Skin metabolism of steroid hormones as endogenous compounds?

Van Luu-The
Department of Molecular Medicine
Laval University
Québec, Canada

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Steroid hormones are produced by two types of tissues:

A) Endocrine tissues (Adrenals and gonads)

These tissues are able to synthesize from cholesterol active steroid hormones (progesterone, cortisol, aldosterone, testosterone and estradiol) and steroid precursors. The products are delivered in the circulation and can exert their action in tissues away from the site they are synthesized.

B) Peripheral tissues

These tissues cannot use cholesterol to produce active hormones. The latter are synthesized locally from various precursors depending on enzymes that are expressed in the tissue. They will exert their action locally in the tissues they are synthesized.
Steroidogenic Pathways in Endocrine Tissues

CHOLESTEROL

P450SCC

PREG

P450C17

17α-OHPREG

DHEA

3β-HSD

17α-OHPROG

4-DIONE

TESTO

11-DEOXYCORTICOSTERONE

E1

5α-DIONE

DHT

11-DEOXYCOR

TICOSTERONE

E2

5α-Red

17β-HSD1, 5

5α-Red

17β-HSD3, 5

Aromatase

17β-HSD1,7,12

CYP21A1

CYP11B1

CORTICOSTERONE

ALDOSTERONE

CORTISOL
Steroids metabolism in intracrine peripheral tissues
mRNA expression levels of steroid metabolizing enzymes in the skin
mRNA expression levels of steroid metabolizing enzymes in the skin
$[^{14}\text{C}]$DHEA metabolism using cultured SkinEthic
[\textsuperscript{14}C]4-dione metabolism using cultured SkinEthic
[\textsuperscript{14}C]4-dione metabolism in the presence of 5α-reductase inhibitor
$[^{14}\text{C}]$4-dione (A and B) and T (C and D) metabolism
A, 6h incubation; B, 24h; C, 6h; D, 6h + $17\beta$-HSD2 inhibitor
Steroidogenesis: Conventionnal Pathway (A) and revisited pathway (B)

A

DHEA → 3β-HSD1,2 → 4-dione → 17β-HSD3,5 → T → E2 → DHT

B

DHEA → 3β-HSD1,2 → 17β-HSD 3,5 → 4-dione → 17β-HSD 2 → E1 → 5α-dione → 5α-reductase 1, 2, 3 → T

Aromatase → 5α-reductase 1, 2, 3

17β-HSD 1,7,12 → E2

17β-HSD 5, 15 → DHT
Aromatase and 5α-reductases possess higher affinity for 4-dione than T

<table>
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<th>Enzymes</th>
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<th>4-dione Km (μM)</th>
<th>T Km (μM)</th>
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Facts in favor of revisited steroidogenic pathway

- The cloning of estrogen-specific 17\(\beta\)-HSDs
- The higher affinity of aromatase for 4-dione than T (Kellis and Vickery, 1987; Reed and Ohno, 1976)
- The higher affinity of 5\(\alpha\)-reductases for 4-dione than T (Andersson and Russell, 1990; Russell and Wilson, 1994)
- These data strongly suggest that the step catalyzed by 17\(\beta\)-HSDs follows the step catalyzed by aromatase or 5\(\alpha\)-reductases.
To verify whether the step catalyzed by 17β-HSDs precedes or follows the step catalyzed by aromatase or 5α-reductases, we incubate SZ-95 (sebocyte gland), Jeg-3 (choriocarcinoma) cells with [14C]DHEA and DU-145 prostate cancer cells with [14C]4-dione, in the presence of inhibitors of 3β-HSD, 5α-reductase and aromatase.
Transformation of $^{14}$C DHEA in SZ-95, a sebocyte gland cell line

(Data from Samson et al. J Invest Dermatol. 2010 130:602-4)
Transformation of $[^{14}C]$DHEA in SZ-95 cells in the presence of epostane, a $3\beta$-HSD inhibitor

(Data from Samson et al. J Invest Dermatol. 2010 130:602-4)
Steroidogenesis: Conventional Pathway (A) and revisited pathway (B)

**A**
- DHEA
  - $3\beta$-HSD1,2
  - 4-dione
    - $17\beta$-HSD3,5
      - T
    - Aromatase
      - E2
      - 5α-reductase 1, 2, 3
      - DHT

**B**
- DHEA
  - $3\beta$-HSD1, 2
  - 4-dione
    - $17\beta$-HSD 3, 5
    - $17\beta$-HSD 2
    - Aromatase
      - E1
      - 5α-reductase 1, 2, 3
    - 17β-HSD 1,7,12
      - E2
      - DHT
    - 17β-HSD 5, 15
      - T
Transformation of $[^{14}C]$DHEA in SZ-95 cells in the presence of finasteride, a 5$\alpha$-reductase inhibitor

(Data from Samson et al. J Invest Dermatol. 2010 130:602-4)
Transformation of $[^{14}\text{C}]4$-dione in DU-145, a prostate cancer cell line

(Data from Samson et al. Horm Mol Biol Clin Invest. 2010 1:67-72)
Transformation of $[^{14}\text{C}]4$-dione in DU-145 cells, in the presence of finasteride, a 5α-reductase inhibitor

(Data from Samson et al. Horm Mol Biol Clin Invest. 2010 1:67-72)
Transformation of $[^{14}\text{C}]\text{DHEA}$ in JEG-3, a choriocarcinoma cell line

Transformation of $^{14}$C]DHEA in JEG-3 cells
In the presence of 3β-HSD inhibitor
Transformation of $[^{14}C]$DHEA in JEG-3 cells in the presence of aromatase inhibitor

Revisited steroidogenic pathways

- The data using cell culture, clearly, indicate that the steps catalyzed by aromatase and $5\alpha$-reductase precede the step catalyzed by $17\beta$-HSDs and testosterone is not the obligatory intermediate in the biosynthesis of DHT and E2.
Additional data in favor of the pathway that does not require T as an intermediate

To determine whether the data observed using cell culture incubation have physiological relevance, we analyze previous data reported for patient that have testicular 17β-HSD deficiency

1.- Testicular 17β-HSD deficiency is generally associated with gynecomastia (3). Since gynecomastia is most probably due to an excess of E2, the observation strongly suggests that the excess of E2 is coming from the higher level of 4-dione in these patient, thus, it is in favor of the pathway that does not require T as intermediate (9).

(3) Saez et al. (1971) J. Clin. Endocrinol Metab. 32, 604-610
2.- As shown in the figures below, taken from Rosler et al. (4) patients having a defect in testicular 17β-HSD (open bar), possess a higher level of 4-dione and a lower level of T than in normal control (black bar). The profile of stable 5α-reduced steroids, namely ADT-glucuronide (ADT-GL), 3α-diol-GL and DHT, showing equal or higher levels in patient than in normal control is better associated with 4-dione than T. It is thus in favor of the pathway that does not require T as intermediate.

(4) Rosler et al. (1992) J. Clin. Endocrinol Metab. 75, 773-776
3. In addition, the profile of 5α-reduced metabolites (10) that show very high levels of 5α-reduced steroids compared to T in the circulation, in micromolar range, strongly suggest the existence of a pathway where the step of 5α-reduction precedes the step of 17keto-reduction. Indeed, the concentration of 5α-reduced steroids in men between 20 to 30 (20) is highest for androsterone-sulfate (ADT-S) with ~1400 nM, followed by ADT-G (80 nM) > 5α-androstane-3β,17β-diol-G (3β-diol) (~47 nM) > 5α-androstane-3α,17β-diol-G (3α-diol-G) > ADT (5nM) > 3β-diol (3nM) > DHT (2.8 nM) > 3α-diol (2.2 nM). The very large amount of ADT derivatives (ADT-S, ADT-G and ADT) that bear a 17-keto group compared to compound having a 17β-hydroxy group (DHT, 3β-diol-G, 3α-diol-G, 3β-diol and 3α-diol) strongly suggest that the step of 5α-reduction does not require the step of 17keto-reduction by 17β-HSD.

Additional data in favor of the pathway that does not require T as an intermediate

- The higher affinity of testosterone for the androgen receptor (AR) \((K_m=10^{-8}, 10^{-9} \text{ M})\) than \(5\alpha\)-reductases \((K_m=10^{-6} \text{ M})\) is also in favor of the role of testosterone as ligand for AR without requiring its transformation into DHT, since in tissues that express both AR and \(5\alpha\)-reductases, T will preferentially bind to AR and exert its androgenic activity before binding to \(5\alpha\)-reductases.

- These data are also in agreement with the presence of two androgens, namely, T and DHT, that are synthesized by two different pathways and exerted their action in different tissues.
Expression levels of $17\beta$-HSD and $5\alpha$-reductase mRNAs in the testis (A) and muscle (B)

In tissues having low (negligible) expression of $5\alpha$-reductases compared to $17\beta$-HSDs, such as the testis and muscle, the active androgen is essentially T, while in tissues that express high levels of $5\alpha$-reductases (prostate, skin and brain), the active androgen is DHT
It is also important to distinguish endocrine tissues such as the gonads and the adrenals that are able to produce active steroid hormones from cholesterol and to deliver them into the circulation to exert action away from the site where they are produced.

Because of the high dilution in the circulation, high amount of steroids are produced and accordingly very high amount of mRNA (millions copies/ug total RNA have been detected.)
Expression levels of P450scc (CYP11A1) and P450c17 (CYP17A1) in endocrine and intracrine tissues
Need to revise the steroidogenic pathway

- Intracrine tissues do not have the ability to transform cholesterol into active steroid hormones, but depending on enzymes that are expressed in the tissues, active steroids are produced from various steroid precursors. These included DHEAS, DHEA, E1S, E1, androsterone, (ADT), ADTS, epi-ADT, epi-ADTS, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol.
Physiologically, steroid dehydrogenases are not reversible enzymes as conventionally suggested.

Data using purified enzymes or tissue homogenates show that steroid dehydrogenases are reversible enzyme, they are able to catalyse both direction, oxidative or reductive reaction, dependent on oxidative or reductive cofactors.

Transfected cells in culture show that steroid dehydrogenases are enzyme that catalyze unidirectional reaction.

Enzymes that prefer the phosphorylated cofactors (NADP/NADPH) catalyze reductive reaction. While those prefer non phosphorylated cofactors (NAD/NADH) catalyse oxidative reaction.
Enzymatic activities of $17\beta$-HSD types 1, 2 et 3 using subcellular fractions of transfected HEK-293 cells.

Autoradiograph of TLC of the transformation of $^{14}$C-labeled substrate by control mock-transfected cells (C) and by cells transfected (T) with pCMV-$17\beta$-HSDs 1, 2 & 3

(Figure from Luu-The et al. J Steroid Biochem Mol Biol. 1995 55:581-7.)
Enzymatic activity of expressed cDNAs encoding human types 1, 2, 3, 5, 7 and 8 17β-HSDs in intact transfected 293-cells.

(From Luu-The 2001 J Steroid Biochem Mol Biol 76:143-151)
Relationship between cofactor specificity and oxidative or reductive activity catalyzed by some dehydrogenases.

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</table>

+, enzymatic activity.

*From Luu-The 2001 J Steroid Biochem Mol Biol 76:143-151*
Conclusion

- The skin is an intracrine peripheral tissues that possess enzymes able to convert various steroid precursors (DHEA, 4-dione, ADT, E1S, E1) into active hormones.

- Depending on the set of enzymes expressed in different skin compartments and their relative efficiency to transform a substrate, different pathway will be used.