The contributions of oestrogen and growth factors to increased adrenal androgen secretion in polycystic ovary syndrome

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Adrenal hyperandrogenism is prevalent in many women with polycystic ovary syndrome (PCOS), although the expression of this enhanced secretion may be heterogeneous. Since no single factor acts in isolation, this study was performed to assess the influence of oestriadiol (total and unbound), insulin, insulin-like growth factor (IGF)-I, IGF-II and the binding proteins IGFBP-I, and IGFBP-3, on basal and adrenocorticotrophic hormone (ACTH) stimulated adrenal androgen secretion in 25 women with PCOS and 10 matched ovulatory controls. Women with PCOS exhibited elevations of all androgens as well as unbound oestradiol, insulin and non-IGFBP-1 bound IGF-I. Positive correlations were noted between oestrogen and basal and ACTH stimulated Δ⁵ adrenal androgens. Serum IGF-I was only correlated with basal dehydroepiandrosterone sulphate (DHEA-S), while insulin exhibited a strong correlation with the Δ⁴ pathway and androstenedione formation in particular. This correlation was also confirmed by dividing the PCOS group into those women with and without hyperinsulinaemia. The activity of 17,20 lyase favouring androstenedione was increased in the hyperinsulinaemic women. By multivariate analyses, body mass index did not influence these findings. Although there are inherent difficulties in making major conclusions based on correlational analyses, it is suggested that oestrogen may have a greater influence on enhancing Δ⁵ adrenal androgen secretion, and insulin a greater effect on the Δ⁴ pathway. In turn, the relative importance of these influences may contribute to the heterogeneous nature of adrenal hyperandrogenism in PCOS.

Key words: adrenal androgens/IGF-I/insulin/oestrogen/PCOS

Introduction

It is well known that adrenal hyperandrogenism (AH) is a prevalent feature of polycystic ovary syndrome (PCOS) (Hoffman et al., 1984; Lobo, 1984; Gonzalez et al., 1991; Carmina et al., 1992, 1997a). Although many studies have been carried out examining possible causes of AH in PCOS (Lobo, 1984; Carmina, 1997a,b), its pathogenesis remains unclear. Among the several factors which may be involved, hyperoestrogenism and hyperinsulinaemia have been the most studied (Lobo et al., 1982; Ditkoff et al., 1995; Gonzalez et al., 1996; Moghetti et al., 1996; Rosenfield, 1996; Carmina, 1997b; Eldar-Geva et al., 1998). However, conflicting results have been reported regarding the relative importance of these influences (Ditkoff et al., 1995; Rosenfield, 1996). Eldar-Geva et al. (1998) have suggested that with ovarian stimulation, adrenal secretion of progesterone is enhanced, although no specific factors could be implicated. Insulin-like growth factors (IGF-I and IGF-II) also are known to be potent in-vitro stimulators of adrenal secretion (Pham et al., 1991; Mesiano and Jaffe, 1993) and have not been adequately studied. Inhibition of both insulin and IGF-I with somatostatin has been shown to decrease AH in PCOS (James et al., 1994). Therefore the aim of this study was to understand the relative importance of oestrogen and growth factors which may influence adrenal androgens in women with PCOS. Since it is clear that no factor acts in isolation, several of these factors were examined simultaneously in the same patient. Given the difficulties inherent in correlative analyses, in this report different types of relationships have been observed for oestrogen and growth factors; oestrogen correlated with basal and stimulated adrenal androgens of the Δ⁵ pathway while insulin correlated with the adrenal Δ⁴ pathway.

Materials and methods

Subjects

Twenty-five women with PCOS and 10 normal controls, matched for age and body mass index (BMI) were studied. Women with PCOS were diagnosed on the basis of hyperandrogenism and chronic anovulation, after the exclusion of tumours, Cushing’s syndrome and adrenal enzymatic deficiencies. Polycystic ovaries on ultrasound were present in all of the women with PCOS in this study but this was not a required inclusion criterion.

Protocol and measurements

During the mid-follicular phase of the cycle, between days 5 and 8, at 8–9 a.m. after an overnight fasting, a blood sample was obtained for measurements of oestradiol, unbound oestradiol, androstenedione, dehydroepiandrosterone sulphate (DHEA-S), insulin, IGF-I, IGF-II, IGFBP-3 and IGFBP-1. The following day, patients and controls underwent an adrenocorticotrophic hormone (ACTH) stimulation test (ACTH 1–17, 0.25 mg i.v. with blood samples at 0, 60, 120 and 180 min). Dexamethasone (1 mg) was administered orally the night before
the ACTH test (at 11 p.m.). After ACTH, blood samples were obtained for 17-OH pregnenolone (17-OHPe), 17-OH progesterone (17-OHP), androstenedione, DHEA and DHEA-S. After ACTH, steroid ratios were calculated as approximate estimates of various enzymatic activities.

Serum hormone concentrations were quantified by well-established methods which were validated previously in our laboratory. All steroids were measured by specific radioimmunoassay (RIA) after extraction using previously described methods (Lobo and Goebelsmann, 1981; Hoffman et al., 1984). Non-sex hormone-binding globulin (SHBG)-bound oestradiol was measured by adding 500 c.p.m. of tritiated oestradiol to 0.3 ml of serum prior to precipitation with ammonium sulphate. The percentage of tritiated oestradiol in the supernatant was calculated, as previously reported (Lobo et al., 1981). Insulin and IGFBP-3 were quantified by use of direct RIA kits (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). IGF-I and IGF-II were measured by RIA using material provided by Diagnostic Systems Laboratories. Both assays were performed after an acid ethanol extraction step. IGFBP-1 was measured by an immunoreactive assay using a kit provided by Diagnostic Systems Laboratories. The methods for the assay of IGF-I, IGFBP-3, IGFBP-1 have been previously described (Carmina et al., 1995). There was no cross reactivity of the IGF-II antiserum, IGF-I, IGF binding proteins (1, 2, 3, 4, 5) insulin or growth hormone. The sensitivity of the IGF-II assay was 12 ng/ml. The interassay coefficient of variation was 12% and the intraassay coefficient of variation was 6%.

Statistics
Analysis of the data was performed using Student’s t-test for comparisons between the two groups: PCOS and controls. Correlations were analysed by Pearson product moment correlations and stepwise multivariate linear regression analysis with forward selection. P < 0.05 was considered significant. Results are expressed as mean ± SE.

Table I. Basal hormone concentrations mean ± SE in controls and women with PCOS

<table>
<thead>
<tr>
<th></th>
<th>Insulin (µU/ml)</th>
<th>IGF-I (ng/ml)</th>
<th>IGF-II (ng/ml)</th>
<th>Unbound oestradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>9.1 ± 0.8</td>
<td>292 ± 72</td>
<td>702 ± 64</td>
<td>22.2 ± 4.1</td>
</tr>
<tr>
<td>PCOS</td>
<td>14.5 ± 1.9*</td>
<td>279 ± 23</td>
<td>822 ± 49</td>
<td>37.7 ± 5.2*</td>
</tr>
</tbody>
</table>

*P < 0.05.

Results
Controls and PCOS were of similar age (24.1 ± 1 versus 23.6 ± 1.2 years) and BMI (27.4 ± 0.8 versus 28.2 ± 1.6). As depicted in Table I, PCOS patients had increased concentrations of insulin (P < 0.05), while serum IGF-I and IGF-II were similar in the two groups. Total serum oestradiol was also similar but unbound oestradiol was significantly (P < 0.05) higher in PCOS (Table I).

Patients with PCOS had lower concentrations of serum IGFBP-1 (20.4 ± 3 versus 12.4 ± 12 ng/ml, P < 0.01) and higher concentrations of basal DHEA-S (3.4 ± 0.2 versus 1.9 ± 0.2 µg/ml, P < 0.01) and -A (3.8 ± 0.2 versus 1.5 ± 0.2 ng/ml, P < 0.01). Serum IGFBP-3 was similar in controls and PCOS.

Table II depicts the adrenal androgen responses to ACTH. In PCOS, patients exhibited an exaggerated response (P < 0.01) to all steroids measured.

Serum insulin did not correlate with basal DHEA-S or -A but showed a significant (P < 0.05) negative correlation with the increment of 17OHP (Δ17-OHP) after ACTH (Table III). Serum insulin was positively correlated with the ratio of ΔA/Δ17-OHP and ΔA/A 17-OHPe, suggesting the capacity to increase adrenal 17,20 lyase activity (Table IV).

Serum IGF-I was significantly correlated (P < 0.05) with the basal concentrations of DHEA-S but did not show any correlations with the adrenal androgen responses to ACTH (Tables III and IV).

Both total and unbound oestradiol correlated significantly with basal DHEA-S but showed a negative correlation with the androstenedione response to ACTH (Table III). Moreover, total and unbound oestradiol correlated negatively with the ratio of ΔA/ΔDHEA and positively with the ratio (ΔDHEA/Δ17-OHP) (Table IV), suggesting a relative increase in 17,20 lyase activity and reduction in 3β-dehydroxysteroid dehydrogenase activity favouring the formation of Δ5 steroids.

IGF-II did not show any correlation with adrenal androgen secretion.

Thirteen women with PCOS were hyperinsulinaemic (mean insulin 21.3 ± 2 µIU/ml) with values >12 µIU/ml, which was the upper 95% confidence value of the controls. Twelve women with PCOS were normoinsulinaemic (mean insulin 7.1 ± 0.7 µIU/ml, range 3.1–10 µIU/ml). The two groups were significantly different in terms of BMI (hyperinsulinaemic PCOS: 32.6 ± 2, normoinsulinaemic PCOS: 23.4 ± 1, P < 0.01). As shown in Table V, the hyperinsulinaemic patients with PCOS had significantly (P < 0.05) higher ratios of ΔA/Δ17-OHP and ΔA/Δ17-OHPe compared to the normoinsulinaemic group.

The possibility that BMI may have influenced the results was assessed directly. In PCOS, BMI correlated positively with serum insulin (r = 0.57, P < 0.01) and negatively with serum IGF-I (r = 0.55, P < 0.01) but not with unbound oestradiol (r = 0.04) or with IGF-II (r = 0.34). BMI did not correlate with basal or stimulated adrenal androgen concentrations or with any steroid ratios.

Multivariate analysis showed that BMI did not influence the correlations of unbound oestradiol and IGF-I with adrenal androgens. In spite of a strong influence of BMI on serum insulin, BMI did not affect the negative correlation of insulin with Δ17-OHP, or the positive correlation with the ΔΔA/Δ17-OHP ratio. Correcting for BMI, the correlation of insulin with Δ17-OHP was r = 0.43 (P < 0.05) and with ΔA/Δ17-OHP was r = 0.40 (P < 0.05). Multivariate analyses did not show any influence of insulin on the correlation between oestradiol and unbound oestradiol with adrenal androgens.

Discussion
The data in this study confirm that several extra-adrenal hormones may influence adrenal androgen secretion and may participate in the pathogenesis of AH in PCOS. In this study, serum IGF-II did not correlate with circulating adrenal
Table II. Adrenal androgen responses to ACTH in controls and PCOS represented as maximal increments from baseline (Δ)

<table>
<thead>
<tr>
<th></th>
<th>Δ17-OHPe ng/ml</th>
<th>Δ17-OHP ng/ml</th>
<th>ΔA ng/ml</th>
<th>ΔDHEA ng/ml</th>
<th>ΔDHEA-S ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.5 ± 1</td>
<td>0.8 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>8.2 ± 0.6</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>PCOS</td>
<td>13.5 ± 1**</td>
<td>1.3 ± 0.1**</td>
<td>2.4 ± 0.2*</td>
<td>13.8 ± 1**</td>
<td>1.1 ± 0.1**</td>
</tr>
</tbody>
</table>

**P < 0.01.

ACTH = adrenocorticotrophic hormone, PCOS = polycystic ovary syndrome, 17-OHPe = 17-hydroxypregnenolone, 17-OHP = 17-hydroxyprogesterone, A = androstenedione, DHEA = dihydroepiandrosterone and DHEA-S = dihydroepiandrosterone sulphate.

Table III. Correlations between serum concentrations and basal concentrations of DHEA-S and A; and Δ responses to ACTH in PCOS

<table>
<thead>
<tr>
<th></th>
<th>Basal DHEA-S</th>
<th>Basal A</th>
<th>Δ17-OHPe</th>
<th>Δ17-OHP</th>
<th>ΔA</th>
<th>ΔDHEA</th>
<th>ΔDHEA-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>0.21</td>
<td>0.33</td>
<td>−0.35</td>
<td>−0.43*</td>
<td>0.15</td>
<td>−0.36</td>
<td>−0.31</td>
</tr>
<tr>
<td>IGF–I</td>
<td>0.46*</td>
<td>0.23</td>
<td>0.27</td>
<td>0.19</td>
<td>−0.23</td>
<td>0.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>0.47*</td>
<td>−0.27</td>
<td>0.29</td>
<td>−0.32</td>
<td>−0.48</td>
<td>0.28</td>
<td>0.26</td>
</tr>
<tr>
<td>Unbound oestradiol</td>
<td>0.50**</td>
<td>−0.28</td>
<td>−0.22</td>
<td>−0.26</td>
<td>−0.44*</td>
<td>0.27</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*P < 0.05.
For abbreviations, see Table II.

Table IV. Correlations between baseline concentrations and adrenal enzymatic activity

<table>
<thead>
<tr>
<th></th>
<th>3β-Hydroxysteroid dehydrogenase activity</th>
<th>17,20-Lyase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ17-OHP/Δ17OHPe</td>
<td>ΔA/ΔDHEA</td>
</tr>
<tr>
<td>Insulin</td>
<td>−0.29</td>
<td>−0.29</td>
</tr>
<tr>
<td>IGF–I</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>−0.18</td>
<td>−0.46*</td>
</tr>
<tr>
<td>Unbound oestradiol</td>
<td>−0.16</td>
<td>−0.44*</td>
</tr>
<tr>
<td>BMI</td>
<td>−0.34</td>
<td>−0.08</td>
</tr>
</tbody>
</table>

*P < 0.05.
For abbreviations, see Table II.

Table V. 17,20-Lyase activity in normoinsulinaemic and hyperinsulinaemic PCOS

<table>
<thead>
<tr>
<th></th>
<th>ΔDHEA/Δ17OHPe</th>
<th>ΔA/Δ17-OHP</th>
<th>ΔA/Δ17-OHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoinsulinaemic PCOS</td>
<td>1.0 ± 0.11</td>
<td>1.62 ± 0.3</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>Hyperinsulinaemic PCOS</td>
<td>1.09 ± 0.1</td>
<td>2.51 ± 0.3*</td>
<td>0.24 ± 0.11**</td>
</tr>
</tbody>
</table>

*P < 0.05.
**P < 0.01.

Novel implications of findings in this study are that these extra-adrenal hormones correlated differently with various adrenal androgens suggesting that these factors may influence AH by different mechanisms. Serum IGF-1 correlated with basal DHEA-S, but not with adrenal androgen responses to ACTH while insulin did not correlate with basal adrenal androgens but showed a positive correlation with the ΔA/Δ17-OHP ratio and the ΔA/Δ17-OHPe ratio after ACTH stimulation. In evaluating steroid ratios in blood after ACTH, only the effect on true enzyme activity was estimated. Nevertheless, these data suggested an important influence of insulin on adrenal Δ^4 A production. These findings were further enhanced by our findings in the subgroup of hyperinsulinaemic patients with PCOS whose responses were different from the normoinsulinemia group. In this study, single time point measurements of fasting insulin for correlative analyses were used. Clearly, fasting insulin alone is inadequate to characterize the existence of insulin-resistance in these women.

Oestrogen (oestradiol and unbound oestradiol) on the other hand, correlated with both basal DHEA-S and adrenal androgen responses to ACTH (positively with the ratio of ΔDHEA/Δ17-

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gests a positive influence of oestrogen on the \( \Delta^4 \) pathway, predominantly resulting in an enhancement of DHEA/DHEA-S production.

In this study, BMI was similar in patients and controls, and multivariate analyses were carried out. It was shown that BMI did not influence the correlations observed.

In PCOS, it has been shown that the ovarian theca hyper-


References


hormones, specifically oestrogen and insulin, correlate with the presence of AH in PCOS and that these hormones may influence adrenal androgen pathways differently, thus explaining the heterogeneous presentation of AH in PCOS.


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