Androgen Receptor Expression in Human Ovarian and Uterine Tissue of Long Term Androgen-Treated Transsexual Women

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Androgen receptor (AR) modulation in human uteri and ovaries of long term androgen-treated transsexual female patients was investigated. Androgen receptor expression was evaluated immunohistochemically in the ovaries of 11 and the endometria and myometria of six androgen-treated transsexual female patients. This was compared with AR expression in the ovaries and uteri of premenopausal and postmenopausal women not receiving treatment and in 10 ovaries of female patients with polycystic ovarian disease (PCOD). In the normal ovaries germinal epithelium, granulosa cells of antral follicles, corpus luteum, and theca and stromal cells exhibited moderate AR expression. The more intense and uniform staining of ovarian stroma of female transsexual patients and those of patients with PCOD compared with ovarian stroma of normal controls was most remarkable. This similarity in histology and distribution of ARs supports the hypothesis that PCOD is an androgen-mediated disorder. Immunostaining for ARs was only occasionally detectable in the uteri of premenopausal and postmenopausal women. In contrast, myometrial and endometrial stroma of the uteri of female transsexual patients displayed an intense and diffuse staining, but glandular epithelia remained unstained. Western blot analysis of the ovaries and uterine myometrial tissue samples from transsexual female patients confirmed the presence of the 110-kd AR molecule. Because the androgen treatment of some transsexual female patients was discontinued 6 weeks before they underwent hysterectomy, our data indicate a stable and persistent androgen-induced up-regulation of AR expression in ovaries.

Women with female gender dysphoria (transsexualism) who prepare for gender reassignment receive prolonged androgen treatment while awaiting reassignment surgery. This group of patients receiving a high dose of exogenous androgen over a prolonged period of time provides a unique and ethically acceptable opportunity to study the morphological and endocrinological effects of androgen on ovary and uterus. Histological changes in the genital tracts of transsexual female patients who received long term androgen therapy were described by Miller et al and Futterweit et al. Histological examination showed marked changes in cervical epithelia and a variable degree of endometrial atrophy. The ovaries showed an increased number of follicular cysts and an occasional corpus luteum. The finding of similar histological changes in ovaries of women exposed to exogenous androgens and in patients with polycystic ovarian disease (PCOD) suggests that the morphological changes observed in the latter indeed result from excess androgens. It is not clear whether these androgens mediate effects by direct interaction with androgen receptors (AR) in the target cell or after their conversion into estrogens by estrogen receptors.

The production of a monoclonal antibody designated F39,4 specific for human ARs has permitted us to employ a simple immunohistochemical method for light microscopic visualization of ARs in normal human tissues, including testis, prostate, and cervix. A limited number of studies have described the localization of ARs in human endometrium. Hild-Petito et al demonstrated the presence of ARs in the follicle and corpus luteum of primate ovary. Recently, the immunohistochemical localization of ARs in human ovary was studied at various stages of the menstrual cycle and follicular development. We also showed immunohistochemically the presence of ARs in ovarian carcinomas and granulosa cell tumors of ovary. The immunohistochemical findings from the latter studies make it likely that in human ovary androgens may influence follicular recruitment and growth by a direct interaction of androgens with ARs.

This study was designed to investigate whether exposure of ovarian tissue to androgens results in altered AR expression in the different cell types. For that purpose AR expression was evaluated in normal ovaries and compared with that in ovaries of female transsexual patients treated with large doses of androgen and in ovaries of patients with PCOD. The histological changes observed in endometria of androgen-treated women could be the result of either a direct interaction of androgens with ARs or an indirect interaction by way of estrogens produced by aromatization of androgens. To explore the possibility of the former we examined the presence of ARs in endometria and myometria of uteri obtained from androgen-treated female transsexual patients.

MATERIALS AND METHODS

Patients

Thirteen ovaries were obtained from 11 female-to-male transsexuals who underwent hysterectomy and bilateral oo-
phorectomy. Mean age at the time of surgery was 25 years (range, 18 to 35 years). Menstrual cycles were regular in all but three women before androgen therapy was started. Testosterone treatment was given for a mean period of 21 months (range, 11 to 72 months) before surgery. One normal ovary frozen at autopsy, four paraffin-embedded ovaries from premenopausal women 31 to 46 years old, and seven ovaries from postmenopausal women who underwent surgery for nonendocrine reasons provided a total of 12 normal ovaries as controls. Ten ovaries from patients 20 to 49 years old with documented PCOD, including one ovary frozen at autopsy, also were available.

The following testosterone preparations were administered before surgery (Table 1): (1) 250 mg of Sustanon (30 mg of testosterone propionate, 60 mg of T phenylpropionate, 60 mg of T isohexanoate, and 100 mg of T undecanoate; Organon, Oss, The Netherlands) was given intramuscularly once every second week for a mean period of 25 months (range, 12 to 72 months) to three women; (2) 120 to 150 mg/d of Andriol (10 mg of T undecanoate; Organon) was given orally for a mean period of 15 months (range, 12 to 21 months) to seven women; and (3) 125 mg/d of dihydrotestosterone (Vrij Universiteit Hospital Pharmacy, gel preparation) was given orally for a mean period of 15 months (range, 12 to 21 months) to seven women; and (3) 125 mg/d of dihydrotestosterone. Ten ovaries from patients 20 to 49 years old with documented PCOD, including one ovary frozen at autopsy, also were available.

Uterine specimens were available from six female-to-male transsexual patients to whom testosterone treatment was administered for a period of 14 to 96 months until surgery. Their ages ranged from 22 to 37 years (average, 27 years). Menstrual cycles were regular in all but three women before androgen therapy was started. Testosterone treatment was given for a mean period of 21 months (range, 12 to 72 months) to three women; (2) 120 to 150 mg/d of Andriol (10 mg of T undecanoate; Organon) was given orally for a mean period of 15 months (range, 12 to 21 months) to seven women; and (3) 125 mg/d of dihydrotestosterone (Vrij Universiteit Hospital Pharmacy, gel preparation) was given orally for a mean period of 15 months (range, 12 to 21 months) to seven women; and (3) 125 mg/d of dihydrotestosterone.

**Immunohistochemistry**

Cryostat sections, 5-μ thick, air dried, and subsequently fixed in 4% buffered formalin for 10 minutes, were dehydrated in chilled methanol and acetonite. After washing in phosphate-buffered saline (PBS) at pH 7.4, the slides were incubated with 5% nonimmune rabbit serum (Dakopatts, Glostrup, Denmark) diluted in PBS to block nonspecific antibody binding and immediately incubated overnight with a 1:10,000 dilution of F93.4 ascitic fluid in PBS. To compare immunoreactivity of different ovarian sample sections were incubated with different titers of F93.4. Titration of the antibody F93.4 was done by 10-fold dilutions of the original dilution (1:10,000) in PBS. Immunoreactivity was visualized with a two-step peroxidase antiperoxidase technique as described previously. Hydrogen peroxide and diaminobenzidine were used as substrates. In negative controls the primary antibody was replaced by an irrelevant monoclonal antibody or PBS. A semiquantitative estimation of AR positivity was performed by scoring the intensity of staining as focally positive (±), positive (+), or intensely positive (+ + +).

Paraffin sections, 5-μ thick, were cut and mounted on 3-aminopropyltriethoxysilane-coated slides (Sigma, St Louis, MO) to prevent detachment resulting from the high temperature present during antigen retrieval treatment. The sections were placed in a 60°C oven for overnight drying. After deparaffinization and rehydration endogenous peroxidase activity was blocked with 3% H2O2 in methanol. Sections then were placed in a plastic box containing 200 mL of 0.1 mol/L citric acid monohydrate (Merck, Darmstadt, Germany) adjusted to pH 6.0 with NaOH and heated in a microwave oven (Miele M696, Vianen, the Netherlands) for 3 × 5 minutes at 700 W, during which time the fluid level was checked every 5 minutes and filled when necessary. The sections were allowed to cool to room temperature and were rinsed with PBS. They were then preincubated for 15 minutes at room temperature with nonimmune goat serum (Dakopatts, Glostrup, Denmark) diluted in PBS (1:10). Next the primary antibody F93.4, diluted 1:5000 in PBS, was applied for overnight incubation at 4°C. After rinsing in PBS the sections were subsequently incubated with biotinylated goat antirabbit antibody (Dakopatts) diluted in PBS (1:400) for 30 minutes in streptavidin-biotin complex (Strept-ABC; Dakopatts) for 30 minutes.

**Table 1**. Posttreatment and Posttreatment Serum Hormonal Levels from Eight Female Transsexual Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Date of Birth</th>
<th>Start of Androgen Treatment/ Date of Surgery</th>
<th>Androgen Treatment</th>
<th>LH (μ/L)</th>
<th>FSH (μ/L)</th>
<th>E2 (pmol/L)</th>
<th>TE (nmol/L)</th>
<th>PR (μ/L)</th>
</tr>
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<tbody>
<tr>
<td>1*</td>
<td>08-03-63</td>
<td>07-88/12-89</td>
<td>Andriol</td>
<td>7.7</td>
<td>0.1</td>
<td>5.5</td>
<td>2.5</td>
<td>181</td>
</tr>
<tr>
<td>2*</td>
<td>05-11-63</td>
<td>08-85/07-90</td>
<td>Andriol</td>
<td>3.5</td>
<td>2.5</td>
<td>640</td>
<td>181</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>09-14-65</td>
<td>19-88/06-90</td>
<td>Andriol</td>
<td>9.5</td>
<td>10.5</td>
<td>11.9</td>
<td>9.8</td>
<td>2.7</td>
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<tr>
<td>4</td>
<td>06-28-67</td>
<td>08-88/02-90</td>
<td>Andriol</td>
<td>—</td>
<td>9.6</td>
<td>9.4</td>
<td>159</td>
<td>1.9</td>
</tr>
<tr>
<td>5</td>
<td>06-10-55</td>
<td>06-76/05-90</td>
<td>Testoviron</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>12-18 86</td>
<td>01-80/12-89</td>
<td>Andriol</td>
<td>4.5</td>
<td>9.3</td>
<td>18.7</td>
<td>8.9</td>
<td>618</td>
</tr>
<tr>
<td>7</td>
<td>03-22-55</td>
<td>05-84/05-90</td>
<td>Sustanon</td>
<td>4.7</td>
<td>1.6</td>
<td>5.4</td>
<td>6.6</td>
<td>344</td>
</tr>
<tr>
<td>8</td>
<td>03-29-68</td>
<td>08-89/06-90</td>
<td>DHT</td>
<td>8.7</td>
<td>1.2</td>
<td>3.0</td>
<td>1.2</td>
<td>231</td>
</tr>
</tbody>
</table>

**Abbreviations:** LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, estradiol; TE, testosterone; PR, progesterone.

* Pretreatment cycle irregular. Androgen treatment: Andriol 40 mg two tablets twice daily; Sustanon 250 mg IM/2 wk.
† Pretreatment level.
‡ Posttreatment level.
and in 3,3'-diaminobenzidine tetrahydrochloride (Fluka, Basel, Switzerland) for 7 minutes. Between each incubation step the sections were rinsed three times in PBS. Negative controls were included, replacing F39.4 antibody with PBS for all the tissues investigated. Mayer's hematoxylin was used for nuclear counterstaining.

**Western Blot Analysis**

Western blot analysis was performed on ovarian and uterine tissues of androgen-treated female transsexual patients confirming the presence of human ARs. Frozen tissue specimens were pulverized under liquid nitrogen. One hundred milligrams of the resulting powder was suspended in 200 µL of buffer (40 mmol/L of Tris, 1 mmol/L of ethylenediaminetetraacetic acid, 10% [v/v] glycerol, 0.08% [w/v] sodium decylsulfate [SDS], 10 mmol/L of dithiothreitol [DHT], 0.5% [w/v] deoxycholate, and 1% [w/v] Triton X-100) in the presence of inhibitors of proteolytic breakdown (0.6 mmol/L of phenylmethylsulfonyl fluoride, 0.5 mmol/L Betancorin, and 0.25 mmol/L of leupeptine). After lysis for 20 minutes on ice the suspension was centrifuged for 10 minutes at 1,700 g. The supernatant was stored at -80°C until use. From 200 µL of tissue lysate and 50 µL of LNCaP cytosol ARs were immuno-precipitated with AR-specific monoclonal antibody F39.4 complexed to goat-antimouse immunoglobulin G agarose, followed by SDS-polyacrylamide gel electrophoresis, as described previously. Western blotting was performed with monoclonal antibody F39.4. The procedure for protein detection was described in detail previously.

**RESULTS**

**Histomorphological Features of Ovaries and Uterine Tissue From Female Transsexual Patients**

Transsexual ovaries from female transsexual patients were grossly enlarged and displayed an increased number of cystic follicles and atretic follicles (Fig 1); ovarian cortices were collagenized and thicker (Fig 2). Theca interna hyperplasia and luteinization were uniformly observed in cystic follicles. Stromal hyperplasia accompanied frequently by clusters of luteinized cells and diffuse, intense staining of ovarian stroma (Fig 3) was a constant finding. Histological examination of the uteri of female transsexual patients showed inactive endometria. The glands were small and tubular, mostly lined by a single layer of cuboidal or low columnar epithelium, and a few glands displayed slight stratification. No mitoses were seen. In two cases endometria showed atrophy with small, tubular, rather sparse glands. Endometrial stroma showed no significant changes. In one of these cases tubal metaplasia was present. No endometrial samples showed any evidence of secretory activity. The myometria in the uteri of female transsexual patients were histologically unremarkable.

**Androgen Receptor Expression in Normal Ovarian Tissue**

In the seven ovaries from postmenopausal women the germinal (coelomic) epithelia and the epithelial lining of inclusion cysts showed strong nuclear reactivity for ARs. The underlying stroma exhibited a patchy staining reaction for ARs (Fig 3). Cryostat sections of the ovarian tissue obtained at autopsy as well as the four normal paraffin-embedded ovaries from premenopausal women showed positive nuclear staining of granulosa cells and a small proportion (10%) of stromal cells (Fig 4). The corpus lutea demonstrated positive nuclear staining for ARs, but some selective cytoplasmic staining also was observed. The same cytoplasmic staining was found in control sections using a nonspecific antibody indicating that the cytoplasmic staining of lutein cells was nonspecific. Germinal epithelia and stroma always were negative in the control sections.

**Androgen Receptor Expression in Ovarian Tissue of Female Transsexual Patients and Patients With PCOD**

In 13 ovaries obtained from 11 female transsexual patients ovarian stroma showed a diffuse and intense nuclear AR expression. Granulosa and thecal cells of the follicle cysts present in 11 ovarian samples exhibited strong nuclear reactivity as well (Fig 5). Ovarian tissue from one frozen ovary and nine paraffin-embedded ovaries from patients with PCOD showed similar findings with intense nuclear staining of granulosa and thecal cells and diffuse, intense staining of ovarian stroma (Fig 6). Data on AR expression in ovaries of normal controls and patients with PCOD are listed in Table 2.

To substantiate our impression (judging from the intensity of immunohistochemical staining) that ovaries from female transsexual patients had higher levels of ARs than did ovaries from normal postmenopausal women, titration was performed with F39.4 in three ovaries from normal controls and three ovaries from female transsexual patients. In contrast to the ovaries from normal postmenopausal women in which the staining was heterogeneous (with variation in intensity and a lower percentage of positive cells), virtually all ovarian stromal cells from female transsexual patients remained positive even at the greatest dilution of 1:32,000 (Table 2). The intensity of staining of stromal cells from female transsexual patients diminished with higher dilutions showing a dilution response and proving that the staining of stromal cells was not an artifact.
Androgen Receptor Expression in Uterine Tissue of Female Transsexual Patients and Normal Women

Myometrial and endometrial stromal cells from all six uteri of female transsexual patients displayed an intense nuclear reaction for ARs. In contrast, endometrial glandular epithelia remained completely negative (Figs 7 and 8). Uteri of premenopausal women tested generally were negative for AR in myometrium and endometrium, except in two cases in which a focal and faint staining of endometrial stromal cells and of smooth muscle cells of myometrium was seen.

Three uteri of postmenopausal women exhibited a moderately intense AR expression in myometrium, and in one of these endometrial stroma also contained focal areas with a faint nuclear reactivity for F39.4 (Table 3). None of the uterine tissues studied displayed staining of blood vessel walls.

Western Blot Analysis

With F39.4 a 110-kd doublet was observed in uterine tissues of female transsexual patients corresponding to the well established molecular weight of the phosphorylated and nonphosphorylated variants of AR (Fig 9). In the uterine tissue of a nontreated premenopausal woman this 110 kd doublet also was detectable. The large low molecular weight band in the Western blot of the uteri of androgen-treated female transsexual patient represents degradation products. The strong variation in degradation of the 110-kd AR molecule precludes a reliable quantitation of AR on the basis of Western blot analysis.

DISCUSSION

To date a limited number of studies have described the presence of ARs in human endometrium. In our study we detected the presence of ARs in human endometrium and myometrium and compared the localization of AR in normal human ovary with that in the ovaries of female transsexual patients receiving large doses of androgen.

Cytosolic detection of ARs in nuclear fractions of
rat ovaries by classical binding methods was reported by Schreiber et al.\textsuperscript{17} and Schreiber and Ross.\textsuperscript{18} Our results on human ovarian tissue showing the presence of ARs in granulosa-thecal cells, stroma, and corpus luteum confirm those of Horie et al.\textsuperscript{19} in human ovary. In the ovaries of rhesus monkeys ARs were observed in germinal epithelium, stromal fibroblasts, granulosa cells, and corpus luteum.\textsuperscript{8} The results of these studies are consistent with the concept that androgens interacting with ARs play an essential role in follicular growth and maturation, atresia, and luteinization in an either autocrine or paracrine manner. They are thus consistent with the hypothesis that androgens could play a direct role in ovarian function regulation via a receptor-mediated mechanism.\textsuperscript{11,19}

Distinct from ARs, estrogen receptors are selectively localized in germinal epithelium and not in antral follicle, corpus luteum, or stromal cells of normal ovary. Progesterone receptors have been detected in stromal and thecal cells and not in granulosa cells.\textsuperscript{9} Ovaries of the female transsexual patients exhibited an intense AR expression by the granulosa-thecal cells of the follicles as well as by stroma. The presence of AR molecules in the ovaries and uterine tissues of androgen-treated female transsexual patients was further substantiated by Western blot analysis showing the characteristic 110-kd doublet in tissue homogenates. Our titration study demonstrated that AR expression by ovarian stromal cells in the ovaries of androgen-treated female transsexual patients is more uniform and more intense than it is in the ovaries of nontranssexual (premenopausal and postmenopausal) women. We also demonstrated in 10 ovaries from women with PCOD that the stromal cells exhibited a similar diffuse and intense AR staining as is the case in the ovaries of androgen-treated female transsexual patients. The hyperplasia of stromal cells in the ovaries of androgen-treated female transsexual patients and the ovaries of patients with PCOD provides an additional argument for androgen-mediated functional effects in human ovary. In PCOD stromal hyperplasia may be a consequence of either prolonged exposure to elevated androgens or of hypersensitivity of the stromal cells to androgens. The increased presence of ARs in the hyperplastic stroma of ovaries of patients with PCOD and in ovaries of androgen-exposed female transsexual patients implies a functional role of AR in this morphological change.

It should be noted that in most of these female transsexual patients androgen therapy was discon-

**TABLE 2.** Androgen Receptor Immunohistochemical Expression in Ovaries of Normal Controls and Female Transsexual Patients

<table>
<thead>
<tr>
<th></th>
<th>Ovarian Stroma</th>
<th>Granulosa Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ovary</td>
<td>±± (5/12)†; + (7/12) + (12/12)</td>
<td>\n</td>
</tr>
<tr>
<td>transsexual patient</td>
<td>++ (13/13)</td>
<td>+ (5/10); ++ (7/10)</td>
</tr>
<tr>
<td>Ovary from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>patient with PCOD</td>
<td>+ (1/10); ++ (9/10)</td>
<td>+ (1/10); ++ (9/10)</td>
</tr>
</tbody>
</table>

* ±, +, ++, intensity of staining.
† Fraction of examined cases.
AR gene constructs demonstrated that AR half-life is prolonged and persists for a considerable time after removal of the inducing agent. Apparently the androgen-induced upregulation of ARs in ovarian stroma is quite stable and persists for a considerable time after removal of the inducing agent.

Ruizeveld et al. studied AR expression immunohistochemically in several human tissues and showed AR localization in male sex organs, vagina, and cervix, although in a small series of uterine tissues no immunoreactivity for ARs was detectable. In this larger series of uteri of premenopausal and postmenopausal women focal AR expression occasionally was detected with immunohistochemistry in human endometrial stroma and myometrium. Western blot analysis of untreated uterine tissue confirmed the presence of a 110-kD AR molecule but did not allow quantitation. The observed increase in AR expression in the ovaries, and particularly in the endometria and myometria of female transsexual patients receiving long-term androgen therapy, may be the result of androgen-mediated up-regulation of AR expression. Considering the high turnover of endometrial stromal cells, the possibility of replacement of AR-negative stromal cells by AR-positive stromal cells should be considered as well. Because the turnover of myometrial as well as ovarian stroma is low, the observed androgen-induced intense positivity with anti-AR must be a consequence of androgen-induced AR up-regulation within the same cells.

Autoregulation of AR expression has been described in a few experimental models. In a human prostate carcinoma cell line androgen ablation has been shown to down regulate AR expression. Moreover, several investigators showed in animal models that androgens up-regulate AR expression. Autoregulation of ARs may occur at the transcriptional or posttranscriptional level. Studies performed by Kemppainen and coworkers showed that AR gene constructs demonstrated that AR half-life is prolonged and persists for a considerable time after removal of the inducing agent.

In the ovaries of female transsexual patients the presence of numerous cystic follicles is associated with hyperplasia of ovarian stromal and thecal cells. In contrast, the endometria of female transsexual patients showed an inactivation or even atrophy of the glandular compartment. Stromal cells were not significantly affected. The myometria of female transsexual patients examined by us showed no apparent atrophy or hyperplasia. However, functional changes in myometrium may be induced by androgens. Thus, Roy et al. demonstrated a severalfold increase in NAD-dependent uterine prostaglandin 15-hydroxydehydrogenase in androgen-treated female transsexual patients.

The lack of ARs in glandular epithelial cells suggests that the glandular changes are not a consequence of direct interaction of androgen with these epithelial cells. It remains to be established whether the androgen effect on endometrial glands is mediated by a paracrine effect on stromal cells. It is not likely that the inactivation of endometrial glands is caused by an altered exposure to hypophysis-derived gonadotropic hormones. Previous studies showed that androgen treatment does not substantially alter serum levels of follicle stimulating hormone or luteinizing hormone. The variety in morphological effects of androgens on the female genital tract implies that other regulatory proteins also determine the outcome of the interaction of androgens with ARs in these organs.

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REFERENCES


TABLE 3. Immunohistochemical Analysis of Androgen Receptor in Endometria and Myometria of Female Transsexual Patients and Premenopausal and Postmenopausal Women

<table>
<thead>
<tr>
<th></th>
<th>Endometria (Stroma)</th>
<th>Myometria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenenopausal women</td>
<td>2± (n = 7)</td>
<td>2± (n = 7)</td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td>1± (n = 7)</td>
<td>3± (n = 7)</td>
</tr>
<tr>
<td>Female transsexual patients</td>
<td>6± (n = 6)</td>
<td>6± (n = 6)</td>
</tr>
</tbody>
</table>

* Number of cases positive.
| + , ++ +, ++ ++, intensity of staining.
| Number of cases tested.


