17-Hydroxyprogesterone responses to gonadotrophin-releasing hormone agonist buserelin and adrenocorticotropic hormone in polycystic ovary syndrome: investigation of adrenal and ovarian cytochrome P450c17α dysregulation

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Abnormal regulation of cytochrome P450c17α causes the exaggerated secretion of ovarian androgens in polycystic ovary syndrome (PCOS). This enzyme is active in both the ovaries and adrenal glands. We examined whether there is an abnormal regulation of cytochrome P450c17α in the adrenal gland by investigating the relationship of 17-hydroxyprogesterone (17-OH progesterone) hyperresponsiveness to the gonadotrophin releasing hormone (GnRH) agonist, buserelin, testing with 17-OH progesterone response to adrenocorticotrophic hormone (ACTH) in PCOS. In all, 68 women with PCOS and 24 normal women were included in the study. Ultrasound, clinical and hormonal parameters were used to define PCOS. 17-OH progesterone response to ACTH was measured in all the women. In 52 of the 68 women with PCOS, 17-OH progesterone response to buserelin was measured. The mean basal 17-OH progesterone concentrations were similar in both PCOS and control groups. PCOS women had significantly higher net increment in 17-OH progesterone after ACTH administration (P<0.02). No significant correlations were found between the peak 17-OH progesterone values, the net increments in 17-OH progesterone and the area under the 17-OH progesterone–response curves after ACTH stimulation and buserelin test. Although 17-OH progesterone response to ACTH was significantly higher in the patients with PCOS than in the control subjects, the lack of relationship between 17-OH progesterone response to GnRH agonist buserelin and 17-OH progesterone response to ACTH stimulation suggests that the dysregulation of the cytochrome P450c17α enzyme may not play a role in adrenal androgen excess seen in PCOS.

Key words: adrenocorticotrophic hormone/buserelin/cytochrome P450c17α/17-hydroxyprogesterone/polycystic ovary syndrome

Introduction

The role of the adrenal gland in the genesis of polycystic ovary syndrome (PCOS) and the relative contribution of the adrenal gland to androgen overproduction is not well known. Numerous investigators have found elevated androgen production by the adrenal glands in patients with PCOS (Moltz and Schwartz, 1986). Dehydroepiandrosterone sulphate (DHEAS), an androgen formed primarily by the adrenal cortex, was found to be elevated in >50% of patients with PCOS (Hoffman et al., 1984). Using selective catheterization of the adrenal and ovarian vessels, some hyperandrogenic women demonstrate significant adrenal androgen hypersecretion (Moltz et al., 1984). Approximately 40–60% of hyperandrogenic women have excessive responses of adrenal androgens to adrenocorticotropic hormone (ACTH; Ehrmann et al., 1992; Barnes, 1994). The mechanism responsible for the adrenal androgen excess in women with PCOS is still an unresolved problem. In patients with PCOS, studies conducted under basal conditions and in response to a sustained ACTH stimulation revealed significantly higher concentrations of 17-hydroxyprogesterone (17-OH progesterone) as compared to normal women (Lachelin et al., 1979).

Women with PCOS have a high 17-OH progesterone response to gonadotrophin releasing hormone (GnRH) agonist testing with nafarelin (Barnes et al., 1989), buserelin (Şahin and Keleştimur, 1993) or leuprorelin acetate (Suikkari et al., 1995). PCOS is thought to be caused by an increased activity of steroidogenesis through 17-hydroxylation and/or increased but relatively inefficient activity of 17,20-lyase. Both 17-hydroxylase and 17,20-lyase activities arise from the action of the same enzyme, cytochrome P450c17α. This enzyme binds progesterone and converts it to 17-OH progesterone (by 17α-hydroxylase activity) and 17-OH progesterone to androstenedione (by 17,20-lyase activity) (Rosenfield et al., 1990). Cytochrome P450c17α is important for ovarian and adrenal function. 17-OH progesterone is an essential precursor for cortisol. This enzyme is encoded by a single gene on chromosome 10 and is expressed in both the adrenal gland and in ovarian theca cells (Miller, 1988; Takayama et al., 1996). This combined adrenal and ovarian expression of cytochrome P450c17α hyperactivity may explain the well-known enhanced production of androgen by the adrenals in patients with PCOS. Rosenfield et al. (1990) suggested that the abnormal regulation of P450c17α function in both the adrenal cortex and ovary may explain the hyperandrogenic function of both glands. The dysregulation of adrenocortical P450c17α may cause this hyperandrogenic response to ACTH. If the regulation of adrenocortical P450c17α is abnormal, a positive correlation would be expected between the adrenal 17-OH progesterone response to ACTH and the ovarian 17-OH progesterone response to GnRH agonist. To investigate this possibility, the 17-OH progesterone response to ACTH stimulation test (0.25 mg i.v., bolus) and the 17-OH progesterone response to GnRH agonist (buserelin) was evaluated in normal women and in PCOS subjects.
Materials and methods

The study was approved by the Ethical Committee of Erciyes University Medical School, and informed consent was obtained from each woman. A total of 68 women with PCOS (aged 16–36 years, mean ± SEM 23.96 ± 0.59) was randomly selected for study from our outpatient clinic population. The diagnosis of PCOS was made by the presence of polycystic ovaries on pelvic ultrasound examination combined with three or more of the following criteria: oligo/amenorrhea, hirsutism, hyperandrogenaemia, and serum luteinizing hormone (LH)/follicle stimulating hormone (FSH) >2. All patients had hyperandrogenaemia (serum free testosterone >11.1 pmol/l, normal range 2.8–11.1 pmol/l). In all, 68, six and 26% of the women were oligomenorrhoic (intermenstrual interval >35 days), amenorrhoic (no menstrual period for >6 months) and eumenorrhoic (intermenstrual interval between 21 and 35 days) respectively. Of the 68 patients, 64 (94%) had hirsutism (modified Ferriman–Gallwey score >8) (Hatch et al., 1981), and thirty-five (51%) women had elevated serum LH/FSH ratio (LH/FSH >2). Cushing’s syndrome and androgen-secreting tumours were excluded by appropriate test including dexamethasone suppression test.

Pelvic ultrasound examinations were performed by the same investigator (Y.S.) using a 3.5 MHz abdominal transducer in 20 women and a 6.5 MHz vaginal endoprobe (Hitachi, EUB 450, Japan) in 48 women. The ultrasound diagnosis of polycystic ovary was made by the presence of 10 or more cysts 2–10 mm in diameter, arranged around a dense stroma or scattered throughout an increased amount of stroma (Adams et al., 1986; Ardaens et al., 1991). All sonograms were obtained early in the cycle.

The control group consisted of 24 normal women similar in age (range 21–32 years, mean ± SEM 25.0 ± 0.87) and weight to the subjects with PCOS. All had regular menses every 26–32 days, no hyperandrogenaemia, and no evidence of hirsutism. None of them had polycystic ovaries on ultrasound examination. None of the subjects in the PCOS or control groups had received any hormonal medication for at least 8 weeks before the study. A total of 92 women was studied, each in the midfollicular phase (day 5–9) of her cycle in controls and in patients with either regular cycles or oligomenorrhea or in the event that the patient was amenorrhoic, when the serum progesterone concentration was <8.0 nmol/l (2.5 ng/ml).

The ACTH stimulation test was performed in PCOS and control women by administration of a single i.v. bolus of 0.25 mg synthetic ACTH-(1-24) (Synacthen, Ciba, Switzerland) at 0800 h. Venous blood was drawn through an indwelling catheter in the midfollicular phase at 0, 30 and 60 min for determination of serum 17-OH progesterone.

Androstenedione responses to ACTH stimulation were also measured in 28 of the 68 women with PCOS. Buserelin testing (1 mg s.c., Suprefact, Hoechst, Germany) was carried out in 52 women with PCOS as we previously described (Şahin and Keleştimur, 1993). Serum samples were drawn at 6 h intervals for 24 h. The serum samples were stored at −20°C until assayed.

Serum FSH, LH, total testosterone, free testosterone, androstenedione, DHEAS and 17-OH progesterone were measured by radioimmunoassay, using commercial kits (DPC, Los Angeles, CA, USA). Sex hormone-binding globulin (SHBG) (Orion Diagnostica, Finland) was measured by immunoradiometric assay. The intra-assay and interassay coefficients of variation were 6.5 and 5.6% for FSH; 7.0 and 7.9% for LH; <10 and 12.9% for total testosterone; 4.3 and 5.5% for free testosterone; 8.3 and 9.2% for androstenedione; 3.9 and 7.0% for DHEAS; 5.6 and 4.5% for 17-OH progesterone; and 4.0 and 5.5% for SHBG. All samples from the same patients were assayed in the same assay. 17-OH progesterone response to GnRH agonist buserelin and 17-OH progesterone response to ACTH were also determined as area under the curve (AUC) calculated by the trapezoidal rule and expressed as amount of hormone/time (hours for 17-OH progesterone response to buserelin and minutes for 17-OH progesterone response to ACTH). For statistical analysis unpaired t-test and regression analyses were used. Values are expressed as the mean ± SEM. P < 0.05 was regarded as statistically significant.

Results

The clinical and basal hormonal characteristics of PCOS and control subjects are shown in Table I. PCOS women and controls did not differ in mean age or body mass index (BMI) (kg/m²). The hirsutism (Ferriman–Gallwey) score (P < 0.0005), basal serum total testosterone (P < 0.0005), free testosterone (P < 0.0005), androstenedione (P < 0.0005), DHEAS concentrations (P < 0.05) and LH/FSH ratio (P < 0.05) were significantly higher in PCOS subjects than in controls. Basal serum SHBG concentration was significantly lower in the PCOS group than in controls (P < 0.0005).

The mean 17-OH progesterone responses to ACTH in PCOS and control subjects are shown in Table II. The mean basal 17-OH progesterone level in the women with PCOS was slightly, but not significantly, higher than in the control group. 17-OH progesterone responses to ACTH at 30 (P < 0.05) and 60 min (P < 0.02) were significantly higher in PCOS than in control women.

There were significant differences between the women with PCOS and controls in terms of the net increment (P < 0.02) in 17-OH progesterone and in the area under the 17-OH progesterone–response curve from 0 to 60 min (P < 0.02). None of the 17-OH progesterone elevations were in the range expected for late-onset congenital adrenal hyperplasia due to 21-hydroxylase deficiency (New et al., 1983). Androstenedione concentrations (nmol/l) before and after ACTH stimulation in the PCOS women and controls were as follows: basal, 3.36 ± 0.18 vs 2.33 ± 0.12 (P < 0.0005); 30 min, 5.94 ± 0.24 vs 2.98 ± 0.15 (P < 0.0005); 60 min, 6.55 ± 0.27 vs 3.25 ± 0.15 (P < 0.0005); and peak, 6.67 ± 0.25 vs 3.38 ± 0.12 (P < 0.0005), respectively. Net increment (difference between the peak and the basal values) in androstenedione concentration was higher in the women with PCOS (3.31 ± 0.23 nmol/l) than in controls (1.06 ± 0.14 nmol/l) (P < 0.0005).

17-OH progesterone concentrations (nmol/l) before and after buserelin testing in the PCOS women were as follows: 4.95 ± 0.28 (basal), 7.66 ± 0.42 (6 h), 10.83 ± 0.59 (12 h), 14.03 ± 0.72 (18 h), 15.98 ± 0.87 (24 h), and 16.85 ± 0.87 (peak). Net increment (nmol/l, difference between the peak and the basal values) and AUC (nmol/l×24 h) concentrations were 11.84 ± 0.89 and 257 ± 10.83 respectively.

There was no significant correlation between the peak 17-OH progesterone values after ACTH stimulation and buserelin testing (r = 0.044, Figure 1). The relationship between the net increments in 17-OH progesterone after ACTH stimulation and buserelin administration was not significant in PCOS women (r = 0.014). The relationship between the area under the 17-OH progesterone–response curves to ACTH stimulation and buserelin was also not significant (r = 0.024).
suggesting its non-adrenal origin. Nevertheless, the role of androgen production by adrenal glands in the cause and perpetuation of PCOS remains controversial. On the other hand, numerous investigators have found elevated androgen production by the adrenal glands in patients with PCOS and have suggested that the adrenal glands may play a role in the genesis of PCOS (Moltz and Schwartz, 1986).

Ehrmann et al. (1992) have found increased 17-OH progesterone and androgen responses when the adrenal is stimulated with ACTH or when the ovary is stimulated with the GnRH agonist nafarelin and they have suggested that 17-hydroxylase and 17-20-lyase are excessively active in both the ovaries and the adrenal glands in PCOS. We have also reported that the dysregulation of cytochrome P450c17α may be responsible for hyperandrogenism seen in PCOS (Sahin and Kelesstimur, 1993; Sahin et al., 1997). In these studies, we have found that the concentrations of 17-OH progesterone after buserelin stimulation in the patients with PCOS were significantly higher than in the control women. White et al. (1995) also found that 17-OH progesterone level after buserelin testing was higher in PCOS than in healthy women. For this reason, we think that GnRH stimulation with buserelin is a reliable method to investigate cytochrome P450c17α enzyme activity in women with PCOS, and we did not repeat buserelin stimulation among healthy women in the present study. In this study we investigated the regulation of cytochrome P450c17α in the adrenal gland and the role of adrenal androgen production in PCOS. Maximal adrenal stimulation with an ACTH analogue has been the principal challenge test for estimating the relative activity of adrenocortical enzymes (Azziz et al., 1990). For this reason, we have used the ACTH stimulation test to evaluate adrenal androgen production. In our study the 17-OH progesterone concentrations 30 and 60 min after ACTH, the net increment in 17-OH progesterone and the area under the 17-OH progesterone–response curve after stimulation were significantly higher in the PCOS group than in normal women. Our findings show that the patients with PCOS may have increased adrenocortical 17-OH progesterone secretion which may mean the dysregulation of the enzyme cytochrome P450c17α in adrenal gland in response to ACTH stimulation.

Some authors did not find any significant difference between the androgen concentrations in PCOS and control groups when the ACTH-stimulating test was performed (Lucky et al., 1986; Hague et al., 1989; Fulghesu et al., 1991; White et al., 1995). Turner et al. (1992) reported that basal 17-OH progesterone concentrations were significantly higher in the PCOS subjects than in the controls, but the net increments in 17-OH pro-gesterone after ACTH stimulation were similar between the two groups. They also demonstrated that 21-hydroxylase, 11β-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies in women with PCOS are rare, but that other abnormalities of adrenal function are common. Azziz et al. (1990) suggested that the basal and ACTH-stimulated 17-OH progesterone concentrations were significantly higher in hyperandrogenic women than in normal subjects, but there was no significant difference in the net change in 17-OH

### Table I. Clinical and basal hormonal characteristics of polycystic ovary syndrome (PCOS) and control subjects

<table>
<thead>
<tr>
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<th>PCOS (n = 68)</th>
<th>Controls (n = 24)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.96 ± 0.59</td>
<td>25.00 ± 0.87</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.01 ± 0.52</td>
<td>24.37 ± 0.78</td>
<td>NS</td>
</tr>
<tr>
<td>Ferriman–Gallwey score</td>
<td>17.39 ± 0.81</td>
<td>3.00 ± 0.35</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>3.02 ± 0.13</td>
<td>1.37 ± 0.13</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Free testosterone (nmol/l)</td>
<td>21.46 ± 0.93</td>
<td>7.83 ± 0.45</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>16.13 ± 0.91</td>
<td>9.08 ± 0.66</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>DHEAS (µmol/l)</td>
<td>6.67 ± 0.37</td>
<td>5.52 ± 0.29</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>37.44 ± 4.21</td>
<td>62.55 ± 4.27</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>LH:FSH ratio</td>
<td>1.46 ± 0.09</td>
<td>0.86 ± 0.07</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

NS = not significant, BMI = body mass index, DHEAS = dehydroepiandrosterone sulphate, SHBG = sex hormone-binding globulin, LH = luteinizing hormone, FSH = follicle stimulating hormone.

### Table II. 17-OH progesterone (nmol/l) response to ACTH in PCOS and control subjects

<table>
<thead>
<tr>
<th></th>
<th>PCOS (n = 68)</th>
<th>Controls (n = 24)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>5.39 ± 0.27</td>
<td>4.72 ± 0.36</td>
<td>&gt;0.05</td>
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<tr>
<td>30 min</td>
<td>9.60 ± 0.43</td>
<td>8.11 ± 0.49</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>60 min</td>
<td>10.37 ± 0.42</td>
<td>8.59 ± 0.47</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Peak</td>
<td>10.91 ± 0.42</td>
<td>8.94 ± 0.45</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Net increment (nmol/l)*</td>
<td>5.52 ± 0.33</td>
<td>4.22 ± 0.44</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>AUC (nmol/l × 60 min)</td>
<td>524.30 ± 21.06</td>
<td>442.85 ± 23.69</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*Net increment is difference between the peak and the basal values.

AUC = area under the curve.

**Figure 1. The relationship between the peak 17-OH progesterone values after adrenocorticotropic hormone (ACTH) stimulation and buserelin testing (r = 0.044; P > 0.05).**

### Discussion

Although PCOS characterized by menstrual abnormalities, hyperandrogenism, hirsutism and obesity is a common disorder, its pathogenesis is still unclear. The main source of hyperandrogenism seen in PCOS is the ovary (Chetkowski et al., 1984). The exaggerated 17-OH progesterone response after acute GnRH agonist nafarelin (Barnes et al., 1989) or buserelin (Sahin and Kelesstimur, 1993) stimulation also suggested that the elevated circulating 17-OH progesterone concentrations are a product of ovarian secretion. Lachelin et al. (1979) observed that the elevated basal 17-OH progesterone concentration responded poorly to short-term glucocorticoid suppression, suggesting its non-adrenal origin. Nevertheless, the role of androgen production by adrenal glands in the cause and perpetuation of PCOS remains controversial. On the other hand, numerous investigators have found elevated androgen production by the adrenal glands in patients with PCOS and have suggested that the adrenal glands may play a role in the genesis of PCOS (Moltz and Schwartz, 1986).
progesterone from 0 to 30 min after adrenal stimulation between normal and hyperandrogenic women. In this study, we have found that basal and ACTH-stimulated androstenedione concentrations and net increment in androstenedione after ACTH were significantly higher in PCOS than in controls. We have previously shown that there is an adenocortical hyperactivity in hyperandrogenic women (Kelestimur et al., 1996). It seems that there is no specific enzyme defect in the adrenal glands in PCOS but rather a general up-regulation of adrenal cortical steroidogenic function (Homburg et al., 1996).

In the present study, no subject showed concentrations of 17-OH progesterone, either basally or in response to the ACTH test, as high as those described in patients with the non-classical form of congenital adrenal hyperplasia due to 21-OH deficiency (New et al., 1983). So, non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency was not responsible for the elevated 17-OH progesterone found in these patients.

Aziz et al. (1995) recently reported that the steroidogenic profile observed in hyperandrogenic women before and after ACTH stimulation is not consistent with dysregulation of cytochrome P450c17α in adrenal glands. Our findings show that 17-OH progesterone responses to ACTH stimulation were higher in the patients with PCOS than in healthy subjects. This study does not rule out the contribution of ovarian 17-OH progesterone secretion to the high 17-OH progesterone concentrations found in patients with PCOS. We have not found a positive correlation between 17-OH progesterone concentrations after ACTH stimulation and buserelin challenge. This shows that adrenal glands may have some role in the genesis of hyperandrogenism but dysregulation of cytochrome P450c17α may not be responsible for adrenal hyperandrogenism in PCOS.

In conclusion, although 17-OH progesterone response to ACTH was significantly higher in the patients with PCOS than in the control subjects, the lack of relationship between 17-OH progesterone response to GnRH agonist buserelin and 17-OH progesterone response to ACTH stimulation suggests that the dysregulation of the cytochrome P450c17α enzyme may not play a role in adrenal androgen excess seen in PCOS. The adrenal glands may contribute to the hyperandrogenism to a lesser extent when compared with ovarian hyperandrogenism, and they are not primarily responsible for hyperandrogenism.

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References


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