The 5alpha-reductase type 1, but not type 2, gene is expressed in anagen hairs plucked from the vertex area of the scalp of hirsute women and normal individuals

Abstract

The aim of the present study was to determine the expression of the genes for type 1 (SDR5A1) and type 2 (SDR5A2) 5α-reductase isoenzymes in scalp hairs plucked from 33 hirsute patients (20 with polycystic ovary syndrome and 13 with idiopathic hirsutism) and compare it with that of 10 men and 15 normal women. SDR5A1 and SDR5A2 expression was estimated by RT-PCR using the gene of the ubiquitously expressed protein β2-microglobulin as an internal control. The results are expressed as arbitrary units in relation to β2-microglobulin absorbance (mean ± SEM). SDR5A2 expression was not detected in any hair samples analyzed in this study. No differences were found in SDR5A1 mRNA levels between men and normal women (0.78 ± 0.05 vs 0.74 ± 0.06, respectively). SDR5A1 gene expression in the cells of hair plucked from the scalp of normal women (0.85 ± 0.04) and of women with polycystic ovary syndrome (0.78 ± 0.05) and idiopathic hirsutism (0.80 ± 0.06) was also similar. These results indicate that SDR5A1 gene expression in the follicular keratinocytes from the vertex area of the scalp seems not to be related to the differences in hair growth observed between normal men and women and hirsute patients. Further studies are needed to investigate the expression of the 5α-reductase genes in other scalp follicular compartments such as dermal papillae, and also in hair follicles from other body sites, in order to elucidate the mechanism of androgen action on the hair growth process and related diseases.

Key words
- Hair follicle
- Hirsutism
- 5α-Reductase
- Polycystic ovary syndrome
Introduction

Androgens are the main regulators of human hair growth and are associated with one of the major clinical hair growth disorders, namely hirsutism. This condition corresponds to excessive body hair growth in women with a male pattern of body hair distribution. The presence of hirsutism can signal conditions associated with increased androgen secretion by ovaries and/or adrenals such as polycystic ovary syndrome (PCOS), androgen-secreting tumors, and nonclassic adrenal hyperplasia or can result from peripheral hypersensitivity to circulating androgens (idiopathic hirsutism, IH) (1-3). Although in general this condition is not life threatening, it is greatly distressing to patients and has a significant negative psychological impact. Investigating the effects of androgens on hair growth in the presence of hirsutism should improve our knowledge of human hair follicle biology.

The effect of all active androgens on target cells is mediated by their binding to the same nuclear androgen receptor. Previous studies on androgen resistance syndromes have revealed the importance of the androgen receptor for androgen-dependent hair growth (4-6). More recently, an increased androgen binding capacity was observed in scalp hair cells of balding men (7). However, no consistent difference has been found thus far in the number or function of the androgen receptor in hirsute patients compared to normal subjects (8,9).

Hair follicles have autonomous control over androgen metabolism, adjusting the production and degradation of steroid hormones according to local requirements (10). Under normal conditions, 5α-reductase has a key role in the action of androgens on hair follicles, converting testosterone to the more potent androgen dihydrotestosterone (11,12). Studies of molecular cloning have characterized two genes that encode the type 1 and type 2 5α-reductase isoenzymes (13,14). The 5α-reductase isoenzyme predominant in the skin is type 1 (SDR5A1) (15), which has 60% homology with 5α-reductase type 2 (SDR5A2) that is characteristic of the prostate gland (14). An increase in 5α-reductase activity was demonstrated in genital and pubic skin fibroblasts from hirsute patients in comparison to the skin of normal women (8,16). These studies have reported an increase in 5α-reductase activity even in IH, which is characterized by the absence of elevated plasma androgen levels (17). Moreover, pubic skin of hirsute patients expresses the same SDR5A1 isoform as pubic skin of normal subjects, whereas SDR5A2 is mainly expressed in genital skin from both normal subjects and hirsute patients (18). However, the physiological role of 5α-reductase isoenzymes is not completely understood and their distribution in different skin compartments is still unclear. Some immunohistochemical and enzyme activity studies have suggested a predominant expression of SDR5A1 enzyme in sebaceous glands, but also in sweat glands, epidermal cells, root sheath and dermal papilla cells from hair follicles (19-21), whereas SDR5A2 is only expressed in these compartments at very low levels. In contrast, other studies have demonstrated a different distribution of these isoenzymes inside the pilosebaceous unit (22-24). There seems to be a higher distribution of SDR5A1 in hair follicle compartments compared to SDR5A2. Furthermore, since root sheath keratinocytes show high expression of the SDR5A1 gene, they probably play an important role in androgen metabolism in hair follicles.

The aim of the present study was to assess the expression of the SDR5A1 and SDR5A2 genes in hair root sheath cells of the vertex area of scalp from hirsute patients and compare it with normal subjects of both sexes.

Patients and Methods

Subjects

The study population included women
consulting for hirsutism seen consecutively during a 6-month period at the Gynecological Endocrinology Unit of Hospital de Clínicas de Porto Alegre, Brazil. Thirty-three patients ranging in age from 12 to 42 years were selected for the study. Twenty patients were diagnosed as having PCOS and 13 as having IH. The diagnosis of PCOS was based on the physical features of hyperandrogenism, disturbed menstrual cycles, elevated serum luteinizing hormone (LH) levels or LH/follicle-stimulating hormone ratio, increased levels of total testosterone and/or free androgen index (FAI), ultrasound evidence of bilateral enlarged polycystic ovaries (25,26), and absence of ovarian or adrenal neoplasm or Cushing’s syndrome. IH was diagnosed as previously described (27) in hirsute patients with regular ovulatory cycles (luteal phase progesterone levels higher than 3.8 ng/ml), normal androgen levels, and without any known underlying disease.

Late-onset (nonclassic) congenital adrenal hyperplasia patients were not considered for the study on the basis of a high plasma level of 17-hydroxyprogesterone (>5 ng/ml) and/or its marked increase after ACTH stimulation (>12 ng/ml) (28,29). Patients with hyperprolactinemia (serum prolactin levels higher than 20 µg/l on two different occasions) were also excluded.

Fifteen normal women with regular menstrual cycles aged 16-37 years and ten men aged 16-29 years were also selected for the study, which was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre. Informed consent was obtained from each subject. None of the subjects had received any drugs known to interfere with androgen, estrogen or gonadotropin serum levels for at least 3 months before the study.

SDR5A1 and SDR5A2 mRNA levels were estimated by the reverse transcription-polymerase chain reaction (RT-PCR) in hair cells plucked from the vertex portion of the scalp of normal men, normal women and hirsute patients.

**Study protocol**

Anthropometric measurements included body weight, height and body mass index (BMI = current measured weight in kg divided by height in m²). Hirsutism score was graded by the Ferriman-Gallwey method (30), excluding the lower leg and forearm areas.

Hormonal assessment was performed between day 2 and 10 of the menstrual cycle or on any day when the patients were amenorrheic. After an overnight fast, blood samples were drawn from an antecubital vein for determination of LH, sex hormone-binding globulin (SHBG) and total testosterone. All samples were obtained between 8 and 10 am. The FAI was estimated by dividing total testosterone (nmol/l) by SHBG (nmol/l) x 100.

**Assays**

Total testosterone was measured by double-antibody radioimmunoassay (ICN, Costa Mesa, CA, USA), with an assay detection limit of 0.04 ng/ml and intra- and inter-assay coefficient of variance (CV) of 10 and 15%, respectively; SHBG was measured by an immunochemiluminometric assay (ICMA; DPC, Los Angeles, CA, USA), with a detection limit of 0.2 nmol/l, and intra- and inter-assay CV of 5.0 and 8.0%, respectively. LH was measured by ICMA, with a detection limit of 0.7 mIU/ml and intra- and interassay CV of 5.2 and 8.0%, respectively.

**RT-PCR protocol**

Plucked anagen hairs were collected from the vertex of the scalp of all subjects and were immediately frozen in liquid nitrogen and transported to the laboratory for assays. The extraction of total RNA and the synthesis of cDNA were carried out as previously described (31). The plucked hair roots were homogenized in phenol-guanididine isothio-
cyanate (Trizol, Gibco-BRL, Gaithersburg, MD, USA). Total RNA was extracted with chloroform and precipitated with isopropanol by 12,000 g centrifugation at 4°C. The RNA pellet was washed twice with 75% ethanol, resuspended in diethylpyrocarbonate-treated water, and quantified by absorbance at 260 nm.

First strand cDNA was synthesized from 5 μg total RNA for all reactions using the SuperScript Preamplification System (Gibco-BRL). After denaturing the template RNA and primers at 70°C for 10 min, reverse transcriptase was added in the presence of 20 mM Tris-HCl, pH 8.4, plus 50 mM KCl, 2.5 mM MgCl₂, 0.5 mM dNTP mix and 10 mM dithiothreitol, and incubated at 42°C for 55 min. The mixture was heated to 70°C to stop the reaction and then incubated with *E. coli* RNAse for 20 min at 37°C to destroy untranscribed RNA. The template (cDNA) used in the different PCR assays was obtained from the same reverse transcription reaction. PCR was carried out in a final volume of 50 μl. Two microliters of the first strand synthesis reaction (with an expected cDNA yield of 10 ng) was denatured at 94°C for 3 min (2 min only for β₂-microglobulin) in the presence of 20 mM Tris-HCl, pH 8.4, plus 50 mM KCl and 1.5 mM MgCl₂. After this hot start, 1.25 U of *Taq* DNA polymerase was added together with the same Tris-HCl buffer, 1.5 mM MgCl₂, 0.4 μM sense and antisense primers and 0.2 mM dNTP mix.

A 368-bp fragment of the SDR5A1 (24) and a 566-bp fragment of the SDR5A2 (14) cDNA sequence were amplified using primers designed to span intron-exon borders in order to prevent the amplification of any contaminating genomic DNA. A 623-bp cDNA fragment corresponding to the ubiquitously expressed protein β₂-microglobulin (32) was amplified to normalize the cDNA amounts in each sample. The cDNA sequences of SDR5A1 and SDR5A2 and β₂-microglobulin primers are listed in Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5’-3’)</th>
<th>Size of fragment (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDR5A1 Sense</td>
<td>TGGCGCTTCTCTATGGACTT</td>
<td>368 bp (21)</td>
</tr>
<tr>
<td>Antisense</td>
<td>GGAAGCAACACTGCAGTTGA</td>
<td></td>
</tr>
<tr>
<td>SDR5A2 Sense</td>
<td>TACTTCTGGGCCTCTTCTGCG</td>
<td>566 bp (14)</td>
</tr>
<tr>
<td>Antisense</td>
<td>TTTCATCAGCATTGTTGGGACG</td>
<td></td>
</tr>
<tr>
<td>β₂-microglobulin Sense</td>
<td>ATCCAGCGTACTCAGAAGATTCAG</td>
<td>623 bp (32)</td>
</tr>
<tr>
<td>Antisense</td>
<td>AAATTGAGATGTTACTATGCACGC</td>
<td></td>
</tr>
</tbody>
</table>

PCR was standardized by testing a number of cycles (20 to 45) and amplification was performed in the linear range. Final PCR conditions were as follows: 35 cycles (45 s at 94°C, 45 s at 60°C, 90 s at 72°C, 10 min at 72°C) for SDR5A1, 40 cycles (1 min at 94°C, 1 min at 65°C, 2 min at 72°C, 5 min at 72°C) for SDR5A2, and 30 cycles (1 min at 94°C, 1 min at 55°C, 1 min at 72°C, 5 min at 72°C) for β₂-microglobulin. cDNA from dissociated cells of human prostate gland was used as a positive control for all PCR reactions. No cDNA was added to the negative reactions. A sample of the PCR mixture (15 μl) was size-fractionated on 1.5-2.0% agarose gel stained with ethidium bromide, run

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**Table 1. Sequence of the primers related to SDR5A1, SDR5A2 and β₂-microglobulin cDNA sequences and expected sizes of amplified fragments.**

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</tr>
<tr>
<td>Antisense</td>
<td>AAATTGAGATGTTACTATGCACGC</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 2. Clinical and hormonal data for hirsute patients with polycystic ovary syndrome (PCOS) and idiopathic hirsutism (IH).**

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>IH</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)²</td>
<td>23.0 ± 1.2</td>
<td>24.3 ± 1.7</td>
<td>-</td>
</tr>
<tr>
<td>BMI (kg/m²)²</td>
<td>30.7 ± 1.4</td>
<td>26.8 ± 1.1</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Clinical score for hirsutism³</td>
<td>15.1 ± 1.3</td>
<td>13.9 ± 0.9</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Testosterone⁴ (ng/ml)</td>
<td>0.89 ± 0.07</td>
<td>0.64 ± 0.06</td>
<td>0.20-0.81</td>
</tr>
<tr>
<td>FAI⁵</td>
<td>15.3 ± 1.73</td>
<td>8.8 ± 1.34</td>
<td>5.50-11.20</td>
</tr>
<tr>
<td>SHBG⁶ (nmol/l)</td>
<td>24.4 (13.3-37.3)</td>
<td>33.9 (22.5-45.2)</td>
<td>20-118</td>
</tr>
<tr>
<td>LH⁷ (IU/l)</td>
<td>4.6 (2.1-9.1)</td>
<td>2.7 (2.1-6.6)</td>
<td>1.6-8.3</td>
</tr>
</tbody>
</table>

²Data are reported as mean ± SEM. ³Values are reported as median and 95% confidence interval. BMI, body mass index; FAI, free androgen index; SHBG, sex hormone-binding globulin; LH, luteinizing hormone. *P < 0.05 compared to PCOS patients (ANOVA followed by the Duncan test; Mann-Whitney test).
Gene expression of 5α-reductase isoenzymes in plucked hairs at 100 V and visualized under UV light. The expected bands were quantified by densitometric analysis using an image-processing system (ImageMaster VDS, Pharmacia Biotech, Uppsala, Sweden).

Statistical analysis

Data are reported as means ± SEM, unless otherwise noted. Group means were compared by the Student t-test or by one-way analysis of variance (ANOVA) followed by the Duncan test, and median values were compared by the Mann-Whitney test. Differences were considered to be statistically significant at P < 0.05. All analyses were performed using the Statistical Packages for the Social Sciences (SPSS, Inc., Chicago, IL, USA).

Results

Table 2 summarizes the anthropometric and hormonal data for patients with PCOS and IH. No significant differences were observed between the two groups of hirsute patients regarding age or clinical score for hirsutism. However, hirsute patients with PCOS presented higher BMI and showed significantly higher levels of testosterone, FAI and LH than the IH group. SHBG concentrations were lower in the PCOS group than in the IH group.

SDR5A2 gene expression was not detected in any scalp hair samples analyzed by RT-PCR in the present study (Figure 1).

Figure 2 shows SDR5A1 mRNA levels in hair cells plucked from the scalp of normal subjects. SDR5A1 expression, presented as arbitrary units in relation to β2-microglobulin absorbance, was similar in men (0.78 ± 0.05) and normal women (0.74 ± 0.06). Furthermore, no significant differences were observed in follicular keratinocyte SDR5A1 expression between normal women (0.85 ± 0.04) and the PCOS (0.78 ± 0.04) or IH (0.80 ± 0.06) hirsute groups (Figure 3).
Discussion

Although skin 5α-reductase activity is associated with hirsutism, the specific role and the identification of the isoenzyme involved in this clinical hair growth condition still need to be better defined. In the present study, we investigated mRNA expression of both types of 5α-reductase in plucked anagen scalp hair cells of hirsute patients.

The SDR5A1 gene was expressed in plucked hair cells obtained from the vertex of the scalp of normal subjects. Other studies have demonstrated similar results, even when SDR5A1 mRNA was investigated in cultured follicular keratinocytes (24,33). Plucked anagen hairs are mainly constituted by keratinocyte cells forming both outer and inner root sheaths. The connective tissue sheath, the lower bulb, the dermal papilla cells and the sebaceous gland are absent in plucked hairs. Therefore, our data confirm the gene expression of SDR5A1 in follicular keratinocytes, and are in agreement with others, which showed 5α-reductase immunoreactivity in root sheath cells of hair follicles (20,23).

In the present study, SDR5A1 mRNA levels from plucked scalp hairs did not differ between normal men and women. Previous studies have also described a similar 5α-reductase activity in hair follicles from men and women (12,34). In contrast, a higher 5α-reductase activity in pubic skin samples was detected in normal men than in normal women (1). Taken together, these results suggest that in normal individuals 5α-reductase regulation seems to differ between follicular keratinocytes of scalp and pubic skin fibroblasts.

Scalp hair follicle cells seem not to be the main target of hirsutism. However, there are some ethical difficulties in obtaining samples from the face of hirsute patients. Furthermore, there are only few literature reports about the molecular mechanisms of scalp hair follicle cells in the presence of hirsutism (9). The scalp region is a well-known androgen-sensitive site in both sexes and it is relatively easy to obtain a sample of scalp hairs. Therefore, it is interesting to investigate some aspects of androgen metabolism in plucked hair cells from the scalp, particularly when it is possible to compare subjects with an endogenous exposure to higher (PCOS) or normal (IH) circulating androgen levels.

We did not observe any differences in SDR5A1 mRNA levels in the follicular keratinocytes either between hirsute patients and normal women or between normal men and women. Moreover, patients with high serum androgen levels (PCOS group) presented the same SDR5A1 gene expression as those with IH and normal androgen levels. While SDR5A1 is the isoenzyme predominating in the skin (14), the present results indicate that circulating androgens probably do not contribute to SDR5A1 gene expression in scalp follicular keratinocytes, suggesting that SDR5A1 is not the key isoenzyme in the local androgen metabolism of scalp skin. On the other hand, the efficacy of the 5α-reductase inhibitor finasteride in the treatment of male pattern hair loss (35), as well as in IH has been demonstrated (36,37). Finasteride is an orally active inhibitor that preferentially blocks 5α-reductase type 2, but may also inhibit the type 1 isoenzyme, causing a marked decline in dihydrotestosterone and 3α-androstenediol glucuronide levels. It has neither affinity for the androgen receptor nor androgenic, estrogenic, progestational, or other steroidal effects (38). In agreement with our results, these studies indicated that 5α-reductase type 2 probably has a more critical role in the hair growth process and related clinical conditions.

The present results show that the SDR5A2 gene was not expressed in the follicular keratinocytes from any subject, men or normal women or hirsute patients. Previous studies have described the preferential localization of SDR5A2 mRNA and enzyme activity...
(39,40) in the dermal papilla cells, although SDR5A2 immunoreactivity was also found in the keratinocytes of hair follicle (20,22). The apparent discrepancies in protein expression among these studies may be explained, at least in part, by the different methods of immunohistochemical analysis of qualitative data. Concerning the absence of SDR5A2 gene expression, described in the present study, more sensitive methods of gene expression analysis, e.g., real time PCR, will possibly clarify this question in the future.

We have not investigated the gene expression of 5α-reductase isoenzymes in other follicular compartments like dermal papillae because these cells are not present in plucked isolated hairs. The dermal papilla cells are obtained only by excision biopsy, which is an invasive and stressing method. However, it will be very interesting to investigate 5α-reductase isoenzymes in the dermal papilla cells from hirsute patients, since it has been suggested that these cells can be the direct target of androgens in hair follicles, regulating the activity of the hair matrix, melanocytes and keratinocytes with paracrine signals (10).

SDR5A1 gene expression in the follicular keratinocytes from the vertex area of the scalp seems not to be related to differences in hair growth observed between normal men and women and hirsute patients. More investigations about the regulation of 5α-reductase gene expression in hair follicle cells at different body sites may help to elucidate the intriguing mechanism of androgen action on the hair growth process and associated diseases.

References

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