THE INHIBITION OF ENZYMES RELATED TO PULMONARY FATTY ACID AND PHOSPHOLIPID SYNTHESIS BY DIETARY DEPRIVATION IN THE RAT

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Summary - Acute nutritional deprivation results in significant reduction in the activities of acetyl-CoA carboxylase, fatty acid synthetase, microsomal fatty acid elongation and choline phosphotransferase in rat lung. This data establishes the enzymatic basis for the known inhibition of pulmonary surfactant production by acute starvation.

Pulmonary surfactant is believed to lower surface tension at the air alveolar interface thereby preventing collapse of the alveoli of the lung (1). Recent studies have suggested that there is a relationship between dietary intake and surfactant synthesis. Faridy (2) has shown that surface tension of lung extracts is significantly elevated in malnourished rats. Food deprivation resulted in an increased tendency of the lungs to collapse, associated with a lowered content of phosphatidyl choline, the major surface active lipid. Garbagni et al. (3) have shown that the bubble stability ratio of pulmonary alveolar fluid, an index of surfactant content, is decreased following fasting in rabbits. Scholtz and Rhoades (4) examined the incorporation of glucose into lipid by lung slices. They found a 40% decrease in lung lipid synthesis from glucose in fasted rats. The decrease in lipid synthesis was greatest in the fatty acid fraction.

We have investigated the enzymatic basis of this relationship by studying the effect of food deprivation on enzymes involved with the synthesis of pulmonary phospholipids and their fatty acid components.

METHODS

In each experiment, 10 three week old weaned Sprague-Dawley rats were

totally deprived of food for four days, but allowed to drink water ad lib. An equal number of littermates were fed both rat chow and water and served as controls. After four days, all animals were weighed and killed by decapitation. The lungs were excised and chilled immediately in 0.01M Tris Cl pH 7.4 buffer containing 0.25M sucrose and 0.001M EDTA. The lungs were then homogenized, filtered through cheesecloth, and fractionated by differential centrifugation as described previously (5). The filtered homogenate, 100,000g supernatant and microsomal fractions were retained for enzyme assays.

Enzyme Assays - Acetyl-CoA carboxylase (EC 6.4.1.2) activity was assayed in a partially purified gel-filtered supernatant fraction by measuring the rate of [^{14}C]sodium bicarbonate incorporation into malonyl-CoA by the method of Greenspan and Lowenstein (6). Fatty acid synthetase and microsomal fatty acid elongation activity was assayed by measuring the rate of incorporation of [2-^{14}C]malonyl-CoA into pentane-extractable fatty acid as described previously (5). Choline phosphotransferase (EC 2.7.8.2), glycerolphosphate phosphatidyltransferase (EC 2.7.8.5) and CDPdiglyceride-inositol phosphatidyltransferase (EC 2.7.8.11) were assayed in the filtered cell-free homogenate by measuring the rate of incorporation of CDP[methyl-^{14}C]choline, L[^{14}C]glycerol-3-phosphate and [2-^{3}H]myo-inositol into lipid essentially by the procedures of Mudd et al. (7), Kiyasu et al. (8), and Paulus and Kennedy (9), respectively. Aliquots of the reaction mixtures were removed at intervals and processed on filter paper disks prior to counting (10).

RESULTS AND DISCUSSION

De novo fatty acid synthesis, which is the most active pathway for fatty acid synthesis in lung, occurs in a two-step reaction catalyzed by acetyl-CoA carboxylase and fatty acid synthetase (11). The end product this reaction is palmitic acid (5), the fatty acid component of dipalmityl phosphatidyl choline, which is the major surface active phospholipid. As

is shown in Table I, food deprivation resulted in a 55% decrease in acetyl-CoA carboxylase activity and a 36% decrease in fatty acid synthetase activity.

Table I. Influence of starvation on the enzymes of lipid synthesis in rat lung

Enzyme	Enzyme activity		Starved/ Control	p
	Control	Starved		
Acetyl-CoA carboxylase	944±180	426±54	0.45	< 0.01
Fatty acid synthetase	776± 98	498±71	0.64	< 0.025
Microsomal fatty acid elongation	45.6±3.4	22.1±2.3	0.48	< 0.001
Choline phosphotransferase	82.2±3.1	45.9±2.5	0.56	< 0.001
Glycerolphosphate phosphatidyltransferase	12.8±1.7	13.0±1.7	1.02	N.S.
CDPdiglyceride-inositol phosphatidyltransferase	1021±186	853±187	0.84	N.S.

Enzyme activity expressed as pmoles/min/mg.

Each value represents the mean of 6-9 experiments S.E. Statistical analysis was by paired t test. N.S. = not significant.

Another pathway of fatty acid synthesis is by chain elongation of pre-existing fatty acids. By this mechanism, long chain fatty acids may be synthesized from shorter chain precursors. In lung, fatty acid elongation occurs in both the microsomal and mitochondrial fractions (5,11). As is shown in Table I, we observed a 52% decrease in pulmonary microsomal fatty acid elongation activity, following 4 days of starvation.

In the lungs from the starved animals, the activity of choline phospho-transferase, an important enzyme in the de novo synthesis of phosphatidyl-

choline, was only 56% that of the controls. The activities of glycerol-phosphate phosphatidyltransferase, an enzyme involved in the synthesis of phosphatidylglycerol, which is the second most abundant surface active phospholipid in pulmonary surfactant (12), and CDPdiglyceride-inositol phosphatidyltransferase, an enzyme involved in the synthesis of phosphatidylinositol, a non-surface active lipid, were not altered by starvation.

As is shown in Table II, there was a significant reduction in both lung and total body weight in the starved animals. The decrease in lung weight was proportional to the reduction in body weight. This observation is consistent with the findings of Jackson (13).

Table II. Influence of starvation on lung and body weight

	Body weight (g)	Lung weight (g)	Lung weight / Body weight
Control	77.3±11.6	0.65±0.08	0.87±0.05
Starved	42.8± 5.2	0.39±0.06	0.91±0.04
p	<0.05	<0.025	N.S.

Our data establishes the enzymatic basis for the decreased pulmonary lecithin and fatty acid synthesis associated with food deprivation. It is possible that other enzymes involved with phosphatidyl choline synthesis are also inhibited by dietary deprivation, although the activity of the enzymes of phosphatidylglycerol and phosphatidylinositol synthesis were not influenced by starvation.

It is often difficult to achieve an adequate dietary intake in

infants with the respiratory distress syndrome, a disorder characterized by inadequate surfactant production. Although there is no evidence that nutritional deprivation is a cause of this syndrome, it is conceivable that, after it has developed, it may be further aggravated by an inadequate diet.

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REFERENCES

1. Gluck, L. (1972) Pediat. Clin. N. Am. 19, 323-332.
2. Faridy, E.E. (1970) J. Appl. Physiol. 29, 493-498.
3. Garbagni, R., Coppo, F., Grassini, G. and Cardellino, G. (1968) Respiration 25, 458-464.
4. Scholz, R.W. and Rhoades, R.A. (1971) Biochem. J. 124, 257-264.
5. Gross, I. and Warshaw, J.B. (1974) Pediat. Res. 8, 193-199.
6. Greenspan, M. and Lowenstein, J.M. (1967) Arch. Biochem. Biophys. 118, 260-263.
7. Mudd, J.B., Van Golde, L.M.G. and Van Deenan, L.L.M. (1969) Biochim. Biophys. Acta 176, 547-556.
8. Kiyasu, J.Y., Pierenger, R.A., Paulus, H. and Kennedy, E.P. (1963) J. Biol. Chem. 238, 2293-2298.
9. Paulus, H. and Kennedy, E.P. (1960) J. Biol. Chem. 235, 1303-1311.
10. Goldfine, H. (1969) in Methods in Enzymology (Lowenstein, J.M., ed.) Vol. 14, pp 649-651, Academic Press, New York and London.
11. Schiller, H. and Bensch, K. (1971) J. Lipid Res. 12, 248-255.
12. Rooney, S.A., Canavan, P.M. and Motoyama, E.K. (1974) Biochim. Biophys. Acta 360, 56-57.
13. Jackson, C.M. (1915) Am. J. Anat. 18, 75-108.