0.

[1-^{14}C]linoleate were purchased from the Radiochemical Centre, Amersham (Great Britain). [1-^{14}C]Palmitoyl-CoA, [9,10-$^{3}H_2$]-oleoyl-CoA and [1-^{14}C]linoleoyl-CoA were synthesized according to the methods of GOLDMAN AND VAGELOS[41]. ATP was bought from Sigma (U.S.A.) and Coenzyme A from Boehringer(Germany).

RESULTS AND DISCUSSION

The present work concerns a comparative study on the biosynthesis of the various molecular species of lecithin from rat lung and lecithin and phosphatidylethanolamine from rat liver. Table I shows the fatty acid composition and weight percentages of the disaturated, mono-,di-, tri-, tetra and hexaenoic fractions of these phospholipids.

Monoenoic and, particularly, disaturated molecules are encountered in only small quantities in lecithin and phosphatidylethanolamine from rat liver. On the other hand, lung lecithin is very rich in dipalmitoyl lecithin and monoenoic molecules. Both liver phospholipids comprise high levels of arachidonic acid containing molecules and, especially phosphatidylethanolamine, a conspicuous amount of hexaenoic species. These data are in good agreement with previous results of several investigators on the molecular compositon of phosphatidylcholine[2,3,4,27] and phosphatidylethanolamine from rat liver [21,23,27,28] and phosphatidylcholine from rat lung[3,4,42]. In order to investigate which pathways contribute to the biosynthesis of the various molecular species of lecithin of lung, the *in vivo* incorporation of [1(3)-^{3}H]glycerol into the various molecular species of this phospholipid was followed. The incorporation of glycerol into the species of lecithin and phosphatidylethanolamine of liver was, for comparison, investigated as well. Table II presents the percentage distribution of isotope among the different molecular species of these phospholipids at the indicated periods after injection of [1(3)-^{3}H]glycerol. Moreover the relative specific activities of the various molecular species at the different time intervals are presented. The relative specific activity of a given species is defined as the percentage of isotope incorporated into that species divided by the weight percentage of that species (both percentages with respect to total lecithin or phosphatidylethanolamine). Under these conditions the glycerol is not significantly converted into fatty acids: at 60 min after the injection of [1(3)-^{3}H]-glycerol, 97% of the radioactivity incorporated into lecithin and phosphatidylethanolamine of both rat lung and liver was found in the glycerol moiety of these phospholipids. This observation is supported by previous work of OMURA *et al.*[43] who showed that, even at 23 h after injection of glycerol, less than 10% of the radioactivity incorporated into rat liver phospholipids was present in the fatty acyl constituents.

In rat liver, at 3.5 min after injection of the [^{3}H]glycerol, the linoleic acid containing molecules (Δ 2 and Δ 3) of lecithin showed a much higher relative specific activity than the other species of this phospholipid. These observations support previous studies *in vitro* by HILL *et al.*[21], VAN GOLDE *et al.*[22] and KANOH[23] and *in vivo* studies by ÅKESSON *et al.*[24-26] which showed that a *de novo* synthesis *via* CDP-choline[16] is primarily operating in the formation of these linoleic acid containing species. In a very elegant study ÅKESSON[26] demonstrated that in particular the palmitoyl–linoleoyl molecules are produced by synthesis *de novo*. In contrast to the linoleic acid containing molecules, the tetraenoic species of rat liver lecithin revealed a much lower relative specific activity in agreement with the concept that arachidonic acid containing

TABLE II

ISOTOPE DISTRIBUTION (%) AND RELATIVE SPECIFIC ACTIVITY OF THE VARIOUS MOLECULAR SPECIES OF LECITHIN FROM LUNG AND LIVER AND PHOSPHATIDYLETHANOLAMINE FROM LIVER AT DIFFERENT TIME INTERVALS AFTER INJECTION OF [1(3)-^{3}H]-GLYCEROL

	Molecular species	*Isotope distribution* (%)			*Weight* %	*Relative specific activity**		
		5.5 min	*29 min*	*60 min*		*5.5 min*	*29 min*	*60 min*
Lung phosphatidylcholine	Δ 0	20.9	28.6	31.8	32.2	0.65	0.85	0.99
	Δ 1	25.1	32.6	33.8	27.9	0.90	1.17	1.21
	Δ 2	26.6	17.5	16.6	24.1	1.10	0.73	0.69
	Δ 3	10.9	4.4	3.8	4.3	2.53	1.02	0.88
	Δ 4	16.3	16.9	14.1	11.5	1.42	1.47	1.23
		3.5 min	*29.5 min*	*60 min*		*3.5 min*	*29.5 min*	*60 min*
Liver phosphatidylcholine	Δ 1	10.8	12.9	17.2	7.8	1.38	1.65	2.20
	Δ 2	56.9	54.4	52.9	26.0	2.19	2.09	2.03
	Δ 3	7.8	5.1	+	2.9	2.69	1.77	+
	Δ 4	18.1	21.4	20.3	51.6	0.35	0.41	0.39
	Δ 6	6.4	6.2	7.9	11.7	0.55	0.53	0.67
		3.5 min	*29.5 min*	*60 min*		*3.5 min*	*29.5 min*	*60 min*
Liver phosphatidylethanolamine	Δ 1+Δ 2**	30.1	43.0	43.7	24.3	1.24	1.77	1.80
	Δ 4	12.0	13.9	18.3	48.9	0.25	0.28	0.37
	Δ 6	58.0	43.1	37.1	26.8	2.16	1.61	1.38

* The relative specific activity of a given species is defined as the percentage of isotope incorporated into the species divided by the weight percentage of that species (both percentages with respect to total lecithin or phosphatidylethanolamine).

** Not always a clear separation was accomplished between the dienoic (21.2%) and the monoenoic (3.1%) fraction of phosphatidylethanolamine.

species are synthesized predominantly *via* acylation of monoacyl-*sn*-glycero-3-phosphorylcholine rather than *via de novo* synthesis[21-26]. At prolonged times after injection the relative specific activity of the dienoic molecules decreased slowly whereas a more rapid decrease was observed for that of the trienoic fraction. A concomitant increase was observed for the relative specific activity of the monoenoic molecules.

In the case of phosphatidylethanolamine from rat liver, the highest relative specific activity at 3.5 min was attained in the hexaenoic fraction. This suggests that for phosphatidylethanolamine, the *de novo* synthesis *via* CDP-ethanolamine is primarily responsible for the formation of the hexaenoic molecules.

At prolonged times a strong decline is observed in the relative specific activity of the hexaenoic phosphatidylethanolamine which is accompanied by an increase in that of the other phosphatidylethanolamine fractions.

Similar *in vivo* studies on the incorporation of glycerol into the molecular species of rat liver lecithin and phosphatidylethanolamine have been reported by ÅKESSON *et al.*[24]. These authors followed the isotope distribution among the various molecular species of these phospholipids at very short time intervals after injection of [2-^{3}H]glycerol. Their findings are in good agreement with the isotope distribution found at the shortest time studied in the present work. However, the alterations after more prolonged periods, as studied in the present work, are more pronounced, particularly in the case of phosphatidylethanolamine.

The observation, that the relative specific activity of the hexaenoic phosphatidylethanolamines reaches a high value shortly after injection and subsequently diminishes quickly, indicates that this species reveals a higher turn-over than the other phosphatidylethanolamines. In this connection it is, however, important to realize that the absolute specific activity of the total phosphatidylethanolamine increased over the whole period studied (at 3.5 min 75, at 29.5 min 148, and at 60 min 226 counts/min per μg P).

The findings that the mono-, di- and tetraenoic molecules of phosphatidylethanolamine show an increasing relative specific activity concomitantly with the decrease of that of the hexaenoic species can be interpreted in several ways: (a) The mono-, di- and tetraenoic 1,2-diacyl-*sn*-glycerols may be converted into phosphatidylethanolamine at a much lower rate than the hexaenoic diacylglycerols. This would be in agreement with observations *in vitro* by KANOH[44] and *in vivo* by ÅKESSON *et al.*[24]. that hexaenoic diacylglycerols are much more rapidly incorporated into phosphatidylethanolamine than the other species. On the other hand, one has to realize that the hexaenoic diacylglycerol[24] or phosphatidic acid pools[24,25] are much smaller than those of the other species. (b) An additional explanation is that the *de novo* synthesis is not responsible, or only partially responsible, for the increase of the relative specific activity of the mono-, di-, and tetraenoic phosphatidylethanolamines after that of the hexaenoic fraction has reached its maximum: the initially *de novo* synthesized hexaenoic species may be quickly degraded, possibly *via* a phospholipase A_2 which releases the hexaenoic acid. In this context it is interesting that endogenous hexaenoic phosphatidylethanolamine appears to be preferentially hydrolyzed by the mitochondrial phospholipase A (G. L. SCHERPHOF, unpublished results) when compared to the other phosphatidylethanolamine species. The remaining 1-acyl-*sn*-glycero-3-phosphorylethanolamine may then be acylated with oleic, linoleic and arachidonic acid which might cause the continued increase of the relative specific activities of the molecular

species containing these acids. The involvement of the deacylation–reacylation mechanism particularly in the formation of tetraenoic phosphatidylethanolamine was also suggested by the findings of KANOH[23] and ÅKESSON *et al.*[25]. Hexaenoic phosphatidylethanolamines are also known to be the preferential substrates in the methylation pathway. This could, in fact, explain the decrease in relative specific activity of the hexaenoic species, but does not explain the increase in specific activity of the other species of phosphatidylethanolamine.

The results obtained with lecithin from lung show that, at prolonged periods after injection of [1(3)-^{3}H]glycerol, the relative specific activities of the linoleic acid containing species strongly decrease. This indicates that, also in lung, dienoic and especially trienoic lecithins show a higher turn-over than the other molecular species. This does not agree with the findings of TIERNEY *et al.*[42]. These authors followed the radioactivity of disaturated, mono-, di- and polyenoic lecithin fractions between 3 and 40 h after injection of [^{3}H]glucose and [^{14}C]palmitate. Though the turn-over of the dienoic lecithins appeared to be slightly higher than that of the disaturated and monoenoic lecithins, these authors found similar turn-over rates for both the glycerol and the fatty acid portions of all lecithin species. It is interesting to note that the difference in relative specific activity between the disaturated and dienoic species in lung (0.65 *versus* 1.10) at the shortest time is much smaller than that between the tetraenoic species of rat liver lecithin, which are known to be synthesized mainly *via* acylation[21–26] and the linoleic acid containing species of this phospholipid (0.35 *versus* 2.19).

This would suggest that a *de novo* synthesis *via* CDP-choline, when compared to the deacylation–reacylation cycle, contributes relatively more to the formation of disaturated lecithins in lung than to the arachidonic acid containing lecithins in liver. However, at prolonged times after injection a considerable decrease in relative specific activity of the di-, and trienoic lung lecithins occurs which is accompanied by a still continued increase of the relative specific activity of the monoenoic and particularly of dipalmitoyl lecithin. This observation could be explained by a slower rate of conversion of monoenoic and disaturated 1,2-diacyl-*sn*-glycerols into lecithin or by a significant contribution of a deacylation–reacylation mechanism to the formation of the monoenoic and disaturated lecithins.

In contrast to lecithin and phosphatidylethanolamine from rat liver, no pronounced low relative specific activity was observed for the tetraenoic species of rat lung lecithin.

The *in vivo* studies described above indicate that, in lung, a *de novo* synthesis is primarily responsible for the formation of the linoleic acid and arachidonic acid containing lecithins and contributes also significantly to the synthesis of monoenoic and disaturated lecithins. However, a deacylation–reacylation mechanism may contribute an additional formation of the latter two species. In previous studies[18–20] on the acylation of monoacyl-*sn*-glycero-3-phosphorylcholines in rat liver, it was found that the acyltransferases catalyse predominantly the uptake of unsaturated fatty acids by 1-acyl-*sn*-glycero-3-phosphorylcholine. Only a limited uptake of saturated acyl constituents was observed. (For an excellent review, see ref. 2.). If, however, as indicated by the *in vivo* studies described above, in lung this acylation mechanism would play an important role in the formation, or maintaining of dipalmitoyl lecithin, one would expect that the acyltransferases in this tissue catalyse a significant uptake of palmitic acid at the 2-position of lecithin.

TABLE III

ACYLATION OF 1-PALMITOYL-*sn*-GLYCERO-3-PHOSPHORYLCHOLINE WITH LABELED FREE FATTY ACIDS AND LABELED ACYL-CoA ESTERS IN THE PRESENCE OF LUNG AND LIVER MICROSOMES FROM RAT*

Added fatty acid or acyl-CoA esters	*Fatty acid incorporated into lecithin (nmoles·min^{-1}·mg^{-1} protein)*	
	Liver	*Lung*
16:0	10.8	5.6
18:1	18.4	6.4
18:2	53.2	7.6
16:0-CoA	5.2	10.4
18:1-CoA	15.2	11.6
18:2-CoA	22.0	–

* The detailed composition of the incubation mixtures is presented in EXPERIMENTAL.

Table III shows the results obtained after incubation of microsomes from lung and liver with various labeled free fatty acids in the presence of 1-palmitoyl-*sn*-glycero-3-phosphorylcholine. In agreement with previous observations[18–20] acyltransferases from rat liver catalysed a preferential uptake of unsaturated fatty acids into the 2-position of lecithin. The rate of linoleic acid uptake (expressed in nmoles·min^{-1}·mg^{-1} protein) exceeded that of palmitate by a factor of about 5. In strong contrast to this, no preferential uptake of unsaturated fatty acids was observed for acyltransferases from lung microsomes: the uptake of palmitate into the 2-position of lung lecithin was almost equal to that of oleic and linoleic acid. Similar results were obtained when exogenous labeled acyl-CoA esters were used instead of free fatty acids: whereas a pronounced preferential uptake of unsaturated acyl-CoA esters was observed for liver, palmitoyl-CoA was almost equally incorporated into lung lecithin as oleoyl-CoA. Similar findings have recently been reported by FROSOLONO *et al.*[35], who studied the

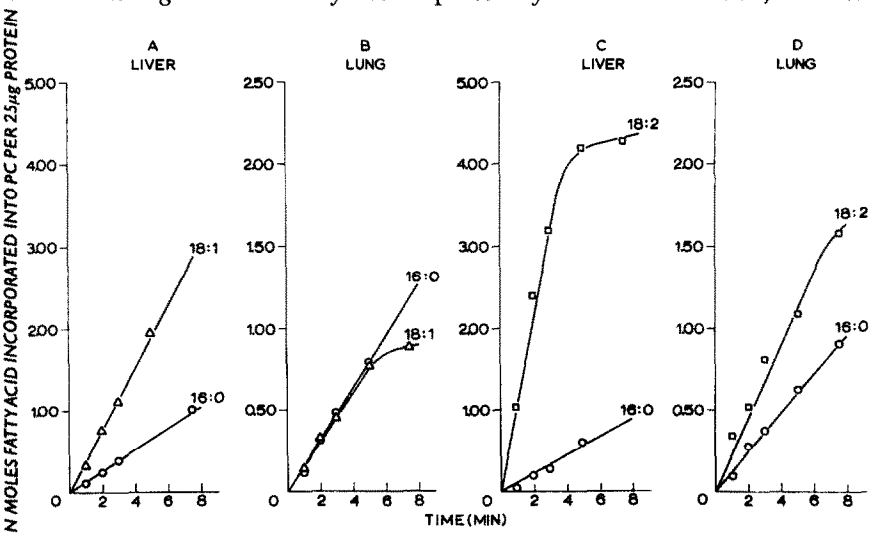

Fig. 1. A+B. Acylation of 1-palmitoyl-*sn*-glycero-3-phosphorylcholine with a 1:1 mixture of [1-^{14}C]palmitic acid (○——○) and [9, 10-$^{3}H_2$]oleic acid (△——△) in the presence of liver (A) and of lung (B) microsomes. C+D. Similar to A and B except that a 1:1 mixture of [9, 10-$^{3}H_2$]palmitic (○——○) and [1-^{14}C]linoleic acid (□——□) was used in the presence of liver (C) and of lung (D) microsomes. The detailed composition of the incubation mixture is presented in EXPERIMENTAL. PC = phosphatidylcholine.

incorporation of palmitic, hexadecenoic, stearic and oleic acid, added as acyl-CoA esters, into lecithin of dog lung and liver as stimulated by various monoacylphosphoglycerides. In order to extend the observations described above and those by FROSOLONO *et al.*[35] it was thought of interest to follow the incorporation of mixtures of fatty acids into lecithin. Fig. 1 A–D show the simultaneous incorporation of mixtures of [1-^{14}C]palmitate and [9,10-$^{3}H_2$]oleate and of [9,10-$^{3}H_2$]palmitate and [1-^{14}C]-linoleate into lecithin of rat liver (A + C) and rat lung (B + D). In agreement with the experiments described above and those by FROSOLONO *et al.*[35] where only 1 fatty acid or acyl-CoA ester was added to the incubation mixture, Fig. 1B shows that oleate and palmitate incorporate equally well into the 2-position of rat lung lecithin also when added as a mixture. This is in contrast to the preferential uptake of oleate into the 2-position of rat liver lecithin (Fig. 1A). A similar tendency was observed for the incorporation of palmitate and linoleate. Whereas a small preference for linoleate was observed in lung (Fig. 1D) when compared to palmitate (a factor of 1.9), a much more selective uptake of linoleate was found in rat liver microsomes (a factor of 10; Fig. 1C).

These studies show that the acylation of 1-acyl-*sn*-glycero-3-phosphorylcholine may play an important role in the introduction of palmitic acid at the 2-position of rat lung lecithin. It may partially account for, or maintain, the high amount of dipalmitoyl lecithin encountered in lung tissue. In contrast to this, palmitic acid ap peared to be a poor substrate for 1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase in rat liver microsomes in line with the observation that almost no palmitate occurs at the 2-position of rat liver lecithins[3,4].

Theoretically a transacylation mechanism, first discovered in rat liver supernatant by ERBLAND AND MARINETTI[46] might represent a mechanism involved in the synthesis of dipalmitoyl lecithin of lung. This mechanism has also been shown in rat lung supernatant by VAN DEN BOSCH *et al.*[47]. Experiments carried out with homogenates from rat liver[47,48] and rat lung[47] and also microsomes from rat liver[48] have shown that the incorporation of doubly labeled 1-acyl-*sn*-glycero-3-phosphorylcholine into lecithin occurs, in the presence of excess non-labeled fatty acids and ATP and CoA, almost exclusively *via* a direct acylation rather than *via* transacylation between two monoacyl-*sn*-glycero-3-phosphorylcholines. The contribution of the transacylation mechanism, detectable *in vitro* only under energy poor conditions in the cytosol to the production of dipalmitoyl lecithin *in vivo* is probably very small.

It has been suggested by MORGAN[29] that the methylation of phosphatidylethanolamine may represent an important pathway in the formation of dipalmitoyl lecithin of lung. This suggestion is not supported by SPITZER *et al.*[31], WEINHOLD[32] and WOLFE *et al.*[33]. These authors found in agreement with previous observations of BJØRNSTAD AND BREMER[30] that the methylation of phosphatidylethanolamine is a minor contributor to the synthesis of lecithin in lung. Table IV shows the fatty acid patterns of phosphatidylethanolamine and phosphatidylcholine of rat lung. It is interesting to note that the palmitic acid content of phosphatidylethanolamine is much lower than that of lecithin. In fact the palmitic acid content of lung phosphatidylethanolamine is equal to the level of this acid observed for lecithin and phosphatidylethanolamine from liver. Moreover, no significant amount of disaturated phosphatidylethanolamine could be detected after thin-layer chromatography on $AgNO_3$-impregnated silica. This observation supports the findings of BJØRNSTAD AND BREMER[30], SPITZER *et al.*[31], WEINHOLD[32] and WOLFE *et al.*[33] that the methylation of phos-

TABLE IV

THE FATTY ACID COMPOSITION OF PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE FROM RAT LUNG

Fatty acid	*Mol. percentage*	
	Phosphatidylethanolamine	*Phosphatidylcholine*
16:0	25.1	59.2
16:1	+	1.6
18:0	18.9	7.1
18:1	21.0	11.3
18:2	6.9	12.9
20:3	5.1	–
20:4	23.0	7.9

phatidylethanolamine does not play an important role in the formation of dipalmitoyl lecithin. The present work shows that dipalmitoyl lecithin of lung can be synthesized, at least partially, *via* the CDP-choline pathway whereas a deacylation–reacylation cycle may represent an additional mechanism in the biosynthesis or maintaining of this important compound of the lung surfactant.

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