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IDENTIFICATION AND ONTOGENY OF β-ADRENERGIC RECEPTORS IN FETAL RABBIT LUNG

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SUMMARY

High affinity (Kd=0.2 nM), low capacity (48 fmoles per mg protein), stereospecific binding sites, with properties characteristic of the β_1-subtype of β-adrenergic receptors, have been detected in fetal rabbit lung membranes as early as the 22nd day of gestation. The concentration of the receptor did not change significantly between the 22nd and 26th day of gestation, but increased 3-fold between the 26th and 29th day, reaching a level of 198 fmoles per mg protein. A further increase (from 198 to 315 fmoles per mg protein) in receptor concentration was observed in adult female rabbits. The increase in the levels of pulmonary β-adrenergic receptors between the 26th and 29th day of gestation is temporally related to the increase in surfactant release into the alveolar spaces of the fetal lung. Thus β-adrenergic agonists may act directly on the fetal lung to regulate surfactant secretion.

INTRODUCTION

Secretion of surfactant from storage sites within the type II cells of the lung is an important step for the maintenance of alveolar stability. A defect in surfactant secretion may be the primary cause of surfactant deficiency in infants with respiratory distress syndrome, in addition to a defect in surfactant synthesis (1-6). Recent evidence from both *in vivo* and *in vitro* studies have suggested that surfactant secretion may be regulated by β-adrenergic mechanisms. When added to type II cells of adult rats in primary culture, β-adrenergic agonists caused a moderate but significant increase in the release of disaturated phosphatidylcholine into the culture medium (7). Administration of β-adrenergic agonists into fetuses in late gestation increased lung stability accompanied by an increase in phospholipid content in lung lavage and a decrease in the number of lamellar bodies per type II cell, suggesting release of surfactant into alveolar spaces (8-12).

In order to determine whether the onset of surfactant secretion towards the end of pregnancy is associated with development of the capacity of the

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fetal lung to interact directly with β-adrenergic agonists, we have searched for the presence of β-adrenergic receptors in fetal rabbit lung and have examined developmental changes in receptor concentration and affinity from the fetal to the adult stage.

MATERIALS AND METHODS

Nonpregnant adult female rabbits (3-5 Kg), immature rabbits 10-30 days of age, or pregnant New Zealand white rabbits at 22-29 days of gestation were killed by a blow on the neck. Fetuses from pregnant animals were removed by uterotomy and immediately decapitated. The lungs were washed in cold isotonic phosphate-buffered saline (pH 7.4), dissected free of large vessels and bronchi, frozen in liquid nitrogen, and stored at -85° for up to two months. Receptor levels in tissue that was frozen and stored under these conditions were not significantly different from receptor levels in fresh tissue. On the day of the assay the tissue was minced in 10 volumes of ice-cold buffer (50 mM HEPES, 0.25 M sucrose, 10 mM $MgCl_2$, ph 7) and homogenized with 2 x 10-sec bursts of a polytron PT-10 homogenizer (setting 5) with 20 sec allowed between pulses for cooling. The homogenate was filtered through cheesecloth and centrifuged at 840 xg for 10 min. The supernatant was centrifuged at 30,000 xg for 10 min and the pellet was washed twice with binding assay fuffer (50 mM HEPES, 10 mM $MgCl_2$, 0.1% sodium ascorbate, 1 μM pargyline, pH 7). The pellet was dispersed with a teflon pestle in binding assay buffer and used directly in the binding studies.

β-Adrenergic receptors were assayed with the radiologand [^{125}I]IHYP* (New England Nuclear Corp.) (2200 Ci/mmol), a potent β-adrenergic antagonist. Aliquots of lung membranes (50-70 mg protein) were incubated for 60 min at 37° with increasing concentrations (25-500 pM) of [^{125}I]IHYP in the presence or absence of 10 μM (-)-propranolol in a final volume of 250 μl assay buffer in 12 x 75 mm polypropylene tubes. Binding was terminated by diluting the incubation mixture with 1.5 ml of 50 mM HEPES, pH 7, containing 10 mM $MgCl_2$ and 0.1 mM (+)-propranolol at 37° and immediately filtering the sample through Gelman A-E glass fiber filters. The filters were then washed for 30 sec with 30 ml of 20 mM K_2HPO_4, pH 7, at 37° and counted in a gamma counter. Specific binding was defined as the difference between the cpm observed in the absence (total binding) and presence (nonspecific binding) of 1 μM (-)-propranolol. The apparent equilibrium dissociation constant (Kd) and the number of binding sites were determined by the method of Scatchard (13).

β-Adrenergic agonists and antagonists were tested for their abilities to compete with [^{125}I]IHYP for the binding sites. The Ki value (inhibition constant) for the interaction of each compound with the binding site was calculated from the concentration of the compound which caused 50% inhibition of [^{125}I]IHYP binding (IC_{50}) using the equation of Cheng and Prusoff (14) $Ki = IC_{50}/(1+S/Kd)$, where S is the concentration of [^{125}I]IHYP used in the assay and Kd is the apparent equilibrium dissociation constant of [^{125}I]IHYP binding determined by Scatchard analysis.

Protein in particulate fractions was measured by the method of Lowry et al (15) using bovine serum albumin as the standard.

(-)-Propranolol HCl and (+)-propranolol HCl were gifts from Ayerst Research Laboratories. (-)-Isoproterenol bitartrate, (+)-isoproternol bitartrate, (-)-epinephrine bitartrate and (-)-norepinephrine bitartrate were purchased from Sigma. Solutions of all drugs were prepared in binding assay buffer shortly before use.

*Abbreviation: IHYP, iodohydroxybenzylpindolol.

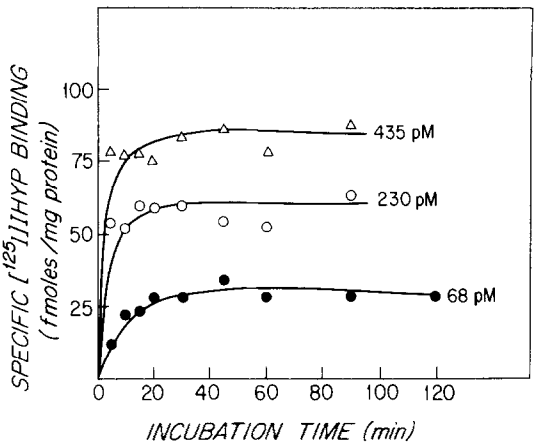

Fig. 1. Specific binding of [^{125}I]IHYP to fetal lung membranes as a function of incubation time. Aliquots of fetal lung membranes (50-70 µg protein) were incubated at 37° for the times indicated with various concentrations (68,230 or 435 pM) of [^{125}I]IHYP in the absence or presence of 10 µM (-)-propranolol. Specific binding, defined as the difference between binding in the presence and absence of 10 µM (-)-propranolol, was determined as described in "Materials and Methods".

RESULTS AND DISCUSSION

Presence of the receptor in fetal lung: Specific binding of [^{125}I]IHYP to fetal lung membranes was similar in HEPES, phosphate or Tris buffer and was linear between 10 and 100 µg of protein. Therefore, 50-70 µg protein per assay tube was used in most experiments. Maximal binding was observed between pH 6.5 and 7.5 in the absence of $MgCl_2$. However, 10 mM $MgCl_2$ was used in the binding reaction mixture to prevent membrane aggregation. In the presence of 10 mM $MgCl_2$, binding was approximately 80% of that observed in the absence of $MgCl_2$.

The forward rate of the binding reaction at 37° was rapid, maximum binding being observed within 40 min even at very low [^{125}I]IHYP concentrations or within 5 min at higher concentrations of ligand (Fig. 1). Under the conditions of the assay, the receptor was stable for at least 2 hrs. Therefore, incubations were carried out for 1 hr, to insure equilibrium of binding at all [^{125}I]IHYP concentrations used in the assay.

Specific binding was saturable, whereas nonspecific binding increased linearly with increasing [^{125}I]IHYP concentrations and ranged between 2 and 7% of the total binding (Fig. 2). For determination of the Kd and the number of receptor sites, saturation analysis was routinely performed using 8 different concentrations of [^{125}I]IHYP in the 25-500 pM range. Within this range of [^{125}I]IHYP concentrations, Scatchard analysis of the binding data

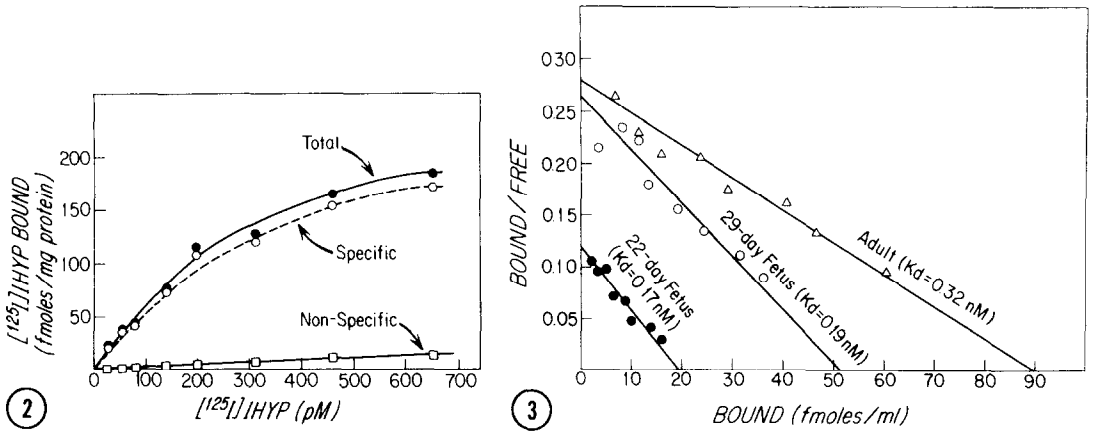

Fig. 2. Binding of [^{125}I] IHYP to fetal lung membranes as a function of [^{125}I]-IHYP concentrations. Aliquots of fetal lung membranes (50-70 µg protein) were incubated for 60 min at 37° with increasing concentrations of [^{125}I] IHYP in the absence (total binding) or in the presence (nonspecific binding) of 10 µM (-)-propranolol. After incubation, the amount of bound [^{125}I] IHYP was measured as described in "Materials and Methods". Specific binding is the difference between the total and the nonspecific binding.

Fig. 3. Scatchard plots of [^{125}I] IHYP binding to lung membranes from rabbit fetuses at 22 or 29 days of gestation and from adult female rabbits. Aliquots of lung membranes (50-70 µg protein) were incubated for 60 min at 37° with increasing concentrations (25-500 pM) of [^{125}I] IHYP in the absence or presence of 10 µM (-)-propranolol. Specific binding was determined as described in "Materials and Methods" and the binding data was plotted by the method of Scatchard (13). Each point is the mean of duplicate samples. In the experiments shown, the concentration of binding sites was 52 fmoles per mg protein in the 22-day fetus, 222 fmoles per mg protein in the 29-day fetus and 344 fmoles per mg protein in the adult rabbit.

gave a straight line indicating the presence of a single class of high affinity binding sites (Fig. 3). The apparent Kd of [^{125}I] IHYP binding to such sites in fetal lung membranes was about 0.2 nM.

The active stereoisomers (-)-propranolol and (-)-isoproterenol were 70 to 1000 times more potent in inhibiting [^{125}I] IHYP binding than the (+)-stereoisomers of propranolol and isoproterenol (Fig. 4). Thus the binding of agonists and antagonists to fetal lung membranes exhibits the stereospecificity expected for β-adrenergic receptors. The Ki's calculated from competition experiments (Fig. 4) were 0.7 nM for (-)-propranolol, 50 nM for (+)-propranolol, 0.25 µM for (-)-isoproterenol, 250 µM for (+)-isoproterenol, 4.6 µM for (-)-epinephrine and 3.6 µM for (-)-norepinephrine. The order of potency of agonists in inhibiting [^{125}I] IHYP binding was (-)-isoproterenol > (-)-epinephine $\tilde{=}$ (-)-norepinephrine. This profile of drug binding is characterisitic of the β_1 subtype of adrenergic receptors and is similar to the profile of drug binding to adrenergic receptors of the adult rabbit lung (16).

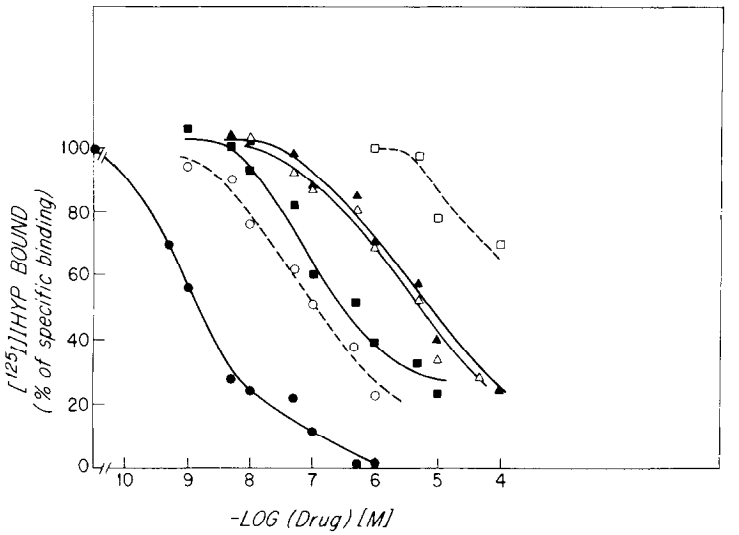

Fig. 4. Competition for [^{125}I] IHYP binding sites by β-adrenergic agonists and antagonists. Fetal lung membranes (50-70 μg protein) were incubated at 37° for 60 min with 200 pM [^{125}I] IHYP in the absence or presence of increasing concentrations of (-)-propranolol (●——●), (+)-propranolol (○----○), (-)-isoproterenol (■——■), (+)-isoproterenol (□---□), (-)-epinephrine (▲——▲) or (-)-norepinephrine (△——△) and specific binding was determined. Data are plotted as the percentage of specific binding in the absence of the drug and are a composite of data from several (3-5) experiments. Specific binding is the difference between binding in the presence and absence of 10 μM (-)-propranolol.

Ontogeny of the pulmonary receptor: The Scatchard plots of [^{125}I] IHYP binding to lung membranes from rabbit fetuses at 22 days of gestation, rabbit fetuses at 29 days of gestation, and adult female rabbits are compared in Fig. 3. The apparent Kd of the [^{125}I] IHYP-receptor interaction was similar in 22- and 29-day rabbit fetuses and appeared to be somewhat higher in adult animals. The concentration of binding sites calculated from the X-intercept was highest in adult animals and lowest in 22-day fetuses. These observations suggested a maturation-dependent increase in the concentration of pulmonary β-adrenergic receptors and prompted more detailed studies to determine developmental changes in the number and affinity of receptor sites in the rabbit lung from the fetal to the adult stage. As shown in Fig. 5, the concentration of the receptor was about 50 fmoles per mg of membrane protein in fetuses at 22 days of gestation, did not change significantly between the 22nd and the 26th day of gestation, and then increased about 3-fold between the 26th and the 29th day reaching a level close to 200 fmoles per mg of protein. This increase in pulmonary β-adrenergic receptor concentration towards the end of pregnancy parallels the increase in surfactant secretion of the type II cells of the fetal lung (5). In postnatal animals of 10-30 days of age the concen-

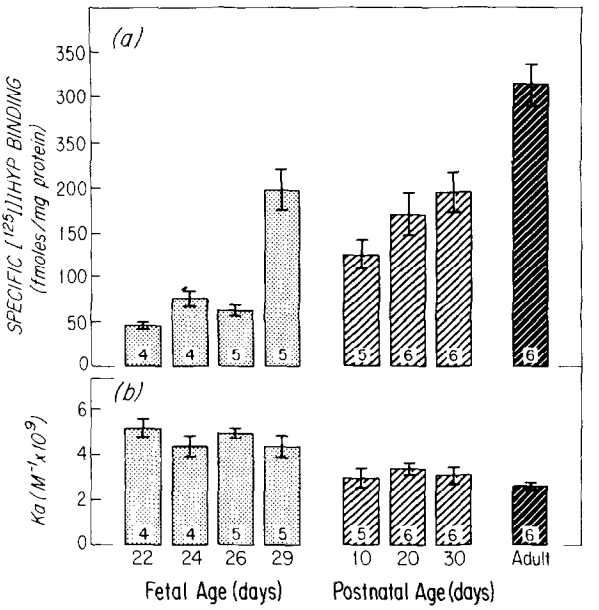

Fig. 5. Concentrations (fmoles/mg protein) and affinity (Ka) of β-adrenergic receptors in lung membranes from rabbit fetuses at 22, 24, 26 and 29 days of gestation, immature rabbits of 10-30 days of age, and adult female rabbits. The concentration and the affinity of the receptor sites were determined by Scatchard analysis of the type illustrated in Fig. 3. The numbers in the bars indicate the number of determinations for each group of animals. Each value represents the mean ± SEM.

tration of receptor remained at about the same level found in 29-day fetuses, but increased significantly to about 350 fmoles per mg of protein in adult female animals. The affinity of the receptor did not change during fetal life but appeared to decrease somewhat after birth (Fig. 5).

Conclusion: The present studies have demonstrated that fetal rabbit lung cells contain saturable, high affinity, stereospecific binding sites which have the characteristics expected of β-adrenergic receptors. The receptors are present in fetal lung cells long before the onset of surfactant secretion and their concentration increases significantly towards the end of pregnancy at about the same time that surfactant begins to be secreted into the alveolar spaces. These data favour the hypothesis that β-adrenergic agonists interact directly with β-adrenergic receptors on the surface of type II cells to stimulate surfactant secretion. It should be pointed out, however, that the lung is a very heterogeneous organ containing over 40 different cell types (17). Therefore, confirmation of this model of the hormonal regulation of surfactant secretion must await the demonstration of β-adrenergic receptors in isolated homogeneous type II cells.

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