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Androgen Formation and Metabolism in the Pulmonary Epithelial Cell Line A549: Expression of 17β-Hydroxysteroid Dehydrogenase Type 5 and 3α-Hydroxysteroid Dehydrogenase Type 3*

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ABSTRACT

Surfactant synthesis within developing fetal lung type II cells is affected by testosterone and 5α-dihydrotestosterone (5α-DHT). The pulmonary epithelial cell line A549, isolated from a human lung carcinoma, like normal lung type II cell, produces disaturated phosphatidylcholines and has been widely used for studying the regulation of surfactant production. Androgen receptor has been detected in A549 cells; however, the capacity of these cells for androgen synthesis and metabolism has not been investigated at molecular level. This study was undertaken to identify the steroidogenic enzymes involved in the formation and metabolism of androgens from adrenal C19 steroid precursors in A549 cells. When cultured in the presence of normal FCS, A549 intact cells converted DHEA to androstenediol, androstenedione principally to testosterone, and 5α-DHT to 5α-androstane 3α,17β-diol. High levels of 17β-hydroxysteroid dehydrogenase (HSD) and 3α-HSD activities were detected in both cytosol and microsomes isolated from homogenates. Analysis of A549 RNA indicated the presence of 17β-HSD type 4 and type 5, and of 3α-HSD type 3 messenger RNAs. Very low levels of 3β-HSD type 1 and 5α-reductase type 1 messenger RNAs and activities were detected. With regard to active androgen formation, there was little or no capacity for the conversion of DHEA to 5α-DHT. In contrast, androstenedione was rapidly transformed to testosterone. The pattern of steroid metabolism was not affected by the use of charcoal-stripped FCS or by the synthetic glucocorticoid dexamethasone. Together, our findings show that A549 cells express a pattern of steroid metabolism in which 17β-HSD type 5 and 3α-HSD type 3 are the predominant enzymes. The level of androgens is regulated at the level of catalysis in intact cells such that the intracellular level of testosterone is stabilized, whereas 5α-DHT is rapidly inactivated by reduction to 3α,17β-diol. This pattern of androgen metabolism has implications for the relative importance of testosterone and 5α-DHT in normal lung development and surfactant production. (*Endocrinology* **141:** 2786–2794, 2000)

DURING fetal lung development, the production of pulmonary surfactant by alveolar type II cells is under multihormonal control. Glucocorticoids accelerate the synthesis of surfactant phospholipids and proteins, whereas sex steroids exert opposing effects, with estrogens accelerating and androgens inhibiting (1–4). Testosterone and 5α-dihydrotestosterone (5α-DHT) have been shown to delay pulmonary surfactant production in glucocorticoid-treated and untreated human fetal lung tissue samples in organ culture (*e.g.* Ref. 5). Similar deleterious effects of androgens on fetal pulmonary surfactant production have been also demonstrated *in vivo* in 5α-DHT-treated pregnant rats (6) and in pregnant rabbits treated with 4-MA, a potent inhibitor of the enzyme 5α-reductase (7). Current findings suggest that these steroid effects are mediated by the interaction of testosterone or 5α-DHT with androgen receptors in lung fibroblasts and/or alveolar type II cells.

Analyses of C19-steroid metabolism in whole lung tissue in the 60s and 70s (8, 9) provided the first evidence of the presence of a number of steroidogenic enzyme activities. From their studies of lung slices, Milewich *et al.* (10) concluded that 3α-hydroxysteroid dehydrogenase (3α-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) were the principal C19-steroid-metabolizing enzymatic activities in the human lung with a cofactor milieu that favored reduction of 17-ketosteroids to 17β-hydroxysteroids. The detection of low 5α-reductase and 3β-hydroxysteroid dehydrogenase/Δδ5-Δ4 isomerase (3β-HSD) activities (10–12) suggested further that human lung tissue may have limited ability to produce *in situ*, the hormonally-active androgen 5α-DHT from inactive adrenal C19-steroid precursors.

It is now established that there are multiple isoforms of hydroxysteroid dehydrogenases and 5α-reductases. These are products of separate genes and differ in their substrate and cofactor specificity, subcellular and tissue localization,

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and catalytic behavior within intact cells (13–15). These enzymes are widespread in human tissues and it is currently believed that, within a hormone-responsive tissue, the pattern of steroidogenic enzyme gene expression determines steroid hormone action (13). Four enzymatic activities regulate the formation and inactivation of testosterone and 5α-DHT from adrenal-derived DHEA or androstenedione in human peripheral tissues (Fig. 1). These activities are carried out by the enzymes 3β-HSD, 5α-reductase, 17β-HSD, and 3α-HSD. The latter two-enzymatic activities are of particular interest with regard to regulation of active androgen levels because they are reversible. As a result, the net direction of 17β-HSD reaction will influence the ratio of biologically active 17β-hydroxysteroids to inactive 17-ketosteroids within a tissue. In fact, the 17β-HSD and the 3α-HSD activities depend on the balance between specific isoform of each enzyme, which is believed to determine the amount of hormone that can bind to members of the nuclear steroid receptor superfamily and thus, ultimately, modulate the expression of androgen responsive genes.

The A549 epithelial cell line was established from a lung adenocarcinoma isolated from an adult man (16). These type II epithelial-like cells, like normal lung type II cells synthesize disaturated phosphatidylcholines (DSPC) *in vitro* (17–19), and they have been used in many studies as a model system for studying the regulation of pulmonary surfactant production (20–23). Although A549 cells contain androgen receptor at a relatively high level (24), the enzymology regulating androgen receptor occupancy in these cells has not been examined at molecular level. This investigation was undertaken to estimate the intracrine capacity of A549 cells for testosterone and 5α-DHT formation and metabolism and to identify genes responsible for these activities. The effect of the synthetic glucocorticoid dexamethasone on steroid metabolism was also investigated.

In this report, we describe an enzymology in A549 cells wherein 17β-HSD type 5 and 3α-HSD type 3 are the predominant steroidogenic enzymes and their activities, combined with low 5α-reductase, integrated within intact cells to maintain high levels of testosterone while rapidly inactivating 5α-DHT.

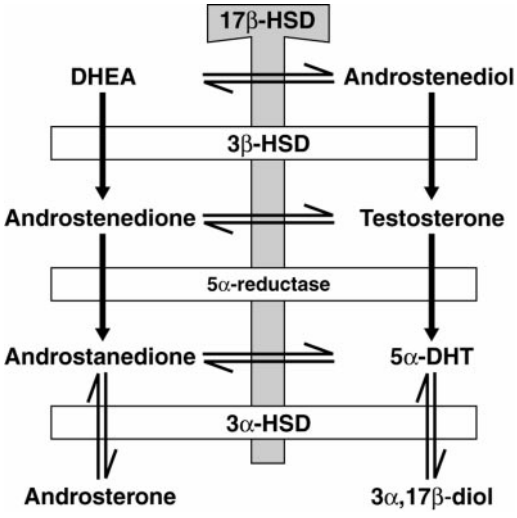

FIG. 1. Biosynthetic steps involved in the formation in peripheral target tissues of the androgens testosterone and 5α-DHT.

Materials and Methods

Chemicals

Reagents that were purchased: [1,2,6,7-^{3}H]testosterone (95.0 Ci/mmol), [6,7-^{3}H]estradiol 17β (51.0 Ci/mmol), [1,2,6,7-^{3}H]androstenedione (93.0 Ci/mmol), [2,4,6,7-^{3}H]estrone (114 Ci/mmol), [9,11-^{3}H]5α-androstane-3α,17β-diol, (40–60 Ci/mmol), [1,2-^{3}H]androst-5-ene-3β,17β-diol, (40–60 Ci/mmol) and 5α-dihydro[1,2-^{3}H]testosterone (60 Ci/mmol) from Amersham Pharmacia Biotech (Arlington Heights, IL); [1,2,6,7-^{3}H]dehydroepiandrosterone (100 Ci/mmol) from NEN Life Science Products (Boston, MA); unlabeled steroids from Steraloids (Wilton, NH); HEPES, Bicine, NAD(P), NAD(P)H and BSA from Sigma (St. Louis, MO); FCS from Life Technologies, Inc./BRL (Burlington, Ontario, Canada) and from HyClone Laboratories, Inc. (Logan, UT); Ecolume from ICN Radiochemicals (Irvine, CA).

Cell cultures

A549 cells (CCL 185; American Type Culture Collection, Manassas, VA) were grown in DMEM-HEPES-high glucose medium (Life Technologies, Inc., Gaithersburg, MD) containing, 2.2 g/liter NaHCO3, 100 IU/ml penicillin, and 50 μg/ml streptomycin (DMEMHG) supplemented with heat-inactivated (56 C/20 min) FCS (10%, vol/vol). The medium was changed every 2 days.

Steroid metabolism by A549 cultures

Intact 10^5 cells in culture were incubated with 1 ml of DMEMHG containing [^{3}H]-steroid (2×10^6 dpm; 9.6 nM) for the indicated time periods and steroids measured in the medium. Steroids were extracted twice with 5-ml diethyl ether, evaporated and applied to silica gel-coated TLC plates and resolved in toluene-acetone-chloroform (8:2:5, vol/vol). Androsterone and 5α-DHT were separated on silver nitrate-conditioned aluminiumoxid 60 F254 neutral TLC plates (VWR, Montréal, Québec, Canada) developed with toluene-acetone (4:1, vol/vol) (25). Experiments were repeated with three different cultures and each time-point was assayed in triplicate.

Effects of different batches of FCS and of dexamethasone on A549 cells

Experiments with Dex (2.5×10^{-7} M) were conducted with cells cultured in DMEMHG supplemented with 10% (vol/vol) FCS or with dextran-coated and charcoal treated FCS (FCS-A). Steroid stripping was done by adding 1 g of activated charcoal (Norit A, Fisher Inc., Burlington, Ontario, Canada) and 2 ml of 5% (wt/vol in water) of dextran T-70 (Pharmacia & Upjohn, Baie d'Urfé, Québec, Canada) to 100 ml of FCS. After stirring (overnight at 4 C), charcoal was removed by two run of centrifugation at $6000 \times g$, 30 min at 4 C. After a second step of adsorption (3 h, RT), the FCS-A was filtered (0.22 μM) and stored at −20 C until used. Three different lots of FCS were used. Intact cells (10^5 cells/0.8 cm^2) were plated. The following day, the medium was changed and replaced either with DMEMHG-FCS or DMEMHG-FCS-A. After 24h, [^{3}H]-androstenedione (2×10^6 dpm, 9.6 nM) was added to the cells in the presence or absence of Dex, and incubations pursued for 24 h. The identity of each steroid, in particular 5α-DHT was confirmed by HPLC analysis using a Shimadzu model 10A chromatograph and a C18 column (Ultra sphere, 0.5 μM, 4.6 × 150 mm). An isocratic elution by water-acetonitrile-tetrahydrofuran (65:23:12, vol/vol) was used as previously described (26).

Cortisol determination in FCS and FCS-A

The levels of cortisol were determined by a heterogeneous competitive magnetic separation assay according to the manufacturer instructions (Bayer Corp., Tarrytown, NY). The lower limit of detection was 6.0 nM.

Subcellular fractionation

10^7 cells were resuspended in 1 ml of ice-cold buffer containing 20% (vol/vol) glycerol, 1.0 mM EDTA, and 4 mM potassium phosphate, pH 7.0 (KPBS). Samples were homogenized by hand in an all-glass Dounce homogenizer fitted with a B-pestle, centrifuged at 1000 × *g* to remove cell debris, and again at 105,000 × *g* for 1 h. Supernatants were saved as cytosol. The microsome-enriched pellets were washed, subjected to a second round of ultracentrifugation, and resuspended in 1 ml KPBS. Completeness of homogenization was evaluated by light microscopy. Proteins were measured by the method of Bradford (27).

Enzymatic assays on cytosolic and microsomal fractions

17β-HSD activity was measured as we described previously (28, 29). Briefly, a 10-μl aliquot of cytosol or microsomes was combined with 10 μl of reaction mixture containing 0.5 mM nicotinamide nucleotide cofactor and 1.0 μM ^{3}H-labeled steroid substrate in 0.08 M HEPES, pH 7.2, for both reductase and dehydrogenase activities. Assays were run at 37 C. Reaction mixtures (total) were transferred to the adsorbant layer of silica gel HL plates and analyzed by TLC (30, 31). The activities of 3α-HSD, 5α-reductase and 3β-HSD were respectively assayed with tritiated 5α-DHT, testosterone or DHEA. Following TLC, substrate and products were localized by a light misting with water and scrapped into 10 ml of Ecolume for scintillation counting. Percent conversion was calculated as the ratio of cpm recovered in product/cpm in substrate plus products. This value was transformed into picomoles per mg protein per 30 min incubation as described previously (32).

TABLE 1. Metabolism of A549 intact cells incubated with several steroids for 24 h

Steroid incubated [^{3}H]	Steroid recovered from the medium at the end of the incubation	% of radioactivity present in the medium after 24 h of incubation[a]
DHEA	DHEA	31 ± 1
	Androstenediol	69 ± 2
Androstenedione	Androstenedione	30 ± 4
	Testosterone	44 ± 3
	3α,17β-diol	9 ± 2
	Androsterone	10 ± 1
5α-DHT	5α-DHT	6.0 ± 0.5
	3α,17β-diol	95 ± 2
Testosterone	Testosterone	77 ± 2
	3α,17β-diol	14 ± 1

The values are the percentage (%) of the total specific radioactivity recovered from the medium in substrate and products.

[a] Data are the means ± SD of three different experiments.

RNA preparation and complementary DNA probes

Cellular RNA was prepared from 10^7 cells by lysis in 5 ml of Tri-Reagent, a mixture of phenol and guanidine thiocyanate in a monophasic solution (Molecular Research Center, Cincinnati, OH). RNA was separated from DNA and proteins by the addition of 1 ml of chloroform. RNA was recovered by precipitation with 2.5 ml of isopropanol. RNA (25 μg) was glyoxalized, resolved by 1% (wt/vol) agarose gel electrophoresis and transferred to Nytran$^+$ membrane (Schleicher & Schuell, Inc., VWR) (33). Three different membranes with cells recovered from different passages were separately prehybridized, hybridized, and washed under high stringency conditions (34). Each Northern blot was successively hybridized with each of the following human complementary (c) DNA probes: 3β-HSD type 1 *Eco*RI/*Pvu*II 1038-bp fragment (35); 17β-HSD type 1 *Eco*RI/*Sac*I 964-bp segment (35); 17β-HSD types 2, 3, and 5 full-length fragments (1.3 kb) (13, 31); 17β-HSD type 4 *Eco*RI/*Eco*RI 1.4-kb fragment (36); and 5α-reductase types 1 (2.1 kb) and 2 (2.4 kb) both full-length (37). Probes were labeled with (α^{32}P)deoxy-CTP to 2 × 10^6 dpm/ng with random primers (38). Different human tissues were used as positive control. Term villous was used for the 3β-HSD-1, 17β-HSD-1, -2, and -4; testis for the 17β-HSD-3 and prostate for the 5α-reductase.

Ribonuclease protection assay

Full-length human complementary DNA (cDNA) fragments of 17β-HSD type 5 and 3α-HSD type 3 were subcloned into the *Eco*RI/*Bam*HI of the Bluescript KS$^+$ (BSKS$^+$) vector (Stratagene, La Jolla, CA). The recombinant plasmid carrying 17β-HSD type 5 cDNA was linearized with *Hin*dIII to generate a cRNA (antisense) probe of 188 nts specific for 17β-HSD type 5 messenger RNA (mRNA). The plasmid carrying 3α-HSD type 3 was linearized with *Sal*I to generate a cRNA probe of 250 nucleotides (nts) specific for 3α-HSD type 3 mRNA. The protected regions correspond to nts 36–286 of the 3α-HSD type 3 cDNA and to nts 831-1019 of the 17β-HSD type 5 cDNA. RNase protection assays for the presence of human 17β-HSD types 1 and 2 mRNAs were also performed as described previously (30, 35).

Results

Steroid metabolism by A549 intact cells

As an initial approach to estimating the capacity of A549 cells for 5α-DHT and testosterone synthesis and metabolism, intact cells in culture were exposed to tritiated DHEA, 5α-

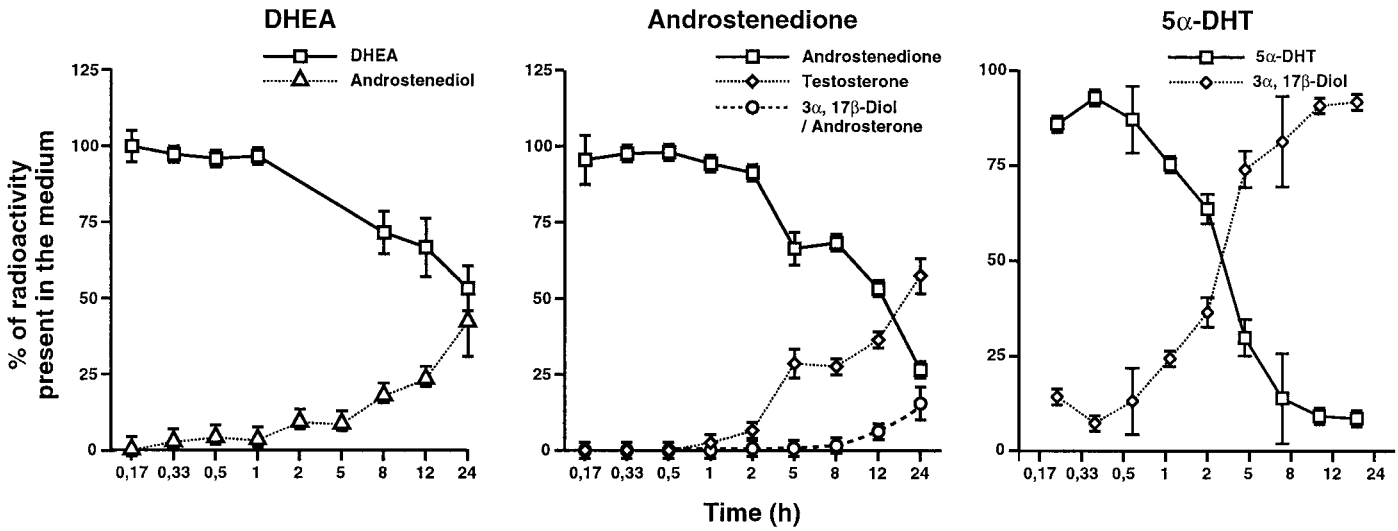

FIG. 2. Time-course of DHEA, androstenedione, and 5α-DHT metabolism by A549 intact cells incubated with FCS. Enzymatic reactions were stopped at different time intervals, steroids were extracted and analyzed as described in *Materials and Methods*. The values are the percentages of the total specific radioactivity recovered from the medium in substrate and products (mean ± SD of triplicate cultures).

DHT, androstenedione, and testosterone for 24 h (Table 1). DHEA was converted to a single product, androstenediol. There was no evidence of androstenedione formation from DHEA. When 5α-DHT was the substrate, there was almost complete conversion into 3α,17β-diol. With androstenedione as substrate, testosterone was the major androgen formed accounting for about 44% of the total radioactivity recovered. 3α,17β-diol and androsterone were also formed, but at levels consistent with the presence of a low level of 5α-reductase activity. This was confirmed when testosterone was tested. 3α,17β-diol accumulated in the medium to the same low level (14 ± 1%) as when androstenedione was the substrate (9 ± 2%).

To confirm that DHEA, 5α-DHT, and androstenedione were metabolized principally into single products, time-course experiments were performed in which enzymatic reactions were stopped at different time periods (Fig. 2). Formation of a single product accounted for DHEA and 5α-DHT metabolism with about 50% conversion into 3α,17β-diol in less than 5 h for the latter. With androstenedione as substrate, testosterone formation was detected within 1 h, with a small amount of 3α,17β-diol and androsterone formed in agreement with results from Table 1.

17β-HSD, 3β-HSD, 3α-HSD, and 5α-reductase activities in A549 cytosol and microsomes

The patterns of metabolism observed with intact cell were suggestive of the presence of relatively high levels of 17β-HSD and 3α-HSD and low levels of 3β-HSD and 5α-reductase. In addition, reductase activity clearly predominated over dehydrogenase activity in intact cells. To clarify the basis for these patterns, cells were fractionated into cytosol and microsomes and NAD(P)-dependent dehydrogenase and NAD(P)H-dependent reductase activities with estradiol-17β (E_2) and estrone (E_1), testosterone and androstenedione, and DHEA and 5α-DHT as substrates assayed. The pattern of oxidation and reduction at C17 of E_2, E_1, testosterone, and androstenedione in cytosol and microsomes is shown in Fig. 3. Most (>75%) of the 17β-HSD activity was recovered in the cytosol and NADP or NADPH were the preferred cofactors. When assayed at pH 7.2, dehydrogenase activity with E_2 and testosterone exceeded reductase activity with E1 and androstenedione. The pattern of DHEA metabolism is shown in Fig. 4. Conversion of DHEA to androstenedione was detected in both cytosol and microsomes. There was a significantly greater NADPH-dependent reduction of DHEA at the C17 position to androstenediol, consistent with the pattern observed with intact cells. Here again, the majority of the activity was recovered in the cytosol. Both cytosol and microsomes also exhibited a large capacity for the reduction of 5α-DHT to 3α,17β-diol (Fig. 5), predicted on the basis of the metabolic pattern seen with intact cells. NADPH was the preferred cofactor. In contrast, conversion of 5α-DHT to androstanedione was low. 5α-reductase activity was assayed at pH 8.0 (type 1 isoform) and pH 5.0 (type 2 isoform) in microsomes and cell sonicates. In agreement with the metabolic activity noted with intact cells, activity was at the limit of detection in each case and radioactivity was recovered as

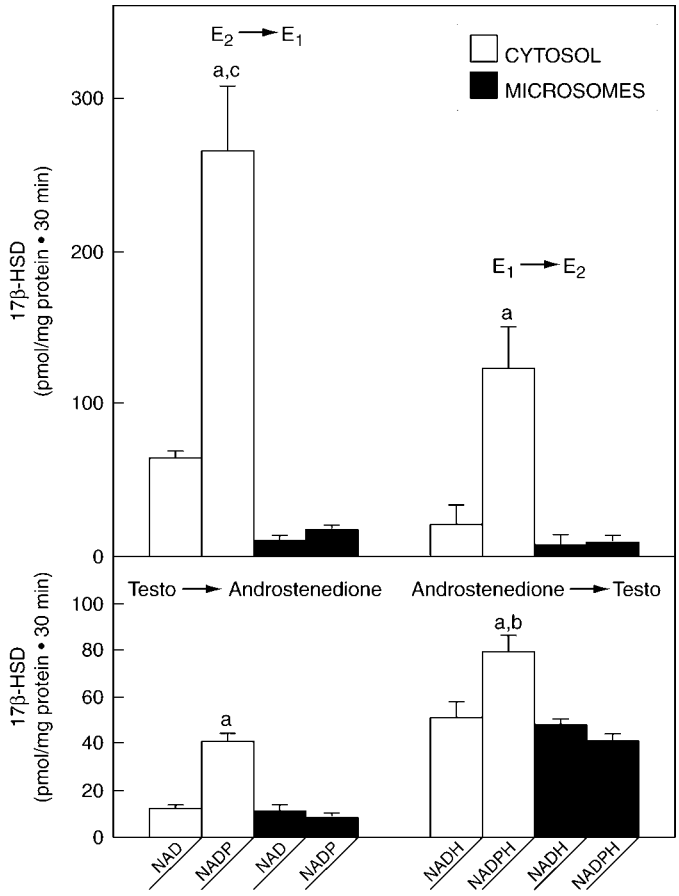

FIG. 3. Enzymatic 17β-HSD activity of cytosol and microsomes from A549 cells. Experimental conditions were as described in *Materials and Methods*. Dehydrogenase activity was assayed with E2 and testosterone with NAD or NADP as the cofactor. Reductase activity was assayed with E1 and androstenedione with NADH or NADPH as the cofactor. The values are the mean (± SD) of cytosol or microsomes from three separate cultures. Data were analyzed by ANOVA in combination with the Student's-Newman-Keuls posttest. [a]$P < 0.01$ or less for NADP > NAD and NADPH > NADH; [b]$P < 0.01$ for NADPH > NADP; [c]$P < 0.01$ for NADP > NADPH.

3α,17β-diol consistent with the high level of 3α-HSD and 17β-HSD type 5 (data not shown).

Presence of steroidogenic enzyme mRNAs in A549 cells

When Northern blots with A549 total RNA were probed with each of 3β-HSD type 1, 5α-reductase types 1 and 2, and 17β-HSD types 1 to 5, only mRNAs that corresponded to 17β-HSD type 4 (Fig. 6, lane 2) and 17β-HSD type 5 (Fig. 6, lane 3) were detected. The full-length cDNA probe of 17β-HSD type 5 used in Northern blot analysis does not discriminate between 17β-HSD type 5 and the 3α-HSDs, because more than 80% sequence identity exists between these cDNAs (13). Thus, we designed cRNA probes for 17β-HSD type 5 and 3α-HSD type 1 and type 3. When RNase protection assays were performed with these probes, RNA protected fragments specific to 17β-HSD type 5 (Fig. 7A) and 3α-HSD type 3 (Fig. 7B) were detected. We have also conducted RNase protection analysis with 17β-HSD type 1, type 2, and type 3 RNA probes. Again, with this sensitive method, no

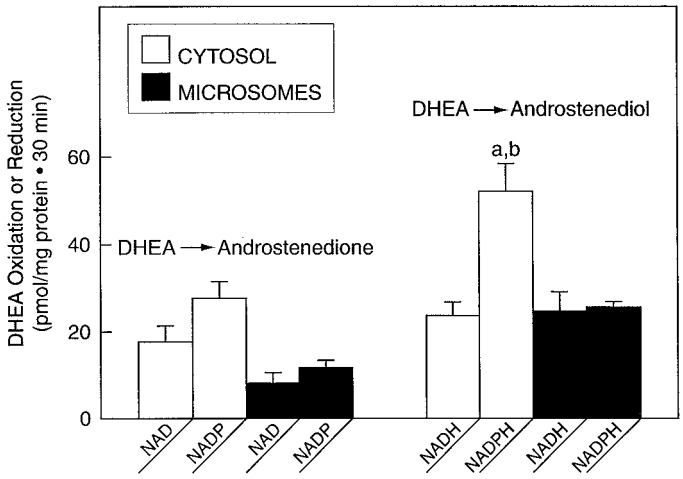

FIG. 4. Oxidation and reduction of DHEA by cytosol and microsomes from A549 cells. Assay conditions and data analysis were as described in the legend to Figure 2 with [^{3}H]-DHEA as the substrate. $^aP < 0.01$ for NADPH > NADH; $^bP < 0.001$ for NADPH > NADP.

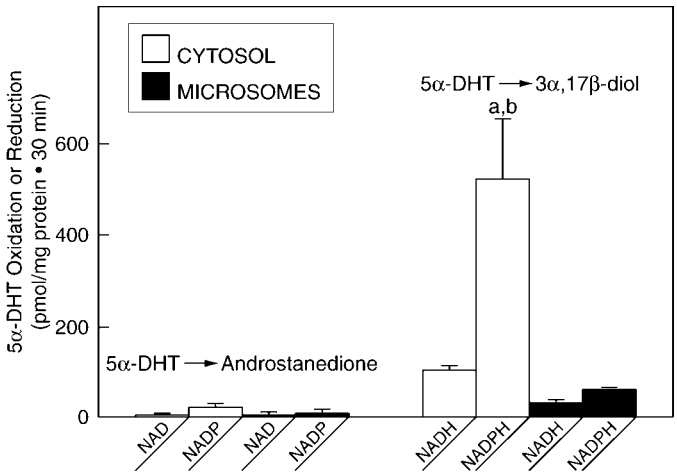

FIG. 5. Oxidation and reduction of 5α-DHT by cytosol and microsomes from A549 cells. Assay conditions and data analysis were as described in the legend to Fig. 2 with [^{3}H]-5α-DHT as the substrate. $^aP < 0.001$ for NADPH > NADH; $^bP < 0.001$ for NADPH > NADP.

corresponding mRNAs were detected (data not shown). 5α-reductase type 1 and 3β-HSD type 1 mRNAs were detected but at very low levels by RNase protection assays (data not shown).

Regulation of 5α-reductase activity

The observation of 5α-reductase activity and mRNA at the limit of detection was a surprising result considering the known effect of 5α-DHT on surfactant production (21, 39). We reasoned that the low level of 5α-reductase mRNA and activity in A549 cells could be due to a down-regulatory factor present in the culture medium. Therefore, we compared three different lots of serum. In addition, to study the possible effects of FCS-low molecular weight factors, we used charcoal-stripped serum. As Dex has been shown to stimulate the incorporation of tritium-labeled choline into DSPC in A549 cells cultured in serum-free medium (40), we studied the effects of 0.25 μM of Dex, the optimal concentration in those studies. The pattern of androstenedione metabolism in A549 intact cells was independent of the serum lot (data not shown). The removal of small molecules such as cortisol by charcoal had no major impact on the steroidogenic pattern and no increase in DHT formation was observed (Fig. 8A). Moreover, when Dex was added to normal (data not shown) or charcoal-stripped FCS (Fig. 8B), there was no significant effect on the metabolism of androstenedione. Similar levels of 5α-DHT and 3α,17β-diol were detected. Because the amounts of 5α-DHT formed from androstenedione were very low, the identity of each metabolite formed was confirmed by HPLC analysis (Fig. 8C). The inset represents a spike experiment in which HPLC-purified [^{3}H]-5α-DHT was added to the sample. Both the 5α-DHT added and the metabolite produced from androstenedione had the same retention time.

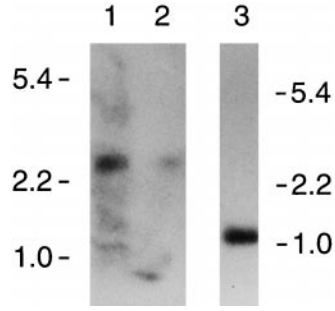

FIG. 6. Northern blots analysis of 17β-HSD type 4 and type 5 expression in A549 cells. Twenty-five micrograms of total RNA isolated from A549 cells cultured with DMEMHG supplemented with 10% FCS were hybridized with each of the cDNA probes described in *Materials and Methods*. Three membranes were made and each was used in successive rounds of hybridization with all probes. Only autoradiograms with signals are presented. Positions of the molecular weight standards (5.4, 2.2, and 1.0 kb) are shown. RNA samples are from term villi (positive control; lane 1) or A549 cells (lanes 2 and 3) and were hybridized with 17β-HSD type 4 (lanes 1 and 2) or 17β-HSD type 5 (lane 3) cDNA probes.

Discussion

The experiments reported here were undertaken to relate the pattern of C19-steroid metabolism by intact A549 epithelial lung cell line to mRNA expression and specific enzymatic activities. With regard to the potential of these type II-like cells to synthesize the active androgen 5α-DHT from the inactive precursor DHEA, we found that under widely used cell culture conditions, DHEA was metabolized principally to androstenediol. There was no evidence of testosterone formation from DHEA, even though a low level of 3β-HSD activity was detected *in vitro* (Figs. 2 and 4). When androstenedione was the substrate for intact cells in 24-h incubations, 5α-DHT was barely detectable and the major end-product was 3α,17β-diol (Fig. 8), indicative of a slight capacity for net 5α-reduction in agreement with the observations that 5α-reductase activity was at the limit of detection by direct assay *in vitro* and that the level of 5α-reductase mRNA was very low. Our results of C19-steroids metabolism by intact A549 cells are in agreement with those of Milewich (41). Thus, although, they are androgen receptor positive (24), A549 cells cultured in normal 10% FCS appears to have a minimal capacity for net 5α-DHT formation from DHEA.

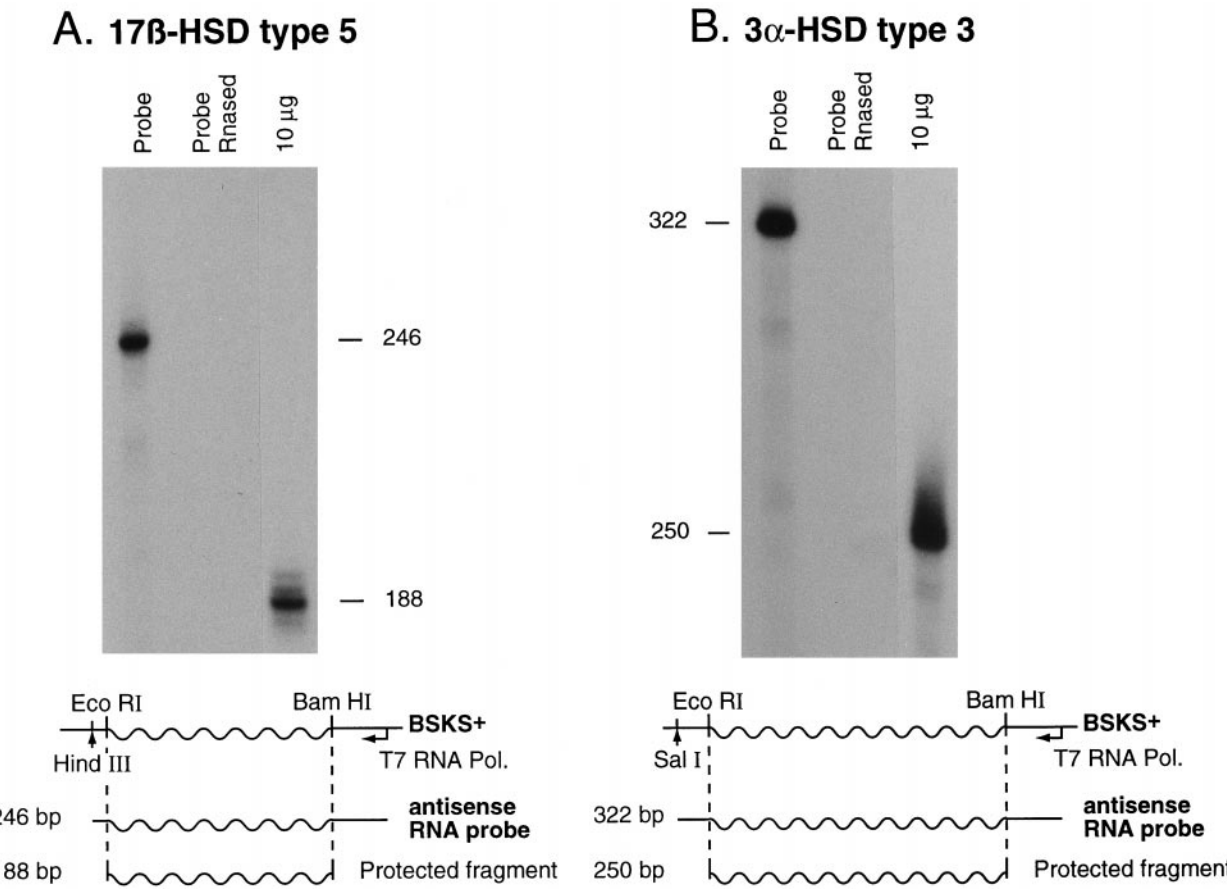

FIG. 7. RNase protection analysis of 17β-HSD type 5 and 3α-HSD type 3 expression in A549 cells. Twenty-five micrograms of total RNA isolated from A549 cells cultured with DMEMHG supplemented with 10% FCS were subjected to RNase protection assay using a 17β-HSD type 5 (A) and a 3α-HSD type 3 (B) specific antisense RNA probes. The lengths of each probe and protected fragments are illustrated below the autoradiographs.

It is of particular interest that A549 intact cells show a large capacity to convert androstenedione to testosterone but have a very limited relative capacity to metabolize testosterone to either androstenedione or 5α-DHT. This raises the possibility that, at least under the culture conditions described here, testosterone rather than 5α-DHT may be the active androgen acting on DSPC production in these cells (17–19). Relevant to this, Nielsen (7) showed that testosterone by itself, *e.g.* in the absence of conversion into 5α-DHT, modulated fetal rabbit lung development as measured by the production of DSPC.

The pattern of E_2, E_1, testosterone, and androstenedione metabolism *in vitro* (Fig. 3) with NADP and NADPH as the preferred cofactors and with the majority of activity recovered in the cytosol is characteristic of 17β-HSD type 5 (42). If considered along with the mRNA analyses, these data indicate 17β-HSD type 5 is the predominant isoform of 17β-HSD in A549 cells. Our observation that 17β-HSD type 5 activity is reversible *in vitro* but acts predominantly as a reductase in intact cells is also in accord with the report of Dufort *et al.* (42). They found that when 17β-HSD type 5 cDNA was transfected into human embryonic 293 cells, the expressed enzyme acted as both dehydrogenase and reductase in cell homogenates but essentially only as reductase in intact transfected cells. The presence of other isoforms of 17β-HSD may account for the low level of activity with both C18 and C19 steroids in the microsomal fraction. Microsomal metabolism of E_2, in particular, can be accounted for by the presence of 17β-HSD type 4. This isoform is highly specific for E_2 and androstenediol and acts almost exclusively as a dehydrogenase (36). The more strongly favored reduction of 5α-DHT to 3α,17β-diol can be accounted for by the presence of the 3α-HSD type 3. 17β-HSD type 5 also catalyzes reduction of 5α-DHT to 3α,17β-diol (42) and thus, would also contribute to the rapid inactivation of 5α-DHT as an active androgen by these cells.

Our findings with A549 cells concur with the results that Milewich *et al.* (10) obtained with human lung tissue and suggest that 17β-HSD type 5 and 3α-HSD type 3 play a major role in the metabolism of androgens in the human lung. In addition, our results establish that 17β-HSD type 5 and 3α-HSD type 3 mRNA expression, in combination with regulation at the level of catalysis to favor reductase activity in intact cells, can form the basis for a pattern of metabolism that would stabilize the intracellular level of testosterone and rapidly metabolizes 5α-DHT. To our knowledge, this is the first description of such a pattern in androgen receptor-rich cells.

It is well established that androgens influence fetal lung development. Findings from a number of laboratories are consistent with a role for 5α-DHT in the regulation of sur-

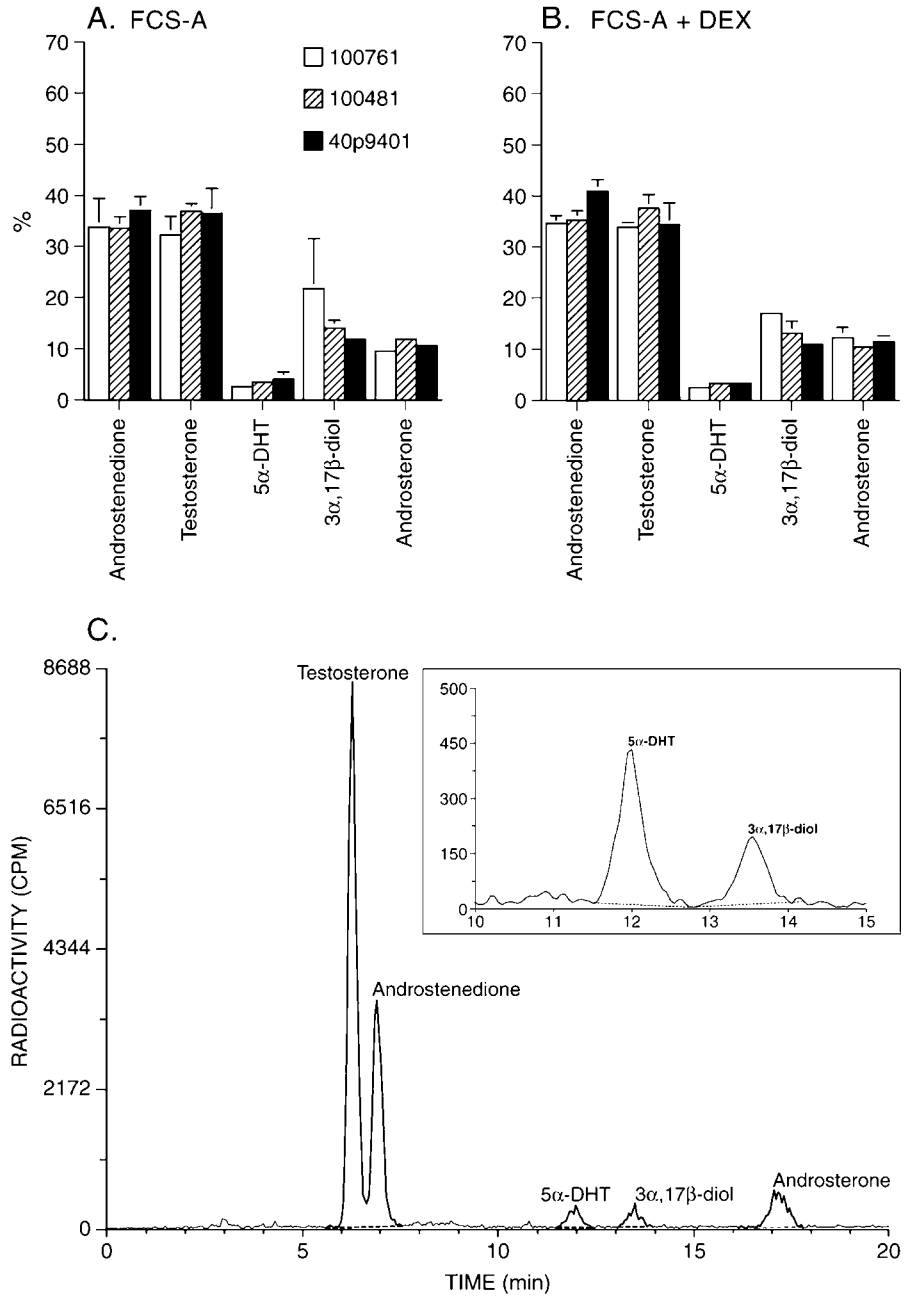

FIG. 8. Effects of three different lots of FCS and of Dex on the metabolism of androstenedione by A549 intact cells. A549 cells were cultured with DMEMHG supplemented with the different lots of FCS-A in the absence (panel A) or the presence (panel B) of 2.5×10^{7} M Dex. For each condition, cells were incubated with [^{3}H]-androstenedione for 24 h. (Mean ± SD of triplicate assays). Similar results were obtained with cells incubated with the same three lots of FCS in the same round of experiment (not shown). (C), The identity of each steroid was also verified by HPLC. Retention times of 6.30, 6.95, 12.00, 13.55, and 17.30 min were obtained for testosterone, androstenedione, 5α-DHT, 3α-,17β-diol, and androsterone, respectively. The insert corresponds to the zoom of an area from a graph obtained with the same sample spiked with an equal amount in dpm of [^{3}H]-5α-DHT to confirm the co-elution of the latter with the biological sample.

factant formation during fetal lung development (5, 7, 21, 43). Because this hormone is produced locally from testosterone in 5α-DHT-dependent tissues, as it is case in the prostate (44), we expected to find a significant level of 5α-reductase activity in A549 cells. The apparent near-absence of 5α-reductase mRNA and activity led us to examine the possibility that serum factors may be influencing the levels of 5α-reductase mRNA. Because glucocorticoid receptor is present in A549 cells (18, 40, 45), we examined the possibility that cortisol in FCS might be affecting 5α-reductase activity in our cultures. Our initial experiments were all done with cells cultured in medium supplemented to 10% with FCS. Subsequent analysis revealed that various lots of FCS contained cortisol at levels between 1.2 and 1.4 μM and that the steroid could be removed by charcoal treatment (cortisol was undetectable after the charcoal-stripping procedure). On that basis, we compared the effects of untreated and charcoal-treated FCS as well as the addition of the synthetic glucocorticoid dexamethasone on steroid metabolism by intact cell. The absence of an effect of either charcoal-stripping or dexamathasone on the metabolic pattern indicates that cortisol and other steroids removed by charcoal treatment are not inhibiting the expression of 5α-reductase mRNA or the mRNA levels of the other steroidogenic enzymes we assayed in our cultures.

Our findings raise questions about the nature of the active androgen (testosterone or 5α-DHT) and the mechanism of androgen action on surfactant production. If pneumocyte type II epithelial cells in the normal fetal developing lung or

in the adult lung not only lack the capacity to generate 5α-DHT but can rapidly inactivate it as well, a direct effect of 5α-DHT on these cells is unlikely. In fact, our results are suggestive that the testosterone produced by type II cells could be the active androgen in the lung, and/or be transformed in 5α-DHT by the lung fibroblasts before acting on surfactant synthesis. Such a mesenchymal-epithelial interaction is consistent, for example, with the inhibitory effect of 5α-DHT on dexamethasone-stimulated surfactant production by explant cultures of human lung tissue reported by Torday (5). In addition, Floros and co-workers (46) have shown that exposure of rat fetuses to 5α-DHT *in utero* inhibits fibroblast-pneumocyte factor (FPF) production by lung fibroblasts cultured *in vitro*. More recent reports have confirmed that lung fibroblasts can be directly affected by 5α-DHT (47–49). However, even though effects of 5α-DHT administration *in vivo* and *in vitro* have been demonstrated, there is also evidence that testosterone may be the active androgen in the lung. Relevant to this, Nielsen (7) showed that treatment of pregnant rabbits with a 5α-reductase inhibitor which eliminated 5α-DHT from the fetal lung, had no effect on male:female differences in phosphatidylcholine: spingomyelin or saturated phosphatidylcholine:spingomyelin ratios in fetal lung. In addition, testosterone in the presence of the same 5α-reductase inhibitor inhibited the release of FPF by fetal lung fibroblasts in culture.

In summary, we have characterized a complex steroid enzymology in a widely studied cellular model of human lung pneumocyte type II function. Our results demonstrate a pattern of 17β-HSD type 5 and 3α-HSD type 3 activities in which testosterone level is maintained and 5α-DHT is rapidly metabolized. This has important implications for the nature of the active androgen affecting surfactant production in the lung.

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