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New Frontiers in Androgen Biosynthesis and Metabolism⁺

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Abstract

Purpose of review—To summarize recent advances in androgen biosynthesis and metabolism in peripheral tissues (e.g. liver and prostate) and how these can be exploited therapeutically.

Recent findings—Human liver catalyzes the reduction of circulating testosterone (T) to yield four stereoisomeric tetrahydrosteroids. Recent advances have assigned the enzymes responsible for these reactions and elucidated their structural biology. Data also suggests that for 5 α -dihydrotestosterone (5 α -DHT), conjugation reactions (phase II) may precede ketosteroid reduction (phase I) reactions. Human prostate is the site of benign prostatic hyperplasia and prostate cancer which occur in the aging male. While the importance of local androgen biosynthesis in these diseases is accepted, recent advances have identified enzymes that regulate ligand access to the androgen receptor; a “backdoor pathway” to 5 α -DHT that does not require T acting as an intermediate; and the finding that castrate resistant prostate cancer has undergone an adaptive response to androgen deprivation, which involves intra-tumoral T and 5 α -DHT biosynthesis that can be targeted using inhibitors of (CYP17-hydroxylase/17,20-lyase), aldo-keto reductase 1C3, and 5 α -reductase type 1 and type 2.

Summary—Enzyme isoforms responsible for the biosynthesis and metabolism of androgens in liver and prostate have been identified and those responsible for the biosynthesis of androgens in castrate resistant prostate cancer can be therapeutically targeted.

Keywords

castrate resistant prostate cancer; aldo keto reductase; abiraterone acetate; 5 α / β -reductase

Introduction

Androgen biosynthesis (testosterone (T) production in the Leydig cells of the testis under Luteinizing Hormone (LH) control; and 5 α -dihydrotestosterone (5 α -DHT) production under local control in peripheral tissues) is essential for sex-determination and mature sexual development in man. In the absence of these androgens hypogonadism and pseudohermaphroditism occurs [1, 2]. As males age there is a slow decline in circulating testosterone which can lead to deleterious effects (e.g. loss of libido, osteoporosis and loss of muscle mass). These are symptoms of the so called “andropause” which do not affect all men equally [3^{*}, 4].

Diseases of the prostate e.g. benign prostatic hyperplasia (BPH) and prostate cancer are prevalent in the aging male, and are androgen dependent at the time when circulating T from the gonads wanes. This suggests that the local or intracrine production of androgens may

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drive these diseases [5, 6]. Importantly, BPH affects 90% of all men above the age of 80; while prostate cancer is the second leading cause of cancer mortality in the US population and generally occurs in men > 50 years of age. It is estimated that 200,000 new cases of prostate cancer will be diagnosed annually in the USA and that this will result in 40,000 deaths per year.

Androgen insufficiency syndromes can be treated by providing replacement androgen therapy (androgen receptor agonists) while BPH and prostate cancer can be treated by androgen deprivation e.g. the use of agents that deprive the androgen receptor (AR) of its ligand. These agents include inhibitors of steroidogenesis and androgen receptor antagonists. The former approach requires a detailed knowledge of the extra-gonadal biosynthesis and metabolism of androgens and the discrete enzymes involved. The purpose of this article is to review recent breakthroughs in this knowledge and how it can be used to improve therapy.

Hepatic Androgen Metabolism

Testosterone is a C₁₉ Δ^4 -3-ketosteroid and undergoes clearance in the liver by sequential reduction by either 5 α -reductase type 1 (*SRD5A1*) or steroid 5 β -reductase (*AKR1D1*) to yield 5 α -DHT and 5 β -DHT, respectively [2, 7]. Each of these dihydrosteroids are further reduced by 3 α or 3 β -hydroxysteroid dehydrogenases (HSD) to yield four stereoisomeric tetrahydrosteroids e.g. 5 α -androstane-3 α ,17 β -diol (3 α -diol), 5 α -androstane-3 β ,17 β -diol (3 β -diol), 5 β -androstane-3 α ,17 β -diol and 5 β -androstane-3 β ,17 β -diol, Figure 1. It is now recognized that human liver contains four Aldo-Keto Reductase (AKR) isoforms (AKR1C1-AKR1C4) which are NADPH-dependent ketosteroid reductases. These enzymes display different ratios of 3 α -/3 β -HSD activity [8, 9], and couple with either *SRD5A1* or *AKR1D1* to produce the four isomeric products observed. For example, *SRD5A1* and *AKR1C1* couple to produce 3 β -diol and *SRD5A1* and *AKR1C2* couple to produce 3 α -diol [9].

Until recently no structural information existed on any mammalian steroid double bond reductase. Recently the structures of several AKR1D1•NADP⁺•Steroid (testosterone, progesterone, cortisol, 5 β -dihydroprogesterone, finasteride) ternary complexes were reported [10–13]. This enzyme has the (α/β)₈-barrel structural motif shared by the AKR superfamily, and contains three large loops at the back of the structure that determine substrate specificity, Figure 2A. The cofactor binding site is highly conserved within the superfamily and straddles the lip of the barrel in an extended *anti*-conformation that determines 4-proR-hydride transfer to C5 of the steroid. By conducting hydride transfer to the β -face of the steroid the A/B *cis*-ring junction is introduced and generates a 90° bend into the planar steroid structure, Figure 1. This AKR has a substitution in its catalytic tetrad where E120 replaces a histidine. The structure reveals that this substitution allows the steroid substrate to penetrate further into the active site so that the hydride donor (NADPH) and C5 of the steroid are optimally aligned. In addition, the side-chain of E120 is in a fully protonated *anti*-conformation which forms hydrogen bonds with the C3-ketone of the ketosteroid, Figure 2B. Thus the mechanism of steroid double-bond reduction has been revealed for the first time.

Finasteride (a selective mechanism-based inactivator of *SRD5A2*) is a competitive inhibitor of 5 β -reductase $K_i = 2.1 \mu\text{M}$. However, finasteride is not a mechanism based inactivator of *AKR1D1* and the structure of the AKR1D1•NADP⁺•Finasteride complex explains why this is the case [12**]. The NADPH is on the wrong side of the steroid pocket in *AKR1D1* and thus reduction of the $\Delta^{1,2}$ -ene of finasteride observed in *SRD5A2* to produce a bisubstrate analog is not possible. The K_i value of finasteride for *AKR1D1* is only one order of magnitude higher than that observed for *SRD5A1* suggesting that high doses of this drug

would inhibit both enzymes. In this instance finasteride would inhibit its own metabolism. 5 β -Pregnanes produced by AKR1D1 are ligands for the orphan nuclear receptors PXR and CAR which when activated induce CYP3A4 [14, 15]. This CYP isoform is the major enzyme involved in finasteride metabolism [16].

The route of hepatic androgen metabolism so far described leads to tetrahydroandrostanes which are conjugated as either glucuronides or sulfates for excretion; i.e., reduction of the ketosteroid (phase 1 reactions) precede conjugation reactions of the alcohol (phase 2 reactions). In recent studies, evidence for the reverse sequence was found to exist [17^{**}]. AKR1C1-AKR1C4 which reduce 5 α - and 5 β -DHT to their corresponding 3 α - and 3 β -diols were found to have high catalytic efficiencies towards 5 α -DHT-17 β -glucuronide or 5 β -DHT-17 β -sulfate. The products of the reactions were verified by liquid chromatography-mass spectrometry (LC-MS). Interestingly, although uridine glucuronosyl transferase (UGT)2B7 preferentially catalyzes the 3'-OH glucuronidation of 3 α -diol and is highly expressed in liver [18] no 3'-glucuronide is formed in the circulation, suggesting the dominant pathway is conjugation at the 17 β -position followed by reduction of the 3-ketone [17], Figure 1 (inset). Others have proposed that steroid metabolism could occur on steroid conjugates [19, 20] but these recent studies revive this issue with strong supporting data.

Prostate Androgen Biosynthesis and Metabolism

BPH and prostate cancer are two separate diseases. The former originates in the transitional zone where outgrowth of the stromal and epithelial cells leads to obstruction of the urinary bladder and difficulty in voiding the urine. By contrast prostate cancer is a disease of the peripheral zone (outer prostate) and aggressive disease can metastasize into the adjacent lymph glands and bone. Both are dependent upon intra-tumoral conversion of T [$K_d = 10^{-9}$ M for the androgen receptor (AR)] to the higher-affinity ligand 5 α -DHT ($K_d = 10^{-11}$ M for AR) and can be treated by blocking 5 α -DHT synthesis. For this approach to be effective requires a detailed knowledge of androgen biosynthesis in both disease states with the understanding that there may be paracrine influences between epithelial and stromal cells.

The route to 5 α -DHT production in the prostate starting from circulating dihydroepiandrosterone (DHEA) is shown in Figure 3. Major progress in this area has led to the precise annotation of the enzyme isoforms involved in the process as follows: (i) DHEA is converted to Δ^4 -androstene-3,17-dione via 3 β -hydroxysteroid dehydrogenase/ketosteroid isomerase type 1 and type 2 (*HSD3B1* and *HSD3B2*) [21, 22]; (ii) Δ^4 -androstene-3,17-dione is reduced to T by 17 β -HSD type 5 (*AKR1C3*) [23–26]; (iii) T is reduced to 5 α -DHT by both 5 α -reductase type 1 and type 2 (*SRD5A1* and *SRD5A2*) where the type 2 enzyme plays the more dominant role [2, 27]; (iv) 5 α -DHT is reduced by 3 α -HSD type 3 (*AKR1C2*) to produce 3 α -diol and by 3(20 α)-HSD (*AKR1C1*) to produce 3 β -diol [9, 23, 28, 29]; and (v) 17 β -hydroxysteroids can be conjugated by glucuronidation by UGT2B15/17 [18, 30^{**}].

Several features are worthy of mention. First, the pathway from DHEA is emphasized since in the aging male the influence of adrenal androgens acting as precursors becomes pronounced. Second, AKR1C3 is the peripheral 17-ketosteroid reductase responsible for testosterone production. It has been shown to be expressed in prostate at the RNA, protein and functional level [24, 25, 31]. The enzyme is more highly expressed in epithelial cells than stromal cells and it is upregulated in prostate cancer [23]. By contrast although Leydig cell specific 17 β -HSD type 3 (*HSD17B3*) transcripts have been detected in the prostate its role in testosterone production in this tissue remains to be elucidated [32^{*}]. Third, the inability of finasteride to reduce prostatic volume by more than 30% and intraprostatic 5 α -DHT levels by greater than 90%, suggests that 5 α -DHT still forms in sufficient amounts to sustain prostate size [33]. Fourth, AKR1C2 is the dominant 3-ketosteroid reductase that will

convert 5 α -DHT ($K_d = 10^{-11}$ M AR) to 3 α -diol ($K_d = 10^{-6}$ M) and will prevent 5 α -DHT from binding to its receptor [9, 23, 29]. Fifth, the highly related AKR1C1 (98 % sequence identity) instead converts 5 α -DHT to 3 β -diol, where 3 β -diol is a pro-apoptotic ligand for estrogen receptor β [9, 34, 35].

Studies in castrate dogs [36], the tamar wallaby [37, 38], and humans [39] all support the back conversion of 3 α -diol to 5 α -DHT with growth consequences for the prostate. Five different short-chain dehydrogenase genes (*HSD17B10*, *HSD17B6*, *RDH5*, *DHRS9*, *RODH4*) have been invoked as being the oxidative 3 α -HSDs responsible for this back conversion. However, detailed kinetic analysis using mammalian expression systems and expression profiling have provided unequivocal evidence that the enzyme responsible is “RoDH like 3 α -HSD” (*HSD17B6*) [40]. Remarkably, *HSD17B6* is more highly expressed in stromal versus epithelial cells providing a paracrine influence on the later. These data beg the question as to the circulating source of 3 α -diol. Recently, a “backdoor pathway” to 5 α -DHT was proposed starting with adrenal steroidogenesis to generate progesterone [41, 42]. In this pathway progesterone is converted to 17 α -hydroxy-progesterone by CYP17 α -hydroxylase/17,20 lyase and then reduced by SRD5A1 to yield 5 α -pregnane-17 α -ol-3,20-dione. This is then reduced by AKR1C2 to yield 5 α -pregnane-3 α ,17 α -diol-20-one. Subsequent reaction by CYP17 α -hydroxylase/17,20-lyase would produce androsterone which is then reduced by AKR1C3 to 3 α -diol. Thus a new molecular switch that controls ligand access to the AR has been identified as AKR1C2 which reduces 5 α -DHT to 3 α -diol (in epithelial cells) and HSD17B6 which oxidizes 3 α -diol back to 5 α -DHT (in stromal cells) [43, 44**].

Targeting the Androgen Axes in Prostate Disease

Finasteride was originally used to treat BPH but despite the beneficial affects on reducing prostatic volume and intraprostatic 5 α -DHT levels symptomatic relief was disappointing [33]. This led to the concept that dutasteride a dual SRD5A1 and SRD5A2 inhibitor would provide greater benefit [45*]. In aside by side comparison of finasteride and dutasteride symptomatic relief seems comparable [33]. While compounds such as terazosin and tamsulosin (α -adrenergic receptor antagonists) may be superior compounds to increase urine flow [46] they do not halt the natural progression of the disease that can be achieved with 5 α -reductase inhibitors.

Androgen ablative therapy is a major treatment for prostate cancer. The disease can be treated by surgical castration and prostatectomy or by the combined use of a LH-RH agonist (leuprolide) and an AR antagonist (bicalutamide/flutamide). Leuprolide blocks LH production at the level of the anterior pituitary and reduces circulating T levels to those seen in the castrate male (<0.2 ng/mL) [47]. By contrast bicalutamide prevents binding of 5 α -DHT to the AR with an $EC_{50} = 160$ nM [48*]. Dual inhibition of both SRD5A1 and SRD5A2 with dutasteride may also be effective in preventing or delaying the growth of prostate cancer and is the focus of the ongoing 4-yr REDuction by DUtasteride of prostate Cancer Events (REDUCE). Irrespective of the treatment regimen, in about 30–40% of cases the cancer can re-emerge (2–3 yr later) with a tell-tale increase in prostatic specific antigen (PSA). This recurrent cancer has been described as Castrate Resistant Prostate Cancer (CRPC). Consensus has built that CRPC is not androgen-independent and that both adaptive responses in AR signaling [49–51] and increased dependence on intra-tumoral androgen biosynthesis [32**, 52*, 53] may drive the disease and by-pass the form of castration used. Recently, a phase I/II clinical trial of abiraterone acetate (a CYP17 α -hydroxylase/17,20-lyase inhibitor) in advanced CRPC was shown to reduce PSA levels and bone metastases in a dramatic fashion [54**, 55**]. These studies added weight to the importance of intra-tumoral formation of androgens [54**]. Abiraterone acetate would not only block the adrenal

production of DHEA but also *de novo* synthesis of DHEA in the prostate and together these mechanisms would prevent the formation of T and 5 α -DHT in the tumor.

Evidence for the importance of intra-tumoral formation of androgens in CRPC has come from studies in patients and xenograft models. Affymetrix microarray analysis and confirmatory real-time PCR in both primary prostate tumors and “androgen independent prostate cancer” showed the following fold increases in gene expression in the latter disease *AR* (5.8) *HSD3B2* (1.8); *AKR1C3* (5.3); *SRD5A1* (2.1); *SRD5A2* (0.54); *AKR1C2* (3.4 x); *AKR1C1* (3.1 x) and *UGT15* (3.5x). The increase in *AKR1C3* expression was also confirmed by immunohistochemistry [53]. In a subsequent study, levels of testosterone and 5 α -DHT were measured in prostate cancer and CRPC using stable-isotope dilution LC-MS and this was related to transcript level. Interestingly, prostate cancer gave 0.23 ng/g T and 2.75 ng/g 5 α -DHT; but in CRPC the levels were reversed to 0.74 ng/g T and 0.25 ng/5 α -DHT. These changes were reflected by increases in the transcripts for *CYP17A1*, *HSD3B1*, *AKR1C3* and decreases in *SRD5A2* [32**]. Taken together there seems to be an adaptive response for CRPC to synthesize their own androgens and that there may be a shift towards the production of T over 5 α -DHT.

The dependence of CRPC on intra-tumoral androgens has also been modeled in LNCaP (AR positive cancer cell line) xenografts in castrate immunodeficient mice [52, 56**, 57**]. A caveat of this model is that *CYP17 α* -hydroxylase/lyase is not expressed in the murine adrenal gland and these tumors may be under additional selection pressure to produce their own androgens. Nevertheless *ex vivo* experiments on the recurrent tumors provided evidence of the conversion of [¹⁴C]-acetate into 5 α -DHT and the conversion of [³H]-progesterone into pregnane intermediates in the “backdoor” pathway to 5 α -DHT, Figure 4 [52, 56**]. These experiments have been recapitulated to show that T production is accompanied by increases in StaR (steroidogenic acute regulatory protein) and side-chain cleavage enzyme (*CYP11A1*) which suggest that *de novo* synthesis from cholesterol could be occurring [52, 58*]. In other studies on the LNCaP xenograft model a coordinated response to increase cholesterol influx and cholesterol biosynthesis was observed (including altered expression of LDL-r, SR-BI, HMGCoA reductase, ACT1,2 and ABCA1 proteins) [56*]. Thus CRPC can find away to produce its own androgens.

The dramatic response to abiraterone acetate suggests a role for targeting androgen biosynthesis in CRPC. Because this drug inhibits *CYP17 α* -hydroxylase/17,20-lyase there are concerns that this will have the unintended consequence to cause the accumulation of the mineralocorticoid desoxycorticosterone in the adrenal. This will be exacerbated by the lack of cortisol to cause feedback inhibition of ACTH at the level of the anterior pituitary. Mineralocorticoid excess is currently prevented by the co-administration of dexamethasone [55**]. The success of abiraterone acetate has led to the development of both steroidal and nonsteroidal inhibitors of this enzyme [59, 60**]. One such compound VN/124-1 not only inhibits the target enzyme but also increases the rate of AR degradation [60**]. An alternative attractive target for CRPC which is down stream from *CYP17* is *AKR1C3* which catalyzes the final step in prostate T biosynthesis. Compounds that target this enzyme would *not* have to be co-administered with dexamethasone. Indomethacin and indomethacin analogs that do not inhibit COX-1 and COX-2 are lead compounds in targeting *AKR1C3* [61*]. Importantly, these compounds do not inhibit the highly related *AKR1C1* and *AKR1C2*. All these *AKR1C* isoforms are inhibited by the N-phenylanthranilic acids including flufenamic acid [62]. Recently, flufenamic acid analogs were reported to be AR antagonists [63*]. Thus another strategy for the treatment of CRPC would be to develop flufenamic acid analogs that target *AKR1C3* and AR but leave *AKR1C1* and *AKR1C2* unaffected.

Conclusions

Reductive androgen metabolism in extragonadal tissues is now understood relative to the genes and enzyme isoforms involved. In liver a case can be made for reduction occurring after phase II conjugation. In castrate resistant prostate cancer adaptive local androgen biosynthesis may surmount androgen deprivation and the enzymes involved may be drug targets for advanced disease.

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Figure 1.

Reductive androgen metabolism in human liver. The reductive enzymes responsible for the metabolism of testosterone to its four stereoisomeric tetrahydrosteroids are shown (genes are italicized). The inset shows the preferred pathway of 5α-DHT metabolism with glucuronidation (a phase II reaction) preceding 3-ketosteroid reduction (a phase I reaction). AKR1C4 is shown preferentially reducing 5α-DHTG to 3α-diol-17-G since it has the highest catalytic efficiency for this reaction among the AKR1C isoforms: 5α-DHT = 5α-dihydrotestosterone; 3α-diol = 5α-androstane-3α,17β-diol; 3α-diol-3G = 3α-diol-3α-glucuronide; 5α-DHTG = 5α-DHT-17β-glucuronide; 3α-diol-17G = 3α-diol-17β-glucuronide.

Figure 2.

Structure and mechanism of human steroid 5 β -reductase (AKR1D1). (A) (α/β)₈-Barrel structure of an AKR1D1•NADP⁺ binary complex. NADP⁺ is in a stick presentation, a water molecule (red ball) is hydrogen bonded to Y58 and E120 of the catalytic tetrad which also contains (D53 and K87); and the three loops A, B and C which comprise the steroid binding site are in blue. (B) Catalytic mechanism of steroid double bond reduction.

Figure 3.

Androgen biosynthesis in the prostate. Traditional pathway converting DHEA to 5 α -DHT is shown in blue. Arrows and genes in blue show altered expression in castrate resistant prostate cancer (CRPC). Arrows and genes in red show the molecular switch that regulates ligand access to the AR. AKR1C2 is also overexpressed in CRPC. Arrows in grey show *de novo* routes to DHEA and Δ^4 -androstene-3,17-dione that may contribute to adaptive androgen biosynthesis in CRPC. Open orange arrows show the backdoor pathway to 5 α -DHT.