Vol. 96, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1980 Pages 1209-1215

COORDINATE INCREASES IN THE ENZYME ACTIVITIES RESPONSIBLE FOR PHOSPHATIDYLGLYCEROL SYNTHESIS AND CTP:CHOLINEPHOSPHATE CYTIDYLYLTRANSFERASE ACTIVITY IN DEVELOPING RAT LUNG

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Received July 21,1980

SUMMARY: The enzymes responsible for the biosynthesis of phosphatidylglycerol, CTP:phosphatidate cytidylyltransferase, CDP-diacylglycerol:glycerophosphate phosphatidyltransferase and phosphatidylglycerophosphate phosphatase demonstrated a coordinate increase in activity in fetal rat lung at term when the demand for pulmonary surfactant increases. The activity of CTP:cholinephosphate cytidylyltransferase, the enzyme responsible for CDP-choline production also increased in the perinatal period. The activity of cholinephosphate cytidylyltransferase in fetal and neonatal cytosol was stimulated by the addition of phosphatidylglycerol but no effect was noted with cytosol from adult lung. These results are consistent with the suggestion that the activity of cholinephosphate cytidylyltransferase, a potential rate-determining enzyme in pulmonary phosphatidylcholine synthesis, may be regulated in the perinatal period both through an activation by phosphatidylglycerol and by an increase in total enzyme units.

Phosphatidylcholine (PC) is a major constituent of the pulmonary surfactant which lines the alveoli and prevents their collapse by reducing the surface tension (1,2). The production of PC and disaturated PC (DSPC) for surfactant is augmented late in gestation (1,2). Recent evidence indicates that the production of CDP-choline by CTP:cholinephosphate cytidylyltransferase (E.C. 2.7.7.15) has a regulatory role in PC biosynthesis (2-6).

Although the incorporation of choline into PC and DSPC with rat lung slices rises as term approaches, the major increase (3-fold) occurs between the 21st day of gestation (term 22 days) and the first day after birth (6,7). Cholinephosphate cytidylyltransferase activity also increases during this period (6,7). The major component of this enzymatic activity is

Abbreviations: DSPC, disaturated phosphatidylcholine; PC, phosphatidylcholine; PG, phosphatidylglycerol.

0006-291X/80/191209-07$01.00/0

localized in the cytosol and exists in two states: a low molecular weight form, and a high molecular weight form (3,4). Recent studies have demonstrated that in fetal rat cytosol, cholinephosphate cytidylyltransferase is activated by anionic phospholipids, in particular phosphatidylglycerol (PG), and this activation is accompanied by a conversion from the low to the high molecular weight form (4). PG is the second most abundant phospholipid in pulmonary surfactant (1,2). This relationship between PC and PG in surfactant, coupled with the observation that PG activates cholinephosphate cytidylyltransferase, has led to speculation that the production of PC for surfactant may be coordinated by PG synthesis (2,4,5). The present investigation demonstrates a simultaneous increase in the enzymes responsible for PG synthesis which parallels the elevation in cholinephosphate cytidylyltransferase activity. The results suggest that both an activation of cytidylyltransferase by PG and an increase in total enzyme may be responsible for the increased production of PC for surfactant during the perinatal period.

EXPERIMENTAL PROCEDURES: Unless otherwise stated, all biochemicals were purchased from Sigma Chemical Company, St. Louis, MO., U.S.A., and radioactive compounds from New England Nuclear (Canada) Ltd., Dorval, Quebec. All other reagents were obtained from Fisher Scientific Co., Toronto, Ont. Phosphatidic Acid (Na salt) and PG were prepared from egg PC as previously described (8). CDP-diacylglycerol was purchased from Serdary Research Laboratory, London, Ont.

Fertilization of female Wistar rats was confirmed by the presence of sperm in the vagina. The day of mating was considered as day zero. Rats were sacrificed by decapitation and subcellular fractions prepared as described in (9).

CTP:phosphatidate cytidylyltransferase (E.C. 2.7.7.41), glycerophosphate phosphatidyltransferase (E.C. 2.7.8.5), and phosphatidylglycerophosphate phosphatase (E.C. 3.1.3.27) activities were determined in whole lung homogenate as previously described (10,11,12), except that for the phosphatidate cytidylyltransferase, the pH was adjusted to 6.25. Preliminary studies were conducted to establish that the concentrations of the substrates were optimal and that the reactions were linear with respect to time and protein concentration over the ranges studied. CTP:cholinephosphate cytidylyltransferase (E.C. 2.7.7.15) activities in whole lung homogenate and cytosol franctions were determined as described in (5).

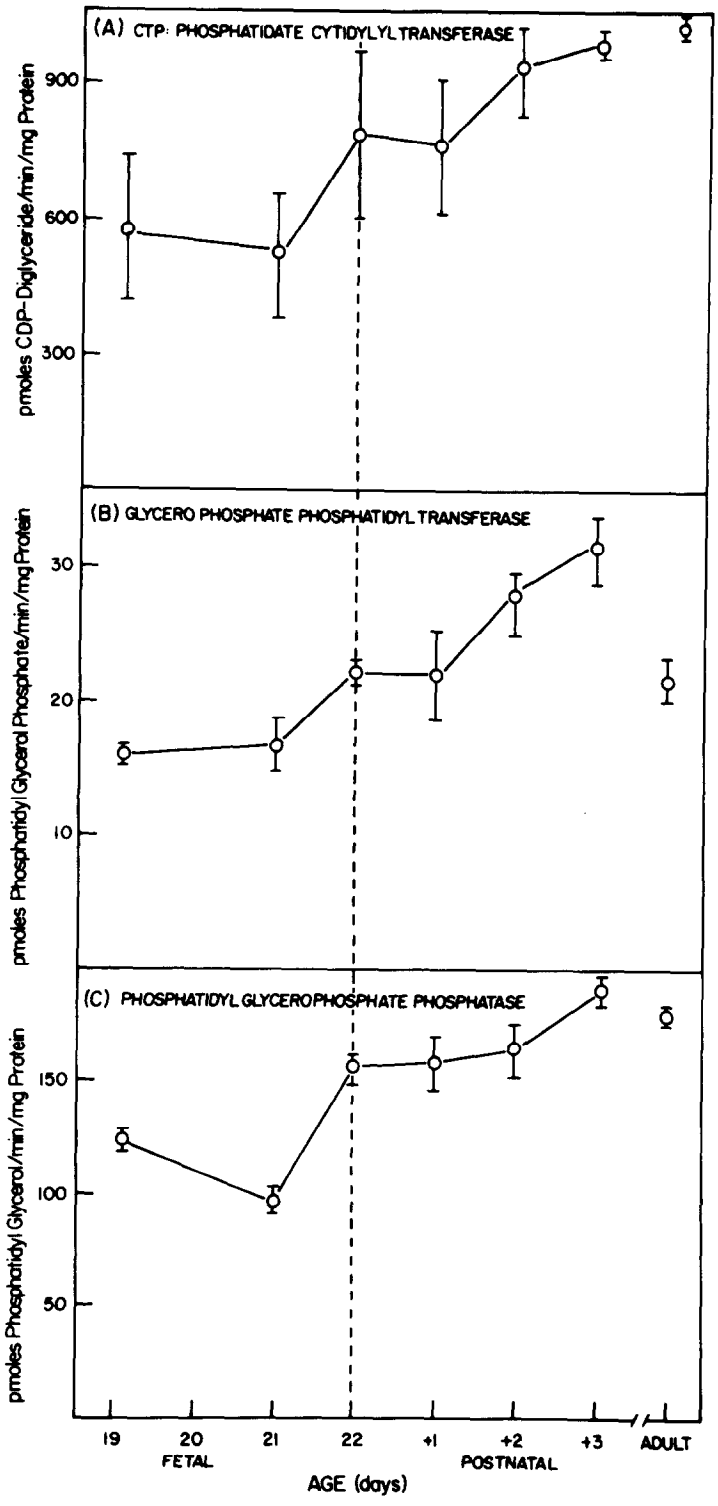

FIGURE 1: The activities of CTP:phosphatidate cytidylyltransferase (A), glycerophosphate phosphatidyltransferase (B) and phosphatidylglycerophosphate phosphatase (C) in rat lung measured as a function of gestational age. Activities were determined as described in materials and methods. Each point represents the mean ± SEM of 2-4 separate estimations with pooled homogenates from 2-3 litters being used for the fetal and neonatal samples.

RESULTS: Developmental Changes in PG Synthesizing Enzymes. The developmental changes in the enzyme activities responsible for conversion of phosphatidic acid to PG in rat lung are shown in Fig. 1. There was a coordinate increase in the specific activities of all enzymes at term (22 days), which continued after birth. In each case, the activities 3 days after birth were significantly greater ($P<0.05$) than those observed in the fetuses on day 19.

Developmental Changes in Cholinephosphate Cytidylyltransferase Activities. Cytidylyltransferase activities measured in whole homogenates increased slightly at term (not significant) and markedly after birth to attain a specific activity at day 3 in the neonate which was 1.8-fold greater ($P<0.01$) than the activities observed with the fetus prior to term (Fig. 2).

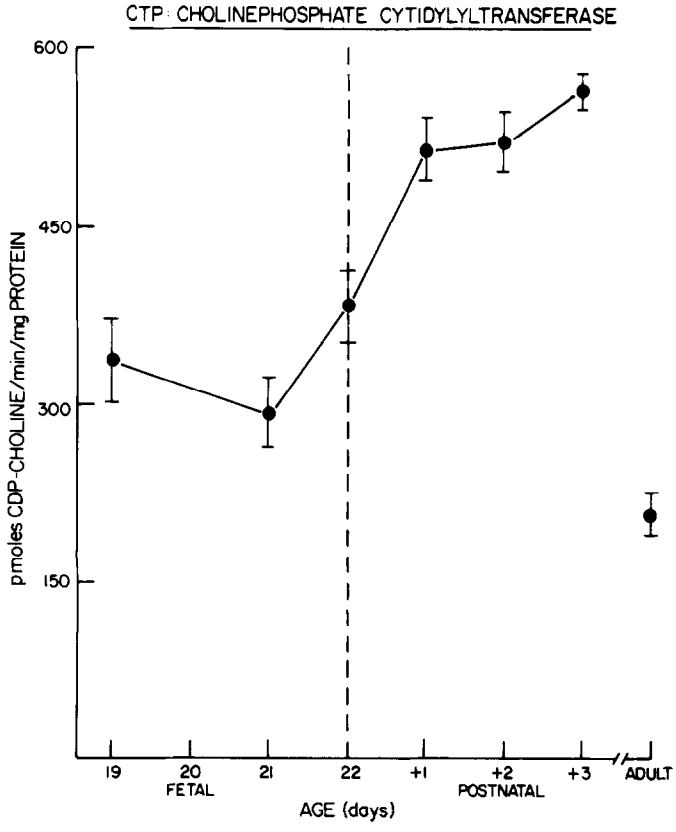

FIGURE 2: The activities of cholinephosphate cytidylyltransferase measured in homogenates of rat lung as a function of gestational age. Each point represents the mean ± SEM of 2-4 separate estimations with pooled homogenates from 2-3 litters being used for the fetal and neonatal samples.

TABLE 1. Effects of PG on cytosol cholinephosphate cytidylyltransferase activities in rat lung through development

Age (days)	n	Specific Activity n moles/min per mg protein -PG	Specific Activity n moles/min per mg protein +PG	Enzyme Activity n moles/min per gram lung -PG	Enzyme Activity n moles/min per gram lung +PG
21	(3)	0.21 ± 0.01	0.67 ± 0.02	6.81 ± 0.40	22.17 ± 0.61
+1	(3)	0.43 ± 0.01	1.23 ± 0.00	15.10 ± 1.98	42.93 ± 0.12
Adult	(3)	0.53 ± 0.01	0.48 ± 0.05	32.21 ± 1.07	31.01 ± 2.21

Cholinephosphate cytidylyltransferase activities were determined in rat lung cytosol as described in the text. The data are means ± SEM with homogenates from 2 - 3 litters being pooled for each fetal and neonatal sample (n). PG prepared (from egg PC) was sonicated into clear suspension in 0.9% NaCl and added to a final concentration of 1.6 mM.

The increases in the activity of this enzyme roughly paralleled the increases in the activities of the enzymes involved in the synthesis of PG. The specific activities in the neonate were significantly greater ($P<0.01$) than those observed with adult lung. The effect of PG on the cytosol cytidylyltransferase activities is shown in Table 1. Addition of PG to the fetal and neonatal cytosols increased the specific activity values by approximately 3-fold. On the other hand, the activities in adult cytosol were not stimulated by PG. The total enzyme units per gram lung both in the absence and presence of PG were greater in the neonates than in the fetuses. Chromatography on Bio-Gel A 5m (Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ont.) revealed the presence of low and high molecular weight forms in fetal and neonatal cytosols but only the high molecular weight form with cytosol from adult lung (data not shown).

DISCUSSION: It has become apparent that cholinephosphate cytidylyltransferase may play an important role in PC synthesis in lung and other tissues (2,13). The observation that the specific activity of cholinephosphate cytidylyltransferase increases in cytosol from rat lung

at birth has led a number of investigators to suggest that this elevation might be responsible for the increased PC synthesis for surfactant (2-7). Weinhold and coworkers (3,4) have presented evidence indicating that an activation by lipid factors such as PG are responsible for at least part of the increase in cholinephosphate cytidylyltransferase activity. These workers (14) have also noted an increase in the incorporation of radioactive precursors into PC in prematurely delivered rats, but it is not known whether cholinephosphate cytidylyltransferase activity is increased under these circumstances. Rooney et al. (15) have observed that the specific activity of cholinephosphate cytidylyltransferase in the cytosol of fetal rabbits declines before birth but increases after delivery. The increase in the specific activity of the cytidylyltransferase also occurs after premature delivery (16).

The studies cited above were consistent with the view that the ability of PG to activate cholinephosphate cytidylyltransferase might be involved in the increase in the activity of this enzyme at birth. In the present investigation, it has been observed that the enzymes responsible for PG synthesis increase in a coordinated fashion in rat lung near term and this increase is paralleled by an increase in the level of cholinephosphate cytidylyltransferase activity in the whole homogenates and in the cytosol. It will be necessary to establish that the levels of PG in cytosol also increase during this period. The role of the cytidylyltransferase activity in the microsomal fraction must also be evaluated. The ability of PG to stimulate cholinephosphate cytidylyltransferase activity in cytosol from fetal and neonatal (but not adult) lung suggests that a substantial proportion of the enzyme in this fraction is in an inactive form. On the other hand, the total cytosol activity in the presence of PG also increases during development indicating that an increase in the amount of enzyme also occurs concomitantly with the activation.

ACKNOWLEDGEMENTS: The authors are grateful to S. Tokmakjian, W. Ross, and R. Layden for breeding the rats used in this study and to K.P. Strickland for helpful discussion concerning the phosphatidate cytidylyltransferase assay. This work was supported by the Medical Research Council of Canada.

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