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# Hamster SRD5A3 lacks steroid 5α-reductase activity *in vitro*

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ABSTRACT

According to current knowledge, two steroid 5α-reductases, designated type 1 (SRD5A1) and type 2 (SRD5A2), are present in all species examined to date. These isozymes play a central role in steroid hormone physiology by catalyzing the reduction of 3-keto-4-ene-steroids into more active 5α-reduced derivatives, including the conversion of testosterone (T) to dihydrotestosterone (DHT). A third 5α-reductase (SRD5A3, -type 3), which is overexpressed in hormone-refractory prostate cancer cells, has been identified; however, its enzymatic characteristics are practically unknown. Here, we isolated a cDNA encoding hamster Srd5a3 (hSrd5a3) and performed functional metabolic assays to investigate its biochemical properties. The cloned cDNA encodes a 330 amino acid protein that is 87% identical to the homologous protein in mice and 78% to that in humans. However, hSrd5a3 exhibits low sequence homology with its counterparts hSrd5a1 (19%) and hSrd5a2 (17%). A fusion protein consisting of hSrd5a3 and green fluorescent protein provided evidence for cytoplasmic localization in transfected mammalian cells. Real-time PCR analysis revealed that, Srd5a3 mRNA was present in nearly all hamster tissues, with high expression in the cerebellum, Harderian gland and testis. Functional assays expressing hSrd5a3 cDNA in HEK-293 cells revealed that this isozyme is unable to reduce T into DHT. Further expression assays confirmed that similar to testosterone, progesterone, androstenedione and corticosterone are not reduced by hSrd5a3 or human SRD5A3. Together, these results indicate that hSrd5a3 lacks the catalytic activity to transform 3-keto-4-ene-compounds; therefore 5α-reductase type 3 may not be involved in 5α-reduction of steroids.



## 1. Introduction

5α-Reductases (3-oxo-5α-alpha-steroid 4-dehydrogenase; EC 1.3.99.5) are NADPH-dependent microsomal enzymes involved in the 5α-reduction of testosterone (T), progesterone (P), corticosterone (B), $\Delta^4$-androstenedione, and other 4-ene-3-keto steroids into their respective 5α-dihydro-3-keto derivatives (i.e., 5α-DHT, 5α-DHP and 5α-DHB), and this irreversible step is thought to play catabolic and anabolic roles in steroid hormone metabolism [1,2]. The best studied activity for these enzymes is the transformation of T into 5α-DHT, which is the androgen responsible for the differentiation of male external genitalia and the prostate in addition to virilization at puberty [3].

5α-Reduction also appears to play a role in the action of other steroids, including the boar pheromones androstanol and androstenol, the brassinosteroid campesterol and possibly cortisol and aldosterone [4–6].

In most species studied thus far, only two 5α-reductase isozymes (type 1 and type 2), which are encoded by separate genes (*SRD5A1/Srd5a1* and *SRD5A2/Srd5a2*), have been well characterized [7]. These isozymes share moderate (~50%) sequence homology, show similar substrate preferences and have identical gene structures (five exons, four introns), but they differ with respect to their biochemical properties, sensitivity to certain steroidal 5α-reductase inhibitors, physiological role and tissue distribution [7,8]. For example, 5α-reductase type 2 is active over a narrow acidic pH range (4.5 to 5.5), whereas the type 1 enzyme has a broad neutral to basic (6.5 to 8.5) pH optimum. Similarly, the type 2 isozyme is predominantly expressed in male reproductive tissues such as the epididymis, seminal vesicles, prostate, fetal genital skin and the Harderian gland (HG), whereas 5α-reductase type 1 is highly expressed in the liver and moderately expressed in several other tissues including ovaries, HG and skin [9–11].

In Syrian hamsters, the HG displays marked sexual dimorphism, including differences at the histological, biochemical and ultrastructural levels. Male HGs contain two forms of alveolar cells (types I and II), produce high amounts of specific hemeproteins and are heavier than HGs in females. Upon gross examination, HGs appear to be pale because of their low porphyrin content. Conversely, HGs in females

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possess only type I cells and are darkly speckled because of their high content of porphyrins (almost 1000 times more than in males). Interestingly, male castration in hamsters eliminates type II acinar cells, which is accompanied by an increase in the synthesis of intraglandular protoporphyrins [12,13]. Studies have shown that androgen administration induces the formation of male-type glands in males and females [14]. It is also known that HGs in hamsters efficiently transform T to DHT, and this conversion is necessary for maintaining the male glandular pattern. In fact, the activity of 5α-reductase in the HG displays a marked androgen-regulated sexual dimorphism. This species contains both isozymes, and its HGs have high 5α-reductase activity as shown in previous studies aimed at determining the predominant isozyme within the HG [10,15].

Recently, a third 5α-reductase enzyme (type 3) which is encoded by *SRD5A3*, was described by Uemura et al. [16]. Although several studies have demonstrated expression of SRD5A3 (at the mRNA and protein level) in practically all human tissues, including several cancer cell lines [17,18], its enzymatic activity has been poorly studied and its natural steroid substrates are largely unknown. Because the presence of a third isozyme may account for sex differences in 5α-reductase activity in the HG, we sought to determine the specific biochemical characteristics of 5α-reductase-3 in this study.

After extensive functional assays using cultured human cells transiently expressing hSrd5a3 or SRD5A3, we found that hamster and human enzymes are unable to catalyze the conversion of testosterone and other $\Delta^{4,5}$-3-keto steroids to their corresponding 5α-reduced derivatives. These findings suggest that 4-ene-3-keto steroids are not natural substrates for 5α- reductase type 3, which is thus likely not a steroidogenic enzyme. Our results are in line with the data of Cantagrel et al. [19] who reported that the primary function of SRD5A3 may not be related to the metabolism of steroids but rather to the *N*-glycosylation of proteins by mediating the α-reduction of polyprenol to dolichol.

## 2. Experimental

### 2.1. Animals and tissues

Twelve-week-old male and female Syrian hamsters (*Mesocricetus auratus*) were used throughout the study. The hamsters were housed under controlled conditions (temperature; 21 ± 2 °C; lighting; 14:10 h/light:darkness) with free access to food and water. Tissue samples from different organs were dissected from the body, frozen on dry ice and stored at −75 °C until they were assayed. All experimental procedures involving the use of animals were approved by the Ethical Committee for Research in Animals at our institution (INCMNSZ).

### 2.2. RNA isolation and reverse transcription

Total RNA was isolated from hamster tissues using the TRIzol reagent (Invitrogen, Carlsbad, CA). The purity and integrity of the RNA were evaluated spectroscopically (at 260/280 nm) and by gel electrophoresis before reverse transcription. First-strand cDNA was synthesized from total RNA (1–1.5 μg) using the Transcriptor First-Strand cDNA Synthesis kit (Roche Diagnostics, Mannheim, Germany) following the supplier's guidelines.

### 2.3. Isolation of full-length hamster Srd5a3 cDNA

Specific Srd5a3 cDNA fragments were obtained by RT-PCR using two degenerate primers, HSR35F (forward) 5′-GCGMTCTTCCAGGACCTGMTCCGCTA-3′and HR33F (reverse)5′-CTAACTACYTAG CAGAGCTGATGATC-3′, which were designed by comparing the reported sequences with that of 5α-reductase-3 from mice (GenBank accession No. BC_086584) and humans (GenBank accession No. NM_025592). Gene-specific primers (GSPs) derived from these fragments were used to determine the 5′- and 3′-end regions by rapid amplification of cDNA ends (RACE). First-strand cDNA synthesis for the RACE reactions was performed for total RNA from male HGs using the SMART RACE cDNA amplification kit (Clontech Inc., Palo Alto, CA). The complete cDNA sequence was obtained by PCR with the GSPs 3R19, 5′-TAACTCGTTCCCG GGCCAT-3′, and P919, 5′-TGTCTCCAGGAATGGAATCTGTCC-3′, which correspond to nucleotides −17 to +2 and 1049 to 1026 in Srd5a3 cDNA. After 30 cycles of amplification (94 °C for 1 min, 67 °C for 1 min, and 72 °C for 3 min with a 7 min final extension at 72 °C), the PCR products (1065 bp) were purified and then ligated into the pcDNA3.1/NT-GFP-TOPO TA expression vector (Invitrogen Co., Carlsbad, CA). Positive clones were analyzed by restriction-enzyme digestion and DNA sequencing to verify the orientation and integrity of the inserts. The nucleotide sequence in the final constructs (pcDNA3.1/hSrd5a3) was determined from both strands using an ABI-PRISM 3100 automatic DNA Sequencer (PE Applied Biosystems, Foster City, CA).

### 2.4. Real-time quantitative RT-PCR (RT-qPCR)

Total RNA extracted from different hamster tissues, and transiently transfected mammalian cells was reverse transcribed using oligo-d(T) primers as described above. Real-time PCR was conducted using a LightCycler 2.0 system from Roche (Applied Science) with LightCycler TaqMan master mix and pre-validated TaqMan hydrolysis probes (Roche Diagnostics, Mannheim, Germany). The relative level of hSrd5a3 mRNA (*sense*: 5′-GTG CCC ATG GAT GAC AAG A-3′ and *antisense* 5′-ATC ATC ATT CCG AGG ACG TG-3, 86 bp) was normalized based on the level of hamster β-actin mRNA (*sense*: 5′-AGC TAT GAG CTG CCT GAT GG-3′ and *antisense*: 5′- CAG GAA GGA AGG CTG GAA A-3′; 87 bp) [GenBank accession No. AJ312092]. Primers were designed to generate amplicons (80–160 bp) that crossed intron/exon boundaries to prevent the amplification of contaminating genomic DNA, and the product of hamster Srd5a3 spanned the boundary of exons 3 and 4. Transcripts of Srd5a3 and β-actin were detected using the universal fluorogenic probes #60 (04-688-589-001) and #9 (04-685-075-001), respectively. The mRNA level of hSrd5a1 and hSrd5a2 in transfected cells was assessed using previously described vectors and primers (Ramos et al., 2010), whereas the mRNA level of human SRD5A3 was assessed using the primers QRT3F, 5′-GCTTCATGGTTTGCTCAGAA-3′, and QRT3R 5′-CGTAGAGCCACTCGAAGAGCT-3′ (128 bp, located between exons 2 and 3), and normalized against the mRNA expression level of GAPDH [GenBank accession No. NM_002046]. PCR amplifications were performed by preheating at 94 °C for 10 min followed by 40 cycles of 94 °C for 10 s, and 72 °C for 1 s with a final cooling step at 40 °C. The RT-qPCR data were analyzed using the relative quantification method provided by LightCycler software (Version 4.5), and they were expressed in arbitrary mRNA units as the median value of six independent PCR runs.

### 2.5. Fluorescence analysis, confocal microscopy and Western blotting

To ascertain the subcellular location of overexpressed hSrd5a3, HEK-293 and HeLa cells were transfected with the pcDNA3.1/hSrd5a3 plasmid. The HeLa and HEK-293 cells were plated one day before transfection in chamber slides at a density of $1 \times 10^5$ cells in Dulbecco's Modified Eagle Medium without phenol red (DMEM-HG) supplemented with 5% stripped FBS under 5% $CO_2$ at 37 °C. Transfections were performed using Lipofectamine reagent (Invitrogen Co., Carlsbad, CA) and 1 μg of pcDNA3.1/hSrd5a3