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THYROID DEPENDENT MATURATION OF β-ADRENERGIC RECEPTORS IN THE RAT LUNG

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SUMMARY β-Adrenergic receptors were identified in membranes of fetal and postnatal rat lung with (-)-[^{3}H]dihydroalprenolol, [^{3}H]DHA. β-Receptor number (Bmax) increased 11-fold from day 18 of gestation to adult levels by day 28 of postnatal life. The increase of β-adrenergic receptors occurring between postnatal days 15 and 28 was dependent on thyroxine (T4) in propylthiouracil treated pups. β-Adrenergic receptors on day 28 were identical in euthyroid (PTU + T4) as compared to normal control pups (489±31 and 491±30 femtomoles·mg^{-1}) however receptors were markedly reduced in 28 day hypothyroid pups (PTU alone), Bmax = 294±21.5, m±S.E. $p<0.01$. Treatment of the hypothyroid pups with T4 for three days on postnatal day 25 increased β-adrenergic receptors approximately two-fold by day 28. This thyroid hormone dependent increase in lung β-adrenergic receptors occurs between postnatal days 15 and 28 coincident with the known increase in thyroid gland activity in the rat pup.

INTRODUCTION β-Adrenergic agonists are mediators of surfactant release and smooth muscle tone in the lung. These effects are presumably mediated by β-adrenergic receptors present on the plasma membrane of various pulmonary cell types which stimulate adenylate cyclase. Increase in lung c-AMP *in vivo* and *in vitro* by the addition of dibutyryl c-AMP, phosphodiesterase inhibitors or β-adrenergic agonists results in smooth muscle relaxation, increased phospholipid release, and the prevention of respiratory distress in the prematurely delivered rabbit fetus (1-4). These latter effects are presumably mediated by the action of catecholamines on alveolar type II pneumocytes (4). In addition, several studies have demonstrated a role of thyroid hormones in lung metabolism. Thyroxine enhances phospholipid synthesis and the morphologic maturation of type II pneumocytes in the rabbit and a relationship exists

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between low serum thyroxine concentration and hyaline membrane disease in the human neonate (5,6). Considerable evidence supports the role of catecholamines in the mediation of pulmonary function; however the development of β-adrenergic receptors, their β-adrenergic subtypes and their possible regulation by thyroid hormone have not been previously characterized in developing mammalian lung.

METHODS: Animals Timed-bred Wistar rats were purchased from Charles River Corp., Mass., and treated as follows: Group I - hypothyroid rats were treated by adding 0.05% propylthiouracil (PTU) to the dam's drinking water from day 18 of gestation until sacrifice. Group II - euthyroid rats were maintained on 0.05% PTU and injected daily with thyroxine (T4) as described by Hamburgh (7). Group III controls were untreated rat pups. Pups from all groups were nursed in litters of less than 10 pups and were sacrificed by cervical dislocation. The lungs were removed, diced and pooled from each litter of fetal rat in iced buffer containing 250 mM sucrose, 1 mM EGTA, and 5 mM Tris-HCl (pH 7.2). All postnatal samples were obtained from individual animals. The washed tissue was homogenized with a Tekmar Tissuemizer (Tekmar Co., Cincinnati, O.) in 20 volumes of this sucrose buffer per tissue weight at 4° with three 5-second bursts at high setting. The homogenate was poured through four layers of gauze and centrifuged at 3000 x g for 5 min. The supernatant was centrifuged at 40,000 x g for 20 min, and this pellet was resuspended by gentle homogenization in iced buffer and centrifuged again at 40,000 x g for 20 min. This washed pellet was resuspended in the same sucrose buffer to a final membrane protein concentration of 2 mg/ml, by the method of Lowry with bovine serum albumin as standard. Protein, DNA and [^{3}H]DHA binding were also determined in the crude lung homogenate.

Binding Studies: Binding studies were performed in triplicate assays by filtration as previously described (8) using 40-60 μgm membrane protein in buffer containing 10 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.4) and [^{3}H]DHA, 56 Ci/mmole (New England Nuclear). Assays were terminated by rapid filtration through Whatman glass fiber filters which were washed and counted by standard liquid scintillation technique at 25% efficiency. Nonspecific binding was determined in triplicate assay containing 1 μM (-)-alprenolol and was subtracted from total binding to determine specific binding. Equilibrium data were obtained at five or six [^{3}H]DHA concentrations and the K_D and Bmax were determined by the method of Scatchard. The linear regression correlation coefficient (r) was in general greater than 0.90 for individual Scatchard plots in these studies. Deoxyribonucleic acid (DNA) was determined by the diphenylamine reaction with calf thymus DNA as standard.

RESULTS (-)-[^{3}H]Dihydroalprenolol bound to rat lung membrane with the characteristics of a β-adrenergic receptor. Specific binding was saturable to a single class of sites, Bmax = 490±10 fmole·mg^{-1}, K_D = 0.74±0.17 nM, mean ± SE, n = 4 in the adult. Binding was stereoselective for (-)-isoproterenol and not altered by 10 μM phentolamine. Binding was complete at the lowest [^{3}H]-DHA concentration before 20 min. at 30° and was linearly related to membrane protein (40-400 μgms). In membranes from untreated control pups

[^{3}H]DHA binding (Bmax) increased from 46±7 femtomoles per mg protein on day 18 of gestation to 491±30 m±SE n=5 on postnatal day 28. Receptor affinity did not vary significantly with advancing age. The properties of the β-adrenergic receptor were similar in the adult and fetal rat lung and were characteristic of a β_2-adrenergic receptor subtype by classical agonist inhibition of β-adrenergic receptor binding. Agonists competed for the receptor site in fetal rat lung in the order of potency (-)- isoproterenol > (-)-epinephrine > (-)-norepinephrine.

EFFECTS OF HYPOTHYROIDISM Rat pups nursed by dams on propylthiouracil (Group I) manifested the characteristics of congenital hypothyroidism. Somatic growth was nearly normal until after day 15 of age but by day 28 hypothyroid pups were significantly smaller than euthyroid (PTU + T4 treated) or control pups. Lung weight, protein and DNA content were significantly reduced in the hypothyroid pups. Serum T4 was below limits of detection (<1.0 μg/dl) in Group I. β-Adrenergic receptors in both the crude homogenate and washed lung membrane were similar in Groups II and III controls on postnatal days 5, 15 and 28 but were significantly reduced in Group I - hypothyroid pups at 28 days of age, $p < 0.01$ (Figures 1a, 1b and Table 1). Pulmonary β-adrenergic receptors in hypothyroid pups were not altered on postnatal day 5 and were only slightly decreased on day 15 as compared to controls (Table 1). While lung weight, protein, and DNA content were decreased in hypothyroid pups on day 28, the protein to DNA ratio was not altered by thyroid status at any age. β-Adrenergic receptors in crude lung homogenates and washed membrane preparations were increased nearly two-fold (to control levels) on day 28 in hypothyroid rats treated with 5.0μgm T4 on days 25-27, Bmax in the crude lung homogenate was (122±8 fmoles per mg, m±S.E., n=4) as compared to saline treated hypothyroid pups (70±12, n=4) $p<0.01$. Thyroxine treatment of these hypothyroid pups was associated with only a small increase (19%) in total lung weight and DNA content. Neither the affinity for β-adrenergic antagonists nor the β_2-adrenergic subtype was dependent on age or thyroid status. Lastly,

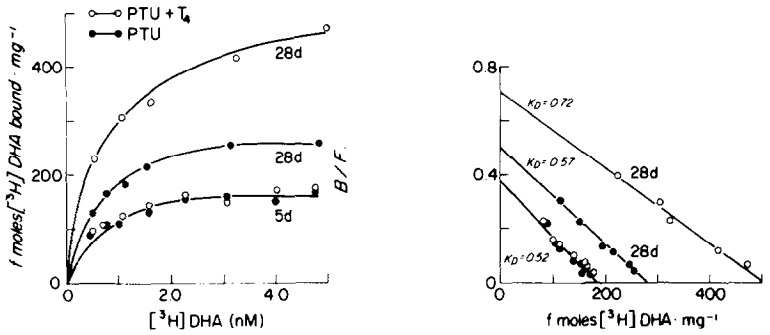

Figure 1a Specific [^{3}H]DHA binding in rat lung membranes in PTU treated hypothyroid ●—● and euthyroid ○—○ , (T4 and PTU treated) rat pups at 5 and 28 days of postnatal age. Experiments are representative of 4-8 preparations at each age in each group as listed in Table 1. Bmax from lungs of animals in Groups II and III increased with age and were identical at days 5, 15 and 28, Table 1.

Figure 1b Scatchard analysis of the saturation data from Figure 1a. K_D did not vary with age or thyroid status. Correlation coefficients, r, were in general greater than 0.90 in all of these experiments. Units of the y axis are ml per mg.

treatment of mature (220g) female rats with PTU for six weeks did not alter β-adrenergic receptor binding capacity or affinity, Bmax = 478±64.5, m±S.E. n=4, K_D=0.72.

DISCUSSION The present study demonstrates the effects of hypothyroidism and replacement thyroxine on lung growth and on the ontogeny of β-adrenergic receptors in rat lung. Thyroid hormone is required for the normal postnatal

[^{3}H]DIHYDROALPRENOLOL BINDING (BMAX)

Group		5 Days		15 Days		28 Days	
		femtomoles per mg protein					
I	PTU	199±15	n = 4	243±25*	n = 4	294±22**	n = 8
II	PTU + T4	188±4.8	n = 4	295±18	n = 4	489±31	n = 8
III	Control	193±7.0	n = 4	304±27	n = 4	491±30	n = 5

Table 1 [^{3}H]DHA binding, Bmax, in rat lung membranes on postnatal days 5, 15, and 28. Values are m ± S.E., *$p < 0.05$, **$p < 0.01$, Group I compared to Group II or III. K_D, approximately 0.85nM [^{3}H]DHA did not change with age or thyroid status.

increase in pulmonary β-adrenergic receptor which occurs between days 15 and 28 of postnatal life. This period coincides with the increase in serum TSH, T3, and T4 concentrations which occurs late in neonatal life in this species as compared to man and strongly supports the role of thyroid hormones or other thyroid hormone dependent factors in the normal physiologic maturation of the β-adrenergic system in the rat (10). The effects of hypothyroidism on lung β-adrenergic receptors are readily reversed by thyroxine treatment. Whether this thyroid dependent maturation of pulmonary β-adrenergic receptors plays a role in the regulation of postnatal pulmonary function remains to be clarified.

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