Acta Paediatrica Hungarica, 24 (1), pp 67—72 (1983)

Maturation of the fetal lung I. Phosphatidic acid phosphohydrolase in the fetal and newborn rat

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Phosphatidic acid phosphohydrolase (PAPase) activity was measured in the lungs, brain, liver and kidneys of fetal, newborn and adult rats. The highest activity was found in the kidneys. In one-day-old rats pulmonary enzyme activity was nearly as high as in the renal tissue. In four-days-old rats the highest activity was found in the 10% homogenate of the lung, the highest PAPase specific activity could be demonstrated in the mitochondria. In the fetal lung, the enzyme activity moderately increases from the 18th day of gestation up to birth; at birth 39—77% of the normal adult value was measured. Immediately after birth 150% activity is attained, thereafter a slow decrease can be shown throughout the first week of life but the values remain above the average adult value.

Immediately before birth a marked increase in the phospholipid content of the fetal lung can be shown. This is due to the activity of various enzymes. Two main pathways of pulmonary lecithin synthesis are known; choline incorporation, and methylation [3]. In the rat [15], rabbit [5], monkey [4] and in man [7] the major route is choline incorporation. A key enzyme of this pathway is phosphatidic acid phosphatase or phosphohydrolase (PAPase; EC. 3.1.3.4). It catalyses the hydrolytic cleavage of phosphatidic acid (PA) to diglyceride and orthophosphate (P_i): 1.2-diacyl-glycerol-3-phosphate+ + H_2O → 1.2-diacyl-glycerol + Pi (Fig. 1).

PA is a precursor of 1.2-diglyceride, this in turn plays a key role in phospholipid biosynthesis. PAPase has been isolated from animal tissues [6], and its presence in the cytoplasmic membrane of Bacillus subtilis 168 has been demonstrated [8].

The aim of this work was to study the dynamics of lecithin synthesis in the neonatal lung, with special reference to PAPase activity and to the role of enzyme induction.

MATERIAL AND METHOD

The experiments were carried out in Wistar rats having a gestation period of 22 days. Fetal age was calculated from the first sperm positive day. The fetuses were excised by Caesarean section under sterile conditions after short ether anaesthesia, placed in ice for some minutes, then killed before the first extrauterine respiratory movement. The newborn animals, 1, 2, 3, 4 resp. 6 days old, and the adult rats weighing 180—200 g were killed

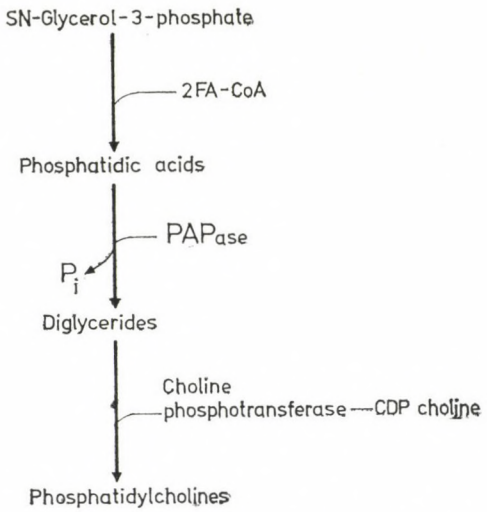

FIG. 1. Biosynthesis of phosphatidylcholine via CDP-choline pathway

by exsanguination. The lungs, liver, kidneys and brain were placed in ice immediately after removal and each organ was weighed on an analytical scale. The fetal and newborn lungs and kidneys were pooled; each pooled sample contained 4—6 fetal lungs or kidneys originating from one litter removed at the same gestational age. From the newborn animals 2—3 individual organs were pooled. Mean values for each five pooled samples were calculated.

For the studies L-alpha-phosphatidic acid (Sigma) isolated from egg-yolk and synthetic L-alpha-phosphatidic acid dipalmitoyl (Sigma) were used.

A 10% homogenate was prepared from the freshly removed organs. The organs were homogenized in 0.25 M sucrose in a teflon-headed homogenizer (Tissue grinder, Thomas, USA), then sonicated for 30 minutes (Lab. Sonic. 1510, Braun-Melsungen). The samples were kept in ice until starting the enzyme reaction. Subcellular particles were separated by fractionated centrifugation after Ravinuthala et al [11].

PAPase assay was carried out according to the method of Coleman and Hübscher [2] modified by ourselves: 1.5 μmol PA, 60 μmol maleate buffer pH 6.0 and the enzyme in a total volume of 250 μl were incubated at 37°C for 60 minutes. The reaction was stopped by addition of 250 μl 10% trichloroacetic acid. Inorganic phosphate was determined in the supernatant. All reactions were performed in duplicate. The blank did not contain PA. In previous assays we showed that incubation of PA without enzyme does not result in inorganic phosphate efflux. One unit of PAPase reflects 1 μmol inorganic phosphate liberated during one hour. Specific enzyme activity was calculated for 1 mg protein and one minute. The phosphatidic acid emulsion was prepared as follows: 10 μmol PA was dissolved in 0.1 ml hexane, then maleate buffer pH 6 was added and the solution sonicated for one minute. This resulted in a milky emulsion. The hexane was evaporated by fluid nitrogen.

Protein was measured according to Lowry et al [9]; for inorganic phosphate estimation the method of Bartlett [2] was used.

Results

Table I shows the PAPase activity found in 10% homogenates of various organs removed from newborn rats. The maximum of enzyme activity was found between pH 6 and 7. Over 60 minutes there was a linear increase of liberated inorganic phosphate if the protein content was about 1 mg/ml. There was no difference between the two phosphatidic acid preparations; the use of L-alpha--phosphatidic acid of egg-yolk origin resulted in about 5% higher activity values.

In one-day-old newborn rats there was a considerable variation of enzyme activity between the kidneys, liver, lungs and brain. The highest activity was found in the kidneys while in the brain it was the lowest. The pulmonary enzyme activity hardly differed from that found in the renal tissue.

Table II indicates the subcellular distribution of PAPase in lungs of four-day-old rats. The highest activity was found in the 10% homogenate: 782 $\pm$ 28 nmol/min/g lung tissue while the highest specific activity was observed in the mitochondrial fraction: 21.3 $\pm$ 1.1 nmol/min/mg protein. No measurable inorganic phosphate was liberated into the supernatant.

The changes in PAPase activity during development of the lung are

Table I

Distribution of PAPase in tissues of newborn rats. The results show the average $\pm$ S. D. for five separate experiments. Newborn tissues were taken from one-day-old rats

Tissue	P_i formed μmol/h
Kidney	0.62$\pm$0.01
Lung	0.6 $\pm$0.02
Liver	0.4 $\pm$0.016
Brain	0.34$\pm$0.012

Table II

Subcellular distribution of PAPase. The results are the average $\pm$ S. D. for four separate experiments. Newborn lungs were taken from 4-day-old rats

Fraction	nmol/min/g lung	nmol/min/m_o protein
Homogenate	782$\pm$28	7.2$\pm$1.2
Nuclei and debris	353$\pm$18	6.9$\pm$0.9
Mitochondria	287$\pm$20	21.3$\pm$1.1
Microsome	120$\pm$18	10.1$\pm$3
Supernatant	0	0

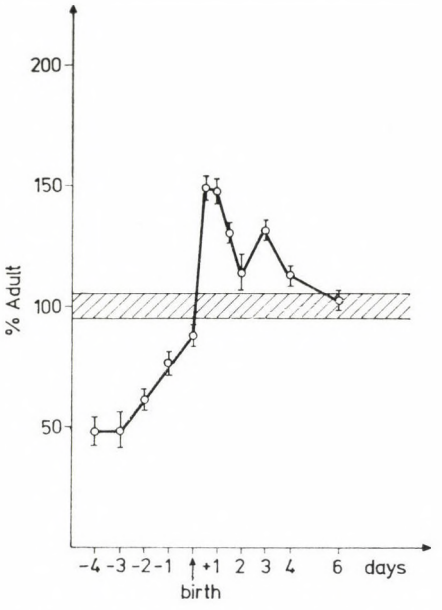

FIG. 2. PAPase activity during lung development. Each value is the average of 5 separate litters ± S. D. The activity is presented per lung weight. The adult values come to 699 nmol/min/g lung. The activity was measured in 10% homogenate

shown in Fig. 2. Enzyme activity increased moderately from the 18th day of gestation to term; at birth about 39—77% of the adult value was measured. A very sharp increase ensued immediately after birth, values as high as 150% of the adult level were attained. Thereafter a slow decrease was observed during the first week of life, but the levels remained higher than the adult mean all over this period.

DISCUSSION

The key role of PAPase in fetal pulmonary metabolism was described by Schultz et al [12] in 1974. In rabbits, they found a fourfold increase of specific PAPase activity of lung homogenates and microsomes between the 23rd and 30th days of gestation. This was followed by a decrease after birth, and there were no changes in CDP-choline diglyceride transferase activity over this period of time.

Smith et al [13] were the first to demonstrate PAPase activity in the heart, kidneys, brain, liver and striate muscle of rats and in the liver of chickens. In our experiments PAPase activity was determined also in pulmonary tissue.

Meban [10] localized PAPase activity in the type II alveolar cells of the lung by histochemical methods. Ravinuthala et al [11] investigated the PAPase activity of subcellular fractions extracted from fetal and

adult rat lungs. They found the highest specific activity in the mitochondria in fetal lung cells while in adults the microsomes had the highest specific activity. In our experiments, specific activity was the highest in the mitochondria of four-day-old newborn rats (21.3 ± 1.1 nmol/min/mg protein).

Spitzer et al [14] found that about 5—10% of all pulmonary cells are alveolar cells of type II. According to their studies in adult pigs, more than 40% of the total activity is concentrated in the lamellar bodies of type II alveolar cells. They assume that phosphatidylcholine is stored in the lamellar bodies after being synthesized on the perilamellar surface. In addition, PAPase activity is high also in the microsomes. In our experiments, a PAPase activity of 10 ± 3 nmol/min/mg protein was measured in the lungs of 4-day-old newborn rats, thus a marked activity was demonstrated both for mitochondria and microsomes. The highest PAPase activity calculated for wet pulmonary weight was observed in the homogenate. For this reason the comparative measurements performed in fetuses and newborns of various age were carried out in 10% homogenates of freshly removed lungs. Ravinuthala et al [11] found an about 70% activity in fetal lungs between the 18th day of gestation and term as compared with the corresponding adult activity level. In our experiments the corresponding figures were lower, between 40 and 77% of the adult values.

It is interesting that the events of birth induce a marked increase in enzyme activity speeding up lecithin synthesis in the alveolar membrane, this is then followed by a slight decrease on the 5th or 6th day of life. Presumably, the high oxygen content of the air perfusing the alveoli plays here an important role. The results of the present experiments may serve as a basis for further research into the role of postnatal changes in oxygen concentration.

Acknowledgements

I am indebted to Dr P. Rády, Institute of Hygiene and Epidemiology, for valuable help.

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Received 26 June 1982

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