High serum luteinizing hormone levels induce ovarian $\Delta^4$ cytochrome P450c17$\alpha$ down-regulation in hirsute women: complete effect on 17-hydroxylase and partial effect on 17,20-lyase

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Abstract

It is well known that normal and mildly elevated luteinizing hormone (LH) levels induce increased activity of ovarian $\Delta^4$17-hydroxylase and $\Delta^4$17,20-lyase, the cytochrome P450c17$\alpha$ (P450) enzymes. This leads to increased ovarian $\Delta^4$17$\alpha$-hydroxyprogesterone (17-OHP) and androstenedione production. In contrast, it has been shown in both in vitro and in vivo studies in animals and in in vitro studies in women that high LH concentrations have opposite effects on these enzymes. These LH down-regulating effects appear to be more marked on $\Delta^4$17,20-lyase than on $\Delta^4$17-hydroxylase. Finally, these LH effects have not been reported in vivo in women. Therefore, we investigated the relationships between serum LH levels and serum 17-OHP and androstenedione concentrations in 263 consecutive hirsute women (HW) with normal serum 17-OHP responses to acute adrenocorticotropin (ACTH) stimulation.

The patterns of basal serum steroid concentrations differed according to the basal serum LH levels. Indeed, for relationships between LH and 17-OHP concentrations, a positive correlation ($P < 0.001$) was found between the levels of these parameters when LH levels ranged from 0.2 to 9.0 IU/l. Conversely, for LH levels greater than 9.0 to 21.0 IU/l, LH values were negatively correlated ($P < 0.001$) with 17-OHP concentrations. Similar results were observed for relationships between LH and androstenedione levels but the LH peak level related to decreasing androstenedione concentrations was 12.0 IU/l. Finally, the mean 17-OHP level in patients with LH levels which induced marked P450 down-regulation (i.e. more than 12 IU/l) was similar to that in patients with LH levels within the normal range (i.e. less than 6 IU/l). In contrast, the mean androstenedione level in the former patients was markedly higher ($P < 0.001$) than that in the latter patients. In conclusion, as previously reported in in vitro studies, this in vivo study indicates that LH induces stimulating and down-regulating effects on both ovarian $\Delta^4$17-hydroxylase and $\Delta^4$17,20-lyase activities as serum LH levels gradually increase. However, in contrast to in vitro studies, LH levels which induce P450 down-regulation appear to be less effective on $\Delta^4$17,20-lyase than on $\Delta^4$17-hydroxylase in HW. This strongly suggests that serum factors induce, in most HW, a marked increase in $\Delta^4$17,20-lyase, but not in $\Delta^4$17-hydroxylase, activity leading to both partial impairment of LH-induced $\Delta^4$17,20-lyase down-regulation and complete LH-induced $\Delta^4$17-hydroxylase down-regulation in these patients.

Introduction

It is well known that normal and mildly elevated serum luteinizing hormone (LH) levels induce an increase in ovarian cytochrome P450c17$\alpha$ (P450) activities and, therefore, in ovarian $\Delta^4$17$\alpha$-hydroxyprogesterone (17-OHP) and androstenedione production (1–5). This LH effect is related in rats to both increased LH receptor sites and stimulation of cyclic AMP production leading to increased activity of both 17-hydroxylase and 17,20-lyase, the P450 enzymes. In contrast, it has been shown in both in vitro and in vivo studies in animals and in in vitro studies on ovaries from hyperandrogenic (HA) and non-HA women that high LH concentrations have opposite effects on this enzyme leading to decreased ovarian 17-OHP and androstenedione production (6–15). These LH-induced down-regulating effects appear to be more marked on 17,20-lyase than on 17-hydroxylase (12–15). In vivo testicular P450 down-regulation by very high serum human chorionic gonadotropin (hCG) concentrations has also been reported both in normal men (16) and in men with hCG-producing tumors (17). In vivo LH-induced ovarian $\Delta^4$ P450 down-regulation has not been reported in women. Hirsute women (HW) as a whole provide a study model for the assessment of the stimulating and down-regulating effects of LH on ovarian $\Delta^4$ P450 activities.
in vivo since all serum LH ranges are observed in these patients. Indeed, women with the so-called idiopathic hirsutism have LH levels within the normal range, whereas mildly increased or high LH levels are frequently encountered in patients with polycystic ovarian syndrome (PCOS) (5). Therefore, we investigated the relationship of serum LH levels to serum 17-OHP and androstenedione concentrations in a large series of HW.

Patients and methods

Patients

This cross-sectional study included 303 consecutive HW (age range, 18–40 years). Patients with prolactinoma, thyroid dysfunction, Cushing’s syndrome, androgen secreting tumor and non classic congenital adrenal hyperplasia caused by 21-hydroxylase deficiency were excluded from this study. The latter was ruled out by a serum peak response of 17-OHP to acute adrenocorticotropic (ACTH) stimulation of less than 30.3 nmol/l (18). Amenorrhoeic HW were also excluded from this study, because of the unknown phase of their cycle: unfortunately, their serum progesterone levels were not measured. No patient had received hormonal treatment for at least 6 months prior to evaluation. Investigations were performed in the early follicular phase of the menstrual cycle (days 1–7), between 0830–0930 h in the fasting state. Basal blood samples were obtained for LH, 17-OHP, androstenedione, total testosterone and 17β-estradiol (E₂) measurements. Then, an i.m. bolus (0.25 mg) of ACTH-1–24 (Synacthen, Ciba-Geigy Laboratories, Rueil-Malmaison, France) was administered, and blood samples were collected 60 min after injection for 17-OHP measurements. The delta (Δ) 17-OHP response to ACTH corresponds to the difference between the 17-OHP value at 60 min and the basal 17-OHP level after ACTH injection. Basal serum 17-OHP and androstenedione are mainly of ovarian origin in patients with normal 17-OHP and/or androstenedione responses to ACTH (19–21). Two hundred and sixty-three patients exhibited a normal Δ 17-OHP response to ACTH (less than 3.3 nmol/l, i.e. the mean + 3 s.d. in 21 controls: age range, 18–40 years) (Table 1). Among these patients, 116 had the so-called idiopathic hirsutism (i.e. basal LH, 17-OHP, androstenedione and testosterone levels within the normal ranges), whereas 147 had PCOS (i.e. increased basal LH, 17-OHP, androstenedione or testosterone levels). In contrast to patients with normal 17-OHP and/or androstenedione responses to ACTH, basal serum 17-OHP and androstenedione are, at least partly, of adrenal origin in HW with exaggerated 17-OHP and/or androstenedione responses to ACTH (19–21). This may hide the genuine LH effects on ovarian P450 activities in these patients. Forty HW exhibited an exaggerated Δ 17-OHP response to ACTH and, therefore, were excluded from this study. Furthermore, polynomial regression analysis (second-order) was performed between LH and 17-OHP levels in HW as a whole, and the curve had a parabolic pattern. The virtual peak value of LH was calculated according to the equation of the curve and was 9.0 IU/l. A similar procedure was used for studying the relationships between LH and androstenedione levels in these patients. The curve also had a parabolic pattern, but the virtual peak of LH was 12.0 IU/l. Because of the difference of 3.0 IU/l in virtual LH peak levels in the relationship of LH to androstenedione (i.e. 12.0 IU/l) and in that of LH to 17-OHP (i.e. 9.0 IU/l), patients with LH ranges of 3.0 IU/l deviation were included in order to compare the mean 17-OHP and androstenedione levels according to the LH range (i.e. from 0.2–3.0 IU/l (n = 122), from >3.0–6.0 IU/l (n = 74), from >6.0–9.0 IU/l (n = 23), from >9.0–12.0 IU/l (n = 20), and >12.0 IU/l (n = 24)).

Assays

Serum LH levels were measured by immunometric assay, as described previously (22). The intra- and the interassay coefficients of variation were 3.8% and 7.9% respectively. The detection limit was 0.1 IU/l. Serum 17-OHP, androstenedione and testosterone levels were measured by RIA after chromatography on a Celite column (Touzet Matignon, Paris, France) and serum E₂ was determined by RIA, as previously described (17). For all these steroid hormones, intra- and interassay coefficients of variation were less than 6% and less than 9% respectively. The detection limit was 0.18 nmol/l for both 17-OHP and androstenedione. These steroids showed <0.1% cross-reactivity. The upper limit of the normal range (mean+ 3 s.d.) in 21 controls during the early follicular phase was <6 IU/l for LH, <2.4 nmol/l for 17-OHP, <8.4 nmol/l for androstenedione, <1.7 nmol/l for testosterone and <345 pmol/l for E₂.

Table 1 Mean (± S.E.M.) values or prevalence (% of clinical and serum hormonal parameters in hirsute women.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>263</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>26.1 ± 0.4</td>
</tr>
<tr>
<td>Oligomenorrhea (%)</td>
<td>58.0</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>4.96 ± 0.28</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.7 ± 0.3</td>
</tr>
<tr>
<td>17-OHP (nmol/l)</td>
<td>1.43 ± 0.06</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>6.53 ± 0.19</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.51 ± 0.05</td>
</tr>
<tr>
<td>E₂ (pmol/l)</td>
<td>147 ± 6</td>
</tr>
<tr>
<td>Δ 17-OHP (nmol/l)</td>
<td>1.79 ± 0.06</td>
</tr>
</tbody>
</table>

Δ 17-OHP, delta 17-OHP response to ACTH corresponds to the difference between the 17-OHP value at 60 min and the basal 17-OHP level.
Statistics

Statistical analysis was performed using one-way analysis of variance. Results are expressed as the mean ± S.E.M. Differences in mean levels were determined by applying the Mann-Whitney U test. The relationships of LH to steroids were evaluated by multiple linear regression analysis (second order) or Spearman’s rank test. The level of significance was taken as \( P < 0.025 \) for multiple linear regression analysis according to Bonferroni’s correction and as \( P < 0.05 \) for Spearman’s rank test.

Results

The mean \( \Delta 17\text{-OHP} \) level after ACTH injection was 1.79 ± 0.06 nmol/l. The correlation between LH and 17-OHP levels had a parabolic pattern \( (r = 0.47, r^2 = 0.21, F = 36.17, P < 0.001) \) (Fig. 1), and the virtual peak value of LH was 9.0 IU/l. A positive correlation was found between LH and androstenedione levels \( (r = 0.48, r^2 = 0.23, F = 39.72, P < 0.001) \) (Fig. 1), but the virtual peak value of LH was 12.0 IU/l. A positive correlation was found between LH and androstenedione levels when LH levels were between 0.2 and 12.0 IU/l \( (r = 0.36, r^2 = 0.12, F = 18.49, P < 0.001) \) and \( E_2 \) \( (r = 0.26, r^2 = 0.06, F = 8.90, P < 0.001) \) levels also had parabolic patterns.

The mean 17-OHP levels were enhanced \( (P < 0.001) \) when LH values increased from 0.2–3.0 IU/l to 6.0–9.0 IU/l (Fig. 2). Thus, the mean 17-OHP level associated with LH levels ranging from 6.0–9.0 IU/l was increased 115% relative to its level when LH was in the range 0.2–3.0 IU/l. Likewise, the mean androstenedione levels were enhanced \( (P < 0.01 \text{ at least}) \) when the range of LH concentrations increased from 0.2–3.0 to 9.0–12.0 IU/l (Fig. 2). Thus, the mean androstenedione level associated with LH in the range from 9.0–12.0 IU/l was increased 98% relative to its level when LH was in the range 0.2–3.0 IU/l. Furthermore, the mean 17-OHP level when the LH concentration was more than 12.0 IU/l was markedly lower \( (P < 0.001) \) than that when the LH concentration was between 6.0 and 9.0 IU/l and became similar to that in patients with normal LH levels (Fig. 2). All 17-OHP levels associated with an LH concentration greater than
12.0 IU/l were below the upper limit of the normal range. In contrast, the mean androstenedione level associated with an LH concentration greater than 12.0 IU/l was only slightly lower (P < 0.05) than that when the LH concentration was between 9.0 and 12.0 IU/l and remained markedly higher (P < 0.001) than that in patients with normal LH levels (Fig. 2). Thus, only 33% of androstenedione levels in the LH range greater than 12.0 IU/l were below the upper limit of the normal range. Finally, the prevalence of increased androstenedione levels (22%) was twofold higher than that of 17-OHP concentrations (11%) in HW as a whole.

**Discussion**

As previously reported in *in vitro* studies (1–15), our results indicate that LH induces *in vitro* stimulation and down-regulating effects on ovarian Δ⁴ P450 activities as serum LH levels gradually increase. Indeed, for relationships between LH and 17-OHP concentrations, a positive correlation was found between these parameter levels when LH levels ranged from 0.2–9.0 IU/l. Conversely, for LH levels ranging from 9.0–21.0 IU/l, LH values were negatively correlated with 17-OHP concentrations. Similar results were observed for relationships between LH and androstenedione levels. However, the LH peak level related to decreasing androstenedione concentrations was 12.0 IU/l. The upper limit of normal serum LH levels was 6.0 IU/l. Taken together, these data indicate that normal and mildly elevated serum LH levels induce an increase in both ovarian Δ⁴ 17-hydroxylase and Δ⁴ 17,20-lyase activities in HW. In contrast, high serum LH levels, as observed in the preovulatory surge (5), induce opposite effects on P450 activities in these patients. Very high serum LH levels are rarely observed in HW. Whether these LH levels continue down-regulating ovarian Δ⁴ P450 activities in these patients requires further studies. Interestingly, very high serum LH levels obtained during gonadotropin-releasing hormone (GnRH) agonist stimulation induce increased ovarian P450 activities in PCOS women (23, 24). Finally, the number of patients with high LH levels was markedly lower than that of women with normal or mildly increased LH levels. Whether the use of an increased number of patients with high LH levels would show some changes in the parabolic relationships of LH to 17-OHP and androstenedione requires further studies.

It has been reported in *in vitro* studies that LH has less effect on 17,20-lyase than on 17-hydroxylase activity (12–15, 23). Moreover, LH-induced down-regulation of 17,20-lyase begins at a lower LH concentration than that of 17-hydroxylase (12–15, 23). Many of our data indicate that, in contrast to LH effects on Δ⁴ 17-hydroxylase, LH effects on Δ⁴ 17,20-lyase in HW are different from those observed in *in vitro* studies. Indeed, in contrast to *in vitro* studies, the mean androstenedione and 17-OHP levels in HW with mildly elevated LH levels increased in similar importance relative to their respective mean levels in HW with low LH levels. Furthermore, in contrast to *in vitro* studies, LH-induced down-regulation of Δ¹ 17,20-lyase began at higher LH levels than that of Δ¹ 17-hydroxylase in HW. Moreover, in contrast to *in vitro* studies, high LH levels induced markedly decreasing 17-OHP levels which became similar to those in patients with normal LH levels. In contrast, high LH levels induced only slightly decreasing androstenedione levels which remained higher than those in patients with normal LH levels. Thus, LH-induced down-regulation of Δ¹ 17,20-lyase, but not that of Δ¹ 17-hydroxylase, is partially impaired in HW. Finally, in contrast to *in vitro* studies, the prevalence of increased androstenedione levels was much greater than that of 17-OHP levels in HW as a whole. Taken together, these data indicate that all the discrepancies between studies in HW and *in vitro* studies relating to the effect of LH on P450 activities are related to a marked increase in Δ¹ 17,20-lyase, but not in Δ¹ 17-hydroxylase, activity in HW. This strongly suggests that serum factors induce a marked increase in Δ¹ 17,20-lyase activity in most of these patients. Indeed, these serum factors could act in concert with LH to induce markedly increased androstenedione levels in HW, with LH levels stimulating Δ¹ 17,20-lyase. They could induce delay in LH-induced down-regulation of this enzyme in HW. Finally, they could partially counteract LH-induced Δ¹ 17,20-lyase down-regulation leading to increased androstenedione levels in HW with high LH levels. Interestingly, very high serum LH levels obtained during GnRH agonist stimulation induce increased P450 activities, together with a relative impairment of Δ¹ 17,20-lyase, in PCOS women (23, 24). This also strongly suggests that serum factors act on Δ¹ 17,20-lyase, but not on Δ¹ 17-hydroxylase, leading to partial impairment of LH-induced stimulating effects on Δ¹ 17,20-lyase, but not on Δ¹ 17-hydroxylase, in PCOS women with GnRH-induced very high LH levels (23). Insulin and bioavailable insulin-like growth factor-I in obese women, and growth hormone in lean PCOS women seem to be likely candidates to act on ovarian Δ¹ 17,20-lyase (25).

In summary, as previously reported in *in vitro* studies, this *in vivo* study indicates that LH induces stimulating and down-regulating effects on both ovarian Δ¹ 17-hydroxylase and Δ¹ 17,20-lyase activities as serum LH levels gradually increase. However, in contrast to *in vitro* studies, in HW the activity of Δ¹ 17,20-lyase is increased more than Δ¹ 17-hydroxylase activity, irrespective of LH levels. This strongly suggests that serum factors induce these P450 activity changes in most of these patients. These serum factor effects on ovarian P450 activity could explain all the discrepancies observed between studies in HW and in *in vitro* studies regarding the effects of LH on this enzyme and, especially, both partial impairment of LH-induced Δ¹ 17,20-lyase down-regulation and
complete LH-induced Δ417-hydroxylase down-regulation in these patients.

References


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