Source of Lung Surfactant Phospholipids: Comparison of Palmitate and Acetate as Precursors

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ABSTRACT

The phospholipids and the fatty acid compositions of major phospholipids in rat lung parenchyma, microsomes, lamellar bodies and alveolar wash were quantified. Adult rats were injected simultaneously with [^{3}H]palmitate and [^{14}C]acetate into the femoral vein. The appearance of labeled phosphatidylcholine (PC), disaturated phosphatidylcholine (DSPC) and phosphatidylglycerol (PG) in each lung fraction was measured during short periods of time (5 min to 2 hr) after isotope administration. Relatively more PC, DSPC and PG labeled with acetate radioactivity in lung microsomes entered lamellar body and alveolar wash fractions than those labeled with palmitate radioactivity. However, there was no difference between palmitate and acetate labeled phospholipids in the transport from microsomes to lamellar bodies by phospholipid exchange proteins. On the other hand, prior injection of colchicine resulted in decrease in the transport of PC from microsomes to alveolar space to a relatively greater extent in the acetate radioactivity than in the palmitate radioactivity.

Lipids 17:884-892, 1982.

INTRODUCTION

1,2-Dipalmitoyl species of phosphatidylcholine (PC) and phosphatidylglycerol (PG) are the main constituents of lung surfactant (1), which has been implicated in the maintenance of lung compliance (2). In order to supply sufficient amounts of palmitate for the synthesis of these dipalmitoyl species, the lung tissue actively takes up palmitate from the circulation and synthesizes palmitate endogenously de novo. Labeled palmitate administrated intravenously is rapidly taken up by the lung and incorporated preferentially into phospholipids, particularly into PC (3-5). On the other hand, lung slice experiments with labeled acetate have shown that palmitate accounts for greater than 80% of the radioactivity incorporated into phospholipids (6). Namely, the major product of the de novo fatty acid synthesis from acetate appears to be palmitate (7,8). This notable characteristic in the de novo fatty acid synthesis in the lung permits a comparison of the endogenously synthesized palmitate as the acetate radioactivity and the exogenously supplied palmitate as palmitate radioactivity in the metabolism of lung phospholipids. Studies on their relative differences revealed that they were metabolized differently in the transfer into alveolar space (9,10), as membrane bound substrate for microsomal phospholipase A_2 (11), or in the synthesis of dipalmitoyl PC by the effect of essential fatty acid deficiency (12). Jobe (9) demonstrated that the relative specific activities of surfactant PC and DSPC labeled with acetate radioactivity were ca. twice those measured using the palmitate radioactivity.

The present study was made to confirm the preferential appearance of palmitate synthesized de novo in the surfactant phospholipids, and further to study possible differences between the endogenously synthesized and exogenously supplied palmitate in the transfer mechanisms of phospholipids from microsomes to the surfactant related fractions.

MATERIALS AND METHODS

Materials

[9,10-^{3}H]Palmitic acid (sp act, 500 mCi/mmol), [1-^{14}C]palmitic acid (sp act, 50 mCi/mmol), [1-^{14}C]acetic acid (sp act, 60 mCi/mmol) and [^{3}H]acetic acid (sp act, 300 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, England. The labeled palmitic acids were complexed with fatty acid-free bovine serum albumin (Sigma Co.) according to the method described by Åkesson et al. (13).

In vivo Experiments

Male Wistar rats weighing ca. 250 g were fasted for 16 hr before isotope injection. 0.25 ml of 4% albumin-0.9% saline solution containing 50 μCi (26 μg) of [9,10-^{3}H]palmitic acid and 200 μCi (50 μg) of [1-^{14}C]acetic acid were injected within 5 sec into the femoral vein of the rats under slight ether anesthesia. Some rats were pretreated by the intraperitoneal injection of colchicine (10 mg) in 0.9% saline (0.5 ml)

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TABLE 1

Phospholipid Composition of Rat Lung Fractions

	Parenchyma (n=5)	Microsomes (n=5)	Lamellar bodies (n=4)	Alveolar wash (n=4)
Total phospholipid content (μmol/g wet tissue)	18.1 ± 2.5	1.93 ± 0.6	0.23 ± 0.04	1.16 ± 0.3
Phosphatidylcholine	45.5 ± 4.4	41.7 ± 5.2	74.2 ± 4.5	81.7 ± 2.0
Phosphatidylethanolamine	25.6 ± 2.8	23.1 ± 2.1	11.5 ± 1.7	4.4 ± 1.0
Phosphatidylglycerol	2.0 ± 0.3	4.6 ± 4.7	8.5 ± 0.2	9.4 ± 0.9
Phosphatidylinositol	9.0 ± 1.9	9.6 ± 1.6	0.6 ± 0.8	1.6 ± 0.7
Phosphatidylserine	2.6 ± 1.5	3.6 ± 2.4	1.5 ± 1.7	
Sphingomyelin	13.1 ± 3.4	12.3 ± 2.1	3.0 ± 0.6	1.9 ± 0.9
Lysophosphatidylcholine	1.6 ± 1.3	3.2 ± 0.1	0.6 ± 1.0	0.2 ± 0.3
Lysophosphatidylethanolamine	0.2 ± 0.2	1.8 ± 1.5	tr	tr
Lyso-*bis*-phosphatidic acid	0.4 ± 0.2	0.1 ± 0.1	0.1 ± 0.6	0.8 ± 0.8

The results are presented as mean percent composition ± standard deviation (n).

according to the description by Delhunty and Johnston (14). After 3 hr, the isotopes were injected into the animals in the same manner as above. After different time intervals, the rats were killed by bleeding through the abdominal aorta. The trachea was opened in the neck and cannulated with a polyethylene tube connected to a syringe. The lungs were washed 4 times with the same 5 ml of 0.9% saline. The effluent volume recovered was about 14 ml. The alveolar wash was not centrifuged to remove cellular materials, as in the method for rabbit lung alveolar wash described by Jobe (15).

Isolation of Rat Lung Fractions

The isolation procedure for rat lung fractions was carried out generally according to the method described for rabbit lung fractions by Jobe (15). The recovery of phospholipid in the lamellar body fraction and the microsomes isolated from rat lung by this procedure was 1.3% and 10.7% of the total lung phospholipid. The ratio of phospholipid (μmol) to protein (mg) was 0.33 ± 0.6 for the microsomes and 2.15 ± 0.52 for the lamellar body fraction.

Phospholipid-transfer Experiments

Labeled microsomes from rat lung were prepared by lung slice experiments with [1-^{14}C] palmitic acid and [^{3}H]acetic acid; tissue slices from two rat lungs (2.4 g) were incubated at 37 C in 20 ml of Krebs-Ringer medium containing [1-^{14}C]palmitic acid (500 μ Ci, 2.6 mg) complexed with bovine serum albumin or [^{3}H] acetic acid (5 mCi, 1.0 mg). The lung slices were removed after 1.5 hr incubation, rinsed in cold 0.25 M sucrose/1 mM EDTA/10 mM Tris-HCl (pH 7.4) (buffer A), and the microsomes were isolated as mentioned above except for the use of buffer A. The isolation procedures for unlabeled mitochondria and lamellar bodies from rat lung, and the assay procedures for phospholipid-transfer activity were essentially the same as described by Engle et al. (16). The 100,000 × g supernatant fractions, isolated as described by Vereyken et al. (17), were adjusted to pH 5.1 with 3 M HCl. After standing in ice for 30 min with occasional stirring, the suspensions were centrifuged at 15,000 × g for 15 min. The supernatants were then adjusted to pH 7.4 with solid Tris. In this study, they were designated as pH 5.1 supernatant, which were used as phospholipid exchange proteins in phospholipid-transfer experiments.

Lipid Analysis

Analytical procedures for lipids were generally the same as described in our previous paper (12,18,19). Lipids of the lung parenchyma, microsomes, lamellar bodies and alveolar wash were extracted by the method of Bligh and Dyer (20), after which they were subjected to two-dimensional thin layer chromatography (TLC) to separate individual lipid classes (21). For analysis of phospholipid composition, the spots on the plates were detected by charring and analyzed for phosphorus. The spots were also detected by fluorescein spray and each phospholipid was recovered from the gel by the method of Arvidson (22). PC isolated was converted to 1,2-diacyl-3-acetylglycerol and separated into molecular classes using the procedures described by Okano et al. (19). The PC was also subjected to permanganate/periodate oxidation to isolate desaturated phosphatidylcholine species according to the method of Shimojo et al.

TABLE 2

Fatty Acid Composition of Phosphatidylcholine and Phosphatidylglycerol from Rat Lung Fractions

	Phosphatidylcholine				Phosphatidylglycerol		
	Parenchyma (n=5)	Microsomes (n=5)	Lamellar bodies	Alveolar wash (n=5)	Parenchyma	Microsomes	Alveolar wash
Total							
14:0	1.3 ± 0.3	1.1 ± 0.2	2.2	2.3 ± 0.2	0.6	0.4	2.4
16:0	51.8 ± 2.1	49.5 ± 1.2	65.4	66.1 ± 2.2	46.5	38.7	57.2
16:1	3.7 ± 1.5	4.0 ± 1.7	6.5	10.1 ± 0.6	2.3	2.8	8.9
18:0	9.5 ± 0.4	10.2 ± 0.7	4.0	3.2 ± 0.5	7.0	8.4	4.0
19:1	13.4 ± 1.6	13.1 ± 0.5	7.4	6.9 ± 1.0	18.4	20.1	12.3
18:2	8.8 ± 0.8	9.1 ± 0.5	8.5	7.4 ± 1.2	15.3	17.8	7.2
20:3	0.3 ± 0.1	0.4 ± 0.1	0.5	tr	tr	tr	tr
20:4	7.1 ± 1.0	8.3 ± 0.9	4.7	3.1 ± 0.8	5.1	6.0	3.3
20:5	0.5 ± 0.1	0.6 ± 0.05	0.3	0.4 ± 0.2	0.3	tr	tr
22:5	0.8 ± 0.1	0.9 ± 0.1	tr	tr	0.7	0.8	tr
22:6	1.0 ± 0.2	1.0 ± 0.2	0.4	tr	2.2	2.8	1.1
Others	1.8 ± 0.5	1.8 ± 0.5	0.1	0.5 ± 0.2	1.6	2.2	3.6
2-Position							
14:0	1.2 ± 0.4	0.9 ± 0.3	2.9	3.1 ± 0.6	tr	–	–
16:0	34.8 ± 4.1	32.3 ± 2.3	45.5	49.0 ± 4.3	24.5	–	–
16:1	6.3 ± 2.9	9.0 ± 0.7	12.2	18.7 ± 0.9	4.1	–	–
18:0	2.0 ± 0.5	1.7 ± 0.2	1.5	0.3 ± 0.2	tr	–	–
18:1	17.2 ± 6.7	18.0 ± 1.4	11.4	8.5 ± 1.1	22.6	–	–
18:2	15.0 ± 1.2	15.0 ± 0.01	13.6	12.9 ± 2.4	29.0	–	–
20:3	0.6 ± 0.3	0.6 ± 0.4	1.0	tr	0.4	–	–
20:4	14.3 ± 2.0	15.8 ± 1.1	9.3	6.2 ± 1.7	10.2	–	–
20:5	0.9 ± 0.3	1.2 ± 0.1	0.6	0.8 ± 0.8	1.4	–	–
22:5	1.5 ± 0.3	1.8 ± 0.2	tr	tr	0.6	–	–
22:6	1.9 ± 0.3	1.9 ± 0.5	0.7	0.1 ± 0.1	4.4	–	–
Others	3.1 ± 0.8	2.8 ± 0.9	1.3	1.3 ± 0.8	2.8	–	–

The results are presented as mean percent composition ± standard deviation (n). Where no standard error is given, the values were obtained from single analyses of samples pooled from 8 rats. tr, trace amounts; –, not determined.

(23). The positional distribution of the fatty acids of PC and PG was analyzed by hydrolysis with phospholipase A_2 (*Crotalus adamanteus* and *Crotalus atrox*). The free fatty acids and lysophospholipids prepared were separated by TLC. The fatty acid methyl esters prepared by BF_3/CH_3OH (24) were analyzed by gas liquid chromatography (GLC).

A part of the labeled phospholipids from lung parenchyma was transmethylated by BF_3/CH_3-OH (24). The fatty acid methylesters prepared were separated into saturates, monoenes, dienes and polyenes by argentation TLC (25). The saturates were further separated according to the carbon numbers by reverse-phase TLC (25).

Protein was determined by the method of Lowry et al. (26). Phosphorus was determined by the method of Bartlett (27). The amount of 1,2-diacyl-3-acetylglycerol was estimated by glycerol determination according to the method of Van Handel and Zilversmit (28). Radioactivity counting was carried out with a Packard Liquid scintillation spectrometer using a toluene based scintillator as described by Snyder (29), and Aquasol (New England Nuclear) when determined with silica gel.

RESULTS

The phospholipid composition of four lung fractions, lung parenchyma, microsomes, lamellar bodies and alveolar wash, are presented in Table 1. As noted earlier (30,31), the phospholipid profiles of the lung parenchyma and microsomes were quite similar, but significantly different from those of the lamellar bodies and alveolar wash. Compared to the phospholipid profiles in microsomes, PC and PG increased approximately 2-fold in the surfactant related fractions, e.g., lamellar bodies and alveolar wash. In contrast, sphingomyelin and phosphatidylethanolamine (PE) decreased significantly in the surfactant related fractions. The fatty acid compositions of the major phospholipids, i.e., PC and PG, in the surfactant related fractions are given in Table 2. In these 2 phospholipids, palmitic acid was the major fatty acid, not only in the total but also in the 2-position. Its relative concentration increased in the order of microsomes, lamellar bodies and alveolar wash. Concomitant decreases in the relative concentration of oleic and arachidonic acids were also found in the supernatant related fractions.

Table 3 provides a comparison of the proportion of the molecular classes of PC from the 4 lung fractions. Consistent with the findings on their fatty acid patterns, significant increases were found in the disaturated classes in the surfactant related fractions and, conversely, de-

TABLE 3

Composition of Molecular Species of Phosphatidylcholine and Phosphatidylglycerol from Rat Lung Fractions

	Phosphatidylcholine				Phosphatidylglycerol	
	Parenchyma (n=5)	Microsomes (n=5)	Lamellar bodies (n=4)	Alveolar wash (n=4)	Parenchyma	Alveolar wash
Pool size (μmol/g wet tissue)	8.25 ± 1.2	0.78 ± 0.2	0.17 ± 0.03	0.95 ± 0.2	0.36 ± 0.05	0.11 ± 0.03
Saturated	34.3 ± 4.7	29.9 ± 0.8	52.8 ± 0.9	54.2 ± 5.5	22.3	36.4
Monoenoic	21.5 ± 3.8	26.1 ± 5.6	27.1 ± 0.2	26.3 ± 3.6	28.5	35.6
Dienoic	20.6 ± 3.9	19.7 ± 4.2	11.2 ± 0.6	11.8 ± 1.2	21.2	18.5
Trienoic	3.8 ± 0.7	3.9 ± 1.0	2.4 ± 0.6	2.0 ± 0.3	7.7	2.5
Tetraenoic	16.5 ± 1.8	17.1 ± 4.8	4.3 ± 1.8	4.0 ± 1.1	11.1	5.3
Polyenoic	3.2 ± 1.4	3.3 ± 1.3	2.2 ± 1.6	1.6 ± 0.4	7.3	1.7

The results are presented as mean percent composition ± standard deviation (n). Where no standard error is given, the values were obtained from duplicate analyses of samples pooled from 8 rats.

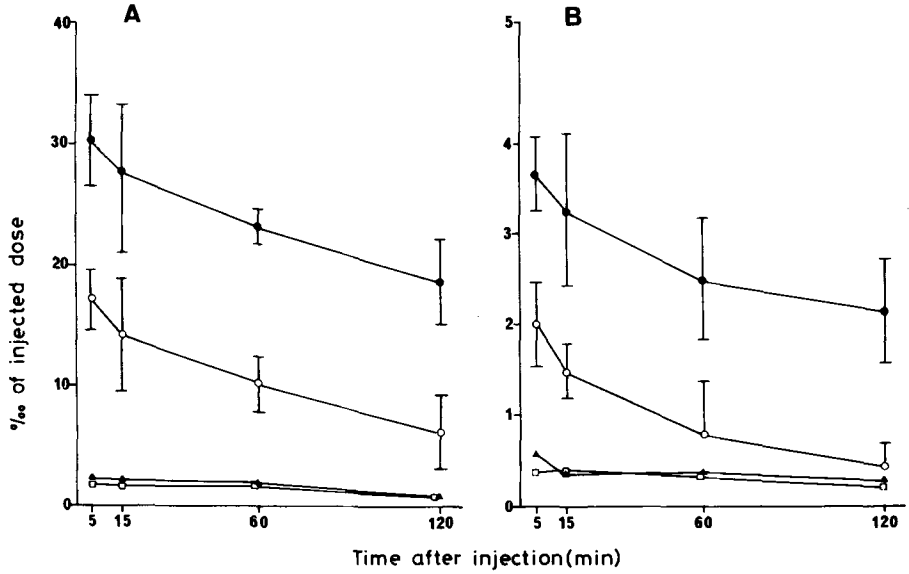

FIG. 1. Incorporation of [9,10-^{3}H]palmitic acid (A) and [1-^{14}C]acetic acid (B) into each lipid clss of rat lung parenchyma after intrafemoral injection. For phosphatidylcholine and triacylglycerol, the data are given as the average values ± SD for 3 independent experiments. The range of deviation was omitted in other fractions. •, phosphatidylcholine; ○, triacylglycerol; ▲, phosphatidylglycerol; □, phosphatidylethanolamine.

creases were observed in the dienoic and tetraenoic classes. Our analytical results were essentially in agreement with those reported in rabbits (31) and rats (30).

In order to elucidate the source of palmitate acylating the main phospholipids in the surfactant related fractions, a comparison of the labeling profiles was made using [9,10-^{3}H]palmitate and [1-^{14}C]acetate as the sources of surfactant phospholipids. Following the injection of the above-mentioned isotopes, the radioactivity distribution among fatty acids acylating in the total phospholipids from lung parenchyma was determined. Ten min after injection, ^{14}C-activity was 85.4% for palmitate and 6.0% for unsaturated acids. There was little change in these percentages during the experimental time periods, which seems to indicate that palmitate was primarily synthesized from the acetate radioactivity in the lung tissue. On the other hand, ca. 95% of the ^{3}H-activity was recovered as palmitate.

Figure 1 shows the appearance of [^{3}H]palmitate and [^{14}C]acetate in various lipid classes of rat lung parenchyma during short periods of time after the simultaneous injection of both radioactivities. The labeling profiles of lipid classes were similar for both radioactivities. The ^{3}H and ^{14}C activities in lipid classes rapidly decreased from 5 min to 120 min after the injection. Among lipid classes, PC was predominantly labeled with both precursors. These results indicate that both radioactivities incorporated into lung lipids with similar kinetics.

Figure 2 shows the time-dependent changes in specific radioactivities of PC, DSPC and PG in 3 lung fractions after injection of [^{3}H]palmitate and [^{14}C]acetate. In order to compare labeling profiles of both precursors, the specific radioactivities of these phospholipids are shown as specific activities relative to the unity of the specific radioactivities of microsomal PC at 60 min after injection of each isotope. The specific activities in lung microsomes were highest at the initial time point of 5 min after the injection, after which they rapidly decreased. The appearance in the lamellar body fraction of the labeled phospholipids was also rapid. Maximal specific activities were achieved within 1 hr after injection. The specific activities of these phospholipids in lamellar bodies exceeded those in microsomes 2 hr after injection. This seems to indicate that the microsomal subpool of phospholipids destined for the lamellar bodies may exist in the lung. The labeled phospholipids appeared in the alveolar space in a linear fashion for a period of 2 hr, although their specific activities were still lower compared to those in lamellar bodies. These labeling profiles were similar in both precursors. It should, however,

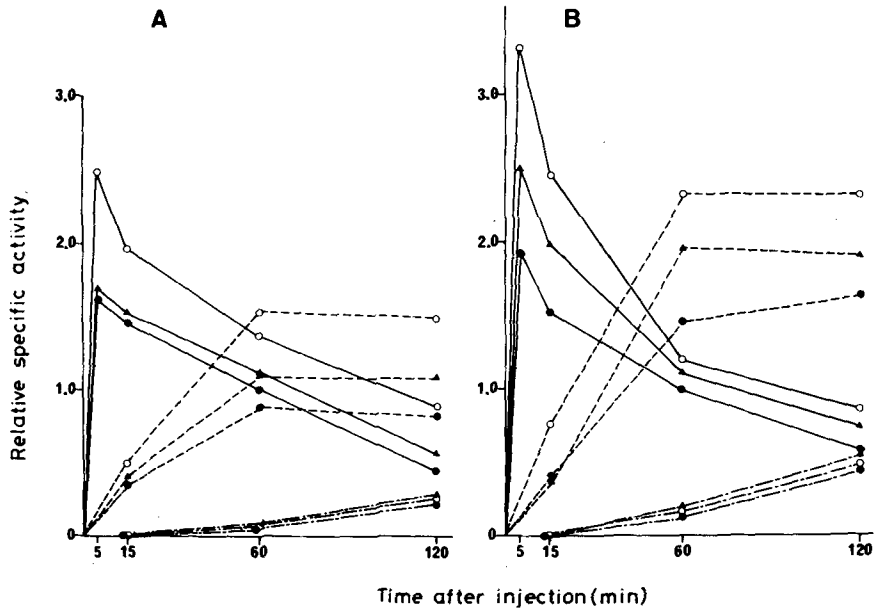

FIG. 2. Changes of specific radioactivity of phosphatidylcholine, disaturated phosphatidylcholine and phosphatidylglycerol in microsomes, lamellar bodies and alveolar wash of rat lung after intrafemoral injection of [9,10-^{3}H]palmitic acid (A) and [1-^{14}C]acetic acid (B). The data are given as relative specific activity calculated as unity of the specific radioactivity of microsomal phosphatidylcholine at 60 min after injection of each isotope, which was 21.6 ± 1.7 dpm/nmol for ^{3}H-activity and 9.3 ± 0.8 dpm/nmol for ^{14}C-activity. Each mark is the average value from 2 to 3 animals. The range of deviation was omitted for technical reasons. —— line, microsomes; ------ line, lamellar bodies; - - - -line, alveolar wash. •, phosphatidylcholine; ○, disaturated phosphatidylcholine; ▲, phosphatidylglycerol.

be noted that the relative specific activities of these phospholipids in the surfactant related fractions were significantly higher in the acetate radioactivity than those in the palmitate radioactivity.

In order to compare the rate of appearance of PC, DSPC and PG labeled with both [^{3}H] palmitate and [^{14}C]acetate from microsomes to the surfactant related fractions, the ratios of ^{14}C-activity to ^{3}H-activity of these phospholipids in the 3 lung fractions were calculated (Table 4). The results showed that, when the ^{14}C/^{3}H ratios of these phospholipids in lamellar body and alveolar wash fractions were calculated as the unity of those in microsomal phospholipids, ^{14}C-activity was ca. from 1.5 to 1.8 times of ^{3}H-activity in the lamellar body fractions and ca. from 1.6 to 2.7 times in alveolar wash in the 3 phospholipids. These data indicate that relatively more PC, DSPC and PG labeled with [^{14}C] acetate in lung microsomes entered the surfactant related fractions than those labeled with [^{3}H] palmitate. These metabolic findings strongly suggest that PC and PG utilize both palmitates synthesized de novo and supplied exogenously for their synthesis in lung microsomes with similar kinetics, but that phospholipids acylating palmitate synthesized de novo appear preferentially in the surfactant related fractions.

Possible differences in metabolic fate between palmitate and acetate radioactivities were further studied with regard to the transport of surfactant phospholipids from microsomes to the surfactant related fractions. A fraction containing phospholipid exchange proteins catalyzed the transfer of various phospholipids from microsomes labeled with palmitate and acetate to unlabeled mitochondria or lamellar bodies. However, there was no difference between palmitate and acetate radioactivities in the transport from microsomes to mitochondria or lamellar bodies (data not shown). It is, therefore, likely that the phospholipid exchange proteins do not distinguish between the palmitate and acetate labeled phospholipids in their transport from microsomes. Another transport system, the microtubular system, was tested using colchicine-treated rats. The results are shown in Table 5. The prior injection of colchicine resulted in marked decreases in the secretion of PC and DSPC labeled with both precursors into the alveolar space. This decrease was more signifi-

TABLE 4

Comparison of Labelings with [^{3}H] Palmitate and [^{14}C] Acetate in Phosphatidylcholine, Disaturated Phosphatidylcholine and Phosphatidylglycerol of Rat Lung Fractions

Time after injection (min)	Lung fraction	$^{14}C/^3H$ ratio of relative specific activity[a]		
		Phosphatidyl-choline	Disaturated phos-phatidylcholine	Phosphatidyl-glycerol
60	Microsomes	1.00	0.87 (1.00)	1.02 (1.00)
	Lamellar bodies	1.65	1.62 (1.86)	1.77 (1.74)
	Alveolar wash	2.23	2.07 (2.38)	2.81 (2.75)
120	Microsomes	1.34	0.98 (1.00)	1.23 (1.00)
	Lamellar bodies	2.02	1.54 (1.57)	1.77 (1.44)
	Alveolar wash	1.93	1.87 (1.91)	2.00 (1.63)

Values in parenthesis are the values calculated as unity of the $^{14}C/^3H$ ratio in microsomes. The results are means from three independent experiments.

[a]Relative specific activities were calculated as unity of specific activity of phosphatidylcholine in microsomes at 60 min after the injection of [^{3}H] palmitate and [^{14}C] acetate.

cant in the acetate labeled phospholipids than in the palmitate labeled phospholipids. These findings suggest that the lung microtubular system may participate in the secretion of surfactant phospholipids into alveolar space. They also suggest that phospholipids that acylate palmitate de novo synthesized may be transfered preferentially to the alveolar space by the microtubular system.

DISCUSSION

Jobe (9) demonstrated that in in-vivo experiments to determine the specific activities of PC and DSPC, more palmitate synthesized from labeled acetate than that supplied from circulation was preferentially incorporated into lung PC and DSPC destined to become surfactant. The present study confirmed the results of earlier reports by Jobe (9) and Jobe et al. (10) in experiments carried out during relatively short periods of time (from 5 min to 2 hr) after simultaneous administration of labeled palmitate and acetate. In those experiments and ours, relatively more PC, DSPC and PG acylating de novo synthesized palmitate than that acylating exogenously supplied palmitate entered the surfactant related fractions. There, however, has been no conclusive evidence presented to explain this preferential appearance of phospholipids labeled with acetate in the surfactant fractions.

There are several possible explanations for this preferential appearance: (a) whole lung microsomes are derived from many different cell types. Compared to other lung cell types, alveolar type II cells may utilize more acetate for the surfactant phospholipid synthesis than plasma-born plamitate. It has been reported, however, that a relatively greater amount of exogenous palmitate is utilized by alveolar type II cells for the synthesis of dipalmitoyl PC and PG than is utilized by other lung cell types (32). In addition, the metabolic profiles in PC, such as that for positional distribution, are almost the same for palmitate synthesized de novo from acetate as for that supplied exogenously (33). (b) There may be metabolically different palmitate pools in the lung microsomes. De novo synthesized palmitate may enter preferentially a pool destined for the surfactant phospholipids. In contrast, exogenously administrated palmitate may be primarily acylated to membrane phospholipids. The existence of different palmitate pools in lung microsomes was suggested by Longmore et al. (11), who reported that microsomal PC labeled with [1-^{14}C] acetate were utilized as substrate by microsomal phospholipase A_2, while those labeled with [9,10-^{3}H] palmitate were not degraded by this enzyme. Groener and van Golde (34) also showed in studies with isolated rat hepatocytes that palmitate synthesized endogenously from acetate did not mix completely with exogenously supplied palmitate. (c) There may be a preferential transport of phospholipids acylating de novo synthesized palmitate from the microsomes to the surfactant fractions. If this is the case, there may be a specific transfer system available for the specific phospholipid components including preferentially de novo synthesized palmitate in the surfactant fractions. This possibility has not been tested previously.

The mechanisms by which surfactant phospholipids are transported from their synthetic site in alveolar type II cells to the lamellar bodies are unknown. The phospholipid exchange proteins, which are present in cytosol and cata-

lyzed the intracellular transfer of phospholipids, have been demonstrated in several mammalian tissues (35-39). In the lung, the occurrences of such soluble proteins have been described for PC (40,41) and PG (42,43). However, the present study showed that there was no difference between palmitate and acetate labeled phospholipids in the transport by phospholipid exchange proteins from labeled microsomes to mitochondria or lamellar bodies. This would seem to indicate that phospholipid exchange proteins do not distinguish between microsomal phospholipids having endogenously synthesized palmitate and palmitate exogenously supplied during intracellular transport. On the other hand, the results of our experiments with colchicine-treated rats showed that the microtubular system in the lung might participate in translocation of PC from microsomes to surfactant fractions, and that the phospholipids acylating palmitate which was synthesized de novo from acetate might be preferentially transferred from microsomes to surfactant fractions by the microtubular system. Namely, the present observation strongly suggests that, when certain microsomal phospholipids are directed toward the surfactant fractions, the microtubular system may have a relative specificity for phospholipids acylating palmitate synthesized endogenously compared to phospholipids acylating palmitate supplied exogenously.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Education, Science and Culture, Japan, and a grant from Hokkaido Geriatric Institute.

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TABLE 5

Effects of Colchicine on the in-vivo Transport of Phospholipids Labeled with [9.10^{-3}H]Palmitate and [1-^{14}C]Acetate in Rat Lung

		Specific radioactivity (dpm/nmol-lipid)					
		Palmitate label-^{3}H			Acetate label-^{14}C		
		Control	Treated	Treated/control	Control	Treated	Treated/control
Phosphatidylcholine	Microsomes	13.7	48.5	3.54	12.0	45.2	3.78
	Lamellar bodies	34.4	55.6	1.62	36.1	45.6	1.26
	Alveolar wash	2.0	0.9	0.45	3.5	0.7	0.20
Saturated phosphatidylcholine	Microsomes	31.7	68.8	2.17	28.0	84.2	3.01
	Lamellar bodies	32.4	94.2	2.90	33.6	82.6	2.45
	Alveolar wash	2.6	1.7	0.65	4.1	1.2	0.29

The rats were pretreated by the intraperitoneal injection of colchicine (10 mg) per rat in saline (0.5 ml). After 3 hr, the animals were injected simultaneously with [9.10^{-3}H]palmitate (50 μCi) and [1-^{14}]acetate (200 μCi) complexed with 4% bovine serum albumin into the femoral vein. 2 hr after the isotope administration, the animals were killed and the specific radioactivities of phospholipids in rat lung fractions were determined. Data are averages of 2 experiments.

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[Received March 15, 1982]