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EVIDENCE FOR THE SYNTHESIS OF LUNG SURFACTANT DIPALMITOYL PHOSPHATIDYLCHOLINE BY A "REMODELING" MECHANISM

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SUMMARY: Synthesis of dipalmitoyl phosphatidylcholine in the isolated perfused rat lung has been studied using radio labeled glucose and palmitate. Following subsequent isolation of lamellar bodies, microsomes and surfactant and nonsurfactant fractions, evidence in support of at least two pools of palmitate, one synthesized de novo from glucose and one supplied by exogenous palmitate for dipalmitoyl phosphatidylcholine synthesis has been obtained. Further, the results indicate that palmitate of dipalmitoyl phosphatidylcholine is derived to a greater extent from the de novo synthesized pool of palmitate than from exogenous palmitate and that dipalmitoyl phosphatidylcholine synthesis involves a "remodeling" mechanism.

INTRODUCTION: Pulmonary surfactant is a complex mixture of lipids and proteins, consisting primarily of dipalmitoyl phosphatidylcholine, which lowers the surface tension in alveoli at end expiration and prevents lung collapse. Autoradiographic studies have suggested that surfactant phospholipids or their precursors are synthesized in the endoplasmic reticulum of type II pneumocytes (1). The lipids are then transported to the Golgi apparatus, and finally to unique organelles, lamellar bodies, for storage and eventual secretion into the alveolar space.

The extent to which the palmitate moiety of dipalmitoyl phosphatidylcholine in lung surfactant is derived from exogenous palmitate obtained from serum lipids in contrast to that synthesized in the surfactant producing type II epithelial cells of lung is not known. In addition, the mechanism of synthesis of dipalmitoyl phosphatidylcholine in lung is not established as indicated by recent conflicting reports in which data from various in vivo

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and in vitro models have been gathered (2-6). The synthesis of dipalmitoyl phosphatidylcholine may be the result of initial synthesis by the CDP-choline pathway (7) or the result of remodeling of the unsaturated phosphatidylcholines synthesized by that pathway. Acyl rearrangement of phospholipids may take place via a phospholipase A_2-reacylase mechanism in the endoplasmic reticulum (8,9) or in the lamellar bodies (10) or by a transacylation reaction between two molecules of lysophosphatidylcholine in the cytoplasm of lung cells (11). The predominant site and method of acyl remodeling, if it is of importance, is also open to question at this time.

The present experiments, using the isolated perfused adult rat lung as a model, were performed to determine if certain substrates were used preferentially by the lung for the fatty acids of lamellar body and surfactant phosphatidylcholine and to detect any post-microsomal remodeling of lamellar body and surfactant phosphatidylcholine fatty acids.

MATERIALS AND METHODS: Lungs from fed, adult male Wistar-strain rats were used in all experiments. The rats were anesthetized with pentobarbital (50 mg/kg) and the lungs removed and perfused as described by Godinez and Longmore (12) in a water-saturated cabinet maintained at 37° C. The lungs were ventilated 40 times per min at a tidal volume of 2.5 ml with 90% O_2:10% CO_2. A recirculating perfusion medium composed of Krebs-Ringer bicarbonate buffer (pH 7.35) (13) with 3% (w/v) bovine serum albumin (Fraction V, Miles Laboratories) was oxygenated with 90% O_2:10% CO_2 and gravity fed to the lungs at a flow rate of 10 ml per min with a pressure of 20 cm of H_2O. All media contained 5.6 mM glucose and 0.5 mM palmitate. Single isotope studies were performed with [U-^{14}C]glucose or [1-^{14}C]palmitate while dual label studies employed [1-^{14}C]palmitate and 0.5 mM [1,3-^{3}H]glycerol or [U-^{14}C]glucose and [9,10-^{3}H]palmitate. The rates of incorporation of the labeled substrates were linear during the 1 hr perfusion period.

All radioactive substrates were purchased from New England Nuclear Corporation, Boston, MA. The specific activity of each radiolabeled substrate in the various perfusion media was determined from media samples taken at regular intervals during the perfusion.

Following perfusion, the lungs were immediately processed to yield either lamellar body and microsomal or surfactant and nonsurfactant fractions. For the surfactant preparation, the lungs were immediately chilled and homogenized in cold (4° C) 0.154 M NaCl, 0.001 M EDTA, 0.010 M Tris-HCl, pH 7.40. The resulting homogenate was then centrifuged to isolated surfactant and nonsurfactant fractions as previously reported (4). This procedure isolates the intra- and extracellular surfactant together in a single fraction. Tissue to be utilized for the preparation of lamellar bodies and microsomes was placed in ice-cold 0.33 M sucrose, 0.010 M Tris-HCl, pH 7.40. The lamellar bodies and microsomes were then isolated as described previously (10).

Lipids were extracted from the four isolated fractions by homogenization in chloroform:methanol (2:1, v/v). Non-lipid contaminants were removed by the method of Radin (14). The phospholipids were further separated by conventional column and thin-layer chromatographic methods (10). Phospholipid phosphorous was determined by a modification of the Bartlett procedure (15).

For some of the dual-label perfusion experiments, both the α- and β-fatty acids were removed from the glyceride backbone using the mild alkaline hydryolysis method of Dittmer and Wells (15).

RESULTS AND DISCUSSION: Comparison of the rates of incorporation of [U-^{14}C]glucose and [1-^{14}C]palmitate into the phosphatidylcholine of the four subcellular fractions is presented in Table I. Palmitate labeled phosphatidylcholine of the microsomal fraction at a rate approximately three times that of the rate into phosphatidylcholine of the lamellar bodies. Glucose carbons were also incorporated into the microsomal phosphatidylcholine at a higher rate than their incorporation into lamellar body phosphatidylcholine; this isotope labeled the microsomal phosphatidylcholine at a rate twice that for lamellar body phosphatidylcholine. The labeling pattern of lamellar bodies and microsomes is very similar to that found for surfactant and nonsurfactant. For these fractions palmitate was found to label nonsurfactant phosphatidylcholine at a rate approximately twice that for surfactant phosphatidylcholine. Glucose incorporation was found to be 50% greater into nonsurfactant phosphatidylcholine than into surfactant phosphatidylcholine. These data demonstrate that the incorporation of glucose and palmitate into

TABLE I

Incorporation of [1-^{14}C]palmitate and [U-^{14}C]glucose into Phosphatidylcholine of Rat Lung Subcellular Fractions[a]

Subcellular fraction	Incorporation of: (DPM/nmole/hr) Palmitate	Glucose
Lamellar body	13.2 ± 2.0 (5)[b]	13.3 ± 1.3 (4)
Microsome	35.8 ± 1.8 (5)	28.6 ± 1.4 (4)
Surfactant	19.5 ± 1.2 (5)	13.5 ± 1.0 (6)
Nonsurfactant	41.6 ± 1.9 (5)	18.5 ± 0.8 (6)

[a]Perfusion conditions were as described in Materials and Methods.

[b]Values are the mean ± S.E.M. for (N) determinations.

the surfactant phosphatidylcholine correlates well with the incorporation obtained into the phosphatidylcholine of lamellar bodies. Thus studies of surfactant synthesis are a good approximation of lamellar body synthesis. Furthermore, the incorporation of precursors into nonsurfactant phosphatidylcholine is a good approximation of microsomal synthesis.

Previous studies in this laboratory (4) have shown that palmitate and glucose are incorporated to a greater degree into the β-position palmitate as compared to the α-position palmitate of surfactant and nonsurfactant phosphatidylcholine. This relationship also holds true for the incorporation of glucose and palmitate into the α- and β- fatty acids of microsomal and lamellar body phosphatidylcholine (data not shown). Another recent study supports the preferential incorporation of palmitate into the β-position of dipalmitoyl phosphatidylcholine of lung microsomes (16). These data would indicate that there are at least two fatty acid pools in the lungs, and that the α- and β- fatty acids are esterified at different times. This implies that fatty acid remodeling of lamellar body and surfactant phosphatidylcholine is occurring subsequent to its de novo synthesis in the endoplasmic reticulum. This remodeling could take place in the endoplasmic reticulum, or at a site after de novo synthesis and prior to surfactant release into the alveolar space.

Perfusions were therefore designed to determine if the lamellar body and surfactant phosphatidylcholines are remodeled after leaving the endoplasmic reticulum. Perfusions were performed with [1-^{14}C]palmitate (0.5 mM) and [1,3-^{3}H]glycerol (0.5 mM), and 5.6 mM glucose. The lamellar body and microsomal phosphatidylcholine fractions were then isolated. If the phospholipids of the lamellar body were synthesized entirely in the microsomes, then the ratio of the lamellar body (^{14}C/^{3}H) to the microsomal (^{14}C/^{3}H) should equal one. Any deviation from one would indicate that the phospholipids were being modified. The results of these experiments are shown in Table II. When [1-^{14}C]palmitate and [1,3-^{3}H]glycerol are used as substrates,

TABLE II

Comparison of the Ratios of Incorporation of [1-^{14}C]palmitate and [1,3-^{3}H]glycerol or [U-^{14}C]glucose and [9,10-^{3}H]palmitate into the Phosphatidylcholine of Rat Lung Subcellular Fractions[a]

Substrate	Ratios	
	Lamellar Body (^{14}C/^{3}H) / Microsome (^{14}C/^{3}H)	Surfactant (^{14}C/^{3}H) / Nonsurfactant (^{14}C/^{3}H)
[1-^{14}C]Palmitate[b] / [1,3-^{3}H]Glycerol	0.78 ± 0.04 (8)[d]	---
[U-^{14}C]Glucose[c] / [9,10-^{3}H]Palmitate	1.46 ± 0.13 (6)	1.56 ± 0.10 (6)

[a]Perfusion conditions were as described in Materials and Methods.

[b]Incorporation of radioactivity was determined in phosphatidylcholine.

[c]Incorporation of radioactivity was determined in phosphatidylcholine fatty acids only.

[d]Values are the mean ± S.E.M. for (N) determinations.

the ratio for whole molecule phosphatidylcholine was 0.78. The ratio could fall below one only if another unlabeled substrate, besides exogenous palmitate, were being used for the fatty acids of lamellar body phosphatidylcholine. Since the nonradioactive glucose present in the perfusion medium could be incorporated into fatty acids, and thus influence the ratio, the relative incorporation of [U-^{14}C]glucose (5.6 mM) and [9,10-^{3}H]palmitate (0.5 mM) into the fatty acids of phosphatidylcholine was studied. Surfactant and nonsurfactant phosphatidylcholine were also isolated after separate but similar perfusions. With these substrates the lamellar body (^{14}C/^{3}H)/microsomal (^{14}C/^{3}H) ratio for phosphatidylcholine fatty acids was 1.46, while the surfactant (^{14}C/^{3}H)/nonsurfactant (^{14}C/^{3}H) ratio was 1.56 supporting this value. In this case a ratio greater than one indicates that relatively more glucose is being used for the fatty acids of the phospholipids of the lamellar body and surfactant than those of the microsomes and nonsurfactant fractions. These results imply that fatty acids synthesized from glucose may be preferentially utilized for surfactant dipalmitoyl phosphatidylcholine synthesis.

Since the α and β fatty acids of the lamellar body and surfactant phospholipids are being esterified at different times, there must exist at least two pools of fatty acids in the lung. At least one pool could be involved in the synthesis of nonsurfactant phospholipids, and the other would be used for the esterification of surfactant lipids. These pools may be differentiated on the basis of the origin of the fatty acids. These experiments also indicate that a significant percentage of the phosphatidylcholine of the lamellar body and surfactant fractions is remodeled after leaving the endoplasmic reticulum.

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