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A ROLE FOR CYTIDINE MONOPHOSPHATE IN THE REGULATION OF THE GLYCEROPHOSPHOLIPID COMPOSITION OF SURFACTANT IN DEVELOPING LUNG

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SUMMARY

The concentration of cytidine monophosphate (CMP) in lung tissue was found to increase during rabbit lung development from 12 nmol/g of tissue on day 23 of gestation to 52 nmol/g in the adult. The concentrations of cytidine triphosphate in lung tissue decreased from 113 nmol/g of tissue on day 23 to 63 nmol/g of tissue in the adult. The concentration of CDP-choline increased from 14.9 nmol/g at day 21 of gestation to 38.4 at day 26 and decreased subsequently in the newborn (6.5 nmol/g) and adult (9.7 nmol/g). The increase in the concentration of CMP in lung appeared to be organ and nucleotide specific since there was no increase in the concentration of CMP in liver tissue, and the concentration of AMP in the lung tissue did not increase with development. A function for CMP in regulating the availability of CDP-diacylglycerol is proposed to account for the changes in the glycerophospholipid composition of lung surfactant which occur during development.

INTRODUCTION

Augmented production of surfactant by fetal lungs does not commence until gestation is about 80% complete, and even then the glycerophospholipid composition of surfactant synthesized by immature lungs is different from that produced by mature lungs. Surfactant in immature lung tissue contains dipalmitoylphosphatidylcholine as the major surface-active component, but is deficient in phosphatidylglycerol which is the second most abundant glycerophospholipid of surfactant in mature lungs (1). In place of phosphatidylglycerol, surfactant from immature lungs contains a correspondingly higher level of phosphatidylinositol (2). This developmental change from the production of a surfactant rich in phosphatidylinositol to one rich in phosphatidylglycerol occurs after increased dipalmitoylphosphatidylcholine biosynthesis has commenced (3). Importantly,

Abbreviations: PAPase - phosphatidate phosphohydrolase (E.C. 3.1.3.4.)

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infants born before the level of phosphatidylglycerol in their lung surfactant rises may be at increased risk of succumbing to the respiratory distress syndrome (3,4,5). However, the mechanism(s) whereby the glycerophospholipid composition of surfactant is regulated during fetal lung maturation is unknown.

The reciprocal changes in the phosphatidylinositol and phosphatidylglycerol content of surfactant likely are brought about by regulation of the metabolism of a common precursor, e.g., CDP-diacylglycerol. Evidence has been presented which is supportive of the view that both phosphatidylinositol and phosphatidylglycerol in rat pancreatic tissue (6) and in rabbit lung tissue (7) are synthesized from the same pool of CDP-diacylglycerol, and that competition for available CDP-diacylglycerol can occur. In this regard, we have suggested that the reversibility of the reaction catalyzed by CDP-diacylglycerol:inositol phosphatidyltransferase (EC 2.7.8.11) in lung tissue (8) is of critical importance in the metabolism of CDP-diacylglycerol. We found that the reverse reaction proceeded readily in microsomes prepared from rabbit lung tissue and that the rate of formation of CDP-diacylglycerol from phosphatidylinositol was accelerated as the concentration of CMP was increased.

CMP is a product of the reaction catalyzed by choline phosphotransferase (EC 2.7.8.2), the final step in phosphatidylcholine biosynthesis. For this reason we hypothesized that intracellular levels of CMP in fetal lung would rise as a consequence of increased phosphatidylcholine production which occurs during the latter stages of gestation. CMP, in increased concentrations, would then promote the reverse reaction catalyzed by CDP-diacylglycerol:inositol phosphatidyltransferase (8), an event that could lead to an increase in the amount of CDP-diacylglycerol available for phosphatidylglycerol biosynthesis. Such a series of events could account for the changing pattern of glycerophospholipid composition of surfactant that is seen with fetal lung development. For these reasons we quantified CMP, CTP, CDP-choline and CDP-ethanolamine in rabbit lung tissue obtained at various stages of development.

MATERIALS AND METHODS

Pregnant New Zealand white rabbits were anesthetized by the intraperitoneal administration of Ketamine (40 mg/kg body weight) and anesthesia was maintained with

Metofan/O_2. Maternal blood gases did not change during this procedure. Following laparotomy, fetuses were removed individually. The fetal lungs and livers were excised rapidly and freeze-clamped to a wafer at -196°C. The average time from uterine incision to the time of freeze-clamping of the fetal lung was 30s. No differences in CMP levels were found in different samples of the same tissue when the freeze-clamping time varied from 20s to 50s. The tissue wafers were weighed, ground to a powder at -70° C, and homogenized in 2.8% (v/v) perchloric acid (3 ml/g tissue). [^{3}H]CMP, of high specific radioactivity, was added in picomole amounts as an internal standard. Extraction of the tissues and neutralization of the extracts was conducted as described by Khym (9).

The nucleotides in the neutralized extracts were separated by high performance liquid chromatography employing a Whatman Partisil 10-SAX column according to the procedure of Chen et al (10). Cytidine and adenosine nucleoside phosphates were quantified by measurement of ultraviolet absorption at their absorbance maxima (280 nm and 254 nm, respectively) employing solutions containing authentic nucleotides (11). The recoveries of CMP and CTP were determined to be 90% and 85% respectively. The quantity of nucleotide measured in any particular sample was corrected for procedural losses according to the amount of [^{3}H]CMP recovered in that sample. To quantify CDP-choline and CDP-ethanolamine, an initial purification of the neutral tissue extracts was performed according to the method of Moran et al (12). Aliquots (1 ml) of the neutral extract were applied to a DEAE-cellulose column (4 x 1cm). CDP-ethanolamine was eluted quantitatively with 50 mM ammonium formate (8 ml); CDP-choline was eluted quantitatively with 100 mM ammonium formate (8 ml). The eluates were lyophilized and reconstituted in a known volume of ammonium dihydrogen phosphate (10mM). The components of the eluted fractions were separated by high performance liquid chromatography utilizing a Du Pont Zorbax-NH column. The mobile phase was 10 mM ammonium dihydrogen phosphate (pH 4.5). All separations were conducted at 36°C with a flow rate of 2 ml x min^{-1}. CDP-choline and CDP-ethanolamine were quantified at 280 nm. Utilizing authentic nucleotides, recoveries of each compound were determined to be 90-95%. The data were subjected to an analysis of variance. Differences among means were determined by the Newman-Keuls multiple range comparison test (13).

RESULTS

The levels of CMP found in fetal, neonatal and adult rabbit lung and liver tissues are given in Figure 1A. The gestational period of the rabbit is 31 days. From day 21 to day 30 of gestation, CMP levels in fetal lung tissue doubled. A further increase in CMP levels in lung occurred postnatally, a maximal value of 56 nmol/g lung tissue being attained in the adult. There was no increase in the level of CMP in liver tissue during development. The concentration of AMP in lung tissue (510 $\pm$ 30 nmol/g) did not change during fetal development or during the first seven days of neonatal life.

It was considered important to ascertain whether the observed increase in CMP content of lung tissue was accompanied by an associated decrease in CTP levels. Between day 21 and day 30 of gestation, CTP concentrations fell to levels which were maintained throughout the remainder of development (Fig. 1B). Since this decrease in CTP content

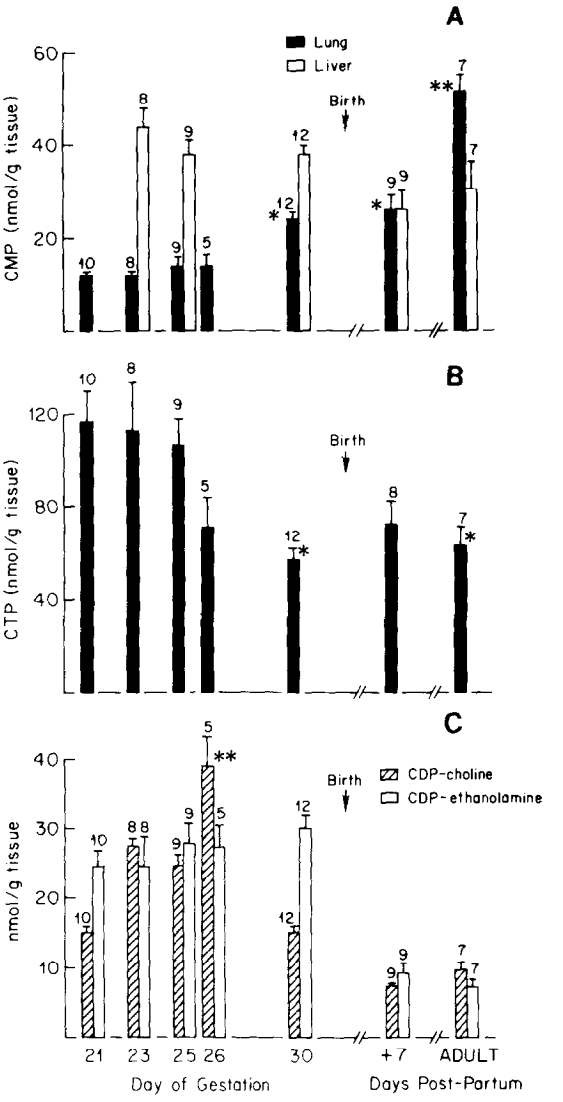

Figure 1. Cytidine nucleoside phosphates in fetal, neonatal and adult rabbit tissues. (Mean ± S.E.). The gestational period of the rabbit is 31 days. Nucleoside phosphates contained in a neutralized acid-soluble extract were separated by high performance liquid chromatography. The cytidine containing nucleoside phosphates were quantified at 280 nm. The data were subjected to an analysis of variance. Differences among means were determined by the Newman-Keuls multiple range comparison test (see Methods). The number above each bar represents the number of individual determinations. A. CMP content of lung and liver tissues. CMP in lung tissue increased (*$p<.05$; **$p<.01$) with development. B. CTP content of lung tissue decreased (*$p<.05$) by day 30 of fetal development. C. CDP-choline and CDP-ethanolamine content of lung tissues. CDP-choline increased from day 21 to day 26 of fetal development (**$p<.01$), subsequently decreasing by day 30.

did not coincide with the increase in CMP content of developing lung tissue, the levels of CDP-choline and CDP-ethanolamine in lung tissue were measured throughout development. The CDP-choline content of fetal lung tissue increased from 14.9 nmol/g at day 21

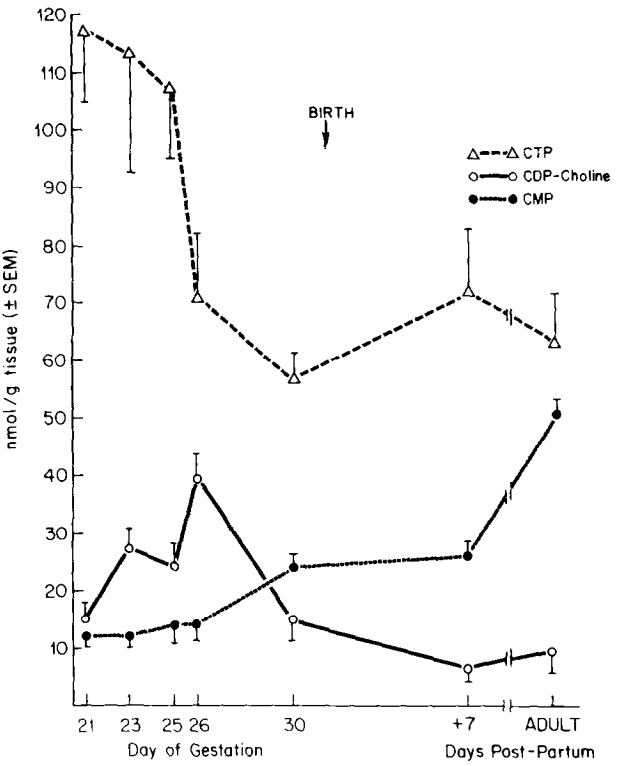

Figure 2.: The temporal relationship of the developmental changes in the levels of CTP, CDP-choline and CMP in the rabbit lung.

of gestation to 38.4 nmol/g at day 26 (Fig. 1C). Thereafter, the CDP-choline levels decreased to levels found in the adult (9.7 nmol/g). At a time in lung development when CDP-choline levels fall, the levels of CMP rise. No significant change was found in the content of CDP-ethanolamine during fetal lung development.

The temporal relationship of the developmental changes in the levels of CTP, CDP-choline and CMP is illustrated in Figure 2. Early in lung development, at a time before augmented phosphatidylcholine synthesis for surfactant has begun, CTP levels are high. There is a time in fetal lung development when the CTP level has fallen and the CDP-choline level has risen. This occurs by day 26, a time which precedes by two days the time of augmented surfactant phosphatidylcholine synthesis in the fetal rabbit lungs (14). The subsequent decrease in CDP-choline levels coincides, in time, with the surge in phosphatidylcholine synthesis for surfactant and likely comes about by the rapid

incorporation of CDP-choline and diacylglycerol into phosphatidylcholine. The concentration of the co-product of phosphatidylcholine biosynthesis viz., CMP, increases in lung tissue as the rate of phosphatidylcholine biosynthesis increases.

DISCUSSION

The developmental changes in the levels of CTP, CDP-choline and CMP in rabbit lung tissue found in the present investigation are indicative of a flux of cytidine nucleotides from CTP to CMP as a consequence of augmented phosphatidylcholine biosynthesis. The maximum levels of CDP-choline are attained on day 26 of gestation, two days before the reported increase in surfactant phosphatidylcholine biosynthesis (14), therefore, it seems likely that at this stage of development it is not the availability of CDP-choline, but rather the availability of diacylglycerol which may limit phosphatidylcholine biosynthesis. This finding is supportive of the proposed role of phosphatidate phosphohydrolase (PAPase) in the regulation of phosphatidylcholine biosynthesis during lung development (15). The finding that the specific activity of PAPase in rabbit lung tissue increases at this stage of gestation (16) is consistent with these data.

The changes in the levels of CMP reported here, likely are reflective of enhanced glycerophospholipid metabolism in fetal lung tissue late in gestation. Mandel and Edel-Harth (17) found marked increases in CMP levels in association with the enhanced glycerophospholipid metabolism that accompanies myelination in neonatal rat brain. We suggest that the levels of CMP not only reflect an increase in glycerophospholipid biosynthesis but that this nucleotide plays a regulatory role in glycerophospholipid metabolism during lung development.

Early in fetal lung development there is limited capacity for surfactant phosphatidylcholine biosynthesis in the type II pneumonocyte. Later in gestation, PAPase activity increases (16) giving rise to increased levels of diacylglycerol for the biosynthesis of phosphatidylcholine. The rate of phosphatidylcholine synthesis may also be enhanced by increased levels of CDP-choline which arise from increasing CTP:phosphocholine cytidylyltransferase activity (EC 2.7.7.15). Recently it was found that the activity of this

enzyme increases during fetal lung development and that the increase is brought about by an activation of pre-existing enzyme, a process which is facilitated by acidic glycerophospholipids (18). The importance of CTP:phosphocholine cytidylyltransferase in the regulation of phosphatidylcholine biosynthesis in other tissues has been emphasized (19). Since CMP is also a product of phosphatidylcholine biosynthesis, it follows that with increased phosphatidylcholine synthesis, intracellular levels of CMP will rise. CMP, in increased concentrations, affects the reversible reaction catalyzed by CDP-diacylglycerol:inositol phosphatidyltransferase (8) bringing about a decrease in net biosynthesis of phosphatidylinositol and an increase in the amount of CDP-diacylglycerol available for phosphatidylglycerophosphate biosynthesis. In the biosynthesis of phosphatidylglycerophosphate, CMP is regenerated, thus maintaining the elevated intracellular levels of CMP via this cyclic process. Phosphatidylglycerophosphate is then converted to phosphatidylglycerol in an irreversible reaction catalyzed by PAPase. The specific activity of PAPase is elevated at this stage of lung development and we have shown previously that phosphatidylglycerophosphate is a substrate for PAPase (20). This latter finding has been confirmed recently (21).

Of the different cell types of the lung, the type II pneumonocytes are likely to be the most active in glycerophospholipid biosynthesis because of their role in surfactant production. If the developmental changes in the levels of CMP observed in this study were confined to type II pneumonocytes, then the changes are especially remarkable since these cells constitute only 10-15% of the total cell population of the lung (22).

The concentration of CMP required to stimulate the incorporation of glycerol-3-P into phosphatidylglycerophosphate and phosphatidylglycerol (7) are similar to those found in the present investigation. CMP in such concentrations is also known to promote the reverse reaction catalyzed by CDP-diacylglycerol:inositol phosphatidyltransferase (8), i.e., concentrations of CMP below saturation levels. This becomes an important consideration if CMP were to participate in the regulation of phosphatidylinositol and phosphatidylglycerol metabolism. We propose that the existence of such a CMP cycle provides an explanation for the developmental changes in the glycerophospholipid composition of surfactant that accompany fetal lung maturation.

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