Differential regulation of rat testicular 5α-reductase type 1 and 2 isoforms by testosterone and FSH

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Abstract

Testosterone is metabolised to the more potent androgen, dihydrotestosterone, by the 5α-reductase (5αR) enzyme. We previously showed that 5α-reduced androgens are important for maintaining androgen action on rat spermatogenesis when testicular testosterone concentrations are reduced. This study investigated expression and activity of the 5αR isoforms, type 1 (5αR-1) and type 2 (5αR-2), in the rat during hormone manipulation in order to understand the factors that regulate the testicular concentration of 5αR and testicular 5α-reduced androgen biosynthesis. Testicular 5αR-1 and 5αR-2 mRNA and enzyme activity were measured by real-time PCR and specific enzyme assays respectively. Hormone levels were first suppressed using two models of gonadotrophin suppression: testosterone and oestriadiol treatment (LH/testosterone deficiency) or GnRH immunisation (LH/testosterone and FSH deficiency). Hormones were then either restored or suppressed for 6 days by a variety of hormonal treatments. 5αR-1 mRNA and enzyme activity increased when testosterone was suppressed, yet restoration of testosterone decreased 5αR-1 mRNA and enzyme activity, suggesting that testosterone negatively regulates 5αR-1. Suppression of FSH decreased 5αR-1 mRNA yet FSH administration increased 5αR-1 mRNA, but no changes in 5αR-1 activity were observed within the 6 day period. In contrast to 5αR-1, testosterone did not affect the testicular concentration of 5αR-2 mRNA or activity, but there was evidence for modulation of 5αR-2 activity by FSH. Measurement of testicular androgens revealed that 5αR-1 was primarily responsible for the production of 5α-reduced metabolites. It is concluded that the 5αR isoforms in rats testis are differentially regulated by testosterone and FSH: testosterone negatively regulated 5αR-1 mRNA and enzyme activity but had no effect on 5αR-2, whereas FSH positively regulated 5αR-1 mRNA and appeared to regulate 5αR-2.

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Introduction

Testosterone is the predominant androgen involved in the regulation of normal spermatogenesis in the rat due to its high intratesticular concentration (Wright & Frankel 1979). The process of spermatogenesis is critically dependent on testosterone and the amount of sperm produced is dose-responsive to the level of testosterone in the testis (Awoniyi et al. 1989, McLachlan et al. 1994). The 5α-reduction of testosterone to the more potent androgen, 5α-dihydrotestosterone (DHT), is postulated to be important for maintaining spermatogenesis when intratesticular testosterone concentrations are low (Anderson et al. 1996, O’Donnell et al. 1996, 1999).

Testosterone has been the target for suppressing spermatogenesis in numerous contraceptive trials. In both primates (Narula et al. 2002) and men (McLachlan et al. 2002), gonadotrophin suppression by exogenous testosterone administration caused a marked reduction in testicular testosterone levels yet maintenance of 5α-reduced androgen levels. It is thought that maintenance of testicular 5α-reduced androgen levels may be responsible for continued low levels of sperm production in the ~30% of normal men whose sperm counts do not fall to zero (azoospermia) after testosterone contraceptive treatment (Anderson et al. 1996, 1997). In the rat model of spermatogenic suppression, administration of a 5α-reductase (5αR) inhibitor to prevent DHT production suppressed spermatogenesis (O’Donnell et al. 1996, 1999). Furthermore, administration of the androgen receptor (AR) antagonist flutamide during spermatogenic suppression in the rat increased the production of 5α-reduced metabolites in the testis, suggesting that 5αR may be hormonally regulated (O’Donnell et al. 1999). Taken together, these studies suggest that testicular 5αR may be regulated by gonadotrophins and/or testosterone and that 5αR activity is important for determining the amount of bioactive androgen in the testis.
Two isoforms of 5αR, type 1 (5αR-1) and type 2 (5αR-2), have been cloned in rats and humans (Andersson & Russell 1990, Normington & Russell 1992). These isoforms are products of different genes and possess different biochemical properties: 5αR-1 has a micromolar affinity for steroid substrates with peak activity extending over a broad neutral pH range, whereas 5αR-2 has a nanomolar affinity for steroid substrates with peak activity at pH 5·0 (Andersson & Russell 1990). 5αR isoforms have not been extensively investigated in rat testis. Viger & Robaire (1995) used Northern blots to show expression of 5αR-1 in postnatal and adult rat testis, whereas 5αR-2 was not detected. In contrast, Normington & Russell (1992) used RNA blot hybridisation to show that 5αR-2 was the predominant isoform expressed in adult rat testis. The development of enzyme activity assays for measuring the R isoforms being lower in adult testis (Pratis et al. 2000).

Understanding the regulation of the testicular 5αR isoforms is important, given their central role in androgen biosynthesis, and thus the potential for influencing sperm production. Thus, the aim of this study was to investigate the hormonal regulation of 5αR-1 and 5αR-2 mRNA and enzyme activity in adult rat testis, using two in vivo models of luteinizing hormone (LH)/testosterone and/or follicle-stimulating hormone (FSH) suppression. LH/testicular testosterone was suppressed with testosterone and oestradiol implants, and LH/testosteron and FSH was suppressed by active immunisation of rats against gonadotrophin-releasing hormone (GnRH). In both models, the AR antagonist, flutamide, was used to suppress residual levels of androgens, and residual FSH levels were suppressed with an FSH antisemur. Testosterone was partially or fully restored with high doses of testosterone (using implants) and human chorionic gonadotrophin (hCG) respectively. FSH action was restored by administering recombinant human FSH (rhFSH). The 5αR-1 and 5αR-2 mRNA levels were quantified by real-time PCR, and enzyme activities were measured with an in vitro activity assay.

Materials and Methods

Reagents, hormones and drugs

Organic solvents were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and thin layer chromatography plates (ITLC-SA) were obtained from Gelman Sciences (NSW, Australia). Non-radioactive steroids were purchased from Sigma (St Louis, MO, USA), radioactive steroids were purchased from Du Pont-New England Nuclear (Boston, MA, USA). [4-14C]-3α-androstanediol (3α-Adiol) was prepared as described previously (Pratis et al. 2000). Flutamide (Sigma) was dissolved in methylene chloride and added to peanut oil (methylene chloride:oil, 1:2 v/v). The methylene chloride was evaporated by bubbling with nitrogen and warming the solution to 37 °C. The FSH.Ab was an immunoglobulin fraction of anti-rat FSH antibody raised in sheep (FSH.Ab #1792; Meachem et al. 1998). rhFSH (Gonal-F) was obtained from Serono (Sydney, Australia). hCG (Pregnyl) was obtained from Organon (Sydney, Australia).

Animals

Adult male Sprague–Dawley rats were obtained from the Monash Central Animal House (Clayton, Australia) and housed under a 12 h light:12 h darkness cycle with free access to food and water. Animals were killed by CO2 asphyxiation, blood was removed by cardiac puncture and the testes quickly removed, trimmed of fat, weighed and frozen in liquid nitrogen. Blood was stored at −20 °C and testes were stored at −70 °C until required. These studies were approved by the Monash Medical Centre Animal Ethics Committee.

Experimental design

Two in vivo models of gonadotrophin suppression were used to investigate the regulation of 5αR by testosterone and FSH. All animals except controls received two injections, one in oil and the other in normal rat serum (NRS). All preparations (except flutamide) were prepared in 15% NRS and filtered through a 0·2 µm filter (Millipore, Bedford, MA, USA). Vehicle oil injections were similarly prepared except the drug/hormone was omitted.

Testosterone and oestradiol suppression model

Testosterone (3 cm) and oestradiol (0·4 cm) (TE) implants were prepared by filling medical-grade polydimethylsiloxane Silastic tubing (outer diameter (o.d.) 3·18 mm; inner diameter (i.d.) 1·98 mm; Dow–Corning, Midland, MI, USA) with testosterone and oestradiol respectively. Testosterone and oestradiol implants, and LH/testosterone and FSH were prepared except the drug/hormone was omitted.

GnRH immunisation model

Animals were actively immunised against GnRH with a proprietary GnRH
immunogen preparation (BA-1666–4; Biotech, Roseville, NSW, Australia) incorporating an adjuvant free of myobacterial components (Stewart et al. 1992). Animals received 100 µl GnRH immunogen every 4 weeks at a single s.c. site over a 12-week period as previously described (McLachlan et al. 1994, 1995, Meachem et al. 1998). Five groups of adult rats received GnRH immunisations (Table 2). During the treatment phase animals received either vehicle, FSH, Ab (2 mg/kg per day), or rhFSH (25 IU/kg per day), and two GnRH-immunised groups were treated with hCG (2.5 IU/kg per day). Controls did not receive the immunogen or any treatment during the treatment phase.

**5αR mRNA**

Total RNA was extracted from rat testis using the Qiagen RNeasy kit (Qiagen, VIC, Australia) and treated with DNase (DNA-free; Ambion, Austin, TX, USA) to remove any contaminating DNAs. Extracted RNA (2 µg) was reverse transcribed with either 100 ng random primer (for 5αR–1 mRNA) or 125 ng oligo (dT)12–18 (for 5αR–2 mRNA) at 42 °C for 50 min using 50 U of Expand reverse transcriptase (Roche Molecular, Mannheim, Germany). Following reverse transcription, the reaction was terminated by heating at 70 °C for 15 min. The cDNAs were stored at 4 °C until required for use in real-time PCR.

5αR mRNA was quantified by real-time RT-PCR amplification in a LightCycler (Roche) instrument (Bustin 2000) using SYBR Green I fluorescence detection of amplified products. The forward (5′-TCC TGG TCA CCT TTG TCT TGG C-3′) and reverse (5′-GGT TCC CCT GGT TTT CTC AGA TTC-3′) primers for 5αR–1 were designed to produce a 128 bp amplicon. The forward (5′-ACA TCC ACA GTG ACT ACA CCC TGC-3′) and reverse (5′-ATT ATTCAA TAA TCT CGC CCA G-3′) primers for 5αR–2 were designed to produce a 207 bp amplicon. A 20 µl PCR reaction was used and included PCR grade water (10·8 µl), 5 mmol/l magnesium chloride, specific reverse and forward primers (0·5 µmol/l final concentration for 5αR–1 and 0·375 µmol/l final concentration for 5αR–2), SYBR Green dye (2 µl) and reverse-transcribed cDNAs (2 µl, diluted 1:10). An initial denaturation step of 10 min was used for both 5αR–1 and 5αR–2. PCR amplification for 5αR–1 consisted of 38 cycles at 95 °C for 15 s (denaturation), 55 °C for 5 s (annealing), and 72 °C for 10 s (elongation). PCR amplification for 5αR–2 consisted of 40 cycles at 95 °C for 15 s (denaturation), 62 °C for 5 s (annealing), and 72 °C for 10 s (elongation). The DNA standards were highly purified and sequence-verified cDNAs, which were identical to the real-time PCR products to ensure equal amplification efficiency between standards and PCR products. For each quantification, standard curves consisting of twofold dilutions of the appropriate 5αR cDNA were included, and these ranged between 1 and 0·0625 fg for 5αR–1 and 0·2 and 0·0125 fg for 5αR–2. Following PCR, melting curve analysis was performed on the amplified product to ensure that only specific PCR amplicons were obtained and quantified. This was also confirmed by nucleotide sequencing. The mRNA data obtained from the LightCycler were normalised against 28S ribosomal RNA, which was quantified by electrophoresing equivalent RNA on an agarose gel and the 28S ribosomal RNA fluorescence measured using a CCD camera with Quantity One 5.1 software (Bio-Rad, NSW, Australia). All measurements were done within the linear range under non-saturating pixel conditions. The electrophoresed RNA was also used to confirm the integrity of all RNAs used in the real-time PCR analysis. All samples were measured in eight separate assays. Between-assay variation as determined from the reproducibility of a quality control sample was 8·9% for 5αR–1 and 8·8% for 5αR–2. 5αR–1 and 5αR–2 mRNA was measured in all animals except for the T24-treated animals in the TE suppression study due to insufficient sample amounts.

**5αR enzyme activity**

The remainder of the left testis was homogenised in 0·25 mol/l sucrose, centrifuged at 10 000 g for 20 min and the supernatant used for measuring 5αR–1 and 5αR–2 enzyme activity with enzyme assays validated previously (Pratis et al. 2000). 5αR–1 activity was measured at pH 7·0

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**Table 1** TE suppression model. Animals received T24 implants on day 2 of the treatment phase

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Suppression phase (8 weeks)</th>
<th>Treatment phase (6 days)</th>
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<tbody>
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<td>1</td>
<td>8</td>
<td>Untreated control</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>TE</td>
<td>TE + vehicle</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>TE</td>
<td>TE + flutamide</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>TE</td>
<td>TE + FSH, Ab</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>TE</td>
<td>T24 + vehicle</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>TE</td>
<td>T24 + flutamide</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>TE</td>
<td>T24 + FSH, Ab</td>
</tr>
</tbody>
</table>

**Table 2** GnRH immunisation (GnRH<sub>imm</sub>) model

<table>
<thead>
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<th>n</th>
<th>Suppression phase (12 weeks)</th>
<th>Treatment phase (6 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>Untreated control</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>GnRH&lt;sub&gt;imm&lt;/sub&gt;</td>
<td>GnRH&lt;sub&gt;imm&lt;/sub&gt; + vehicle</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>GnRH&lt;sub&gt;imm&lt;/sub&gt;</td>
<td>GnRH&lt;sub&gt;imm&lt;/sub&gt; + FSH, Ab</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>GnRH&lt;sub&gt;imm&lt;/sub&gt;</td>
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<td>GnRH&lt;sub&gt;imm&lt;/sub&gt; + hCG</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>GnRH&lt;sub&gt;imm&lt;/sub&gt;</td>
<td>GnRH&lt;sub&gt;imm&lt;/sub&gt; + hCG + FSH, Ab</td>
</tr>
</tbody>
</table>
and 5αR-2 activity was determined at pH 5.0. The contribution of the 5αR-1 isoform at pH 5.0 in the 5αR-2 activity measured (i.e. overlap) was determined and subtracted to give specific 5αR-2 enzyme activity.

Briefly, the two assays measure the conversion of \([^{3}H]\)testosterone to \(^{3}H[\text{DHT}]+^{3}H[\text{3α-Adiol}]\) in 0.1 mol/l Tris-citrate buffer at their respective pH, followed by steroid extraction with toluene:ether (1:5), steroid separation with TLC, and steroid quantification by scintillation β-counting. 5αR activity was expressed as the amount of DHT and 3α-Adiol produced after corrections for procedural losses, determined using \([^{14}C]\)steroids (testosterone, DHT and 3α-Adiol) as internal standards. The blank activity for the 5αR assay (in the absence of testicular supernatant) was 0.010 ± 0.003 pmol DHT+3α-Adiol/min (n=6), which corresponded to less than 0.12% conversion of substrate.

**LH, FSH and testicular steroids**

Serum LH was measured by immunofluorometric assay (Haavisto et al. 1993) using rat LH (rLHRP-3; NIDDK, Bethesda, MD, USA) as a standard. All samples were assayed in two assays with a detection limit of 0.02 ng/ml.

Serum FSH was measured by RIA using iodinated rat FSH (rFSH17; NIDDK) as tracer, rFSH antiserum (anti-rFSH S11; NIDDK), and rFSH RP2 as standard. All samples were assayed in two assays with a detection limit of 1.5 ng/ml.

The right testis from each rat was kept for measurement of testicular steroids. Testicular testosterone, DHT and 3α-Adiol were extracted with acetonitrile, separated by HPLC and measured by RIA, as described previously (O’Donnell et al. 1996). The within-assay variations for testosterone, DHT and 3α-Adiol were 8, 13 and 12% respectively, and the between-assay variations were 8, 3 and 8% respectively (n=6 assays).

**Statistics**

Differences between groups (data with equal variances, as assessed by Bartlett’s test) were assessed by ANOVA and independent t-tests. Data with unequal variances were assessed by the Kruskal–Wallis test and Dunn’s multiple comparison test between selected treatment groups. All data were expressed as means ± S.E.M. (n=7 or 8) and considered significant when \(P<0.05\) was achieved.

**Results**

**TE suppression model**

**Serum LH and FSH** Serum LH was suppressed (\(P<0.05\)) in all TE-treated groups compared with controls and administration of flutamide to TE- and T24-treated groups increased (\(P<0.05\)) LH levels above corresponding vehicle-treated animals (Table 3). Serum FSH was not affected by TE or T24 treatment; however, TE+flutamide treatment significantly increased FSH above TE+vehicle (Table 3).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>1.15 ± 0.15</td>
<td>3.65 ± 0.12</td>
</tr>
<tr>
<td>TE+ vehicle</td>
<td>0.12 ± 0.02*</td>
<td>3.06 ± 0.11</td>
</tr>
<tr>
<td>TE+ flutamide</td>
<td>0.69 ± 0.16†</td>
<td>6.47 ± 0.84†</td>
</tr>
<tr>
<td>TE+ FSH Ab</td>
<td>0.08 ± 0.01*</td>
<td>NA</td>
</tr>
<tr>
<td>T24+ vehicle</td>
<td>0.11 ± 0.02*</td>
<td>4.02 ± 0.33</td>
</tr>
<tr>
<td>T24+ flutamide</td>
<td>0.41 ± 0.09†</td>
<td>5.59 ± 0.52</td>
</tr>
<tr>
<td>T24+ FSH Ab</td>
<td>0.08 ± 0.01*</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA denotes not assayed due to interference of FSH:Ab in the FSH RIA.

*Significant difference compared with untreated control. †Significant difference compared with corresponding TE+vehicle or T24+vehicle.

**Testicular 5αR-1 and 5αR-2 mRNA and enzyme activity** There was no difference in the housekeeping gene 28S ribosomal RNA between any of the treatment groups (Fig. 1A). 5αR-1 mRNA (Fig. 1B) and enzyme activity (Fig. 1C) in TE-suppressed animals increased (\(P<0.05\)) above controls levels, while administration of flutamide to TE-suppressed rats further increased 5αR-1 mRNA and enzyme activity levels (\(P<0.05\), Fig. 1B and C). T24 treatment did not change 5αR-1 enzyme activity compared with TE+vehicle treatment; however, T24+flutamide treatment increased (\(P<0.05\)) 5αR-1 enzyme activity above the T24+vehicle group (Fig. 1C). TE+FSH:Ab treatment decreased (\(P<0.05\)) 5αR-1 mRNA compared with TE+vehicle treatment but did not change 5αR-1 enzyme activity.

5αR-2 mRNA was suppressed below control by TE suppression (\(P<0.05\)), while acute FSH:Ab treatment further suppressed 5αR-2 mRNA levels by ~4.5-fold (\(P<0.05\), Fig. 1D). 5αR-2 enzyme activity was not affected by these treatments (Fig. 1E).

**Testicular steroids** TE treatment suppressed testicular testosterone to <3% of control levels (\(P<0.05\), Fig. 2A), whereas DHT and 3α-Adiol (Fig. 2B and C) respectively were suppressed to 26 and 53% of controls (\(P<0.05\)) respectively. Flutamide administration significantly increased DHT and 3α-Adiol levels compared with TE treatment, in the absence of a significant increase in testosterone. Acute FSH:Ab administration did not significantly alter any of the androgens. TE suppression significantly increased the conversion of testosterone to 5α-reduced metabolites (DHT+3α-Adiol/testosterone, Fig. 2D). Co-administration of flutamide significantly (\(P<0.05\)) increased this conversion while FSH had no effect.
GnRH immunisation model

Serum LH and FSH  GnRH immunisation significantly suppressed both serum LH and FSH below control levels and administration of hCG to GnRH-immunised animals partially restored (P<0.05) serum FSH (Table 4). Administration of hCG to GnRH-immunised animals partially restored serum FSH, presumably as a consequence of a rise in circulating testosterone, which stimulates FSH in a GnRH-independent manner (Rea et al. 1986, Bhasin et al. 1987).

Testicular 5αR-1 and 5αR-2 mRNA and enzyme activity  There was no difference in the housekeeping gene 28S ribosomal RNA between any of the treatment groups (Fig 3A). 5αR-1 mRNA levels (Fig. 3B) decreased to 49% of control following GnRH immunisation (P<0.05), which returned to control levels following treatment with rhFSH or hCG (P<0.05). The hCG-induced increase in 5αR-1 mRNA was inhibited (P<0.05) by the co-administration of FSH.Ab. 5αR-1 enzyme activity (Fig. 3C) was significantly increased above control by GnRH immunisation, and this increase was not affected by administration of FSH.Ab or rhFSH. However, hCG treatment, with or without FSH.Ab treatment, suppressed (P<0.05) 5αR-1 enzyme activity below the corresponding GnRH immunisation+vehicle treatment.

5αR-2 mRNA (Fig. 3D) appeared to be lower in GnRH-immunised animals; however, this was not significant. Co-administration of FSH.Ab with hCG treatment increased 5αR-2 mRNA compared with corresponding vehicle-treated animals (P<0.05). 5αR-2 enzyme activity (Fig. 3E) was suppressed (P<0.05) by GnRH immunisation and restored to control levels with rhFSH or hCG treatment (P<0.05). The hCG-induced increase in 5αR-2 enzyme activity was prevented by co-administration of FSH.Ab (P<0.05).

Testicular steroids  GnRH immunisation suppressed testicular testosterone to <2% of control (P<0.05, Fig. 4A). hCG treatment (with or without FSH.Ab) significantly increased testosterone levels. Testicular DHT (Fig. 4B) and 3α-Adiol (Fig. 4C) in GnRH-immunised animals did not change with rhFSH or FSH.Ab treatment.

GnRH immunisation increased the conversion of testosterone to 5α-reduced metabolites (DHT+3α-Adiol/
testosterone) and this increase was not affected by FSH Ab or rhFSH treatment (Fig. 4D). Administration of hCG to GnRH-immunised animals decreased the conversion of testosterone to 5α-reduced metabolites compared with GnRH-immunised+ vehicle-treated animals.

**Discussion**

This study aimed to investigate the effects of testosterone and FSH on 5αR-1 and 5αR-2 mRNA and enzyme activity in adult rat testis in order to understand the hormonal regulation of the testicular 5αR isoforms and their relative importance in maintaining spermatogenesis. Two in vivo models of spermatogenic suppression were used: TE implants selectively suppressed LH/testosterone but not FSH, whereas GnRH immunisation suppressed both testosterone and FSH. This study showed that the testicular 5αR isoforms are differentially regulated by FSH and testosterone at the level of gene expression, and provides a better understanding of the control of testicular androgen biosynthesis.

**Effect of testosterone on 5αR-1 and 5αR-2**

When the TE suppression model was used to suppress testicular testosterone, 5αR-1 mRNA and enzyme activity increased, and this stimulatory effect was enhanced further by blocking residual androgen levels with the AR antagonist, flutamide. These data suggest that testosterone negatively regulates the expression of the 5αR-1 isoform. In support, restoration of testosterone by administration of hCG in combination with FSH.Ab (which neutralised the hCG-induced concomitant increase in FSH in this species) to GnRH-immunised animals decreased 5αR-1 mRNA and enzyme activity. Partial restoration of testosterone with T24 treatment, however, did not suppress 5αR-1 as did hCG, presumably because the concentration of testosterone was not increased sufficiently to inhibit 5αR-1. Evidence in support of this is seen from testicular steroid levels in these model systems. The amount of testosterone

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**Table 4**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
</tr>
</thead>
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<tr>
<td>Untreated control</td>
<td>0.74 ± 0.19</td>
<td>3.92 ± 0.23</td>
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<td>0.09 ± 0.01*</td>
<td>2.1 ± 0.08*</td>
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<td>GnRHimm + FSHAb</td>
<td>0.05 ± 0.01†</td>
<td>NA</td>
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<td>GnRHimm + rhFSH</td>
<td>0.11 ± 0.01*</td>
<td>NA</td>
</tr>
<tr>
<td>GnRHimm + hCG + vehicle</td>
<td>0.06 ± 0.01*</td>
<td>2.95 ± 0.08*</td>
</tr>
<tr>
<td>GnRHimm + hCG + FSHAb</td>
<td>0.06 ± 0.01*</td>
<td>NA</td>
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</table>

NA denotes not assayed due to interference of FSH.Ab or rhFSH in the FSH RIA. *Significant difference compared with untreated control. †Significant difference compared with GnRHimm + vehicle.

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**Figure 2**

Testicular androgens in the TE model. Testicular concentration of testosterone (A; note log scale on y axis), DHT (B) and 3α-Aldiol (C) in untreated control and TE-suppressed animals. Formation of 5α-reduced androgens as a percentage of testosterone [(DHT 3α-Aldiol/testosterone) × 100] represented in (D). *Significant difference (P<0.05) compared with untreated control. ††Significant difference (P<0.05) compared with TE vehicle. Data expressed as means ± S.E.M. (n = 7 or 8). Note log scale on y axis in (A) and (D).
in a normal adult rat was \(~ 100 \text{ ng/g testis} \) and this was suppressed to \(~ 2 \text{ ng/g testis} \) with GnRH immunisation. Whereas hCG treatment fully restored testosterone (\(~ 300 \text{ ng/g testis} \), T24 treatment only partially restored testicular testosterone (\(~ 12 \text{ ng/g testis} \); O’Donnell et al. 1999). Thus the suppression of 5αR-1 mRNA and activity by testicular testosterone presumably requires levels greater than 12 ng/g testis. Negative regulation of 5αR-1 isoform by testosterone is consistent with our hypothesis that a reduction in testicular testosterone leads to an increase in the metabolism of testosterone to the more potent metabolite, DHT. Thus, when testicular testosterone levels are low and unable to maintain sperm production, amplification of the androgenic stimulus via conversion of testosterone to DHT would enable continued low levels of spermatogenesis. Negative regulation of 5αR-1 by testosterone has been demonstrated in a number of tissues, such as liver (Yates et al. 1958, Lopez-Solache et al. 1996) and adrenal cortex (Lephart et al. 1991). Unlike the 5αR-1 isoform, which was shown to be regulated by testosterone, the 5αR-2 isoform in rat testis was not regulated by testosterone. Thus, neither suppression nor restoration of testosterone affected 5αR-2 mRNA or enzyme activity. This is in contrast to the ventral prostate where 5αR-2 is positively regulated by androgens (Andersson & Russell 1990). The reason for the differential regulation of the 5αR isoforms in different tissues is unknown, but suggests that factors that regulate the 5αR isoforms may be tissue-specific.

Effect of FSH on 5αR-1 and 5αR-2

Studies in the literature that have investigated the regulation of 5αR-1 are controversial, and have measured only enzyme activity in immature rat testis. Some studies showed an effect of FSH on 5αR-1 enzyme activity (Nayfey et al. 1975, Welsh & Wiebe 1976) whereas others did not (Dorrington & Fritz 1975, Murono & Payne 1979). The present study showed that acute (6 day) FSH suppression, by administering FSH, decreased 5αR-1 mRNA, and that FSH restoration, by rhFSH or hCG treatment to GnRH-immunised animals, increased 5αR-1 mRNA. These findings demonstrate that FSH positively regulates the 5αR-1 isoform in rat testis at the mRNA level.

Figure 3 5αR-1 and 5αR-2 mRNA (B and D respectively) and enzyme activity (C and E respectively) in the GnRH immunisation model. Untreated control animals are represented by the open bars and GnRH-immunised (GnRHimm) and GnRHimm+hCG-treated rats administered vehicle, FSH,Ab or rhFSH are represented by closed bars. The housekeeping gene (A) and testis weight (F) are also represented. *Significant difference \( (P < 0.05) \) compared with untreated control. **Significant difference \( (P < 0.05) \) compared with GnRHimm+vehicle. Significant differences between treatment groups represented at \( P < 0.05 \). Data are expressed as means ± S.E.M. \((n=8)\).
neither suppression nor restoration of FSH changed 5αR-1 enzyme activity, suggesting that the regulation of the 5αR-1 isoform by FSH in these studies occurred at the gene expression level and did not affect the translation of 5αR-1 mRNA into protein within the time-frame studied here. There are numerous reports in the literature that demonstrate that the level or change in 5αR-1 mRNA does not necessarily follow 5αR-1 protein levels. For example, in humans 5αR-1 mRNA expression is clearly detectable whereas there are undetected levels of 5αR-1 protein (Jenkins et al. 1992, Thigpen et al. 1993b). Viger & Robaire (1995) showed that in the rat, 5αR-1 mRNA levels at day 90 was about one-third of that of day 30, whereas 5αR-1 enzyme activity at day 90 was ~30-fold less than that at day 30 (Rivarola et al. 1972, Pratis et al. 2000). Thus, there appears to be post-transcriptional or translational modification of the 5αR-1 isoform. In rat testis, the 5αR-1 transcript has been demonstrated to change in size with age, such that a 2-5 kb 5αR-1 transcript is expressed during immaturity when 5αR-1 activity is high, and a 2-7 kb transcript is expressed in the adult, which coincides with the period when 5αR-1 activity levels are low, suggesting that 5αR-1 enzyme activity may depend on the transcript size of the 5αR-1 mRNA (Viger & Robaire 1995).

There are several studies showing discordant patterns of mRNA and protein expression. For example, DHT decreased AR mRNA but increased AR protein in LnCaP and MDA453 cell lines (Yeap et al. 1999) and in rat prostatic smooth muscle cells, androgen withdrawal increased steroid sensitive gene 1 (SSG1) mRNA whereas SSG1 protein decreased (Marcantonio et al. 2001). The discordant response between mRNA and activity for this study (described above) could relate to differences in the half-life of the mRNA and protein. The half-life of recombinant 5αR-1 protein is ~30 h (Thigpen et al. 1993a) whereas the half-life of 5αR-1 mRNA is unknown. A short half-life would indicate that mRNA and enzyme activity levels would be critically dependent on the time-point at which measurements were taken, suggesting that further studies are required to determine the changes in 5αR-1 mRNA and activity at different time-points following treatment.

5αR-2 enzyme activity was suppressed by GnRH immunisation, and restoration of FSH by rhFSH or hCG treatment following GnRH immunisation, increased 5αR-2 enzyme activity to control levels. These findings suggest that FSH positively regulates 5αR-2 enzyme activity. In support, blocking the concomitant rise in FSH that occurs with hCG treatment prevented the restoration of 5αR-2 activity, providing further evidence that FSH positively regulates 5αR-2 enzyme activity. Whereas FSH affected 5αR-2 enzyme activity, FSH did not change the levels of 5αR-2 mRNA. The FSH-induced increase in 5αR-2 enzyme activity but not mRNA could be due to the time-point at which samples were collected after treatment, as described above for 5αR-1. It is conceivable that FSH caused an initial increase in mRNA within

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**Figure 4** Testicular androgen concentrations in the GnRH immunisation model. Testicular concentration of testosterone (A; note log scale on y axis), DHT (B) and 3α-Adiol (C) in control and GnRH-immunised (GnRH<sub>imm</sub>) animals. Formation of 5α-reduced androgens as a percentage of testosterone ((DHT + 3α-Adiol)/testosterone) × 100) represented in (D). *Significant difference (P<0.05) compared with untreated control. **Significant difference (P<0.05) compared with GnRH<sub>imm</sub> +vehicle. Data are expressed as means ± S.E.M. (n=8).
1–2 days of treatment, which then declined to control levels. The half-life of recombinant 5αR–2 protein is similar to that of recombinant 5αR–1 (30 h; Thigpen et al. 1993), whereas the half-life of 5αR–2 mRNA is unknown. As for 5αR–1, further studies are required to measure 5αR–2 mRNA and activity over several time-points. Additional studies are also required to determine the effect of other hormones on the regulation of the 5αR isoforms. For example, in genital skin fibroblasts, which exclusively express the 5αR–2 isoform, 5αR–2 activity has been shown to be regulated by DHT and insulin-like growth factor-I (Horton et al. 1993), transforming growth factor-β1 and -β2 (Wahe et al. 1993) and activin A (Antonipillai et al. 1995).

As mentioned above, 5αR–2 enzyme activity decreased when FSH.Ab was co-administered with hCG. Surprisingly, co-administration of FSH.Ab with hCG increased 5αR–2 at the mRNA level. However, in the TE suppression model, FSH.Ab administration decreased 5αR–2 mRNA. The discrepancy between these findings is unexplained but it is possible that the increase in 5αR–2 mRNA after FSH suppression in hCG-treated animals is partly due to the presence of testosterone in these animals. A difference between these in vivo models is that GnRH immunisation produces more severe spermatogenic regression and FSH is chronically suppressed in GnRH-immunised animals, whereas FSH is only acutely suppressed in the TE-suppression model.

5αR isoform mRNA and activity were expressed on a per gram of testis basis in order to investigate the testicular concentrations of these enzymes, and their relationship to the testicular androgen concentration. The significant changes in 5αR–1 and 5αR–2 induced by hormonal treatment of TE-treated or GnRH-immunised rats, in the absence of changes in testis weight, may be a consequence of modulation of the regulation and expression of these enzymes in Leydig and Sertoli cells, both of which have been demonstrated to contain at least 5αR–1 (Dorrington & Fritz 1975, Nayfey et al. 1975, Welsh & Wiebe 1976, Murono & Payne 1979, Viger & Robaire 1995). Since restoration of testosterone and/or FSH in these models cause increases in some germ cell populations (Meachem et al. 1998), it is also possible that the changes in the testicular concentration of 5αR isoforms is related to the appearance of certain germ cell types. Although it seems that 5αR–1 is present predominantly in Leydig cells (Viger & Robaire 1995) there is also evidence for 5αR activity (probably 5αR–1) in seminiferous tubules, in Sertoli cells as well as germ cells (Dorrington & Fritz 1975). The localisation of 5αR–2 in the testis is unknown.

Interpretation of changes in the concentration of 5αR isoforms between untreated controls and treated (TE-treated or GnRH-immunised) animals is complicated by the fact that there are large changes in testis weight induced by these treatments. These differences in testis weight are related to the disappearance of various germ cells from the testis (Meachem et al. 1998) and are not likely to be related to changes in Sertoli cell numbers (McLachlan et al. 1995). The data expressed per gram of testis show that TE-treated and GnRH-immunised animals have an increase in the concentration of 5αR–1 activity; however, if the data were expressed on a per testis basis no changes would be seen. Nevertheless, analysis of the testicular androgen levels showed that the ability of the testis to produce 5α-reduced androgens is increased when the testis is in a hypogonadotrophic state. This is demonstrated by an increase in the amount of DHT and 3α-Adiol produced when taking into account the large decrease in substrate (testosterone) concentration. Thus, TE treatment or GnRH immunisation increased the capacity of the testis to produce 5α-reduced androgens. This increased capacity correlated with an increase in the testicular concentration of 5αR–1 activity, suggesting that the concentration of this enzyme in particular is important for the production of 5α-reduced androgens in the testis.

Thus, this study showed that the concentration of 5α-reduced metabolites in the testis is influenced by expression of 5αR–1 rather than 5αR–2, and that the 5αR–1 isoform appears to be negatively regulated by testosterone. Thus 5αR–1, which is the predominant 5αR isoform in rat testis (Pratis et al. 2000), is important for testicular 5α-reduced androgen biosynthesis. We have shown previously in rats that synthesis of 5α-reduced androgens in the testis is particularly important for the maintenance of androgenic effects on spermatogenesis when testicular testosterone levels are experimentally lowered in a model of contraception (O’Donnell et al. 1996, 1999). The results presented here extend the previous studies to suggest that when testicular testosterone levels are low, the concentration of 5αR–1 in the testis is increased, thereby allowing increased production of the more potent androgen DHT, and hence amplification of the androgenic stimulus on spermatogenesis. In terms of relevance to clinical contraceptive studies, the maintenance of testicular 5α-reduced androgen levels during testosterone-based contraception has been suggested to be associated with the continued low level of sperm production in oligospermic responders (Anderson et al. 1996, 1997). Therefore we speculate that increases in testicular 5αR–1 activity in the human testis may occur during contraceptive treatment, as a consequence of the negative regulation of 5αR–1 expression by testosterone, and may underlie the maintenance of spermatogenesis in oligospermic men. Further studies are required to assess 5αR–1 expression and activity in the human testis. Considering that 5αR–1 appears to be the predominant 5αR expressed in human testis (Aumuller et al. 1996) and that administration of specific 5αR–2 inhibitors failed to induce complete suppression of spermatogenesis in men undergoing hormonal contraception (McLachlan et al. 2000, Kinniburgh et al. 2001), it is possible that selective inhibition of 5αR–1 or dual inhibition of both 5αR-
isofoms may be warranted to improve the degree of spermatogenic suppression during testosterone-based contraceptive administration. In addition, our studies show that FSH can influence $5\alpha$R-1 and $5\alpha$R-2, suggesting that circulating levels of FSH could also modulate $5\alpha$-reduced androgen biosynthesis in the testis. In summary, this study suggests that the concentrations of testicular $5\alpha$ isoforms are hormonally regulated, and highlights the contribution of the $5\alpha$R-1 isofom to $5\alpha$-reduced androgen biosynthesis.

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