Acyl transferase activities in dog lung microsomes

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ABSTRACT Mammalian lung has a high concentration of dipalmitoyl phosphatidylcholine and other phospholipids in which both fatty acid ester chains are saturated, as opposed to the usual asymmetric phospholipid (one saturated fatty acid and one unsaturated fatty acid). The acyl transferase system in dog lung microsomes was studied by determining the reactivities of various acyl CoA derivatives with 1-lyso-2-acyl- and 1-acyl-2-lyso-phosphatidylcholine. The 16:0 derivative had equal reactivity for both the 1- and 2-lyso positions. The 18:0 derivative also exhibited marked reactivity toward both positions, although the specific activity of the enzyme when palmitoyl CoA was used was approximately twice that compared to when stearoyl CoA was used. The 16:1 derivative showed approximately the same reactivity toward the 1-lyso position as did 16:0 but both 16:1 and 18:1 were more active with the 2-lyso position. These results suggest that acyl transferases may be important in the lung to insure that sufficient amounts of dipalmitoyl phosphatidylcholine will always be present for use in pulmonary surfactant biosynthesis. It is also conceivable that the acyl transferase system described acts on 1- and 2-lyso-palmitoyl phosphatidylcholine (produced by phospholipase hydrolysis of dipalmitoyl phosphatidylcholine) in order to produce phosphatidylcholine species needed for cellular purposes other than surfactant function.

SUPPLEMENTARY KEY WORDS unilateral pulmonary artery occlusion · pulmonary surfactant

THE CONCENTRATION of phospholipids in which both fatty acid ester chains are saturated is higher in the lung than in any other mammalian tissue (1). The presence of dipalmitoyl-PC in the lung is thought to be related to the surface properties of pulmonary surfactant, which presumably functions to reduce alveolar surface forces at low lung volumes during respiration, thereby preventing alveolar collapse (2–7). We recently described the isolation of a surface-active material from dog lung which meets all of the proposed theoretical criteria expected for pulmonary surfactant (8). The PC fraction isolated from this preparation contained, as a maximum value, 68% total disaturated molecules and, of these, a maximum of 80% were dipalmitoyl-PC (9). A deficiency of surface activity of lung extracts has been observed in pathological states (3, 6). UPAO is an experimental model for inducing such changes (10–12).

The typical distribution of fatty acid residues in mammalian phospholipids is asymmetric, i.e., saturated fatty acid esters are predominantly found on the 1-carbon of the glycerol moiety and unsaturated fatty acid esters predominantly on the 2-carbon (13). Lands has speculated that this asymmetric fatty acid distribution may be the result of the operation of a combined phospholipase–acyl transferase cycle (14):

$$\text{diacyl phosphatides} \underset{\text{acyl transferase(s)}}{\overset{\text{phospholipases}}{\rightleftharpoons}} \text{lysophosphatides}$$

When initially synthesized, the diacyl phosphatides might have a random fatty acid distribution which would then subsequently be replaced, as a result of the cycle, by the very nonrandom and asymmetric distribution found in vivo. It has not yet been clearly established if the acylation of glyceryl-3-phosphate, which can be considered as the first step in phospholipid biosynthesis, is random or nonrandom (15).

The presence of phospholipase A in rat lung has previously been reported (16, 17). Lands (18) and Webster (19) have demonstrated acyl transferase activity in rat lung but these studies were not detailed, and most subsequent detailed work was concerned with

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; Pal-C, phosphatidalcholine; 16:0, palmitic; 16:1, palmitoleic; 18:0, stearic; 18:1, oleic; DTNB, 5,5′-dithio-bis-(2-nitrobenzoic acid); DFP, diisopropylfluorophosphate; UPAO, unilateral pulmonary artery occlusion; TLC, thin-layer chromatography.

transferases found in liver. The purpose of the present communication is to describe the presence and some of the properties of the acyl transferase activity in dog lung microsomes in relation to the biosynthesis of phosphatidylcholine.

MATERIALS AND METHODS

Synthesis of Acyl Coenzyme A Derivatives

Palmitoyl, palmitoleoyl, stearoyl, and oleoyl coenzyme A derivatives were prepared by a modification of the procedure of Lands, Blank, Nutter, and Privett (20). 100 mg of coenzyme A (Calbiochem, Los Angeles, Calif.) was dissolved in 25 ml of 50% tetrahydrofuran in water. It was essential to use tetrahydrofuran freshly distilled from $LiAlH_4$. For the derivatives prepared in this study, it was not necessary to use an added antioxidant (20). The pH was adjusted to 7.5–8.0 with 1 N NaOH. The free -SH content was estimated by the DTNB procedure (21). 100 mg of the appropriate acyl chloride (Hormel Institute, Austin, Minn.) was added as the neat liquid. The pH was maintained at 7.5–8.0. The free -SH content was again estimated. More acyl chloride was added in small portions until there was no further decrease in free -SH content. The pH was adjusted to 6.5 with HCl. In order to remove the tetrahydrofuran, the reaction mixture was lyophilized until approximately one-fourth of the original volume remained. The mixture was allowed to thaw but the temperature was maintained at 0°C. Ice-cold 10% $HClO_4$ was added to acidify the mixture and to precipitate the acyl CoA ester. At this point, there was also fatty acid present which was then removed by three extractions with diethyl ether. After each ether extraction, the mixture was centrifuged at 2000 rpm to sediment the acyl CoA ester. If large amounts of water remained after the lypophilization, the acyl CoA ester was found at the interface between the ether and water layers. In this case, both the ether and aqueous layers were removed after the centrifugation. Any remaining ether was removed with nitrogen. The acyl CoA ester was dissolved in water and the pH was adjusted to 6.0 with dilute $NaHCO_3$. We have found it convenient to carry out the entire procedure in a 50-ml centrifuge tube.

Yields were generally on the order of 50–70% based on the phosphorus content of the original coenzyme A and the final product. Reactivity of the acyl CoA esters was estimated by comparing the amount of free -SH groups produced in known acyl transferase systems (21) with the phosphorus content. Generally, reactivity was better than 90%. The following ultraviolet absorbance ratios were found: 250 nm:260 nm, 0.84–0.86; 280 nm:260 nm, 0.19–0.25; and 232 nm:260 nm, 0.72–0.77 (22).

Preparation of Lysophosphatides

Egg-PC was obtained and characterized as described previously (8). Phosphatidalcholine was isolated from dog heart choline phosphatide by the procedure of Gottfried and Rapport (23) and gave the following analysis: 3.76% phosphorus; molar ratio aldehydogenic groups to phosphorus, 0.88; molar ratio ester groups to phosphorus, 1.00. The material was shown to be homogeneous by TLC at the 100-μg level. Egg-PE was isolated from egg yolk total lipid in a manner similar to that used for the isolation of egg-PC (8), except that the desired fraction was eluted from Unisil with chloroform–methanol 7:3. After recrystallization from diethyl ether–acetone, the product gave the following analysis: 4.25% phosphorus; molar ratio ester groups to phosphorus, 1.97. It was homogeneous by TLC at the 100-μg level.

1-Acyl-2-lyso-PC. Egg-PC was hydrolyzed by phospholipase A (24) and the reaction products were separated by column chromatography on Unisil. The desired fraction was eluted with methanol. After recrystallization from ethanol–hexane, the material contained 6.00% phosphorus and had a molar ratio of ester to phosphorus of 1.00. TLC at the 100-μg level indicated that the material was homogeneous.

1-Lyso-2-acyl-PC was obtained by treating Pal-C with 90% acetic acid at 37°C for 60 min in a nitrogen atmosphere. In order to minimize the possibility of acyl group migration, the resultant product was used immediately after the acetic acid was removed under N_2. The TLC pattern of the reaction mixture indicated that hydrolysis of the Pal-C was complete.

Lysophosphatidalcholine (*Lyso-Pal-C*) was prepared from dog heart choline phosphatide by the procedure of Frosolono, Kisic, and Rapport (25). The material gave the following analysis: 6.10% phosphorus; molar ratio of aldehydogenic groups to phosphorus, 0.88; and a molar ratio of ester to phosphorus, 0.00. The material was shown to be homogeneous by TLC at the 100-μg level.

1-Acyl-2-lyso-PE was obtained after phospholipase A hydrolysis of egg-PE (24). The reaction products were separated by column chromatography on Unisil, and the desired fraction was eluted with chloroform–methanol 1:3. After recrystallization from absolute ethanol, the material gave the following analysis: 6.25% phosphorus and a molar ratio of ester to phosphorus, 1.00. TLC at the 100-μg level indicated the material was homogeneous.

Preparation of Microsomes

Adult mongrel dogs were killed by exsanguination. Microsomal fractions were prepared from dog lung and liver according to the procedure of Lands and Hart (21), except that the microsomes were washed one additional time with the 0.25 M sucrose solution. The sucrose solution was then decanted from the sedimented microsomes

TABLE 1 ACYL TRANSFERASE ACTIVITIES IN DOG LUNG AND LIVER

Microsomal Preparation	Phosphatide Substrate	Acyl Coenzyme A Derivative							
		16:0*	Lyso-2/lyso-1†	16:1	Lyso-2/lyso-1	18:0	Lyso-2/lyso-1	18:1	Lyso-2/lyso-1
		nmole/min/mg protein‡							
Lung, normal	1-Lyso-2-acyl-PC	23.58 (3.38)		25.99 (5.62)		9.98 (2.57)		7.37	
	1-Acyl-2-lyso-PC	23.53 (3.50)	1.00	34.57 (5.47)	1.33	12.04 (0.98)	1.20	30.32	4.11
	Lyso-Pal-C	2.64		2.99					
	1-Acyl-2-lyso-PE	1.91		1.53					
Lung, UPAO									
Nonoccluded	1-Lyso-2-acyl-PC	25.02 (3.00)		27.88 (2.79)					
	1-Acyl-2-lyso-PC	17.77 (1.82)	0.70	29.70 (5.34)	1.06			29.26	
Occluded	1-Lyso-2-acyl-PC	27.93 (1.29)		30.57 (2.49)					
	1-Acyl-2-lyso-PC	21.07 (1.87)	0.75	32.87 (3.48)	1.07			28.14	
Liver§	1-Lyso-2-acyl-PC	54.87		14.67		57.13		0.00	
	1-Acyl-2-lyso-PC	22.77	0.41	57.07	3.88				
	1-Acyl-2-lyso-PE	9.52		8.68					

Acyl transferase activity was estimated by the DTNB spectrophotometric assay at 37°C. Incubation mixtures contained 0.1 mg of microsomal protein in 0.8 ml of 0.088 M Tris–Cl, pH 7.4; 25 nmole of acyl CoA; 100 nmole of phosphatide substrate, 0.1 ml of 0.01 M DTNB, pH 7.4, and 0.1 ml of water. Corrections for acyl CoA hydrolase activity were made (17).

* Number of carbon atoms : number of double bonds.

† Refers to ratio of transferase activity, with 1-acyl-2-lyso-PC and 1-lyso-2-acyl-PC.

‡ Values given are averages of 6–13 different microsomal preparations (±SD), each from a different dog. If standard deviations are not given, values are averages of 2–4 preparations.

§ Microsomes treated with DFP.

and the pellet was suspended in 0.088 M Tris–Cl, pH 7.4. The preparation could be stored at −20°C for up to one month without loss of activity. Repeated freezing and thawing did, however, lead to a loss of activity. When required, microsomes were treated with DFP (21).

Dog lung is extremely difficult to homogenize due to the presence of large amounts of connective tissue. Prior to the homogenization, the lung sample must be thoroughly scissor-minced and then passed through a tissue press (Harvard Apparatus Co., Millis, Mass.). These steps are essential in order to obtain a good yield of lung microsomes. Indeed, it was found to be impossible to use a Potter–Elvehjem homogenizer without these preliminary operations. Microsomal protein was estimated by the procedure of Lowry, Rosebrough, Farr, and Randall (26).

Unilateral Pulmonary Artery Occlusion (UPAO)

To assess the effect of hypoperfusion, UPAO was accomplished in adult mongrel dogs with a balloon-tipped catheter introduced via the femoral vein under fluoroscopic control, as described by Charms (27). The balloon was then inflated in the right or left main pulmonary artery and the catheter was sealed, allowing the animal to return to normal activity. The animals were killed after 72 hr of occlusion, the lungs were removed, and the microsomal fraction was obtained from the occluded and nonoccluded lungs. Lungs were used only from animals in which the balloon remained inflated throughout the 72-hr period as verified fluoroscopically and at autopsy.

RESULTS

Relative Acyl Transferase Activities (Table 1)

With 1-acyl-2-lyso-PC and 16:0-acyl CoA, 50–60% of the acyl transferase activity in the lung homogenate was recovered in the microsomal fraction used in these studies. No acyl transferase activity was found in any other tissue fraction obtained in the isolation procedure. Although the acyl coenzyme A hydrolase activity was extremely low for lung, appropriate corrections were made for each assay (21). The hydrolase activity could be completely removed by treatment of the microsomes with DFP, and this was routinely done with liver preparations. The assay procedure gave reproducible results as can be seen from the standard deviations shown in Table 1. The acyl transferase reactions were linear with respect to time during the assay period which was usually 2–10 min.

Liver

Dog liver microsomes gave similar results to those previously reported for other animals (21). The 16:1-acyl CoA

reacted faster with the 1-acyl-2-lyso-PC, and 16:0-acyl CoA reacted faster with the 1-lyso-2-acyl-PC. This can easily be seen from the lyso-2/lyso-1 ratios of transferase specific activity. This ratio is computed directly from the values shown in the table for each acyl CoA with 1-acyl-2-lyso-PC (lyso-2) and 1-lyso-2-acyl-PC (lyso-1).

Normal Dog Lung

The results with normal lung microsomes indicate that there is no distinction made between the 1- and 2-lyso positions with the 16:0-acyl CoA derivative. This is demonstrated by the fact that the lyso-2/lyso-1 transferase specific activity ratio for 16:0 is unity. There is also no clearly significant positional distinction seen with the 18:0 derivative. These results are quite different from the findings with liver microsomes. There is, however, a marked preference shown by the 16:1 and 18:1 derivatives for the 2-lyso position (1-acyl-2-lyso-PC) and, in this respect, lung microsomes are qualitatively similar to liver microsomes.

If one now compares the specific activities of the transferase when the 16:0 and 16:1 derivatives were used with 1-lyso-2-acyl-PC, it can be seen that there is no significant preference for esterification of the saturated or unsaturated fatty acid at the 1-position. The same results were obtained with the 18:0- and 18:1-acyl CoA's. These findings are, again, quite different from what is seen in the liver. A comparison of 16:0 with 16:1 and of 18:0 with 18:1 with 1-acyl-2-lyso-PC demonstrates a definite preference toward esterification at the 2-position with unsaturated fatty acids. This latter finding is similar to the situation in liver microsomes in the present study and in other studies (21).

Lyso-Pal-C and 1-acyl-2-lyso-PE were not effective acyl transferase substrates under the conditions of this study.

UPAO

Application of Student's "*t*" test to the values obtained with microsomes from the occluded lung and non-occluded control lung indicated that any differences between these data were not statistically significant. These findings are clearly demonstrated by comparing the lyso-2/lyso-1 esterification ratios in both groups of microsomes for each set of substrates. If, however, the lyso-2/lyso-1 esterification ratios for the normal microsomes are compared with those found for the microsomes from the occluded and nonoccluded lungs, it is seen that the ratios are significantly lower after UPAO.

Effect of pH on Acyl Transferase Activity

The effect of pH on both acyl transferase and hydrolase activity is shown in Fig. 1. Although the figure presents data for only one combination of acyl CoA and lysophosphatide, similar results were obtained with 16:0- and 16:1-acyl CoA and 1-acyl-2-lyso-PC and 1-lyso-2-acyl-PC. The pH optimum was at 7.4. At alkaline pH the total activity was constant, and an increasing hydrolase blank was responsible for the decreased acyl transferase activity. At acid pH the production of total -SH groups decreases while the hydrolase activity increases, although at a smaller rate than at alkaline pH. This indicates that the drastic decrease in acyl transferase activity is not due to the increasing hydrolase blank. The hydrolase activity appears to be minimal at neutral pH, the region where acyl transferase activity is maximal.

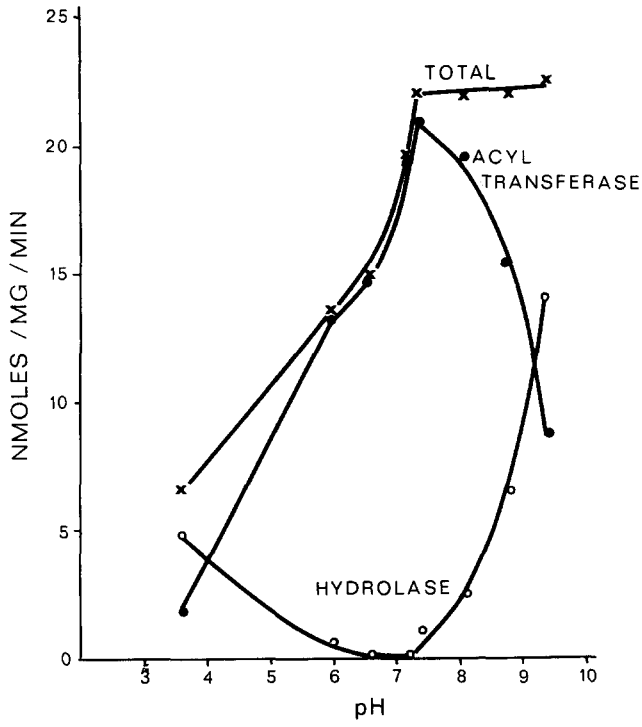

FIG. 1. Effect of pH on acyl transferase activity in dog lung microsomes. Experimental conditions as in Table 1 except that pH was adjusted to values indicated. The figure presents data for 16:0 CoA and 1-acyl-2-lyso-PC. Hydrolase activity was assayed in the absence of 1-acyl-2-lyso-PC; transferase activity is the difference between total activity in the presence of all components and the hydrolase activity.

Fatty Acid Distribution in Lung Phosphatidylcholine

The distribution of fatty acids on the 1- and 2-positions of lung PC is given in Table 2. The fatty acids found on the 1-position are predominantly saturated, and 16:0 accounts for 60% of the total fatty acids esterified at this position. Although approximately 54% of the total fatty acids esterified on the 2-position are saturated, the majority of the unsaturated fatty acids are, however, found at this position. As expected, 16:0 was found in highest concentration of the total at each position. The 2/1 fatty acid distribution ratios clearly demonstrate

TABLE 2 DISTRIBUTION OF FATTY ACIDS IN PHOSPHATIDYLCHOLINE ISOLATED FROM DOG LUNG

Position Occupied in Phosphatidylcholine Molecule	Fatty Acids										
	14:0	15:0	15:1	16:0	16:1	16:2	17:0	17:1	18:0	18:1	19:0
	% of total fatty acids										
1-Linked (α)	1.9	0.5	0.2	60.3	2.6	0.7	1.3	0.2	21.4	10.4	0.5
2-Linked (β)	2.7	0.7	0.4	38.6	11.3			0.5	4.2	34.4	7.1
2/1 ratio				0.64	4.35				0.20	3.31	

Procedures used for the isolation of phosphatidylcholine from the lung, phospholipase A hydrolysis, and GLC analysis have previously been described (8).

these findings. If one compares the 2/1 fatty acid distribution ratios (Table 2) with the lyso-2/lyso-1 acyl transferase specific activity ratios (Table 1), no immediately obvious quantitative correlation can be made between the two sets of values.

DISCUSSION

Dog Liver

The results shown in Table 1 indicate that acyl transferase activities in dog liver microsomes are qualitatively similar to those found in the livers of other animals (21). The specific activities reported in this present communication, however, are higher than those reported previously. This may be due in part to the fact that we washed our microsomal preparations an additional time. Moreover, we found that hydrolase activity was very low in comparison to transferase activity.

The 16:0 derivative was preferentially esterified on the 1-position and 16:1 preferentially on the 2-position as expected (21). This is clearly seen in Table 1. The reactivity found for 16:1 at the 1-position, while considerably less than the 16:0, was not particularly low in comparison with results previously reported for rat liver (16, 20, 21). This can be taken as an indication that there are species differences for acyl transferase activities.

In addition to demonstrating that dog liver microsomal acyl transferases have specificities qualitatively similar to those reported from other animals, the present findings indicate that very little acyl migration took place with the 1-lyso-2-acyl-PC. If acyl migration had occurred, there would have been no difference in specificities with the two lysophosphatides. This is especially important in considering the results obtained with dog lung.

Normal Dog Lung

The pH studies, Fig. 1, indicate the optimum pH for acyl transferases is 7.2–7.7. In this region, the hydrolase shows no significant activity. At both acid and alkaline pH, the hydrolase activity increases while the transferase activity decreases. Preliminary experiments demonstrated that any hydrolase activity was completely removed by treatment of the microsomes with DFP (21). Effros and Chinard (28) have recently calculated from experiments using indicator dilution techniques that the pH of the pulmonary extravascular space is 6.69. From Fig. 1 it can be seen that in vivo at this pH acyl transferase activity would be expected to proceed at approximately 80% of the maximum in vitro rate at pH 7.4. This, of course, assumes that all other parameters would be equal in vivo and in vitro, an assumption that may not be warranted.

It has been reported that lyso-Pal-C was not as effective an acyl transferase substrate as was 1-acyl-2-lyso-PC in rat liver (29) and in erythrocytes and muscle (30). Our results confirm that finding for lung. Interestingly, 1-acyl-2-lyso-PE does not appear to serve as an efficient substrate for lung microsomal acyl transferases.

As might be expected from the reported high concentration of dipalmitoyl-PC in the lung (1) and from the fatty acid distribution results shown in Table 2, the acyl transferase specific activity with the 16:0-acyl CoA is identical with both 1-lyso-2-acyl-PC and 1-acyl-2-lyso-PC. The specific activity with 18:0-acyl CoA, while approximately one-half that with the 16:0 derivative, showed a similar lack of phosphatide positional specificity. (Alternatively, this could be expressed as positive specificity for each position.) On the other hand, 18:1 exhibited a very high degree of specificity for the 2-position. The positional specificity of 16:1 was not as pronounced as that for 18:1.

It is also interesting to compare the activity of 16:0 with 16:1 and of 18:0 with 18:1 toward the 1-position, that is, horizontally in Table 1 rather than vertically. The lack of a clearly expressed difference between saturated and unsaturated acyl derivatives is demonstrated. A similar comparison with 1-acyl-2-lyso-PC does, however, indicate a decided preference for unsaturated fatty acids at the 2-position.

Two assumptions are implicit in interpreting our results. The specificity of the acyl transferase is due to

the acyl CoA derivative and the position to be acylated rather than to the composition of the acyl groups already present in the lysophosphatide substrate. Secondly, no acyl migration takes place when 1-lyso-2-acyl-PC is used as a substrate. Brandt and Lands (31) have presented strong evidence indicating that the position to be acylated is much more important than the composition of the acyl group of the lysophosphatide. Preliminary experiments with lung microsomes in this laboratory indicated no significant differences in acylation rates when 1-palmitoyl-2-lyso-PC and the 1-acyl-2-lyso-PC prepared from egg-PC were used as substrates. With regard to acyl migration as a possible mitigating factor in our experimental design, our results with 1-lyso-2-acyl-PC and liver microsomes were similar to those previously reported (21). Therefore, our results with liver microsomes serve as a control for our results with lung microsomes. Another consideration which rules out any significant acyl migration with lung microsomes is that, while 16:0 exhibited equal reactivity toward both lysophosphatides, the other acyl CoA derivatives showed definite positional specificity. One would not expect positional specificity with any acyl CoA if significant acyl migration had taken place in the lysophosphatide.

There is no apparent correlation between the whole lung 2/1 fatty acid distribution ratios (Table 2) and the lyso-2/lyso-1 acyl transferase specificity ratios (Table 1). A definite qualitative correlation between these values has been shown to exist in liver (20, 32). The isolation and characterization of a fraction from dog lung which meets all of the theoretical criteria of pulmonary surfactant was recently reported by this laboratory (8). Calculation of the phosphatidylcholine 2/1 fatty acid distribution ratios for this material as in Table 2 gives the following: 16:0, 0.80; 16:1, 1.85; 18:0, 0.27; and 18:1, 2.51. Except for 18:1, these ratios are in much closer qualitative agreement with the lyso-2/lyso-1 acyl transferase specific activity ratios (Table 1) than those found for whole lung PC. It must be emphasized, however, that these values are in agreement only in direction, and are not strictly quantitative.

The above results, in conjunction with what is already known concerning lung metabolism, have led us to speculate on a possible role for acyl transferase systems in the lung. The metabolism of lung has been considered to be directed largely toward the production of palmitic acid and dipalmitoyl-PC (33). The acylation of glyceryl-3-phosphate to form phosphatidic acid may, as reported for other tissues (34), be random with regard to positional specificity; however, the availability of large amounts of palmitate would nevertheless tend to give preponderant amounts of dipalmitoyl-phosphatidic acid and, subsequently, dipalmitoyl-PC. As expected, the acyl transferases acting upon 1-lyso-2-acyl-PC and 1-acyl-2-lyso-PC are rather active with 16:0-acyl CoA. The operation of the phospholipase–acyl transferase cycle would, because of the high level of 16:0-acid present, further insure that sufficient amounts of dipalmitoyl-PC would always be present for use in pulmonary surfactant biosynthesis. This would be true even though the specific activities with 16:1 and 18:1 derivatives are greater than for the reaction of the 16:0-acyl CoA with 1-acyl-2-lyso-PC. Abrams and Wigglesworth (35) have obtained evidence from isotopic labeling studies that the PC present in pulmonary surfactant has a biological half-life of about 28 hr as opposed to 35 hr for PC in total lung lipid. That portion of the PC not used for pulmonary surfactant synthesis would be expected to have a fatty acid distribution similar to that found in other tissues, i.e., saturated on the 1-position, unsaturated on the 2-position. Consequently, it is not inconceivable that the acyl transferase system(s) described in this report also acts on a 1- and 2-lyso-palmitoyl-PC (produced by phospholipase hydrolysis of dipalmitoyl-PC) in order to produce PC species needed for cellular purposes other than surfactant function. The lung is composed of many cell types, and our microsomal preparation is, of course, markedly heterogeneous with respect to cellular origin. Consequently, the assignment of a specific metabolic role to microsomal acyl transferases is somewhat speculative.

UPAO Studies

Occlusion of one of the main pulmonary arteries has been reported to lead to a decrease in the surface activity in the occluded lung in comparison to the unoccluded "control" (10–12). The occluded lung is not completely deprived of its blood supply by this procedure since the bronchial circulation maintains the blood flow at a level sufficient to prevent necrosis. The occluded lung becomes atelectatic (collapsed) and hemorrhagic in areas but appears morphologically normal in adjacent areas. This phenomenon has been termed "focal" atelectasis (10). After ligation of one of the pulmonary arteries, the bronchial circulation increases and the atelectasis resolves. The exact mechanisms leading to a decrease in the surface forces in the occluded lung have not yet been elucidated, and most studies in this area have lacked biochemical precision. It has, however, been reported that ligation of a pulmonary artery leads to a decreased phospholipid and dipalmitoyl-PC content and alterations in fatty acid composition of phospholipids in the atelectatic areas (12). The occlusion technique (27) used in this study avoids the necessity of a drastic surgical procedure, and the animals were allowed to return to normal activity. Grossly atelectatic areas were not used as tissue sources for two reasons. The presence of an active acyl transferase system has been

reported in erythrocytes (36). The collapsed areas contain large amounts of blood which cannot be removed by exsanguination. Secondly, at the time period used, 72 hr, atelectasis is not maximum, but it was presumed that biochemical alterations leading to a decreased surface activity should be evident even in areas which had normal gross morphology.

The lyso-2/lyso-1 acyl transferase specific activity ratios appear to be essentially identical in the occluded and nonoccluded lung samples, although the individual specific activities are not identical. However, the lyso-2/lyso-1 ratios for both the occluded and nonoccluded samples are clearly significantly decreased in comparison to the normal lung. This would tend to indicate that, if there is an effect of UPAO on the acyl transferase system(s) at this time period, the effect is not limited to the occluded lung. Starvation did not appear to greatly affect acyl transferase systems in the liver, and it was surmised that these systems are stable cellular components (20). In this respect, i.e., denial of extracellular metabolites, occlusion of the primary blood supply to a lung would resemble starvation, and our present results could indicate that the acyl transferases in the lung are also stable cellular components and would not be expected to undergo a drastic change at this time period. Work is currently in progress to investigate the effect of UPAO on these systems at other time periods and also in the grossly atelectatic areas of the occluded lung obtained after a lung lavage prior to homogenization.

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