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# Androgen biosynthetic pathways in the human prostate

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It is well recognized that there are two androgens, namely testosterone (T) and dihydrotestosterone (DHT); T plays an important role in the testis and muscle, and DHT is crucial for the development, function and pathology of the prostate. It is generally thought that DHT is produced from the  $5\alpha$ -reduction of circulating T before being inactivated by  $3\alpha$ -hydroxysteroid dehydrogenase ( $3\alpha$ -HSD) that converts DHT into  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol ( $3\alpha$ -diol). However, the presence of various steroidogenic enzymes in the prostate as well as the availability at high levels of various steroid precursors such as dehydroepiandrosterone sulphate (DHEAS), dehydroepiandrosterone (DHEA) and 4-androstenedione (4-dione) strongly suggest the existence of additional pathways involved in the biosynthesis and metabolism of DHT. Because steroidogenesis could be different in different species, data from the literature obtained from various human, dog, rat and mouse prostate tissues, as well as primary cells and prostatic cancer cell lines, provide a somewhat confusing picture. In the present chapter, we review the data in order to provide a clearer picture of the pathways involved in DHT biosynthesis and metabolism in the human prostate.

**Key words:** prostate; sex steroids; androgen; oestrogen; dihydrotestosterone; oestradiol; steroidogenesis; intracrinology; androgen biosynthesis.

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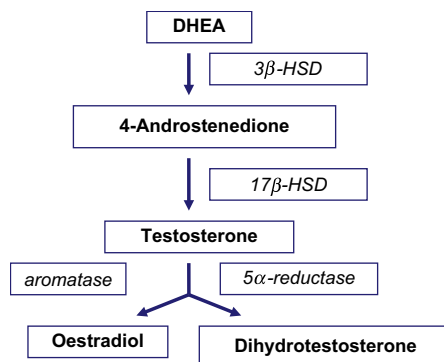
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Androgens play a crucial role in the development, growth, and maintenance of the prostate. They are thus involved in frequent diseases, especially benign prostatic hyperplasia (BPH) and prostate cancer. One of the main targets for the treatment of these diseases is, therefore, to reduce androgen levels in prostatic cells. It is worth noting that prostate cancer is the most frequently diagnosed cancer and the second cause of death due to cancer in North America.<sup>1</sup> Surgical castration has been the method of treatment uniformly used<sup>2,3</sup> until the discovery of chemical castration using gonadotropin-releasing hormone agonists.<sup>4,5</sup> The testicles are considered as the main provider of serum T that is delivered to all tissues of the body through the blood circulation.<sup>6</sup> Indeed, it is generally believed that circulating T is the precursor of the biosynthesis of the most potent natural androgen and oestrogen, namely DHT and oestradiol (E<sub>2</sub>), via the enzymes 5 $\alpha$ -reductase and aromatase, respectively (Figure 1). This well recognized biosynthetic pathway of active sex steroid formation is supported by the clinical observation of pseudohermaphroditism due to the deficiency of testicular 17 $\beta$ -hydroxysteroid dehydrogenase and 5 $\alpha$ -reductase.<sup>6</sup> These observations could suggest that there is a unique steroidogenic pathway for the sex steroids produced by the gonads.

The cloning and molecular characterization of multiple forms of enzymes involved in the biosynthesis of sex steroids in the human, namely 3 $\beta$ -hydroxysteroid dehydrogenases types 1 and 2 (3 $\beta$ -HSDs)<sup>7-11</sup>, 17 $\beta$ -hydroxysteroid dehydrogenases types 1-15<sup>12-26</sup> and 5 $\alpha$ -reductases types 1 and 2<sup>27-30</sup> expressed in tissues other than the gonads, show that the conventional testicular sex steroid biosynthetic pathway should be revised in the light of the identification of additional sources of steroids and the tissue-specific expression of steroidogenic enzymes. In this chapter we review the characteristics of the steroidogenic enzymes expressed in the human prostate, and we propose a new androgen biosynthetic pathway that does not require T synthesis.

## ANDROGEN BIOSYNTHESIS

The expression of the male phenotype is driven by androgens which exert their action during male sexual differentiation, development and maintenance of secondary male characteristics as well as during the initiation and maintenance of spermatogenesis.<sup>6</sup> Studies in patients having a defect in androgen biosynthesis allow to clearly identify two androgens, namely T and DHT. T is produced in the testis by the conversion of 4-dione into T by the enzyme 17 $\beta$ -HSD type 3.<sup>15</sup> T acts directly to promote the formation of



**Figure 1.** Schematic representation of the three last steps of the biosynthetic pathways of sex steroids as generally accepted. DHEA, dehydroepiandrosterone; HSD, hydroxysteroid dehydrogenase.

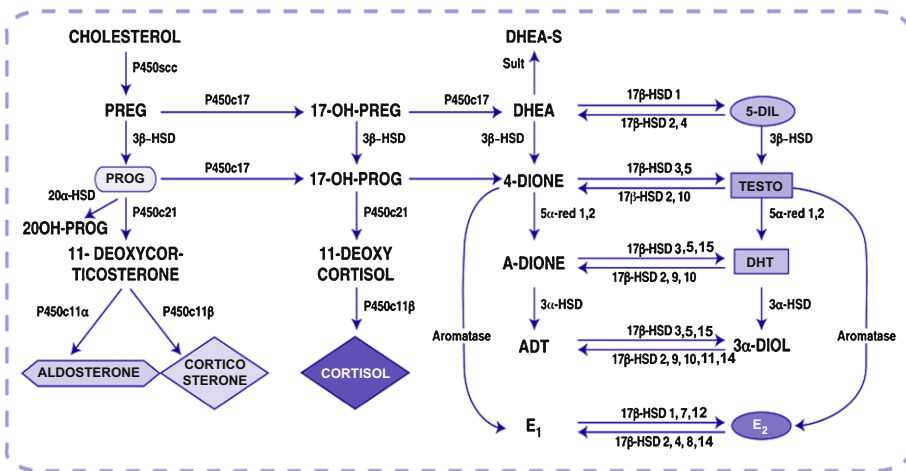
the internal male reproductive structures (epididymes, seminal vesicles and vas deferens). A defect in  $17\beta$ -HSD type 3 causes disorders of human intersex generally called male pseudohermaphroditism.<sup>15</sup> The typical features of  $17\beta$ -HSD deficiency show a 46XY individual having ambiguous female external genitalia and virilization at puberty.<sup>6</sup> At surgery, the testes and epididymes are found in the inguinal canals. The lower Wolffian duct structures are male in character, including the seminal vesicles and ejaculatory ducts.

The production of DHT requires a step of  $5\alpha$ -reduction catalysed by the enzyme  $5\alpha$ -reductase. A defect in  $5\alpha$ -reductase also causes male pseudohermaphroditism<sup>28</sup> with ambiguous external genitalia. However, in contrast with  $17\beta$ -HSD type 3 deficiency, the Wolffian structures are normally differentiated.<sup>31</sup>

A defect in androgen receptor (AR) causes a X-linked androgen-insensitive syndrome (AIS)<sup>32,33</sup> in which the development of both the internal and external male structures in 46, XY individuals are altered, thus indicating that both T and DHT act via the same androgen receptor. The concept of two hormones and one receptor to explain the different actions of androgens has been generally accepted. It is thus strongly suggested that depending on the presence of steroid precursors and the enzymatic machinery within the cells, the androgenic effect exerted by AR could be modulated by T, DHT or by both compounds.

## ANDROGEN SOURCES IN THE HUMAN

The enzyme  $17\alpha$ -hydroxylase/ $17,20$ -lyase (P450c17, CYP17) is a crucial enzyme in the steroidogenic pathway (Figure 2). It possesses a dual enzymatic activity, namely  $17\alpha$ -hydroxylase and  $17,20$ -lyase, that convert C21-steroids (pregnenolone and progesterone) into  $17\alpha$ -hydroxypregnenolone and  $17\alpha$ -hydroxyprogesterone that are the precursors of cortisol biosynthesis in the human, as well as into C19-steroids (DHEA and 4-androstenedione) that are the precursors of sex steroid biosynthesis. It is worth noting that P450c17 in the human and in rodents possesses different substrate specificity. In the rodent, P450c17 is more active on 4-ene steroids<sup>34–37</sup>, while in the human and primates, it is more active on 5-ene steroids.<sup>34–39</sup> This probably explains why rodents do



**Figure 2.** Schematic representation of general steroidogenic pathways. DHEA, dehydroepiandrosterone; DHEAS, DHEA sulphate; DHT, dihydrotestosterone; HSD, hydroxysteroid dehydrogenase.

not produce DHEA and cortisol but 4-androstenedione and corticosterone, instead. The steroidogenic pathways in the rodent and human are thus quite different.

In addition, while P450c17 is expressed in the gonads of all mammals including human and rodents, it is absent in the adrenals of many animals, including rodents, but it is expressed at high levels in the human and primate adrenals.<sup>40–42</sup> The presence of high levels of CYP17 in human and primate adrenals is most probably the cause of the high levels of steroid precursors of adrenal origin found in the circulation of the human and primate, while they are almost absent in rodents. In fact, the high level of circulating adrenal androgen precursors (DHEA and DHEAS) in the human is well recognized as an additional source of active sex steroids in peripheral tissues. It represents a new field of hormone action termed intracrinology.<sup>43–46</sup>

Indeed, the normal development of sexual secondary characteristics observed in congenital adrenal hyperplasia patients having a deficit in  $3\beta$ -HSD type 2<sup>47</sup> (exclusively expressed in human adrenals and gonads) is a natural proof of the conversion of adrenal DHEA by  $3\beta$ -HSD type I that is expressed exclusively in peripheral tissues. Another proof is the virilization at adulthood of young men deficient in  $17\beta$ -HSD type 3 that have impaired testicular T biosynthesis.<sup>48</sup>

Furthermore, in men whose testicles have been removed, it is observed that although there is a 90–95% decrease in T levels in the blood, the intra-prostatic DHT level is only reduced by 50%, thus indicating the presence in the prostate of a DHT biosynthetic pathway that does not require testicular T.<sup>45,46,49</sup>

Another natural example of the biosynthesis of active sex steroids using DHEAS as precursor is the biosynthesis of oestradiol in the placenta. In this tissue, there is a high level of P450scc,  $3\beta$ -HSD, sulphatase, aromatase and  $17\beta$ -HSD type I, but the enzyme  $17\alpha$ -hydroxylase/ $17,20$ -lyase is absent, thus preventing the formation of DHEA or 4-dione. The precursor for  $E_2$  biosynthesis in the placenta is, indeed, DHEAS that is produced by the adrenals of the fetus. In the placenta, DHEAS is transformed into DHEA by steroid sulphatase, and subsequently converted to 4-dione by type I  $3\beta$ -HSD, then  $E_1$  and  $E_2$  by aromatase and  $17\beta$ -HSD type I, respectively.

These are many observations demonstrating that sex steroids, in addition to the gonadal source that is the almost exclusive source of sex steroids in children and animal models such as rodents, are also synthesized in peripheral tissues, including the prostate, in adult men and women, using inactive adrenal precursors.

## EXPRESSION OF STEROIDOGENIC ENZYMES IN THE HUMAN PROSTATE

### $3\beta$ -HSD

The cDNA encoding  $3\beta$ -HSD<sup>7</sup> was first isolated and characterized by cloning using antibodies raised against human placental  $3\beta$ -HSD purified in our laboratory.<sup>50</sup> Using the DNA fragment of the first  $3\beta$ -HSD cDNA, namely  $3\beta$ -HSD type I, we have isolated a second cDNA encoding  $3\beta$ -HSD type 2<sup>9</sup> that shares 94% amino acid identity with  $3\beta$ -HSD type I. Using cDNA as probe, we have then characterized the structures of the types I and 2  $3\beta$ -HSD genes (HSD3B1 and HSD3B2).<sup>10,11</sup> Knowledge of the structures and sequences of HSD3B1 and HSD3B2 permits to study the genetics of  $3\beta$ -HSD deficiency in affected patients. HSD3B2 expressed almost exclusively in the adrenals and gonads<sup>9</sup> is found to be responsible for congenital adrenal hyperplasia due to  $3\beta$ -HSD deficiency.<sup>47</sup> No phenotype has yet been associated with type I  $3\beta$ -HSD deficiency (Ref.<sup>51</sup> for review).

It is noteworthy that  $3\beta$ -HSD type 2 that catalyses the same reaction as  $3\beta$ -HSD type 1 but with 10-fold less efficiency (lower  $K_m$  and  $V_{max}$ )<sup>9</sup>, is responsible for the congenital disease.

Using RNase protection assay, it has been found that  $3\beta$ -HSD type 1 is specifically expressed in the human prostate as well as in the skin, mammary gland and brain.<sup>9</sup> Later, using in-situ hybridization and immunocytochemistry, we have shown that  $3\beta$ -HSD expression co-localizes with  $17\beta$ -HSD type 5 in the glandular epithelium of the prostate with the highest levels observed in the basal cells relative to luminal cells.<sup>52</sup>

## 5 $\alpha$ -Reductases

The enzyme  $5\alpha$ -reductase catalyses the  $5\alpha$ -reduction of 4-dione, T, progesterone as well as other 4-ene-3-ketosteroids into their corresponding  $5\alpha$ -dihydro-3-ketosteroids. The most widely described activity of this enzyme is its ability to convert T into DHT, the most potent natural androgen responsible for the differentiation of male organs including the prostate, and for the virilisation of boys at puberty.<sup>6</sup>

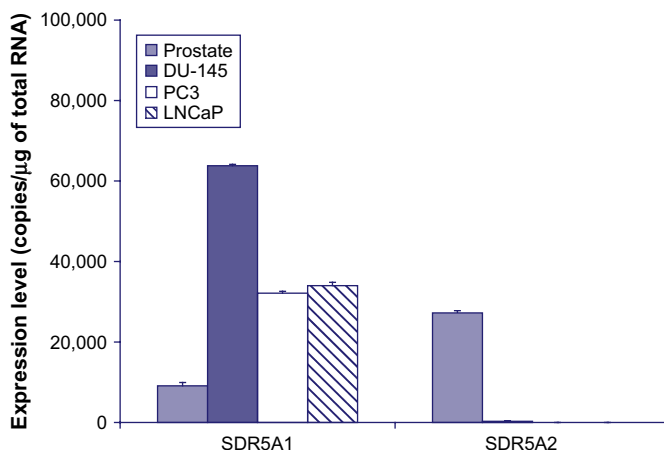
cDNAs encoding two types of  $5\alpha$ -reductase, chronologically named  $5\alpha$ -reductase types 1 and 2, have been cloned and characterized from human prostate cDNA libraries.<sup>27,28</sup> The genomic structure of the corresponding genes has also been elucidated.<sup>29,30</sup> The two types of  $5\alpha$ -reductase share 50% amino acid sequence identity and possess similar substrate specificity. However, the two enzymes show different pH optima and sensitivity to inhibitors. The type-1 isoenzyme has a broad pH optimum (pH 6–8.5), while the type-2 isozyme has a narrow acidic pH optimum centered around 5.<sup>27,29</sup> In addition,  $5\alpha$ -reductase type 1 is approximately 10-fold less sensitive to finasteride (Proscar) than the type-2 enzyme<sup>27</sup>, while it is more sensitive to cations.<sup>53</sup> It has also been shown that human  $5\alpha$ -reductases catalyse 4-dione more efficiently than T.<sup>27,53</sup>

$5\alpha$ -Reductase type 2 is the major form expressed in the human prostate as evidenced by the pH optimum of the enzymatic activity of prostate extracts<sup>54</sup> and mRNA expression<sup>55</sup> (Figure 3). However, the major form expressed in DU-145, LNCaP and PC3 prostatic cancer cell lines, is  $5\alpha$ -reductase type 1;  $5\alpha$ -reductase type 1 is also expressed in primary prostatic epithelial cells in culture (PreC). It is suggested that the expression of  $5\alpha$ -reductase type 2 in differentiated epithelial cells is mediated by factors coming from stromal cells.<sup>56</sup> A similar situation has been observed in the rat.<sup>57</sup> In epithelial cell culture, where the stromal component is absent, it is likely that  $5\alpha$ -reductase type 2 is not expressed, and progressively,  $5\alpha$ -reductase type 1 becomes a major expressed form such as observed in primary<sup>58</sup> and cancer<sup>59,60</sup> cells in culture (Figure 3). Analysis of the data of steroid metabolism using cell culture should thus be done with much care in order to avoid misinterpretation.

Studies of the distribution of the  $5\alpha$ -reductase isoforms in the human prostate have provided controversial results. While some authors have reported the presence of only type-2  $5\alpha$ -reductase in the prostate, a few reports have shown that both types of  $5\alpha$ -reductase can be detected.<sup>27,28,30,61,62</sup> Using in-situ hybridization<sup>63</sup>, we have also confirmed the expression of  $5\alpha$ -reductase types 1 and 2 in both the stromal and epithelial cells of the prostate.

## 17 $\beta$ -Hydroxysteroid dehydrogenases

Active sex steroids (T, DHT and E2) are characterized by the presence of a hydroxyl group at position  $17\beta$  on the steroid nucleus. The reduction of the  $17$ -keto into



**Figure 3.** mRNA expression levels of  $5\alpha$ -reductase types 1 and 2 in human prostate and in prostatic cancer cell lines. Total RNA extracted from a pool of prostate sample of men aged 20–54 was obtained from Ambion Inc (Austin, T). Total RNA of prostatic cancer cell line DU-145, PC-3 and LNCaP, was extracted using RNA extraction kit from Qiagen Inc. (Mississauga, Ontario, Canada). mRNA expression levels were quantified using Realtime PCR as described.<sup>42</sup> The data are expressed as mean  $\pm$  SEM of duplicate measurements.

a  $17\beta$ -hydroxy group is catalysed by  $17\beta$ -hydroxysteroid dehydrogenases ( $17\beta$ -HSDs). To date, 15 types of  $17\beta$ -HSD have been identified.<sup>12–26</sup> It is evident, however, that many of these enzymes are associated with functions other than sex steroid production. Using a transgenic mouse model to express human  $17\beta$ -HSD type 2, Zhongyi et al<sup>64</sup> have provided evidence indicating that this enzyme has a role independent from its action on sex steroids. Most probably, it is associated with retinol metabolism.

Patients having altered genes for  $17\beta$ -HSD types 4 and 10 also show that these two genes are not involved in sex steroid biosynthesis.  $17\beta$ -HSD type 4 is mainly a peroxisomal D-hydroxyacyl-CoA dehydrogenase catalysing fatty acid  $\beta$ -oxidation.<sup>65</sup> Its deficiency causes accumulation of branched long-chain fatty acids and very-long-chain fatty acids, and a disturbed synthesis of bile acids.<sup>66</sup> Deficiency of  $17\beta$ -HSD type 10, on the other hand, causes 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency: an X-linked inborn error of isoleucine metabolism.<sup>67</sup>

$17\beta$ -HSDs types 6, 9, 10 and 11 use mainly  $5\alpha$ -reduced steroids as substrates.<sup>21–24</sup> Their exact functions are still ill-defined, and some of their orthologues in the human – such as  $17\beta$ -HSD types 6 and 9 – have not yet been found.<sup>68</sup>  $17\beta$ -HSD type 7 was cloned and characterized as an oestrogenic  $17\beta$ -HSD catalysing the transformation of E1 into E2.<sup>18,19,69</sup> Recently, this enzyme was found to possess a 3-keto reductase activity catalysing the transformation of DHT into  $5\alpha$ -androstane- $3\beta$ , $17\beta$ -diol<sup>70,71</sup>, and is one of the main enzymes involved in the cholesterol biosynthetic pathway.<sup>72</sup>

$17\beta$ -HSD type 8 is an enzyme that possesses oestrogenic  $17\beta$ -HSD activity catalysing the transformation of E2 into E1.<sup>20,73</sup> It is expressed in the ovary, testicles, kidney and liver.<sup>20</sup> Using in-situ hybridization, the mouse enzyme was detected in granulosa cells of growing follicles and luteal cells, in the seminiferous tubules of the testicles and in epithelial cells of proximal convoluted tubules of the kidney.<sup>74</sup>

$17\beta$ -HSD type 13 was recently cloned from a human liver cDNA library.<sup>75</sup> It is expressed in the liver, lung and intestine, but is absent in the prostate. Sequence analysis indicates that it shares 78% homology with  $17\beta$ -HSD type 11.

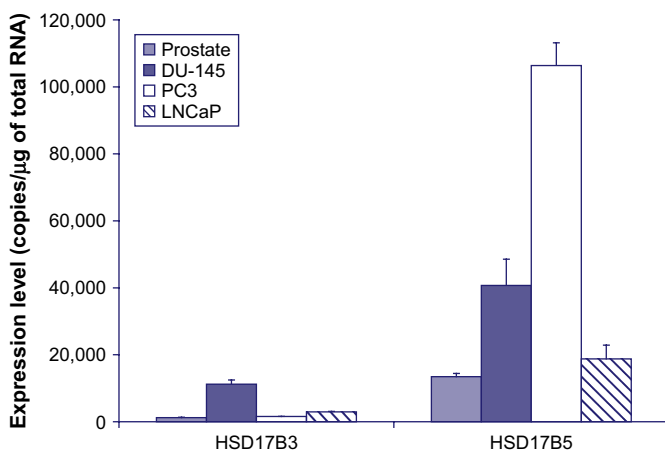
17 $\beta$ -HSD type 14 is a recently characterized 17 $\beta$ -HSD.<sup>26</sup> It catalyses the oxidative reaction using C19- as well as C18-steroids as substrates. This enzyme shows similar substrate specificity as 17 $\beta$ -HSD type 2.<sup>14</sup> Sequence analysis indicates a high sequence homology with the retinol dehydrogenase family.

The 17 $\beta$ -HSDs that are most likely to be involved in sex steroid biosynthesis are types 1, 3, 5 and 12. 17 $\beta$ -HSD type 1 is a cytosolic enzyme that catalyses the transformation of E1 into E2 in the placenta<sup>12,76,77</sup>, and probably also in the ovary<sup>78</sup> where it acts as a partner of aromatase in the formation of E<sub>2</sub>. This enzyme is most likely linked to reproduction.

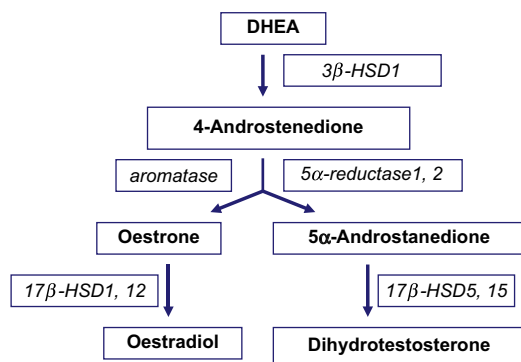
17 $\beta$ -HSD type 3 catalyses the transformation of 4-dione into T.<sup>15</sup> It is mainly expressed in the testis. As mentioned above, its deficiency causes male pseudohermaphroditism in young boys<sup>15</sup> but is asymptomatic in girls<sup>79</sup>, thus suggesting that the formation of androgens in the ovaries is catalysed by an enzyme different from the type 3 active in men's testicles. Until now, 17 $\beta$ -HSD type 5 is the only other enzyme able to catalyse the transformation 4-dione into T<sup>17</sup>, an activity similar to 17 $\beta$ -HSD type 3. The localization of 17 $\beta$ -HSD type 5 in the theca cell layer<sup>80</sup> strongly suggests that this enzyme is involved in the formation of T in women.<sup>81</sup>

As illustrated in Figure 4, comparison of the expression levels of 17 $\beta$ -HSD types 3 and 5, quantified by RealTime PCR, indicates that 17 $\beta$ -HSD type 5 is an enzyme involved in the formation of active androgens in the prostate. We have recently identified a new 17 $\beta$ -HSD that is expressed in the prostate and is able to catalyse the transformation of 5 $\alpha$ -dione into DHT (Luu-The, unpublished data). This enzyme is most likely involved in the biosynthesis of DHT in the prostate. We tentatively name this enzyme type 15 17 $\beta$ -HSD (Figures 5 and 6).

Human 17 $\beta$ -HSD type 12 possesses the same genomic structure as 17 $\beta$ -HSD type 3.<sup>25</sup> These two enzymes also show conserved active and cofactor binding sites. They are most likely duplicate genes. Both genes, however, have evolved to acquire different substrate selectivity, androgens for 17 $\beta$ -HSD type 3 and oestrogens for 17 $\beta$ -HSD type 12 in both humans and primates.<sup>25,82</sup> It is noteworthy that the mouse type 12 17 $\beta$ -HSD

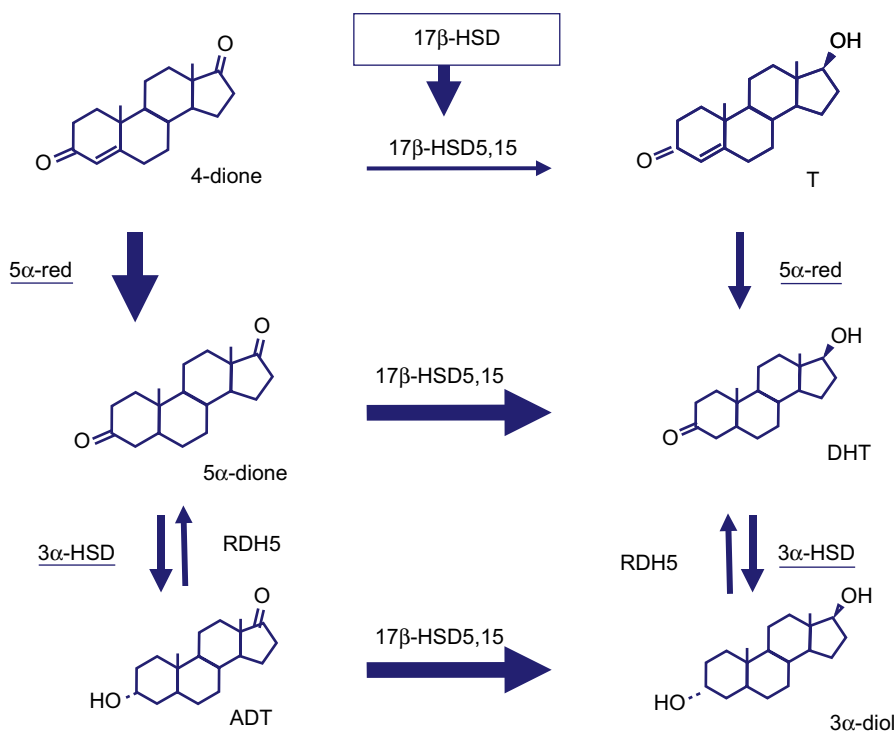


**Figure 4.** mRNA expression levels of 17 $\beta$ -HSD types 3 and 5 in human prostate and in prostatic cancer cell lines. Experimental procedure was as described in the legend to Figure 3.



**Figure 5.** Schematic representation of the three last steps of the biosynthetic pathways of sex steroids as in a newly proposed pathway. DHEA, dehydroepiandrosterone; HSD, hydroxysteroid dehydrogenase.

catalyses the transformation of both androgens and oestrogens.<sup>83</sup> In agreement with their major role in the formation of active sex steroids in the human,  $17\beta$ -HSD type 3 is strongly expressed in the testis, while type 12 is expressed strongly in the ovary and mammary gland.<sup>25</sup>



**Figure 6.** Proposed new metabolic pathways for the biosynthesis and metabolism of DHT that does not require T synthesis. Line thickness indicates the preferred steps. DHEA, dehydroepiandrosterone; HSD, hydroxysteroid dehydrogenase; T, testosterone; DHT, dihydrotestosterone; ADT, androsterone.

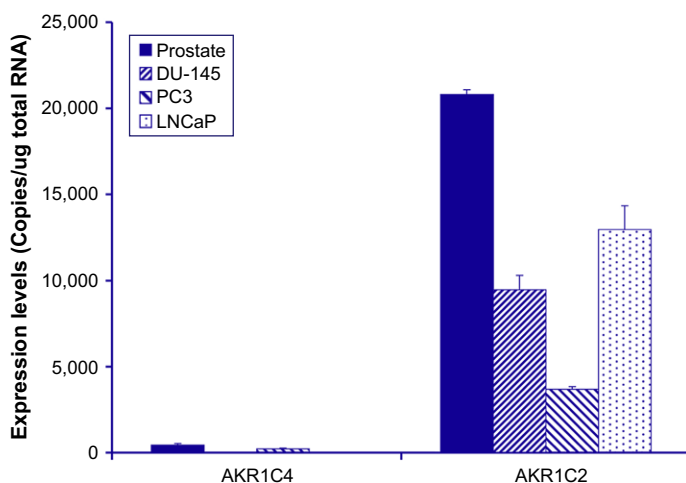
### 3 $\alpha$ -Hydroxysteroid dehydrogenases

3 $\alpha$ -HSD catalyses the conversion of 3-keto-5 $\alpha$ -saturated steroids into their corresponding 3 $\alpha$ -hydroxy compounds. For example, 5 $\alpha$ -androstane-3-one and DHT are transformed into ADT and 3 $\alpha$ -diol, respectively. This enzymatic activity has been found in various mammalian tissues including the liver<sup>84</sup>, prostate<sup>85</sup>, brain<sup>86</sup>, and epididymis.<sup>87</sup> In the human, two types of 3 $\alpha$ -HSDs have been isolated<sup>88-90</sup>, chronologically named type 1 and type 3 3 $\alpha$ -HSD.<sup>90,91</sup> Type 2 3 $\alpha$ -HSD<sup>89,92</sup> is now recognized as 17 $\beta$ -HSD type 5<sup>17</sup>, since its ability to transform 4-dione into T in intact transfected cells in culture is much higher than its transformation of DHT into 3 $\alpha$ -diol. According to the new nomenclature for the aldo-keto reductase family<sup>93</sup>, human 3 $\alpha$ -HSD types 1 and 3, 20 $\alpha$ -HSD, and 17 $\beta$ -HSD type 5 are now named AKR1C4, AKR1C2, AKR1C1 and AKR1C3, respectively.

As illustrated in Figure 7, 3 $\alpha$ -HSD type 3 is the form that is expressed in the prostate, while 3 $\alpha$ -HSD type 1 is absent. Such findings are in agreement with previous data<sup>91</sup> showing that human 3 $\alpha$ -HSD type 1 is exclusively expressed in the liver.

### PROPOSED BIOSYNTHETIC PATHWAY OF DHT IN THE PROSTATE THAT DOES NOT REQUIRE T BIOSYNTHESIS

It is well accepted that 17 $\beta$ -HSD and 5 $\alpha$ -reductase are the main enzymes involved in the last steps of androgen biosynthesis. Testicular androgenic 17 $\beta$ -HSD, namely 17 $\beta$ -HSD type 3, catalyses the transformation of 4-dione into T, while 5 $\alpha$ -reductase catalyses the formation of DHT from T (4-dione  $\rightarrow$  T  $\rightarrow$  DHT). According to this well-accepted pathway, the step of 17 $\beta$ -HSD activity precedes 5 $\alpha$ -reductase activity. However, the evidence that 5 $\alpha$ -reductase prefers 4-dione as substrate compared to T<sup>27,53</sup>, the low free T levels in the circulation compared to 4-dione due to binding to SHBG, the very high level of C-19 5 $\alpha$ -reduced steroids in the circulation ( $\mu$ M range)



**Figure 7.** mRNA expression levels of 3 $\alpha$ -HSD type 1 (AKR1C4) and type 3 (AKR1C2) in human prostate and in prostatic cancer cell lines. Experimental procedure was as described in the legend to Figure 3.

and the higher affinity of T towards AR than 5 $\alpha$ -reductases strongly suggest that the 5 $\alpha$ -reductase activity step precedes the 17 $\beta$ -HSD activity step according to the pathway: 4-dione  $\rightarrow$  5 $\alpha$ -dione  $\rightarrow$  DHT. Because of the lack of identification of the presence of an enzyme exclusively able to catalyse the transformation of 5 $\alpha$ -dione into DHT, the second pathway has not been studied. The finding that 17 $\beta$ -HSD type 5 catalyses efficiently the transformation of 5 $\alpha$ -dione into DHT<sup>17,94</sup>, and the recent identification of a new 17 $\beta$ -HSD that we tentatively chronologically name type 15 17 $\beta$ -HSD that is also able to catalyse this activity allows us to propose a new DHT biosynthetic pathway in the prostate that does not require the synthesis of T (Figure 5).

## CONCLUSION

The androgen physiology, in which T, an active androgen, is a precursor in the biosynthesis of another more active androgen, DHT, which are both able to activate the same androgen receptor, has been so far considered an unsolved problem.<sup>95</sup> The present proposed DHT biosynthetic pathway that does not require T biosynthesis (Figures 5 and 6) is a potential solution to this problem. Indeed, we propose that there are two separate biosynthetic pathways for T and for DHT. In tissues in which 5 $\alpha$ -reductase is absent but type 3 and/or 17 $\beta$ -HSD type 5 are present, the main androgen is T is produced by the conversion of 4-dione by 17 $\beta$ -HSD type 3 (testicles) or type 5 (muscle). In the tissues where 5 $\alpha$ -reductase is present, such as the hair, skin and prostate<sup>63</sup>, 4-dione is converted to 5 $\alpha$ -dione that is further converted to DHT by 17 $\beta$ -HSD types 5 and 15, or to ADT by 3 $\alpha$ -HSD types 1 and 3. DHT is further converted to 3 $\alpha$ -diol by 3 $\alpha$ -HSD types 1 and 3, while ADT is transformed to 3 $\alpha$ -diol by 17 $\beta$ -HSD types 5 and 15. Interestingly, the presence of a DHT biosynthetic pathway that does not require T synthesis has also been described in the marsupial.<sup>96</sup>

It is noteworthy that 3 $\alpha$ -diol could also be converted into DHT by enzymes that possess oxidative 3 $\alpha$ -HSD activity, such as RDH5.<sup>97</sup> In the testes from immature mouse<sup>98</sup>, rat<sup>99</sup> and tammar wallaby<sup>96</sup>, it has been shown that there is a 3 $\alpha$ -diol biosynthetic pathway that involves 5 $\alpha$ -reduced C21-steroids (5 $\alpha$ -pregnane) without T synthesis; 3 $\alpha$ -diol could thus be a source of DHT biosynthesis through oxidative 3 $\alpha$ -HSD.

In the light of the cloning and characterization of multiple oestrogenic and androgenic 17 $\beta$ -HSDs, the general belief that testosterone is a precursor for oestradiol and DHT biosynthesis (Figure 1) should be revised. In fact, although aromatase and 5 $\alpha$ -reductases could use T as substrate, 4-dione is a better substrate for these enzymes.<sup>27,53,100</sup> The existence of oestrogen-specific 17 $\beta$ -HSDs, such as 17 $\beta$ -HSD types 1 and 12, that transform selectively E1 into E2, is an additional proof that the aromatization step precedes the oestrogenic 17 $\beta$ -HSD step. On the other hand, in addition to the fact that 4-dione is better substrate than T for 5 $\alpha$ -reductase<sup>27</sup>, the higher affinity of T for the androgen receptor ( $K_m \sim 10^{-8}$ – $10^{-9}$  M) than 5 $\alpha$ -reductases ( $K_m \sim 10^{-6}$  M) strongly suggests that T is not substrate for 5 $\alpha$ -reductases, since the later are often expressed in the same cells as the androgen receptor.<sup>52</sup> Further studies are necessary to obtain more knowledge about the new DHT biosynthetic pathway as well as inhibitors of enzymes involved in the formation of DHT (17 $\beta$ -HSD types 5 and 15 and oxidative 3 $\alpha$ -HSD) for the treatment of androgen-dependent diseases.

### Research agenda

- further research is needed to characterize the effectiveness of DHT biosynthesis by 17 $\beta$ -hydroxysteroid dehydrogenase type-15 in the prostate as well as other peripheral tissues
- studies to address the significance of the new DHT biosynthetic pathway that does not require the step of testosterone biosynthesis in peripheral tissues are ongoing in our laboratory

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