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Activity and Inhibition of 3-Beta-Hydroxysteroid Dehydrogenase/Delta-5-4-Isomerase in Human Skin

Key Words
Androgen activity
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Enzymes
Human skin

Abstract
Activity and inhibition of 3β-hydroxysteroid dehydrogenase/Δ^{5-4}-isomerase, a key enzyme of biosynthesis of androgenic steroids, in human skin were studied. Whole-width dermal tissue specimens excised from various regions of the male and female body were investigated with an in vitro radioenzyme assay method using dehydroepiandrosterone as substrate. The Michaelis-Menten constant of the enzyme was found to be K_m=10 n*M* and the maximal velocity was V_{max} = 0.625 pmol produced 4-androstene-3,17-dione/mg protein/20 min. Activity of 3β-hydroxysteroid dehydrogenase/Δ^{5-4}-isomerase in male inguinal skin (n = 8) was 0.132–0.412, in female abdominal skin (n = 4) 0.140–0.255, in perineal skin (n = 4) 0.138–0.962 pmol/mg protein/20 min. The synthetic steroids cyproterone acetate, 4-MA and epostane proved to be potent inhibitors, IC_{50} values were 150, 6.2 and 1.45 n*M*, respectively.

Introduction

In previous investigations we found that normal male and female skin slices intensively metabolized [4-^{14}C]dehydroepiandrosterone and/or [4-^{14}C]5-androstene-3β,17β-diol ([4-^{14}C]Δ^5-diol) in vitro. Among the metabolites, significant amounts of 4-androstene-3,17-dione (4-ene-dione) or testosterone were identified [1–4]. The identified steroids proved that both female and male skin contains 3β-hydroxysteroid dehydrogenase/Δ^{5-4}-isomerase (Δ^5-3β-hydroxysteroid dehydrogenase) besides numerous other enzymes [5–

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10]. The metabolic processes of the androgenic steroids in the skin take part in the androgenic turnover [11–13] and in the development of pathologic alterations in the skin [13–15]. All the steroids containing the Δ^5-3β-hydroxy moiety [pregnenolone, 17β-hydroxypregnenolone, dehydroepiandrosterone (DHEA) and Δ^5-diol] are transformed into 3-keto-4-ene steroids under the influence of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD), and thus they are precursors of biologically active steroid hormones [16, 19].

Recently, several methods have been developed for measurement of the activities of 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD), Δ^5-3β-HSD and 5α-reductase (5α-R) involved in the biosynthesis of steroid hormones in various endocrine glands, and inhibitors of these enzymes have been developed in the hope of attaining new therapeutic possibilities. These investigations seem to be particularly promising in the treatment of hyperandrogenism [18–24]. We set out to measure the activity of Δ^5-3β-HSD from various regions of the human skin. With radioactive DHEA as substrate, we also examined the effects of various steroids on the activity of this complex enzyme. The experimental results are described in the present paper.

Materials and Methods

Materials

The solutions applied in the investigation were made in water distilled from a glass flask; 1 m*M* dithiothreitol was dissolved in Krebs-Ringer phosphate 200 mg% glucose buffer (KRPG-DTT, pH = 7.4). *d,l*-Dithiothreitol and NAD were purchased from Sigma Co. (USA). The radioactive steroids [1,2-^{3}H]dehydroepiandrosterone ([1,2-^{3}H]DHEA), SA = 51.5 Ci/mmol, [4-^{14}C]dehydroepiandrosterone ([4-^{14}C]DHEA), SA = 51.0 mCi/mmol, and [4-^{14}C]4-androstene-3,17-dione ([4-^{14}C]4-ene-dione), SA = 53 mCi/mmol were purchased from Amersham Co. (England). The non-radioactive ('cold') steroids DHEA and 4-ene-dione were products of Makor Chemical Ltd. (Jerusalem, Israel). The florisil employed for column chromatography (Nymco, 60/100 mesh) was purchased from Floridin Co. (Tallahassee, USA), the glass plates (28 × 5 cm) and the Al_2O_3 adsorbent employed for thin-layer chromatography were from Merck Co. (nach Stahl, type 60/E, Germany), while the n-hexane, ethyl acetate, absolute ethanol and glacial acetic acid were of analytical quality. Cyproterone acetate was a product of Schering AG (Berlin), while 17β-N,N-diethyl-carbamoyl-4β-methyl-4-aza-5α-androstan-3-one (4-MA) and epostane (4α,5α-epoxy-17β-hydroxy-4β,17α-dimethyl-3-oxoandrostene-2α-carbonitrile) were obtained from G. Richter Co. (Budapest, Hungary).

Measurement of the Activity of Δ^5-3β-Hydroxysteroid Dehydrogenase

Skin Samples. Skin samples of 8 male and 8 female patients were obtained via surgery for appendicitis, inguinal hernia and gynaecological diseases. The skin samples, consisting of dermis and epidermis, were purified from antiseptic agents and accessory fatty tissue and cut into 1- to 2-mm slices with scissors. The slices were homogenized within 60 min after operation, or stored at –70 °C.

Preparation of Skin Homogenates. Skin slices (0.6–5.0 g) were homogenized in KRPG-DTT buffer (ratio 1:3) for 8 × 1 min under constant ice-cooling with an Ultra-Turrax homogenizer. The homogenate was then centrifuged at 2,000 *g* for 10 min. The protein concentration of the supernatant was determined by the method of Lowry et al. [25] against bovine serum albumin as standard.

Incubation. 3 n*M* [1,2-^{3}H]DHEA was incubated with an aliquot of the homogenate (0.4 ml = 2.5–5.5 mg protein) in the presence of 1 m*M* NAD for 20 min, by shaking in air at 37 °C in a total volume of 1 ml. The enzymatic reaction was stopped by the addition of ethyl acetate (5 ml) containing carrier steroids (DHEA and 4-ene-dione, 30 μg each).

Extraction and Purification. Steroids were extracted from the incubation mixture with 4 × 5 ml ethyl acetate (recovery 93–99%) and the extract was purified on a 1.5-gram Nymco florisil column (recovery of radioactive steroids 84–94%) [26].

Separation of 4-ene-dione and DHEA. After florisil column purification, the radioactive and non-radioactive DHEA and 4-ene-dione were separated by Al_2O_3-G thin-layer chromatography. The thin-layer plate was developed in absolute benzene to separate traces of fat from the steroids, then dried in hot air and repeatedly chromatographed in the system n-hexane:ethyl ace-

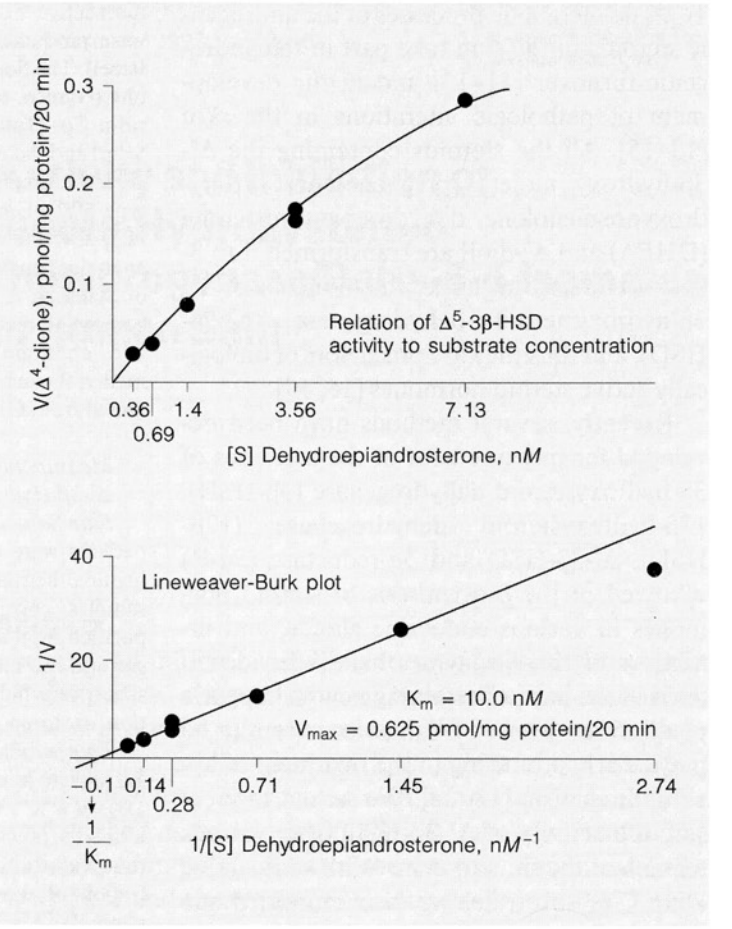

Fig. 1. Determination of the kinetic parameters of Δ^5-3β-HSD in the human skin homogenate.

tate:absolute ethanol:glacial acetic acid (120:13:1:2; v/v/v/v). Steroid spots were localized on the thin-layer plate in iodine vapour or in ultraviolet light and also with a Packard Radiochromatogram Scanner. The steroids were dissolved from the thin layer with 4 × 2 ml methanol. In a portion of the steroid extracts, the radioactivities of the substrate and the product were measured by scintillation spectrometry. 71–81% of DHEA and 4-ene-dione were recovered from the layer and a loss correction was applied in the calculation of the results.

4-ene-dione Identification. The 4-ene-dione formed and the residual DHEA substrate were identified and determined via the quotient $^3H/^{14}C$ [27]. The quotients for the steroids and their acetylated or oxidized derivatives did not differ by more than 5%.

Calculation of Δ^5-3β-HSD Activity. A Packard Liquid Scintillation Spectrometer was used to measure the radioactivities of the 4-ene-dione formed and of the residual DHEA substrate with quench correction. The Δ^5-3β-HSD activity was expressed in terms of the quantity of [1,2-^{3}H]4-ene-dione formed per milligram protein per 20-min incubation, with correction (4-ene-dione pmol/mg protein/20 min).

Investigation of Inhibitors

For the investigation of materials inhibiting the activity of Δ^5-3β-HSD, the incubation conditions were as described above. Besides the control incubation, four quantities of each inhibitor were applied so that the inhibition should be above 50% with two doses, and below 50% with two further doses. The results were expressed in IC_{50}.

Results

Methodological Investigations

Preliminary investigations were performed to determine the optimum conditions for human dermal Δ^5-3β-HSD activity. With DHEA as substrate, a 20-min incubation time, a 2.5 to 5.5-mg protein/ml concentration of the skin homogenate, 3 n*M* DHEA as substrate and 1 m*M* NAD as cofactor seemed optimal. With this method, and under these experimental conditions, the coefficient of variation of the Δ^5-3β-HSD activity of 0.6- to 5-gram skin pieces from various regions of the body varied between 1.0 and 13.8%, with a mean of 3.55%.

Kinetic Parameters of the Δ^5-3β-HSD Activity of the Skin

Details of the Michaelis-Menten constant (K_m) and the maximum reaction velocity (V_{max}) determinations are shown in figure 1. The calculation of these parameters was based on the correlation of the enzyme activity and the DHEA substrate concentrations according to the Lineweaver-Burk [28] reciprocal representation method. For K_m = 10 n*M* and V_{max} = 0.625 pmol/mg protein/20 min values, data were obtained from an inguinal skin specimen.

Δ^5-3β-HSD Activity of Various Skin Samples

Δ^5-3β-HSD activity was examined in inguinal skin obtained from 8 males undergoing inguinal hernia operations. The activity varied between of 0.132 and 0.412 pmol 4-ene-dione/mg protein/20 min, with a mean of 0.244. The activity in the abdominal skin samples from 4 females undergoing gynaecological operations varied between 0.140 and 0.255 pmol 4-ene-dione/mg protein/20 min, with a mean of 0.187 and in the perineal skin obtained from 4 females undergoing cholecystectomy are between 0.138 and 0.962 pmol 4-ene-dione/mg protein/20 min, with a mean of 0.426. The results are shown in table 1. The number of cases of the two skin types is small; however, the Δ^5-3β-HSD activity tends to be higher in the perineal skin.

Table 1. Δ^5-3β-HSD activity in inguinal skin of males, and in abdominal and perineal skin of females

Patient	Age years	4-ene-dione formed pmol/mg protein/20 min
Inguinal skin		
Sz.J.	32	0.218
Ö.J.	38	0.132
D.I.	56	0.201
R.K.	58	0.155
K.B.	61	0.191
Sz.P.	61	0.412
R.A.	69	0.345
N.J.	80	0.297
Mean value		0.244
Abdominal skin		
I.E.	39	0.194
N.M.	49	0.158
M.I.	58	0.255
N.Gy.	60	0.140
Mean value		0.187
Perineal skin		
T.Sz.	44	0.138
P.S.	46	0.328
B.J.	48	0.962
L.S.	63	0.276
Mean value		0.426

Inhibition of Δ^5-3β-HSD Activity

We studied the effects of increasing quantities of cyproterone acetate, epostane and 4-MA as inhibitors on the Δ^5-3β-HSD activity of the skin (fig. 2). The inhibitions with DHEA as substrate resulted in the dose-response plots given in figure 3. The IC_{50} values were determined from plots of log inhibitor concentrations vs. logit percentage control activity: 150 n*M* for cyproterone acetate, 6.2 n*M* for epostane and 1.45 n*M* for 4-MA.

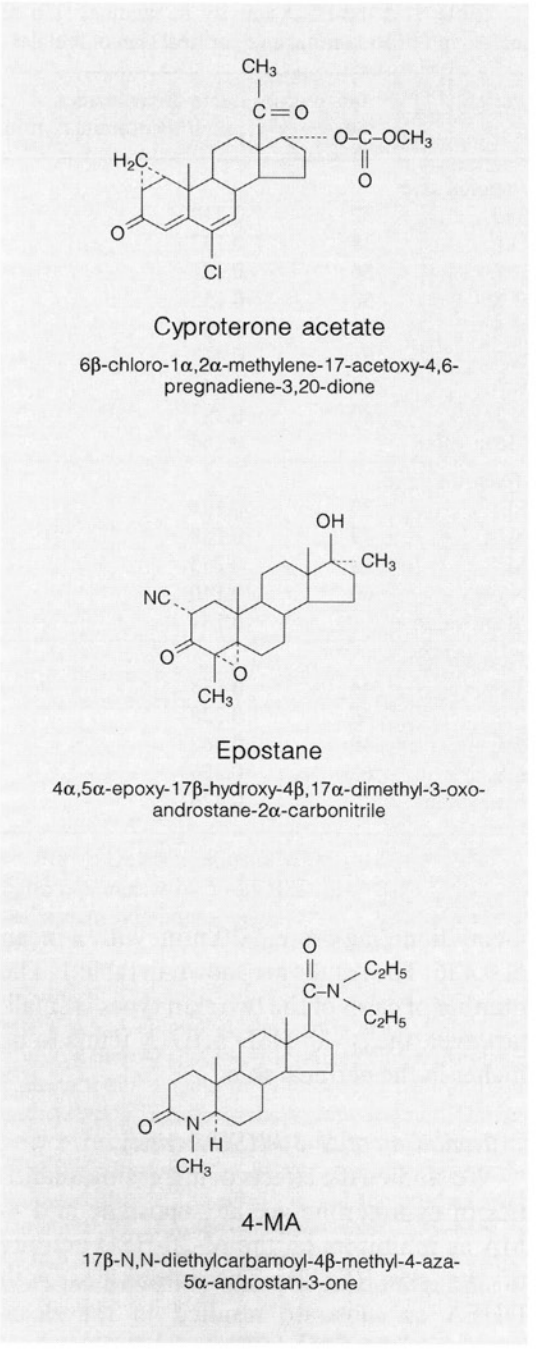

Fig. 2. Inhibitors of Δ^5-3β-HSD in the human skin.

Discussion

In the biosynthesis of steroid hormones in general and androgenic steroids in particular, Δ^5-3β-HSD plays a key role, catalysing the conversion of 3β-hydroxy-5-ene-steroids into 3-oxo-4-ene-steroids. The presence of this important enzyme has been proved in the human adrenal cortex, the testis and even the placenta [16–20, 29].

The incubation of [4-^{14}C]DHEA with skin slices led to significant 4-ene-dione formation, and it therefore seemed worthwhile to develop a method for the measurement of Δ^5-3β-HSD activity of the human skin. Our investigations suggested that the described method was suitable for this purpose. The results exhibited an interassay variation coefficient of 1.0–13.8%, which meets international requirements.

The role of DHEA in the human body has not yet been clarified in many respects. A great amount of DHEA is to be found in the skin [13, 30, 31]. Together with its water-soluble sulphate ester (DHEA-S), it also occurs in the lipids of the axillary hair [32]. Moreover, DHEA-S is secreted by the apocrine sweat glands [33]. In adrenogenital syndrome and in idiopathic hirsutism, the concentrations of DHEA and DHEA-S in the skin are increased [13–15, 34]. During incubation with skin slices, radioactive DHEA is transformed to 4-ene-dione [2, 12, 35], then to testosterone [11, 12, 36] and even to DHT [11, 37]; this fact supports the hypothesis that DHEA entering the skin from the blood stream plays a significant role in the androgenic metabolism processes of the skin [38]. In these events, the enzymes Δ^5-3β-HSD, 17β-HSD and 5α-R are of major importance [4, 39–44] (fig. 4).

Our investigation revealed Δ^5-3β-HSD activity in the skin of males and females. Its Michaelis-Menten constant K_m = 10 n*M* (1 × 10^{-8} *M*), as referred to DHEA in the inguinal

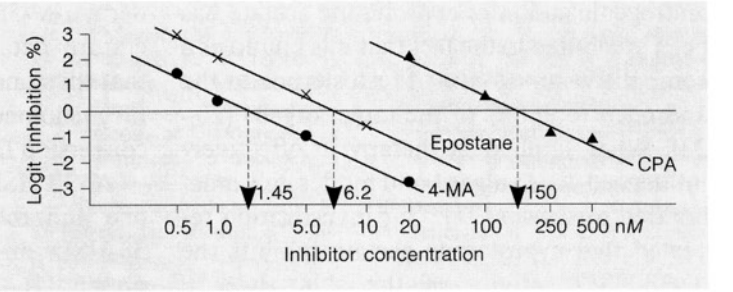

Fig. 3. Determination of IC_{50} values of the Δ^5-3β-HSD inhibitors.

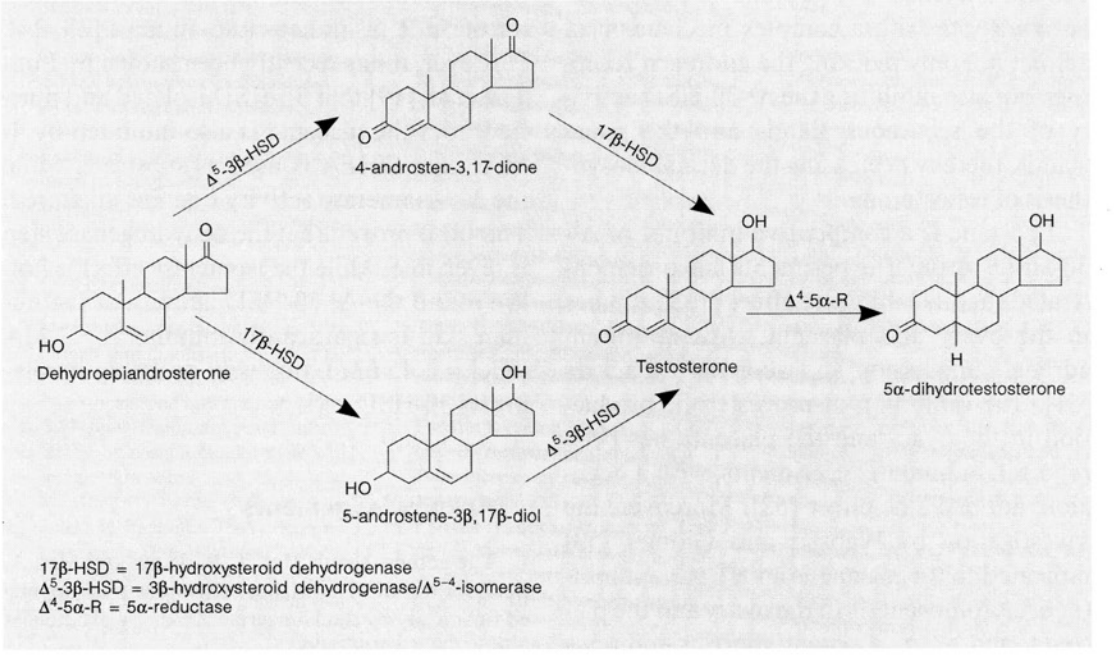

Fig. 4. Androgen metabolism in human skin.

skin, is much lower than the value measured by Voigt et al. [39], Voigt and Hsia [40] and Hsia and Voigt [41] for 5α-R: $K_m = 1.1 \times 10^{-6}$ *M*, as referred to testosterone. The affinity of inguinal skin DHEA for Δ^5-3β-HSD is much higher than the affinity of its testosterone for 5α-R.

The Δ^5-3β-HSD activity is higher in male inguinal skin than in the abdominal skin of females. The greatest Δ^5-3β-HSD activity was found in female perineal skin. These findings clearly demonstrate that the enzyme activities of skin samples from various regions of the body differ considerably [4, 14, 15, 42, 45–50].

Enzymes of the androgen metabolism in the human skin are believed to play an important role in the pathological alterations exhibited in hyperandrogenisms. Inhibition of the activity of Δ^5-3β-HSD seems to be of promise in the treatment of acne, hirsutism and also prostatic carcinoma [20–22, 37]. The anti-

androgenic action of cyproterone acetate has been attributed to the fact that it is bound as a competitive antagonist of testosterone to the androgen receptors of the target organs [20–23]. When applied in therapy, it effectively influenced acne vulgaris and moderately ameliorated hirsutism [21]. Our investigation revealed that cyproterone acetate inhibits the Δ^5-3β-HSD activity of the skin (IC_{50} = 150 n*M*). Hence, cyproterone acetate is an antiandrogen with a complex mechanism of action, not only blocking the androgen receptors, but also inhibiting the Δ^5-3β-HSD activity of the sebaceous glands and the sweat glands, thereby decreasing the dermal biosynthesis of testosterone.

Epostane is a competitive inhibitor of Δ^5-3β-HSD. Animal experiments have demonstrated that its inhibitory effect predominates in the ovary and placenta, with minimum adrenal suppression and abortion capacities [51]. The inhibition of progesterone production in the ovary and the placenta has been reported in human experiments, with a negligible adrenal side-effect [52]. Moreover, the investigation by Webster and Gillmer [53] indicated that epostane is an effective inhibitor of steroidogenesis in the ovary and the placenta, and exerts a potent abortion-inducing effect in early pregnancy. Our studies showed epostane to be a very effective inhibitor of dermal Δ^5-3β-HSD, too. In harmony with literature data, our result provided evidence that epostane inhibits not only the conversion pregnenolone→progesterone, but also the conversion DHEA→4-ene-dione [51–53].

Δ^5-3β-HSD is a complex enzyme: within one and the same protein molecule, both 3β-HSD and $\Delta^{5\text{-}4}$-isomerase functions are present [16–18, 29]. The synthetic steroid 4-MA has long been known to be a good inhibitor of 5α-R in humans and animals [23, 24]. However, it has recently been shown by Luu-The et al. [19] that 3β-HSD isolated and purified from the placenta is also inhibited by 4-MA when DHEA is used as substrate, while the $\Delta^{5\text{-}4}$-isomerase activity remains unaltered. They also proved that the dehydrogenase step is reversible, while the isomerase effect is not. We found the Δ^5-3β-HSD activity of the human skin is significantly inhibited by 4-MA (IC_{50} = 1.45 n*M*), this very probably involving its 3β-HSD action.

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