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METABOLISM OF ADRENAL ANDROGENS BY HUMAN ENDOMETRIUM AND ADRENAL CORTEX

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Summary—The enzyme 17β-hydroxysteroid dehydrogenase (17OHSD) was studied in human endometrium and adrenal cortex with respect to the metabolism of 5-androstene-3β,17β-diol (androstenediol) and dehydroepiandrosterone (DHA). The aim was to provide further information concerning the origin and biological significance of these androgens in endometrium, particularly the increased concentrations of the secretory phase and to compare the characteristics of the enzyme in the two tissues.

In both endometrium and adrenal cortex the metabolism of androstenediol to DHA was linear with time and increasing enzyme concentration. The preferred cofactor was NAD and the apparent K_m values were 3.4 ± 0.2 (SD) μM ($n = 3$) for endometrium and 30.5 ± 6.1 μM ($n = 3$) for adrenal cortex. In endometrium DHA was not metabolised to androstenediol in the presence of either NADH or NADPH whereas in the adrenal cortex both cofactors were utilised. However, the concentration of NADH required to achieve maximum enzyme activity was 10-fold higher (1 mM) than for NADPH (0.1 mM) and maximum activity with NADH was only 30% of that using NADPH. The apparent K_m was 125 μM DHA ($n = 2$).

The study indicates that androstenediol in endometrium does not arise from DHA metabolism but that its presence could be due to a binding protein particularly during the secretory phase. Our findings also suggest that the enzyme of endometrium differs from that of the adrenal cortex and that the kinetic properties may be related to the physiological requirements of the two tissues.

INTRODUCTION

Our earlier studies have demonstrated that 5-androstene-3β,17β-diol (androstenediol), a weak androgen with oestrogenic properties, and the metabolically related dehydroepiandrosterone (DHA) are present in human endometrium and that concentrations increase 4-fold during the secretory phase without any corresponding increase in the plasma concentrations [1, 2]. The origin and biological significance of these androgens in endometrium is unknown although androstenediol has been shown to bind to the oestradiol receptor in both rat [3] and human uterine tissue [4] and to exert an oestrogenic effect. However, the concentration of oestrogen receptors in endometrium are at a maximum in the proliferative phase [5, 6] and thus, this is unlikely to be responsible for the increase in androstenediol concentrations in secretory phase tissue. A high affinity specific nuclear binding protein for androstenediol has been demonstrated in rat vaginal and uterine tissue [7] but similar proteins in human endometrium have yet to be identified.

In order to obtain information about the origin of androstenediol and DHA in endometrium we have studied the enzyme 17β-hydroxysteroid dehydrogenase (17OHSD) with an aim to determining whether androstenediol arises from DHA metabolism or *vice versa*. The current interest in the diversity of 17OHSD enzymes in different tissues led us to compare the characteristics of the enzyme in endometrium with that of the adrenal cortex, a tissue which is considered to be the major source of these androgens.

EXPERIMENTAL

Steroids

[4-^{14}C]Dehydroepiandrosterone (sp. act. 51.9 mCi/mmol) and [1,2-^{3}H[*N*)]-androst-5-ene-3β,17β-diol (sp. act. 45 Ci/mmol) were purchased from New England Nuclear, Dreieich, W. Germany. Purity was checked by thin layer chromatography (TLC). Unlabelled steroids were obtained from the Sigma Chemical Company, Poole, Dorset, U.K.

Tissue

Secretory phase endometrium was obtained at hysterectomy or curettage from women undergoing surgery for non-malignant conditions. The tissue was either used immediately or frozen within 30 min of collection and stored at −20°C until required. It was possible to store tissue for at least 1 month without loss of activity.

Tissue from the adrenal cortex was obtained at 6–8 h post mortem from 2 female subjects aged 76 and 85 years. The cause of death was congestive cardiac failure and pneumonia respectively. The tissue was dissected free of medulla and fat and used immediately or stored at −20°C until required. There was no effect of storage for up to 1 month.

Enzyme studies

The characteristics of the enzyme 17OHSD with respect to adrenal androgen metabolism were investigated in both endometrium and adrenal tissue using a modification of a method described for oestrogen metabolism studies [8]. Conditions of time,

enzyme concentration, cofactor requirement and substrate concentration were examined for both the oxidation reaction (androstenediol → DHA) and the reduction reaction (DHA → androstenediol).

Enzyme preparation

Tissue was weighed and homogenized briefly (using a Polytron homogenizer) in ice-cold 0.1 M phosphate buffer pH 7.4 at a concentration of 50–100 mg tissue per ml buffer. The homogenate was then centrifuged at 600 *g* for 10 min and the supernatant decanted and retained for enzyme studies. Protein was measured by the method of Lowry *et al.*[9] using bovine serum albumin as standard and the results were expressed as pmol product per mg protein per min.

Incubation procedure

Tissue homogenates (50–100 μl) were incubated in 100 × 15 mm glass tubes in 0.1 M phosphate buffer pH 7.4 containing either [1,2-^{3}H]androstenediol (4×10^5 cpm) and unlabelled androstenediol (for the oxidation reaction) or [4-^{14}C]DHA (10^5 cpm) and unlabelled DHA (for the reduction reaction). The final incubation volume of 350 μl included cofactors as described below. Procedural losses were monitored by the addition of [^{14}C]DHA (4×10^3 cpm) or [^{3}H]androstenediol (4×10^4 cpm) to the incubation medium during oxidation and reduction reactions respectively. All steroids were dispensed in ethanol, evaporated to dryness and reconstituted in buffer. The reaction was initiated by the addition of cofactors. The incubation was carried out at 37°C in a shaking water bath for the appropriate time and the reaction terminated by the addition of 3 ml diethyl ether. Enzyme blanks were included in each study.

Extraction and separation of products

Androstenediol and DHA were extracted with 3 ml diethyl ether and separated by chromatography on silica gel thin-layer plates (E. Merck, Darmstadt, W. Germany) using the system dichloromethane–dioxane (94:6, v/v). Each plate was developed twice in the same system. The area corresponding to the product was located by a radiochromatogram scanner (Panax). Radioactivity was measured in a Beckman LS7500 dual label scintillation counter using a toluene based scintillant. The counting efficiency was 50% for ^{14}C and 32% for tritium.

Tissue incubation studies

Duplicate (100 mg) aliquots of fresh endometrium were washed in Medium 199 with Earle's salts and 0.2% sodium bicarbonate (Flow Laboratories, U.K.) chopped finely and incubated in mini scintillation vials containing either [^{3}H]androstenediol or [^{3}H]DHA (2×10^6 cpm) which has been reconstituted in 1 ml Medium 199. The medium also contained 1 mM NAD or NADH respectively. The vials were gassed with 95% O_2–5% CO_2, capped immediately and incubated for 1 h at 37°C. Vials without tissue were included at each incubation to check any spontaneous decomposition of substrate. At the end of the incubation time the tissue and medium were extracted with 2 × 5 ml diethyl ether and the products separated by TLC with double development using the system dichloromethane–dioxane (94:6, v/v). Radioactive and unlabelled reference steroids were used for identification of the reaction products. Radioactive metabolites were detected by a Panax radiochromatogram scanner and identified by comparison of the calculated R_f value with that of the authentic steroid. Unlabelled reference steroids were visualized by spraying the plate with 10% phosphomolybdic acid in ethanol. Radioactivity was counted as described above.

RESULTS

Enzyme studies

Time course. Adrenal tissue homogenate (100–150 μg protein) was incubated in the presence of 14 μM androstenediol and 2 mM NAD for periods of time up to 120 min. The metabolism of androstenediol to DHA was found to be linear with time for 45 min (Fig. 1a) after which the conversion of DHA to further metabolites became significant. A similar relationship with time was found for endometrium (Fig. 1b).

The reduction reaction was investigated by incubating adrenal tissue homogenate with 6 μM DHA and 0.5 mM NADPH for a similar period of time. Androstenediol production from DHA was shown to be linear for 90 min. However, when endometrial tissue homogenate was incubated for up to 120 min with 15 μM DHA and either NADH or NADPH (1 mM) no conversion of DHA to androstenediol could be detected. Similar results were obtained with both fresh and frozen tissue.

Enzyme concentration

Linearity with respect to enzyme concentration was determined by incubating aliquots of tissue homogenate containing up to 0.9 mg protein in the presence of the relevant substrates and cofactors. When either endometrial or adrenal tissue homogenates were incubated for 30 min with 14 μM androstenediol and 2 mM NAD, a linear relationship between the amount of enzyme present and the production of DHA was demonstrated over the range 30–160 μg protein (Fig. 2a,b). Incubation of similar amounts of adrenal tissue homogenate with 6 μM DHA and 0.5 μM NADPH for 60 min established a linear relationship with androstenediol production over the same range of enzyme concentrations (Fig. 2c).

Cofactor requirements

The conversion of androstenediol to DHA by adrenal tissue homogenate as a function of cofactor concentration is shown in Fig. 3a. The enzyme preparation was incubated for 30 min with 14 μM

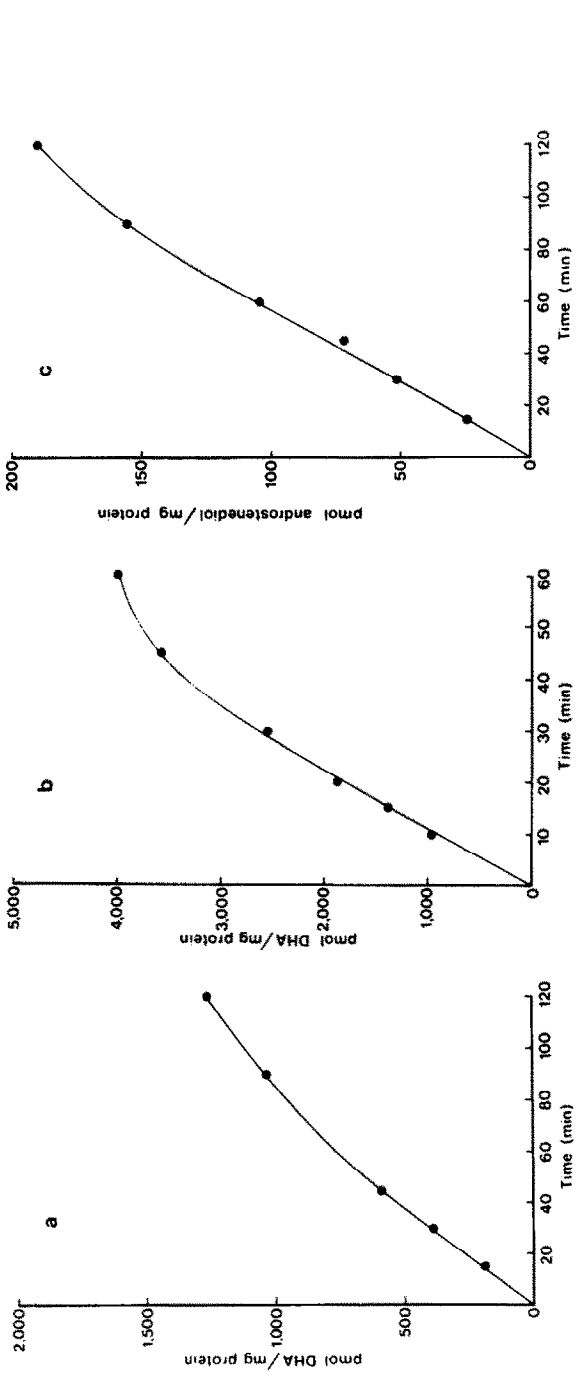

Fig. 1. Time course of the conversion of androstenediol to DHA by (a) adrenal cortex (b) endometrium and (c) of the conversion of DHA to androstenediol by adrenal cortex. The activity of 17OHSD is expressed as pmol androstenediol or DHA formed per mg protein.

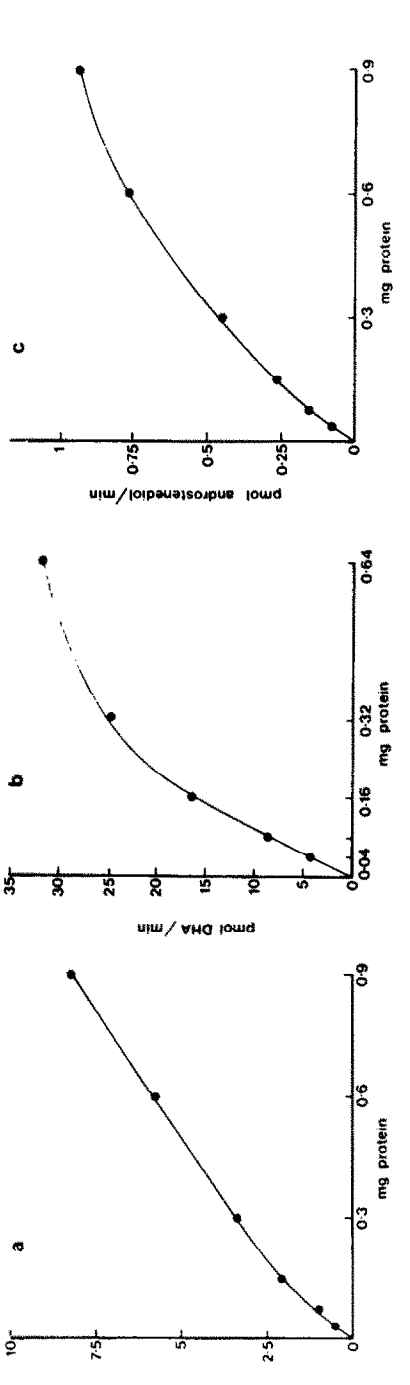

Fig. 2. The effect of protein concentration on the conversion of androstenediol to DHA by (a) adrenal cortex (b) endometrium and (c) of the conversion of DHA to androstenediol by adrenal cortex. The activity of 17OHSD is expressed as pmol androstenediol or DHA formed per mg protein.

androstenediol and a range of concentrations of either NAD or NADP between 2.0 and 0.125 mM. The enzyme was able to utilize both cofactors but the maximum velocity in the presence of NADP was only 50% of that of NAD. A concentration of 1 mM NAD or NADP was required for maximum enzyme activity. When endometrial tissue homogenate was incubated under the same conditions using a range of cofactor concentrations between 1.0 and 0.0625 mM the enzyme was shown to be maximally active at 0.5 mM NAD but was unable to use NADP as a cofactor (Fig. 3b).

The cofactor requirements for the conversion of DHA to androstenediol by adrenal tissue homogenate were assessed in a similar manner using a range of concentrations of NADH and NADPH between 2.0 and 0.0125 mM. The incubation period was 60 min. The maximum velocity in the presence of NADH was only 30% of that in the presence of NADPH (Fig. 3c). Furthermore, the concentration of

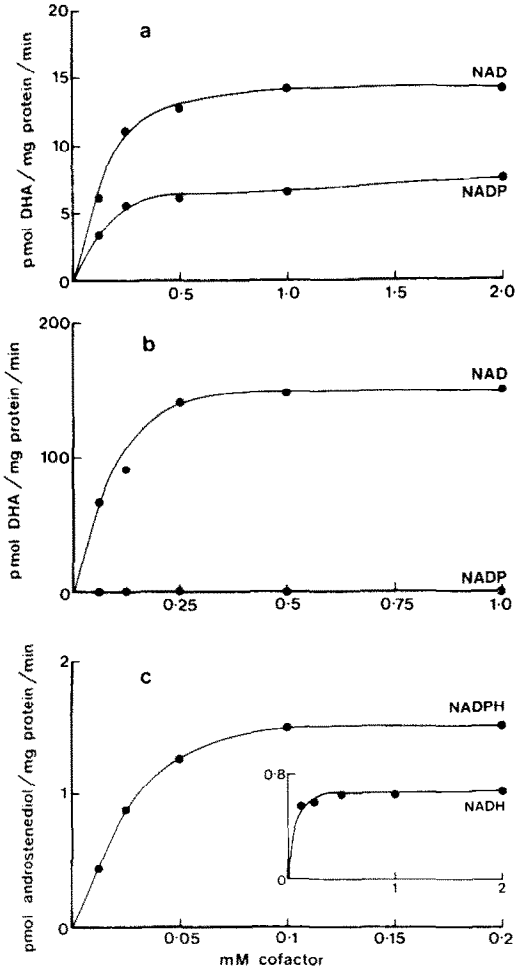

Fig. 3. The conversion of androstenediol to DHA by (a) adrenal cortex (b) endometrium and (c) of the conversion of DHA to androstenediol by adrenal cortex as a function of cofactor concentration. Enzyme activity is expressed as pmol androstenediol or DHA formed per mg protein.

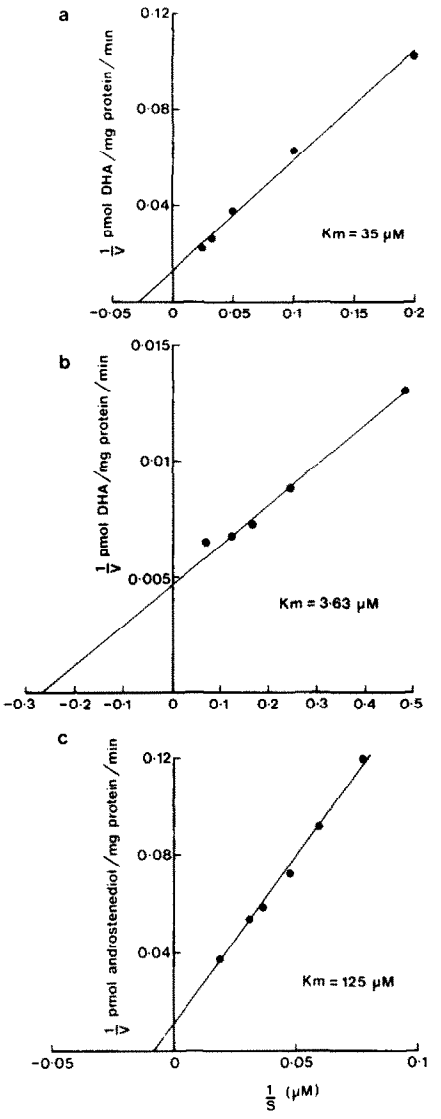

Fig. 4. Lineweaver–Burk plot of the initial velocities of the conversion of androstenediol to DHA in (a) adrenal cortex (b) endometrium and (c) of the conversion of DHA to androstenediol in adrenal cortex at various concentrations of substrate.

NADPH required for maximum activity was 0.1 mM which was 10-fold lower than the requirement for NADH (1 mM).

Apparent Michaelis Menton constant

The apparent K_m was determined by the method of Lineweaver and Burk[10] using duplicate measurements of initial velocity over a range of substrate concentrations at a single time point of 10 min in the presence of excess cofactors. Typical Lineweaver–Burk plots are shown in Fig. 4. The apparent K_m for the conversion of androstenediol to DHA by adrenal tissue was 30.5 ± 6.1 (SD) μM ($n = 3$) and for DHA to androstenediol, 125 μM ($n = 2$). A much lower K_m ($3.4 \pm 0.2\,\mu$M, $n = 3$) was found for the conversion of androstenediol to DHA by endometrium.

Table 1. Androstenediol and DHA metabolism by endometrium

R_f value	Steroid	% cpm recovered	
		Androstenediol	DHA
0.06	7β-Hydroxy DHA	6.6	0.0
0.21	—	5.4	4.0
0.27	Androstenediol	51.4	2.8
0.36	Testosterone	1.0	3.2
0.47	DHA	35.3	89.0
0.59	Androstenedione	0.3	0.1

Tissue incubation studies

Incubation of endometrium with [^{3}H]androstenediol resulted in the production of several identifiable metabolites of which DHA was predominant [35.3%]. A scan of the radioactive metabolites formed is shown in Fig. 5a. The other products as listed in Table 1 were 3β,7β-dihydroxyandrost-5-en-17-one (7β-hydroxy DHA) [6.6%], testosterone [1.0%], androstenedione [0.3%], and an unidentified metabolite with an R_f value of 0.21 [5.4%]. The total recovery from the plate was 74%. On the other hand, [^{3}H]DHA was not metabolised to any significant extent since 89% of the total radioactivity recovered remained unchanged (Table 1, Fig. 5b). The only metabolite present in any quantity was the unidentified steroid noted above [4.0%]. Androstenediol, testosterone and androstenedione were present in small amounts, namely, 2.8, 3.2 and 0.1% respectively but 7β-hydroxy DHA was not present. 3β,7α-Dihydroxyandrost-5-en-17-one (7α-hydroxy DHA) was not identified in either case. There was no spontaneous metabolism of either [^{3}H]androstenediol or [^{3}H]DHA as evidenced by the blanks included in the study.

DISCUSSION

The majority of studies of 17OHSD activity in human endometrium have been concerned with the metabolism of oestrogens [11–14]. It is well established that the enzyme is able to interconvert oestradiol and oestrone using NAD and NADH as cofactors and that the kinetics of the reaction favour the oxidation of oestradiol to oestrone [15]. Some studies have investigated a range of other steroids as substrates, namely testosterone, androstenedione, androstenediol and DHA [15, 16] with the purpose of determining whether or not a single dehydrogenase is responsible for the metabolic transformation of all these steroids in endometrium. Tseng and Gurpide[16] demonstrated that the steroid dehydrogenase activities for the four substrates are all stimulated by progestins but did not provide conclusive evidence of the existence or otherwise of four different enzymes. Tseng *et al.*[15] quote apparent K_m values of 0.6 and 0.8 μM for the transformation of androstenediol and DHA respectively but the V_{max} for the conversion of DHA to androstenediol was extremely low indicating that the enzyme would be unlikely to catalyse the reduction of DHA to any significant extent. Our findings in the present study are essentially in agreement. The preferred cofactor for the oxidation reaction was NAD and the average apparent K_m was 3.6 μM. Such variation in apparent K_m values between tissues might be expected when studying crude enzyme preparations. It was not possible to detect any significant metabolism of DHA to androstenediol in the presence of either NADH or NADPH indicating a non-reversible reaction. Further confirmation of this finding was obtained by incubating endometrium with tritiated substrates. Under these conditions the predominant metabolite of androstenediol was DHA whereas radioactivity measured in the area corresponding to androstenediol was negligible. These observations indicate that androstenediol present in human endometrium is unlikely to be derived from DHA and that the reverse is probably the case. Evidence suggests that androstenediol could enter the endometrium directly from the plasma to be concentrated within the tissue, particularly during the luteal phase, by a binding protein. The presence of a specific nuclear binding protein for androstenediol in rat vaginal tissue and to some extent uterine tissue [7] lends weight to the concept of such a binding protein in human endometrium. Although there is at present no evidence to support this, there have been reports of other steroid induced proteins in endometrium. For instance, Ciocca *et al.*[17] demonstrated the presence

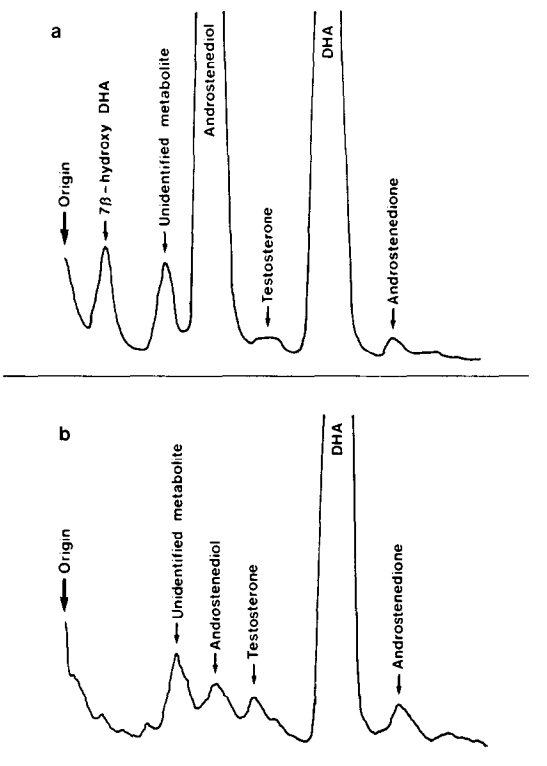

Fig. 5. Radiochromatogram scan of the radioactive metabolites formed from (a) [^{3}H]androstenediol (b) [^{3}H]DHA during incubation with endometrial tissue pieces.

of a 24 K protein of unknown function present in late proliferative and secretory phase endometrium indicating an association with oestrogen and progesterone. Also, a progesterone dependent endometrial protein has been described by Joshi *et al.*[18] and its presence in endometrium shown to correlate with increased secretory activity.

The 17OHSD of endometrium is thus able to interconvert oestradiol and oestrone but not androstenediol and DHA. Furthermore, the enzyme is able to utilize NADP as a cofactor for the oxidation of oestradiol [14] but not androstenediol. Whether a single enzyme is responsible for both transformations in endometrium is uncertain from the evidence available to date. However, the androgen metabolising 17OHSD of adrenal cortex does appear to differ from the endometrial enzyme. For instance, the adrenal enzyme is able to use both NAD and NADP for the oxidation of androstenediol and the apparent K_m (mean value 30 μM) is 10-fold higher than that of the endometrial enzyme (mean value 3.4 μM). The adrenal 17OHSD is also able to catalyse the reduction reaction, albeit at high concentrations of DHA (apparent K_m 125 μM) and is saturated at comparatively low concentrations of NADPH. In this case the preferred cofactor is NADPH where as oestradiol dehydrogenase of endometrium requires NADH [15]. Thus substantial differences exist between the two enzymes although studies with purified enzyme preparations are necessary to confirm these observations.

The physiological requirements of the two tissues demand different enzymic properties. The adrenal cortex is an important site of both DHA and androstenediol production and the emphasis on generation of either steroid is likely to depend on substrate and cofactor availability. The V_{max} is similar for both reactions and therefore at high concentrations of DHA in the presence of comparatively low concentrations of NADPH conditions will favour the production of androstenediol. On the other hand, when concentrations of DHA are low and NADPH is unavailable, there will be a tendency towards DHA production. Thus a mechanism may exist for the regulation of adrenal androgen production by the adrenal cortex via the direction of 17OHSD activity. Since the endometrium is unlikely to be an important site of adrenal androgen production a similar mode of regulation would be unnecessary.

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REFERENCES

1. Bonney R. C., Scanlon M. J., Reed M. J. and James V. H. T.: Endometrial tissue and plasma concentrations of 5-androstene-3β,17β-diol during the menstrual cycle in normal premenopausal and perimenopausal women. *J. steroid Biochem.* **19** (1983) 1627–1634.
2. Bonney R. C., Scanlon M. J., Jones D. L., Reed M. J. and James V. H. T.: Adrenal androgen concentrations in endometrium and plasma during the menstrual cycle. *J. Endocr.* **101** (1984) 181–188.
3. van Doorn L. G., Berenschot-Roozendaal J., Poortman J. and Thijssen J. H. H.: Binding characteristics of 5-androstene-3β,17β-diol and estradiol-17β to the cytoplasmic estrogen receptor of the immature rat uterus. *J. steroid Biochem.* **16** (1982) 661–671.
4. Kreitman B. and Bayard F.: Androgen interaction with the oestrogen receptor in human tissues. *J. steroid Biochem.* **11** (1979) 1589–1595.
5. Evans L. H., Martin P. J. D. and Hahnel R.: Estrogen receptor concentration in normal and pathological human uterine tissues. *J. clin. Endocr. Metab.* **38** (1974) 23–32.
6. Rodgers N. T. and Kaufman D. G.: The measurement of cytosolic estrogen receptors in human endometrial tissue and organ cultures. *J. steroid Biochem.* **14** (1981) 801–806.
7. Shao T., Castaneda E., Rosenfield R. L. and Liao S.: Selective retention and formation of a Δ^5-androstenediol receptor complex in cell nuclei of the rat vagina. *J. biol. Chem.* **250** (1975) 3095–3100.
8. Bonney R. C., Reed M. J. and James V. H. T.: Inhibition of 17β-hydroxysteroid dehydrogenase activity in human endometrium by adrenal androgens. *J. steroid Biochem.* **18** (1983) 59–64.
9. Lowry Q. H., Rosenbrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
10. Lineweaver H. and Burk D.: The determination of enzyme dissociation constants. *J. Am. chem. Soc.* **56** (1934) 658–666.
11. Tseng L. and Gurpide E.: Estradiol and 20α-dihydroprogesterone dehydrogenase activities in human endometrium during the menstrual cycle. *Endocrinology* **94** (1974) 419–423.
12. Tseng L. and Gurpide E.: Induction of human endometrial estradiol dehydrogenase by progestins. *Endocrinology* **97** (1975) 825–833.
13. Pollow K., Lübbert H., Boquoi E., Kreutzer G., Jeske R. and Pollow B.: Studies on 17β-hydroxysteroid dehydrogenase in human endometrium and endometrial carcinoma. I. Subcellular distribution and variations of specific enzyme activity. *Acta endocr., Copenh.* **79** (1975) 134–145.
14. Pollow K., Lübbert H., Jeske R. and Pollow B.: Studies 17β-hydroxysteroid dehydrogenase in human endometrium endometrial carcinoma. II. Characterization of the soluble enzyme from secretory endometrium. *Acta endocr., Copenh.* **79** (1975) 146–156.
15. Tseng L., Mazella J. and Tseng L.: Kinetic studies of human endometrial hydroxysteroid dehydrogenase. *J. steroid Biochem.* **14** (1981) 437–442.
16. Tseng L. and Gurpide E.: Stimulation of various 17β and 20α-hydroxysteroid dehydrogenase activities by progestins in human endometrium. *Endocrinology* **104** (1979) 1745–1748.
17. Ciocca D. R., Asch R. H., Adams D. J. and McGuire W. L.: Evidence for the modulation of a 24 K protein in human endometrium during the menstrual cycle. *J. clin. Endocr. Metab.* **57** (1983) 496–499.
18. Joshi S. G., Henriques E. S., Smith R. A. and Szarowski D. H.: Progestogen dependent endometrial protein in women: Tissue concentration in relation to developmental stage and to serum hormone levels. *Am. J. Obstet. Gynecol.* **138** (1980) 1131–1136.