INFLUENCE OF THYROID HORMONE ON THE PHOSPHOLIPID COMPOSITION OF LUNG TISSUE AND SURFACTANT OF RATS

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Summary : Thyroid hormone is an important regulator of lipid metabolism *in vivo*. The effect of thyroid hormone on the phospholipid composition of lung tissue and surfactant has been studied in hypothyroid and hyperthyroid rats in comparison with the control rats. Rats were made hyperthyroid by administering 1 *mg* of L-thyroxine/*kg* body weight for six days. Another group of rats was rendered hypothyroid by injecting 1 mci of Na I^{131} to each rat. Phosphatidyl choline, lysophosphatidyl choline, lysophosphatidyl ethanolamine, phosphatidyl ethanolamine, and sphingomyelin, were estimated by thin layer chromatography. A decrease in phospholipids in hypothyroid and an increase in the hyperthyroid rats was observed. This can be attributed to the altered thyroid activity.

Key words : thyroid phospholipids lung tissue surfactant

INTRODUCTION

Lung performs active metabolic functions (9) in addition to its respiratory functions. One of its metabolic functions that has received special attention in recent years is the synthesis of fatty acids and phospholipids that are important constituents of the alveolar surfactant which is essential to maintain alveolar stability and normal fluid balance (3). The large alveolar cells in the alveolar epithelial lining are the active sites of the surfactant synthesis. The mitochondrial fraction of these cells contain the necessary enzymes for its synthesis and metabolism (11).

The thyroid gland is responsible for the optimal level of cellular metabolism, normal growth and maturation. Thyroid hormones stimulate the oxygen consumption and help in regulating lipid and carbohydrate metabolism. It is reported that in the rats, L-thyroxine may be a potent regulator of lung surfactant metabolism (10).

The present study was, therefore, undertaken to assess the phospholipid composition of lung tissue and surfactant in rats with altered thyroid activity.

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MATERIAL AND METHODS

Twenty eight adult male albino rats were used in this study. Rats were divided into three groups. Ten rats were made hyperthyroid by daily subcutaneous injections of 1 *mg* of L-thyroxine per kilogram of body weight for six days and on the seventh day, they were sacrificed. Ten rats were made hypothyroid by administration of 1 mci of Na I^{131} to each rat intraperitoneally. They were sacrificed after one month. Eight rats were used as controls for the comparison of hyperthyroid and hypothyroid rats. The rats were sacrificed by exsanguination via abdominal aorta after intrapertitoneal injection of sodium pentabarbitone (40 *mg/kg* body weight). Lungs were dissected out and lavaged with 25 *ml* isotonic saline. The infusion and withdrawal of fluid was done five times to ensure maximum extraction of surfactant. The lung lavage was centrifuged at 300 *g* for ten minutes to remove tissue elements. The supernatant was separated out and recentrifuged at 4°C at 2000 *g* for sixty minutes. The sediment fraction was separated, out. The supernatant was treated with an equal volume of chilled 25% TCA. The precipitate was centrifuged out and supernatant was discarded. Thus lavage fluid was separated into two surfactant fractions (*i*) white sediment fraction representing lipid rich component and (*ii*) TCA precipitate fraction containing lipoprotein following the method of Fujiwara (6). Lipids were extracted from these two fractions according to the method of Folch (5).

The lung tissues were homogenized in chloroform : methanol mixture (2:1 v/v) so as to give 10% w/v homogenate, final extract was obtained by the method of Folch (5).

Total lipids were analysed gravimetrically. Thin layer chromatography was done to fractionate different type of phospholipids on Silica Gel G using chloroform : methanol :7 M Ammonia 115:45:7.5 (v/v/v) as solvent (1). The phospholipid spots were visualised by exposing the plates to iodine vapours. Spots of individual phospholipids were marked and scrapped off from the plate for estimation of phosphorus by the method of Bartlett (2). The values of phosphorus were converted into phospholipid values by the multiplication factor 25.

RESULTS

The changes in body weights and lung weights before and after the experimental procedure in hyperthyroid, hypothyroid and of control rats are given in Table I. The body weight of hypothyroid rats increased significantly ($P<0.001$) while the body weights of hyperthyroid rats decreased significantly ($P<0.001$). There was no significant change in the lung weights of hypothyroid, hyperthyroid and control rats.

TABLE I : Body weights and lung weights.

Body weights (gms)				*Lung weights (gms)*		
Hypothyroid		*Hyperthyroid*		*Control*	*Hypothyroid*	*Hyperthyroid*
Before	*After*	*Before*	*After*			
200.0±1.7	250.4±3.5	239.4±4.3	214.3±4.2	1.208±0.016	1.257±0.032	1.257±0.031
Significance P<0.001		P<0.001			NS	NS

Values are mean ± S.E.M.

Lung tissue phospholipids :

Total lipids, phospholipids, phosphatidyl ethanolamine, phosphatidyl choline, lysophosphatidyl choline and sphingomyclin fractions of lung tissue in control, hypothyroid and hyprerthyroid rats are given in Table II. In hypothyroid rats there was significant decrease in total lipids (P<0.001), total phospholipids (P<0.001), phosphatidyl ethanolamine (P<0.05), phosphatidyl choline (P<0.001) and lysophosphatidyl choline (P<0.01), fractions. In hyperthyroid rats there was a significant increase in total phospholipids (P<0.05), phosphatidyl ethanolamine (P<0.001), phosphatidyl choline (P<0.01) and sphingomyelin+lysophosphatidyl ethanolamine (P<0.01) fractions.

Lung surfactant phospholipids :

As mentioned earlier the alveolar surfactant system lavaged from the lungs was divided into two fractions (i) sediment fraction and (ii) TCA fraction.

(i) *Sediment fraction.* In hypothyroid rats there was significant decrease in total phospholipids (P<0.05) and phosphatidyl ethanolamine (P<0.05). In hyperthyroid rats there was significant (P<0.01) increase in phosphatidyl ethanolamine fraction.

(ii) *TCA fraction.* In hypothyroid rats there was significant decrease in total phospholipids and phosphatidyl choline (P<0.001) and (P<0.001) respectively while no significant change was observed in hyperthyroid rats (Table III).

DISCUSSION

A progressive body weight gain was observed in hypothyroid rats. In hyperthyroid rats, there was a significant decrease in body weights. These are some of the known

TABLE II : Lung tissue phospholipids.

Parameter	*Control rats*	*Hypothyroid rats*	*Hyperthyroid rats*
TL *mg/gm* wet tissue	35.145±0.533	28.811±0.507***	36.762±0.755
TP " " "	21.734±0.333	15.884±0.442***	23.928±0.502**
PE " " "	6.212±0.221	5.326±0.210**	7.371±0.203***
PC " " "	9.348±0.214	7.422±0.254***	10.372±0.235*
Sph+LPE " "	0.794±0.058	0.820±0.031	0.958±0.041*
LPC " "	0.766±0.044	0.607±0.022*	0.800±0.061

Values are mean ± S.E.M.

TL = Total lipids
PE = Phosphatidyl ethanolamine
Sph = Sphingomyelin
LPC = Lysophosphatidyl choline
TP = Total phospholipids
PC = Phosphatidyl choline
LPE = Lysophosphatidyl ethanolamine

* = P<0.01
** = P<0.05
*** = P<0.001

TABLE III : Lung surfactant phospholipids.

	TCA fraction			*Sediment fraction*		
mg/gm wet tissue	*Control rats*	*Hypothyroid rats*	*Hyperthyroid rats*	*Control rats*	*Hypothyroid rats*	*Hyperthyroid rats*
TP wet tissue	1.044±0.062	0.055±0.030***	1.208±0.070	0.442±0.036	0.359±0.021**	0.469±0.017
PE " "	0.107±0.010	0.105±0.005	0.110±0.007	0.078±0.009	0.059±0.005**	0.105±0.004*
PC " "	0.491±0.032	0.257±0.011***	0.503±0.031	0.271±0.018	0.261±0.014	0.297±0.012
Sph+LPE "	0.078±0.009	0.059±0.006	0.085±0.005	--	--	--
LPC "	0.077±0.011	0.096±0.009	0.070±0.005	--	--	--

Values are Mean ± S.E.M.

TP = Total phospholipid
PC = Phosphatidyl choline
PE = Phosphatidyl ethanolamine
Sph = Sphingomyelin
LPE = Lysophosphatidyl ethanolamine
LPC = Lysophosphatidyl choline

* = P<0.01
** P<0.05
*** = P<0.001

effects of hypothyroidism and hyperthyroidism. Similar observations have been made by Redding (10).

In hypothyroid rat lung tissue there was a significant decrease in total lipids, total phospholipids, phosphatidyl choline, lysophosphatidyl choline and phosphatidyl ethanolamine. In TCA fraction of surfactant there was reduction in total phospholipids and phosphatidyl choline. In the same way sediment fraction also exhibited a decrease in total phospholipids and phosphatidyl ethanolamine. The decrease of phospholipid fractions in lung tissue and surfactant may be due to the general effects of hypothyroidism which results in reduced metabolic rate. These changes appear as a part of physiological adjustment to the reduced O_2 consumption of the tissue due to diminished metabolic rate (4). It is reported that hypothyroidism results in arterial hypoxemia due to a low arterial PO_2(8). Hypoxic condition has been shown to slow the rate of metabolism due to decreased levels of ATP (12,14). The observed decrease in phospholipids, may be due to inhibition of phospholipid synthesis which is oxygen dependent, in the type II cells of the lung.

In the hyperthyroid rats, the lung tissue was found to have a increase in total phospholipids, phosphatidyl ethanolamine, phosphatidyl choline and sphingomyelin+lysophosphatidyl ethanolamine fractions. In the TCA fraction of the surfactant there was no significant change in any phospholipid fraction. In sediment fraction a significant increase in phosphatidyl ethanolamine fraction was observed.

The observed increase in tissue phospholipid in hyperthyroid state may be due to rise in O_2 consumption (13) with concomittant rise in ATP levels. It is also reported that thyroxine treatment results in increased activity of malic enzyme, Glucose-6-phosphate dehydrogenase, citrate cleavage enzymes (16), NADP-linked hexose mono phosphate (HMP) shunt enzymes and L-α-glycerophosphate dehydrogenase which makes available more glycerol-3-P and NADPH for phospholipid biosynthesis (15). In de novo synthesis of lung phospholipids malonyl-Co A is an intermediate compound and these units are successively condensed on an acetyl-Co A nidus until a 16 or 18 carbon saturated fatty acid is formed (7). These condensations are accompanied by additions of hydrogen and the reduced pyridine nucleotide, NADPH is the specific source for this reduction. So the increase in malic enzymes and HMP-shunt activity gives rise to increased consentration of NADPH and ATP, which is a prerequisite of phospholipid synthesis.

The surfactant of hyperthyroid rats did not reveal any significant change except an increase in the phosphatidyl ethanolamine in sediment fraction. It indicates that despite the increased phospholipid biosynthesis in lung tissue, the incorporation of this fraction in lung surfactant is not altered. The concomittant decrease in the phospholipid content in lung tissue and lavage in hypothyroid rats is probably brought about by

reduced synthesis and release. The increase observed in the phospholipid content of the lung tissue in hyperthyroid state is likely to be due to an accelerated synthesis of lipids.

It is also likely that the catabolism of the lung phospholipids may also be different in the two experimental groups of rats. But the present study has not been primarily focussed on this aspect and this requires further studies with labelled fatty acid derivatives.

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REFERENCES

1. Abramson, D. and M. Blescher. Quantitative two dimensional thin layer chromatography of naturally occurring phospholipids. *J. Lipid. Res.*, **5** : 628–631, 1964.
2. Bartlett, G.R. Phosphorus assay in column chromatography. *J. Biol. Chem.*, **334** : 466–468, 1959.
3. Clements, J.A., R.F. Hustead, R.P. Johnson and J. Gribetz. Pulmonary surface tension and alveolar stability. *J. Appl. Physiol.*, **16** : 444–450, 1961.
4. Das, K.C. Erythropoiesis and erythropoietin in hypo and hyperthyroidism. *Proc. Vth Asia Congress of Endocrinology, India.* pp. 78, 1974.
5. Folch, J., M. Lees and G.H. Sloane-Stanley. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226** : 497–509, 1957.
6. Fujiwara, T., F.H. Adams, M. Nozaki and G.B. Dermer. Pulmonary surfactant phospholipids from turkey lung: comparison with rabbit lung. *Am. J. Physiol.*, **218** : 218–225, 1970.
7. Haflen, W.R. Jr and S.I. Said. Selected aspects of lung metabolism. The Biological Basis of Medicine, *Academic Press, London*, **6** : 369–370, 1969.
8. Koenig, M.P. and M. Scherrer. Respiratory functional abnormalities in hypothyroidism. In thyroid Research (ed. by Robbins J. and L.E. Braverman) Excerpta Medica, Amsterdam Oxford, American Elsevier Publishing Co. Inc., N.Y. pp. 527–531, 1976.
9. Nasr, K. and H.O. Heinemann. Lipid synthesis by rabbit lung tissue *in vitro*. *Am. J. Physiol.*, **208** : 118–121, 1965.
10. Rooring, R.A., W.H.J. Douglas and M. Stein. Thyroid hormone influence upon lung surfactant metabolism. *Science*, **175** : 994–996, 1972.
11. Said, S.I., R. M. Klein, L.W. Norrell and Y.T. Maddox. Metabolism of alveolar cells : Histochemical evidence and relation to pulmonary surfactant. *Science*, **152** : 657–659, 1966.
12. Sanders, A.P., D.M. Hale and A.T. Miller. Some effects of hypoxia on respiratory metabolism and protein synthesis in rat tissue. *Am. J. Physiol.*, **208** : 443–446, 1965.
13. Swanson, H.E. Inter relationship between thyroxine and adrenaline in regulation of oxygen consumption in the albino rats. *Endocrinology*, **59** : 217–219, 1956.
14. Upsenskii, V.I. and S. Chou. Some aspects of fat metabolism in hypoxia. Patal fiziol eskperim terapiya. **7** : 60, 1963 *(Chem. Abstr.)* **60** : 7245–h, 1964.
15. Ya Pin Lee and Henry A. Lardy. Influence of thyroid hormones on L-a-glycerophosphate dehydrogenases and other dehydrogenases in various organs of the rat. *J. Biol. Chem.*, **24** : 1427–1436, 1965.
16. Young, J.M. Effect of D and L-thyroxine on enzymes in liver and adipose tissues of rats. *Am. J. Physiol.*, **214** : 378–383, 1968.