Testosterone Regulation of Sex Differences in Fetal Lung Development (43379)

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Abstract. **Fetal lung development, in particular surfactant synthesis, exhibits a sexual dimorphism. Dihydrotestosterone (DHT) has been shown to delay fetal pulmonary surfactant production, but the potential role for testosterone is unknown. Both testosterone and DHT are potent masculinizing hormones, yet in some instances, an end organ specificity for DHT is present. We hypothesized that the delay in fetal lung surfactant production is dependent upon DHT such that inhibition of the synthesis of DHT from the precursor hormone testosterone would eliminate the sex difference by allowing the male fetus to produce surfactant at the female level. We tested this hypothesis using 17β-*N,N*-diethylcarbamoyl-4-aza-4-methyl-5α-androstane-3-one (4-MA), a potent inhibitor of the enzyme 5α-reductase, which converts testosterone into DHT. First, studies were performed *in vivo*. 4-MA (20 mg/kg/day) or an equivalent volume of vehicle was injected into pregnant rabbits from Day 12 through Day 26 of gestation. On Day 26, the fetuses were delivered, the lungs were lavaged, and fetal sex was noted. Treatment with 4-MA resulted in a lack of any male-female difference in the anogenital distance and no DHT was detected in the serum of any treated fetus. Phosphatidylcholine (PC), saturated phosphatidylcholine (SPC), and sphingomyelin (S) were measured in the lung lavage, and were expressed as the ratios of PC to sphingomyelin (PC:S) and SPC to sphingomyelin (SPC:S). Sex differences in the PC to sphingomyelin ratio of 4-MA-treated fetuses (female PC:S ratio, 1.43 ± 0.14; male PC:S ratio, 1.00 ± 0.13 [mean ± SE]; $P = 0.04$) and in the SPC:S ratio of the 4-MA-treated group (female SPC:S ratio, 0.68 ± 0.10; male SPC:S ratio, 0.35 ± 0.10; $P = 0.03$) were present after treatment with 4-MA. The effect of testosterone and of 4-MA on fibroblast pneumonocyte factor (FPF) production was studied *in vitro*. Fetal rat lung fibroblasts were cultured to confluence with either no added androgen, DHT, testosterone, or testosterone plus 4-MA, and conditioned media for FPF were prepared. Conditioned media were added to fetal Type II cell cultures and FPF activity was measured as the degree of stimulation of the incorporation of [^{3}H] choline into SPC. The conversion of radiolabeled testosterone to DHT by the fibroblasts was inhibited by 4-MA (10^{-5} *M*). Conditioned media from untreated female fibroblasts stimulated with cortisol exhibited significant FPF activity ([^{3}H]choline incorporation into SPC, 140 ± 17% of control). Conversely, conditioned media prepared from the fibroblasts treated with DHT, testosterone, or testosterone plus 4-MA exhibited no FPF activity. These data indicate that the mechanisms leading to delayed fetal lung development are regulated by testosterone as well as dihydrotestosterone.** [P.S.E.B.M. 1992, Vol 199]

Several aspects of fetal lung development exhibit a sexual dimorphism, including Type II cell differentiation, fibroblast-epithelial cell communications, and surfactant phospholipid production (1–3). The mechanism that causes the sex difference in surfactant synthesis is related to the process of fetal sexual differentiation and is under dihydrotestosterone (DHT) regulation (4, 5). The response of the fetal lung to DHT is dependent upon the presence of androgen receptors (5). Classically, both testosterone and DHT bind with high affinity to the same population of receptors. In some androgen target tissues, such as the wolffian duct, seminal vesicle, and skeletal muscle, testosterone and DHT are equally capable of inducing the androgen response. Other tissues, for example, the urogenital tubercle, external genitalia, and skin, specifically require DHT for an androgen response (6). Even though

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cytosolic androgen receptors in these tissues bind testosterone with normal affinity, this elicits no metabolic response. Testosterone is converted to dihydrotestosterone by the enzyme 5α-reductase. In general, 5α-reductase is present in tissues that are DHT dependent. The fetal lung fibroblast has 5α-reductase activity (7).

The studies that demonstrate DHT influences on fetal lung development do not indicate whether DHT is the necessary androgen for producing the effects. This question is not easily addressed using testosterone either *in vivo* or *in vitro*. For example, *in vivo*, the rabbit placenta aromatizes testosterone from the maternal circulation to estrogen (8). In addition, testosterone injections cause the pregnant rabbit to abort. *In vitro*, the 5α-reductase activity of the fetal lung tissue obscures the ability to separate the effects of testosterone and DHT in a tissue culture model.

The steroid analog 17β-*N,N*-diethylcarbamoyl-4-aza-4-methyl-5α-androstane-3-one (4-MA) specifically inhibits 5α-reductase by competition for the enzyme (9). This compound is saturated in the 5α-position and therefore is not metabolized by the 5α-reductase enzyme. It is the most potent 5α-reductase inhibitor currently known and does not exhibit intrinsic androgen, estrogen, or antiestrogen effects.

We postulated that the fetal lung is a DHT-dependent organ, and that blocking DHT synthesis would eliminate the sex difference by allowing the male fetus to produce surfactant at the female level. We used 4-MA to test this hypothesis *in vivo* and *in vitro*.

Methodology

In Vivo Studies. 4-MA was obtained as a gift from Dr. Jerry R. Brooks, Merck Institute (Merck, Sharpe, and Dohme, Rahway, NJ), and dissolved in triolein and ethanol (90:10, 75 mg/ml). Timed-pregnancy New Zealand White rabbits (Hickory Hill Farms, Flint, TX) were used; the day of mating was denoted as Day 0 of gestation. The pregnant does were injected subcutaneously with either 4-MA (20 mg/kg/day) or with an equivalent volume of vehicle beginning on Day 12 and continuing through Day 26 of gestation, at which time the does were sacrificed and the fetuses were delivered and weighed. Day 26 was studied because it is the date on which a sex difference in tracheal lavage surfactant first appears (2). This also was the day previously studied in a similar experimental protocol, in which it was found that DHT caused inhibition of surfactant production (4). Blood was obtained from the fetal neck veins and centrifuged to obtain serum for assay of testosterone and DHT. A tracheostomy tube was placed and the lungs were lavaged with five aliquots each of 0.5 ml of ice-cold normal saline, as in previous studies (2, 4). After lung lavage, the fetal sex was identified by inspection of the gonads (10). Lavages and serum were stored at −40°C until assayed.

The lung lavage of each fetus was extracted with chloroform and methanol (11) and split into two equal aliquots. Lavages that were grossly bloody were not assayed. One aliquot was reacted with osmium tetroxide to isolate saturated phosphatidylcholine (12). The other aliquot was used for measurement of phosphatidylcholine and sphingomyelin. Phosphatidylcholine (PC), saturated phosphatidylcholine (SPC), and sphingomyelin (S) were separated by thin layer chromatography on silica gel H sheets (Eastman Kodak, Rochester, NY) in chloroform, methanol, and water (65:25:4). The resulting spots were scraped and assayed by phosphorus analysis (13). The results were expressed as PC:S and SPC:S ratios (2, 4).

The effectiveness of 4-MA treatment on testosterone metabolism in the fetal rabbit was assessed both by measuring DHT levels and, in some fetuses, by measuring the anogenital distance, a DHT-dependent feature of sexual differentiation. Serum collected from individual fetuses was extracted with ethyl acetate. Testosterone and dihydrotestosterone were separated by column chromatography and measured by radioimmunoassay (lower limit of sensitivity, 10–20 pg/ml) courtesy of Frederick W. George, Ph.D., at the University of Texas Health Science Center at Dallas. The characteristics and standardization of this assay were published previously by Dr. George and colleagues (14). Studies by Dr. George showed that 4-MA does not compete for binding to the antibody when added in 100–1000-fold excess (14). The anogenital distance was measured in duplicate using an eyepiece micrometer in a dissecting microscope at magnification ×10. The duplicate measurements were averaged to give a single value per fetus.

In Vitro Studies. Fibroblast and Type II cell cultures were prepared from male and female Day 19 fetal rats using procedures that we have described previously in detail (15, 16). Fibroblasts were cultured to confluence in minimal essential medium (MEM) + 10% charcoal-stripped fetal calf serum. During this culture time, all fibroblast cultures received one of the following treatments daily: (i) no added hormone; (ii) DHT (10^{-8} *M*); (iii) testosterone (10^{-8} *M*); (iv) testosterone (10^{-8} *M*) + 4-MA (both 10^{-8} *M* and 10^{-5} *M*); or (v) testosterone (10^{-8} *M*) + 4-MA carboxylate (both 10^{-8} *M* and 10^{-5} *M*). The hormones DHT, testosterone, and 4-MA were dissolved in ethanol at 10^{-6} *M* and 10^{-3} *M*. Controls received an equivalent volume of ethanol except in one experiment. We have shown previously that ethanol at this volume has no effect on these cultures (15, 16). The total amount of ethanol added to all cultures was the same and was not more than 1% of the total culture media volume. The 4-MA carboxylate is the water-soluble analog to 4-MA and was dissolved in distilled water at 10^{-6} *M* and 10^{-3} *M*. When fibroblasts reached confluence, conditioned media for fibroblast pneumonocyte factor (FPF) activity were prepared by changing all culture media to serum-free MEM +

10^{-8} M cortisol. These conditioned media were collected 24 hr later.

Conditioned media prepared from male and female fibroblasts from each of the above five conditions were tested for FPF activity using the Type II cell cultures. The ability of conditioned media to stimulate the incorporation of [^{3}H]choline into SPC was determined as the index of FPF activity, as described previously (16). Culture media on the Type II cells were replaced with 0.5 ml of MEM, 0.5 ml of conditioned media, and 1 μCi of [^{3}H]choline (sp act 30–50 Ci/mmol; Amersham, Arlington Heights, IL). After incubation for 24 hr, the Type II cells were harvested and counted by electronic particle counting. SPC was isolated from cell homogenates by the procedure described above for lavage fluids, and labeled SPC was measured by scintillation counting. The results were normalized to those of simultaneous control Type II cells (which received only MEM with no conditioned media) to give an index of the activity of FPF to stimulate SPC synthesis in Type II cells. Six experiments were performed, with three to five observations per experiment. The data are reported as the mean ± SE of the averages of each experiment.

The ability of 4-MA and of 4-MA carboxylate to block the conversion of testosterone to DHT by the fetal lung fibroblast cultures was studied using the methodology of Sultan *et al.* (7) and Brooks *et al.* (9), as modified by us. Fetal rat lung fibroblasts (Day 19 of gestation, separated by fetal sex) were obtained and cultured as above in six-well culture plates. Cells were grown to confluence in the same five conditions for male and female as above. At confluence, the cells were washed with serum-free MEM, then exposed for 24 hr to MEM containing 1 μCi/ml of [1α,2α-^{3}H(N)]-testosterone (sp act 40 Ci/mmol; NEN Research Products, Dupont, Wilmington, DE). At this time, the media were collected and the cells were washed with phosphate-buffered saline, which was added to the collected media. The steroids were extracted from the media with carbon tetrachloride and plated on silica gel H thin layer chromatography sheets, along with standards for testosterone, DHT, and androstenedione. The steroids were separated by chromatography in chloroform and ether (90:10) and visualized by iodine staining. The silica gel spots were scraped into scintillation vials and the amount of labeled steroid was measured by scintillation counting. Two to three experiments were performed, with three replicates per experiment. Again, the data are reported as the mean of the averages of each experiment.

Results

In Vivo Experiments. Injection of 4-MA into pregnant rabbits did not appear to cause an increase in fetal abortions or otherwise lead to increased fetal wastage. Four fetuses were eliminated from any studies, one because of apparent multiple congenital anomalies (control) and three because of marked reduction in birth weight (two control group, one 4-MA group: birthweight <58% of mean body wt of all other control or 4-MA fetuses). The mean body weights of the remaining males and females in the control and treatment groups were not different (mean birth wt ± SD, 23.3 ± 3.9 and 24.3 ± 3.8 g for females and 24.7 ± 2.4 and 24.4 ± 2.7 g for males in the control and 4-MA groups, respectively).

The effectiveness of 4-MA in blocking 5α-reductase activity was studied at the metabolic level by measuring serum levels of testosterone and DHT, and in an additional group of fetuses at the physiological level by measuring the anogenital distance. This distance normally exhibits a sexual dimorphism (male > female) that is dependent upon DHT and is a sensitive measure of the biologic activity of DHT (17–19). Administration of 4-MA appeared highly effective in blocking 5α-reductase activity, inasmuch as no DHT was found in the serum of any treated fetus (Fig. 1). Estimation of a P-value for the comparison of the DHT levels in the 4-MA treatment group to those in the control group was done by assuming a mean serum DHT level at the lower limit of detection by this assay, with a variance equal to the control group. Under this assumption, the reduction in DHT levels in the 4-MA treatment group was significant at $P = 0.03$. This assumption may underestimate the true magnitude of the significance of the difference. The mean testosterone value was somewhat higher in the 4-MA-treated group, but the difference was not significant. Measured values of serum testosterone and dihydrotestosterone in control fetuses tended to be quite variable from fetus to fetus, apparently because of inherent difficulties in measuring serum androgens in fetal rabbits (20). The anogenital distance in control fetuses exhibited the typical sex difference (male, 0.53 ± 0.020 mm; female, 0.40 ± 0.005 mm [mean ± SE]; n = six males and five females; $P = 0.02$). On the other hand, the anogenital distance of 4-MA-treated fetuses showed no male-female difference (male, 0.44 ± 0.015 mm; female, 0.43 ± 0.012

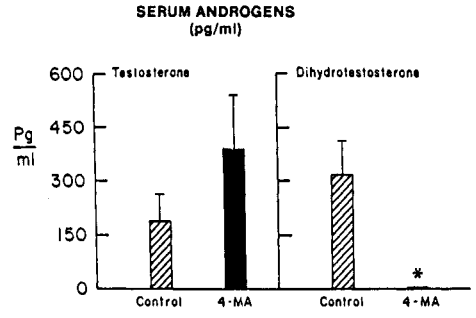

Figure 1. Serum testosterone and dihydrotestosterone levels for control and 5α-reductase inhibitor (4 MA)-treated fetuses, mean ± SE. n = 23 for the control values and n = 18 for the 4 MA values. Asterisk denotes 4-MA treatment group less than controls, $P = 0.03$ by Student's two tail t test (see Results for details of this comparison).

mm; $n = 12$ males and 15 females). These treated male and female values were not significantly different from untreated females and were significantly less than untreated males ($P < 0.01$).

The results for the PC to sphingomyelin ratio are shown in Figure 2. As in our previous studies, a sex difference in the PC:S ratio was not apparent in the control fetuses. There was a significant male-female difference in the PC:S ratio of female and male fetuses from 4-MA-treated does (female PC:S ratio, 1.43 ± 0.14; male PC:S ratio, 1.00 ± 0.13; $P = 0.03$); however, these values were not significantly different from those for controls of the same sex.

The results for the SPC:S ratio are shown in Figure 3. In agreement with all previous studies, a sex difference in the SPC:S ratio of control fetuses was noted (female SPC:S ratio, 0.65 ± 0.07; male SPC:S ratio, 0.35 ± 0.08; $P = 0.009$). Blocking the formation of DHT did not affect the observed sex difference. The 4-MA treatment group continued to exhibit the same male-female difference (female SPC:S ratio, 0.68 ± 0.10; male SPC:S ratio, 0.35 ± 0.10; $P = 0.03$), in spite of the fact that no DHT was measured in the serum of any of these fetuses and that no biologic activity of DHT was noted.

In Vitro Experiments. Studies were done in tissue culture to further confirm a role of testosterone in delaying lung maturation and to test a potential mechanism by which this occurs. Previous studies of the production of FPF by the fetal lung fibroblast demonstrated that this factor is made earlier in gestation by female fibroblasts than by male fibroblasts (Day 19 of gestation in the fetal rat), and that FPF synthesis is blocked by DHT (15). Therefore, we studied whether FPF synthesis would be blocked by testosterone when the conversion of testosterone to DHT was blocked by 4-MA. The water-soluble carboxylate form of 4-MA (4-MA carboxylate) was also used in these studies to help show that a toxic effect of the ethanol carrier on the culture conditions was not involved.

The conversion of radiolabeled testosterone to DHT was studied to determine the effectiveness of 4-MA and of 4-MA carboxylate at inhibiting 5α-reductase in the fetal lung fibroblast cultures. The results are shown in Figure 4. At the equimolar dose of 10^{-8} M, neither 4-MA nor 4-MA carboxylate had any significant effect on the conversion of testosterone to DHT. At a 1000-fold molar excess dose of 10^{-5} M (chosen to effectively compete for the enzymatic reaction), there was a significant reduction of conversion of testosterone to DHT. The differences in conversion of testosterone

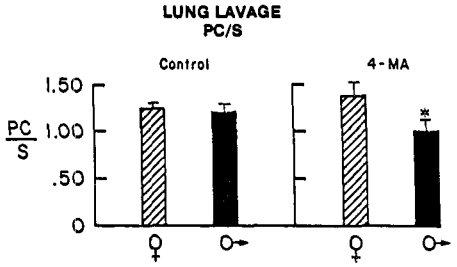

Figure 2. Phosphatidylcholine to sphingomyelin ratio in lung lavage of females and males in the control and 5α-reductase inhibitor (4-MA) treatment groups. Each bar represents the mean ± SE. $n = 9$–16. Asterisk denotes males less than females, $P = 0.03$ by Student's two tail t test.

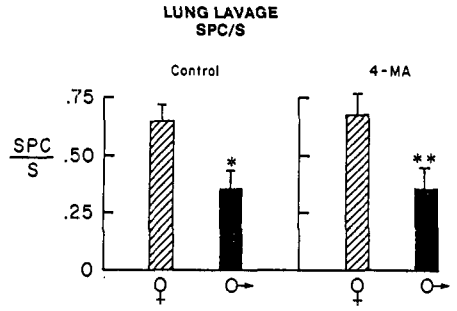

Figure 3. Saturated phosphatidylcholine to sphingomyelin ratio in lung lavage of females and males in the control and 5α-reductase inhibitor (4-MA) treatment groups. Each bar represents the mean ± SE. $n = 9$–16. Asterisks denote males less than females, $P = 0.03$ by Student's two tail t test, and ** males less than females, $P = 0.009$ by Student's two tail t test.

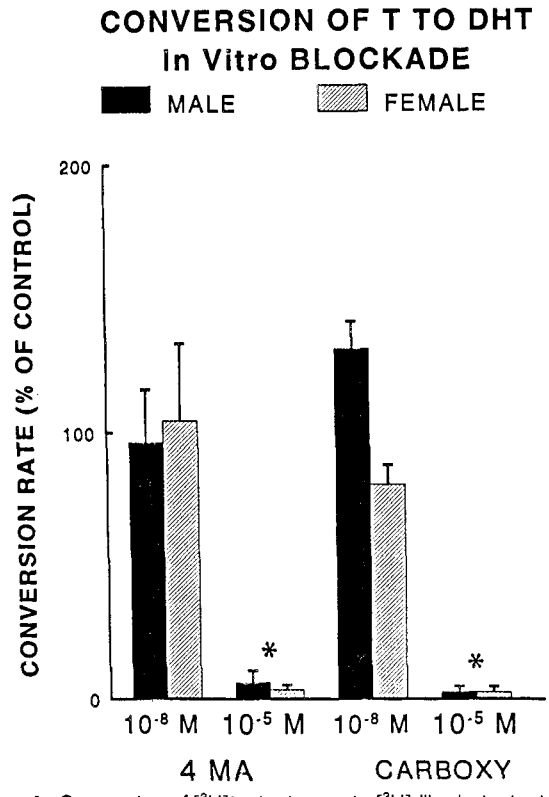

Figure 4. Conversion of [^{3}H]testosterone to [^{3}H]dihydrotestosterone by male and female fetal rat lung fibroblasts. Data are expressed as the percentage of conversion of testosterone to DHT in the presence of either the 5α-reductase inhibitor 4-MA (10^{-5} M and 10^{-8} M), or of its water-soluble analog 4-MA carboxylate (10^{-5} M and 10^{-8} M) compared with simultaneous controls. Each bar represents the mean ± SE of two to three experiments, each done in triplicate. Asterisk denotes 10^{-5} M treatment less than 10^{-8} M treatment, $P < 0.05$ by Student's two tail t test.

to DHT between these two doses were significant at $P < 0.05$. The conversion of testosterone into androstenedione was extremely small in all experiments and was not affected by 4-MA or 4-MA carboxylate (data not shown).

The stimulation by fibroblast conditioned media of [^{3}H]choline incorporation into SPC in fetal lung Type II cells was studied as the index of FPF production by the fibroblast. Neither male nor female conditioned media, which received no hormone treatment (particularly, no glucocorticoid treatment), exhibited any FPF activity. FPF activity was observed in the conditioned media of cortisol-treated female fibroblasts ([^{3}H]choline incorporation into SPC, 140 ± 17% of control; $P = 0.06$), but not in the media of male fibroblasts ([^{3}H] choline incorporation into SPC, 114 ± 19% of control). Fibroblasts grown in either DHT or testosterone produced no FPF activity in their conditioned media when stimulated by cortisol (female: DHT condition, 118 ± 15% of control; testosterone condition, 115 ± 10% of control; male: DHT condition, 112 ± 20% of control; testosterone condition, 82 ± 15% of control). When either 4-MA or 4-MA carboxylate in low dose (10^{-8} *M*) was added to cultures containing testosterone, there continued to be no evidence of FPF activity (for example, female: 4-MA condition, 103 ± 14% of control; 4-MA carboxylate condition, 115 ± 18% of control; male results were similar). These results were expected, since there was no significant blockade of 5α-reductase activity at this dose. At the higher dose of 4-MA and 4-MA carboxylate (10^{-5} *M*), at which significant reduction of DHT formation was present, there was still no presence of FPF activity in the conditioned media (for example, female: 4-MA condition, 91 ± 7% of control; 4-MA carboxylate, 101 ± 2% of control). The data from these experiments are consistent with previous descriptions of the female advantage in appearance of FPF activity and with the observed effect of DHT as an inhibitor of FPF synthesis (15).

Discussion

Testosterone and dihydrotestosterone each play important roles in the regulation of fetal growth and differentiation of both reproductive and nonreproductive tissues. Both compounds have high affinities for the same androgen receptor, but the two hormones are not necessarily interchangeable in function. Several organs require dihydrotestosterone to become androgenized. Evidence suggests that this target specificity may be a result of two factors: a somewhat higher affinity of the androgen receptor for DHT (6), and an apparent greater ease in activating the receptor-DHT complex to bind DNA (21). Therefore, it has been suggested that DHT may serve as a signal amplifier for specific types of events. Since maximal male-female differences in serum DHT and testosterone levels are relatively small (2–4-fold in fetal rabbit) (20), such signal magnification by DHT might be a convenient explanation of why androgenic delay of lung maturation is manifested in males. Although other, non-receptor-mediated effects of DHT and testosterone on development are known (22, 23), these are unlikely to be active in the fetal lung, as shown by studies in the androgen receptor-deficient Tfm mouse (5).

A critical issue in interpreting the present studies is whether 4-MA effectively prevented the synthesis of enough DHT to cause a metabolic effect on the lung. The effectiveness of 4-MA at blocking 5α-reductase activity in the *in vivo* experiments is best demonstrated by the lack of masculinization of the anogenital distance in males and further confirmed by the failure to detect any DHT in the serum. It is, however, possible that very minute amounts of DHT may have been present in the lung or genital tissue of the 4-MA-treated fetuses, since inhibition of 5α-reductase by substrate competition probably cannot be expected to be 100% effective. Nevertheless, the results of the present study indicate that even if very small levels of tissue DHT are present in the female fetal lung, surfactant production is unaffected and the premature male lung remains less mature than the premature female lung.

The values reported here for fetal rabbit lavage PC to sphingomyelin and SPC to sphingomyelin ratios in male and female fetuses are consistent with those reported earlier by us (2, 4). The role of DHT as an inhibitor of fetal lung maturation has been clearly shown in a variety of experiments in rabbits (4), as well as in rats and mice (5, 15). We have shown previously that the antiandrogen flutamide reverses the effects of DHT on the developing fetal rabbit lung *in vivo* (4). A dose-response relationship between DHT and the degree of inhibition of lung maturation in the fetal rabbit was also demonstrated; it was found that female circulating androgens needed to be elevated at least to normal male levels in order to delay female lung surfactant production (4). With these points in mind, the observed sex difference in surfactant production in the 4-MA-treated fetuses must be attributed to some regulator other than DHT. A primary role of testosterone in causing the sex difference is very likely when this study is viewed in the context of the above-mentioned work relating fetal sexual differentiation, dihydrotestosterone, antiandrogen, and androgen receptors to the expression of the sex difference.

We used rabbits for the *in vivo* and rats for the *in vitro* aspects of this study because of their suitability for those particular studies. Fetal rabbit lungs are more readily cannulated and lavaged. Fetal rat fibroblast and Type II cell cultures have been much better characterized in terms of development of FPF activity, Type II cell culture purity, and sex differences in culture responses. Overall, studies of rabbit and rat lung development, especially in regard to sex differences and cell-cell interaction mechanisms, are very similar between

the species, and studies in one are generally confirmed in the other (24).

The *in vitro* experiments are consistent with known effects of androgen on cell-cell communications in fetal lung development. Fibroblasts were cultured in medium containing fetal calf serum stripped twice with charcoal, which we have previously reported contains less than 10^{-12} *M* concentrations of both testosterone and DHT. Therefore, only the added androgen could affect the production of FPF. Studies of testosterone and DHT were done at 10^{-8} *M* concentrations because this is the level demonstrated in several previous studies to effectively inhibit FPF synthesis in tissue culture (5, 15). Since 4-MA inhibits 5α-reductase by competing for the enzyme, we studied two different concentrations of 4-MA and 4-MA carboxylate: an equimolar amount, at which we expected little inhibition of 5α-reductase, and a 1000-fold excess, which we expected to effectively out-compete testosterone for the reductase enzyme. The results of our assays of conversion of radiolabeled testosterone to DHT indicate that this did indeed occur. The subsequent assays of fibroblast conditioned media for FPF activity are consistent with our results obtained *in vivo* in the fetal rabbit. Even when testosterone is converted to DHT at only a very low rate, there continues to be inhibition of mechanisms involved in stimulating surfactant synthesis, suggesting that testosterone is an active hormone for this androgenic effect. This refutes the hypothesis we made when starting these studies, that the lung would be a DHT-dependent organ.

Other explanations of observed delays in FPF and surfactant production might be possible. For example, one alternative is that other metabolites of testosterone are the major active agents causing delayed surfactant synthesis (25). The fetal lung fibroblast is capable of metabolizing testosterone into its weak androgenic metabolites and to aromatize testosterone into estrogen. However, the rate of conversion is relatively low, such that *in vitro*, only about 12% of testosterone is metabolized to estrogen and very much smaller amounts to the weak androgens (J. S. Torday, personal communication, 1989). We observed no increase in the relative production of radiolabeled androstenedione with 5α-reductase inhibition in this study, and the amount converted was very small. The proportions of these metabolites in the fibroblast cultures (one to two orders of magnitude less than the level of testosterone) are very unlikely to have any significant effect on FPF synthesis or surfactant production (26).

A second alternative is raised by the work of Catlin and colleagues (27) showing that müllerian inhibiting substance (MIS) inhibits fetal lung SPC synthesis. MIS is also an important regulator of fetal sexual differentiation. Its major effect is to cause regression of the müllerian duct in the male fetus, a process not regulated by androgen. MIS may have elicited the male delay observed in this study *in vivo*. However, it is unlikely to have caused inhibition of FPF production by fibroblasts treated with testosterone *in vitro*. The mechanism by which MIS inhibits lung development is not clear and requires further study.

The modulation by androgen of the development of the fetal lung is incompletely understood; nevertheless, the results of this study may assist in forming a more clear picture of the process. Testosterone-mediated events frequently involve interactions between mesothelial or mesenchymal structures (such as the fibroblast) and the epithelium. In keeping with this, we found that testosterone, like DHT, inhibits the production of FPF by the fetal lung fibroblast. Since the fetal lung fibroblast has 5α-reductase activity, the use of 4-MA to competitively block this activity was important to show that testosterone itself affected lung development. These results are consistent with the reports that DHT affects fetal lung development and cell-cell communications through an androgen receptor-mediated mechanism (5, 15). DHT has also been shown to exert a growth-promoting effect on the fetal lung while delaying lung maturation (28). Such an effect is also consistent with testosterone-masculinizing actions on other systems.

Thus, androgens (testosterone and dihydrotestosterone) appear to influence the cell-cell interactions that bring about the development and differentiation of the fetal lung. Some of the mechanisms by which testosterone regulates cellular growth in other organs are similar to those proposed here for the fetal lung, that is, influences on growth and differentiation via cell-cell interactions. The regulatory mechanisms may be sufficiently sensitive that signal amplification by DHT is not required. In fact, these data allow the speculation that the delay of lung development by androgen is not an unfortunate byproduct of the masculinization process, but rather that androgen has an important, as yet undefined, role in both male and female lung development that is more pronounced in the male consequent to the higher levels of androgens (28). Furthermore, these results, when fitted into the overall mechanistic concept, emphasize the importance of determining what process is initiated by the androgen-activated receptor complex which results in delayed lung maturation. A more clear understanding of this process will be of great use in understanding the controls of fetal growth and differentiation.

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