Androgen Regulation of Epidermal Growth Factor Receptor Binding Activity during Fetal Rabbit Lung Development

Jonathan M. Klein* and Heber C. Nielsen‡

**Department of Pediatrics, University of Iowa, Iowa City, Iowa 52242; and ‡Boston Perinatal Center, Department of Pediatrics, New England Medical Center, Boston, Massachusetts 02111*

Abstract

Fetal lung development progresses in a sex-specific manner with male fetuses exhibiting delayed maturation. Androgens, both exogenous and endogenous, inhibit while epidermal growth factor (EGF) enhances fetal lung development. We hypothesized that one mechanism responsible for the delay in male fetal lung development is an androgen-induced delay in EGF receptor binding activity. We measured EGF binding in sex-specific fetal rabbit lung plasma membranes isolated from control fetuses (days 21, 23, 25, 27, 29, and 30 of gestation) and from androgen-treated fetuses (days 21, 23, and 27 of gestation) that had been continuously exposed in vivo to exogenous 5α-dihydrotestosterone from day 12 through 27 of gestation. Specific binding of EGF was significantly lower in male than in female fetal lung tissue isolated from controls at day 21 of gestation. Scatchard analysis revealed that this decrease in EGF binding was associated with decreased EGF receptor density without any significant change in affinity. Prenatal exogenous androgen treatment led to decreased EGF binding in fetal rabbit lung tissue from both sexes secondary to a decrease in EGF receptor density. These findings suggest that one mechanism responsible for the delay in male fetal lung maturation is an androgen-induced delay in EGF receptor binding activity during fetal lung development. (*J. Clin. Invest.* 1993. 91:425–431.) Key words: epidermal growth factor receptor • sex-specific • fetal lung maturation • 5α-dihydrotestosterone • autophosphorylation

Introduction

Epidermal growth factor (EGF)[1] is a 6-kD polypeptide hormone involved in the regulation of growth, development, and differentiation in the fetus (1). EGF stimulates fetal lung maturation both in vivo: pulmonary morphology (sheep [2]), compliance (rabbit [3]), and surfactant synthesis (rabbit [4]) and in vitro: surfactant phospholipid synthesis (rat fetal lung explants [5]), fibroblast-pneumonocyte factor synthesis (mouse fetal lung fibroblasts [6]), and surfactant-associated protein A synthesis (human fetal lung explants [7]).

EGF mediates its effects through binding to its receptor, a 170-kD transmembrane glycosylated phosphoprotein with intrinsic tyrosine kinase activity (8). EGF binding leads to the activation of the receptor's tyrosine kinase, which is essential for the receptor to function (9). The intracellular effects of a functional EGF receptor include both autophosphorylation and the phosphorylation of various substrates. These substrates function as second messengers during the process of signal transduction through which receptor binding leads to the increased transcription of specific genes, which in turn influence growth and maturation (10).

Fetal lung development progresses in a sex-specific manner with the male fetus having delayed type II cell maturation (11). This is manifested, in the male human neonate, by both an increased incidence (12, 13) and severity (14–16) of surfactant deficiency disease (respiratory distress syndrome) (17). Androgens have been shown to delay fetal lung maturation both in vitro (18) and in vivo (19, 20). Androgen receptors have been identified in both human (21) and rabbit fetal lung tissue (22). Furthermore, in nonpulmonary tissue (rat prostate), androgens have been shown to decrease EGF receptor levels (23, 24). We have previously shown that one of the mechanisms responsible for the sex-specific difference in rabbit fetal lung maturation is a delay in the onset of EGF activity in the male fetal lung (25). At day 21 of gestation, female fetal lung explants but not male explants responded to EGF stimulation with increased surfactant phospholipid synthesis (25). Furthermore, at day 21 of gestation, the difference in plasma androgen levels (5α-dihydrotestosterone) between male and female rabbit fetuses is fivefold (26). We hypothesized that one mechanism responsible for the male lag in fetal lung maturation would be an androgen-induced delay in EGF receptor binding activity during fetal lung development.

To test our hypothesis we performed three studies. First, we measured the ontogeny of EGF binding in sex-specific fetal rabbit lung plasma membranes isolated during fetal lung development at days 21, 23, 25, 27, 29, and 30 of gestation (term 31 d). Second, we exposed fetuses in vivo to exogenous androgens and then measured EGF binding in sex-specific androgenized fetal rabbit lung plasma membranes at days 21, 23, and 27 of gestation. Third, we measured EGF-induced autophosphorylation of EGF receptor in sex-specific fetal rabbit lung plasma membranes at day 21 of gestation.

Methods

Animals. Pregnant New Zealand White rabbits of known gestation (term 31 d; Pine Acres Rabbitry, West Brattleboro, VT) were killed with an intravenous injection of sodium pentobarbital (600 mg) on days 21, 23, 25, 27, 29, and 30 of gestation. The uteri were removed and

Address correspondence to Jonathan M. Klein, Department of Pediatrics, University of Iowa, Iowa City, IA 52242.

Received for publication 5 June 1992 and in revised form 21 August 1992.

1. *Abbreviations used in this paper:* B_{max}, maximum binding capacity; DHT, dihydrotestosterone; EGF, epidermal growth factor; LPM, lung plasma membranes.

J. Clin. Invest.

0021-9738/93/02/0425/07 $2.00
Volume 91, February 1993, 425–431

the fetuses were collected from multiple pregnancies (12 litters at day 21 to 2 litters at day 30 of gestation). The sex of each fetus was identified (27) and the lungs were removed, pooled by sex, and stored briefly at 4°C until subsequent preparation of lung plasma membranes.

Maternal androgen treatment. Osmotic pumps (model 2ML2; Alza Corp., Palo Alto, CA) containing dihydrotestosterone (5α-Androstan-17β-ol-3-one (DHT); Steraloids, Inc., Wilton, NH) were implanted subcutaneously under sterile conditions into pregnant does on day 12 of gestation. The osmotic pumps were loaded under sterile conditions with 2 ml per pump of DHT (9 mg/ml) dissolved in dimethyl sulfoxide:glycerol (50:50). A single pump was placed into each pregnant doe via a dorsal incision using lidocaine as a local anesthetic. This concentration of DHT allowed the pumps to deliver ~ 1 mg of DHT per day to each pregnant doe; a dose chosen from previous studies that produces female androgen levels of the same order of magnitude as those of male fetuses (19). On days 21, 23, and 27 of gestation the does were killed and the fetuses removed.

Isolation of fetal lung plasma membranes. Crude lung plasma membranes were prepared from pooled fetal lung tissue using a modification of the method of Morishige et al. (28). All buffer solutions used during the isolation of the lung plasma membranes contained freshly prepared protease inhibitors (leupeptin, 8 μg/ml; soybean trypsin inhibitor, 50 μg/ml; and phenylmethylsulfonyl fluoride, 0.1 mM) (Sigma Chemical Co., St. Louis, MO) (29). Isolation procedures were conducted at temperatures between 0 and 4°C. Fetal lung tissue was cleaned by trimming off visible bronchial tissue and then blotting the remaining tissue with filter paper to remove excess blood. The tissue was weighed and then homogenized in 5–10 vol (wt/vol) of ice cold 0.3 M sucrose buffer containing 25 mM Tris-HCl (pH 7.6) and 10 mM $MgCl_2$. The homogenate was centrifuged at 800 *g* for 10 min; the resulting supernatant was decanted off and then centrifuged at 20,000 *g* for 20 min. The pellet containing the plasma membranes was resuspended in 10–20 vol of isolation buffer (25 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.6) and centrifuged again at 20,000 *g* for 10 min. The final plasma membrane pellet was resuspended in 1–2 vol of isolation buffer without protease inhibitors and stored in aliquots at −70°C until used for subsequent binding assays. Plasma membrane protein concentration was measured (30) in 5-μl aliquots of this suspension.

EGF binding assay. EGF binding was measured by incubating fetal lung plasma membranes (400 μg of protein per tube) with mouse [^{125}I]-EGF (0.4 ng, sp act 100 μCi/μg; ICN Radiochemicals, Irvine, CA, or Amersham Corp., Arlington Heights, IL) for 60 min at 22°C. The binding reaction was carried out in a total vol of 0.25 ml consisting of fetal lung plasma membranes, radiolabeled EGF, and binding buffer (128 mM NaCl, 5 mM KCl, 3 mM $MgSO_4$, 0.9 mM $CaCl_2$, 0.1 mM Na_2HPO_4, pH 7.4), 0.4% BSA, and protease inhibitors as described above (29). The reaction was terminated by rapid vacuum filtration of the incubation mixture through a filter (GFC; Whatman Inc., Clifton, NJ) using a sampling manifold (Millipore Corp., Bedford, MA). This method rapidly separates the free radiolabeled EGF from the receptor-bound which remains on the filter (31). The filters were rinsed with 10 ml of binding buffer while under vacuum on the manifold, dried, and then counted for 1 min in a gamma counter. Total binding was determined by measuring the amount of radiolabeled EGF that remained bound to the fetal lung plasma membranes that were trapped on the filter. Nonspecific binding was measured as described above after preincubation of the membrane preparation in the presence of 500-fold excess of unlabeled EGF (Gibco Laboratories, Life Technologies, Inc., Grand Island, NY) for 10 min before the label was added. Specific binding of EGF was determined by subtracting nonspecific from total binding. Specific binding assays were performed a minimum of three times in triplicate for each gestational age except for days 29 and 30, when the assays were run twice.

In preliminary studies, EGF binding was measured using increasing amounts of membrane protein (100–500 μg/250 μl assay vol) to confirm the linearity of binding as a function of membrane protein concentration (data not shown). Subsequent binding studies were then performed with 400 μg of lung plasma membrane protein. Additional preliminary studies were performed to confirm that EGF binding with 400 μg of lung plasma membrane (LPM) protein reached equilibrium during 60 min of incubation at 22°C (data not shown).

Competitive binding assays were performed by measuring [^{125}I]-EGF binding to fetal lung plasma membranes in the presence of increasing concentrations of unlabeled EGF (0.1 nM–1.0 μM) or, in one experiment, increasing concentrations of DHT (10 nM–100 μM). Each EGF competition assay was performed three times in duplicate. EGF receptor density and affinity were calculated by Scatchard analysis (32) using the computer program LIGAND (33) as modified for microcomputers by G. A. McPherson (34).

We used a logarithmic scale for the competitor concentration (unlabeled EGF) when graphing the results of the competitive binding experiments. This was done to ensure that we had obtained binding data at ligand concentrations above the inflection point (half-maximum binding), validating our use of Scatchard analysis to estimate receptor density and affinity (35).

EGF-induced autophosphorylation of EGF receptor. Sex-specific fetal rabbit lung plasma membranes isolated at day 21 of gestation as described above were used to study EGF-induced autophosphorylation of EGF receptor using a modification of the method of Freidenberg et al. (36). Fetal lung plasma membranes (120 μg of protein) were incubated with or without EGF (100 nM) at 22°C for 10 min (total vol of 40 μl). The phosphorylation reaction was then initiated by incubating the plasma membranes with [^{32}P]ATP (18 μM) (sp act 50 μCi/μg; ICN Radiochemicals, Irvine, CA) in a reaction mixture containing 5 mM $MnCl_2$, 5 mM $MgCl_2$, 100 μM sodium orthovanadate, and 45 mM NaF for 10 min at 22°C (total vol of 60 μl). The phosphorylation reaction was terminated by heating the mixture with Laemmli sample buffer at 95°C for 5 min. The phosphoproteins were separated by electrophoresis on a 6% polyacrylamide gel (37) and visualized by autoradiography. The band corresponding to the EGF receptor, a 170-kD EGF-stimulated phosphoprotein, was cut from the gel and the amount of ^{32}P incorporated into the receptor in the presence and absence of EGF was quantitated in a liquid scintillation counter.

Statistical analysis. The significance of the effects of sex and gestational age on EGF binding were evaluated by two-way analysis of variance (38). Differences in EGF binding between control and androgen-treated groups were evaluated by the unpaired Student's *t* test. Data from each polyacrylamide gel were normalized by calculating the percent increase in EGF induced incorporation of ^{32}P into the EGF receptor as compared with its sex-specific control. Differences in the level of EGF induced autophosphorylation of EGF receptor between males and females were evaluated by the unpaired Student's *t* test. Values were expressed as either the mean±SD or, where appropriate, the mean±SEM, and were considered statistically significant if the two-tailed *P* values were < 0.05.

Results

Ontogeny of EGF binding during fetal lung development. Specific binding of EGF to fetal rabbit lung plasma membranes varied significantly in both sexes with gestation ($P < 0.001$), initially increasing in males (stable in females), reaching maximal binding in both sexes at day 23 of gestation, then decreasing as term approached (days 25 through 30, Fig. 1). A sex-specific difference in the binding of EGF to fetal rabbit lung plasma membranes was clearly seen at day 21 of gestation (Fig. 1). At this early point in gestation EGF binding to male fetal lung plasma membranes was only 58% of female binding ($P < 0.05$).

Scatchard analysis (Fig. 2) of competitive binding experiments (Fig. 3) performed at day 21 of gestation revealed a significant difference in receptor density between male and female fetal lung plasma membranes. EGF receptor density was significantly lower in males than in females (157±15 versus

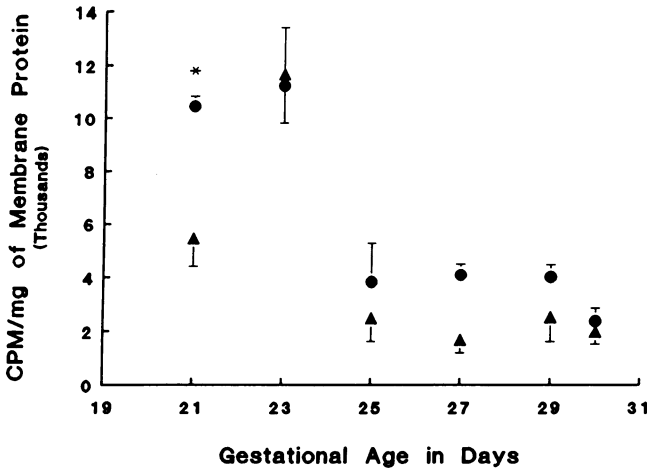

Figure 1. Specific binding of [^{125}I]EGF to fetal rabbit LPM. Fetal rabbit LPM were isolated at days 21, 23, 25, 27, 29, and 30 of gestation. The data are the mean±SEM of two to four experiments (two experiments at days 29 and 30) performed in triplicate. Male (▲); female (●). *EGF binding to female LPM is significantly greater than male at day 21 of gestation ($P < 0.05$). Specific binding of EGF to fetal rabbit LPM isolated from both sexes varies significantly with gestation ($P < 0.001$).

206±25 fmol/mg membrane protein, mean±SD, $P < 0.05$) (Table I). Analysis of EGF receptor binding affinity as described by the K_d was not significantly different between males and females (7.1±1.1 versus 10.5±2.4 nM, mean±SD) (Table I). Thus the decreased binding of EGF to male fetal lung plasma membranes at day 21 of gestation is caused by a decrease in receptor density rather than by a decrease in affinity (i.e., increased K_d).

EGF binding after maternal androgen treatment. We measured EGF binding in sex-specific fetal rabbit lung plasma membranes isolated at days 21, 23, and 27 of gestation from fetuses that had been treated prenatally with androgens. Maternal androgen treatment produced a significant decrease in EGF binding in females at day 21 of gestation as compared with controls ($P < 0.05$, Fig. 4). Maternal androgen treatment eliminated the sex-specific difference in EGF binding at day 21 of gestation by lowering the level of specific binding in females to the male level (Fig. 4 *A*). Furthermore, at day 23 of gestation, maternal androgen treatment continued to inhibit the development of EGF binding. EGF binding was decreased by 63% in the androgen-treated males as compared with the male controls ($P < 0.05$, Fig. 4 *B*). Females at day 23 of gestation also showed a consistent decrease of 43% in EGF binding after maternal androgen treatment (Fig. 4 *B*). However, at day 27 of gestation, there was no significant inhibitory effect of androgens on EGF binding (Fig. 4 *C*). Scatchard analysis (Fig. 2) of

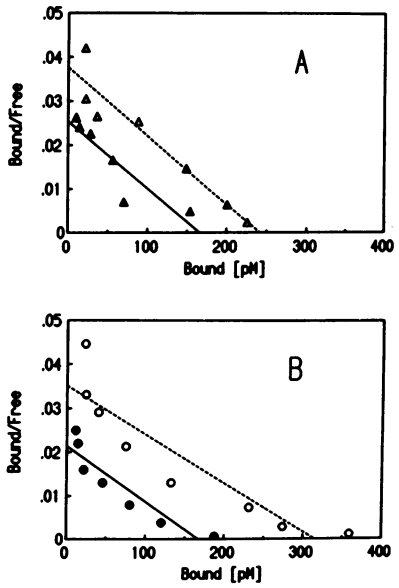

Figure 2. Scatchard plots of competitive [^{125}I]EGF binding to fetal rabbit LPM isolated at day 21 of gestation. Scatchard analysis was performed using mean binding data from competitive binding experiments. Control males (△); androgen treated males (▲); control females (○); androgen treated females (●). Bound/free [^{125}I]EGF is plotted versus bound [pM]. Binding to the EGF receptor was linear by regression analysis in all four conditions: control male ($r = -0.95$), control female ($r = -0.92$), androgen-treated male ($r = -0.90$), androgen-treated female ($r = -0.93$). (*A*) Male day 21 fetal rabbit LPM. (*B*) Female day 21 fetal rabbit LPM. EGF receptor B_{MAX} of control females (*B*) is significantly greater ($P < 0.05$) than control males (*A*). *A* shows that EGF receptor B_{MAX} of androgen-treated males (▲) is significantly lower ($P < 0.05$) than control males (△). *B* shows that EGF receptor B_{MAX} of androgen-treated females (●) is significantly lower ($P < 0.05$) than control females (○).

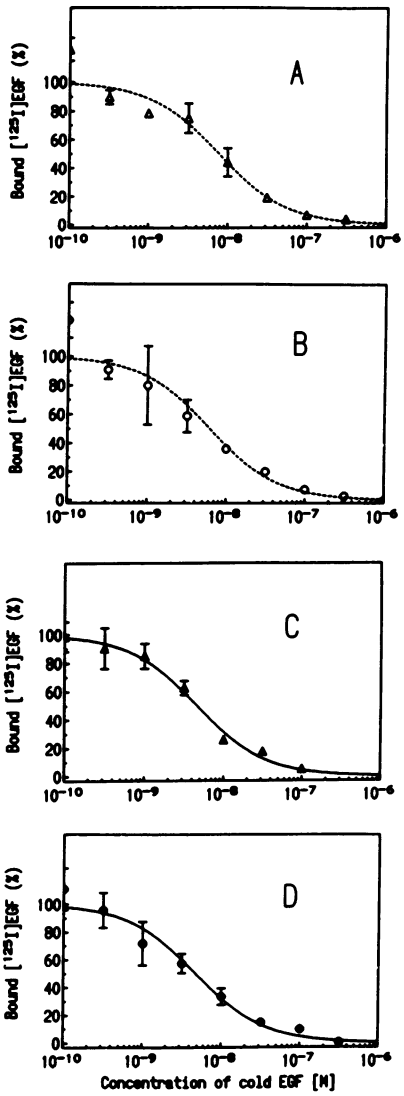

Figure 3. Competitive binding of [^{125}I]EGF versus unlabeled EGF. Competitive [^{125}I]EGF binding curves were obtained from experiments performed with fetal rabbit lung plasma membranes isolated at day 21 of gestation. Percent [^{125}I]EGF bound is plotted versus increasing concentrations of unlabeled EGF expressed on a logarithmic scale. Curve fitting to a one component competitive binding curve was done by nonlinear regression with all r values > 0.99. Values represent mean±SEM ($n = 3$). (*A*) Control male (△). (*B*) Control female (○). (*C*) Androgen-treated male (▲). (*D*) Androgen-treated female (●).

Table I. Scatchard Analysis of [^{125}I]EGF Competitive Binding Experiments

	EGF receptor density	Dissociation constant (K_d)
	fmol/mg membrane protein	*nM*
Control		
Male	157±15*‡	7.1±1.1
Female	206±25*§	10.5±2.4
Androgen treated		
Male	105±10‡	6.9±2.5
Female	150±19§	12.6±3.3

EGF receptor density and affinity (dissociation constant K_d) as determined by Scatchard analysis of competitive binding experiments performed with fetal rabbit LPM isolated at day 21 of gestation (mean±SD, n = 3). EGF receptor density is significantly lower in male fetal LPM than female (*$P < 0.05$), in androgen-treated female fetal LPM than control female (§$P < 0.05$), and in androgen-treated male fetal LPM than control male (‡$P < 0.01$). There was no significant difference in the affinity of the EGF receptor among the groups studied.

competitive binding experiments (Fig. 3) performed with androgenized fetal lung membranes prepared at day 21 of gestation revealed a significant difference in EGF receptor density between control and androgen-treated female fetal lung plasma membranes. EGF receptor density was lower in androgen-treated female fetal lung tissue as compared to control tissue (150±19 versus 206±25 fmol/mg membrane protein, mean±SD, $P < 0.05$) (Table I). Analysis of EGF receptor binding affinity as measured by the K_d was not significantly different between androgen-treated and control female fetal lung tissue (12.6±3.3 versus 10.5±2.4 nM, mean±SD) (Table I). Thus, the decreased binding of EGF to androgen-treated female fetal lung plasma membranes at day 21 of gestation was due to a significant decrease in receptor density rather than a decrease in affinity.

The lowest level of EGF binding was seen in the androgen-treated males (Fig. 4). Scatchard analysis of EGF binding again revealed decreased receptor density in fetal rabbit lung plasma membranes isolated from day-21 androgen-treated males as compared with control males (105±10 versus 157±15 fmol/mg membrane protein, mean±SD, $P < 0.01$) (Table I). Furthermore, EGF receptor binding affinity was not different between day-21 androgen-treated and control male fetal lung tissue (K_d 6.9±2.5 versus 7.1±1.1 nM, mean±SD) (Table I). Thus the decreased binding of EGF to androgen-treated male fetal lung plasma membranes at day 21 of gestation was caused by a decrease in receptor density rather than to a decrease in affinity.

EGF-induced autophosphorylation of EGF receptor. EGF-induced autophosphorylation of EGF receptor was measured by the percent increase in ^{32}P incorporation into EGF-stimulated fetal lung plasma membranes as compared with control membranes using sex-specific fetal lung plasma membranes isolated at day 21 of gestation. EGF-induced autophosphorylation of EGF receptor was significantly greater in females than in males (25.8±4.9% versus 10.9±4.2%, mean±SEM, n = 7, $P < 0.05$). This difference in EGF-induced autophosphorylation of the EGF receptor is consistent with the increased EGF receptor density found at day 21 of gestation in female fetal lung plasma membranes.

Competitive binding with DHT. The binding of [^{125}I]EGF to female fetal rabbit lung plasma membranes isolated at day 23 of gestation was measured in the presence of increasing concentrations of 5α-dihydrotestosterone or unlabeled EGF (Fig. 5). The binding of [^{125}I]EGF was displaced by excess unlabeled EGF, but not by excess DHT. Thus, the EGF receptor is highly specific for EGF and DHT does not compete against EGF for receptor binding.

Discussion

Sex-specific differences in fetal lung development exist, with male fetuses having a delay in lung maturation. Androgens, both endogenous and exogenous, inhibit fetal rabbit lung development (19). Androgens are known to decrease EGF receptor levels in nonpulmonary tissue, i.e., adult male rat prostate (23, 24). The data presented here show that androgens decrease pulmonary EGF receptor levels during fetal lung development indicating that one of the mechanisms for the androgen-induced delay in fetal rabbit lung maturation is an inhibition of EGF receptor binding activity.

The level of EGF receptors present in fetal lung was determined through EGF binding studies. The binding of [^{125}I]EGF

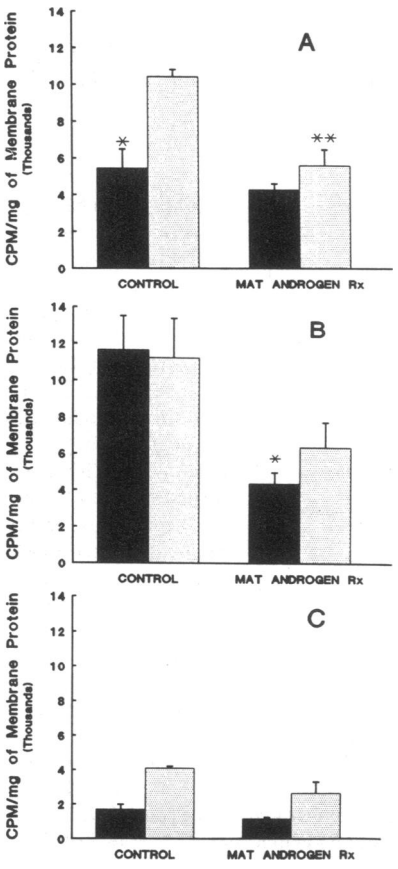

Figure 4. Specific binding of [^{125}I]EGF to androgen-treated fetal rabbit LPM. (*A*) Specific binding of [^{125}I]EGF to fetal rabbit LPM isolated at day 21 of gestation from both control and maternal androgen-treated groups. Mean±SEM of two to three experiments performed in triplicate. EGF binding to female LPM is significantly greater than male binding (*$P < 0.05$). EGF binding to androgen-treated female LPM is significantly lower than control female binding (**$P < 0.05$). (*B*) Specific binding of [^{125}I]-EGF to fetal rabbit LPM isolated at day 23 of gestation from both control and maternal-androgen treated groups. Mean±SEM of two to three experiments performed in triplicate. EGF binding to androgen-treated male LPM is significantly lower than control male binding (*$P < 0.05$). (*C*) Specific binding of [^{125}I]EGF to fetal rabbit LPM isolated at day 27 of gestation from both control and maternal androgen-treated groups. ■, Male; □ female.

to the fetal lung plasma membrane preparation was specific, since the binding was displaced by excess unlabeled EGF, but not by dihydrotestosterone (Fig. 6). The ontogeny of EGF binding seen in fetal rabbit lung, with maximal binding occurring before lung maturation, agrees with studies in the mouse (39) with maximal EGF binding occurring between days 13 and 14 of gestation (term 19 d). In the fetal lung EGF binding increases with the initiation of type II cell differentiation and diminishes with subsequent progression towards maturation.

The ontogeny of EGF binding in the fetal rabbit lung is sex-specific with significantly higher levels of binding occurring earlier in female (day 21 of gestation) than in male fetal lung plasma membranes (day 23 of gestation). This greater level of EGF binding in female than in male fetal lung at day 21 of gestation is consistent with the earlier EGF-stimulated enhancement of female explant surfactant phospholipid synthesis previously reported (25). The male lag in EGF binding had resolved by day 23 of gestation, at which time EGF binding had increased to a level similar to females. This was consistent with our previous work (25), in which male fetal lung explants did not respond to EGF-stimulated enhancement of maturation until day 24 of gestation.

Scatchard analysis of competitive binding experiments from day 21 of gestation revealed a single class of high affinity receptors. Scatchard binding from Fig. 2 could also have been interpreted using a two-compartment model of EGF binding with separate low and high affinity sites by resolving the binding data into two distinct straight lines asymptotic to the shallowest and steepest portions of a curvilinear versus a linear plot of the data. Some authors have done this and used these separate portions of their EGF receptor binding data to generate two distinct receptor subtypes with different affinities. We feel that it is equally valid to use a single-compartment model of affinity for the EGF receptor when interpreting EGF binding data since there is no biochemical or molecular evidence for the existence of two distinct purified EGF receptor subtypes (8, 10). This approach of analyzing all of the binding data with a single linear regression was also felt to be appropriate since the analysis resulted in very tight fit (all r values ≥ -0.9). Furthermore, the interpretation of our binding data to reflect a single class of high affinity EGF receptors is in agreement with similar K_d values that are reported in the literature (1, 8, 10). A potential cause for the curvilinear appearance of the binding data in Fig. 2 *B* could instead be the existence of negative cooperativity rather than two receptor subtypes (31). Negative cooperativity implies that the affinity of the receptor population as a whole continuously decreases as one increases the percentage of occupied or bound receptors leading to an upward concave (curvilinear) appearance of the Scatchard plot as maximum binding capacity (B_{max}) is approached (31). The concept of negative cooperativity may be applicable to our data.

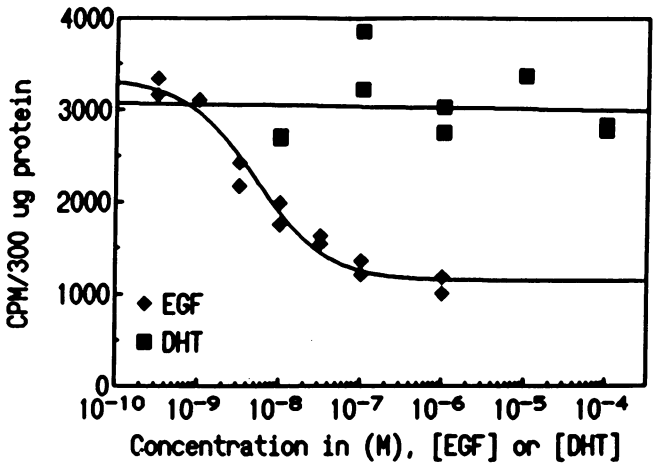

Figure 5. Competitive [^{125}I]EGF binding versus unlabeled EGF or DHT using female fetal rabbit lung plasma membranes isolated at day 23 of gestation. [^{125}I]EGF bound (cpm/300 μg of membrane protein) versus increasing concentrations of unlabeled EGF (◆) or DHT (■) expressed on a logarithmic scale. The binding of [^{125}I]EGF is highly specific since it is displaced by excess unlabeled EGF, but not by excess DHT.

EGF receptor density, as determined by Scatchard analysis, was significantly lower in male than in female fetal lung tissue with no difference in K_d (Table I, Fig. 2). The decreased EGF receptor density in males was consistent with their lower level of EGF binding at day 21 of gestation. Although previous studies (40, 41) have also identified a high affinity EGF receptor in fetal rabbit lung, they reported no difference in EGF receptor number or density between the sexes. However, these studies were performed with lung tissue isolated from either mature fetuses at term (40) or maturing fetuses at day 27 of gestation (41), not with lung tissue isolated early in development, i.e., day 21 of gestation. Both studies are consistent with our data that showed no sex differences in EGF binding in late gestation. Androgen-induced differences in EGF binding between the sexes occurred when the maximal difference in circulating levels of testosterone was present. The difference in plasma testosterone levels between male and female rabbit fetuses is five-fold at day 21 of gestation when the difference in EGF binding is seen, versus only twofold at day 27 of gestation (26) when no difference exists. Thus, endogenous fetal androgens present in normal male fetuses can modulate the level of EGF receptors available for binding at a discrete point (day 21) in rabbit fetal lung development.

Treatment with prenatal androgens inhibited the development of EGF binding activity in day-21 female fetal lung plasma membranes and minimized the sex difference in EGF binding. This inhibition of EGF receptor binding activity with exogenous androgens was also seen at day 23 of gestation in both sexes, blunting the natural rise in EGF binding. However, the androgen-induced inhibition of EGF receptor binding activity was no longer evident by day 27 of gestation. This is consistent with the natural ontogeny of EGF binding in the fetal rabbit lung which drops by over threefold between day 23 and 27 of gestation (Fig. 1). This normal developmental decrease in EGF binding during fetal lung maturation could mask any additional inhibitory effect due to exogenous androgens at day 27 of gestation. Scatchard analysis revealed that the inhibitory effect of exogenous androgens on EGF binding in both male and female androgen-treated fetuses was due to a decrease in EGF receptor density, not affinity.

The sex-specific difference in EGF binding was associated with a concurrent sex-specific difference in EGF induced autophosphorylation of EGF receptor. EGF-stimulated receptor autophosphorylation was lower in day-21 male fetal lung plasma membranes than in female membranes. This finding was consistent with the lower EGF receptor density and bind-

ing in male fetal lung tissue. Furthermore, this result was consistent with the EGF-stimulated enhancement of surfactant disaturated phosphatidylcholine synthesis in female but not male day-21 fetal lung explants (25), thus demonstrating a relationship between increased EGF receptor binding and EGF-induced autophosphorylation of EGF receptor with accelerated fetal lung differentiation.

The site of action for the inhibitory effect of androgens on EGF binding in fetal rabbit lung plasma membranes is not known. Androgens do not compete directly with EGF for the EGF receptor, since excess amounts of DHT did not displace bound [^{125}I]EGF (Fig. 5). This lack of competition for the receptor was seen even with DHT levels in vitro as high as 30 μg/ml (10^{-4} M), which greatly exceeds both the normal level of DHT (0.2 ng/ml) in the plasma of male fetal rabbits at day 21 of gestation (26) and the level of DHT in male fetal rabbits treated prenatally with exogenous DHT (0.9 ng/ml) (19).

The use of lung plasma membranes in our binding studies prevents us from addressing the issue of cell-specific effects of androgens on EGF receptor downregulation. The EGF receptor is known to be present in conducting airways (i.e., tracheobronchial epithelium) from both ovine (42) and human fetal lung tissue (43). The EGF receptor has also been detected in isolated cells from peripheral lung tissue (i.e., isolated adult rat type II cells (44). More importantly, in terms of fetal lung maturation, the EGF receptor has been detected in situ in prealveolar epithelium from late midtrimester human fetal lung tissue (45) and in human alveolar type II cells from differentiating fetal lung explants (46). The above immunolocalization studies of EGF receptor in peripheral lung tissue lend further support for a connection between the EGF receptor and fetal lung development.

The present study demonstrates that 5α-dihydrotestosterone decreases EGF receptor density and EGF induced autophosphorylation of EGF receptor in male fetal rabbit lung. This androgen-induced decrease in pulmonary EGF receptor binding activity may be one of the mechanisms responsible for the male delay in fetal lung development. We speculate that androgens decrease EGF receptors by decreasing steady-state levels of EGF receptor mRNA through either decreased transcription or increased degradation of message. Other hormones have been shown to affect levels of EGF receptor mRNA or EGF binding. Thyroid hormone increases EGF receptor mRNA levels in adult mouse liver (47, 48) and the absence of thyroid hormones (congenital hypothyroidism) delays EGF receptor binding in neonatal mouse liver (49). Similarly, estrogens increase EGF receptor mRNA levels in mouse uterine tissue (50). Future efforts will be directed towards evaluating the hormonal regulation of EGF receptor mRNA levels during fetal lung development.

Acknowledgments

This work was supported by a research training fellowship grant from the American Lung Association and National Institutes of Health grants R01 HL37930, R01 HL43407, and P30 HD27748.

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