

Biosynthesis of Dihydrotestosterone by a Pathway that Does Not Require Testosterone as an Intermediate in the SZ95 Sebaceous Gland Cell Line

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TO THE EDITOR

Sex steroids have an important role in the development and physiological functions of human skin (Labrie *et al.*, 2000; Chen *et al.*, 2002; Luu-The *et al.*, 2005; Zouboulis *et al.*, 2007), which has been shown to be not only a hormone-sensitive tissue but also a peripheral organ that possesses the ability to synthesize locally significant amounts of sex steroids with intracrine or paracrine actions (Labrie *et al.*, 2000; Zouboulis, 2004). The sebaceous and sweat glands are major sites of local or intracrine (Labrie, 1991) steroid production (Deplewski and Rosenfield, 2000; Fritsch *et al.*, 2001). In addition, the function of sebaceous glands and hair follicles is strongly dependent on androgens, and the inhibition of androgen formation thus represents an interesting target for the treatment of skin androgen-sensitive diseases such as acne seborrhea and androgenic alopecia (Sansone and Reisner, 1971; Puerto and Mallol, 1990).

The last two steps of dihydrotestosterone (DHT) biosynthesis using the precursor 4-dione involve the enzymes 17 β -hydroxysteroid dehydrogenase (17 β -HSD) and 5 α -reductase (5 α -Red). These enzymes can act in two different pathways: 4-dione \rightarrow 17 β -HSD \rightarrow T \rightarrow 5 α -Red \rightarrow DHT (the conventional pathway) or 4-dione \rightarrow 5 α -Red \rightarrow 5 α -dione \rightarrow 17 β -HSD \rightarrow DHT (a newly proposed pathway). It is noteworthy that the second pathway does not require testosterone (T) as an intermediate.

To determine which of these two pathways has an effect in sebocytes, we

incubated immortalized human SZ95 sebocytes (Zouboulis *et al.*, 1999) with [¹⁴C]DHEA (dehydroepiandrosterone) (Fritsch *et al.*, 2001; Chen *et al.*, 2009) and with the inhibitors of 3 β -hydroxysteroid dehydrogenase (3 β -HSD) and 5 α -Red to determine the pathway of DHT biosynthesis.

As illustrated in Figure 1a, SZ95 sebocytes possess high 3 β -HSD activity, which causes a rapid decrease in DHEA (almost totally eliminated after 12 hours of incubation) with a concomitant increase in 4-dione and 5 α -dione levels, 5 α -dione being the product of transformation of 4-dione by 5 α -Red. However, there is no detectable synthesis of T. After 24 hours of incubation, 4-dione decreases with a concomitant increase in 5 α -dione and, to a lesser extent, in DHT and androsterone, which are found in equal amounts (data not shown). In the presence of epostane, a potent 3 β -HSD activity inhibitor (Takahashi *et al.*, 1990), there was no transformation of DHEA (Figure 1b). The data thus confirm that the production of 4-dione and 5 α -dione observed in Figure 1a requires prior transformation of DHEA into 4-dione by 3 β -HSD (Fritsch *et al.*, 2001).

To further characterize 5 α -Red activity, we added 1 μ M finasteride, a well known 5 α -Red inhibitor, to the incubation medium (Figure 1c). Although finasteride is eightfold less efficient in the inhibition of type 1 compared with type 2 5 α -Red and is identified in the literature as a specific inhibitor of type 2 5 α -Red (Andersson *et al.*, 1991), we

found, using human embryonic kidney (HEK)-293 cells stably expressing types 1, 2, and 3 5 α -Reds, that this compound inhibits these enzymes with IC₅₀ (half maximal inhibitory concentration) values of 107, 14, and 17 nM, respectively, thus indicating that finasteride is still a relatively potent inhibitor of type 1 5 α -Red. Moreover, finasteride inhibited type 1 5 α -Red in higher concentrations in SZ95 sebocytes (Seiffert *et al.*, 2007). As illustrated in Figure 1c, the levels of 5 α -dione and DHT are almost completely abolished in the presence of finasteride, with a concomitant accumulation of 4-dione. The amounts of androsterone and DHT are undetectable in the presence of finasteride but in their absence are found at 12.9 \pm 1.1% of the control. Their levels in the presence and absence of finasteride are statistically different ($P < 0.001$). The present data thus indicate that 5 α -dione is produced by the 5 α -reduction of 4-dione. We have also observed reduced 3 β -HSD activity in the presence of finasteride, an effect probably due to the inhibitory effect of finasteride on 3 β -HSD activity. Indeed, we have previously shown that 4-MA (N, N-dimethyl-4-methyl-3-oxo-4-aza-5 α -androstane-17 β -carboxamide), a potent inhibitor of 5 α -Red that has a structure somewhat similar to that of finasteride, is also a potent inhibitor of 3 β -HSD activity (Takahashi *et al.*, 1990; Luu-The *et al.*, 1991).

The present data clearly show that the pathway of DHT biosynthesis from the DHEA precursor in SZ95 sebocytes does not always require T as an intermediate. The same pathway is also found in the prostate cancer cell line DU-145 (M. Samson *et al.*, 2009). Interestingly, DHEA was recently

Abbreviations: 17 β -HSD, 17 β -hydroxysteroid dehydrogenase; 3 β -HSD, 3 β -hydroxysteroid dehydrogenase; 4-dione, 4-androstenedione; 5 α -dione, 5 α -androstane-3,17 dione; 5 α -Red, 5 α -reductase; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; T, testosterone

shown to be the major androgen precursor in SZ95 sebocytes (Chen *et al.*, 2009). Our findings are partially in contradiction with the traditional literature, which indicates that DHT is produced by the 5 α -reduction of T, thus suggesting that the step catalyzed by 17 β -HSD precedes the step catalyzed by 5 α -Red (DHEA \rightarrow 4-dione \rightarrow T \rightarrow DHT). The pathway described in this report suggests that the step catalyzed by 5 α -Red precedes the step catalyzed by 17 β -HSD (DHEA \rightarrow 4-dione \rightarrow 5 α -dione \rightarrow DHT). Because 4-dione possesses a higher affinity than T for 5 α -Red (Andersson and Russell, 1990; Sugimoto *et al.*, 1995), the proposed pathway corresponds better to the thermodynamic law. In addition, given that T binds to the androgen receptor efficiently and could activate the receptor without requiring its transformation to DHT, the transformation of T to DHT is not essential.

The concept of two androgens and one receptor accounting for the different actions of T and DHT has been generally accepted, on the basis of the differences observed in type 3 17 β -HSD (Geissler *et al.*, 1994), type 2 5 α -Red (Andersson *et al.*, 1991), and AR (Peterson *et al.*, 1977) deficiencies.

To assess specific isoforms of steroidogenic enzymes in SZ-95 cells, we quantified the mRNA expression levels of specific types of steroidogenic enzymes using a second derivative and the double-correction real-time PCR method (Luu-The *et al.*, 2005). As shown in Figure 2, we confirmed that type 1 3 β -HSD (Dumont *et al.*, 1992; Fritsch *et al.*, 2001) and type 1 5 α -Red (Luu-The *et al.*, 1994; Chen *et al.*, 1998) are expressed. In addition, we found high expression levels of a newly identified type 3 5 α -Red (Uemura *et al.*, 2008), thus suggesting that this previously unreported type 3 5 α -Red sensitive to finasteride could have an important role in sebocytes.

A better understanding of the revised DHT biosynthetic pathway that does not require T as an intermediate should permit the design of more specific inhibitors of the enzymes involved in this pathway and should thus improve treatment and prevention

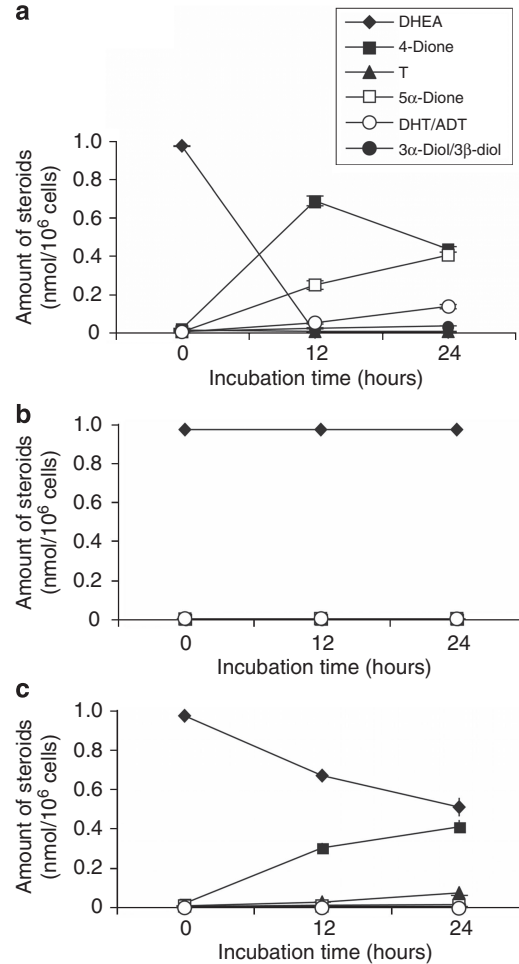


Figure 1. Quantification of [¹⁴C]metabolites of dehydroepiandrosterone (DHEA) metabolism in SZ95 sebocytes in culture. Curves showing metabolic profiles of 0.1 μ M [¹⁴C]DHEA incubated for 12 and 24 hours with SZ-95 cells in the absence (a) and presence of epistane, an inhibitor of 3 β -hydroxysteroid dehydrogenase (b), and finasteride, an inhibitor of 5 α -reductase (c). Substrates and metabolites were separated by thin-layer chromatography, identified by a comparison with reference steroids and quantified by a PhosphorImager Storm 860 system (Molecular Dynamics, Sunnyvale, CA). Data are expressed as means \pm SD of triplicate experiments.

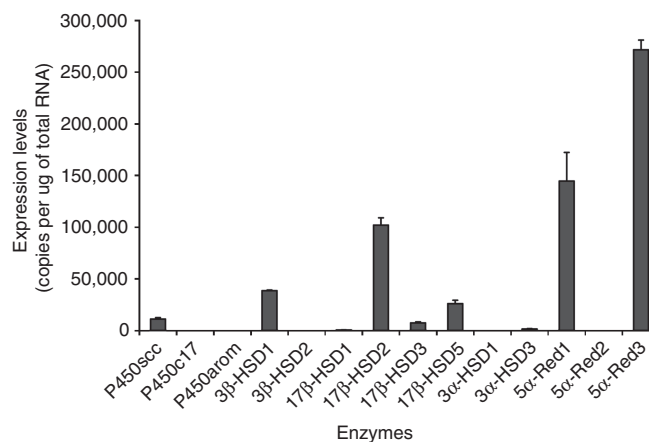


Figure 2. mRNA expression levels of steroidogenic enzymes in SZ-95 cells. Graph showing mRNA expression levels of steroidogenic enzymes in SZ-93 cells quantified by real-time PCR using the double-correction method (Luu-The *et al.*, 2005). Data are expressed as means \pm SD of triplicate measurements. Some error bars are hidden by the labels.

of androgen-sensitive diseases, especially acne and seborrhea.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Human Skin Aging Is Associated with Reduced Expression of the Stem Cell Markers β 1 Integrin and MCSP

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TO THE EDITOR

Intrinsic and UV-induced human skin aging involves a number of changes, including reduced epidermal prolifera-

tion, impaired melanocyte function, and decreased collagen biosynthesis (Yaar et al., 2002; McCullough and Kelly, 2006). To ameliorate or inhibit

these effects it is important to identify the principal factors that influence skin aging.

Reduced stem cell abundance or self-renewal ability is a feature of aging in a number of different tissues (Flores et al.,

Abbreviations: MCSP, melanoma chondroitin sulfate proteoglycan