Effect of CO_2 concentration on phosphatidylcholine and phosphatidylglycerol metabolism in surfactant and residual lung fractions

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Abstract An investigation of the effect of change of total CO_2 concentration from 7 to 43 mM at pH 7.35 in the medium perfusing isolated rat lungs on [U-^{14}C]-glucose incorporation into lung phospholipids has been carried out. The incorporation of [U-^{14}C]glucose into phosphatidylcholine and phosphatidylglycerol of the surfactant fraction and of the remaining lung tissue (residual fraction) was observed. Increased CO_2 concentration increased [U-^{14}C]glucose incorporation into phosphatidylcholine of the surfactant fraction and residual fraction by 43 and 50%, respectively, during a 2 hr perfusion. Likewise, incorporation of [U-^{14}C]glucose into phosphatidylglycerol was increased 22 and 34% into the surfactant and residual fractions, respectively. The percentage of [U-^{14}C]glucose incorporated into the fatty acid moieties of phosphatidylcholine of both fractions increased as a result of increased CO_2 concentration. The increase in the incorporation of [U-^{14}C]glucose into the fatty acid moieties of phosphatidylcholine was confirmed by an average increase of 56 and 77% in the specific activity of palmitic acid isolated from phosphatidylcholine of the surfactant and residual fraction, respectively, as a result of increased CO_2 concentration. The results suggest that alteration in extracellular CO_2 concentration affects the de novo synthesis from glucose of phosphatidylcholine and phosphatidylglycerol of the surfactant–lipoprotein fraction of lung.

Supplementary key words [U-^{14}C]glucose · palmitic acid · diplamitoyl phosphatidylcholine

Variation of medium CO_2 concentration within physiological limits of pH has been previously reported to alter the incorporation of [U-^{14}C]glucose into phospholipids of the isolated perfused rat lung (1). In those studies, glucose incorporation into the phosphatidylcholine and phosphatidylethanolamine fractions of whole lung was measured. Increasing the medium CO_2 concentration from 6 to 45 mM was found to increase the incorporation of [U-^{14}C]-glucose into phosphatidylcholine fatty acids over 100%. Recent studies have shown that phosphatidylglycerol, in addition to phosphatidylcholine, is an important lung phospholipid. It has been reported to be a major component of rat and dog lung surfactant (2, 3) and to be a metabolically active phospholipid in rat lung (3, 4).

Continued interest in factors that may regulate or otherwise alter the synthesis of the specific phospholipids of the surfactant fraction has prompted the present study. In this study, the effect of medium CO_2 concentration on [U-^{14}C]glucose incorporation into phosphatidylcholine and phosphatidylglycerol of the rat lung surfactant complex and into the same phospholipids present in the remaining lung tissue are compared. The surface-active lipoprotein complex of lung is separated from whole lung after ex vivo perfusion using a discontinuous sucrose density gradient centrifugation procedure (4). The surfactant fraction isolated by this procedure differs from that obtained by lavage of lung in that it contains both the intracellular and extracellular components. These two pools of surfactant material have been found to have a similar chemical composition and surface activity (5).

EXPERIMENTAL PROCEDURE

Male Wistar strain rats (Hilltop Lab Animals, Inc., Scottdale, PA) were used. They were fed commercial laboratory chow ad libitum. Preparation of the lungs for perfusion, perfusate preparation, and procedures of the perfusion have been previously reported (6). The perfusion medium was adjusted to contain approximately 7 or 43 mM total CO_2 by the addition of the appropriate ratio of $NaHCO_3$–NaCl to the medium so that equilibration with CO_2–O_2 gas mixtures in the range of 2–13% CO_2 (and 98–87% O_2) gave initial pH values of 7.32–7.40. Following removal of lung tissue during or at the termination of perfusion, the surfactant and residual fractions were separated by the method of Frosolono et al. (7) as modified by Sanders and Longmore (4). Lipids were extracted from these fractions

with 45 ml of chloroform–methanol 2:1 (v/v). Preliminary separation of lipids was carried out by silicic acid column chromatography using the previously reported procedure designated method 2 (3). The fraction containing primarily phosphatidylglycerol and phosphatidylethanolamine was then separated by thin-layer chromatography on silica gel H with tetrahydrofuran–methylal–methanol–2 M aqueous ammonia 10:5:5:1 (v/v). The fraction containing phosphatidylcholine was likewise isolated by thin-layer chromatography using a solvent system containing chloroform–methanol–acetic acid–water 12.5:7.5:2.1 (v/v).

Procedures concerning pH and CO_2 measurements, analysis of medium glucose concentration, tissue phosphorus and protein content, phospholipid hydrolysis, and the preparation, separation, quantitation, and trapping of fatty acid methyl esters by gas–liquid chromatography were as previously reported (1, 6).

The amount of glucose incorporated into the phospholipid fraction was calculated from the specific activity of glucose in the medium and the pool size and specific activity of the phospholipid fraction at the time of sampling. [U-^{14}C]Glucose was purchased from New England Nuclear Corp., Boston, MA. Radioactivity was determined using scintillation counting techniques with efficiency calculated using the channels ratio method.

RESULTS

The experimental conditions represented as nearly as possible a physiological environment. Blood pH in the lung is normally near the pH used, 7.34, and medium total CO_2 concentration was maintained in the range of 6–45 mM, which is taken as the extreme of physiological conditions. Lung lobes were taken for analysis at 60 and 120 min during the perfusion in order to judge the effect of CO_2 concentration on a time basis and as a check of linearity of incorporation of [U-^{14}C]glucose.

The incorporation of [U-^{14}C]glucose into phosphatidylcholine in the surfactant fraction increased approximately 44 and 42% at 60 and 120 min of perfusion as a result of the increased CO_2 concentration as shown in **Table 1.** A slightly greater increase of 52 and 49%, respectively, for the two intervals was observed for phosphatidylcholine of the residual fraction. Less marked increases were observed in [U-^{14}C]glucose incorporation into phosphatidylglycerol of both the surfactant and residual fractions. The increase in [U-^{14}C]glucose incorporation into phosphatidylcholine was significant at no less than the 95% confidence level.

As glucose is incorporated into both the glycerophosphorylcholine and fatty acid moieties, it was of interest to determine the effect of CO_2 concentration on the distribution of [U-^{14}C]glucose incorporation in phosphatidylcholine in the surfactant and residual lung fractions. The results shown in **Table 2** indicate that, in both fractions at each time interval, the effect of increased CO_2 concentration was to increase the percentage of glucose incorporation into the fatty acids as compared to the glycerophosphorylcholine moiety. It was not possible to provide similar data on the distribution of glucose incorporation in phosphatidylglycerol because of its low concentration in each of the two isolated lung fractions.

TABLE 1. Effect of CO_2 concentration on incorporation of [U-^{14}C]glucose into phosphatidylcholine and phosphatidylglycerol of the surfactant and residual lung fractions[a,b]

	Perfusion Medium				Incorporation of [U-^{14}C]glucose into:			
	Total CO_2 Time Elapsed (min)		pH Time Elapsed (min)		Phosphatidylcholine Time Elapsed (min)		Phosphatidylglycerol Time Elapsed (min)	
	0	120	0	120	60	120	60	120
	mM				*μmol × 10/g protein*		*μmol × 10³/g protein*	
A. Surfactant fraction								
1)	7.28	6.37	7.33	7.33	1.88 ± 0.24[c]	4.58 ± 0.41	4.61 ± 0.59	11.47 ± 1.24
2)	43.17	41.39	7.34	7.36	2.70 ± 0.35	6.50 ± 0.62	5.79 ± 0.80	13.55 ± 1.32
	Increase line 1 to line 2				43.6%	41.9%	25.6%	18.1%
B. Residual fraction								
1)	7.28	6.37	7.33	7.33	2.27 ± 0.26	4.87 ± 0.45	6.92 ± 0.81	11.87 ± 1.54
2)	43.17	41.39	7.34	7.36	3.44 ± 0.41	7.23 ± 0.62	9.10 ± 0.83	16.05 ± 1.31
	Increase line 1 to line 2				51.5%	48.5%	31.5%	35.2%

[a] See Experimental Procedure for isolation of fractions.
[b] Media glucose concentrations 5 mM.
[c] Values are means ± SE of 8 experiments.

TABLE 2. Effect of CO_2 concentration on the incorporation of [U-^{14}C]glucose into the fatty acid moiety of the surfactant and residual fractions[a]

Perfusion Media				% Radioactivity of Phosphatidylcholine Found in Fatty Acid Moieties			
Total CO_2 Time Elapsed (min)		pH Time Elapsed (min)		Surfactant Fraction Time Elapsed (min)		Residual Fraction Time Elapsed (min)	
0	120	0	120	60	120	60	120
mM							
7.28	6.37	7.33	7.33	55.4 ± 2.7[b] (8)	59.5 ± 2.3 (8)	51.9 ± 2.1 (7)	49.9 ± 3.8 (8)
43.17	41.39	7.34	7.36	65.5 ± 2.4 (6)	64.7 ± 3.8 (8)	60.5 ± 2.8 (6)	65.5 ± 4.0 (6)

[a] See Experimental Procedure for isolation of fractions.
[b] Values are means ± SE of number of experiments in parentheses.

The results in Table 2 indicated that the major effect of increased CO_2 concentration was to increase the incorporation of [U-^{14}C]glucose into the fatty acid moieties. If that were the case, then the specific activities of fatty acids isolated from phosphatidylcholine of both fractions should reflect a maximum difference. As palmitic acid represents 85% of the fatty acid present in the surfactant fraction phosphatidylcholine and 67% in the residual fraction (4), the specific activity of palmitate was determined. In addition, because it was of interest to determine whether the effect of CO_2 concentration on [U-^{14}C]glucose was essentially linear between the chosen lower and upper CO_2 concentrations, a series of perfusions was carried out at 27 mM CO_2 and pH 7.34. Such an effect should most easily be demonstrated where the maximum effect of CO_2 concentration is observed. The results (**Table 3**) show significant increases of palmitate specific activity between CO_2 concentrations of 7 and 42 mM, 42 and 70% in the surfactant fraction, and 85 and 68% in the residual fraction, respectively, after 60 and 120 min of perfusion. Values for the specific activity of palmitate at 27 mM CO_2 were intermediate at each time interval for both fractions, indicating an approximately linear response between the limits studied.

DISCUSSION

The continued production of the surfactant–lipoprotein complex that is responsible for alveolar stability is known to be necessary for normal lung function (7–9). The dependence of the synthesis of phosphatidylcholine and phosphatidylglycerol, major

TABLE 3. Effect of CO_2 concentration on incorporation of [U-^{14}C]glucose into palmitic acid of phosphatidylcholine of surfactant and residual fractions[a]

Perfusion Medium[b] Total CO_2 Time Elapsed (min)		Specific Activity of Methyl Palmitate			
		Surfactant Fraction Time Elapsed (min)		Residual Fraction Time Elapsed (min)	
0	120	60	120	60	120
mM		*dpm/nmol*			
7.28	6.37	2.12 ± 0.22[c] (6)	5.35 ± 0.54 (7)	2.82 ± 0.15 (6)	6.30 ± 0.55 (6)
28.10	27.37	2.61 ± 0.62 (8)	6.14 ± 0.97 (8)	3.10 ± 0.43 (8)	7.30 ± 1.02 (8)
43.17	41.39	3.00 ± 0.31 (6)	9.06 ± 0.85 (7)	5.22 ± 0.53 (6)	10.59 ± 1.15 (6)

[a] See Experimental Procedure for isolation of fractions.
[b] Average pH of perfusion medium was 7.34.
[c] Values are means ± SE of number of experiments in parentheses.

components of the surfactant fraction (4), on glucose has not been accurately measured. However, glucose is readily used by the lung (10, 11) and incorporated into lung phospholipid fatty acids even in the presence of physiological levels of fatty acid (1, 12). Unpublished data from this laboratory indicate that a minimum of 20% of the fatty acids of phosphatidylcholine in lung appear to be synthesized de novo from glucose. Of particular importance is that the palmitic acid in the 2 position of the predominant 1,2-dipalmitoyl-*sn*-3-glycerophosphorylcholine of the surfactant fraction appears to be derived to a greater extent from de novo synthesis of palmitate than the 1-position palmitate (4). In addition, synthesis of lung phospholipids from [U-^{14}C]glucose has been reported to be decreased in both alloxan and streptozotocin diabetic rats and reestablished to normal by insulin treatment in vivo (13)

It has been shown previously that increased medium CO_2 concentration increased [U-^{14}C]glucose incorporation into the total phosphatidylcholine fraction of isolated perfused lung (1). The present data extend those findings to show that the effect of increased CO_2 concentration increases the incorporation to about the same extent in phosphatidylcholine isolated from both the surfactant fraction and in the remaining phosphatidylcholine of the lung. The earlier finding (1) of a CO_2 concentration effect on [U-^{14}C]glucose incorporation appeared prior to the identification of phosphatidylglycerol as a significant lung phospholipid and as a component rapidly labeled by [U-^{14}C]glucose (4). In the first report of the effect of CO_2 concentration, phosphatidylglycerol was isolated as a component of the phosphatidylcholine fraction. The data indicate that the effect of increased CO_2 concentration produces a similar effect on the incorporation of [U-^{14}C]-glucose into phosphatidylglycerol. Because of the small amounts present, it was not possible to degrade the phosphatidylglycerol to determine with sufficient accuracy the percent of [U-^{14}C]glucose incorporated into the glycerophosphorylglycerol and fatty acid moieties or to determine the fatty acid specific activities. It would seem reasonable to expect that the same approximate 70% increase in palmitate isolated from phosphatidylglycerol after 120 min of perfusion would occur in both fractions, as was found in palmitate isolated from phosphatidylcholine. The increases in [U-^{14}C]glucose incorporation in the fatty acid moiety and palmitate specific activity agree reasonably well with those previously reported for whole lung (1). Evidence was presented in that report suggesting that the effect of CO_2 concentration was directly upon de novo fatty acid synthesis. That concept was based in part on previous studies carried out in liver (14–16).

The findings reported here suggest that abnormal extracellular CO_2 concentration may alter significantly the synthesis of palmitate from glucose in the synthesis of dipalmitoyl phosphatidylcholine and that of phosphatidylglycerol as well. As the lung is often exposed to low extracellular CO_2 concentrations in the newborn infant with respiratory distress syndrome or to abnormal retention of CO_2, as in conditions such as emphysema, the synthesis of the disaturated phospholipids could be affected.

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