Role of progesterone deficiency in the development of luteinizing hormone and androgen abnormalities in polycystic ovary syndrome

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The aetiology of polycystic ovary syndrome (PCOS) is unknown. It is uniquely characterized by oligomenorrhea or amenorrhea associated with normal or high oestrogen levels. This prospective clinical study was designed to examine the possible role of the lack of cyclical exposure to progesterone in the development of gonadotrophin and androgen abnormalities in PCOS. Gonadotrophin, androgen and oestrogen levels were measured in 15 PCOS patients and 10 normal subjects untreated and following treatment with the progesterone medroxyprogesterone acetate (MPA). When compared to control subjects, PCOS patients had significantly higher luteinizing hormone (LH) pulse height, pulse amplitude, integrated LH levels, LH response to gonadotrophin-releasing hormone (GnRH) and LH/FSH ratio; LH pulse frequency was similar in the two groups. In addition, the testosterone/sex hormone binding globulin ratio (T/SHBG), androstenedione and oestrogen concentrations in the plasma were significantly higher in PCOS than in control subjects. When PCOS patients were treated with MPA for 5 days, there were significant decreases (p < 0.02–0.001) to values no longer different from normal: from 8.7 ± 1.2 to 5.6 ± 0.8 IU/l for integrated LH levels (untreated and MPA-treated PCOS); from 31.2 ± 3.5 to 12.9 ± 1.5 IU/l for LH response to GnRH; from 2.4 ± 0.26 to 1.3 ± 0.2 for LH/FSH ratio; and from 10.4 ± 0.63 to 8.5 ± 0.7 nmol/l for androstenedione. Significant decreases (p < 0.05–0.005) to values that still remained significantly higher than in normal subjects occurred for: LH pulse height, 11.05 ± 1.3 to 6.88 ± 0.79 IU/l (untreated and MPA-treated PCOS); LH pulse amplitude, 2.8 ± 0.5 to 1.8 ± 0.2 IU/l; total testosterone, 2.5 ± 0.2 to 2.0 ± 0.2 nmol/l; T/SHBG ratio, 14.1 ± 1.7 to 11.1 ± 1.5; and oestrone, 265 ± 24 to 208 ± 29 pmol/l. These results are consistent with the concept that ovulation failure and progesterone deficiency play a facilitatory role in the development of the hypothalamic–pituitary abnormality giving rise to disordered LH secretion in PCOS.

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Polycystic ovary syndrome (PCOS) is the commonest cause of anovulatory infertility and accounts for approximately 15–20% of all cases of infertility (1). Clinical features of PCOS cover a wide spectrum, including hirsutism, oligomenorrhoea or amenorrhoea, infertility and obesity (2). Hormonal abnormalities in PCOS patients include elevated androgen and oestrogen levels, elevated basal and GnRH-stimulated LH levels, elevated LH/FSH ratio, hyperinsulinaemia and insulin resistance (3–5). The pathogenesis of this disorder has not been established definitively and it is the subject of on-going review (3, 6, 7). Available treatment is non-specific and frequently unsatisfactory (3, 6, 8, 9). Polycystic ovary syndrome may be associated with several abnormalities occurring singularly or together, including disorders of the ovaries (10), the adrenals (11, 12), the hypothalamic–pituitary axis (13) and hyperinsulinaemia with insulin resistance (14, 15). However, whether these abnormalities are pathogenic in the development of PCOS or occur as a consequence of the disorder is unknown. In some instances clearly distinct abnormalities, e.g. congenital adrenal hyperplasia (16), Cushing’s syndrome (17) or androgen-secreting ovarian tumours (18), appear to be primary to the development of PCOS. Progesterone deficiency, due to decreased frequency of ovulation, may play an important role in the modulation of gonadotrophin secretion (19). When CC or human menopausal gonadotrophin (hMG) and human chorionic gonadotrophin (hCG) are used to induce ovulation, improvement in the hormonal milieu occurs following ovulation (20–22). These observations hint at a possible role of progesterone in bringing about these hormonal corrections. In the present study we have examined the response of gonadotrophin and androgen levels in PCOS to treatment with the progesterone medroxyprogesterone acetate (MPA) and contrasted the values obtained with the hormonal profile found in normal women.
Subjects and methods

Patients
Fifteen PCOS patients participated, all of whom had hirsutism, oligomenorrhea defined as eight or less menses per year and an LH/FSH ratio >2. Ultrasound findings in all patients were consistent with polycystic ovaries with increase stroma and multiple cysts (23). Cushing’s disease was excluded using the overnight dexamethasone suppression test (24) and all patients had ACTH-stimulated 17-hydroxyprogesterone levels less than 5.0 nmol/l, thereby excluding congenital adrenal hyperplasia of the most common type, 21-hydroxylase deficiency. None of these patients had used any hormonal treatment before the study.

Normal subjects
Ten non-hirsute subjects with regular ovulatory menstrual cycles and normal androgen, oestrogen and LH levels were also studied. Ovulation was confirmed by the demonstration of normal luteal-phase progesterone levels. All these volunteers had normal ovarian ultrasound appearances and none had used hormonal contraception before entering the study.

Study protocol
The LH secretory characteristics, androgen and oestrogen levels were examined before and in response to treatment with the synthetic progestogen MPA (6α-methyl-17-acetoxy-pregn-4-ene-3,20-dione) in PCOS patients. Normal subjects were also examined untreated. Each participant was admitted to the Education and Research Centre of St Vincent’s Hospital at 08.00 h on the appropriate study day. An indwelling intravenous catheter was inserted in a forearm vein and the patency was maintained with a low-volume normal saline infusion. On each study day blood sampling for LH assays was performed at 15-min intervals for 6 h, followed by a GnRH test that required blood samples for the measurement of LH and FSH before and at 10, 20, 30, 45 and 60 min following GnRH injection, 100 μg intravenously. Studies were performed in the early follicular phase (days 4–6 of the menstrual cycle) in normal subjects, and in both normal and PCOS subjects plasma progesterone levels excluded a functioning corpus luteum at this time. The PCOS subjects were studied untreated and on the fifth day following the onset of a withdrawal bleed induced by the administration of MPA (10 mg per day for 5 days). Normal subjects were evaluated untreated. In addition, on each study day blood samples were obtained for the measurement of testosterone, androstenedione, sex hormone-binding globulin (SHBG), oestrone and oestradiol. The study protocol was approved by the Ethics and Research Committee of St Vincent’s Hospital and written informed consent was obtained from each participant before enrolment in the study.

Assays
Serum LH and FSH were measured in duplicate employing a two-site fluoroimmunoassay (Delphia system, Wallac, Turku, Finland). The average within-assay reproducibility, estimated from duplicate measurement of three plasma pools in at least 20 assays, was 5.8% (25). Plasma levels of testosterone, androstenedione, oestrone, oestradiol and 17-hydroxyprogesterone were measured by specific radioimmunoassays similar to that described previously for aldosterone (26). These radioimmunoassays were performed following extraction into diethyl ether and, in the case of androgens and oestrogens, after isolation of the steroids by chromatography over celite columns (27). Plasma progesterone was measured by RIA in diluted plasma without extraction using antisera to 11α-hydroxyprogesterone-11α-hemisuccinate-HSA (ICN Biomedicals, Inc., Costa Mesa, CA, Cat. No. 07-170016), progesterone-11α-glucuronide-[3H]iodotyramine (Cat. No. IM.140, Amersham, UK) and progesterone standards (Sigma Chemical Co., Poole, UK) diluted in charcoal-stripped plasma. Incubation of diluted specimens and standards with antisera and labelled progesterone was performed at 37°C for 1.5 h in the presence of excess cortisol, to displace progesterone from plasma binding proteins. Plasma SHBG was measured by immunometric assay (28) and the testosterone/SHBG ratio, (T/SHBG in nmol/l/100 nmol/l) × 100, was used as an indirect index of free testosterone levels (29). The between-assay reproducibility of the methods, estimated by calculating the average coefficient of variation of three to four plasma levels, each measured on at least 20 occasions, was as follows: testosterone, 8.8%; androstenedione, 7.5%; oestradiol, 21%; oestrone, 19%; progesterone, 15%; SHBG, 5.6%; FSH, 6.7%; LH, 7.5%. The minimum LH value to detect a pulse was 1.26 IU/l.

Luteinizing hormone pulse detection
Luteinizing hormone pulse characteristics were analysed employing the cluster analysis program of Veldhuis and Johnson (30). The LH values were analysed in duplicate and a cluster size of 2 × 1 (2 points for a nadir and 1 point for a peak) with a t-statistic of 1 for both upstroke and downstroke were used to provide an optimal sensitivity and specificity over 90% (31). Missing LH values were calculated as a mean of the levels immediately preceding and following the missing value. The LH pulse frequency was defined as the number of LH peaks detected over 6 h. An LH peak was defined as a point flanked on both sides by significant decreases in LH concentrations, and the LH height was the maximum LH value attained within a
peak. The LH pulse amplitude was defined as the LH increment from the preceding nadir to the following peak. For each subject, the means of the LH pulse frequency, height and amplitude over 6 h were used in the final analysis of the data. The integrated LH level is the mean of the LH values over 6 h. The gonadotrope sensitivity to GnRH was defined as the maximum LH increment following GnRH administration in excess of the basal value obtained immediately before GnRH injection.

Statistical analysis

Differences between control and patient groups were analysed by the non-parametric Mann–Whitney U unpaired t-test, and differences in values before and after treatment in the same group were analysed by the Wilcoxon signed-rank paired t-test (StatView II software program for the Apple Macintosh computer, Abacus Concepts, Inc., Berkely, CA). Differences were considered to be significant at p < 0.05. Values are given as the mean and standard error of the mean (SEM), unless stated otherwise.

Results

Clinical features in control subjects and PCOS patients

The PCOS patients had a significantly higher body mass index than control subjects (weight/height$^2 = 33.8 \pm 5.9$ and $22.4 \pm 0.9$, mean±sd, $p < 0.05$) and fewer menses per year ($5.3 \pm 3.6$ and $11.8 \pm 4.0$, $p < 0.05$). Using the hirsutism score system devised by Ferriman and Gallwey (32), this cohort of PCOS patients had a mild degree of hirsutism (PCOS = 7.7 ± 4.6 and control subjects = 1.3 ± 1.0; $p < 0.05$). The two groups were of similar age (22.7 ± 2.3 and 21.9 ± 2.7 years).

Gonadotrophin, androgen and oestrogen profile in untreated control subjects and in untreated and MPA-treated PCOS (Table 1)

When compared to values in normal subjects, PCOS patients demonstrated significantly elevated mean values for LH pulse amplitude, pulse height, integrated LH levels, LH/FSH ratio, maximum LH response to GnRH, testosterone, T/SHBG ratio, androstenedione, oestradiol/SHBG ratio and oestrone, and a decreased mean SHBG level. Normal subjects and PCOS patients had similar LH pulse frequencies. FSH concentrations and FSH responses to GnRH. Treatment of PCOS patients with MPA resulted in normalization of the integrated LH levels, LH response to GnRH, LH/FSH ratio and androstenedione levels. In addition there was a significant reduction in LH pulse height, LH pulse amplitude, testosterone, T/SHBG ratio and oestrone from pre-treatment levels, but these values remained significantly higher than those in untreated normal subjects.

Discussion

This study examined the hypotheses that progesterone deficiency may be primary to hormonal abnormalities in PCOS. Treatment with the progestogen MPA for only 5 days resulted in normalization of the integrated LH levels, LH/FSH ratio, LH response to GnRH and androstenedione. In addition, significant declines in LH pulse height, LH pulse amplitude, total testosterone, T/SHBG ratio and oestrone levels were observed. This beneficial effect of MPA on LH abnormalities and androgen levels in PCOS was evident following just 5 days of treatment, and it was observed on the fifth day after the commencement of MPA-induced withdrawal menstrual bleeding. Therefore, the data presented support the concept that reduced exposure to regular cyclical luteal-phase progesterone levels may contribute to evolution of the abnormalities in LH secretion and LH-dependent androgen excess in PCOS. When CC or hMG and hCG are used in an attempt to induce ovulation, improvement in the hormonal milieu occurs following ovulation (20–22). Blankstein et al.

Table 1. Gonadotrophin, androgen and oestrogen levels in untreated control subjects (N = 10) and in PCOS patients (N = 15) untreated and in response to medroxyprogesterone acetate (MPA).

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
<th>Polycystic ovary syndrome (Untreated)</th>
<th>MPA-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH pulse frequency</td>
<td>3.1 ± 0.38</td>
<td>2.93 ± 0.23</td>
<td>3.13 ± 0.31</td>
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<tr>
<td>(per 6 hours)</td>
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<tr>
<td>LH pulse height</td>
<td>4.77 ± 0.6</td>
<td>11.05 ± 1.3$^a$</td>
<td>6.9 ± 0.8$^b$</td>
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<td>(IU/l)</td>
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<tr>
<td>LH pulse amplitude</td>
<td>1.1 ± 0.1</td>
<td>2.8 ± 0.47$^a$</td>
<td>1.8 ± 0.2$^b$</td>
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<tr>
<td>(IU/l)</td>
<td></td>
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<tr>
<td>Integrated LH level</td>
<td>4.1 ± 0.5</td>
<td>8.73 ± 1.15$^a$</td>
<td>5.6 ± 0.8$^b$</td>
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<tr>
<td>(IU/l)</td>
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<tr>
<td>LH post-GnRH</td>
<td>11.9 ± 2.5</td>
<td>31.2 ± 3.5$^a$</td>
<td>12.9 ± 1.5$^b$</td>
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<tr>
<td>(IU/l)</td>
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<tr>
<td>FSH</td>
<td>4.1 ± 0.4</td>
<td>4.81 ± 0.2</td>
<td>4.1 ± 0.3$^b$</td>
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<tr>
<td>(IU/l)</td>
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<tr>
<td>FSH post-GnRH</td>
<td>2.57 ± 0.5</td>
<td>3.21 ± 0.3</td>
<td>2.7 ± 0.4</td>
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<tr>
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<td>LH/FSH</td>
<td>0.84 ± 0.1</td>
<td>2.39 ± 0.3$^a$</td>
<td>1.33 ± 0.2$^b$</td>
</tr>
<tr>
<td>Total testosterone</td>
<td>1.5 ± 0.2</td>
<td>2.53 ± 0.2$^a$</td>
<td>2.04 ± 0.2$^a$</td>
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<tr>
<td>(nmol/l)</td>
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<tr>
<td>T/SHBG</td>
<td>2.9 ± 0.4</td>
<td>14.1 ± 1.7$^a$</td>
<td>11.0 ± 1.5$^b$</td>
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<tr>
<td>Androstenedione</td>
<td>6.7 ± 1.1</td>
<td>10.4 ± 0.6$^a$</td>
<td>8.5 ± 0.7$^b$</td>
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<td>(nmol/l)</td>
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<tr>
<td>Oestrone</td>
<td>125 ± 14</td>
<td>265 ± 24$^a$</td>
<td>208 ± 29$^b$</td>
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<td>(pmol/l)</td>
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<tr>
<td>Oestradiol</td>
<td>84 ± 10</td>
<td>105 ± 7.6</td>
<td>109 ± 10</td>
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<tr>
<td>SHBG</td>
<td>54 ± 6.0</td>
<td>19 ± 2.1$^a$</td>
<td>18 ± 2.1$^a$</td>
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<tr>
<td>(nmol/l)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Oestradiol/SHBG</td>
<td>1.6 ± 0.2</td>
<td>6.6 ± 0.9$^a$</td>
<td>7.4 ± 1.0$^a$</td>
</tr>
</tbody>
</table>

$^a$ Significantly different from normal subjects, $p < 0.05$.

$^b$ Significantly different from untreated PCOS, $p < 0.05$. 

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reported significantly lower LH and testosterone levels and a lower LH response to GnRH in PCOS following medically induced ovulatory cycles than after exposure to MPA (20). Petsos et al. have demonstrated lowering of the basal and GnRH-stimulated LH values in PCOS subjects treated with MPA for 14 days, with further decline thereafter (19). Berga and Yen reported increased LH pulse amplitude, unchanged mean LH levels and decreased LH pulse frequency after 10 days of MPA treatment in PCOS subjects (33). Treatment of PCOS patients with vaginal progesterone and transdermal oestradiol for 21 days was associated with a significant decline in basal and GnRH-stimulated LH and LH pulse frequency, first noted on the 10th day of treatment (34). While neither abnormalities in LH frequency nor changes in LH pulse frequency following MPA treatment were demonstrated in the present study, these differences may be accounted for by differences in study duration, sampling periods, period of treatment with progestogens, assay methods and, very importantly, the additional use of oestrogen in the study of Christman et al. (34). Whether the activity of the GnRH pulse generator system is deranged, and thereby gives rise to abnormal LH frequency in PCOS, has been the subject of conflicting reports (35–38). The lack of difference in the LH pulse frequency between PCOS patients and normal subjects shown in our study is probably related to less-frequent sampling, because investigators who used a more intensive and prolonged sampling paradigm have shown a greater LH pulse frequency in PCOS patients than in normal subjects (9, 36, 38).

It is clear that MPA treatment did not correct abnormal LH secretion entirely (Table 1). This may merely reflect that, not surprisingly, MPA treatment as used in this study does not duplicate the physiological pattern of luteal-phase progesterone and that repeat cyclical exposure may be necessary to achieve the full progestogen effect. It is possible that treatment with exogenous progestogens may have a significant therapeutic role. There is certainly clear evidence that pretreatment of PCOS patients with a progestogen increases the frequency of ovulation induction by CC (39).

While the findings in the present study are consistent with the concept that abnormalities in LH secretion and ovarian androgen excess occur as a consequence of anovulation, the cause of anovulation remains to be elucidated. When ovulation failure is associated with low oestrogen levels, e.g. hypogonadotrophic hypogonadism and hypergonadotropic hypogonadism, it is not associated with the gonadotrophin and androgen abnormalities seen in PCOS (40). The LH abnormalities that develop in PCOS in response to ovulation failure may depend on the presence of normal or elevated oestrogen levels. An intrinsic ovarian abnormality characterized by failure of the developing graffian follicle to rupture is consistent with this concept. Furthermore, insulin resistance, adrenal hyperandrogenaemia and obesity may all, by different mechanisms, bring about cessation of ovulation, although oestrogen levels are maintained by some mechanism. In this setting abnormal gonadotrophin secretion may develop, giving rise to ovarian androgen hypersecretion and the development of PCOS. Correction of disorders such as obesity (41, 42) and adrenal androgen hypersecretion (11) may allow ovulation to resume, with correction of the hormonal abnormalities. Findings in this study suggest that in order to elucidate whether a primary intra-ovarian abnormality exists in PCOS patients who do not have an extra-ovarian cause, the progestogen replete model should be examined. This will avoid confusion created by attempting to distinguish between abnormalities secondary to ovulation failure and those giving rise to ovulation failure.

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