

The intracrine sex steroid biosynthesis pathways

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Abstract: There is an increasing number of differences reported between the steroidogenesis pathways described in the traditional literature related to gonadal steroidogenesis and the more recent observations achieved using new technologies, especially molecular cloning, pangenomic expression studies, precise quantification of mRNA expression using real-time PCR, use of steroidogenic enzymes stably transfected in cells, detailed enzymatic activity analysis in cultured cell lines and mass spectrometry analysis of steroids. The objective of this chapter is to present steroidogenesis in the light of new findings that demonstrate pathways of biosynthesis of estradiol (E_2) and dihydrotestosterone (DHT) from adrenal dehydroepiandrosterone (DHEA) in peripheral intracrine tissues which do not involve testosterone as intermediate as classically found in the testis and ovary. Steroidogenic enzymes different from those of the ovary and testis act in a tissue-specific manner to catalyze the transformation of DHEA into active sex steroids. These new pathways are especially important in post-menopausal women where all estrogens and practically all androgens are made at their site of action in peripheral tissues from DHEA, the precursor of adrenal origin. In men, on the other hand, from 40 to 50% of androgens are made in peripheral tissues from adrenal DHEA, thus indicating the major importance of the intracrine pathways in both men and women. We also examine the molecular evolution of steroidogenic enzymes which explains the major differences in steroid metabolism observed between laboratory animals and humans.

Keywords: steroidogenesis; menopause; dehydroepiandrosterone; estrogens; androgens

Introduction

In addition to the sex steroids of gonadal origin that are synthesized in the testicles and ovaries before being released in the blood in order to reach all tissues to exert their action in the classical endocrine manner (Fig. 1, left), it is

now well established that an additional source of sex steroids plays a major role in humans of both sexes (Fig. 1, right). For example, in men who have their testicles surgically removed or who are treated with gonadotropin-releasing hormone (GnRH) agonists that completely block testicular androgen secretion (Labrie et al., 1980), it is observed that while the blood levels of testosterone are reduced by 95–97%, the concentrations of intra-prostatic dihydrotestosterone (DHT) is only decreased

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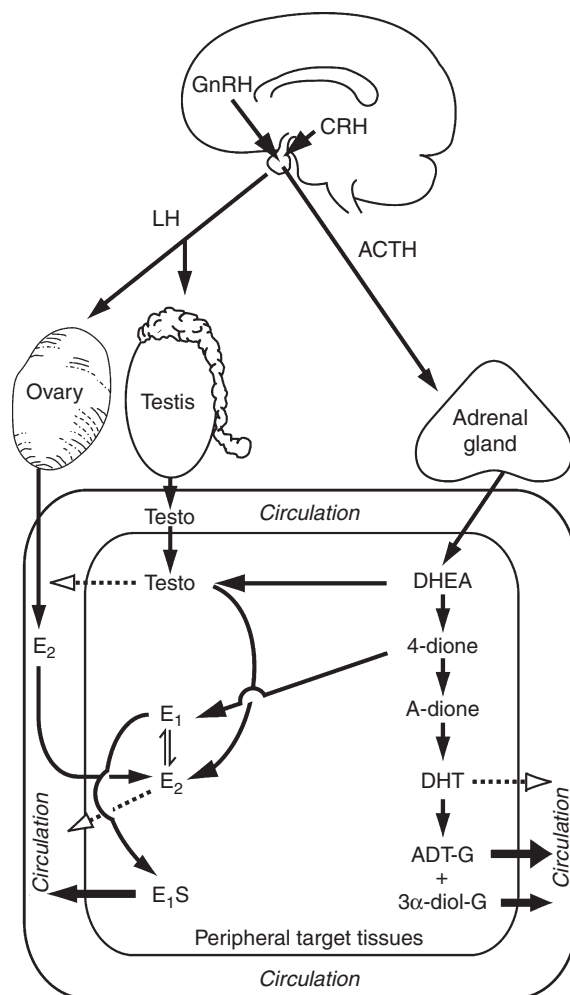


Fig. 1. Schematic representation of the ovarian, testicular and adrenal sources of sex steroids or global sex steroid availability in women and men. After menopause, the secretion of E_2 by the ovaries ceases. Consequently, in post-menopausal women, estrogens and nearly all androgens are made locally in peripheral target intracrine tissues. The pre- and post-menopausal ovary secretes small amounts of testo directly into the circulation, where it has an inhibitory effect (-) on GnRH secretion in the brain. Much larger amounts are secreted by the testis. Conversely, the adrenal glands – as well as secreting cortisol that decreases CRH secretion which stimulates ACTH levels – secrete large amounts of DHEA. This inactive precursor is converted in specific target tissues into androgens and/or estrogens via the process of intracrinology. Only small amounts of these peripherally made sex steroids diffuse into the circulation, thus avoiding the possibility of gaining information about the intra-cellular made and active sex steroids. The active androgens and estrogens are inactivated locally before being released in the circulation. The intra-cellular androgens are metabolized into the metabolites ADT and 3α -diol which are then further transformed into the more water-soluble glucuronide derivatives and released into the blood where they can be measured as parameter of total androgenic activity. Abbreviations: ACTH, adrenocorticotropin; CRH, corticotrophin-releasing hormone; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; E_1 , estrone; E_1S , estrone sulphate; E_2 , estradiol; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; testo, testosterone; ADT-G, androsterone glucuronide; 3α -diol-G, 3α -diol-3 or 17-glucuronide.

by an average of 40% as shown by the various data available in the literature (Labrie, 2007; Labrie et al., 1985). Such findings clearly indicate the existence of an important local

biosynthesis of androgens in the prostate (Fig. 1). Most of the other peripheral tissues such as skin, liver and adipose tissue also show local biosynthesis of sex steroids that exert their

action locally in an intracrine manner (Labrie, 1991; Labrie et al., 2005). In other words, the action of the sex steroids made from dehydroepiandrosterone (DHEA) is exerted in the same cells where the steroids are synthesized and inactivated (Labrie, 1991).

The role of intracrinology or local biosynthesis of sex steroids from adrenal DHEA is even more dramatic in women where, after menopause, all estrogens and practically all androgens are made from DHEA in a tissue-specific manner by the process of intracrinology (Labrie et al., 2005). The importance of the peripheral formation of estrogens and androgens from DHEA has recently been clearly demonstrated in post-menopausal women where a rapid correction of all their symptoms and signs of vaginal atrophy accompanied by improved sexual function was achieved rapidly by intravaginal DHEA administration with no change in the blood levels of estrogens and androgens (Labrie et al., 2008, 2009a, 2009b, 2009c). There are, moreover, a series of data that indicate low DHEA as responsible for the other problems of

menopause related to hormonal deficiency (Labrie, 2007).

The local steroid biosynthesis of sex steroids by the process of intracrinology in peripheral tissues in humans is supported by the cloning and characterization of all the enzymes required which include types 1 (Dumont et al., 1992b; Lachance et al., 1990; Lorence et al., 1990; Luu-The et al., 1989) and 2 (Lachance et al., 1991; Rheume et al., 1991) 3β -hydroxysteroid dehydrogenases (3β -HSDs), types 1, 2, 3, 5, 7, 8, 12, 14 and 15 17β -HSDs (Bellemare et al., 2009; Dufort et al., 1999; Geissler et al., 1994; Krazeisen et al., 1999; Labrie et al., 1995; Luu-The, 2001; Luu-The et al., 2006; Luu The et al., 1989, 1990a; Peltoketo et al., 1988; Wu et al., 1993) as well as types 1, 2 and 3 5α -reductases (Andersson and Russell, 1990; Andersson et al., 1991; Labrie et al., 1992; Uemura et al., 2008; Yamana et al., 2009) that are expressed in a tissue-specific manner (Fig. 2). The virilization of boys having a deficit in type 2 3β -HSD and data in men with aromatase deficiency (Bilezikian et al., 1998; Carani et al., 1997; Morishima et al., 1995) also

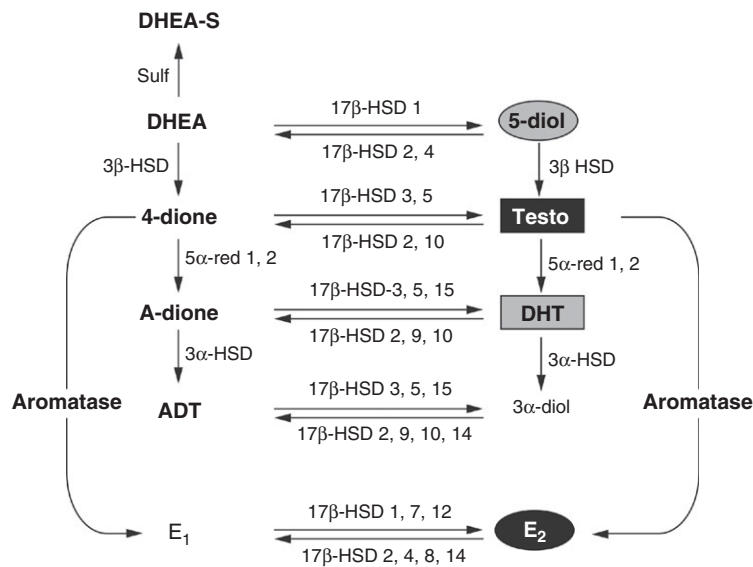


Fig. 2. Schematic representation of the complete steroidogenic endocrine and intracrine pathways in human gonadal and peripheral tissues, respectively.

confirm the importance of local and tissue-specific biosynthesis of sex steroids.

Molecular evolution and different classes of steroidogenic enzymes

Structural studies of the enzymes involved in the formation and degradation of sex steroids indicate that many steroidogenic enzyme families are generated by duplication and divergence such as observed for the 3β -HSDs, 5α -reductases, 3α -HSDs and uridine diphosphate (UDP)-glucuronosyl transferases (UGTs) (Baker, 2001b; Baker, Luu-The, Simard, & Labrie, 1990), while others such as some 17β -HSDs are generated by convergent evolution of activity (Baker, 2001a). In addition, it can be noticed that the enzymes responsible for the formation of estrogens and androgens in peripheral tissues differ markedly between species (Luu-The, 2001).

The steroidogenic enzymes can be divided into three molecular evolutionary classes (Dufort et al., 2001; Luu-The, 2001; Luu-The et al., 2001):

1. The class of 'conserved structures–conserved activities' is composed of genes that are duplicated before divergence between rodent and human. This class includes the genes encoding types 1 and 2 5α -reductases, types 3

and 12 17β -HSDs (Figs. 2 and 3). These genes share a percentage of amino acid sequence homology of less than 70% and possess the same genomic structure. The function and activity of these genes are somewhat conserved, although the amino acid sequences show large differences.

It is worth noting that, in general, the rodent and human genes share a percentage of homology between 70 and 80%. In addition, it should be mentioned that some gene families possess different copy numbers and tissue distribution. Consequently, different steroid metabolic profiles are expected between laboratory animals and humans, in addition to differences in the level of activity of the enzymes present.

2. The 'conserved structures–different activities' class is composed of genes that are more recently duplicated, namely, after the separation between rodent and human, such as the types 1 and 2 3β -HSD that share 94% amino acid identity, many of the enzymes of the aldo-keto reductase family, such as type 5 17β -HSD, types 1 and 3 3α -HSDs, 20α -HSD and the UDP-glucuronosyl transferase (UGT1A family). These genes share a percentage of homology of more than 80% but encode proteins having different activities. For example, human type 5 17β -HSD and types 1 and 3 3α -HSD share ~88% amino acid identity

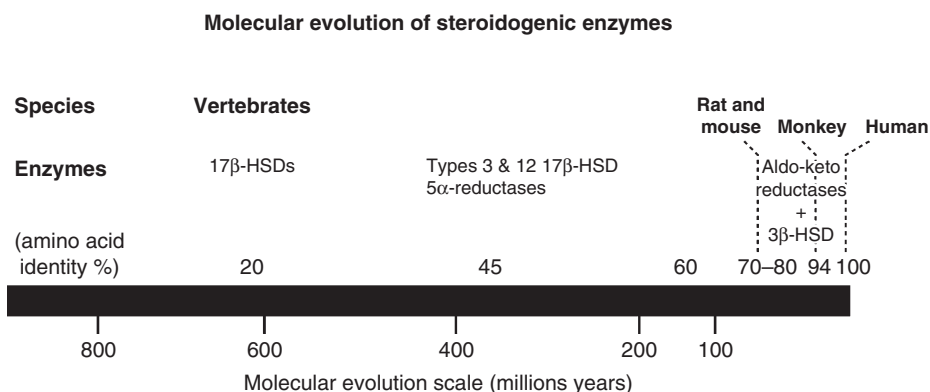


Fig. 3. Schematic representation of the molecular evolution of steroidogenic enzymes.

but catalyze different activities. Similarly, although type 3 3α -HSD and 20α -HSD share 97% amino acid identity, they possess different substrate specificities. Similar structure–activity relationships are also observed with genes of the UGT1A family.

The genes belonging to this evolutionary class encode the main enzymes responsible for the different profiles of steroid metabolism between humans and rodents. In fact, many enzymes existing in humans do not even have their equivalent in rodents.

3. The ‘different structures–conserved activity’ class represents enzymes derived from genes having convergent evolution of activity such as those encoding 17β -HSDs (Baker, 2001a), with the exception of types 3 and 12 17β -HSD that are duplicated genes (Luu-The et al., 2006). These genes share very low homology (less than 25%) and are derived from ancestral genes having different structures and functions. However, all of the enzymes encoded by these genes catalyze the oxidation or reduction of the keto and hydroxyl groups, respectively, at position 17 of the steroid nucleus. These enzymes play a crucial role in the activation and inactivation of androgens and estrogens.

It is noteworthy that some genes encode enzymes specific for C19-substrates such as types 3, 5, 10, 11, 14 and 15 17β -HSDs, while others are specific for C18-substrates such as types 1, 7, 8 and 12 17β -HSDs. On the other hand, type 2 17β -HSD is able to catalyze enzymatic reactions of both C18- and C19-substrates. Many of these enzymes are also capable of transforming substrates other than androgens and estrogens. This ability seems to be acquired from ancestral functions existing in unicellular organisms (yeast) and invertebrates (*Caenorhabditis elegans*) where androgens and estrogens are not functional because of the lack of androgen and estrogen receptors. Indeed, androgen and estrogen receptors and their ligands only appeared just before or during the pre-cambrian period (Baker, 1997; Bertrand et al., 2004; Laudet, 1997).

Does convergent evolution result from modification of an old enzymatic structure or an adaptation of existing activities of a multi-substrate enzyme to a new substrate? Example of type 12 17β -HSD

We have recently isolated and characterized orthologs of type 12 17β -HSD in many species including the nematode *C. elegans* (Desnoyers et al., 2007), mouse (Blanchard and Luu-The, 2007), monkey (Liu and Zheng et al., 2007) and human (Luu-The et al., 2006). The data obtained show that the gene encoding type 12 17β -HSD is highly conserved with a common ancestor found in *C. elegans*, namely, LET-767. This ancestral gene leads, in addition to type 12 17β -HSD, to type 3 17β -HSD, a key enzyme in steroidogenesis in the testis. The deficiency of type 3 17β -HSD impairs testosterone biosynthesis in the human testis and is the cause of male pseudohermaphroditism in boys having the mutated gene (Geissler et al., 1994). LET-767 in *C. elegans* possesses 42 and 40% amino acid identity with human types 3 and 12 17β -HSDs, respectively. The expression of LET-767 in HEK-293 cells shows that the enzyme is able to catalyze the conversion of estrone into estradiol (activity of type 12 17β -HSD) as well as androstenedione into testosterone (activity of type 3 17β -HSD). It is worth noting that estradiol and testosterone are not functional and most probably do not exist in *C. elegans* which does not possess androgen and estrogen receptors (Baker, 1997; Laudet, 1997).

Mutations that inactivate LET-767 lead to a growth inhibition and a reduction of the size of the nematode similar to the observations made in wild-type *C. elegans* cultured in the absence of cholesterol, thus suggesting that LET-767 could be involved in cholesterol metabolism (Kuervers et al., 2003). Others have shown that *C. elegans* having inactivated LET-767 have impaired long-chain fatty acid biosynthesis (Entchev et al., 2008).

Characterization and comparison of the mouse (Blanchard and Luu-The, 2007) and monkey (Liu and Zheng et al., 2007) type 12 17β -HSDs with the human enzyme (Luu-The et al., 2006) show that while the monkey enzyme is estrogen-specific

similar to the human enzyme, the mouse enzyme is able to catalyze both androgen and estrogen substrates, as observed with LET-767. Using molecular modelization confirmed by site-directed mutagenesis, we have identified the involvement of amino acid 234 in the steric hindrance of the entrance of C18- and C19-steroids (Luu-The et al., 2006). Indeed, in the monkey and human enzymes, this amino acid is a phenylalanine, a voluminous amino acid that prevents the entrance of C19-steroids, thus conferring type 12 17 β -HSD selectivity for estrogens. On the other hand, in the *C. elegans* and mouse, the corresponding amino acids are methionine and leucine, respectively. These amino acids are less voluminous than phenylalanine and allow the access of androgens, as well as estrogens, to the binding site.

In a study performed in pre-adipocytes and differentiated adipocytes, Bellemare et al. (Bellemare et al., 2009) have shown that type 12 17 β -HSD expression is significantly increased in differentiated adipocytes. This increase in expression levels of type 12 17 β -HSD corresponds to the increase in the ability to catalyze the transformation of estrone into estradiol in differentiated adipocytes. Since the expression of the two other estrogenic 17 β -HSDs, namely, types 1 and 7 17 β -HSD, are not increased under those circumstances, the data strongly suggest that type 12 17 β -HSD is responsible for the increased conversion of estrone into estradiol in differentiated adipocytes.

The ability of LET-767 to catalyze the formation of estradiol and testosterone, although these compounds are not functional in *C. elegans*, suggests that the convergent evolution of 17 β -HSD activities is due to an adaptation of an existing activity of a multi-substrate enzyme to a newly appearing substrate rather than the evolution of an old enzymatic structure to accommodate a new substrate. It also suggests that if two or more substrates are present in a multi-cellular organism, the enzyme could exert multiple functions, depending upon the intra-cellular environment.

This is most probably the case of the vertebrate type 12 17 β -HSD that possesses dual activities, namely, an ancestral 3-ketoacyl-CoA reductase

activity involved in the elongation of long-chain fatty acids, and a more recent type 12 17 β -HSD activity involved in the formation of estradiol and testosterone in the mouse and estradiol in the monkey and human. The bifunctional activity of type 12 17 β -HSD is in agreement with data obtained by our group showing embryonic lethality of the mouse having homozygous deletion of the type 12 17 β -HSD gene (HSD17B12^{-/-}), while the heterozygous deletion leads to a reduction of androgen and estrogen levels (Bellemare et al., 2009). The cause of embryonic lethality is possibly due to impairment of the formation of long-chain fatty acids, since mice having aromatase (Fisher et al., 1998) or estrogen receptor (Korach, 1994) genes deleted do not show embryonic lethality.

It is noteworthy that while the mouse type 12 17 β -HSD is able to efficiently catalyze the transformation of both androgen and estrogen substrates (Blanchard and Luu-The, 2007), the human enzyme is more selective for estrogen (Luu-The et al., 2006). This difference in type 12 17 β -HSD activity contributes to the important difference of steroid metabolism between laboratory animals and humans.

Another example of convergent evolution in steroidogenic enzymes, in which an ancestor gene is able to catalyze the transformation of a more recent substrate, is the gene *det2* (deetiolated 2) of *Arabidopsis thaliana* and type 2 5 α -reductase. DET2 is a gene that encodes a protein that transforms campesterol (a brassinoid) into campestanol (Noguchi et al., 1999) and also plays an important role in light-regulated development in *Arabidopsis* as well as in cotton fiber cell initiation and elongation (Luo et al., 2007). When expressed in HEK-293 cells, this enzyme is also able to catalyze the transformation of testosterone into dihydrotestosterone (Li et al., 1997), a reaction catalyzed by vertebrate 5 α -reductase. On the other hand, human type 2 5 α -reductase expressed in *det2* mutant plants can substitute for DET2 in brassinosteroid biosynthesis (Li et al., 1997).

Other studies based upon sequence homology suggest that types 3 and 12 17 β -HSD are orthologs of the gene YBR159w in yeast *Saccharomyces cerevisiae* described by Moon and Horton (Moon

and Horton, 2003). The enzyme encoded by this gene in yeast possesses 3-ketoacyl-CoA reductase activity involved in the elongation of long-chain fatty acids. The same authors have shown that the human type 12 17β -HSD expressed in HEK-293 cells is able to catalyze 3-ketoacyl-CoA reductase activity involved in the transformation of palmitic acid into stearic acid.

Marked differences of steroidogenic enzymes between humans, monkeys and rodents

Since sex steroids characterized by the presence of a 17β -hydroxy group and their corresponding androgen and estrogen receptors appeared with the vertebrates some 400,000 million years ago (Colbert and Morales, 1991) (Fig. 3), the common ancestor genes found in yeast, plants, worms and insects from which members of 17β -HSD family are derived should be very different and, most probably, are unlikely to exert the same activities and roles. Taking into account the molecular evolutionary aspects mentioned above that create differences in activity (amino acid changes in the coding region) or tissue distribution (especially nucleotide change (s) in the promoter region), it seems of interest to compare some human, monkey and rodent steroidogenic enzymes (Table 1). It can be seen that marked differences are observed, not only in activity but also in the tissue distribution of the enzymes.

It can be seen in Table 1 that types 1 and 12 17β -HSD selectively catalyze the transformation of E_1 into E_2 in human (Dumont et al., 1992a; Luu-The et al., 1990b, 2006), while, in the rodent, these two enzymes catalyze, in addition, the transformation of 4-dione into testo (Blanchard and Luu-The, 2007; Nokelainen et al., 1996). On the other hand, human type 5 17β -HSD very poorly catalyzes the transformation of E_1 into E_2 (Dufort et al., 1999) while this activity is high with the mouse enzyme (Deyashiki et al., 1995; Liu et al., 2007).

There are also major differences between rodent, sub-human primate and human 3β -HSDs. In humans, the 3β -HSD gene family has two genes

and five pseudogenes grouped in a cluster on chromosome 1 (band 1p13.1) (Berube et al., 1989; Luu The et al., 1992; McBride et al., 1999). In humans, type 1 3β -HSD is expressed essentially in peripheral tissues, namely, the placenta, skin, mammary gland and prostate (Rheaume et al., 1991), while type 2 3β -HSD is exclusively expressed in the adrenal gland, testis and ovary. The deficiency in human type 3 3β -HSD is responsible for congenital adrenal hyperplasia associated with male pseudohermaphroditism (Russell et al., 1994; Simard et al., 2005). In the mouse, on the other hand, there are six functional types of 3β -HSD, while in the *Macaca fascicularis*, there is only one functional 3β -HSD (Liu, 2007).

The above-mentioned data strongly suggest that types 1 and 2 3β -HSD are duplicated after the divergence from the *Macaca fascicularis* 3β -HSD gene. Accordingly, the two human enzymes share 94% amino acid sequence identity, confirming that they are duplicated after the divergence with the rodent and most probably from duplication of the single *Macaca fascicularis* gene.

It is noteworthy that only humans possess two 3β -HSDs which possess a 10-fold difference in activity and affinity. Interestingly, the enzyme that possesses higher activity and affinity is one permitting the intracrine transformation of DHEA into 4-dione and 5-diol into testosterone in peripheral intracrine tissues (Labrie, 1991). Type 2 3β -HSD, on the other hand, has lower affinity and activity, and is responsible for adrenal congenital hyperplasia (Rheaume et al., 1991, 1992). In the mouse adrenal gland and testis, there are two types of 3β -HSDs (Payne and Hales, 2004). Accordingly, the probability of having 3β -HSD deficiency in the mouse is much lower than in humans.

It is noteworthy that in the rodent placenta, there is an absence of enzymes involved in the biosynthesis of E_2 , namely, 3β -HSD, aromatase and type 1 17β -HSD (Payne and Hales, 2004), while these enzymes are expressed at very high levels in humans. In addition, the enzyme 17α -hydroxylase/ 17 , 20 -lyase is absent in the mouse adrenals (Brock and Waterman, 1999; Luu-The

Table 1. Comparison of the activity and tissue distribution of some steroidogenic enzymes between human, monkey and rodents

Enzyme (human)	Percentage homology of amino acid sequence	Enzymatic activity	Site (tissue) of expression
3 β -HSD1	~70% homology with rodent 3 β -HSD	DHEA \rightarrow 4-dione Preg \rightarrow prog High activity and affinity, similar to rodent enzymes	Human: placenta, skin, brain – Could be equivalent to rat type 4 and mouse type 6 that are expressed in the skin, however, mouse type 6 is also expressed in the testis
3 β -HSD2	~94% homology with human type 1 ~70% homology with rodent 3 β -HSD	Low activity – does not have equivalent in rodent, in term of activity	Human: adrenals and gonads – could be equivalent with mouse type 1 that is expressed in the adrenals and gonads
5 α -reductase type 1	~62% homology with rodent ~48.4% identity with human type 2	4-Dione \rightarrow 5 α -dione (high activity) Testo \rightarrow DHT (low activity)	Human: liver, skin Rodent: ventral prostate (epithelial cells), epididymis, liver
5 α -reductase type 2	~75% homology with rodent ~48.4% identity with human type 1	4-Dione \rightarrow 5 α -dione Testo \rightarrow DHT	Human: prostate (stromal cells), epididymis, seminal vesicle, genital skin (fibroblasts) Rodent: prostate (stromal cells), epididymis
Aromatase	81% homology with rodent	4-Dione \rightarrow E ₁ (high activity) Testo \rightarrow E ₂ (weak activity)	Human: placenta, pre-ovulatory follicle, corpus luteum, testis, adipose tissue Rodent: pre-ovulatory follicle, corpus luteum, testis, adipose tissue, absent in placenta
Type 1 17 β -HSD	~63% homology with rodent	E ₁ \rightarrow E ₂ (also 4-dione \rightarrow testo in rodent)	Human: placenta and granulosa cells of human ovary Rodent: granulosa cells of developing follicles, absent in placenta
Type 3 17 β -HSD	~72.5% homology with rodent	4-Dione \rightarrow testo	Human: testis Rodent: testis

Type 5 17 β -HSD	~76% homology	Human: 4-dione \rightarrow testo 5 α -dione \rightarrow DHT Prog \rightarrow 20 α -OHProg Rodent: 4-dione \rightarrow testo 5 α - dione \rightarrow DHT E ₁ \rightarrow E ₂	Human: liver, muscle, small intestine, kidney, skin and mammary gland Rodent: liver
Type 7 17 β -HSD	~85% homology with rodent	E ₁ \rightarrow E ₂ DHT \rightarrow 3 β -diol	Human: liver, ovary Rodent: liver, heart, kidney and epididymis
Type 12 17 β -HSD	~81% with rodent	Human: E ₁ \rightarrow E ₂ Mouse: E ₁ \rightarrow E ₂	Human: liver, mammary gland, uterus, skin, adipose tissue, heart and brain Rodent: adrenals, testis, liver, kidney, epididymis, adipose tissue and brain
Type 15 17 β -HSD	~85% homology with rodent	4-Dione \rightarrow testo DHEA \rightarrow 5-diol 5 α -Dione \rightarrow DHT ADT \rightarrow 3 α -diol 4-Dione \rightarrow testo (weak)	Human: prostate, liver, kidney, brain, heart, pancreas and skin, (very low in testis) Rodent: testis, liver, kidney, (absence in prostate)

The abbreviations and symbols used are: DHEA: dehydroepiandrosterone; 5-diol: 5-androstene-3 β ,17 β -diol; 4-dione: 4-androstenedione; 5 α -dione: 5 α -androstane-3,17-dione; testo: testosterone; DHT: dihydrotestosterone; E₁: estrone; E₂: estradiol; 3 α -diol: 5 α -androstane-3 α ,17 β -diol; 3 β -diol: 5 α -androstane-3 β ,17 β -diol; preg: pregnenolone; prog: progesterone; HSD: hydroxysteroid dehydrogenase.

et al., 2005), while it is highly expressed in humans (Luu-The et al., 2005). These observations can explain the low levels of C19-steroids in the circulation in rodents, while the concentration of circulating C19-steroids, especially DHEA and its sulphate DHEA-S, is very high in humans.

Species differences in sex steroid-inactivating enzymes

In addition to the marked species difference in estrogen and androgen formation between species, there are also major differences between species in tissue distribution and activity of the steroid-inactivating enzymes in peripheral tissues ranging from the absence of glucuronidation enzymes in rodents to very high levels in primates and intermediate concentrations in humans. This observation is most probably due to the fact that most of the inactivating enzymes

(3α -HSDs and UGTs) belong to the class ‘conserved structures–different activities’ described above. These genes diverged after the separation between rodents and primates and also between primates and humans, thus implying that they could not have orthologs in other species. In addition, although showing homology, ortholog genes can encode for enzymes having different substrate specificities.

The major pathway of final androgen and estrogen inactivation in humans is glucuronidation that occurs by the addition of a polar glycosyl group to small hydrophilic molecules, thus facilitating their excretion (Belanger et al., 2003) (Figs. 1 and 4). The enzymes responsible for this transformation are members of the UGT family (Mackenzie et al., 2005, 1997). In humans, UGT enzymes are expressed in the liver and a long series of extra-hepatic tissues, including the kidney, brain, skin, adipose and reproductive tissues (Guillemette et al., 2004) and as expected, androgen glucuronides are present

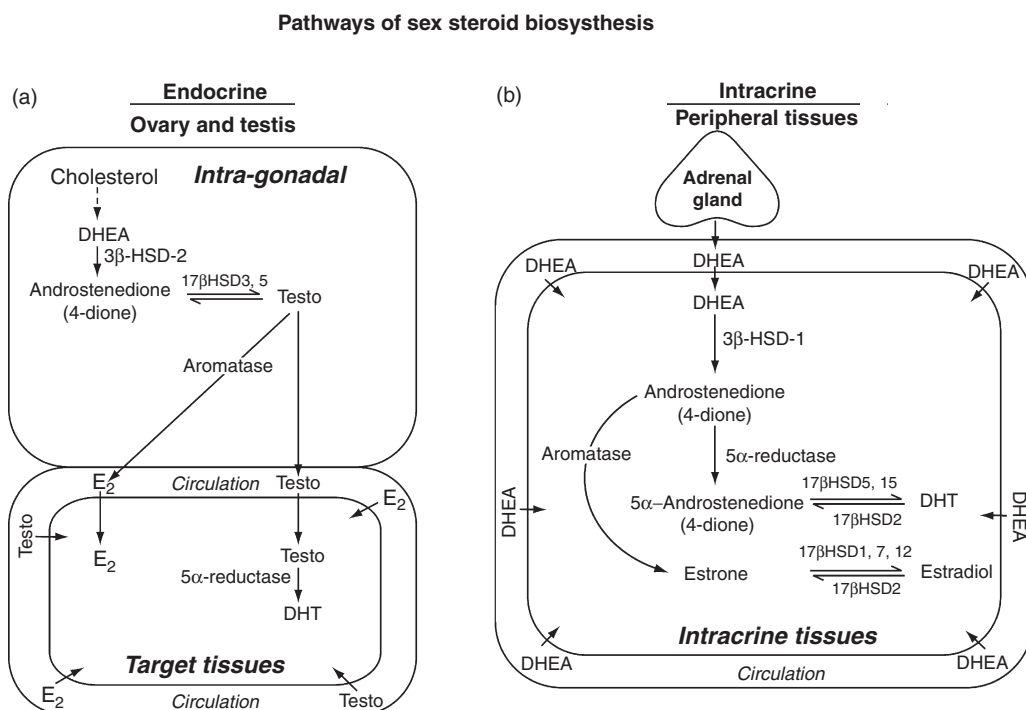


Fig. 4. Schematic representation of pathways requiring (a) and not requiring (b) testosterone as intermediate.

in the circulation which is their route of elimination (Belanger et al., 1991). The extra-hepatic expression and activity of the androgen-conjugating UGT enzymes are major determinants of the local inactivation of androgens and estrogens, thus playing an important role in the regulation of local androgen and estrogen concentration and action or intracrinology (Belanger et al., 2003).

It is important to indicate that the concentrations of the active steroids testosterone and estradiol in the circulation represent the sum of the steroids secreted by the gonads and the concentration of the steroids leaking from the peripheral tissues following DHEA transformation by the intracrine enzymes (Figs. 2 and 4). It is thus important to indicate that the low circulating levels of E₂ or testo observed after castration, in men or after menopause or ovariectomy in women, does not mean that these steroids do not play an important intra-cellular role. Consequently, the conjugated steroids are a better representative of the global exposure to each class of steroids (Labrie et al., 2006).

Does the 17 β -HSD step precede or follow aromatase or 5 α -reductase action in the steroidogenic pathways in peripheral tissues?

The traditional understanding is that estradiol and DHT are always synthesized from the aromatization and 5 α -reduction of testosterone, respectively (Fig. 4a). This pathway indicates that the steps of aromatization and 5 α -reduction follow the step catalyzed by type 3 17 β -HSD and that estrogenic 17 β -HSD is not involved. Contrary to this belief, the cloning of estrogen-specific 17 β -HSD and observation of the higher affinity of aromatase (Kellis and Vickery, 1987; Reed and Ohno, 1976) and 5 α -reductase (Andersson and Russell, 1990; Russell and Wilson, 1994; Sugimoto et al., 1995) for 4-androstenedione than for testosterone are strongly in favour of the biosynthetic pathway in which the step catalyzed by 17 β -HSDs follows the step catalyzed by aromatase (Fig. 4b). On the other hand, in line with this

pharmacokinetic information, DHT is made from A-dione following 5 α -reduction of 4-dione (Fig. 4b). These pathways do not require testosterone as an intermediate as traditionally described.

Using [¹⁴C]-labelled DHEA and 4-androstenedione and inhibitors of aromatase and 5 α -reductase, we have clearly demonstrated that these pathways are the major pathways responsible for the formation of estradiol in JEG-3 cells (Samson et al., 2009b) and DHT in DU-145 (Samson et al., 2009a) and the SZ95 sebaceous gland cell line (Samson et al., 2009c). These pathways are thus specific to intracrinology while, in the testis and ovary, the pathways illustrated in Fig. 4a are operative. Although testosterone can be aromatized into estradiol or 5 α -reduced into DHT *in vitro* with the appropriate enzymes, we believe, in agreement with the recently observed thermodynamics of the reaction, that the main pathways for the biosynthesis of estradiol and DHT in cultured cells and most probably also *in vivo* in humans, in the presence of 4-androstenedione, do not require testosterone as an intermediate.

The reason for the novelty of this observation is most probably due, as mentioned above, to the general belief that steroid concentrations in the circulation represent the concentrations available for transformation in the cells or tissues while, as the more recent information on intracrinology indicates, the circulating concentrations of sex steroids do not represent the intra-cellular concentrations of the same steroids (Labrie et al., 2005).

These data are also in agreement with the presence of two androgens, namely, testosterone and DHT, that are synthesized by two different pathways. The higher affinity of testosterone for the androgen receptor (AR) ($K_m \sim 10^{-8}$, 10^{-9} M) than 5 α -reductases ($K_m \sim 10^{-6}$ M) is also in favour of the role of testosterone as ligand for AR without requiring its transformation into DHT, since in tissues that express both AR and 5 α -reductases, testosterone will preferentially bind to AR and exert its androgenic activity before binding to 5 α -reductases.

Biosynthesis of testosterone

Testosterone is produced from the transformation of 4-androstenedione by types 3 or 5 17 β -HSDs in tissues or cells in which the expression level of 5 α -reductases is low or absent, such as in the testicles and muscle. Type 3 17 β -HSD is mainly expressed in the testis and is responsible for the formation of testosterone necessary for the formation of the internal male reproductive structures. Inactivation of type 3 17 β -HSD in patients who have a mutated gene is responsible for male pseudohermaphroditism (Geissler et al., 1994). The typical features of patients who have a mutated type 3 17 β -HSD gene are ambiguous female external genitalia and virilization at puberty (Wilson, 1978). At surgery, testes and epididymes are found in the inguinal canals, whereas lower Wolffian duct structures are male in character, including the seminal vesicles and ejaculatory ducts. Type 3 17 β -HSD is the main enzyme that produces testicular testosterone that acts as androgen in an endocrine manner in all peripheral target tissues. A defect in type 2 5 α -reductase also causes male pseudohermaphroditism (Andersson et al., 1991) with ambiguous external genitalia. However, in contrast with type 3 17 β -HSD gene deficiency, the Wolffian structures are normally differentiated (Peterson et al., 1977), suggesting that testo and DHT exert their androgenic activity in a different manner via the same AR. It is possible that the co-activators/co-repressors recruited in the presence of testosterone and DHT are different in identity and concentration. Mutations that inactivate the genes encoding AR cause the X-linked androgen-insensitive syndrome (AIS) (Imperato-McGinley et al., 1990; Xu et al., 2003). In these patients, the development of both the internal and external male reproductive structures are altered suggesting that both testosterone and DHT are acting through the same AR.

Another tissue that possesses low levels of 5 α -reductase and high levels of testosterone-producing enzyme is the muscle that expresses high levels of type 5 17 β -HSD. However, type 5 17 β -HSD is a multi-substrate enzyme having the additional ability to convert 5 α -androstane-

3,17-dione into DHT and androsterone (ADT) into 5 α -androstane-3 α ,17 β -diol. Thus, in tissues that also possess 5 α -reductases, type 5 17 β -HSD is involved in the formation of DHT and 5 α -androstane-3 α ,17 β -diol in the pathway that does not require testosterone as an intermediate. In women, the biosynthesis of testosterone in tissues that possess low levels of 5 α -reductase most probably involves type 5 17 β -HSD, as previously described (Luu-The et al., 2001).

Biosynthesis of DHT

In tissues and cells that express 5 α -reductases such as the prostate, skin and liver, DHT, the most potent natural androgen, is most likely to be synthesized, as discussed above, by a pathway that does not require testosterone as an intermediate (Fig. 4b). These tissues are well known as androgen-sensitive tissues and the imbalance of DHT levels could be associated with diseases of the prostate gland, such as benign prostatic hyperplasia (BPH) and prostate cancer, or skin associated diseases such as alopecia, hirsutism and acne seborrea. The above-mentioned data strongly suggest that depending upon the steroid precursors and enzymes present in a specific cell, AR can be modulated by testosterone or DHT.

In addition to 5 α -reductases that catalyze the conversion of 4-androstenedione into 5 α -androstenedione, the conversion of 5 α -androstenedione into DHT requires the presence of 17 β -HSDs. At present, the two most likely candidates among 15 17 β -HSDs identified (Fig. 4b) are types 5 and 15 17 β -HSD (Luu-The et al., 2008).

Biosynthesis of estradiol

Estradiol, the most potent natural estrogen, plays a crucial role in the proliferation of normal and cancerous breast and uterine cells. The role of local E₂ synthesis is increasingly recognized and prevails over the traditional belief that E₂ is exclusively synthesized in the gonads and delivered to peripheral tissues through the circulation. Although the biosynthetic pathway of E₂ was

proposed a few decades ago, there is still, as mentioned above, some controversy about the sequential intervention of aromatase and 17 β -HSD. In fact, traditional literature and textbooks indicate that E₂ is produced by the transformation of 4-androstenedione (4-dione) into testosterone (T) by 17 β -HSD and then by aromati-

zation of T into E₂ (4-dione $\xrightarrow{17\beta\text{-HSD}}$ testo $\xrightarrow{\text{Arom}}$ E₂) (Fig. 4b). However, the four-fold higher affinity of aromatase for 4-androstenedione than for testosterone using microsomes (Reed and Ohno, 1976) and the purified enzyme (Kellis and Vickery, 1987), respectively, are in favour of 4-androstenedione being the substrate for aromatase (Fig. 4b). Accordingly, the aromatase step should naturally precede the action of 17 β -HSD. The role of the pathway is supported by the cloning of estrogen-specific 17 β -HSDs (Krazeisen et al., 1999; Luu The et al., 1989, 1990a, 2006; Peltoketo et al., 1988).

Aromatase represents an interesting case in which the expression of multiple tissue-specific transcripts is driven by different alternative promoters of the same gene (Simpson et al., 1994, 1997). These transcripts, however, possess the same coding sequence and thus encode the same protein structure in all tissues.

It is worth noting that, after menopause, when the ovary is no longer functional, all the active estrogens in women are made in target tissues from inactive adrenal precursors (Labrie et al., 2001, 2005). Ironically, it is in the post-menopausal period that breast cancers are more frequently observed and are, in 75% of cases, efficiently treated by estrogen blockade, thus demonstrating the very important role of intracrine formation of estrogens (Labrie et al., 2005, Mouridsen et al., 2003).

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