Pediatr. Res. *16:* 570–572 (1982)

Pulmonary Superoxide Dismutase Activity in the Euthyroid and Hypothyroid Ovine Fetus

ALLEN ERENBERG,[29] LEE FRANK, ROBERT J. ROBERTS, AND MITCHELL L. RHODES

Departments of Pediatrics and Pharmacology, University of Iowa, Iowa City, Iowa and Department of Internal Medicine, University of Indiana, Indianapolis, Indiana, USA

Summary

Pulmonary superoxide dismutase (SOD) activity was measured in 11 euthyroid and 15 hypothyroid ovine fetuses at 130 days gestation. In the euthyroid fetus, the mean pulmonary SOD activity was similar in saline- and thyroxine-infused groups. Compared to the euthyroid fetus, the mean pulmonary SOD activity was significantly lower in the noninfused or saline-infused athyrotic fetus. Administration of thyroxine to the hypothyroid fetus results in a normalization of pulmonary SOD activity to control values. Thus, thyroxine appears to influence the maturation of pulmonary SOD activity in the ovine fetus during the third trimester.

Speculation

The influence of thyroxine on pulmonary SOD activity was studied in the euthyroid and hypothyroid sheep fetus during the last trimester of gestation.

Superoxide dismutase (SOD), an enzyme which catalyzes the reaction: $2\ O_2 + 2\ H^+ \rightarrow H_2O_2 + O_2$, is found in all cells that metabolize oxygen (9, 14). Despite numerous studies investigating the role of SOD activity in oxygen-induced lung injury, there is little information regarding the mechanisms responsible for endogenous regulation of pulmonary SOD activity (14). It is known that pulmonary SOD activity is lowest in the fetus, with an increase in activity seen in the neonatal and adult lung (2, 23, 26). In contrast to the adult lung, the neonatal lung exposed to a hyperoxic environment will increase pulmonary SOD activity, a response mediated at least in part by a factor(s) in plasma or serum and exposure to oxygen (2, 13, 21, 24).

It has been shown previously that pulmonary oxygen toxicity can be influenced by thyroid function. In the adult, pulmonary oxygen toxicity is enhanced by hyperthyroidism and delayed by hypothyroidism (6, 20, 25). Iodothyronines have also been shown to influence the maturation of the fetal lung (12, 17, 22). If thyroid hormones are used to pharmacologically accelerate fetal lung maturation, the fetus would be, in theory, at greater risk for the development of pulmonary oxygen toxicity. The purpose of this study was to investigate the relationship of iodothyronines and pulmonary SOD activity in the preterm ovine fetus.

MATERIALS AND METHODS

Animal model. Date bred ewes were obtained from a local source, maintained at the University of Iowa vivarium and given free access to alfalfa and water. In fifteen singleton ewes, a surgical thyroidectomy was performed on the fetus at 95–99 days gestation. Term is 147 to 150 days. In nine of the hypothyroid fetuses, catheters were inserted into a fetal artery and vein at 122–124 days gestation. After a 3–5 day stabilization period, five fetuses received a 50 μg bolus intravenous injection of thyroxine (T_4) followed by a continuous intravenous infusion of 100 μg/day of T_4 for 72 h before sacrifice. Four fetuses received a constant infusion of an equivalent volume of saline. These nine fetuses and six noninfused fetuses were sacrificed at 130 days gestation.

Eleven euthyroid ovine fetuses had catheters inserted into a fetal artery and vein at 122–124 days gestation. After a three–five day stabilization period, five fetuses received a 50 μg bolus intravenous injection of T_4 followed by a continuous intravenous infusion of 100 μg/day of T_4 for 72 h before sacrifice. Six fetuses received an equivalent volume of saline by constant infusion. The eleven fetuses were sacrificed at 130 days gestation.

Assays and biochemical studies. Serum samples were taken before and at the end of the study period. In the noninfused fetuses, serum samples were obtained at the time of sacrifice only. At sacrifice, a portion of the lower lobe of the right lung was taken for determination of SOD activity. The lung was perfused to remove cellular elements contained in the pulmonary vascular bed. The lung was homogenized in a 10-fold dilution (w/v) of ice cold isotonic buffer for 30 sec using a Polytron (Brinkman Instruments, Des Plaines, IL) at a power setting of eight. Duplicate aliquots of this homogenate were taken for DNA analysis. The remaining homogenate was centrifuged in a refrigerated Sorvall centrifuge (Iran Sorvall, Norwalk, CN) at 15,000 X *g* for 10 min and the supernatant analyzed for SOD activity.

Serum T_4 concentrations were measured, in duplicate, in 0.025 ml unextracted samples by radioimmunoassay (5). Serum cortisol levels were measured in triplicate on 0.1 ml samples by ligand double antibody radioimmunoassay (reagents obtained from Diagnostics Products Corporation, Los Angeles, CA). Lung protein concentrations were measured by the method of Schacterle and Pollock (19) and lung DNA concentrations were measured by the method of Richards (18). Pulmonary SOD activity was determined by standard assay, which utilizes the inhibition of xanthine and xanthine oxidase-catalyzed reduction of ferricytochrome *c*. Units of SOD activity were derived according to the criteria established by McCord and Fridovich (16). Control studies of boiled supernatant confirmed the enzymatic nature of the SOD activity.

Statistical analysis of the data was accomplished by the Student *t* test.

RESULTS

The mean ± S.E. serum T_4 and cortisol concentrations in the ovine fetuses are shown in Table 1. In the euthyroid fetus, the mean serum T_4 concentration before study was similar in both the saline and T_4-infused groups. At sacrifice, the mean serum T_4 concentration in the T_4-infused fetus had increased to 16.8 ± 1.1 μg/dl, ($P < 0.001$) but did not change significantly in the saline-infused animal. In all athyrotic fetuses, the serum T_4 concentration was below the lower limits of the assay. At the end of the infusion period, the mean fetal serum T_4 concentration had increased to 7.2 ± 1.2 μg/dl in the T_4-infused hypothyroid fetus, a value similar to that found in the saline-infused euthyroid fetus. The mean serum cortisol concentration was less than 2 μg/dl in all fetuses in all groups.

Table 2 shows the mean protein and DNA concentrations, protein/DNA ratio and SOD activity in lung tissue from the euthyroid fetuses. The mean pulmonary SOD activity was similar in both groups of euthyroid fetuses, whether expressed as units per g wet weight of lung or SOD/DNA ratio. Similar data from the hypothyroid fetuses are shown in Table 3. Compared to the noninfused and saline-infused hypothyroid fetuses, the T_4-infused fetus had a significantly lower mean DNA content ($P < 0.005$) and significantly higher mean protein/DNA ratio ($P < 0.05$), similar to values found in the euthyroid fetus. Compared to the euthyroid fetus, the mean pulmonary SOD activity was significantly lower in the hypothyroid noninfused ($P < 0.05$) and saline-infused fetuses ($P < 0.05$). In the T_4-infused athyrotic fetus, the mean pulmonary SOD activity values were similar to those in the euthyroid fetus.

DISCUSSION

In the ovine fetus, fetal thyroid function begins at the start of the second trimester (4). The ovine placenta is relatively impermeable to both T_4 and triiodothyronine (T_3), whether the fetus is euthyroid or athyrotic (10, 11). Thus, the fetal hypothalamic-pituitary-thyroid axis function is autonomous. On the basis of studies in the athyrotic fetus (Table 1), minimal amounts of iodothyronines cross the placenta from the maternal circulation.

The results of this study demonstrate that in the preterm ovine fetus, hypothyroidism is associated with a reduced pulmonary SOD activity, whether expressed on a per gram wet weight of lung or SOD/DNA basis. Administration of T_4 to the athyrotic fetus results in a normalization of pulmonary SOD activity to control values. However, exogenous T_4 given to the euthyroid fetus did not influence pulmonary SOD activity.

Several studies have demonstrated the susceptibility of pulmonary SOD activity to pharmacologic manipulation (6, 25). The normal endogenous regulating mechanisms for pulmonary SOD activity, however, are poorly understood, particularly the mechanisms responsible for the changes observed in pulmonary SOD activity with maturation (23). The relationship between thyroid hormones and the development of fetal pulmonary SOD activity demonstrated by our studies obviously may have significance with respect to endogenous regulatory mechanisms for pulmonary SOD activity. Whether this effect of T_4 is direct or indirect resulting from fetal lung maturation cannot be determined from the results. A secondary effect appears consistent with the fact that exogenous T_4 did not increase pulmonary SOD activity in the euthyroid fetus.

Cortisol has been shown to influence the maturation of the lung and its susceptibility to oxygen toxicity (3, 6). Because the fetal serum cortisol levels were similar in all groups, the results demonstrated in this study were most likely not influenced by cortisol.

If allowed to deliver spontaneously at term, the athyrotic ovine lamb will develop the respiratory distress syndrome (RDS), have delayed synthesis and produce an abnormal type of pulmonary surfactant (8). In the human preterm infant with RDS, postnatal serum concentrations of T_4 and T_3 are lower than in the preterm infant without RDS. There is an increase in the serum levels of both iodothyronines concomitant with improvement of the infants' clinical condition (1, 7, 15). *In vitro* studies have shown that serum from infants with RDS will not support an increase in pulmonary SOD activity (13). Normal serum levels of iodothyronines may be

Table 1. *Mean serum thyroxine and cortisol concentrations in 130 day ovine fetuses*

	Before study		At sacrifice	
	Thyroxine (μg/dl)	Cortisol (μg/dl)	Thyroxine (μg/dl)	Cortisol (μg/dl)
Euthyroid fetus				
Saline infusion (6)[1]	8.4 ± 1.3[2]	<2	8.1 ± 1.1	<2
Thyroxine infusion (5)	10.7 ± 0.7	<2	16.8 ± 1.1	<2
Hypothyroid fetus				
Noninfused (6)			<1	<2
Saline infusion (4)	<1	<2	<1	<2
Thyroxine infusion (5)	<1	<2	7.2 ± 1.2	<2

[1] ( ) = number.
[2] Value = mean ± S.E.

Table 2. *Mean protein and DNA concentrtions, protein/DNA ratio and superoxide dismutase (SOD) activity in lung tissue of 130 day saline and thyroxine-infused euthyroid ovine fetuses*

	Protein (mg/g wet wt)	DNA (mg/g wet wt)	Protein/DNA	SOD (units/g wet wt)	SOD/DNA (units/mg)
Saline infusion (6)[1]	64.6 ± 4.7[2]	7.0 ± 1.1	10.3 ± 1.6	359 ± 24	60.7 ± 14.4
Thyroxine infusion (5)	64.3 ± 7.0	7.6 ± 1.2	10.1 ± 1.7	362 ± 33	61.0 ± 20.1

[1] ( ) = number.
[2] Value = mean ± S.E.

Table 3. *Mean protein and DNA concentrations, protein/DNA ratio and superoxide dismultase (SOD) activity in lung tissue of 130 day saline, thyroxine and noninfused hypothyroid ovine fetuses*

	Protein (mg/g wet wt)	DNA (mg/g wet wt)	Protein/DNA	SOD (units/g wet wt)	SOD/DNA (units/mg)
Noninfused (6)l[1]	76.4 ± 2.4[2]	10.0 ± 0.5	7.7 ± 0.4	306 ± 14	30.7 ± 3.2
Saline infused (4)	80.2 ± 1.3	9.6 ± 0.3	8.4 ± 0.2	296 ± 14	31.0 ± 1.8
Thyroxine infused (5)	63.7 ± 2.6	7.3 ± 0.5	10.7 ± 1.4	394 ± 23	66.4 ± 9.5

[1] ( ) = number.
[2] Value = mean ± S.E.

required for normal maturation of pulmonary SOD activity, and/or for the postnatal pulmonary SOD response seen when the neonatal lung is exposed to a hyperoxic environment. Based on the results of this study, we speculate that progressive or chronic lung disease observed in some infants who present initially with RDS may be related to the interrelationship of lower serum iodothyronine levels and lower or nonresponsive pulmonary SOD activity during the course of exposure to elevated concentrations of oxygen. Further studies are needed to delineate the role of iodothyronines on the maturation of the antioxidant enzyme systems and their response to hyperoxic exposure in both the fetal and neonatal lung.

REFERENCES AND NOTES

1. Abbassi, V., Merchant, K., and Abramson, D.: Postnatal triiodothyronine concentrations in healthy preterm infants and in infants with respiratory distress syndrome. Pediatr. Res., *11:* 802 (1977).
2. Autor, A. P., Frank, L., and Roberts, R. J.: Developmental characteristics of pulmonary superoxide dismutase: relationship to idiopathic respiratory distress syndrome. Pediatr. Res., *10:* 154 (1976).
3. Avery, M. E.: Pharmacologic approaches to the acceleration of fetal lung maturation. Brit. Med. Bull., *31:* 13 (1975).
4. Barnes, C. M., Warner, D. E., Marks, S., and Busted, L. K.: Thyroid function in fetal sheep. Endocrinology, *60:* 325 (1957).
5. Chopra, I. J.: A radioimmunoassay for measurement of thyroxine in unextracted serum. J. Clin. Endocrinol. Metab., *34:* 938 (1972).
6. Clark, J. M. and Lambertsen, C. J.: Pulmonary oxygen toxicity: a review. Pharmacol. Rev., *23:* 37 (1971).
7. Cuestas, R. A. and Engel, R. R.: Thyroid function in preterm infants with respiratory distress syndrome. J. Pediatr., *94:* 643 (1979).
8. Cunningham, M. D., Hollingworth, D. R., and Belin, R. P.: Impaired surfactant production in cretin lambs. Obstet. Gynecol., *55:* 439 (1980).
9. Deneke, S. M. and Fanburg, B. L.: Normobaric oxygen toxicity of the lung. N. Eng. J. Med., *303:* 76 (1980).
10. Dussault, J. H., Hobel, C. J., and Fisher, D. A.: Maternal and fetal thyroxine secretion during pregnancy in the sheep. Endocrinology, *88:* 47 (1971).
11. Erenberg, A., Omori, K., Oh, W., and Fisher, D. A.: The effect of fetal thyroidectomy on thyroid hormone metabolism in maternal and fetal sheep. Pediatr. Res., *7:* 870 (1973).
12. Erenberg, A., Rhodes, M. L., Weinstein, M. M., and Kennedy, R. L.: The effect of fetal thyroidectomy on ovine fetal lung maturation. Pediatr. Res., *13:* 230 (1979).
13. Frank, L., Autor, A. P., and Roberts, R. J.: Oxygen therapy and hyaline membrane disease: the effect of hyperoxia on pulmonary superoxide dismutase activity and the mediating role of plasma or serum. J. Pediatr., *90:* 105 (1977).
14. Frank, L. and Massaro, D.: Oxygen toxicity. Am. J. Med., *69:* 117 (1980).
15. Klein, A. H., Stinson, D., Foley, B., Larson, P. R., and Foley, T. P.: Thyroid function studies in preterm infants recovering from the respiratory distress syndrome. J. Pediatr., *91:* 261 (1977).
16. McCord, J. M. and Fridovich, I.: Superoxide dismutase: an enzymic function for erythrocuprien (hemocuprien). J. Biol. Chem., *244:* 6049 (1969).
17. Redding, R. A., Douglas, W. H. J., and Stein, M.: Thyroid hormone influence upon lung surfactant metabolism. Science, *175:* 994 (1972).
18. Richards, G. M.: Modifications of the diphenylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. Anal. Biochem., *57:* 369 (1974).
19. Schacterle, G. R. and Pollack, R. L.: A simplified method for the quantitative assay of small amounts of protein in biologic material. Anal. Biochem., *51:* 654 (1973).
20. Smith, C. W., Bean, J. W., and Bauer, R.: Thyroid influence in reactions to oxygen at atmospheric pressure. Am. J. Physiol., *199:* 883 (1960).
21. Stevens, J. B. and Autor, A. P.: Induction of superoxide dismutase by oxygen in neonatal rat lung. J. Biol. Chem., *252:* 3509 (1980).
22. Wu, B., Kikkawa, Y., Orzalesi, M. M., Motoyama, E. K., Kaibara, M., Zigas, C. J., and Cook, C. D.: The effect of thyroxine on the maturation of fetal rabbit lungs. Biol. Neonate., *22:* 161 (1973).
23. Yam, J., Frank, L., and Roberts, R. J.: Age-related development of pulmonary oxidant enzymes in the rat. Proc. Soc. Exp. Biol. Med., *157:* 293 (1978).
24. Yam, J. and Roberts, R. J.: Oxygen-induced lung injury in the newborn piglet. Early Human Development, *4:* 411 (1980).
25. Yam, J. and Roberts, R. J.: Pharmacological alteration of oxygen-induced lung toxicity. Toxicol. Appl. Pharmacol., *47:* 367 (1979).
26. Yoshioka, T., Shimada, T., and Sekiba, K.: Lipid peroxidation and antioxidants in the rat lung during development. Biol. Neonate., *38:* 161 (1980).

27. Dr. Erenberg is a recipient of a Research Career Development Award No. 1 KO4 HD00151 from the National Institute of Child Health and Human Development.
28. The authors wish to thank Barbara Kasper and Mary Jo Kline for technical assistance and Kathy Folkmann for preparation of the manuscript.

29. Requests for reprints should be addressed to: Dr. Allen Erenberg, Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IO 52242.

30. This research was supported in part by U.S. Public Health Grant No. 5 RO1 HD09677 from the National Institute of Child Health and Human Development.
31. Received for publication August 4, 1981.
32. Accepted for publication December 3, 1981.

0031-3998/82/1607-0570$02.00/0

Printed in U.S.A.