Elevated Endometrial Androgen Receptor Expression in Women with Polycystic Ovarian Syndrome¹

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ABSTRACT

Androgen receptors (AR) have been identified in human endometrium; however, their role in endometrial cyclic development and function remains poorly understood. The objective of the present study was to investigate the profile of endometrial AR in normal menstrual cycles and in the endometrium of women with polycystic ovarian syndrome (PCOS). This syndrome is characterized by chronic hyperandrogenism and oligo-ovulation, and it is often associated with poor reproductive performance. Using immunohistochemistry and reverse transcription-polymerase chain reaction, we found that women with PCOS exhibited elevated endometrial AR expression compared to normal, fertile controls. This increase was most apparent in glandular and luminal epithelium. Furthermore, when compared to endometrium from fertile women, PCOS endometrium showed other abnormalities in endometrial development, including delay or absence of the αβ v integrin, a well-characterized biomarker of uterine receptivity described previously (Lessey et al., JCI 1992; 90:188–195). To better understand and to gain insights regarding these findings, we used in vitro cell-culture models to study the regulation of AR in primary endometrial stromal and the well-differentiated epithelial cell line (Ishikawa). Based on Western blot analysis, epithelial AR is up-regulated by estrogens and androgens and is inhibited by progesterins and epidermal growth factor (EGF). On the other hand, EGF significantly induced the expression of αβ v, whereas estrogen and androgen treatment inhibited its expression. Collectively, these results suggest that the poor reproductive performance observed in women with PCOS may be due, in part, to the concomitant increase in both serum androgens and elevations in endometrial AR. This combination may reduce endometrial receptivity as judged by the down-regulation of αβ v integrin.

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INTRODUCTION

The primary function of the endometrium is to prepare for and to sustain a pregnancy. As a steroid hormone-responsive tissue, the endometrium undergoes cyclic growth and development, followed by shedding and regeneration. Much is known regarding the effects of estrogen and progesterone in this tissue [1], acting through specific receptor proteins [2, 3]. The secretory phase of the menstrual cycle is controlled largely by progesterone, and this sex steroid hormone is absolutely required for normal implantation and pregnancy [4]. On the other hand, little is known regarding the role of androgens and the androgen receptor (AR) in the cycling endometrium of women. Similarly, still less is known regarding the distribution or role of AR in the endometrium of women with chronic hyperandrogenism and anovulation. This condition, known as polycystic ovarian syndrome (PCOS), was described by Stein and Leventhal in 1935 [5] and is commonly observed in reproductive-aged women [6]. Women with PCOS are often infertile based on ovulation failure [7]. Once ovulation is restored, however, this group exhibits a surprisingly poor reproductive potential [6, 7], with a higher-than-expected rate of spontaneous miscarriage [6–9].

Expression of AR has been detected in endometrium from normal women and from women with endometriosis [10–14]. Some studies have reported relatively constant expression of AR throughout the cycle [12]; other studies have documented variation in endometrial AR, with increased expression during the proliferative phase [13, 14]. Hormonal regulation of AR was recently demonstrated in both primates and in human endometrium [13]. In that study, stromal AR was noted to be higher in the proliferative phase than in the secretory phase, and both stromal and epithelial AR staining increased following treatment with an antiprogestin. In certain endocrinopathies, such as PCOS, both serum estrogen and androgen levels are significantly elevated, and progesterone levels are chronically reduced [6]. Surprisingly little is known regarding the effect of anovulation and/or hyperandrogenism on AR in the endometrium, and to our knowledge, no published reports describe the profile of AR expression in the endometrium of women with PCOS. Because it now appears that endometrial AR are up-regulated by estrogens [15, 16] and inhibited by progesterone [13], we hypothesize that women with PCOS will have demonstrable differences in endometrial AR levels compared with those of normally cycling women.

To investigate this important clinical question, we have examined endometrial samples from normal, fertile women during the menstrual cycle and compared these to endometrium from women with PCOS during the proliferative
and secretory stages of the menstrual cycle. Furthermore, to better understand the regulation and role of AR in human endometrial stroma and epithelium, we used validated and well-defined in vitro cell-culture models, including primary human stromal cells and the well-differentiated adenocarcinoma Ishikawa cell line. This endometrial cell line, first developed by Nishida et al. [17], contains both a functional estrogen receptor (ER) [18, 19] and an estrogen-inducible progesterone receptor (PR) [19, 20]. We have previously shown that the PR is functional in these cells by examining the induction of specific progesterone-responsive genes, such as the α2 integrin subunit [21]. Using this in vitro model, we have recently demonstrated that AR are up-regulated by estrogen and down-regulated by progesterones [16].

In the present study, we investigate the regulation of epithelial and stromal AR by steroid hormones, and we present data to suggest that androgens up-regulate their own receptor in the human endometrium. By examining a well-characterized marker of uterine receptivity, the α2β2 integrin [22], we explored the implications of high endometrial AR using the Ishikawa cell model. We believe that these novel findings provide the groundwork for future studies regarding implantation defects in women with PCOS that will likely advance our understanding of the role of androgens in the female reproductive tract in both health and disease.

MATERIALS AND METHODS

Human Samples

Endometrium was obtained from reproductive-aged women at various times within the menstrual cycle at the time of bilateral tubal ligation or by urinary LH-timed endometrial biopsy. Tissue was obtained from 95 normal, fertile volunteers without ovulatory dysfunction in the early (n = 42), mid (n = 21), and late (n = 21) secretory phase. Endometrial biopsies were also obtained from 31 women with PCOS in the proliferative (n = 7) and secretory (n = 24) phases by pipelle sampling. In these women, PCOS was diagnosed based on a history of oligo-menorrhea, infertility, and/or hirsutism. Although the patients with PCOS were generally heavier in our study, weight was not considered to be a primary diagnostic criteria. To examine the incidence of uterine receptivity defects in PCOS and to compare these samples with those of fertile and infertile moles, we explored the implications of high endometrial AR using the Ishikawa cell model. We believe that these novel findings provide the groundwork for future studies regarding implantation defects in women with PCOS that will likely advance our understanding of the role of androgens in the female reproductive tract in both health and disease.

Immunohistochemistry

Immunohistochemistry for both AR and the β2 subunit was performed on 5- to 6-μm sections of formalin-fixed, paraffin-embedded endometrial biopsies using the Vectastain Elite ABC kits (Vector Laboratories, Burlingame, CA). Diaminobenzidine (DAB; Sigma Chemical Co., St. Louis, MO) was used as the chromogen. Tissue sections were deparaffinized in xylene and hydrated in a series of graded ethanol. Following a PBS rinse, the endogenous peroxidases were quenched on incubation for 30 min with 0.3% (v/v) H2O2 in absolute ethanol, followed by a 10-min rehydration in PBS. For AR, the tissue sections were heated in a microwave for 12 min in 30 min in antigen retrieval solution (10 mM citrate buffer) before the incubation with primary antibody. After initial incubation with blocking serum for 30 min at room temperature (4% normal goat serum), rabbit polyclonal antibody raised against the AR (PG-21; generously provided by Gail Prins, Ph.D., University of Illinois at Chicago, Chicago, IL) or the β subunit of the AR (SSA6; provided by Jim Hoxie, University of Pennsylvania, Philadelphia, PA) were applied at a concentration of 4 μg/ml and 1:400 (v:v), respectively, and incubated at 4°C overnight. The negative controls were run on adjacent sections without the primary antibody (secondary nonimmune rabbit antibody only). The PG-21 antibody has previously been characterized and validated [24]. The specificity and the use of the α2β2 antibody (SSA6) has also previously been reported [22]. Each primary antibody was serially diluted to optimize the concentrations used to achieve maximum sensitivity and specificity. A PBS rinse (pH 7.4) was followed by treatment with secondary antibody consisting of biotinylated goat anti-mouse (for β2) or biotinylated goat anti-rabbit (for AR) for 30 min. After this incubation, sections were washed and incubated with avidin-biotinylated horse radish peroxidase macromolecular complex for 60 min. Visualization of the peroxidase was carried out by adding DAB and then incubating for 10 min to complete the reaction. As a final step, sections were counterstained with hematoxylin, dehydrated in a graded series of ethanol, cleared with xylene, and coverslipped over Permount for evaluation by light microscopy. As a positive control for AR immunostaining, human proximal corpus epididymis was used. The resulting stain was evaluated using a Nikon microscope (Tokyo, Japan) by a single observer.

Assessment of staining intensity and distribution was made using the semiquantitative HSCORE scoring system. The HSCORE was calculated using the following equation: HSCORE = ΣPi(i + 1), where i = intensity of staining with a value of 1, 2, or 3 (weak, moderate, or strong, respectively) and Pi is the percentage of stained endometrial stromal and epithelial cells for each intensity, varying from 0%–100%. Low intraobserver (r = 0.983, P < 0.0001) and interobserver (r = 0.994, P < 0.0001) differences for HSCORE in uterine tissues have been previously reported using this technique [25], and similar results have been obtained in our laboratory (B.A.L.: [26]).

Reverse Transcription-Polymerase Chain Reaction

Total RNA was isolated from endometrial tissues using Tri Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s instructions. The total RNA was then reverse transcribed, and cDNA was subjected to polymerase chain reaction (PCR) using primers specific for AR and glyceraldehyde phosphate dehydrogenase (GAPDH) as an internal control. Total RNA (5 μg) was used for reverse transcription (RT) in a total volume of 20 μl using the RT system (Promega, Madison, WI). The RT mainly included an incubation period of 30 min at 42°C with oligo(dT) primer, followed by incubation for 5 min at 99°C to denature the enzyme. The RT product was aliquoted equally into 2 tubes, and PCR was performed in a 50-μl volume using appropriate AR primers in 1 tube (sense, 5′-AGATGGGCTTGTACTTTTCCCAGAAAG-3′; antisense, 5′-ATGGCGTGTCACTACCTGCTGAG-3′) and GAPDH primer in another tube to serve as an internal control. In preliminary experiments, we optimized the amount of cDNA subjected to PCR as well as the number of cycles, and we showed that the cDNA of interest were amplified linearly between 15 and 30 cycles of PCR. The PCR amplification of AR gave the predicted product size of 545 base pairs (bp), whereas GAPDH yielded a product of 192 bp. The PCR reaction mixture consists of 1× PCR buffer, 2.0 μM MgCl2, 200 μM of each dNTP, 1.25 U of Taq polymerase and 50 μM of each primer. The PCR amplification was carried out as follows: an initial denaturation at 94°C for 10 min; 30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min; and a final extension of 10 min at 72°C. The PCR products were electrophoretically resolved on 1.5% agarose gel, stained with ethidium bromide, and photographed.
Hormone Treatments of Cells

Ishikawa cells, the well-differentiated endometrial carcinoma cell line, and primary endometrial stromal cells were cultured in DMEM/F12 media in the presence of 5% charcoal-stripped FCS to 70% confluence. Cells were then cultured in media with or without various hormones individually or in combination with their antagonists, such as IC182790 (10^{-7} mol/L; Zeneca Pharmaceuticals, Wilmington, DE), RU486 (10^{-5} mol/L; Sigma), hydroxyflutamide (10^{-6} mol/L; Schering Corp., Kenilworth, NJ), and Casodex IC17633 (CAS; 10^{-6} mol/L; Zeneca Pharmaceuticals, Wilmington, DE). Stromal cells were also cultured in the presence of relaxin (10 ng/ml). The hormones were added from 1000-fold concentrated stocks made in absolute ethanol to the desired concentration of 10^{-8} mol/L (10^{-8} mol/L; Sigma), progesterone (10^{-6} mol/L, Sigma), 5α-dihydrotestosterone (DHT; 10^{-6} mol/L; Sigma), diethylstilbestrol (DES; 10^{-8} mol/L; Sigma), and medroxyprogesterone acetate (MPA; 10^{-6} mol/L; Sigma). The DES and MPA were used in addition to or in place of their natural estrogen and progesterone counterpart in an effort to check their efficacy, because DES and MPA are metabolized more slowly in tissue culture. The time of exposure in each case was approximately 3–4 days. In some experiments, cells were also cultured with epidermal growth factor (EGF; 10 ng/ml) in the presence or absence of other steroid hormones.

Western Blot Analysis

Ishikawa and primary stromal cells were cultured in treatment medium consisting of phenol red-free DMEM/F12 media with 0.5% heat-inactivated charcoal-stripped FCS and supplements. Cells treated for 3–4 days with various hormones alone or in combination were harvested individually and pelleted for Western blot analysis. Cell pellets obtained from the respective treatment plates were resuspended in RIPA extraction buffer (10 mM Tris-HCl [pH 8.0], 10 mM EDTA [pH 8.0], 150 mM NaCl, 1% NP-40, and 0.5% SDS) containing protease-inhibitor cocktail (Boehringer Mannheim, Indianapolis, IN). After centrifugation at 10,000 × g for 10 min, protein concentrations were determined using Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA). Total proteins (100 μg from each treatment) were denatured in Laemmli buffer, fractionated using 8% 1D-SDS-PAGE, and transferred to nitrocellulose membrane. Blots were blocked for 1 h at room temperature with peroxidase-conjugated anti-rabbit IgG or anti-goat IgG while rocking. After washing twice for 30 min each time with TBST, the immunoreactive protein complexes were detected using enhanced chemiluminescence (Amersham Pharmacia Biotech Inc., Piscataway, NJ). The blots were exposed to film for 1 h. The bands were identified for endometrial AR expression are shown in Figure 2. In Ishikawa cells, the overall expression of stromal AR is higher than epithelial AR but changed the least during the menstrual cycle. In contrast, both glandular and luminal epithelia exhibited regulated and cycle-dependent expression of AR across the menstrual cycle. The HSCORE is a semiquantitative assessment of the intensity and distribution of staining and was calculated as described in Materials and Methods. Significant differences are indicated by asterisks: early proliferative lumen versus early secretory (P = 0.044) and midsecretory (P = 0.037); late-proliferative stroma versus early secretory, midsecretory, and late-secretory stroma (P < 0.001); and late-proliferative glands versus early secretory (P < 0.001), midsecretory (P = 0.01), and late-secretory (P < 0.005) glands.

Statistical Analysis

Relative levels of AR were estimated using the semiquantitative HSCORE, which is a numerical score ranging from 0 to 4. Comparison between groups was made using ANOVA with the Scheffe correction for multiple comparison. Significance was based on a 95% confidence interval and a P value < 0.05. Comparisons of categorical data was performed using chi-square analysis.

RESULTS

Androgen receptors are known to be expressed in human endometrial cells. In the present study, we first examined the immunohistochemical distribution of AR in normally cycling women. As shown in Figure 1, using the semiquantitative HSCORE assessment of immunohistochemical staining, AR levels were signiﬁcantly increased in the luminal compartment of the early proliferative phase compared to both early secretory (P = 0.044) and midsecretory samples (P = 0.037). Both the stromal and glandular compartment of late-proliferative endometrium expressed greater AR than endometrium in the early secretory (both P < 0.001), midsecretory (P < 0.001 and P = 0.01, respectively), or late-secretory phase (P < 0.001 and P = 0.005). Expression of AR remained higher in the stromal compartment compared to the epithelial compartment throughout the menstrual cycle. The overall staining, however, was reduced in each cell type during the secretory phase compared to the proliferative phase.

Photomicrographs of these immunohistochemical results for endometrial AR expression are shown in Figure 2. In the late-proliferative phase (Fig. 2B), increased epithelial and stromal AR staining was observed (Fig. 2A) compared to that in the early proliferative phase. Human epididymis was used as a positive control for immunohistochemical staining for AR (Fig. 2C). In the early to midsecretory phase, epithelial AR immunostaining was diminished along with reduced stromal AR expression (Fig. 2, D–F). By Day

![Graph: HSCORE values for AR expression in endometrial cells throughout the menstrual cycle.](https://example.com/hscore_chart.png)
FIG. 2. Immunohistochemical localization of AR in human endometria. Immunohistochemical staining was low in the early proliferative phase (A) but increased in the late-proliferative phase (B). During the early secretory (D) and midsecretory phase (E and F), luminal and glandular AR staining were visibly reduced, whereas stromal AR persisted. Also shown are endometria obtained from three different patients with PCOS (G–I) timed to the midsecretory phase (LH + 7–10 days) exhibited increased AR staining on both luminal and glandular epithelium compared to endometria from fertile controls at the same time in the cycle (E and F). Human epididymides were used as a positive control for these studies (C). ×200 (A, B, and D–F) and ×400 (C).

24 (LH + 10 days), AR staining was essentially depleted from the endometrial epithelium (Fig. 2F). We next compared endometrial AR expression in women with PCOS to that in normal, fertile controls. The HSCORE of AR in each cell type was compared between the proliferative and secretory phase, as shown in Figure 3. Overall, a significant increase was observed in epithelial and stromal AR in the endometrium of women with PCOS. Glandular epithelium from PCOS endometrium expressed significantly more AR in both the proliferative and secretory phases (P = 0.012 and P < 0.0001, respectively), but the differences for stromal and luminal cells were only seen in the secretory phase (P = 0.005 and P < 0.0001, respectively). The differences in AR expression as demonstrated by immunohistochemistry are depicted in Figure 2, G–I (representing endometrium obtained from three different women with PCOS during the midsecretory phase), showing a marked increase in epithelial and stromal AR staining compared to that in normal controls. In the control experiments, no immunostaining could be detected in the sections incubated in the absence of primary antibody.

The overall profile of AR mRNA expression in cycling, fertile women and in those with PCOS, as shown by RT-PCR, is depicted in Figure 4. The amount of mRNA used is normalized by GAPDH (Fig. 4, middle panel). For the normally cycling women, a relative increase in AR message was seen during the proliferative phase (labeled P in Fig. 4), with reduced AR expression seen during the secretory phase (Fig. 4, upper panel). Note the apparent increase in overall AR in samples from the endometrium of women with PCOS obtained during the secretory phase compared to that in comparably timed endometrial biopsy specimens from women with normal cycles. A histogram showing the
We have previously reported that women with PCOS exhibit markedly diminished reproductive performance, even once ovulation is restored [7].

FIG. 4. Expression profile of AR mRNA in endometrial tissues obtained throughout the menstrual cycle of normally cycling women and from women with PCOS. Five micrograms of total RNA isolated from tissues at different days of the menstrual cycle were reverse transcribed and subjected to 30 cycles of PCR amplification. Lane C represents the negative control; lanes P (proliferative phase) and 16–28 represent the corresponding days of the secretory phase. The AR is shown (upper panel). As an internal control, the GAPDH mRNA was simultaneously amplified to normalize the RNA concentration (middle panel). Densitometric analysis by stage of the menstrual (proliferative, early secretory, midsecretory, and late secretory) and midsecretory PCOS samples is included (lower panel). PCOS endometria expressed significantly more AR (asterisk) than the amount expressed by proliferative, early secretory, midsecretory, and late-secretory endometria from normal women (P < 0.01 for each).

FIG. 5. Western blot analysis of human AR protein in hormonally treated primary stromal cells and Ishikawa epithelial cells. Western blots were performed as described in Materials and Methods. Stromal cells and the well-differentiated adenocarcinoma cell line (Ishikawa) were cultured in control media containing no hormones or in the presence of various hormone treatments for 3–4 days. Aliquots of 100 μg of total protein were separated on SDS-PAGE and electroblotted on nitrocellulose membrane. The AR protein in stromal cells (upper panel) and in Ishikawa cells (lower panels) was detected by antibody raised against the first 21 amino acids of human AR protein (PG-21). The effect of antiandrogens (hydroxyflutamide and CAS) on DHT-induced AR expression is also shown (lower panel).

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Mean densitometric values for each group demonstrate an increased AR expression in secretory PCOS endometria compared to proliferative (P = 0.004), early secretory (P = 0.001), midsecretory (P = 0.0012), or late-secretory (P = 0.0002) endometria of normally cycling women (Fig. 4, lower panel). On average, by RT-PCR, midsecretory-phase PCOS endometria exhibited a twofold increase in AR message expression compared to that of normally cycling endometria.

To better understand the regulatory mechanisms that account for these noted differences in endometrial AR expression, we studied the regulation of AR in normal endometrial stromal cells and in the well-differentiated Ishikawa cell line. By Western blot analysis, as shown in Figure 5 (upper panel), stromal cells treated with ovarian steroids alone or in combination with their inhibitors showed little regulation of AR expression. Similarly, treatment with relaxin alone or in combination with E2 and progesterone resulted in little change in AR levels. In contrast, Ishikawa cells expressed little AR without treatment, but expression increased significantly after treatment with E2 or DES. This increase was accentuated by the addition of DHT (Fig. 5, lower panel). Increased AR in response to DES was blocked by the addition of progesterone or the estrogen-inhibitor ICI 182780. This increased expression in response to both DES and DHT was further inhibited by the combined addition of the antiestrogen and the antiandrogen hydroxyflutamide in a dose-dependent manner. The increase in AR after DHT was also reduced by an another antiandrogen, Casodex ICI176334 (10−6 M; Bicalutamide). The results shown in each panel of Figure 5 are representative of at least three experiments.

Women with PCOS exhibit markedly diminished reproductive performance, even once ovulation is restored [7]. We have previously reported that women with PCOS appear to have a decreased expression of certain integrins (αvβ3) as markers of uterine receptivity compared to fertile controls [26]. To investigate this observation further, we compared the expression patterns of the αvβ3 integrin in the endometrium of women with PCOS to those found in fertile controls and in women with tubal disease and hydrosalpinges. This second group of infertility patients also exhibit poor reproductive outcome during in vitro fertilization cycles and frequently lack the αvβ3 integrin marker [28]. As depicted in Figure 6, expression of the αvβ3 integrin was frequently absent in the endometrium of PCOS patients (Fig. 6B) compared to fertile controls (Fig. 6A). In comparing the expression patterns of αvβ3 integrin, we were also cognizant of the histologic progression of the endometrium in each group. Because each sample was timed to the midsecretory phase based on a urinary LH surge, a delay in histologic progression by ≥3 days always resulted in a lack of αvβ3 immunostaining based on this histologic delay (type I defects) [29]. Women whose endometrium exhibited “in phase” (expected histology) usually expressed this endometrial marker; those lacking it were recorded as having type II defects [29]. We used a cut-off HSCORE value of ≤0.7, as previously determined, to identify those samples that lacked appropriate expression of the αvβ3 integrin. The cumulative data regarding aberrant expression of this biomarker in these three groups of women are shown in Figure 6C. By chi-square analysis, the three groups were significantly different from each other (P < 0.009). The degree of histologic delay (type I defects) was greater in the hydrosalpinges group compared to that in the normal, fertile controls. The percentage with aberrant αvβ3 expression and “in phase” histology (type II defects) was significantly higher in the PCOS group compared to the fertile control group (P < 0.008). Overall, less than a third of PCOS endometrium would have been judged to be normal by these criteria (histology and endometrial marker protein expression).

Finally, we have previously documented that Ishikawa cells express the αvβ3 integrin in response to EGF and exhibit a reduced level of expression in response to ovarian estrogen [30]. To better understand the results depicted in
Normal and aberrant expression of αvβ3 integrin in the endometria of fertile and infertile women. Immunohistochemical staining for the αvβ3 integrin in endometrium of a normal, fertile woman shows expression mostly in the glandular and luminal (inset) epithelium, with less expression in the stromal compartment (A). In women with PCOS, a large proportion failed to express this integrin, as shown by immunohistochemistry (B; ×200 for both). Overall comparison of putative endometrial receptivity defects in normal endometria of normal, fertile women (Fertile; n = 24), women with hydrosalpinges (Hydrosalpinx; n = 34), and women with PCOS (n = 22) is shown graphically (C). Using a combination of endometrial histology and expression of the αvβ3 integrin as a marker, 71% of normal, fertile endometria were judged to be normal, compared with 43% and 27% of the endometrial samples from hydrosalpinges or PCOS, respectively (P < 0.009). Type I defects were defined as endometria missing the αvβ3 integrin with endometria exhibiting ≥3 days histologic delay. Type II defects were defined as endometria missing the αvβ3 integrin with otherwise “in phase” endometrial histology. The details of this classification system have been previously described [28]. Women with PCOS had a significantly greater prevalence of type I and type II defects compared to normal, fertile controls as determined by chi-square analysis (P < 0.008).

Figure 6, A–C, showing decreased αvβ3 expression in women with PCOS, we cultured Ishikawa cells in control media or in media with added DES, DES plus EGF, DES plus DHT plus EGF, and DHT and EGF alone or in combination. As shown in Figure 7, by Western blot analysis, Ishikawa cells treated with DES demonstrated a trend toward increased AR protein expression, which was further enhanced by the addition of DHT. However, EGF had little or no effect on AR expression in this cell line. Expression of the β3 subunit of the αvβ3 integrin, on the other hand, was substantially increased by treatment with EGF; but this effect was reduced by the addition of DES or DHT. These changes shown in Figure 7 suggest that DHT is a more potent inhibitor of EGF action than is DES using this model.

DISCUSSION

Localization and distribution of AR has been previously documented in human endometrium [10–14]. In the present study, we describe the pattern of expression of endometrial AR in women with PCOS and compare this pattern with that observed in normally cycling women. In agreement with previous reports [13, 14], expression of AR was higher in the stromal compartment than in the epithelial cells of normally cycling endometrium. Similar results were also reported in the uterus of the rhesus monkey [31]. The rise in AR expression observed during the late-proliferative phase was consistent with that described by Fujimoto et al. [15], who reported an increase in endometrial AR in response to estrogen. To our knowledge, what has not been reported previously is the overall increase in AR content in the endometrium of women with hyperandrogenism and PCOS.

We found increased expression of AR in the endometrium of subjects with PCOS using both RT-PCR and immunohistochemistry. By immunostaining, we showed that both epithelial and stromal cells overexpressed AR compared to fertile controls. Using in vitro culture systems, it was further demonstrated that DHT increased the expression or stability of AR in epithelial, but not in stromal, cells. Levels of AR were reduced in the presence of DHT plus the antiandrogens, such as hydroxyflutamide and CAS. Hydroxyflutamide, although an antiandrogen, may also act as an agonist at certain concentrations through stabilization as well as activation [32], and CAS, which possesses high antagonist activity, clearly decreases the DHT-mediated increase in epithelial AR, indicating that DHT up-regulates its own receptor. In sum, the data presented herein concerning the regulation of AR in epithelial cells suggest that the chronic elevation of estrogen and androgens in women with PCOS could account for the overall increase in endometrial AR expression observed in this study.

Hyperandrogenism in women is associated with oligo-
or anovulation, hirsutism, and acne, and it may be present in up to 8% of reproductive-aged women [6]. This disorder was first described by Stein and Leventhal in 1935 [5] and is now commonly referred to as PCOS. Early on, the reproductive outcome of women with this disorder was noted to be poor, with decreased overall cycle fecundity compared to that of normally cycling women [7]. These women are certainly infertile based on ovulatory dysfunction, but once ovulation is restored, these women continue to experience a lower-than-expected rate of conception [33] and a higher-than-anticipated rate of miscarriage [8, 9, 34]. Medical suppression of PCOS in women using gonadotropin-releasing hormone agonists may partially reverse this effect [35], suggesting that this is a hormonal rather than an intrinsic defect. The reason for repetitive pregnancy loss has not been determined, but some believe that it relates to the quality of the endometrium or to possible deficits in progesterone production [36]. The overexpression of endometrial AR, therefore, provides tangible evidence linking the abnormal hormonal milieu to the reported deficiencies in endometrial receptivity [8, 26].

The regulated expression of endometrial steroid receptors appears to be critically important to the establishment of uterine receptivity. In the human, both ER and PR undergo selective down-regulation in the epithelial compartment of the endometrium at the time of implantation [3, 37]. This pattern has now been demonstrated in the endometrium of other mammals, including porcine endometrium [38]. We have postulated that reduced epithelial ER and PR may be a prerequisite for the normal paracrine milieu that triggers normal uterine receptivity [39]. Persistently elevated epithelial PR has been associated with luteal-phase defect and reduced expression of the α3β3 integrin [39]. In normally cycling women, we found that epithelial AR, like its counterparts ER and PR, is normally down-regulated at the time of implantation. Indeed, the levels of AR expression in glandular and luminal epithelium were almost undetectable in most normally cycling women during the secretory phase. In contrast, women with PCOS displayed elevated expression of endometrial AR in glandular and luminal epithelial cells at the time of implantation. This elevation in epithelial AR, analogous to that seen for endometrial PR, is associated with a down-regulation of epithelial integrin expression.

We have previously shown that Ishikawa cells are an excellent model to study hormonally mediated events in endometrial epithelium [16, 20, 21, 30]. These cells maintain functional ER [18, 19] and an estrogen-inducible PR [19, 20]. We have also recently demonstrated that AR is up-regulated by estrogen and down-regulated by progestins in this cell line [16], similar to the pattern of regulation that has been demonstrated both in the present investigation and in others [15]. We and other investigators have also used human stromal cells to study hormone-regulated cell activity [40, 41]. Androgens were shown to increase stromal prolactin [42], similar to the effect seen after treatment with progestins, and apparent regulation of AR by ovarian steroids in primary stromal cells was minimal compared to the regulation of AR in epithelial cells. These results are consonant with other observations of endometrial steroid receptors: 1) ER and PR undergo marked changes in the endometrial epithelial, but not stromal, compartment during the menstrual cycle [3], 2) stromal AR expression, as demonstrated by immunohistochemistry, shows less change over the menstrual cycle compared to epithelial cells, and 3) stromal cells continue to respond to ovarian steroids during and after implantation as part of the decidualization process. Therefore, fundamental differences may exist between the regulatory mechanisms for ER, PR, and AR in endometrial epithelial and stromal cells.

Androgen treatment of estrogen-primed Ishikawa cells demonstrated an antiestrogenic property of DHT, lowering estrogen-induced alkaline phosphatase activity in Ishikawa cells [16]. In contrast, we show here that androgens increase the expression of endometrial AR, suggesting a synergistic effect with estrogens in regulating its own receptor. In the setting of PCOS, in which AR levels are elevated, any endometrial effect of serum androgens is likely to be accentuated compared to that found in normal endometria. As described in this study using in vitro culture systems, androgens potentially down-regulate α3β3 expression, suggesting that the decreased expression of epithelial α3β3 integrin may be one of many potential alterations in endometrial epithelial protein expression that occurs in response to androgens in women with PCOS. Such changes may account for the diminished cycle fecundity that these patients experience.

In summary, patients with PCOS exhibit elevated endometrial AR compared to those seen in fertile controls. An increase in endometrial AR during the putative window of implantation is associated with a decrease in a well-characterized biomarker of endometrial receptivity, the α3β3 integrin. The high miscarriage rate and the low cycle fecundity observed in this group of women may be due, in part, to the combination of elevated serum androgens and higher-than-expected endometrial AR. As a target for steroid hormones, the endometrium is potentially vulnerable to this combined increase in androgenic activity. Future studies will be necessary to fully understand and to appreciate the implications of these observations.
REFERENCES


