Cytoplasmic Receptor for Glucocorticoids in Lung of the Human Fetus and Neonate

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ABSTRACT In fetal animals, glucocorticoids accelerate development of the lung and cause precocious appearance of alveolar surfactant. To determine if the human lung also can respond to corticosteroids, we examined lungs of the human fetus and neonate for both cytoplasmic binding and nuclear uptake of glucocorticoids. In slices of fetal lung incubated with [^{3}H]dexamethasone at 2°C, specific macromolecular binding occurs primarily in the "cytoplasmic" fraction. After further incubation at 37°C. nearly 75% of the radioactivity localizes in the "nuclear" fraction with a concentration of 0.3 pmol/mg DNA at apparent dexamethasone saturation (47 nM). The cytoplasmic receptor binds dexamethasone in vitro with high affinity (dissociation constant = 8.9 nM), and the affinity of various other steroids correlates with their glucocorticoid potency. Receptor was present in lungs of fetuses and neonates of gestational age 12–43 wk, with a mean concentration in hysterotomy specimens of 0.24 pmol sites/mg cytosol protein. Similar binding activity was present at lower concentration in fetal liver, gut, kidney, heart, muscle, and skin. Cytoplasmic receptor was not detected in lung and liver of premature infants with respiratory distress syndrome. This deficit appears to result from increased levels of endogenous steroids (mean cortisol 45.5 μg/100 ml cytosol) as well as inactivation of receptor secondary to the illness. Thus, the lung of the human fetus and neonate contains the receptor mechanism necessary for direct responsiveness to glucocorticoids. These findings support the potential usefulness of these hormones in prevention of respiratory distress syndrome in the premature infant.

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INTRODUCTION

Glucocorticoids trigger specific developmental events in a number of fetal and newborn tissues (2–6). It now appears that these hormones have a similar effect during development of the fetal lung. Administration of corticosteroids to fetal lambs or rabbits causes both accelerated morphological development of the lung and precocious appearance of alveolar surfactant (7, 8). Such treatment increases the survival rate for prematurely delivered animals.

We and others have previously reported (9, 10) that lungs of these fetal animals contain a specific receptor system for glucocorticoids similar to that described in well-recognized target tissues such as liver (11), thymus (12), cultured hepatoma (HTC)[1] cells (13, 14), lymphoid cells (15), and fibroblasts (16). In each of these systems the earliest known event in the interaction of glucocorticoids with their target cell is the binding of steroid by specific cytoplasmic receptor proteins. The receptor-hormone complex thus formed transfers to the nucleus, where it attaches to nuclear acceptor sites and presumably influences specific gene activity. This molecular mechanism of corticosteroid action involving receptors appears to be a necessary event in all glucocorticoid target tissues, since receptor is found in most responsive organs and is not detected in certain physiologically unresponsive tissues (17). In addition, development of steroid resistance in cultured lymphoma cells is associated with a decrease in the concentration of cytoplasmic receptor (15). The detection of receptor in the lungs of fetal rabbits and lambs thus indicates that this organ is capable of responding to glucocorticoids and

[1] *Abbreviations used in this paper:* HTC (cells), hepatoma tissue culture; IRDS, idiopathic respiratory distress syndrome; K_d, equilibrium dissociation constant.

suggests that these hormones directly influence the lung during fetal life.

In the human, development of the lung is perhaps the most critical event determining the health and survival of prematurely delivered infants. Pulmonary immaturity, expressed as a deficiency of alveolar surfactant, is the major cause of idiopathic respiratory distress syndrome (IRDS), or hyaline membrane disease, in premature infants (18). In view of the effects of glucocorticoids on lungs of fetal animals, it has been proposed that administration of these hormones to the human fetus at risk for premature delivery might induce alveolar surfactant and prevent IRDS. In this regard, a beneficial effect of treatment of mothers in premature labor with betamethasone, a potent synthetic glucocorticoid, has recently been reported (19).

To determine if the human lung has the capacity to respond directly to glucocorticoids during fetal and neonatal life, we have examined specimens of lung for both cytoplasmic binding and nuclear uptake of corticosteroids. This communication reports that glucocorticoid receptor is present in human lung. We describe properties of the cytoplasmic and nuclear reactions, as well as studies of the concentration of cytoplasmic receptor in lungs of fetuses throughout gestation and of infants expiring in the neonatal period with and without IRDS.

METHODS

Specimens of human lung and other tissues were obtained after natural death of nonviable or stillborn fetuses after hysterotomy, therapeutic abortions (induced by either intravenous Pitocin [Parke, Davis & Co., Detroit, Mich.] or intrauterine prostaglandin $F_{2\alpha}$), and spontaneous abortions, and from infants dying in the intensive care nurseries of the University of California San Francisco and Mount Zion Hospital, San Francisco. The diagnosis of IRDS in some of the last group of patients was assigned by both clinical and radiological criteria (20). Gestational age was estimated by menstrual history, clinical assessment, and fetal crown-rump and crown-heel measurements (21). Fetal tissues were removed at an autopsy immediately post mortem whenever possible (all hysterotomies and most abortion specimens); otherwise bodies were stored at 4°C for 1½–29 h (average 10½ h) before autopsy. Tissues were removed into isotonic phosphate-buffered saline (pH 7.6) at 2°C and either used within 18 h or frozen at −20°C or −70°C for 1–10 days.

Glucocorticoid receptor activity was measured by the charcoal assay in cell-free cytosol fractions prepared by homogenization and ultracentrifugation as previously described in detail (9). Binding reactions were carried out at 0–2°C for 12–24 h with [^{3}H]dexamethasone (Schwarz Bio Research Inc., Orangeburg, N. Y., 12 Ci/mmol, or New England Nuclear, Boston, Mass., 35 Ci/mmol) with or without a 100–1000-fold excess of unlabeled dexamethasone (gift of Merck Sharp & Dohme, Inc., West Point, Pa.). Radioactivity not absorbed by charcoal in the presence of excess unlabeled steroid is defined as "background binding," and specific macromolecular binding is the total radioactivity bound minus the background value. For determination of receptor site concentration and apparent equilibrium dissociation constants (K_d), specific binding data were analyzed by the Scatchard technique (22).

Uptake of steroid by intact lung cells was studied in slices of fresh lung tissue (approximately 2 mm thick, prepared manually with a razor blade) incubated in Dulbecco's complete medium (Grand Island Biological Co., Grand Island, N. Y.) containing [^{3}H]dexamethasone alone or with excess competing nonradioactive steroid (background binding). Portions of sliced lung (generally 150 mg) were incubated in 3.5-cm diameter petri dishes with 0.75 ml of incubation medium for 10–20 h at 2°C in air. As indicated, some dishes were then exposed for 15–120 min at 37°C in 90% air:10% CO_2. Tissue slices were cooled to 2°C, blotted, and homogenized in 2 vols of cold 0.02 M N-tris-(hydroxymethyl)-methyl-glycine (Tricine)-2 mM $CaCl_2$-1 mM $MgCl_2$ (pH 7.4) (medium 1). The pellet from a 600g centrifugation for 10 min was washed in medium 1 and in medium 1 containing 0.25 M sucrose to provide a "nuclear" fraction (9), which contained only intact nuclei, (mostly devoid of cytoplasmic tags) and some fibrous debris on microscope examination. The nuclear pellet was resuspended in water for assay of radioactivity and DNA. The supernate from the initial 600g centrifugation was recentrifuged for 15 min at 27,000g to provide a "cytoplasmic" fraction, and macromolecular-bound steroid was assayed by the charcoal absorption procedure. The binding activity in this post-mitochondrial cytoplasmic fraction was similar to that obtained in cytosol prepared by centrifugation at 100,000g for 1 h. Radioactivity was determined in both cellular fractions by counting aliquots in a Triton-containing scintillation fluid. Quenching was measured by the external standard method. Protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (23), and DNA was assayed by a modification of the method of Giles and Meyers (14).

Cortisol (and corticosterone) were measured in cytosol preparations by the competitive protein binding microassay of Murphy (24) and by the charcoal absorption assay. Samples were assayed in an ethanol extract both before and after extraction with petroleum ether to also provide an estimate of the level of progesterone and 17α-hydroxyprogesterone (24).

RESULTS

Localization of [^{3}H]dexamethasone in the nuclear fraction of lung slices. When slices of human fetal lung are incubated with dexamethasone, specific uptake of the steroid hormone is detected in both cytoplasmic and nuclear fractions. The uptake in both fractions at 37°C as a function of the free dexamethasone concentration is illustrated in Fig. 1. The dose-response characteristics indicate a limited uptake of labeled steroid in both fractions under these conditions with saturation approached at a dexamethasone concentration below 10^{-7} M. About three times more dexamethasone is bound in the nuclear fraction at all concentrations of free steroid at this temperature. Slices of lung incubated at only 2°C for 10 h, however, accumulate hormone primarily bound to a macromolecule in the cytoplasmic fraction. After 45 min exposure at 37°C there is a depletion of binding in the cytoplasmic fraction and more radioactivity appears in

the nuclear fraction. In four experiments using slices of lung from fetuses of 16-20 wk gestation the average nuclear fraction : cytoplasmic fraction distribution of hormone was 72 : 28% at 37°C and 17 : 83% at 2°C. Saturation of binding in the nuclear fraction occurred at a mean value of 47 nM dexamethasone (range 25–75), and half-saturation occurred at 16 nM (range 6–25). Some additional specific uptake of radioactivity was observed at higher dexamethasone concentrations, apparently reflecting a class of lower affinity sites in the nuclear fraction. The average concentration of dexamethasone bound by the nuclear fraction at apparent saturation was 0.3 (range 0.16 — 0.48) pmol/mg DNA.

Thus, intact cells of the human fetal lung demonstrate specific high-affinity binding of labeled dexamethasone to a macromolecule of the cytoplasmic fraction. Localization of the radioactivity in the nuclear fraction is temperature-dependent and is accompanied by a depletion of bound steroid from the cytoplasmic fraction. Further, the transfer to the nuclear fraction is dependent on cytoplasmic receptor, since specific uptake by the nuclear fraction does not occur in lung slices that lack active receptor nor in isolated lung nuclei incubated with only [^{3}H]dexamethasone (data not shown). Specific nuclear binding does occur in vitro, however, when lung nuclei are exposed to a preformed receptor-steroid complex at 20°C.[2] These findings are consistent with those described in fetal rabbit (9, 10) and lamb lung (9), as well as other glucocorticoid-responsive systems (12–15), and indicate the presence of a specific cytoplasmic glucocorticoid receptor in human lung that is required for migration of hormone to the nucleus.

[2] Ballard, P. L., unpublished data.

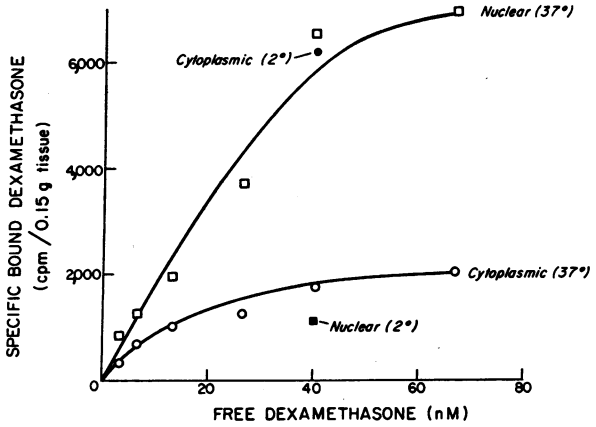

FIGURE 1 Specific nuclear and cytoplasmic binding of dexamethasone by slices of human fetal lung. Slices of lung (150 mg) from a 20-wk gestation fetus were incubated for 10 h at 2°C with various concentrations of 35 Ci/mmol [^{3}H]dexamethasone with or without 8 μM unlabeled dexamethasone. After 45 additional min at 37°C (or 2°C, solid points) the specifically bound radioactivity was determined in nuclear and cytoplasmic fractions (Methods).

Characterization of the in vitro binding of glucocorticoid by lung cytosol. Various properties of the cytoplasmic receptor activity were examined in experiments where lung cytosol was incubated with labeled glucocorticoid. In Fig. 2 the binding of dexamethasone as a function of the free dexamethasone concentration is illustrated for cytosol prepared from lung of a fetus of 17½-wk gestation. Glucocorticoid receptors in this experiment show saturation at a hormone concentration of about 60 nM and half-saturation (K_d) at 9.7 nM. A

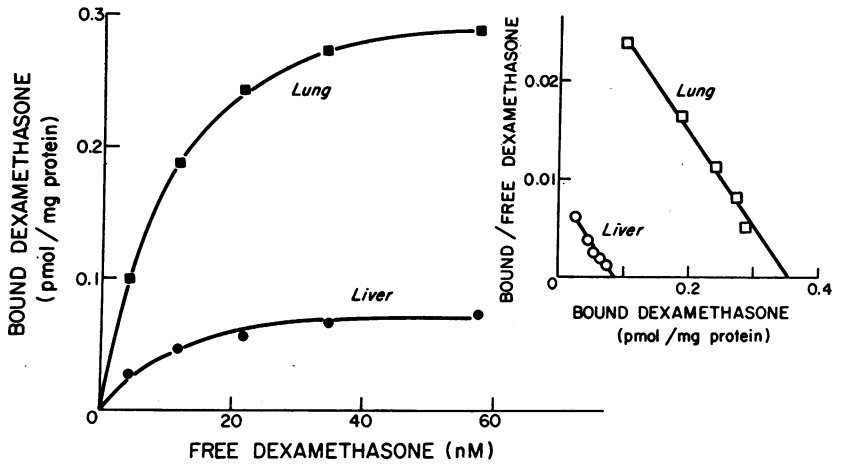

FIGURE 2 Specific binding of dexamethasone by cytosol of human lung and liver. Cytosol was prepared from lung (5.4 mg protein/ml) and liver (12.6 mg/ml) of a 17½-wk gestation fetus and incubated at 2°C for 16 h. The inset shows a Scatchard analysis of the binding data.

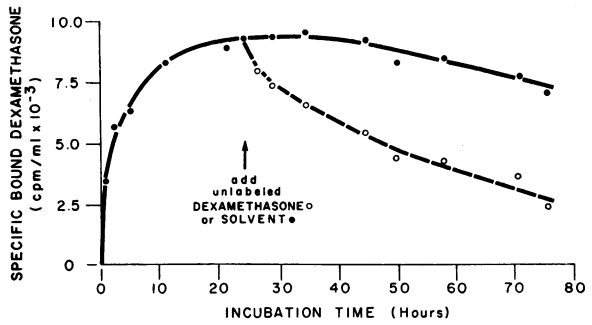

FIGURE 3 Time course of receptor-dexamethasone association and dissociation in cytosol of human lung. After 24 h incubation at 2°C (arrow) 0.01 ml of unlabeled dexamethasone in ethanol was added to half of the reaction mixture for a final concentration of 30 μM. The control system received 0.01 ml of ethanol.

Scatchard plot of the binding data (inset of Fig. 2) is linear, suggesting that lung cytosol contains a single class of receptor sites. In this experiment we also assayed cytosol prepared from liver of the same fetus. While the affinity of receptor in the two tissues is similar, as indicated by the slopes of the Scatchard plots, liver had only 25% of the concentration of receptor sites per milligram of cytosol protein.

The time course of association and dissociation of receptor-steroid complex at 2°C is shown in Fig. 3. In nine experiments of this type with lung specimens from human fetuses between 17 and 43 wk gestational age, the mean time to reach equilibrium in the forward reaction was 13.5 h (range 8-24 h). Similar rates of receptor-steroid association were found with cytosol of human fetal liver (data not presented). The rate of receptor-steroid association at 2°C in these fetal human tissues is slow compared to HTC cells (13) and fetal rabbit lung (9) under similar conditions. These experiments were performed with a [^{3}H]dexamethasone concentration of 20 nM, which is near the half-saturating concentration for many of the receptor preparations. The rate of reaction increased with higher concentrations of hormone and decreased when lower steroid concentrations were used. In one experiment, for example, the times to reach half-equilibrium values at dexamethasone concentrations of 6, 24, and 72 nM were 5.2, 1.6, and 0.8 h, respectively. These results are consistent with a second-order binding reaction and confirm findings in other systems.

TABLE I
Binding of Various Corticosteroids by Cytosol of Human Fetal Lung

[^{3}H]steroid*	Unlabeled competing steroid‡	Binding sites
		nM
Dexamethasone	Dexamethasone	1.46
	cortisol	1.43
Triamcinolone acetonide	Dexamethasone	1.43
	cortisol	1.60
Cortisol	Dexamethasone	1.42
	cortisol	4.64
Corticosterone	Dexamethasone	1.65
	cortisol	3.83

Cytosol was prepared from lungs of a 17½-wk gestation fetus and incubated at a final protein concentration of 5.4 mg/ml for 16 h at 2°C with the steroids listed.
* Final concentration 70 nM.
‡ Final concentration 10 μM.

To evaluate the reverse reaction (i.e., dissociation of receptor-steroid complex) we added a 1000-fold excess of unlabeled dexamethasone to one-half of a reaction system that had reached equilibrium. A control sample received only solvent. The amount of labeled dexamethasone bound to receptor was then determined at intervals, as shown in Fig. 3. Dissociation of the complex is much slower than the forward reaction, consistent with the high affinity of human lung receptor for dexamethasone. In four experiments the mean $t_{\frac{1}{2}}$ at 2°C was 33.5 h (range 31–40) in lungs obtained from fetuses between 17 and 30 wk gestation. A plot (not shown) of percent steroid bound (relative to the control) versus the log of the time is linear, as expected for a first-order reaction dependent only on the concentration of the receptor-steroid complex.

The ability of lung cytosol to bind both natural and synthetic glucocorticoids was examined in the experiment shown in Table I. Cytosol was prepared from lung tissue of a 17½-wk gestation fetus and incubated with a near-saturating level of various tritiated corticosteroids. In each instance background levels of binding were determined in the presence of 140-fold excess of either unlabeled dexamethasone or cortisol. The concentration of specific binding sites competed by dexamethasone is similar for labeled dexamethasone, triamcinolone acetonide, cortisol, and corticosterone. This indicates that the cytosol receptor of human lung binds an equal amount of each glucocorticoid under these conditions. It is noted, however, that the number of binding sites competed by nonlabeled cortisol is 2–3 times greater for [^{3}H]cortisol and [^{3}H]corticosterone. These results reflect the contamination of lung cytosol by transcortin, and perhaps other binding proteins (11), which bind natural corticosteroids but not synthetic steroids (24). For this reason most studies of receptor activity in lung tissue have been carried out with [^{3}H]dexamethasone.

The specificity of lung receptor for various steroids was examined further by testing various unlabeled ster-

oids for their capacity to inhibit binding of [^{3}H]dexamethasone. As shown in Table II, 10 μM dexamethasone, cortisol, corticosterone, 11β-hydroxyprogesterone, and 5α-dihydrocortisol each reduced binding of 10 nM [^{3}H]dexamethasone by lung cytosol to 0–5% of the control value. Testosterone and its 17α-methyl derivative were less inhibitory and both epicortisol and androstenedione had little effect. These results demonstrate a correlation between the affinity of steroids for lung receptor and their glucocorticoid potency in HTC cells. Similar competition data are found with cytosol receptor of fetal rabbit lung and HTC cells (9, 13), suggesting that the glucocorticoid receptors from these different sources have similar binding properties.

The nature of the macromolecular receptor in lung cytosol was investigated in terms of its susceptibility to various hydrolytic enzymes. Cytosol prepared from the lung of a 26-wk gestation fetus was incubated at 5°C for 1 h with various enzymes (0.5 mg/ml), and the capacity to bind dexamethasone was then determined. Deoxyribonuclease and ribonuclease had little effect on the concentration of receptor sites; however, the proteolytic enzymes trypsin, pronase, and collagenase (containing protease activity) abolished binding activity. These results suggest that the cytoplasmic glucocorticoid receptor in fetal human lung, like those macromolecules in certain other target tissues (11–13, 16), is a protein.

Concentration of receptor sites in cytosol of lung and other fetal tissues. In evaluating the ontogeny of receptor activity in human lung, it was important to determine how various storage conditions affected the concentration of binding sites. Full activity was retained in fresh lung tissue left for up to 36 h at 2°C in phosphate-buffered saline; however, there was a variable loss (17–67% of original value) of activity in samples of cytosol stored under similar conditions. Notably, receptor complexed with steroid (eg., Fig. 3) was considerably more stable ($t_{\frac{1}{2}}$ = 80–180 h) than unbound receptor under the same conditions, suggesting, as described elsewhere (13), that binding of hormone stabilizes the receptor molecule. Full activity was retained in most lung samples frozen at −20°C for up to 10 days; however some samples lost activity after 1 wk, and five specimens frozen at −20°C for 6–32 mo had no detectable receptor. For determinations of receptor site concentration, therefore, specimens of lung were assayed fresh whenever possible and otherwise were frozen no more than 4 days.

Glucocorticoid receptor activity was detected in lung cytosol from fetuses and neonates of gestational age 12–43 wk. The concentration of cytoplasmic receptor in 27 specimens of lung, arranged according to the method of obtaining the tissue, are presented in Fig. 4. Lungs from nine fetuses delivered by hysterotomy had a mean

TABLE II

Effect of Various Steroids on Binding of [^{3}H]Dexamethasone by Cytoplasmic Extracts

Nonradioactive steroid added*	Biological activity‡	[^{3}H]dexamethasone bound by cytosol: Human fetal lung	Rabbit fetal lung§	HTC‖
		% of control		
None		100	100	100
Dexamethasone	Optimal inducer	0	0	0
Cortisol	Optimal inducer	0	2	0
Corticosterone	Optimal inducer	2	3	0
11β-hydroxyprogesterone	Suboptimal inducer	5	0	0
5α-dihydrocortisol	Suboptimal inducer	7	5	10
Testosterone	Anti-inducer	21	24	27
17α-methyltestosterone	Anti-inducer	19	18	11
Epicortisol	Inactive	86	82	99
Androstenedione	Inactive	77	91	79

Human lung cytosol was prepared from lungs of a 25-wk gestation fetus and incubated for 12 h at 2°C with 10 nM [^{3}H]dexamethasone at a final protein concentration of 8.6 mg/ml.

* Final concentration 10μM.

‡ Classified by capacity to act as inducer in HTC cells (13).

§ Data from (9).

‖ Data from (13).

receptor site concentration of 0.24±0.03 (SEM) pmol/mg protein with a range of 0.11–0.35. The mean gestational age of these fetuses was 16.5 wk (range 14–25). Other lung specimens were collected after either spontaneous or induced vaginal abortion of previable or nonviable fetuses; these are grouped together since the results were the same for both methods of abortion. The mean gestational age of the 13 fetuses was 21 wk (range 16–30) and both liveborn and stillborn fetuses with detectable receptor activity are included. Mean concentration was 0.17±0.095 (SEM) pmol/mg protein (range

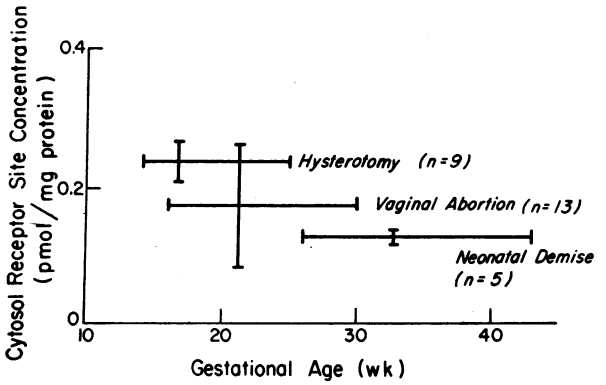

FIGURE 4 Glucocorticoid receptor site concentration in specimens of human fetal and neonatal lung. The range of gestational ages is indicated by the horizontal lines at the mean receptor concentration. Vertical lines represent the SEM at the mean gestational age for the fetuses and infants in each group.

TABLE III

Occurrence of Cytoplasmic Receptor in Various Tissues of a 16-wk Gestation Human Fetus: Site Concentrations and Equilibrium Dissociation Constants

Tissue	Receptor site concentration		Dissociation constant
	pmol/mg protein	*pmol/mg DNA*	*nM*
Lung	0.21	0.78	5.7
Small intestine	0.16	0.64	5.6
Liver	0.13	0.23	4.3
Kidney	0.13	0.60	3.1
Heart	0.07	0.27	—
Muscle*	0.06	0.68	5.5
Skin*	0.015	0.25	—

* Obtained from upper leg.

0.04 − 0.35), which is not statistically different from the hysterotomy group ($P = 0.1$). The wide range of concentrations and SEM in this group results from the generally lower values found in lungs of stillborn fetuses ($n = 7$), apparently reflecting inactivation of receptor after death in utero. Receptor was also assayed in lungs from five infants (mean gestational age 33 wk, range 27–40) who expired in the intensive care nursery 2 h–11 days after birth. Diagnoses included neonatal asphyxia, apnea, intracranial hemorrhage, anencephaly, congenital heart disease, and congestive heart failure. The concentration of binding sites in this group was 0.13±0.02 (SEM) pmol/mg protein with a range from 0.09 to 0.14. This is statistically different from hysterotomy specimens ($P = 0.02$) but not from abortion samples ($P = 0.3$).

Within each of these three groups, which overlap in the range of gestational ages, there was no apparent effect of fetal age on the concentration of receptor sites. Thus, the twofold difference in receptor concentration between young and older fetuses would appear to result from the markedly different conditions of the older fetus before death. Important factors in this regard might include prolonged air breathing, higher cortisol levels, increased pulmonary circulation, and the hypoxemia and stress of a neonatal illness.

TABLE IV

Concentration of Endogenous Steroids in Lung Cytosols

Specimen group	*n*	Steroid concentration
		μg/100 ml cortisol equivalent
Hysterotomy	7	2.0±0.17 (1.4−2.8)
Vaginal abortion	12	4.0±0.83 (1.2−10.5)
Neonatal demise	5	10.5±4.4 (2.4−25.9)
IRDS	6	45.5±14.1 (9.2−104.9)

Mean values with SEM and range are shown.

K_d values for the binding reaction were also determined for most specimens of lung in each group. Mean values were: hysterotomy 8.9±2.1 nM (range 4.2 − 14.4), vaginal abortion 26.2±9 nM (range 8.5–41), and neonatal demise 56.8±22.5 nM (range 21 − 115). The lower K_d values are similar to results in other systems (9, 11, 13, 15, 16). However, the higher values for lungs of many older fetuses suggest the presence of high levels of endogenous corticosteroids, which compete with [^{3}H]dexamethasone for binding to receptors, and artifactually increase the K_d. This possibility was investigated and is discussed in the next section.

Receptor concentration was examined in specimens of liver from 10 fetuses (hysterotomy, $n = 3$; vaginal abortion, $n = 4$; neonatal demise, $n = 3$) and was detected in every instance that receptor was present in the lung. Scatchard plots of binding data for the liver were linear (eg., Fig. 2), and K_d values were similar in each case to those found in the corresponding lung tissue. The mean K_d for liver was 30±11 nM and 29±11 nM for the 10 lung specimens. In each instance, however, a lower concentration of receptor was present in liver (mean 0.09±0.01 pmol/mg protein) than in lung (0.19±0.025 pmol/mg protein.)

It was of interest to examine other human tissues for glucocorticoid receptor activity, since a wide distribution occurs in tissues of certain animals (11, 17). Table III presents results of one experiment in which cytosols prepared from various tissues of a 16-wk gestation human fetus were assayed for binding activity. The highest concentration was found in lung, with lower levels present in small intestine, liver, kidney, heart, skeletal muscle, and skin. Relative tissue concentrations were similar when expressed either as per milligram of protein or per milligram of DNA, except for muscle, which has a large cytoplasmic: nuclear volume distribution. The similarity of the K_d values for these tissues is consistent with the presence of the same binding molecules in various organs. Thus, lung of the human fetus contains a high concentration of receptor sites relative to other tissues, similar to the findings in adult and fetal rabbit (17) and in fetal lamb.[3]

Receptor activity was not detected (< 0.002 pmol/mg protein) in 11 specimens of human lung. Four specimens were obtained from stillborn fetuses, again suggesting that receptor is rapidly inactivated after fetal death in utero. No reason was readily apparent for the failure to detect binding activity in lung tissue of a 40-wk infant with encephalocele and other organ anomalies who expired immediately after birth. It is of interest, however, that receptor was also absent in liver from this infant.

[3] Ballard, P. L., unpublished data.

The remaining cases all involve lung tissue from infants who had IRDS before or at the time of death. One infant of 26 wk gestational age died 18 days after birth, and the remaining patients (gestational age 23–31 wk) expired between 1 and 5 days of life. Additional diagnoses in this group included neonatal asphyxia, apnea, and intracranial hemorrhage. The IRDS patients can be compared to the five nursery infants without IRDS (neonatal demise group in Fig. 4). Receptor was present in every infant expiring in the nursery of causes other than IRDS but was not detected in lung tissue of any infant with this disease. Of interest, however, is the observation that binding activity was also not detected in livers of the three patients with IRDS that were examined.

Levels of endogenous steroids in lung cytosol preparations. We felt that increased levels of endogenous corticosteroids might be responsible for the higher K_d values found for the binding reaction in lungs of infants expiring in the neonatal period. To evaluate this possibility, we measured the concentration of cortisol in various preparations of cytosol. Results for the four groups of lung specimens are presented in Table IV. The steroid values listed primarily reflect the cortisol concentration; however, corticosterone, progesterone, and any other steroids present in an ethanol extract that compete with [^{3}H]cortisol for binding to transcortin would contribute to values obtained. Progesterone and 17α-hydroxyprogesterone were significant components of the extracted steroids only in two of the hysterotomy specimens and five samples obtained after abortion.

Hysterotomy specimens contained a low concentration of steroids, consistent with the low K_d values found for these lungs. Abortion samples had on the average twice the steroid concentration and specimens obtained from infants dying in the nursery had five times the level of hysterotomy specimens. These differences are reflected in the higher K_d values obtained for dexamethasone binding in the latter groups. Lungs from neonates with IRDS contained over 20 times the average value of endogenous steroids compared with hysterotomy specimens. The extremely high levels in three of these six specimens (47, 61.6, and 104.9 μg/100 ml) might be sufficient to mask any receptor activity under the standard assay conditions. However, the other three IRDS specimens contained no more steroid than the higher values in the neonatal demise group that had receptor present. This suggests that other factors, in addition to high endogenous steroid concentrations in the lung, are responsible for the apparent absence of receptor activity.

We also determined steroid levels in 17 samples of cytosol prepared from liver (data not shown). In most instances there was close agreement between the values for lung and liver from the same fetus, and mean values for the four specimen groups were similar. These results suggest that the accumulation of endogenous corticoids in lungs of sick neonates is secondary to increased levels of circulating steroids rather than to preferential localization in the lung.

TABLE V

Effect of Various Cytosol Additions on the Binding of Dexamethasone by Lung Receptor

Addition	Final steroid concentrations	Specific bound [^{3}H]dexamethasone*
	μg/100 ml cortisol equivalent	*% of control*
None	—	100
Unlabeled cortisol	21.5	43
IRDS (no. 33) cytosol	20.5	47
IRDS (no. 17) cytosol	17.5	54
IRDS (no. 13) cytosol	15.5	64
Neonatal demise (no. 21) cytosol	1.2	92
Hysterotomy (no. 7) cytosol	1.0	85

Cytosol preparations are classified as presented in Table IV and Fig. 4.

* Mean value of duplicate determinations with 70 nM [^{3}H]dexamethasone, incubated for 7 h at 2°C.

We tested for inhibitors of in vitro binding activity in lung cytosols from the IRDS group by mixing aliquots with active lung cytosol from a 25-wk gestation fetus. After a preincubation period the amount of binding activity was determined and compared to controls exposed only to buffer solution. It is seen in Table V that three specimens of IRDS lung cytosol each inhibited receptor activity, and that the level of inhibition is proportional to the amount of endogenous steroid present. Two cytosol preparations from non-IRDS fetuses showed less inhibition, consistent with their lower level of steroids. While it is apparent that high levels of steroids in cytosol preparations will lower the apparent receptor concentration under these assay conditions, these data also suggest that there is no additional inhibitory activity present in cytosol of lungs from IRDS patients.

DISCUSSION

We have described the binding of glucocorticoids by a cytoplasmic protein of human fetal and neonatal lung. The specific nature of the binding reaction is indicated by the high affinity for dexamethasone and saturability of binding sites at a relatively low steroid concentration. In addition, there is a specificity for various steroids that correlates with their glucocorticoid potency.

When human lung slices are incubated at 37°C with labeled dexamethasone, the radioactivity is bound primarily in the nuclear fraction. This suggests that recep-

tor-dexamethasone complex localizes in the nuclei of human fetal lung under these conditions. As in other glucocorticoid receptor systems (9, 10, 12, 14, 15), this migration is temperature-dependent, suggesting as proposed elsewhere (12, 14), that activation or modification of the receptor-steroid complex is required before nuclear uptake occurs. A similar temperature dependence was found for in vitro transfer of receptor-steroid complex of fetal rabbit lung to isolated lung nuclei (9). In addition, in both rabbit and human lung approximately 70% of steroid bound in the cytoplasm is transferred to the nuclear fraction. The affinity of binding by the nuclear fraction in lung slices is similar to that found for the association of dexamethasone with cytoplasmic receptor both in vitro and in intact cells at 2°C. This is consistent with a step-wise transfer of hormone to the nuclear fraction, with binding to cytoplasmic receptor being the initial and limiting reaction. We have found in preliminary experiments that in vitro transfer of preformed receptor-steroid complex to isolated human lung nuclei also occurs; the K_d for this reaction (approximately 10^{-10} M) indicates a very high affinity of receptor-steroid complex for the acceptor component in the nuclear fraction, similar to findings with HTC cells (14) and fetal rabbit lung (9).

The binding of 20 nM dexamethasone by human lung cytosol receptor requires 8–24 h at 2°C to reach a plateau value. This time course is considerably slower than for rabbit lung and HTC cells under similar experimental conditions. The kinetics of hormone dissociation from receptor likewise are slower. These in vitro kinetic studies were carried out at a low temperature due to the rapid loss of binding activity on incubation of lung cytosol at 20 or 37°C. The uptake of dexamethasone by slices of lung at 37°C, by contrast, reaches a stable plateau value by 15 min. The faster kinetics under these conditions suggest that binding of glucocorticoids in lung of the intact fetus is also rapid.

One consequence of the slow rates at 2°C is the inability to completely displace endogenous steroids from human lung receptor with labeled dexamethasone in vitro. This results in elevated K_d values and, under certain conditions, falsely low estimates of receptor site concentration when lung specimens contain significant levels of endogenous corticosteroids. Such was the case with some specimens obtained after induced abortion and with most lungs from infants dying after birth. The finding of elevated levels of corticosteroids in these lung tissues is consistent with the high plasma concentration found in premature infants with IRDS (25). The higher apparent K_d values for binding in lungs of older fetuses correlates with the endogenous corticosteroid level; this in turn presumably reflects the increase in fetal adrenal activity during gestation as well as increasing circulation to the lung. Thus it is likely that the actual K_d remains constant during gestation, indicating a constant affinity of receptor for glucocorticoids.

The data presented here indicate that glucocorticoid receptor is present in human lung from early in fetal life to the end of normal gestation. Thus, the presence of an active receptor system is not the limiting factor in the onset of glucocorticoid responsiveness in the fetal lung. We found no apparent change in receptor concentration with gestational age within each group of specimens. This suggests that the lower concentration of sites found in the neonatal demise group is secondary to conditions associated with extrauterine life or neonatal illness. Since there was a higher level of endogenous steroids in these lungs, it is possible that receptor was partially depleted from the cytoplasm because of nuclear uptake in vivo. We conclude, therefore, that the level of cytoplasmic binding sites is probably constant throughout fetal life. The mean value of 0.24 pmol receptor sites/mg protein for human lung from hysterotomy specimens is somewhat less than the level found in fetal rabbit lung (0.43) and fetal lamb lung (0.35) (9). With an average value of 3.5 mg cytosol protein/mg DNA, and assuming 6.5 pg DNA/cell (26), there are 3,200 cytoplasmic receptor sites/fetal human lung cell as a minimal estimate. This compares with a calculated 2,300 high-affinity nuclear acceptor sites/cell. It is estimated that the mammalian lung contains at least two dozen different cell types. Since most of these cells probably are present in the human fetal lung by 14 wk of gestation (27), the binding assay in all experiments sampled a heterogeneous cell population. It is not yet known, however, whether every cell type present in human lung contains both components of the glucocorticoid receptor system.

The level of receptor in lungs of two infants dying 4 and 11 days after delivery at term was similar to that found in infants dying shortly after birth. In this regard the human lung resembles the lung of rabbit and sheep and differs from the rat where receptor levels decrease to low levels soon after birth (17). The maintenance of receptor after birth suggests that cortisol could also affect pulmonary function in the neonatal period.

Receptor was also present in fetal liver, kidney, heart, small intestine, muscle, and skin, although lung consistently demonstrated the highest concentration of binding sites. K_d values for the binding reaction were similar for various tissues, suggesting that a similar receptor is present in many human organs. These findings are the first description, to our knowledge, of the ubiquity of glucocorticoid receptor in tissues of the human fetus. Receptor activity has previously been described in human lymphocytes and leukemic lymphoblasts (28).

The fact that many human tissues have the capacity

to respond to glucocorticoids during development suggests that administration of exogenous corticosteroids might alter the normal developmental pattern of other tissues besides lung. Such a possibility could assume clinical importance if in utero hormonal therapy for IRDS comes into general use. In particular one might question what effects glucocorticoids would have on the fetal thymus and its immunological competence, on the newborn gut and its capacity to absorb proteins and hydrolyze disaccharides, and on cell proliferation in a variety of tissues.

Receptor was not detected in lungs of infants with IRDS. It appears, however, that this deficit is secondary to the disease process and is not involved in the etiology of IRDS. It should be noted, for example, that lungs of previable liveborn fetuses contained receptor activity. Such infants, were they to live for a few hours, would almost certainly have developed IRDS. The inability to detect binding activity in vitro appears to result from greatly elevated endogenous corticosteroids, the slow kinetics of the binding reaction at 2°C in vitro, which restricts displacement of bound steroids, and apparent inactivation of receptor secondary to the hypoxemia or other metabolic and respiratory imbalances associated with IRDS. These mechanisms apparently apply in the liver as well, since we could not detect receptor in that organ also. It is possible that the loss of a functional receptor system in lungs of the infant with IRDS would contribute to the demise of some IRDS patients. This possibility is not supported, however, by the absence of receptor activity in lungs of one premature infant, who died at 18 days of age from an intracranial hemorrhage after being successfully treated for IRDS.

A lower incidence of IRDS in premature infants exposed to betamethasone in utero (19) strongly suggests that exogenous glucocorticoids will stimulate human lung development. The presence of an active glucocorticoid receptor system in human fetal lung indicates that this organ indeed has the capacity to respond directly to corticosteroids and is therefore a target tissue for these hormones. Since receptor is present in lung early in gestation, it may be postulated that the effectiveness of corticosteroids in preventing IRDS would be limited only by the degree of morphological maturity (i.e., formation of alveoli) or by the developmental maturity of enzyme systems or other proteins induced by these hormones. One such enzyme could be choline phosphotransferase, which is stimulated in fetal rabbit lungs by cortisol treatment (29).

The possible role of endogenous glucocorticoids in the normal development of the human lung is still uncertain. At present, there are no data on plasma cortisol levels during the human gestation for comparison with the appearance of surfactant in lung tissue at about the 20th wk (30) and in amniotic fluid around 32 wk of gestation (31). It does appear likely, however, that endogenous cortisol production could be stimulated in the fetus by various stressful conditions (e.g., intrauterine infection, placental insufficiency, maternal disease, prolonged rupture of membranes, or spontaneous labor); this could accelerate surfactant appearance (32) and serve as a protective mechanism for premature adaptation to extrauterine life.

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