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COMPARISON OF THE EFFECT OF CORTISOL ON AROMATASE ACTIVITY AND ANDROGEN METABOLISM IN TWO HUMAN FIBROBLAST CELL LINES DERIVED FROM THE SAME INDIVIDUAL

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Summary—The effect of preincubation with cortisol on estrogen and androgen metabolism was investigated in human fibroblast monolayers grown from biopsies of genital and non-genital skin of the same person. The activity in the cells of aromatase, 5α-reductase, 17β-hydroxysteroid oxidoreductase and 3α-hydroxysteroid oxidoreductase was investigated by isolating estrone, estradiol, estriol, dihydrotestosterone, androstanedione, androsterone, 3α-androstanediol, testosterone and androstenedione after incubation of the cells with [^{14}C]testosterone or [^{14}C]androstenedione. For experiments with ^{14}C-labeled substrate the cells were incubated in medium, charcoal stripped of steroids without Phenol Red.

Preincubation from 6 to 36 h with cortisol in concentrations of 10^{-8}–10^{-6} M showed maximal stimulation of aromatase activity after 12 h preincubation with cortisol in concentrations of 0.5–1.0 × 10^{-6} M in both cell lines. When preincubation with cortisol was omitted no estrogen synthesis was detected. The formation of androgen was not altered after preincubation with cortisol. Pronounced differences were found in estrogen and in androgen metabolism in the two cell lines suggesting a local regulation of the hormonal environment. The aromatase activity, which is low in many tissues could be stimulated by cortisol without altering the androgen metabolism was found to be a suitable system for investigations of the cellular interconversion of androgens and estrogens and for investigations of the *in vitro* regulation of the enzymes involved.

INTRODUCTION

The local production of estrogens from androgen precursors, testosterone and androstenedione via the aromatase system has been demonstrated in a variety of tissues including fat muscle and skin [1–3]. Testosterone and androstenedione can also serve as precursors for a variety of 5α-androstanes by the irreversible reduction of the 4–5 double bond. When studying the physiological and pathophysiological role of estrogens and androgens both the aromatase pathway, the 5α-reduction and the interconversion of estrogens and androgens must be taken into consideration. However, the aromatization of androgens is low or not detectable in many cell types but can be stimulated considerably by glucocorticoids [4–7]. The androgen metabolism in human skin differ greatly, depending on the origin being an androgen responsive site or an area which is not dependent on steroids [8]. In this study we investigated the influence of the natural glucocorticoid, cortisol, on the activity of aromatase, 5α-reductase, 17β-hydroxysteroid oxidoreductase and 3α-hydroxysteroid oxidoreductase was investigated in two strains of human fibroblasts derived from different sites of the same individual. One cell line was derived from scrotal skin, an area known to grow in response to androgens. The other cell line was derived from the forearm which is not considered to be dependent on steroids. The metabolic pathways investigated is specified in Fig. 1. All experiments were carried out in culture medium containing fetal calf serum stripped of steroids and without addition of Phenol Red. The estrogen and androgen metabolites were isolated using modifications of the methods outlined by Perel and Killinger[9] and MacIndoe and Woods[10].

EXPERIMENTAL

Materials

Reference steroids were obtained from Steraloids Inc. (Wilton, N.H.). Radioactive steroids [4-^{14}C]-testosterone (T) (SA 50 mCi/mmol) and [4-^{14}C]-androstenedione (4-AD) (SA 50 mCi/mmol) were

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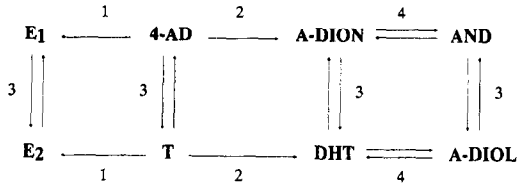

Fig. 1. Enzymatic conversion of testosterone (T) and androstenedione (4-AD). The metabolites are estrone (E_1), estradiol (E_2), androstanedione (A-DION), dihydrotestosterone (DHT), androsterone (AND), and 3α-androstanediol (A-DIOL). The enzymes involved are (1) aromatase, (2) 5α-reductase (5α-R), (3) 17β-hydroxysteroid oxidoreductase (17β-OHSDH), and (4) 3α-hydroxysteroid oxidoreductase (3α-OHSDH).

purified by column chromatography on celite-propylene glycol before use [11]. [1,2,6,7-^{3}H]T (SA 85 Ci/mmol), [1,2,6,7-^{3}H]4-AD (SA 85 Ci/mmol), [9,11-^{3}H]androstane-3α,17β-diol (A-DIOL) (SA 53 Ci/mmol), [9,11-^{3}H]androsterone (AND) (SA 53 Ci/mmol), [1,2,4,6,7-^{3}H]dihydrotestosterone (DHT) (SA 110 Ci/mmol), [2,4,6,7-^{3}H]estrone (E_1) (SA 87 Ci/mmol), [2,4,6,7-^{3}H]estradiol (E_2) (SA 85 Ci/mmol) and [2,4,6,7-^{3}H]estriol (E_3) (SA 90 Ci/mmol) were purified by silica gel thin-layer chromatography before use. All radioactive steroids were purchased from New England Nuclear Corp. (Boston, Mass.). Silica gel precoated thin-layer chromatographic sheets (Kieselgel 60F_{254}), analytical grade reagents and solvents were obtained from Merck (Darmstadt). The scintillation cocktail, Aqualuma, was obtained from Lumac B.V. (The Netherlands).

Cell growth and incubation

The fibroblast strains used in this study were derived from a biopsy of skin of the forearm (LA) and a biopsy of scrotal skin (LS) from a 21-year-old male. Informed consent was obtained.

The cells were grown in 75 mm^2 culture flasks (Costar) in Eagle's minimum essential medium (MEM) enriched by Earle's salt (Gibco), MEM amino acids 10 ml/l and vitamins 10 ml/l (Gibco), L-glutamine 10 ml/l (Gibco), glucose 5 ml/l, 10% fetal calf serum (Gibco), and antibiotics: gentamycin 2 mg/l (Essex Pharma) and mycostatin 10^4 units/l (Squibb). Fibroblasts were stored in liquid nitrogen after the initial passages and were subsequently thawed and utilized prior to the 12th *in vitro* passage.

For experiments, 10^5 fibroblasts in each flask were grown in the medium described above. After one week of growth, when the cultures were subconfluent (10^6 cells), the cells were washed twice in phosphate buffered saline (without Ca^{2+} and Mg^{2+}), and fresh medium charcoal stripped of steroids and without Phenol Red was added. After 24 h the cultures were preincubated with cortisol. Following preincubation the cells were washed by 10 ml phosphate buffered saline, and 10 ml fresh stripped medium without Phenol Red was added together with the experimental substrates for incubation. The experiments were terminated by harvesting the cells by scraping with a rubber policeman, and the cells and media were immediately frozen and kept at −20°C until analysis within one month.

Experimental design

The influence of the following parameters on androgen and estrogen metabolism was investigated: preincubation time with cortisol, the cortisol concentration, the substrate concentration, and the substrate incubation time.

In all experiments both cell lines were incubated with [^{14}C]T or with [^{14}C]4-AD.

The influence of preincubation time was investigated by incubation with 10^{-6} M cortisol for periods lasting from 6 to 36 h followed by incubation with substrate in a concentration of 2×10^{-6} M for 22 h. Cells without addition of cortisol was included. The effect of cortisol concentration was studied by preincubation for 24 h with concentrations of cortisol from 0 to 10^{-6} M and incubation with the substrates (2×10^{-6} M) for 22 h.

After preincubation with 0.25×10^{-6} M cortisol for 6 h the influence of substrate concentration and of substrate incubation time was investigated. The substrate concentration was varied from 0.5×10^{-8} to 3.6×10^{-6} M with an incubation time of 22 h. The incubation time was varied from 2 to 48 h using a substrate concentration of 10^{-6} M.

Isolation of metabolites

The frozen cells in medium were thawed and kept on ice during addition of ^{3}H-labeled T, 4-AD, DHT, A-DIOL, AND, E_1 E_2 and E_3 to correct for losses during subsequent steps. [^{3}H]5α-androstane 3,7-dione (A-DION) was not available, so the same correction as for A-DIOL was used. 100 μg of each unlabeled steroid was added to facilitate identification during purification. The steroids were extracted twice from medium and cells with 15 ml diethyl ether. The dried diethyl ether extract was subjected to phenolic partition by adding 4 ml toluene and extraction of the phenolic fraction, E_1, E_2 and E_3 with 4 × 1 ml NaOH 1 mol/l. The combined NaOH extracts were acidified with 0.25 ml acetic acid and extracted with 2 × 6 ml diethyl ether. The ether was evaporated and the residue was reconstituted in 50 μl ethanol and applied to silica gel thin-layer chromatography sheets. After two ascents in dichlormethane–ethyl acetate–ethanol (85:15:1.5 v/v) E_1, E_2 and E_3 were identified under u.v. light and the areas corresponding to each compound were cut out and the estrogens were eluted with 3 ml ethanol. 0.5 ml ethanol of each fraction was assayed for ^{3}H and ^{14}C. The remaining 2.5 ml ethanol was dried, acetylated in 100 μl pyridine plus 100 μl acetic anhydride for 20 h at 20°C. The resulting acetate derivatives were separately chromatographed on silica gel thin-layer sheets using two ascents of

Table 1. $^3H/^{14}C$ ratios observed during purification of metabolites of [^{14}C]4-AD or of [^{14}C]T after incubation with fibroblasts

Compound	$^3H:^{14}C$			
	TLC 1	TLC 2	ML 5	CR 5
E_1	6.35	12.28	13.41	12.06
E_2	10.02	36.42	36.30	38.35
A-DIOL	3.44	4.66	4.63	5.10
T	0.69	0.93	1.09	1.11
DHT	—	0.61	0.68	0.65
AND	—	0.14	0.10	0.14
4-AD	0.041	0.041	0.45	0.43
A-DION	294 (ML 1)[a]	285 (CR 1)[a]	289[a]	283[a]

[a]The CPM/mg in first and final crystals (CR) and mother liquor (ML) are shown for [^{14}C]A-DION.

dichlormethane–diethyl ether (96:4 v/v). The areas corresponding to the estrogen acetates chromatographed was visualized under u.v. light, cut out and eluted with 3 ml ethanol. 0.5 ml of the ethanol was assayed for ^3H and ^{14}C. The remaining 2.5 ml ethanol was added 40 mg of the appropriate non-radioactive estrogen acetate as carrier and the mixture recrystallized 5 times (Table 1). As no significant change in ^3H to ^{14}C ratios occurred after recrystallizations, also described by Schweikert *et al.*[12] and McIndoe[13], the acetates of E_1 and E_2 were eluted with scintillation cocktail after the second thin-layer chromatography and assayed for ^3H and ^{14}C as standard procedure. As E_3 only was detected in one experiment and in small amounts, the purity was not confirmed by recrystallizations.

The toluene remaining from the phenolic partition, containing the androgen metabolites T, 4-AD, DHT, AND, A-DION and A-DIOL was dried by heating under a stream of air. The residue was dissolved in 50 μl ethanol and applied to thin-layer chromatography sheets. After two ascents in dichlormethane-ethyl acetate T and 4-AD were identified under u.v. light, while AND, DHT, A-DIOL and A-DION were located in marker channels by spraying with 10% sulfuric acid in ethanol and allowing the visible colours to develop at 80°C. Each zone was cut out, and eluted with 3 ml ethanol. AND and DHT were not separated in this system and was eluted together. 0.5 ml ethanol of each fraction was assayed for ^3H and ^{14}C. The androgens were further purified by acetylation, thin-layer chromatography and recrystallizations as described for the estrogens. The fractions containing 4-AD and A-DION were also acetylated in spite of lacking hydroxyl groups, since a better separation from the other androgen metabolites was obtained. R_f values for steroids isolated are shown in Table 2. Only minor changes in the ratio ^3H to ^{14}C in fractions containing T, 4-AD, DHT, A-DIOL and AND were observed after recrystallization, neither did the specific activity of A-DION change (Table 1). Recrystallizations were therefore omitted in subsequent experiments.

Radioactivity measurements were carried out in plastic vials after addition of 10 ml aqualuma and counting was performed in a double label counting program with correction for spill over of ^{14}C into the ^3H-channel using a liquid scintillation counter, Nuclear Chicago system 6880 Mark III. ^3H was counted with an efficiency of 27% and ^{14}C with an efficiency of 70%. The amount of ^{14}C-labeled metabolites was calculated by correcting for per cent recovery measured by the corresponding ^3H-label. The amount of products detected in incubated medium without cells, were subtracted. For calculation of the concentration of A-DION, the recovery obtained in the corresponding A-DIOL fraction was used. The recoveries of androgen and estrogen metabolites ranged from 30 to 50%.

RESULTS

Incubation with cortisol

Estrogens. The optimal stimulation of aromatase activity (Fig. 2) was obtained after 6–12 h preincubation wih 10^{-6} M cortisol. After 24 h preincubation with cortisol the aromatase activity declined in cell line LA but not in LS except when [^{14}C]4-AD was substrate. When [^{14}C]T was substrate in the LS cell line the aromatase activity remained elevated during the whole period. When cortisol was absent no aromatase activity was detected.

Preincubation with varying concentrations of cortisol 0–10^{-6} M resulted in stimulation of aromatase activity, when the concentration of cortisol was 0.25×10^{-6} M in both cell lines with either [^{14}C]T or [^{14}C]4-AD as substrate (Fig. 3). Maximal stimulation of aromatase activity was obtained with cortisol concentrations of $0.5–1.0 \times 10^{-6}$ M.

Androgens. Preincubation with 10^{-6} M cortisol for 6–36 h did not change the concentrations of

Table 2. R_f-values of androgen and estrogen metabolites after two ascents in dichlormethane–ethyl acetate–ethanol (85:15:1.5 v/v) TLC 1 and after acetylation and two ascents in dichlormethane–diethyl ether (96:4 v/v) TLC 2

R_f-values	A-DION	4-AD	DHT	AND	T	A-DIOL	E_1	E_2	E_3
TLC 1	0.73	0.60	0.50	0.46	0.40	0.26	0.71	0.46	0.09
TLC 2	0.31	0.17	0.44	0.54	0.20	0.57	0.54	0.66	0.45

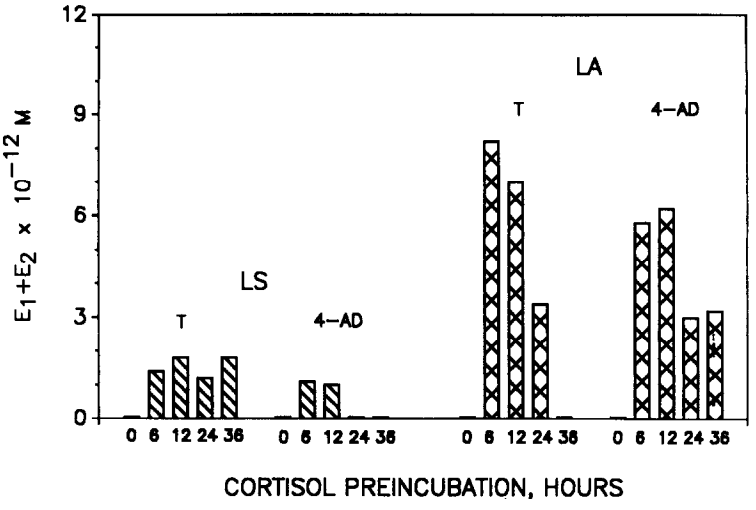

Fig. 2. Aromatase activity of cultured human skin fibroblasts (LS and LA) following 6–36 h preincubation with 10^{-6} M cortisol. After preincubation the cell lines were incubated for 22 h with 2×10^{-6} M [^{14}C]T or [^{14}C]4-AD.

androgens formed from [^{14}C]4-AD or [^{14}C]T in the two cell lines. Figure 4 shows the 5α-reductase (5α-R) activity expressed as 5α-reduced product (A-DIOL + DHT + AND + A-DION) in the two cell lines incubated with 2×10^{-6} M [^{14}C]T or [^{14}C]4-AD in 22 h without cortisol preincubation compared with the 5α-R activity after 6 h preincubation with 10^{-6} M cortisol. The 17β-hydroxysteroid oxidoreductase (17β-OHSDH) was expressed as AND + 4-AD + T + A-DION when [^{14}C]T was substrate and as A-DIOL + T + DHT when [^{14}C]4-AD was substrate. The 3α-hydroxysteroid oxidoreductase (3α-OHSDH) was expressed as A-DIOL + AND. The metabolism of androgens was higher in the LA cell line than in the LS cell line (Fig. 4). 5α-R activity was highest in both cell lines when [^{14}C]4-AD was substrate. The 17β-OHSDH activity indicates intense conversion of [^{14}C]T to 4-AD in LA cells.

Formation of androgen metabolites was not changed by preincubating the cells with varying concentrations of cortisol.

Variation in substrate concentration

Estrogens. Formation of estrogens shown in Fig. 5 was constant in cell line LS when both substrates were used and in LA cells when [^{14}C]4-AD was substrate, indicating saturation conditions. LS cells produced only E_2 from [^{14}C]T, and E_1 from [^{14}C]4-AD. LA cells produced mainly E_1 from 4-AD and a small amount of E_2. The rise in estrogen concentration, mainly E_1 when LA cells were incubated with increasing concentrations of [^{14}C]T, was most likely due to the high 17β-OHSDH activity

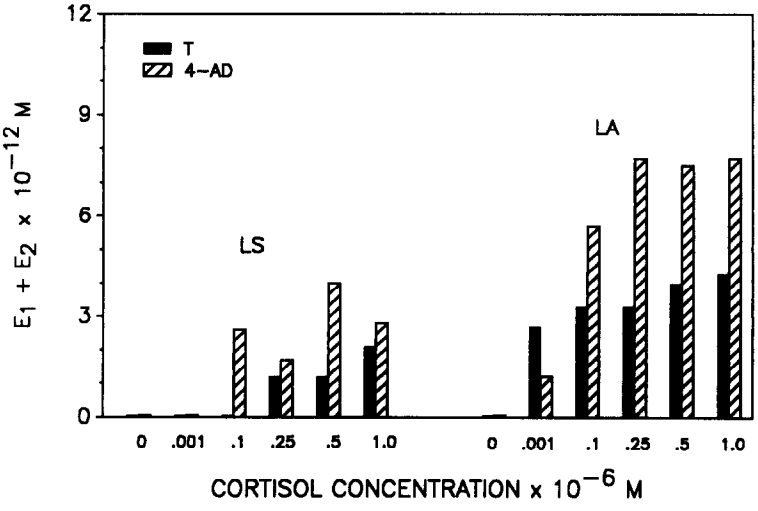

Fig. 3. Aromatase activity of cultured human skin fibroblasts (LS and LA) following 24 h preincubation with different concentrations of cortisol. After preincubation the cell lines were incubated for 22 h with 2×10^{-6} M [^{14}C]4-AD or [^{14}C]T.

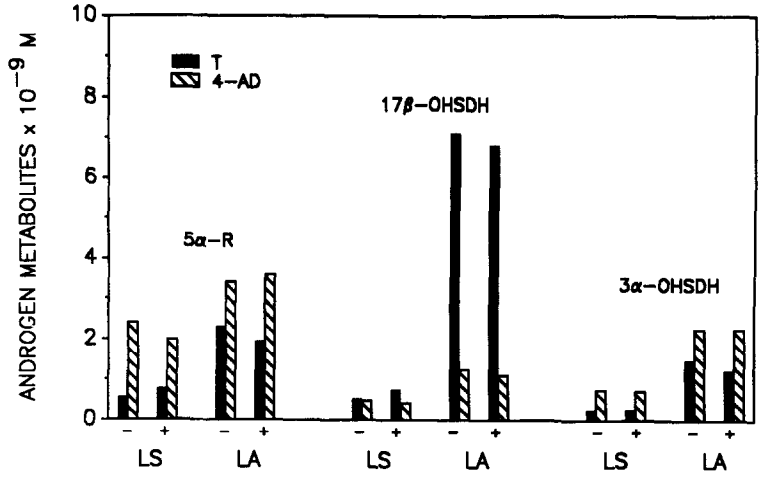

Fig. 4. The activity of 5α-R, 17β-OHSDH, and 3α-OHSDH in cultured human fibroblasts (LS and LA) incubated for 22 h with 2×10^{-6} M [^{14}C]T or [^{14}C]4-AD, without (−) or with (+) 6 h preincubation with 10^{-6} M cortisol. The 5α-R activity was expressed as the sum of 5α-reduced products isolated (A-DION + DHT + AND + A-DIOL). 17β-OHSDH activity was 4-AD + A-DION + AND when [^{14}C]T was substrate, and T + DHT + A-DIOL when [^{14}C]4-AD was substrate. The activity of 3α-OHSDH was expressed as AND + A-DIOL.

giving rise to formation of 4-AD which was aromatized to E_1.

Androgens. Figure 5 shows a linear relationship between the concentration of 5α-reduced products and substrate concentration. The 5α-R activity was highest and similar in both cell lines when [^{14}C]4-AD was used as substrate. Wih [^{14}C]T as substrate LA cells clearly had a higher 5α-R activity than LS cells. Also 17β-OHSDH activity showed a linear relationship to substrate concentrations in both cell lines. In cell line LS the oxidative and reductive function of 17β-OHSDH was similar in contrast to cell line LA where the oxidative function was most active favouring formation of 4-AD. 3α-OHSDH activity was linear until a substrate concentration of approximately 10^{-6} M and hereafter a plateau was reached.

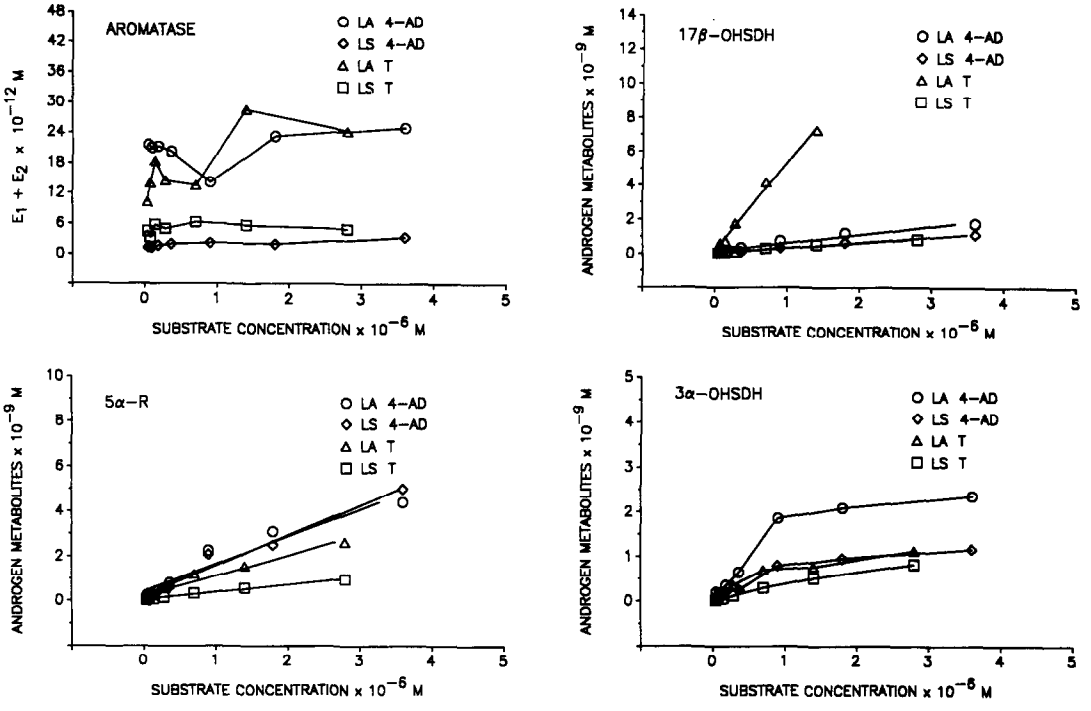

Fig. 5. The activity of aromatase, 5α-R, 17β-OHSDH, and 3α-OHSDH in cultured human fibroblasts (LS and LA) following 6 h preincubation with 0.25×10^{-6} M cortisol, and subsequent incubation for 22 h with different concentrations of [^{14}C]T or [^{14}C]4-AD.

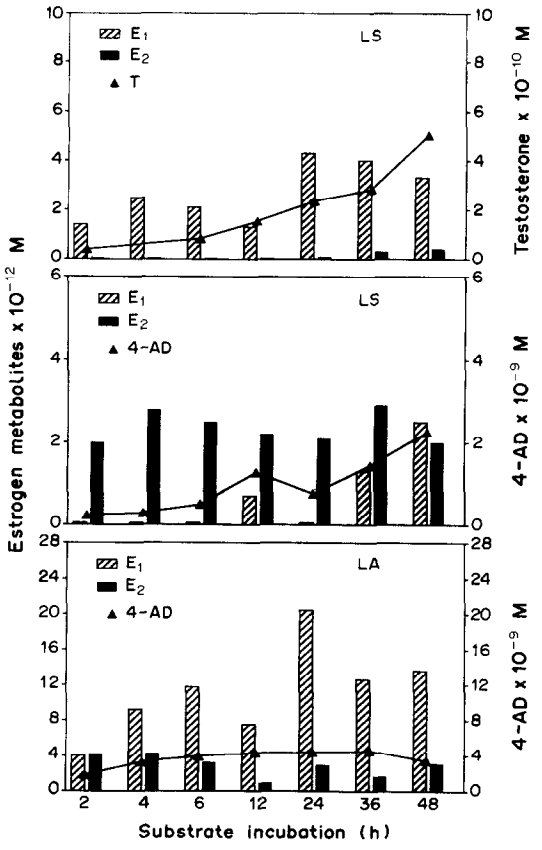

Fig. 6. Formation of E_1, E_2, and T/4-AD in cultured human skin fibroblasts (LS and LA) following 6 h preincubation with 0.25×10^{-6} M cortisol, and subsequent incubation for increasing time with 10^{-6} M [^{14}C]T or [^{14}C]4-AD. T and 4-AD were metabolized when [^{14}C]4-AD and [^{14}C]T, respectively, was substrate.

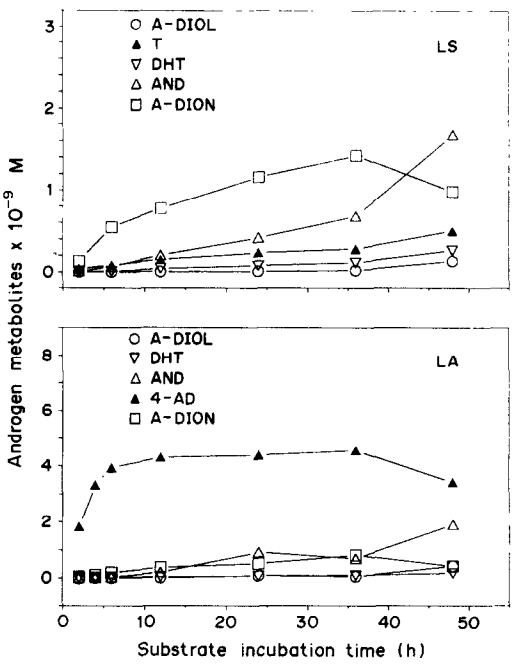

Fig. 7. Formation of androgen metabolites in cultured human skin fibroblasts (LS and LA) following 6 h preincubation with 0.25×10^{-6} M cortisol, and subsequent incubation for increasing time with 10^{-6} M [^{14}C]T or [^{14}C]4-AD. In the LS-experiment shown (top) [^{14}C]4-AD was substrate, while [^{14}C]T was substrate in the LA-experiment (bottom).

The amount of 3α-reduced products was highest when [^{14}C]4-AD was substrate and LA cells were the most active.

Time-course experiments

Estrogens. In both cell lines incubation with [^{14}C]4-AD resulted in aromatization only to E_1. Figure 6 shows aromatization in LS cells with rising values until 24 h incubation and from 24–48 h incubation a constant E_1 level was reached. In LA cells the E_1 level was 3-fold the level in LS cells but the course was identical. When LS cells were incubated with [^{14}C]T the formation of E_2 was constant from 2 to 48 h incubation. E_1 was detected after 36 h incubation simultaneously with the rise in 4-AD concentration, indicating that E_1 was produced from 4-AD and not from oxidation of E_2. In LA cells the time-course for aromatization of [^{14}C]T showed a slight fall in formation of E_2 compared with a rise in E_1 formation. These cells have a high 17β-OHSDH activity so E_1 formation was likely to be derived partly from oxidation of E_2 and partly from aromatization of 4-AD.

Androgens. The enzymatic activity of 5α-R and of 17β-OHSDH had a linear course for 48 h and of 3α-OHSDH for 36 h in both cell lines when 4-AD was used as substrate (not shown). Figure 7 shows the time-course of the individual androgens isolated from incubations with LS cells using [^{14}C]4-AD as substrate. [^{14}C]4-AD was primarily metabolized to A-DION which was further matabolized to AND. Formation of T had a linear course. The metabolites DHT and A-DIOL seem to be derived from T and from AND. The metabolic pattern was similar in LA cells when 4-AD was used as substrate.

The metabolic activity in LA cells is four times higher than in LS cells when [^{14}C]T was used as substrate but the relative amount of metabolites formed was almost identical. So in Fig. 7 only the time-course of the individual androgen metabolites formed in LA cells is shown. A high 17β-OHSDH activity, resulting in formation of 4-AD was seen. The concentration of 4-AD reached a plateau after 6 h as further metabolization to A-DION and AND occurred. DHT and A-DIOL was probably derived from T but also from A-DION and AND. Taking all 5α-reduced metabolites together a linear course was found and the same was observed for the metabolites of 3α-OHSDH. The course of 17β-OHSDH activity followed the formation of 4-AD.

DISCUSSION

Aromatase activity was detected only when the cells were preincubated with cortisol while no effects of cortisol on the interconversion of androgens were

seen. The cortisol concentration required for maximal stimulation of aromatase activity was found to be $0.5–1.0 \times 10^{-6}$ M which is in agreement with other reports [7, 14, 15]. Preincubation with cortisol for more than 12 h with [^{14}C]4-AD as substrate resulted in a decline in aromatase activity in both cell lines. Also in cell line LA incubated with [^{14}C]T as substrate a decline in aromatase activity was observed after preincubation with cortisol for more than 12 h. In contrast to the LS cells, LA cells had a predominant oxidative function of 17β-OHSDH resulting in a high concentration of 4-AD in incubations with [^{14}C]T as substrate. 4-AD could be the main substrate for the aromatase system in cell line LA resulting primarily in formation of E_1 although it cannot be excluded that E_1 was derived partly from oxidation of E_2. In cell line LS, with low 17β-OHSDH activity, incubated with [^{14}C]T as substrate maximal stimulation of aromatase activity was seen after 6–12 h preincubation with cortisol and no decline in aromatase activity was observed after 36 h preincubation indicating a different regulation of the aromatase activity when [^{14}C]T was substrate. Other possibilities are the existence of two aromatase enzyme systems acting specifically on either 4-AD or T as proposed by Osawa *et al.*[16], or as suggested by Fishman and Goto[17] that each of the steps in the aromatization sequence, using different substrates, may be catalyzed by a different catalytic site within the same protein. Recently, Corbin *et al.*[18] isolated a full-length cDNA insert encoding human aromatase, and the expression in COS1 monkey kidney tumor cells, which normally lack steroidogenic capacity, showed that the transfected cells were capable of catalyzing the aromatization of androgens independent of differences in ring D substitution. These findings suggest that only one aromatase enzyme is present in human tissues. Berkovitz *et al.*[19] reported that the corticoid, dexamethasone produced a time-dependent induction of aromatase activity in human skin fibroblasts, incubated with 4-AD as substrate, with peak levels at 12 h followed by a return to baseline, suggesting that the initial stimulation of aromatase was mediated by glucocorticoid receptors which are down-regulated by glucocorticoids and that the aromatase gene is subjected to positive and negative control with a repressor protein involved. The lower formation of 5α-reduced products in cell line LS incubated with [^{14}C]T as substrate may also be of importance for the constant aromatase activity after 36 h preincubation with cortisol as 5α-reduced androgens can inhibit the aromatization of T [7]. Simpson *et al.*[20] reported that dexamethasone increased the aromatase activity to a maximum after 24 h preincubation with no decline after 72 h using stromal cells from human subcutaneous adipose tissue and 4-AD as substrate. These results indicate that glucocorticoids can alter the aromatase activity significantly and in different ways depending on cell type and/or substrate.

Pronounced differences in enzyme activities were found in the LS cells derived from scrotal skin, an area known to grow in response to androgens, and LA cells derived from the forearm, an area which is not considered to be dependent on steroids. In LS cells the oxidative and reductive function of 17β-OHSDH regulating the local concentrations of the biologically active 17β-hydroxysteroids, T and E_2 were low. T secreted from the testes will thus not be transformed to the less active androgen 4-AD in these cells and a high concentration of T can be maintained. Furthermore, the 5α-R mainly converted T to DHT the steroid which together with T mediates the intracellular action of androgens in many target tissues [21]. In LS cells E_2 derived from [^{14}C]T was not converted to the less potent estrogen E_1. It can be concluded that only biologically active androgens and estrogens were synthesized in LS cells.

In LA cells the formation of estrogens was 3-fold the formation in LS cells but the concentration of E_2 was lower caused by 17β-OHSDH favouring formation of the less potent steroids 4-AD and E_1. The major 5α-reduced metabolites of [^{14}C]4-AD and of [^{14}C]T in cell line LA were A-DION and AND while DHT was of less importance. In cell line LA in contrast to cell line LS mainly biologically less potent androgens and estrogens were synthesized. It is not yet certain that the interconversions of 4-AD/T and E_1/E_2 are mediated by the same enzyme rather than related enzymes. The existence of multiple forms of 17β-OHSDH has been suggested [22], but from the present study it can not be deduced if more than one 17β-OHSDH exists.

Little information is available concerning 3α-OHSDH in human tissues and the biological function of AND and A-DIOL in humans is unknown. We found AND as a major metabolite of 4-AD in both cell line LA and in cell line LS, and also of T in cell line LA. The transforation of AND and/or DHT to A-DIOL was low in both cell lines with both substrates. AND was also reported to be a major metabolite of 4-AD in breast cancer cells, and of 4-AD and T in hair roots and in homogenates of tissue from breast and benign hypertrophic prostatic tissue [9, 23, 24].

The striking differences in estrogen and androgen metabolism observed in the two cell lines derived from the same subject suggest a local regulation of the hormonal environment. The source of T and 4-AD are mainly gonadal and adrenocortical secretion. Only T and DHT are bound to the androgen receptor and mediate androgen action in androgen target tissues such as foreskin, scrotum, prostate, hair follicles and sebaceous glands [21]. Thus, the activity of 5α-R and 17β-OHSDH is of interest not only for investigating the matabolic differences among tissues but also whether a given tissue has the capacity to develop response to androgens. Concerning estrogens the peripheral conversion of androgens may account for 50% of the total estrogen produced in young

women depending on the stage of the menstrual cycle [25]. In postmenopausal women and in men, extra glandular tissues such as muscle, fat and skin are considered to be principal sites of estrogen production [26, 27]. The local estrogen production has a possible role in the pathogenesis of gynecomastia, benign prostatic hypertrophy and certain forms of infertility in men [28–30]. In women estrogens influence development and growth of carcinomas of the breast and endometrium [31]. It seems that many tissues are involved in the synthesis of estrogens and that the local modulation of steroid metabolism represent a microendocrine system through which local tissues may be exposed and respond differently. The biological significance of the widespread tissue distribution of aromatase activity and its variation remains to be determined.

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