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ANDROSTENEDIONE IS AN IMPORTANT PRECURSOR OF DIHYDROTESTOSTERONE IN THE GENITAL SKIN OF WOMEN AND IS METABOLIZED VIA 5α-ANDROSTANEDIONE*

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Summary—Androgen action is largely determined by the formation of dihydrotestosterone in target tissues. In women, androstenedione is the major precursor of dihydrotestosterone production in female genital skin. The present study was initiated to determine whether androstenedione is converted to dihydrotestosterone primarily via testosterone or 5α-androstane-3,17-dione (5α-androstanedione), and to examine the pathway of androstenedione metabolism in genital skin. Genital skin was obtained from 9 normal premenopausal women and 2 normal men. Each tissue was incubated with [^{3}H]androstenedione in RPMI-1640 medium for 1 h at 37°C in 95% O_2/5% CO_2. The metabolites were separated and purified by paper partition and thin-layer chromatography. The conversions of androstenedione to 5α-androstanedione and to androsterone were similar (10.45 ± 1.46 and 11.04 ± 2.04%/200 mg tissue), and were approx. 12, 8 and 23 times higher than the conversion of androstenedione to testosterone, dihydrotestosterone and 5α-androstane-3α,17β-diol, respectively. The male samples showed a similar pattern of metabolism. These data indicate that 5α-androstanedione is the most important intermediate in the conversion of androstenedione to dihydrotestosterone. The data also confirm the importance of 5α-reductase activity over that of 17β-hydroxysteroid oxidoreductase activity in the expression of androgen action in women.

INTRODUCTION

The expression of androgenicity is largely determined by the formation of dihydrotestosterone in target tissues. In both normal and hirsute women, the major circulating C_{19} androgen prehormone is androstenedione, and essentially all of the circulating dihydrotestosterone arises from this prehormone [1]. Recently, we have demonstrated *in vitro* that androstenedione is a major precursor of dihydrotestosterone production in genital and pubic skin [2]. These data complement the work of others, suggesting the importance of androstenedione as a precursor of peripheral androgen metabolism in normal and hirsute women [3].

Our data on the conversion of androstenedione to dihydrotestosterone by skin suggested that androstenedione may be first converted to an intermediate other than testosterone. The most likely intermediate for this conversion is 5α-androstane-3,17-dione. The present study was initiated to further investigate the metabolism of androstenedione by genital skin in women. Specifically, we have studied whether androstenedione is converted to dihydrotestosterone primarily via testosterone or 5α-androstanedione. We also examined the conversion of androstenedione to other reduced metabolites, since there is a paucity of data concerning the metabolism of this precursor by genital skin.

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EXPERIMENTAL

Solvents and reagents

All organic solvents (American Burdick & Jackson; Baxter Scientific Products, Chicago, Ill.; EM Science, Cherry Hill, N.J.) were glass distilled. Pyridine and acetic anhydride (J. T. Baker Co., Phillipsburg, N.J.) were reagent grade. Silica gel 60 F_{254} (EM Science) was used for thin-layer chromatography. The medium used for incubation was Roswell Park Memorial Institute (RPMI)-1640 (Gibco Laboratories, Grand Island, N.Y.).

Steroid standards

Authentic steroids used as chromatographic markers and for establishing radiochemical purity of metabolites by reverse isotope dilution were purchased from Steraloids Inc., Wilton, N.H. or Sigma Chemical Company, St Louis, Mo. Each steroid was crystallized three times with different solvent mixtures prior to use.

Radioactive steroids

[1α,2α-^{3}H]androstenedione (sp. act. 42 Ci/mmol, DuPont–NEN Research Products, Boston, Mass) was used as the substrate for incubation studies. [4-^{14}C]androstenedione (sp. act. 52 mCi/mmol), [4-^{14}C]5α-androstanedione (sp. act., 57 mCi/mmol) [4-^{14}C]testosterone (sp. act., 56.9 mCi/mmol; Amersham, Arlington Heights, Ill.), [4-^{14}C]dihydrotestosterone (sp. act., 57 mCi/mmol; Amersham), [4-^{14}C]androsterone (sp. act., 57 mCi/mmol), and [4-^{14}C]5α-androstane-3α,17β-diol (sp. act. 57 mCi/mmol) were used as internal standards to monitor procedure losses. [4-^{14}C]5α-androstanedione, and [4-^{14}C]5α-androstane-3α,17β-diol were prepared from [4-^{14}C]dihydrotestosterone. Synthesis of [^{14}C]5α-androstanedione was carried out by chromic acid oxidation of [^{14}C]dihydrotestosterone as described previously [4]. The product was used to synthesize [^{14}C]androsterone by enzymatic reduction with 3α-hydroxysteroid oxidoreductase [4, 5]. This enzyme was also used to prepare [^{14}C]5α-androstane-3α,17β-diol from [^{14}C]dihydrotestosterone [5]. The radiochemical purity of the radioligands was determined by reverse isotope dilution; the purity of each compound was greater than 95%.

Tissue source and preparation for incubation

Samples of genital skin were obtained from 9 normal, non-hirsute, premenopausal women in their fourth decade of life. These women, who were on no medication and had no medical or endocrinologic illnesses, were undergoing elective laparotomy and/or vaginal surgery for benign gynecological disease. The genital skin samples were taken from the posterior margin of the labia majora as previously described [6]. As a basis for preliminary comparisons, we also obtained scrotal skin from 2 normal men during elective vasectomy. Permission to carry out the present study was received from our Institutional Review Board.

Following its surgical removal, the tissue was placed in RPMI-1640 medium, which was kept on ice. The tissue was then dissected free of fat and minced finely at 4°C as described previously [6]. Wet weights of the prepared skin minces were recorded. All incubations were done with fresh tissue.

Incubation procedure

Optimum substrate concentrations were established. Each tissue was incubated with approx. 100,000 dpm of [^{3}H]androstenedione in 2 ml of RPMI-1640 medium. The substrate was added in 40 μl of ethanol. Incubations were carried out in a Dubnoff metabolic shaking incubator (Precision Scientific Co, Chicago, Ill.) at 37°C for 1 h in an atmosphere of 95% O_2/5% CO_2. Tissueless controls were included in each experiment. The reactions were stopped with 8 ml of cold ethanol, and the products were isolated within one month after the incubation.

Isolation, identification and quantitation of metabolites

Prior to isolating the products, unlabeled and ^{14}C-labeled androstenedione, 5α-androstanedione, testosterone, dihydrotestosterone, androsterone and 5α-androstane-3α,17β-diol were added to the medium. Addition of 50 μg of each unlabeled steroid served to stabilize the radioactive products in the medium. Also, the Δ^4-3-ketosteroid standards facilitated the isolation of ultraviolet-absorbing metabolites. The ^{14}C-labeled internal standards were added to monitor procedural losses. Approx. 10,000 dpm in 0.1 ml of ethanol of each ^{14}C-labeled internal standard were added to each incubation flask.

After evaporating the ethanol *in vacuo* at 40°C, 2 ml of water were added to the residual aqueous fraction (approx. 0.5 ml) and the steroids were extracted twice with 10 ml of ethyl acetate. The ethyl acetate layer (20 ml) was washed with 2 ml of water, dried over sodium

sulfate, filtered, and evaporated *in vacuo* at 40°C. An aliquot of the extract was counted.

Individual steroids were separated by sequential paper partition chromatography (PPC) or thin-layer chromatography (TLC) using the following solvent systems: heptane:methanol:water (5:4:1, by vol; system A); hexane:methanol:water (5:4:1, by vol; system B); benzene:ethyl acetate (6:4, by vol; system C). The residue obtained after evaporation of the ethyl acetate extract was chromatographed on paper in system A for 7 h. This system separated 5α-androstanedione (run-off) from androsterone (fraction I), androstenedione plus dihydrotestosterone (fraction II), and testosterone plus 5α-androstane-3α,17β-diol (fraction III). 5α-Androstanedione was rechromatographed in the same system used for the first chromatography, but was allowed to run for only 4 h. Fraction I did not require further purification. Fraction II was acetylated and subjected to thin-layer chromatography in system C, thereby separating dihydrotestosterone acetate from androstenedione. Fraction III was chromatographed further on paper in system B for 28 h. In an initial experiment, the isolated steroids were identified by recrystallization to constant $^{3}H/^{14}C$ ratio after being diluted with the appropriate authentic carrier steroid. Since there was no significant difference in the $^{3}H/^{14}C$ ratios between each steroid isolated after the chromatographic step(s) described above and the corresponding recrystallized steroid, no recrystallization or additional chromatography was necessary for identification of the steroids in subsequent experiments.

Counting of radioactivity was carried out by standard procedures. The background was subtracted from all counts. Spillover counts from opposite channels (^{3}H or ^{14}C) were subtracted at each channel. All counts were corrected for quenching and converted to dpm. The percentage of counts spilling over into opposite channels, the percentage of quenching, and the efficiency of counting was determined by use of external radioactive standards. The "blank" value (dpm) obtained from the tissueless controls for each metabolite was subtracted from the corresponding value of each metabolite formed from the substrate by genital skin. Conversion of labeled substrates to metabolites was calculated as percent of labeled substrate according to the following formula in which p represents dpm of precursor incubated, t the dpm (^{14}C) of the steroid added as internal standard, and r the $^{14}C/^{3}H$ ratio of the isolated metabolite (c): $c = (t/p) \times (100/r)$.

Statistical analysis

Data were analyzed by the paired t-test.

RESULTS

The percentage conversions of androstenedione to 5α-androstanedione, testosterone, dihydrotestosterone, androsterone and 5α-androstane-3α,17β-diol by genital skin from normal females are shown in Table 1. The conversions of androstenedione to 5α-androstanedione and androsterone were similar (10.45 ± 1.46 and 11.04 ± 2.04%/200 mg/h) and were approx. 12, 8, and 23 times higher than the conversion of androstenedione to testosterone, dihydrotestosterone, and 5α-androstane-3α,17β-diol, respectively. There was no significant difference between the conversion of androstenedione to testosterone (0.86 ± 0.15%/200 mg/h) and that of androstenedione to dihydrotestosterone (1.28 ± 0.20%/200 mg/h). Each of these conversions was significantly greater than the conversion of androstenedione to 5α-androstane-3α,17β-diol.

The percentage conversions of androstenedione by genital skin from 2 males to the same metabolites described above are shown in Table 2. 5α-androstanedione was the most abundant metabolite (18.41 and 13.51%/200 mg/h), which was 2.9 and 1.6 times greater than the second most abundant metabolite, namely, androsterone (6.29 and 8.65%/200 mg/h). The percentage conversion of androstenedione to dihydrotestosterone (1.36 and

Table 1. Percentage conversion of [^{3}H]androstenedione to various metabolites by female genital skin

Metabolite	Experiment No. 1	2	3	4	5	6	7	8	9	$X + SE$
5α-Androstane-3,17-dione	12.48**[a]	4.58	5.86	17.90	7.57	7.37	14.50	12.07	11.69	10.45 ± 1.46
Testosterone	—[b]	0.38	0.84	1.11	0.83	—[b]	1.58	0.75	0.52	0.86 ± 0.15
Dihydrotestosterone	1.35	0.56	0.88	1.24	0.57	1.48	2.20	1.06	2.14	1.28 ± 0.20
Androsterone	12.47	5.17	16.42	22.80	6.04	4.52	14.84	9.20	7.94	11.04 ± 2.04
5α-androstane-3α,17β-diol	—[b]	0.02	0.44	1.40	0.08	—[b]	0.59	0.40	0.32	0.46 ± 0.17

[a]Values are expressed as percent conversion per 200 mg of tissue per hour.
[b]Samples were lost inadvertently.

Table 2. Percentage conversion of [^{3}H]androstenedione to various metabolites by male genital skin

Metabolite	Experiment No. 1	2
5α-Androstane-3,17-dione	18.41*	13.51
Testosterone	0.11	0.17
Dihydrotestosterone	1.36	0.99
Androsterone	6.29	8.65
5α-Androstane-3α, 17β-diol	0.19	0.53

*Values are expressed as percentage per 200 mg of tissue per hour.

0.99%/200 mg/h) was 12.4 and 5.8 times higher than the formation of testosterone (0.11 and 0.17%/200 mg/h) and 7.1 and 1.9 times higher than the formation of 5α-androstane-3α,17β-diol (0.19 and 0.53%/200 mg/h).

DISCUSSION

Androstenedione is an important precursor of androgen formation in women. However, little has been described about its route of conversion to dihydrotestosterone in target tissues. The objective of the present study was to investigate the metabolism of androstenedione by female genital skin, and in particular, to determine the importance of 5α-androstanedione as an intermediate in the formation of dihydrotestosterone from androstenedione. In this study, we assessed the metabolism of androstenedione by male genital skin not only to confirm that the metabolism of androstenedione to dihydrotestosterone is similar in men and women, but also to determine if the pathways of androstenedione metabolism in genital skin from men and women are similar. In men, it is known that testosterone, rather than androstenedione, is the principal precursor of dihydrotestosterone [2]. It is, therefore, not unexpected that the conversion ratios of androstenedione to dihydrotestosterone in men and women are similar, whereas men have higher conversion ratios of testosterone to dihydrotestosterone.

In the present study 5 different metabolites of androstenedione were isolated, identified and quantified, using a relatively simple chromatographic scheme. In addition, we utilized ^{14}C-labeled internal standards, some of which were synthesized chemically or enzymatically from available ^{14}C-labeled steroids, in order to monitor procedural losses. Our results show very clearly that 5α-androstanedione is a quantitatively more important intermediate than testosterone in the formation of dihydrotestosterone by genital skin in both women and men. Since the extent of formation of 5α-androstanedione was about the same as that of androsterone from androstenedione, it appears that the major route of metabolism of androstenedione involves reduction of the double bond between carbons 4 and 5, followed by reduction of the 3-ketone group, i.e. androstenedione → 5α-androstanedione → androsterone. Relatively low conversions were found for testosterone, dihydrotestosterone and 5α-androstane-3α,17β-diol, suggesting that the pathway involving androstenedione → testosterone → dihydrotestosterone → 5α-androstane-3α,17β-diol is a quantitatively minor one. Similar findings were reported by Flamigni *et al.* [7] in an *in vitro* study of the metabolism of labeled androstenedione by genital skin from a single female and male. Their study utilized an indirect method for assessing procedural losses.

Our data clearly indicate that 5α-androstanedione is the most important intermediate in the conversion of androstenedione to dihydrotestosterone by genital skin. Furthermore, these data confirm the importance of 5α-reductase activity over that of 17β-hydroxysteroid oxidoreductase activity in the expression of androgen action in women.

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