Acta Paediatrica Hungarica, 25 (3), pp. 255—261 (1984)

Maturation of the fetal lung III. Effect of transplacental TRH and 2'-thiourea treatment on phosphatidic acid phosphatase and pyruvate kinase activity in rat lung

Terezia KEREPESI, P RÁDY

Department of Paediatrics and Institute of Hygiene and Epidemiology, University Medical School, Debrecen, Hungary

The activity of pyruvate kinase (PK), an enzyme playing a key role in glycolysis, was studied in the lung of Wistar R/A rats in the prenatal, postnatal and adult periods of life. The highest level was measured on the 20th gestational day, a value nearly double the adult mean. In order to elicit fetal hypothyroidism, pregnant rats were treated with 0.1% thiourea solution from the 14th day of gestation up to delivery. The animals were killed on the 21st or 22nd gestational day or on the 2nd postnatal day. A significant increase in PK activity was seen in the treated animals as compared to the control group, while the activity of phosphatidic acid phosphatase (PAPase) remained unchanged. Fetal hyperthyroidism was induced by intravenous injections of TRH to the pregnant mother rat. This resulted in an increase of PAPase and a decrease of PK activity as compared to the control group. The difference was statistically significant in all instances, the level of significance, however, depended on the time when treatment had been initiated.

Glucose is an important fuel in the metabolism of the developing fetus [17]. Pyruvate kinase (PK, EC 2. 7. 1. 40) plays a key part in the breakdown of glucose. PK activity is an important measure of the metabolic state immediately before and after birth [5, 19]. We studied the spontaneous prenatal and postnatal changes in PK activity of the lung and investigated the effect of 2'-thiourea and TRH treatment of the pregnant animal on the activity of PAPase (EC 3.1.3.4) and PK in the lung of the offsping.

MATERIAL AND METHOD

The experiments were carried out in Wistar R/A rats (Institute for Laboratory Animal Breeding, Gödöllő, Hungary); they were fed standard rat chow and tap water ad libitum. These animals have a normal duration of pregnancy of 22 days. The day on which a vaginal sperm plug was first seen was regarded as the first day of gestation. Kertai et al. [9] applied an 0.2% solution of 2'-thiourea to CFY rats, but we have halved this dose in view of its teratogenic effect.

In the first series adult female rats were treated with 0.1% thiourea (Merck) for 6—20 days, and the relative thyroid weight in mg/100 g body weight was registered.

Neonatal hypothyroidism was induced by offering to the pregnant animals drinking water containing 0.1% 2'-thiourea from the 14th day of gestation. The control animals received tap water. The animals were killed on the 21st, 22nd gestational day or the 2nd postnatal day.

To induce neonatal hyperthyroidism,

the pregnant animals received 2 μg/100 g body weight of TRH (Berlin-Chemie) intravenously four times every twelve hours from the 18th, 19th or 21st days of gestation. The control group received four times 0.6 ml physiological saline intravenously. The animals treated from the 18th or 19th day were killed 12h after the last injection, while the animals treated from the 21st gestational day were sacrificed 36 h after the last TRH injection. Removal and work-up of the lungs, and estimation of PAPase and PK activity were performed as described previously [7, 8]. The results were analysed by Student's *t*-test. The Tables show the mean values and the standard deviations.

Results

In untreated animals, PK showed maximum activity on the 20th day of gestation (454 ±11 mU/mg protein), nearly double the normal adult value. A sharp decrease was seen until the second postnatal day, followed by a slight increase by the third day. Thereafter again a decrease was experienced, the values obtained on the eighth postnatal day were already equal to the normal adult values (Fig. 1). Figure 1 also demonstrates the spontaneous changes of PAPase [7].

Figure 2 shows the relative thyroid weights of adult female animals after drinking 0.1% thiourea for 6, 8, 10, 13, 15 and 20 days. 6 days thiourea treatment induced no changes in thyroid weight as compared to the untreated group, between 8 to 15 days the effect depended on the duration of treatment. No further increase could be achieved beyond the 15th day of treatment. Maternal treatment with 2'-thiourea resulted in a statistically significant ($p<0.001$) increase in PK activity measured in the lung of fetuses killed on the 21st or 22nd gestational day. The

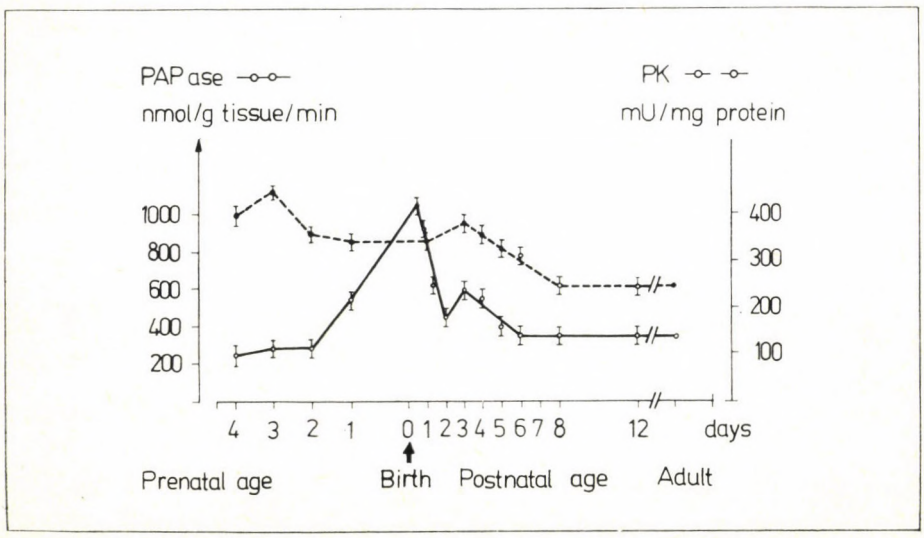

Fig. 1. Spontaneous changes in PAPase and PK activity of rat lungs

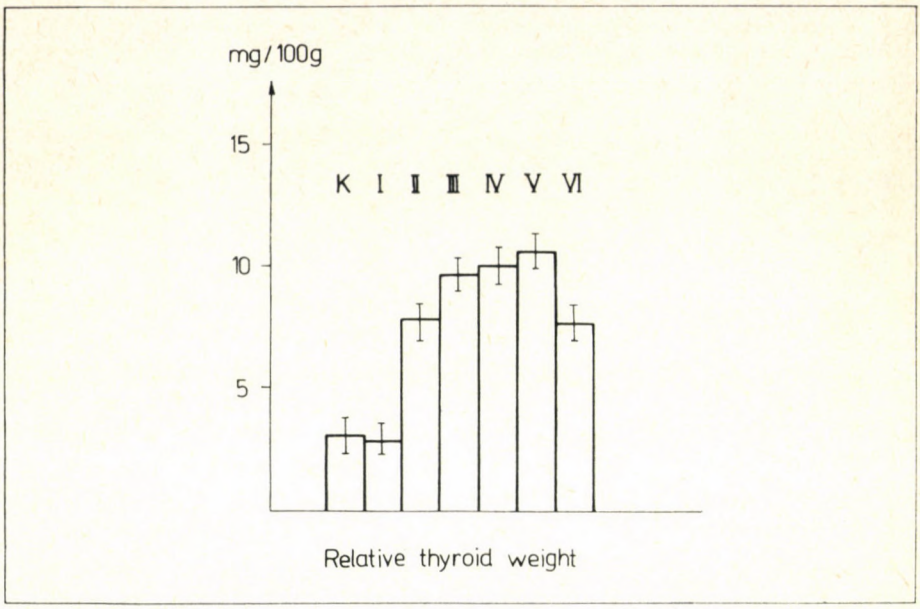

FIG. 2. The effect of 0.1% thiourea on thyroid weight in adult female rats. Each column represents four adult female Wistar RA rats. K: animals that drank tap water. I—VI: animals that drank 0.1% thiourea over 6, 8, 10, 13, 15 or 20 days

difference against the untreated rats was slightly smaller if the newborn animal was killed on the 2nd postnatal day ($p < 0.01$). No changes in PAPase activity were encountered (Table I).

In hyperthyreotic fetal rats sacrificed on the 20th day of gestation, whose mothers received TRH intravenously from the 18th gestational day, pulmonary PAPase showed a marked increase and PK a decrease

TABLE I

The transplacental effect of 2'-thiourea

Age, days[a]	PAPase nmol/g tissue/min		PK mU/mg protein	
	Control	Treated	Control	Treated
21st day of gestation	279±28[b]	342±45	386±36	660±25[c]
22nd day of gestation	560±22	475±44	349± 8	611±36[c]
2nd day after birth	463±26	440±27	354±12	426±34[d]

[a] 0.1% thiourea was administered from the 14th day of gestation, the animals were killed as indicated in the Table

[b] Each value represents the mean of five determinations on samples collected from three lungs each

[c] $p < 0.001$

[d] $p < 0.01$

TABLE II

The transplacental effect of TRH

Age, days[a]	PAPase nmol/g tissue/min		PK mU/mg protein	
	Control	Treated	Control	Treated
18th day of gestation	283±28[b]	578±33[c]	454±11	319±14[c]
19th day of gestation	279±28	535±18[d]	386±36	300±31[d]
2nd day after birth	463±26	547±36[d]	354±12	276±18

[a] TRH treatment was carried out as described under Material and Methods, the animals were killed and the measurements performed on the days indicated in the Table

[b] Each number represents the mean of five determinations on samples collected from three lungs each

[c] $p < 0.001$

[d] $p < 0.01$

as compared to the untreated group. In the untreated group PAPase showed an activity of 283 ±28 nmol/g tissue/min; this value was 578 ±33 in the treated group. For PK the corresponding values were 454 ±11 and 319 ±14 mU/mg protein, respectively. The difference was significant ($p < 0.001$) for both enzymes. A similar but slighter difference was seen if treatment was initiated on the 19th day ($p < 0.01$). A trend of the same direction was observed also in the group treated from the 21st day of gestation. Here PAPase increased from 463 ±25 nmol/g tissue/min to 547 ±36 ($p < 0.01$), and PK activity exhibited a marked decrease, from 354±12 mU/mg protein found in the untreated group to 276 ±18 in the treated group ($p < 0.001$).

DISCUSSION

Greengard et al. [4] studied the activity of phosphotransferases and lysosomal enzymes in fetal rat lungs and in pulmonary tissue obtained from human abortions. They found that the 20th gestational day was a critical point in fetal lung maturation in the rat. They observed a PK value similar to the adult level on the 19th gestational day. The highest activity of this enzyme was observed on the 20th day, and the lowest on the 2nd and 20th postnatal days; the difference between the two latter values was not significant statistically. Our findings were in accordance with most of these results, in that we, too, found the maximum of PK activity on the 20th gestational day, with the only difference that activity was as low as the adult value on the 8th postnatal day. Rády et al [13] studied the maturation of glycolytic enzymes in the murine lung, and observed in fetal, neonatal and adult mice trends similar to those obtained by ourselves in rats. Fluctuations in PK activity are probably due to changes in metabolism ocurring during, immediately before, and after, birth.

It is well known that lung maturation is influenced not only by gluco-

corticoids but also by thyroid hormones since type II pneumocytes contain T_3 receptors [10, 12] in addition to steroid receptors [2]. Kertai et al [9] investigated the effect of maternal thiourea treatment on the thyroid gland of the fetus and the newborn. They found an increase in thyroid weight and a decrease in protein bound iodine in the thyroid and the serum. In their opinion, thiourea or its dervatives occurring in the environment may damage the fetal or neonatal thyroid. Ruel et al. [16] studied the effect of thyroid hormones on the fetal rat lung. Hypothyroidism was induced by a 0.05% solution of 6-n-propylthiouracyl (PTU); they observed a 30—40% decrease in pulmonary phospholipid content but only between the 5th and 30th postnatal days. They concluded that the effect of hypothyroidism was temporary and reversible. The thyroid hormones have a direct effect, they enhance choline incorporation into the phospholipids in fetal lung cells or cell cultures, thus the low level of pulmonary phospholipids encountered in hypothyroidism is not the result of the slow metabolism or the deficient substrate supply [16].

Our experiments were conducted under similar conditions with the difference that we used 0.1% 2'-thiourea instead of PTU. We saw no change in the activity of PAPase, the key enzyme of surfactant synthesis. There was on the other hand an important difference in the method of evaluation of the drug effect: Ruel et al [16] measured the phospholipid content of the neonatal lung between the 5th and 30th postnatal days while in our experiments PAPase activity was determined on the 21st and 22nd gestational days and on the 2nd postnatal day.

A marked change induced by 2'-thiourea was seen in PK activity: it nearly doubled as compared to the controls. PK appeared to be a sensitive marker of the transplacental action of thiourea.

In general, thyroid hormones do not pass accross the placenta; small quantities of T_4, however, cross the barrier in the rabbit and the rat [1]. According to Rooney et al [15] only TRH crosses the rabbit's placenta, the thyroid hormones fail to do so. Kajihara et al [6] demonstrated colloid drop accumulation in the fetal thyroid if the pregnant rat received a single intravenous dose of TRH on the 20th day of gestation. In the present study, under the action of intravenous TRH, PK activity decreased significantly in all instances; this reflects TRH stimulation of the fetal pituitary-thyroid system. This in turn seems to lead to an increase in oxidative metabolism: suppressing thus the relative importance of PK which is an enzyme of anaerobic glycolysis.

There are conflicting data about the effect of thyroid hormones on the lung. According to Akino and Ohno [1], thyroxine had no effect on the enzymes participating in pulmonary phosphatidylcholine synthesis. The placenta is impermeable to thyroxine but if it is injected directly into the

amnionic cavity it accelerates development of the lamellar cells of the fetal lung [14,18]. Mason et al [11] and Rooney et al [15] have speculated that the primary action of T_4 was not directed to the enzymes of surfactant synthesis but only to their secretion; they have not, however, investigated the activity of PAPase. Experiments performed by Gonzales and Ballard [3] in lung cell cultures of fetal rabbits have shown that T_4 stimulates phosphatidylcholine synthesis and leads to glycogen depletion.

In our own studies intravenous TRH administered on the 18th and 19th gestational days caused as significant increase in PAPase activity ($p < 0.001$). The effect was similar but weaker if TRH treatment was initiated on the 19th or 21st day of gestation ($p < 0.01$). The increased phosphatidylcholine synthesis found in tissue cultures [3] may be related to the increased PAPase activity demonstrated by us.

Thus, our experiments have shown that PK and PAPase activity points to the importance of endocrine regulation of the maturation process of the fetal lung and is a good indicator of fetal lung maturity.

It seems noteworthy that we were unable to find any data on the transplacental effect of TRH and 2'-thiourea on pulmonary PK and PAPase activity.

Acknowledgement

We are deeply indebted to Professors L. Karmazsin and P. Kertai for valuable advice.

References

1. Akino T, Ohno K: Phospholipids of the lung in normal, toxic and diseased states. CRC Crit Rev Toxicol 9: 201, 1981
2. Ballard PL, Mason RJ, Douglas WHJ: Glucocorticoid binding by isolated lung cells. Endocrinology 102: 1570, 1978
3. Gonzales LW, Ballard PL: Nuclear 3,5,3'-triiodothyronine receptors in rabbit lung: characterisation and developmental changes. Endocrinology 111: 542, 1982
4. Greengard O, Cayanis E, Bodanszky H: Phosphotransferases and lysosomal enzymes in fetal human and rat lung. J Develop Physiol 2: 291, 1980
5. Imamura K, Tanaka T: Multimolecular forms of pyruvate kinase from rat and other mammalian tissues. J Biochem 71: 1043, 1972
6. Kajihara A, Kojima A, Onaya T, Takemura Y, Yamada T: Placental transport of thyrotropin releasing factor in the rat. Endocrinology 90: 592, 1972
7. Kerepesi T: Maturation of the fetal lung I. Phosphatidic acid phosphohydrolase (PAPase) in the lung of the rat fetus and neonate. Acta Paediatr Hung 24: 67, 1983
8. Kerepesi T, Rády P, Balla Gy, Dauda Gy: Maturation of the fetal lung. II. Effect of hyperoxia on phosphatidic acid phosphatase (PAPase), pyruvate kinase (PK), and superoxide dismutase (SOD) activity in the lungs of newborn rats. Acta Paediatr Hung 25, 1984
9. Kertai P, Remenár I: Effect of 2-thiourea administered to pregnant rats on the thyroid and the protein bound iodine content of the offspring. Acta Med Acad Sci Hung 32: 271, 1975
10. Lindenberg JA, Brehier A, Ballard PL: Triiodothyronine nuclear binding in fetal and adult rabbit lung and cultured lung cells. Endocrinology 103: 1725, 1978
11. Mason RJ, Manganiello V, Vaughan M: Effect of thyroxine on the disaturated lecithin content of lung. Am Rev Respir Dis 106: 767, 1972
12. Morishige WK, Guernsey DL: Triiodothyronine receptors in rat lung. Endocrinology 102: 1628, 1978
13. Rády P, Arany I, Uzvölgyi É, Boján F, Kertai P: Transplacental effects of carcinogens on activities of pyruvate kinase and lactate dehydrogenase as well as isozymic pattern of LDH in mouse lung. Toxicology 24: 251, 1982

14. Redding RA, Douglas WH, Stein M: Thyroid hormoné influence upon the lung surfactant metabolism. Science 175: 994, 1972
15. Rooney SA, Marino PA, Gobran LI, Gross I, Warshaw JB: Thyrotropin-releasing hormone increases the amount of surfactant in lung lavage from fetal rabbits. Pediatr Res 13: 623, 1979
16. Ruel J, Coulombe P, Dussault JH: Thyroid hormones, malnutrition, and biochemical composition of developing rat lung. Am J Physiol 242: (Endocrinol Metab 5) E378, 1982
17. Weber G: Enzymology of cancer cells. New Engl J Med 296: 541, 1977
18. Wu B, Kikkawa Y, Orzalesi MM, Motoyama EK, Kaibara M, Zigas CJ, Cook CD: The effect of thyroxine on the maturation of fetal rabbit lungs. Biol Neonate 22: 161, 1973
19. Yeager H, Massaro D: Glucose metabolism and glycoprotein synthesis by lung slices. J Appl Physiol 32: 477, 1972

Received June 11, 1983

T Kerepesi MD
P.O. Box 32
H-4012 Debrecen, Hungary