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THE ROLE OF ACYL TRANSFERASE IN THE BIOSYNTHESIS OF PULMONARY MICROSOMAL PHOSPHATIDYLGLYCEROL

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Summary: Dog lung microsome contain an enzymatic system capable of catalyzing the transfer of radiolabel from [^{14}C]-acyl CoA, pamitoyl or oleoyl, to 1-acyl-2-lysophosphatidylglycerol thereby forming phosphatidylglycerol. This synthetic activity is consistent with a 1-acyl-2-lyso-phosphatidylglycerol:acyl-CoA acyl transferase. Kinetic analysis suggests the enzyme is preferentially, but not exclusively, orientated to the production of 1-acyl-2-oleoyl-phosphatidylglycerol. When enzymatic activity was examined with palmitoyl-CoA as the donor substrate and either 2-lysophosphatidylglycerol or 2-lysophosphatidylcholine as the acceptor substrates, the perferred product seemed to be phosphatidylglycerol. When the activity was examined with both acceptor substrates present, phosphatidylglycerol formation decreased while phosphatidylcholine formation increased. This observation may represent an effective control mechanism for the differential synthesis of pulmonary phospholipids, according to metabolic or physiological requirements.

Mammalian lungs contain an unusually high amount of phosphatidylglycerol which is primarily associated with the pulmonary surfactant system (1). The acyl groups of lung phosphatidylglycerol are predominantly saturated and it, like disaturated phosphatidylcholine, is surface active (1-3). A high risk of the neonatal respiratory distress syndrome is associated with absence of pulmonary phosphatidylglycerol during fetal development (4-5). Phosphatidylglycerol is synthesized mainly by mitochondrial and endoplasmic reticulum enzyme systems (6-7) in a 3 step sequence; CDP-diglyceride is an intermediate (6-8). It is not clear, however, that the acyl chains of disaturated phosphatidylglycerol are derived solely from saturated CDP-diglycerides. Some reports indicate higher percentages of saturated acyl chains in phosphatidylglycerol than in diglycerides (2,9); another reported the same percentages (10). With respect to the discrepancy in reported percentages, the presence of saturated diglycerides does not necessarily mean the CDP-diglycerides will be saturated.

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The Pulmonary endoplasmic reticulum has the enzymatic apparatus for the production of disaturated phosphatidylcholine through the de novo pathway (11,12) and by means of a deacylation-reacylation cycle involving phospholipase-A_2 and 1-acyl-2-lysophosphatidylcholine:acyl-CoA acyl transferase (acyl transferase) (12,15). An analogous mechanism may also operate in the synthesis of disaturated phosphatidylglycerol by lung. The purpose of this investigation was to determine if lung microsomal acyl transferase is involved in phosphatidylglycerol synthesis.

Materials and Methods

Isolation of lung Microsomes. Mongrel dogs were anesthetized with sodium pentobarbital and killed by severing their abdominal aortas. The lungs were removed and homogenized in 0.25 M sucrose prepared in Krebs-Ringer phosphate, pH 7.4, and the homogenate strained through stainless steel mesh (16). The filtrate was centrifuged sequentially at 1800 x g for 30 min. and 22,400 x g for 10 min. Microsomes were isolated from the 22,400 x g supernatant by the technique of Kamath and Rubin (17). The isolated microsomes were suspended in Krebs-Ringer phosphate, pH 7.4; lyophilized; and stored at -10°C until ready for use (18).

Acyl transferase assay. The required amounts of 1-acyl-2-lyso-phosphatidylglycerol and 1-acyl-2-lyso-phosphatidylcholine in separate chloroform solutions were added to each assay tube and the solvent evaporated under N_2. Lung microsomes based on protein content (9), in 0.2 ml Krebs-Ringer phosphate, pH 7.4, (or other suitable buffers), were added and incubated with constant shaking for 5 min at 37°C. The enzyme reaction was started by addition of 0.1 ml (1-^{14}C)-palmitoyl-CoA (13.62 Ci/mole) or (1-^{14}C)-oleoyl-CoA (16.89 Ci/mole) and the incubation continued for 10 min. The concentrations of acyl-CoA, lung microsomal protein and the incubation time required for assay were determined in preliminary experiments. Addition of 5 volumes chloroform/methanol (2:1) served to stop the reaction and extract the lipids. Lipids were separated by thin layer chromatography using the solvent system chloroform/methanol/concentrated ammonium hydroxide/water (70:30:1:4) and the lipid classes visualized with I_2 vapors. Areas corresponding to standard phosphatidylglycerol and phosphatidylcholine were scraped into scintillation vials, 10 ml Aquasol-2 and 1.5 ml 10% sodium thiosulfite added and the radioactivity associated with each area determined by scintillation counting (corrections were made for quenching using the external standard method). Enzyme-blank and 0-time controls were run. Two blocks of experiments, where all the components were run simultaneously, i.e., (a) palmitoyl-CoA, oleoyl-CoA, and 1-acyl-2-lyso-phosphatidylglycerol and (b) palmitoyl-CoA, 1-acyl-2-lyso-phosphatidylglycerol and 1-acyl-2-lyso-phosphatidylcholine, were carried out.

Radiolabeled palmitoyl-CoA and oleoyl-CoA were purchased from New England Nuclear; 1-acyl-2-lyso-phosphatidylglycerol, 1-acyl-2-lyso-phosphatidylcholine and other phospholipids from Sigma.

Results

Effect of acceptor substrate concentration. The velocity of phosphatidylglycerol synthesis responded much more dramatically to 1-acyl-2-lyso-phosphatidylglycerol over the range of 6.0 to 241.7 μM with oleoyl-CoA than with palmitoyl-CoA (Fig. 1). Computation of least square regression lines after these data were transformed into Lineweaver-Burk plots gave k_m values of 11

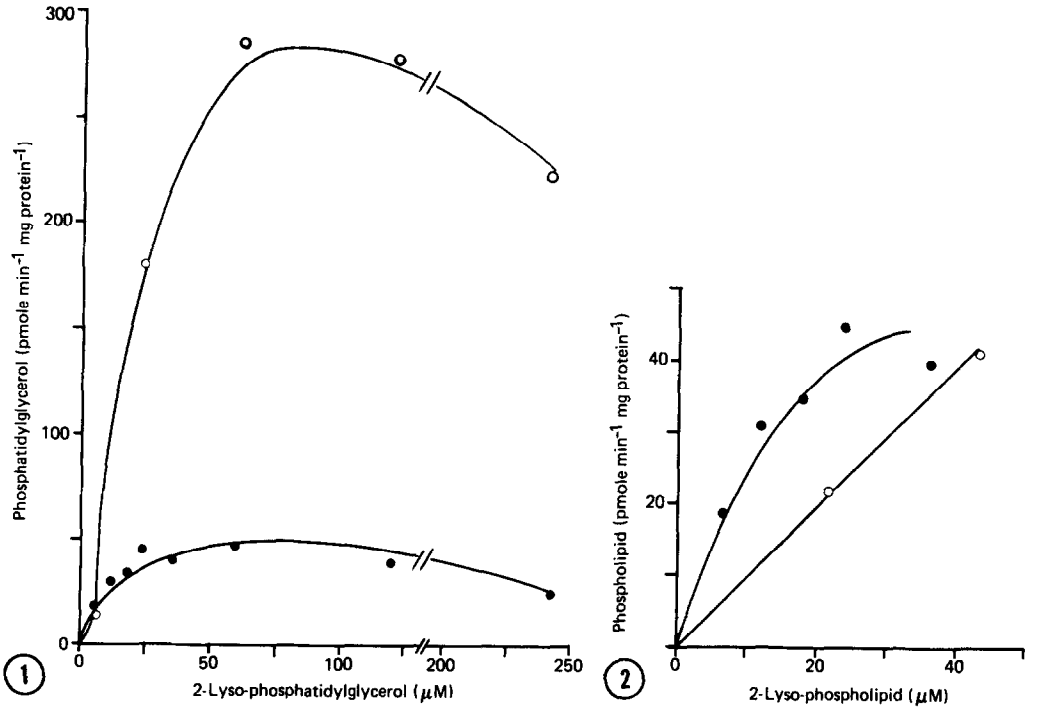

Fig. 1: Effect of 1-acyl-2-lyso-phosphatidylglycerol concentration on the velocity of phosphatidylglycerol synthesis (pmole/min/mg protein). The complete reaction mixtures, 0.6 ml, contained 6.0-241.7 μM 1-acyl-2-lyso phosphatidylglycerol, 2.8 μM [1-^{14}C]-palmitoyl-CoA or 2.3 μM [1-^{14}C]-oleoyl-CoA, and 1.0 mg/ml dog lung microsomal protein. The reaction was carried out for 10 min at 37°C and pH 7.4. Details of the assay procedure are provided under Methods. Results are the pooled data from 3 different determinations in duplicate, normalized for differences in acyl-CoA concentrations radiochemical specific activities. •, [1-^{14}C]-palmitoyl-CoA; o, [1-^{14}C]-oleoyl-CoA.

Fig. 2: Phosphatidylglycerol and phosphatidylcholine synthesis (pmole/min/mg protein) as a function of the appropriate 1-acyl-2-lyso-phospholipid acceptor substrate. The complete reaction mixture, 0.6 ml, for phosphatidylcholine synthesis contained 21.5 or 43.0 μM 1-acyl-2-lyso-phosphatidylcholine, 2.8 μM [1-^{14}C]-palmitoyl-CoA, and 1 mg/ml dog lung microsomal protein. The reaction mixture for phosphatidylglycerol synthesis was the same as given in Fig. 1. The reactions were carried out for 10 min at 37°C and pH 7.4. •, phosphatidylglycerol, o, phosphatidylcholine.

and 46 μM and V values of 57 and 510 pmole of product formed/min/mg microsomal protein for palmitoyl-CoA and oleoyl-CoA, respectively. The coefficients of correlation for the regression lines were 0.96 for palmitoyl-CoA and 0.93 for oleoyl-CoA.

Comparison of phosphatidylglycerol and phosphatidylcholine synthesis. The velocities of phosphatidylglycerol and phosphatidylcholine synthesis with palmitoyl-CoA as the donor substrate are compared in Fig. 2. Throughout most of the range of 1-acyl-2-lyso-phospholipid acceptor substrate concentrations

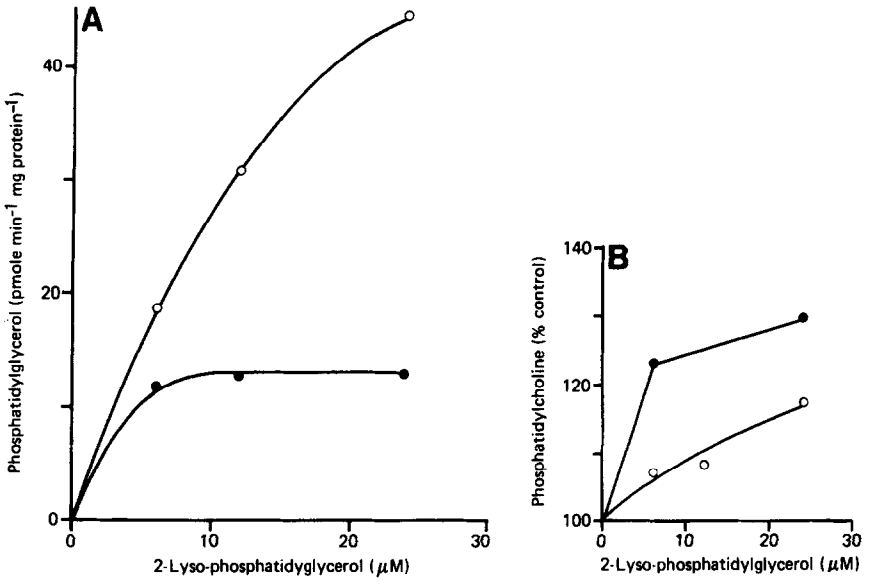

Fig. 3: A. Effect of 1-acyl-2-lyso-phosphatidylcholine on the velocity of phosphatidylglycerol synthesis (pmole/min/mg protein). The complete reaction mixtures, 0.6 ml, contained 6.0-24.0 μM 1-acyl-2-lyso-phosphatidylglycerol, 2.8 μM [1-^{14}C]-palmitoyl-CoA, 1.0 mg/ml dog lung microsomal protein, and 43.0 μM 1-acyl-2-lyso-phosphatidylcholine where indicated. The reaction was carried out for 10 min at 37°C and pH 7.4. Details of the assay procedure are provided under Methods. •, 43.0 μM 1-acyl-2-lyso-phosphatidylcholine; o, no 1-acyl-2-lyso-phosphatidylcholine.

B. Effect of 1-acyl-2-lyso-phosphatidylglycerol on the velocity of phosphatidylcholine synthesis (pmole/min/mg protein) expressed as percent of control. The complete reaction mixtures, 0.6 ml, contained 6.0-24.0 μM 1-acyl-2-lyso-phosphatidylglycerol, 2.8 μM [1-^{14}C]-palmitoyl-CoA, 1.0 mg/ml dog lung microsomal protein, and 43.0 μM 1-acyl-2-lyso-phosphatidylcholine where indicated. The reaction was carried out for 10 min at 37°C and pH 7.4. Details of the assay are provided under Methods. The control was the velocity of phosphatidylcholine synthesis in the absence (i.e., 0 μM) of 1-acyl-2-lyso-phosphatidylglycerol. •, 43.0 μM exogenous 1-acyl-2-lysophosphatidylcholine; the control value was 151 pmole phosphatidylcholine formed/min/mg protein. o, no exogenous 1-acyl-2-lyso-phosphatidylcholine (i.e., only endogenous substrate available); the control value was 109 pmole phosphatidylcholine formed/min/mg protein.

studied, the velocity of phosphatidylglycerol synthesis was approximately twice that observed for phosphatidylcholine.

The velocity of phosphatidylglycerol synthesis with (1-^{14}C)-palmitoyl-CoA as the donor substrate was decreased 37% by addition of 43.0 μM 1-acyl-2-lyso-phosphatidylcholine (Fig. 3A). Whereas phosphatidylglycerol formation was decreased by addition of 1-acyl-2-lyso-phosphatidylcholine, phosphatidylcholine formation from palmitoyl-CoA and 1-acyl-2-lyso-phosphatidylcholine was stimulated by increasing 1-acyl-2-lyso-phosphatidylglycerol concentrations (Fig. 3B). When no exogenous 1-acyl-2-lyso-phosphatidylglycerol was

added to the medium, phosphatidylcholine synthesis still occurred, presumably using the endogenous acceptor substrate present the lung microsomes, and was stimulated by increasing 1-acyl-2-lyso-phosphatidylglycerol concentration. The control values (no added 1-acyl-2-lyso-phosphatidylglycerol) for the rate of phosphatidylcholine synthesis were 109 and 151 pmole/min/mg protein when only endogenous or 43 μM 1-acyl-2-lyso-phosphatidylcholine were present, respectively. The velocity of phosphatidylcholine synthesis increased 23 and 30 percent above control levels at 6 and 24 μM 1-acyl-2-lyso-phosphatidylglycerol when 43 μM 1-acyl-2-lyso-phosphatidylcholine was present and 7 and 18 percent when only endogenous 1-acyl-2-lyso-phosphatidylcholine was present.

Discussion

The present results demonstrate that dog lung microsomes contain an enzymatic system capable of catalyzing the transfer of radioactive label from (1-^{14}C)-oleoyl-CoA to 1-acyl-2-lyso-phosphatidylglycerol thereby forming phosphatidylglycerol. Thus, by virtue of the donor and acceptor substrates involved and the product produced, the synthetic activity is consistent with an 1-acyl-2-lyso-phosphatidylglycerol:acyl-CoA acyl transferase. There is, however, no definitive information which allows a distinction between general or specific acyl transferases in dog lung microsomes. It is not clear that the differences we observed in the synthesis of phosphatidylglycerol with palmitoyl-CoA or oleoyl-CoA, or the pH maximum (centered at 7.4, data not shown) reflect separate enzymes, each with its own substrate specificities, or a single acyl transferase giving broad substrate requirements.

The k_m for 1-acyl-2-lyso-phosphatidylglycerol determined in the presence of palmitoyl-CoA was four times less than that for this acceptor substrate with oleoyl-CoA. V, however, was ten times larger for 1-acyl-2-lyso-phosphatidylglycerol with oleoyl-CoA compared to palmitoyl-CoA. This greater differential in the 1-acyl-2-lyso-phosphatidylglycerol V values (which estimate the actual reaction capabilities) compared to the difference in k_m

values (which reflect substrate affinities for the enzyme) again suggest 2-oleoyl-phosphatidylglycerol, and by inference, other 2-unsaturated-phosphatidylglycerol species would be the predominant products. Since the concentrations of acyl-CoA used were above the critical micelle concentrations, variations in micelle size could contribute to the differences found in k_m and V values. The extent of the putative micelle effect on acyl transferase kinetics was not determined.

Since the lung acyl transferase is involved in the remodeling pathway for the synthesis of disaturated-phosphatidylcholine, further experiments studied only the incorporation of palmitate into phosphatidylglycerol and inhibition of this reaction by lyso-phosphatidylcholine. With palmitoyl-CoA, the initial velocities of phosphatidylglycerol formation were about twice as high as those for phosphatidylcholine synthesis throughout the range of acceptor substrate concentration shown in Fig. 2. We did not attempt to calculate a k_m for phosphatidylcholine synthesis from the limited data shown in Fig. 2; however, values ranging between 18 μM from rat lung to microsomes to 34 μM from rabbit lung microsomes have been reported (15,20,21). These values are similar to the k_m, 11 μM, we found for the formation of phosphatidylglycerol. The presumed similarity in k_m values coupled with the differences in initial velocities (Fig. 2) and, by inference, V values suggest that acyl transferase will preferentially produce more 2-palmitoyl-phosphatidylglycerol than 2-palmitoyl-phosphatidylcholine. These findings differ from results recently presented for rat lung alveolar type II cells (22). The differences may be related to our use of whole lung microsomal preparations rather than a single lung lung cell species.

Modulation or reversal of the suggested preference for 2-palmitoyl-phosphatidylglycerol rather than 2-palmitoyl-phosphatidylcholine synthesis is indicated by the inhibition of phosphatidylglycerol synthesis by 2-lyso-phosphatidylcholine whereas phosphatidylcholine synthesis is stimulated by 2-lyso-phosphatidylglycerol (Fig. 3A and 3B). This type of modulation

could represent an effective control mechanism for the differential synthesis of phospholipids in the lung according to metabolic or physiological requirements.

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