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5α-reduction of epitestosterone is catalysed by human SRD5A1 and SRD5A2 and increases androgen receptor transactivation

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ABSTRACT

Epitestosterone is a stereoisomer of the active androgen testosterone and its circulating concentrations are similar to those of testosterone in women and children. However, its biological function and pathways of metabolism remain unknown. The structural similarity to testosterone suggests a potential function in the modulation of androgen receptor signalling. It is well established that the conversion of testosterone to 5α-dihydrotestosterone enhances local androgen receptor signalling. In this study, we show that epitestosterone is metabolized to 5α-dihydroepitestosterone by both human steroid 5α-reductase isoforms, SRD5A1 and SRD5A2. Using two different variations of a reporter assay for transactivation of the human androgen receptor, we show that epitestosterone is a partial AR agonist and that the 5α-reduction of epitestosterone increases its androgenic activity. In line with this, we show that 5α-reduction of epitestosterone reduces its ability to antagonize 5α-dihydrotestosterone-induced androgen receptor transactivation. In conclusion, we provide evidence that steroid 5α-reductases regulate the modulatory effect of epitestosterone on androgen receptor signalling.

1. Introduction

Testosterone (T) is the most abundant active human androgen in circulation and exerts its androgenic effects by binding and activating the androgen receptor (AR), a ligand-activated nuclear receptor. The androgen signal is further amplified at the pre-receptor level in peripheral tissues expressing either of the two human steroid 5α-reductases, SRD5A1 and SRD5A2, which 5α-reduce T to the most potent human androgen, 5α-dihydrotestosterone (5α-DHT) [1–3]. Indeed, the 5α-reduction of the steroid A-ring allows for maximum transactivation upon binding of the AR [4–6]. The two 5α-reductase isoforms are however characterised by differential substrate preferences and enzyme kinetics and show tissue-specific expression [1, 7, 8]. As opposed to steroid 5α-reduction, 5β-reduction of the steroid A-ring irreversibly abolishes the capability of a steroid to signal via the AR and is exclusively catalysed in the liver by the enzyme aldo-keto reductase 1D1 (AKR1D1) [9–11].

Epitestosterone (EpiT, 17α-hydroxytestosterone, 4-androsten-17α-ol-3-one) is a stereoisomer of T. While circulating EpiT levels in adult men are approximately 10-fold lower than those of T, EpiT circulates at levels similar to that of T in both women and pre-pubertal children [12, 13]. However, despite this quantitative relevance of circulating EpiT in women and children, the biological function and metabolism of EpiT remain largely unexplored. EpiT has been shown to act as a partial AR agonist in a yeast-based promoter reporter assays [14]. Conversely, EpiT has been shown to bind and antagonise AR in rat prostate [15,16]. Notably, EpiT has also been shown to inhibit the generation of 5α-DHT from its precursor T in rat prostate *in vitro* [15,16]. This has led others to hypothesise that EpiT might function as a natural anti-androgen at the level of the AR as well as the pre-receptor level by interfering with the 5α-reduction of T to 5α-DHT [17]. However, the major urinary metabolites of EpiT, 5α- and 5β-androstane-3α,17α-diol, are 5α- and

Abbreviations: 5α-DHT, 5α-dihydrotestosterone; 5α-EpiDHT, 5α-dihydroepitestosterone; 5β-DHT, 5β-dihydrotestosterone; 5β-EpiDHT, 5β-dihydroepitestosterone; AKR1D1, aldo-keto reductase 1D1; AR, androgen receptor; EpiT, Epitestosterone; SRD5A1, steroid 5α-reductase type 1; SRD5A2, steroid 5α-reductase type 2; T, Testosterone; UHPSFC-MS/MS, ultra-high performance supercritical fluid chromatography-tandem mass spectrometry.

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5β-reduced, respectively, with 2–3-fold higher excretion of the 5β-reduced metabolite [18,19], suggestive of active peripheral A-ring reduction of EpiT. 5α-reduction of EpiT has been described *in vitro* for human scalp hair roots [20] and rat liver [21], but the isoform responsible for the observed reaction has not been identified. Fig. 1 highlights the stereochemistry of 5α- and 5β-reduction of T and EpiT.

We hypothesised that the 5α-reduction of EpiT changes its effects on the AR, thereby contributing to the pre-receptor regulation of AR transactivation. The aims of this study were therefore: [1] to investigate the capability of SRD5A1 and SRD5A2 to convert EpiT to a 5α-reduced metabolite (5α-dihydroepitestosterone, 5α-EpiDHT) and [2] to characterise the effect of 5α-reduction on the biocharacter, potency and efficacy of EpiT via the human AR.

2. Materials and methods

2.1. Steroids

Epitestosterone (EpiT, 17α-hydroxytestosterone, 4-androsten-17α-ol-3-one), 5α-dihydroepitestosterone (5α-EpiDHT, 5α-androstan-17α-ol-3-one), 5β-dihydroepitestosterone (5β-EpiDHT, 5β-androstan-17α-ol-3-one) and 5β-dihydrotestosterone (5β-DHT, 5β-androsten-17β-ol-3-one) were purchased from Steraloids. Testosterone (T, 4-androsten-17β-ol-3-one), 5α-dihydrotestosterone (5α-DHT, 5α-androsten-17β-ol-3-one), testosterone-D3 (4-androsten-17β-ol-3-one-16,16,17-D3) and enzalutamide were purchased from Sigma Aldrich. Steroid stocks were prepared at 1 mg/mL in methanol and stored at −80 °C.

2.2. Plasmids

Human SRD5A1 and SRD5A2 in pCMV7 were a generous gift by Prof David W. Russell (UT Southwestern Medical Centre, Dallas, USA). pCMV-XL4 with human AKR1D1 and pcDNA3 with the human AR (pcDNA-AR) were gifted by Prof Jeremy W Tomlinson (University of Oxford, UK). pTAT-GRE-E1b-luc was kindly provided Dr Guido Jenster (Erasmus University of Rotterdam, The Netherlands). pCMV8 was purchased from the American Type Culture Collection ATCC.

2.3. Cell culture

HEK293 were purchased from American Type Culture Collection and were cultured in Minimum Essential Medium Eagle with Earle's salts supplemented with 10% FBS, 100 U/mL penicillin and 0.1 mg/mL streptomycin at 37 °C, 90% humidity and 5% CO_2.

2.4. Steroid conversion assay in HEK293

HEK293 cells were grown in 75-cm^2 flasks. At approximately 80% confluency cells were transfected with 23 μg of the respective plasmid encoding SRD5A1, SRD5A2 or AKR1D1 using FuGENE® HD transfection reagent (Promega). 24 hours after transfection cells were plated in 48-well plates at 5×10^4 cells/well in 250 μL culture medium. After 24 hours, the culture medium was removed and replaced by 250 μL of serum-free Minimum Essential Medium Eagle containing T or EpiT at a concentration of 1 μM. The final methanol concentration in the medium was 0.0288% (vol/vol). Medium samples were collected at different times after substrate addition and were frozen at −20 °C until analysis. Cells of the corresponding wells were lysed in 25 μL passive lysis buffer per well and the total protein content of the cell lysate was determined using the DC Protein Assay (Bio-Rad). Experiments were performed in three independent biological replicates with three technical replicates each.

2.5. Steroid quantification by ultra-high performance supercritical fluid chromatography-tandem mass spectrometry (UHPSFC-MS/MS)

UHPSFC-MS/MS is an emerging technology for steroid analysis with high chromatographic resolution and throughput [22,23]. 200 μL sample or calibrator were mixed with 60 ng of testosterone-D3. Samples were extracted with 2 mL of tert-butyl methyl ether, mixed at 1500 rpm for 10 minutes and incubated at room temperature for 30 minutes. The organic phase was dried under nitrogen and reconstituted in 125 μL acetonitrile. Steroid analytes were separated using an ACQUITY UPC^2 System (Waters) with a Waters Torus 1-AA column (3 mm x 100 mm, 1.7μm particle size) at 60 °C. The injection volume was 2 μL. Supercritical CO_2 was used as primary mobile phase and methanol as modifier. A linear gradient with modifier increasing from 10% to 20% over 3 minutes was used for the separation of the analytes followed by one minute at 10% for re-equilibration. The flow rate was 1.2 mL/min and the back pressure was kept stable 1500 psi. The eluate was mixed with 1% (vol/vol) formic acid in methanol coming from an isocratic solvent pump at 0.15 mL/min. Steroid quantification was performed on a TQ-MS triple quadrupole mass spectrometer (Waters) using electrospray ionisation in positive mode. The capillary voltage was 3.8 kV, desolvation gas temperature was 600 °C, desolvation gas flow was 1000 L/h and the source temperature was 150 °C. Multiple reaction monitoring

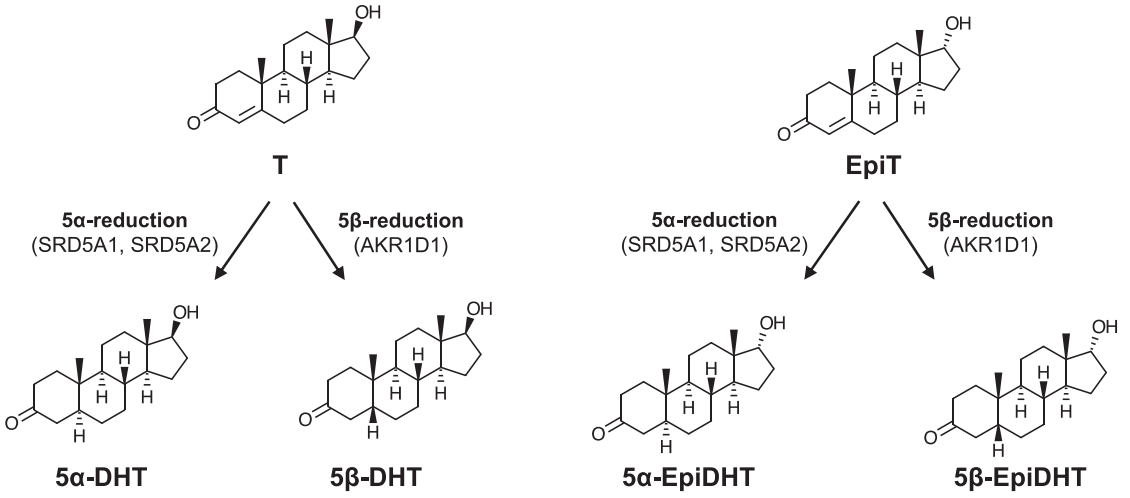


Fig. 1. Structures of testosterone (T) and epitestosterone (EpiT) and their 5α- and 5β-reduced metabolites generated by steroid 5α-reductases type 1 and 2 (SRD5A1, SRD5A2) and aldo-keto reductase 1D1 (AKR1D1). 5α-DHT, 5α-dihydrotestosterone; 5β-DHT, 5β-dihydrotestosterone; 5α-EpiDHT, 5α-dihydroepitestosterone; 5β-EpiDHT, 5β-dihydroepitestosterone.

was used (Table 1). Peak area ratios of analyte and internal standard were plotted against the nominal concentrations of the calibrators and 1/x weighting and linear least square regression were used to produce the calibration curve for quantification.

2.6. Luciferase reporter assay for androgen receptor transactivation

Androgen receptor transactivation was studied in a luciferase reporter assay similar to previously described assays [4,24]. HEK293 were cultured in 75-cm^2 flasks. At approximately 80% confluency cells were co-transfected with 19.2 μg of pTAT-GRE-E1b-luc (containing a glucocorticoid receptor response element, which is also recognised by the AR, and the luciferase reporter under the control of the Eb1 promoter) and 3.8 μg of pcDNA3 with AR using FuGENE® HD transfection reagent (Promega). Twenty-four hours after transfection, cells were plated in 24-well plates at 10^5 cells/well in 500 μL culture medium. After 24 hours the culture medium was removed and replaced by serum-free Minimum Essential Medium Eagle containing increasing concentrations of T, 5α-DHT, EpiT or 5α-EpiDHT (agonist mode) or containing 0.5 nM 5α-DHT and increasing concentrations of EpiT, 5α-EpiDHT or the AR antagonist enzalutamide (antagonist mode). Final methanol concentrations were adjusted across all treatments of an experiment. After 24 hours the medium was removed and cells lysed in passive lysis buffer. Luciferase activity was determined using the Luciferase Assay System (Promega). The results for each well were normalised to total protein content of the lysate as measured using the DC Protein Assay (Bio-Rad). For the agonist assay, normalised luciferase activity is presented relative to the luciferase activity induced by 10^{-6} M (1 μM) 5α-DHT. For the antagonist assay, normalised luciferase activity is presented relative to the luciferase activity induced by 0.5 nM 5α-DHT in the absence of any antagonists. Experiments were performed in three independent biological replicates with four technical replicates each. The data was analysed in GraphPad Prism. A sigmoidal function was used to fit the experimental data of the dose-response experiments. Results of different treatments were compared using a paired, two-tailed t-test. A p-value $\leq$ 0.05 was considered statistically significant.

2.7. Sequential 5α-reduction of T and EpiT and luciferase reporter assay

HEK293 were plated in 12-well plates at 2×10^5 cells/well in 1 mL of growth medium. After 24 hours cells were co-transfected with 0.5 μg SRD5A1 and 0.5 μg SRD5A2 in pCMV7 per well using FuGENE® HD. After another 24 hours the culture medium was removed and replaced by 1 mL/well of serum-free Minimum Essential Medium Eagle containing 1 μM T or EpiT. 1 μM T and EpiT were added to non-transfected cells as negative controls. Medium with vehicle only (without steroids) was added to HEK293 cells to generate conditioned medium without any AR agonists. After 24 hours a fraction of the medium was collected and stored at −20 °C for UHPSFC-MS/MS analysis. 500 μL of the medium was transferred into the wells of a 24-well plate containing HEK293 cells co-transfected with pTAT-GRE-E1b-luc and pcDNA3-AR (or pTAT-GRE-E1b-luc and empty pCMV8) as described in 2.6. As controls, transferred plain conditioned medium (without T or EpiT) was spiked with 1 μM of the test analytes from stock solutions of their standards. For antagonist experiments all wells were spiked with 0.5 nM 5α-DHT after the transfer of the conditioned medium and enzalutamide was used as positive control. After 24 hours cell lysis, luciferase assay and protein quantification were performed as described in 2.6. Luciferase activity was normalised to total protein of the respective well. For the agonist assay, normalised luciferase activity is presented relative to the luciferase activity induced by 1 μM 5α-DHT spiked from a standard solution into conditioned medium. For the antagonist assay, normalised luciferase activity is presented relative to the luciferase activity induced by 0.5 nM 5α-DHT spiked from a standard solution into conditioned medium. Experiments were performed in three independent biological replicates with four technical replicates each. Results of different treatments were compared using a paired, two-tailed t-test. A p-value $\leq$ 0.05 was considered statistically significant.

3. Results and Discussion

3.1. EpiT is 5α-reduced by SDR5A1 and SRD5A2

In order to compare the activity of steroid A-ring reductases in converting EpiT and their well-established substrate T, we incubated HEK293 cells transiently overexpressing SRD5A1, SRD5A2 or AKR1D1 with T or EpiT and analysed the conversion of the substrates to their respective 5α-reduced (SRD5A1 and SRD5A2) and 5β-reduced (AKR1D1) metabolites over time using UHPSFC-MS/MS. We used a substrate concentration of 1 μM for the conversion assay, which is in the range of the K_m values of SRD5A1, SRD5A2 and AKR1D1 for T and, hence, below a concentration that saturates enzyme activity [8, 25–27]. HEK293 cells were used as they do not possess any endogenous steroidogenic or steroid-metabolizing activity [28,29]. After 24 hours, SRD5A1 and SRD5A2 had completely converted both T and EpiT to their respective 5α-reduced products (Fig. 2A and B). Therefore, we show for the first time that EpiT is as a substrate for both steroid 5α-reductases, which likely explains the previously reported effect of EpiT as competitive inhibitor of the 5α-reduction of T [15,16]. Moreover, the comparable rates of 5α-reduction of EpiT and T by both SRD5A isozymes

Table 1
Multiple reaction monitoring for T, EpiT and their metabolites. RT, retention time.

Analyte	RT (min)	Molecular ion (*m/z*)	Cone voltage (V)	Quantifier ion (*m/z*) Collision energy (eV)	Qualifier ion (*m/z*) Collision energy (eV)
T	3.22	289.4	32	97.77 24	109.75 26
EpiT	2.45	289.4	32	97.77 20	109.75 26
5α-DHT	2.76	291.4	28	255.35 16	159.54 24
5α-EpiDHT	2.04	291.16	25	255.2 15	273.2 10
5β-DHT	2.25	291.35	24	255.28 12	273.22 10
5β-EpiDHT	1.73	273.14	25	255.2 15	159.4 20
T-d3	3.22	292.1	30	96.9 20	

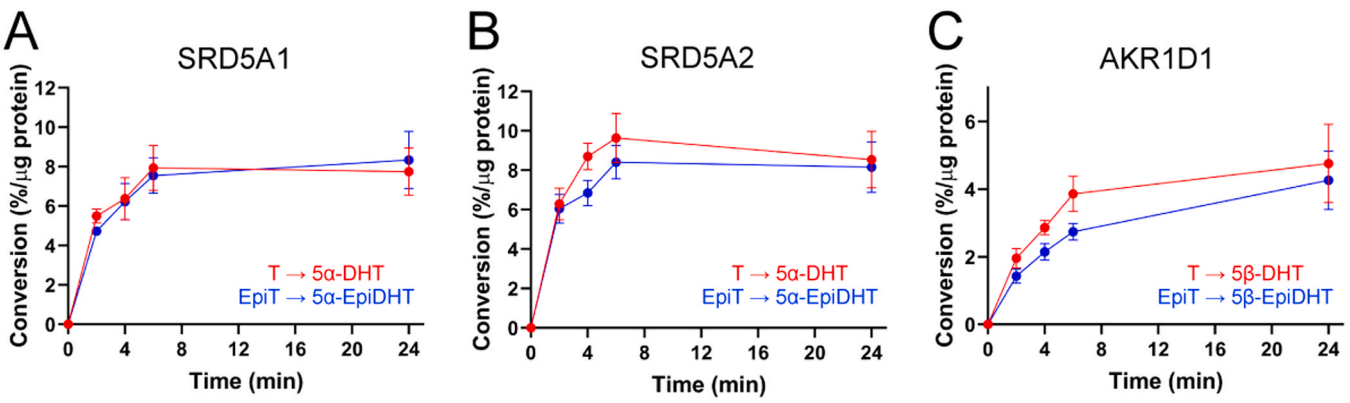


Fig. 2. Time-dependent conversion of T and EpiT by steroid A-ring reductases. SRD5A1 (A), SRD5A2 (B), AKR1D1 (C) were transiently overexpressed in HEK293 cells and incubated with 1 µM T or EpiT. Substrate conversion was quantified by UHPSFC-MS/MS and normalised to total protein content of the reaction. Data represent mean ± SEM of three independent, biological replicates.

suggests that like T [1,3] EpiT can be efficiently 5α-reduced in peripheral tissues with 5α-reductase expression.

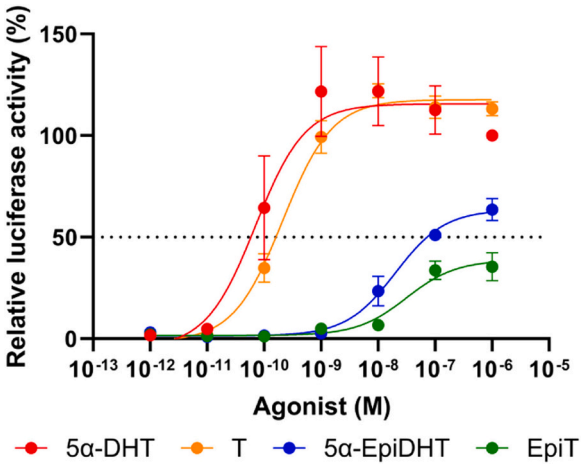
Like T, EpiT was also 5β-reduced by AKR1D1 yielding 5β-dihydroepitestosterone (5β-EpiDHT), though the rate of conversion tended to be slower for EpiT compared to T (Fig. 2**C**). While this difference was not statistically significant in our study, it is consistent with results from studies using recombinant purified AKR1D1 that reported a lower catalytic efficiency for EpiT compared to T [11,25].

3.2. 5α-EpiDHT is a more efficacious AR agonist than EpiT

EpiT has previously been described as a partial AR agonist [14]. Using a luciferase reporter assay we investigated if 5α-EpiDHT shows increased activity via AR. 5β-reduced metabolites were not investigated, as 5β-reduction abolishes the capability to induce AR signalling by introducing a 90° bend at the A/B ring junction of the steran scaffold [9]. We show that both EpiT and 5α-EpiDHT are partial AR agonists; however, the relative efficacy of 5α-EpiDHT (63.5%) is 1.6-fold higher than the efficacy of EpiT (38.7%) (p=0.0364) (Fig. 3). The EC_{50} value determined for 5α-EpiDHT (20.2 nM) was 1.6-fold lower than that for EpiT (31.5 nM) suggesting that 5α-EpiDHT is more potent than EpiT; however, this difference was not statistically significant (Fig. 3). As expected, T and 5α-DHT had similar relative efficacy (117.7% for T, 115.6% for 5α-DHT), but 5α-DHT was substantially more potent that T (EC_{50} 0.07 vs 0.212 nM), with EC_{50} values comparable to previously reported results from mammalian cell line-based reporter assays [30].

3.3. 5α-reduction of EpiT reduces its activity as AR antagonist

Next, we investigated if EpiT and 5α-EpiDHT differentially antagonised the 5α-DHT-induced AR transactivation, as partial agonists are expected to reduce the response induced by full agonists. We performed dose-response experiments to investigate the effect of EpiT and 5α-EpiDHT on 5α-DHT-induced AR transactivation (Fig. 4). We selected a 5α-DHT concentration of 0.5 nM for these assays as this concentration is a similar order of magnitude to the EC_{50} determined in the agonist assay (Fig. 3). Both EpiT and 5α-EpiDHT partially antagonised the transactivation of DHT as expected for partial agonists. While the maximal inhibition tended to be higher for EpiT than 5α-EpiDHT (56.3 vs 43.6%, Fig. 4), this difference was not statistically significant. Similarly, no difference in the IC_{50} values were observed for 5α-EpiDHT (0.15 µM) and EpiT (0.20 µM). In comparison, the potent AR antagonist enzalutamide effectively inhibited 5α-DHT-induced AR transactivation confirming the validity of the assay (Fig. 4).

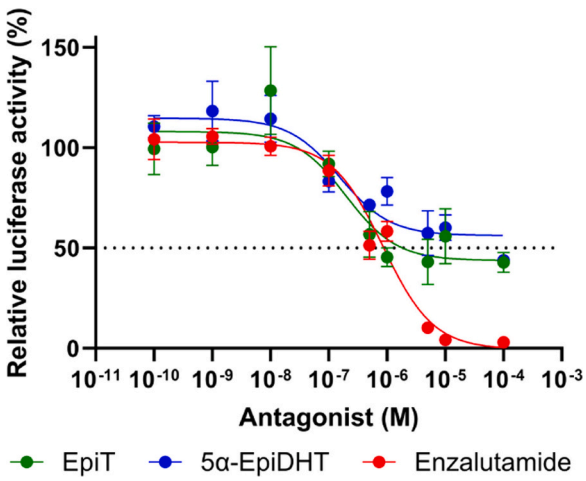


Ligand	EC_{50} (M) (95% CI)	Maximal activity (%) (95% CI)	R^2
5α-DHT	7.05×10^{-11} (2.20×10^{-11} - 2.13×10^{-10})	115.6 (99.9 - 131.5)	0.82
T	2.12×10^{-10} (1.43×10^{-10} - 3.18×10^{-10})	117.7 (111.9 - 123.6)	0.98
5α-EpiDHT	2.02×10^{-8} (1.15×10^{-8} - 3.69×10^{-8})	63.5 (57.3 - 70.4)	0.96
EpiT	3.15×10^{-8} (1.37×10^{-8} - 7.13×10^{-8})	38.7 (32.3 - 45.9)	0.88

Fig. 3. Transactivation of the human AR by T, 5α-DHT, EpiT and 5α-EpiDHT. Luciferase activity is presented relative to the luciferase activity induced by 10^{-6} M (1 µM) DHT (100%). Mean values ± SEM of three independent, biological replicates are shown. EC_{50} and maximal activity with 95% confidence intervals and R^2 values of the fit are summarised below. HEK293 cells were transformed with pTAT-GRE-E1b-luc containing a response element-promoter-luciferase construct and pcDNA3-AR and were exposed to increasing concentrations of the test compounds before determining luciferase activity.

3.4. 5α-reduction of EpiT increases its activity as a partial AR agonist, while reducing its antagonistic properties

To mimic the direct effect of 5α-reduction on AR transactivation, we performed sequential experiments where EpiT and T were 5α-reduced in HEK293 cells transiently co-transfected with SRD5A1 and SRD5A2 and the conditioned medium was transferred to HEK293 cells transiently transfected with the AR and promoter reporter construct to assess the effect of 5α-reduction on activity via the AR (Fig. 4A). UHPSFC-MS/MS analysis confirmed that co-transfection with SRD5A1 and SRD5A2 led to the formation of the 5α-reduced products of the respective substrates,



Ligand	IC_{50} (M) (95% CI)	Maximal inhibition (%) (95% CI)	R^2
EpiT	1.99×10^{-7} (6.05×10^{-8} - 6.27×10^{-7})	56.3 (43.8 - 69.6)	0.72
5α-EpiDHT	1.53×10^{-7} (2.97×10^{-8} - 1.55×10^{-6})	43.6 (32.9 - 59.9)	0.76
Enzalutamide	8.43×10^{-7} (5.06×10^{-7} - 1.39×10^{-6})	100.5 (91.1 - 110.4)	0.95

Fig. 4. Antagonism of DHT-induced AR transactivation by EpiT and 5α-EpiDHT in a luciferase reporter assay. Experiments were performed in the presence of 0.5 nM 5α-DHT and luciferase activity is presented relative to the luciferase activity induced by 0.5 nM 5α-DHT in the absence of any antagonists (100%). Mean values ± SEM of three independent, biological replicates are shown. IC_{50}, maximum inhibition and R^2 values of the fit are summarised below. HEK293 cells were transformed with pTAT-GRE-E1b-luc containing a response element-promoter-luciferase construct and pcDNA3-AR and were exposed to increasing concentrations of the test compounds before determining luciferase activity.

while no substrate conversion was observed for non-transfected control cells (not shown). Conditioned medium from cells co-transfected with SRD5A1 and SRD5A2 and incubated with EpiT tended (p=0.0645) towards eliciting a higher relative AR transactivation response (75.0% ± 6.1) than conditioned medium from control cells incubated with EpiT (47.2% ± 5.1) (Fig. 4B), further confirming that 5α-reduction of EpiT increases the efficacy of transactivation via the AR (Fig. 3). No difference in transactivation was observed for conditioned medium containing T or 5α-DHT. This result was expected, given that T and 5α-DHT are equally efficacious (Fig. 3) and that saturating concentrations were used in this assay (1 μM). None of the conditioned media elicited a response in cells co-transfected with an empty plasmid (no AR) and the promoter reporter construct confirming that the observed effects are AR-dependent.

We next used the same sequential set of experiments to directly integrate the 5α-reduction of EpiT by SRD5A1 and SRD5A2 and its effect on 5α-DHT-induced AR transactivation. Conditioned medium was spiked with 0.5 nM DHT when transferred to the reporter cells (Fig. 4B). Conditioned media from control cells containing EpiT or blank conditioned media spiked with EpiT resulted in similar levels of inhibition (45.9 ± 3.1% vs 37.8 ± 4.5%), confirming the notion that EpiT may function as a partial AR agonist and AR antagonist. However, conditioned media containing 5α-EpiDHT (obtained from the 5α-reduction of EpiT in SRD5A1 and SRD5A2 expressing cells) yielded only 10.9 ± 4.6% inhibition confirming that the 5α-reduction of EpiT reduces its effectiveness as an AR antagonist (p=0.0308, Fig. 4C).

Taken together, using two variations of a reporter assay for AR transactivation, we show that while EpiT and 5α-EpiDHT are both partial AR agonists, 5α-EpiDHT is more efficacious than its precursor EpiT and that EpiT, but not 5α-EpiDHT has the potential to antagonise the AR. Fig 5

4. Conclusion

Our results show that the 5α-reduction of EpiT by both SRD5A1 and SRD5A2 yields 5α-EpiDHT and that 5α-EpiDHT is a more efficacious partial AR agonist than its precursor EpiT. As expected, this increased agonist activity is accompanied by a concomitant decrease in antagonistic properties. Hence, 5α-reduction modulates the biocharacter of EpiT at the AR. Our results are consistent with the hypothesis that EpiT as a partial AR agonist and has the potential to function as an anti-androgen *in vivo* [15–17]. However, we extend this concept by the

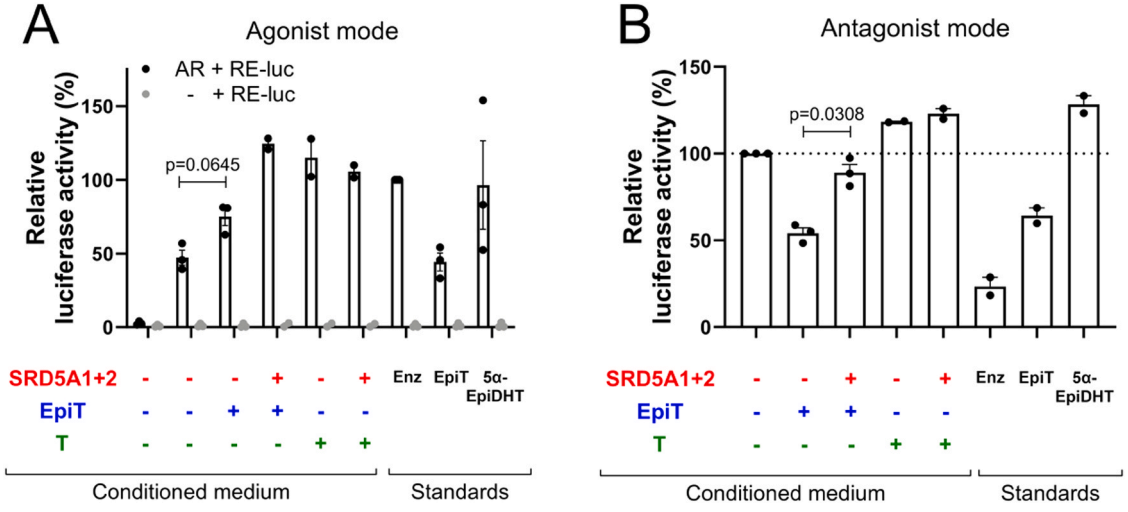


Fig. 5. Effect of the 5α-reduction of EpiT and T on AR transactivation in agonist (A) and antagonist mode (B). HEK293 cells co-transfected with pCMV7_SRD5A1 and pCMV7_SRD5A2 or control cells were incubated with 1 μM EpiT, T or vehicle (-) for 24 hours. The conditioned medium was subsequently transferred to cells co-transfected with pTAT-GRE-E1b-luc (RE-luc) and pcDNA3_AR (AR) or an empty plasmid (-), which were lysed for the determination of luciferase activity 24 hours later (A). Incubations with dilutions of commercial standards in plain conditioned medium were performed in parallel as controls. For antagonist mode (B) the conditioned medium was spiked with 0.5 nM 5α-DHT. Data represent mean ± SEM of three independent biological replicates with four technical replicates each. For the agonist assay (A), luciferase activity is presented relative to the luciferase activity induced by 1 μM 5α-DHT spiked from a standard solution into conditioned medium (100%). For the antagonist assay (B), luciferase activity is presented relative to the luciferase activity induced by 0.5 nM 5α-DHT spiked from a standard solution into conditioned medium (100%). A paired, two-tailed t-test was applied.

finding that steroid 5α-reductases not only increase the potency of the full agonist DHT through its generation from T but that they also increase the efficacy of the partial agonist EpiT through the generation of 5α-EpiDHT. The biological significance of this finding for AR action in men may be challenged by the significantly lower circulating levels of EpiT relative to T [12,13] and the potencies of EpiT and 5α-EpiDHT for AR agonism and antagonism (EC_{50} and IC_{50}) that are significantly lower than the respective potencies of T and 5α-DHT. However, in women, EpiT and T circulate at similar levels [13]. Therefore, we propose the 5α-reduction of EpiT to be a tissue-specific modifier of AR activity in women. In addition, the biological activity of EpiT may differ in tissue-specific fashion as steroid concentrations at the tissue level can vary significantly due to tissue- and cell-specific metabolism [3,31]. Future detailed *ex vivo* and *in vivo* experiments will, however, be required to confirm a biological relevance. In conclusion, steroid 5α-reductases play a complex role in the pre-receptor regulation of AR signalling by not only generating the most potent full AR agonist 5α-DHT from its precursor T, but additionally by modulating the biocharacter of partial AR agonists EpiT and 5α-EpiDHT.

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CRediT authorship contribution statement

Wiebke Arlt: Writing – review & editing, Funding acquisition. **Lina Schiffer:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Karl-Heinz Storbeck:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

No competing interests.

Data availability

Data will be made available on request.

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