Effect of gonadotrophin-releasing hormone analogue (GnRH-A) administration on serum gonadotrophin and steroid levels in patients with polycystic ovarian disease

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Abstract. A gonadotrophin-releasing hormone (GnRH) analogue, D-Ser[TBU]LH-EA10, (GnRH-A), at a dose of 200 μg was given daily for 2 months to 6 women with polycystic ovarian disease (PCO). Prior to therapy the patients presented elevated LH, testosterone (T), oestrone (E1) and dihydrotestosterone (DHT) in the circulation. In response to GnRH-A, these subjects exhibited a marked decrease in circulating T, DHT and androstenedione (A) levels as measured 24 h after GnRH-A injection, by 4 weeks and onwards (P < 0.05). After 2 weeks of daily administration, the serum LH profile, evaluated by sampling at 2, 4, 7 and 24 h after injection of GnRH-A, was not different from baseline, whereas after 4, 6 and 8 weeks the levels were significantly lower (*P < 0.01). The profile of serum T levels was unmodified at the second week, but significantly decreased thereafter (*P < 0.01). At the end of treatment, the E1 concentrations, elevated in pre-injection condition, were markedly decreased.

These data demonstrate that in PCO subjects, GnRH-A significantly lowered the elevated levels of androgens commonly found in these patients. The close correlation observed between reduced serum LH and androgen concentrations suggests that pituitary desensitization could be responsible for the reduction in androgen levels, and may be evidence for a gonadotrophin dependence of the elevated concentrations of T in these patients.

Hormonal findings in polycystic ovarian disease (PCO) are well established. These include elevated LH levels with an increased serum LH/FSH ratio, and elevated serum androgens (DeVane et al. 1975; Chang et al. 1982). Numerous investigators have shown increased concentrations of T (DeVane et al. 1975; Greenblatt & Mahesh 1976) in the peripheral circulation.

The specific glandular source (adrenal or ovarian T) of the excess androgen production in PCO is controversial, although hyperandrogenism in polycystic ovarain disease seems to be partially LH-dependent, since there is a direct temporal correlation between the suppression of LH by oral contraceptive and the decrease in plasma levels of A and T (Givens et al. 1974). The elevated LH levels, then, might stimulate ovarian androgen production by direct stimulation of the ovarian theca cells (Erickson et al. 1979), regardless of whether the initial source of excess androgens is adrenal or ovarian.

It has been widely reported that appropriate administration of gonadotrophin-releasing hormone agonists (GnRH-A) paradoxically, 'down-
regulates the pituitary, resulting in a decrease in gonadotrophins and gonadal steroids (Bergquist et al. 1979a,b; Clayton 1982). Consequently, chronic administration of these analogues has been used in humans for treating diseases in which a lowering of gonadal steroids may be of value (Comite et al. 1981; Koutsilieris & Tolis 1983). This initial observation suggested the suitability of GnRH-A as a tool for suppression of the elevated steroid concentrations in PCO. Reductions in circulating T and A have previously been observed by us (D’Agata et al. 1982) and by others (Chang et al. 1983) in PCO patients following daily injection of potent analogues.

In these studies, however, hormonal evaluation during GnRH-A was limited to one single blood value sampled 24 h following injection. It would appear from previous studies that analogue administration may be followed by a phase of gonadotrophin and steroid stimulation (Faure et al. 1982; Tolis et al. 1983) of a varying duration according to dose and time of treatment.

Therefore, we have been concerned about the possibility that random or single sampling of serum hormones may not adequately reflect the modification of the hormonal pattern induced by GnRH-A. In an attempt to go more deeply into the dynamics of the pituitary-ovarian axis responsiveness to GnRH-A, we evaluated serum gonadotrophin and T concentrations every 2 weeks at various intervals during a 24-h period following morning injection of the analogue in a group of PCO patients treated with a potent GnRH-A for a period of 8 weeks.

### Material and Methods

Six hirsute women with clinical and laboratory data of polycystic ovarian disease were studied. Clinical and laboratory data are shown in Table 1. In 2 women, the presence of bilaterally enlarged polycystic ovaries was confirmed by laparoscopy, and in all patients a pelvic ultrasound revealed a picture consistent with PCO. None were obese, presented acanthosis nigricans or had received medication for at least 3 months before the investigation. Hormone studies included a pretreatment evaluation, in which blood samples were drawn at 09.00 h for 1–3 days for serum LH, FSH, oestradiol-17β (E₂), oestrone (E₁), A, DHT and T concentrations. On the first day of study, serum LH, FSH and T were evaluated at 09.00 h and also at 11.00, 13.00 and 16.00 h (baseline). Each patient then received 3–4 μg/kg of GnRH-A, (D-Ser[TBU]-LRH-EA₁₀), HOE 766.
Hoechst, West-Germany) sc daily at 09.00 h for 60 days. During GnRH-A administration, blood samples were collected every 2 weeks at 2, 4, 7 and 24 h after the daily injection of the drug. LH, FSH and T were evaluated in all samples and A and DHT only in the 24-h sample. Owing to the limited amount of sample allowed us for hormone studies, E₁ and E₂ concentrations could be evaluated only at the end of the study, 24 h after the last analogue injection. These studies were begun at random since the patients' cycles were infrequent and irregular.

**Assay**

Radioimmunoassays were performed for LH and FSH using commercial kits (Biodata, Roma, Italy). All steroids were measured according to previously published RIA techniques, after extraction and separation with LH-20 or celite columns (Auletta et al. 1974; Flamigni et al. 1977; Cutler et al. 1978). Normal ranges with these methods were constructed by collecting samples during the follicular phase of the cycle in 20 normally cycling women. Specimens from any one subject were always measured in the same assay to avoid any inter-assay variations.

**Statistical analysis**

Partial sums of squares were calculated by applying the analysis of variance to all LH, FSH and T concentrations determined throughout the day, partitioning the total variability of the data into two components used as source of variation: treatment and subjects (two-way analysis of variance with interaction. *P). Student's t-test was also used when appropriate (P). All values are expressed as the mean ± SEM.

**Results**

Fig. 1 shows the effects of GnRH-A treatment on circulating androgens as measured 24 h following its administration. Serum T, DHT and A levels, not significantly different from basal values at the second week, were reduced at 4 weeks (P < 0.05) (Fig. 1). At the end of GnRH-A administration, the levels of both T and A were within the ranges observed in normal women. The DHT concentrations, in contrast, remained slightly above normal.

**Oestrogens**

As seen in Table 1, serum E₁ concentrations were increased so the normal E₁ to E₂ ratio was reversed in our PCO patients, as previously reported (Baird et al. 1977; Chang et al. 1982, 1983). In response to GnRH-A, the mean (mean ± SEM) levels of E₁ (93.2 ± 12.2 pg/ml) were down to 48.8 ± 7.8 pg/ml at the end of treatment (P < 0.01). No important changes were noted for the E₂ concentrations (basal 29.6 ± 2.9 vs 30.8 ± 3.2 pg/ml). It must be pointed out that basal concentrations of plasma E₂ in our subjects were markedly low, which may indicate a substantially low ovarian oestrogen activity in these particular patients (Mahesh & Greenblatt 1964; Kirschner & Jacobs 1971). However, since oestrogen levels fluctuate widely in PCO patient, the finding of no E₂ suppression could be the result of limited sampling for E₂ in our experimental conditions.

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**Fig. 1**

Serum T, A and DHT concentrations before and after GnRH-A treatment. Samples during treatment were collected 24 h after each daily sc dose of GnRH-A. All values are mean ± SEM.
Twenty-four h profile of serum LH and FSH before (baseline) and during GnRH-A treatment. Blood samples were collected at 09.00, 11.00, 13.00 and 16.00 h on a single day before and then every 2 weeks during GnRH-A treatment.

The arrow indicates the time of the day when the analogue was administered. All values are mean ± SEM.

**24-h profile of gonadotrophins and testosterone during analogue administration**

Figs. 2 and 3 illustrate the typical profile of serum LH and T levels as measured in the basal condition and 2, 4, 7 and 24 h after administration of GnRH-A. At the second week, the daily serum LH concentration was not different from the basal LH profile (two-way analysis of variance, \( *P < 0.05 \) (Fig. 2), plasma LH in particular being slightly increased above basal 2 and 4 h after injection, and slightly decreased at the two later samplings. At 4, 6 and 8 weeks, the daily levels of LH were significantly lower than baseline (\( *P < 0.01 \) for all these weeks). After 4 weeks, the LH response to analogue measured 2 and 4 h following injection of the peptide, however, was...
consistently more elevated than at any later time throughout the treatment period. This may be an indication of persistence of residual acute pituitary responsiveness to GnRH-A injection. The serum FSH profile shows that treatment with analogue slightly decreased the concentration of this hormone (Fig. 2).

The serum T levels during a 24-h period in analogue-treated subjects are depicted in Fig. 3. Serum concentrations were substantially unmodified at the second week, thereafter the T responses to GnRH-A were characterized by a steady decline, in fact, at all points for each week, when considered together, were found to be significantly different from the 24-h baseline profile (*P < 0.01). In each subject the maximal inhibition of T levels occurred in those samples collected 24 h after daily injection. It is noteworthy that, during month 2 of treatment, the serum concentrations of T remained within the normal range throughout the day.

Clinical and laboratory data for each subject at the end of treatment are shown in Table 2.

### Discussion

Our patients with clinical and laboratory data of PCO had a significant elevation of circulating LH, T and DHT with an increased ratio of serum LH to FSH. E₁ levels were increased and the ratio of E₁ and E₂ was reversed. This hormone picture is consistent with previously reported data for this syndrome (Baird et al. 1977; Chang et al. 1982, 1983). We found in this study that a daily dose of a potent GnRH-A given to 6 women with PCO significantly lowered the serum concentrations of LH; the effect on FSH, however, was less consistent. This LH decrement was associated with marked reduction in circulating T, which was evident by 4 weeks and persisted at the end of the sixth week.

However, it must be pointed out that the analogue administration produced an initial period (~2 weeks) of gonadotrophin and T stimulation. Furthermore, evaluation of temporal changes of gonadotrophin and T for the first 24 h following the daily injection of GnRH-A showed that agonist treatment lowered, but did not abolish, the rise in serum LH and T associated with agonist injection (Faure et al. 1982; Tolis et al. 1983). This indicates that one single blood sample collected 24 h after injection may not necessarily reflect the hormonal changes provoked by the analogue.

LH is known as a potential regulator of ovarian androgen production by a direct stimulation of the ovarian theca cells (Erickson et al. 1979), and GnRH-A administration seems to inhibit the ovarian androgen selectively, but does not affect the adrenal source of these hormones in PCO (Chang et al. 1983). Hence, the concomitant decrease of LH and T in PCO patients, herein reported, may be taken as evidence for a gonadotrophin dependence and an ovarian source of the elevated concentrations of T in the circulation.

Obviously more patients need to be studied to substantiate these findings. GnRH-A administration was also associated with a marked decrease in circulating A, DHT and E₁. The effect on the latter is very likely the consequence of the reduc-

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**Table 2.**

Clinical and hormonal data at the end of a GnRH-A course in patients with PCO.

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Hirsutism score*</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
<th>T (ng/ml)</th>
<th>E₁ (pg/ml)</th>
<th>E₂ (pg/ml)</th>
<th>A (ng/ml)</th>
<th>DHT (ng/ml)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>7.1 ± 0.2</td>
<td>7.0 ± 0.5</td>
<td>43.6 ± 1.6</td>
<td>38.3 ± 0.9</td>
<td>34.5 ± 2.7</td>
<td>127 ± 2.0</td>
<td>39.2 ± 1.1</td>
</tr>
<tr>
<td>2</td>
<td>24.5</td>
<td>6.9 ± 0.14</td>
<td>3.8 ± 0.1</td>
<td>28.5 ± 0.7</td>
<td>85.0 ± 3.0</td>
<td>25.6 ± 0.4</td>
<td>74.5 ± 1.1</td>
<td>38.4 ± 0.8</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>10.2 ± 0.7</td>
<td>7.2 ± 0.2</td>
<td>29.6 ± 0.5</td>
<td>31.0 ± 12</td>
<td>43.8 ± 0.2</td>
<td>163 ± 4.0</td>
<td>36.7 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>14.9 ± 4.1</td>
<td>6.2 ± 0.2</td>
<td>35.0 ± 1.1</td>
<td>46.2 ± 0.7</td>
<td>25.1 ± 0.4</td>
<td>204 ± 3.0</td>
<td>37.2 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>6.9 ± 0.6</td>
<td>6.3 ± 1.1</td>
<td>34.0 ± 0.5</td>
<td>52.1 ± 19</td>
<td>23.3 ± 0.9</td>
<td>87.6 ± 0.7</td>
<td>39.5 ± 0.9</td>
</tr>
<tr>
<td>6</td>
<td>26</td>
<td>4.0 ± 0.14</td>
<td>5.9 ± 0.14</td>
<td>55.0 ± 2.5</td>
<td>40.0 ± 0.4</td>
<td>32.5 ± 2.7</td>
<td>125.1 ± 1.1</td>
<td>42.4 ± 1.5</td>
</tr>
</tbody>
</table>

Normal range: LH, 5–20 mIU/ml; FSH, 5–20 mIU/ml; T, 9–55 ng/dl; A, 90–350 ng/dl; DHT, 5–30 ng/dl. 
E₁ 52 ± 19 pg/ml (early follicular phase); E₂, 64 ± 26 pg/ml (early follicular phase).
* According to the criteria of Ferriman & Gallwey (1961).
tion in serum A concentrations (Siiteri & Mac Donald 1973; Baird et al. 1977) rather than a direct effect on the ovarian axis.

It has been widely known for some time that GnRH-A can diminish gonadotrophin and steroid release in animals and man (Bergquist et al. 1979a; Cusan et al. 1979; Rivier et al. 1979; Linde et al. 1981). The exact mechanism for this action of GnRH-A remains to be established. Several possibilities have been postulated, including down-regulation of gonadotrophin release (Bergquist et al. 1979a; Clayton 1982), desensitization of the gonadal response to persistent non-pulsatile levels of LH in response to GnRH-A treatment (Rabin & McNeil 1980), and/or direct action on the ovary (Hsueh & Jones 1981). The correlation observed between reduced serum LH and androgen concentrations could suggest that a pituitary desensitization may be an important factor in the inhibition of ovarian androgen release observed in our PCO patients. However, the other mechanisms cannot yet be ruled out.

Chang et al. (1983) recently reported that a potent GnRH-A given daily for 4 weeks to patients with PCO brought about a complete suppression of ovarian steroid secretion which was associated with a sustained increase in circulating LH. However, this is not necessarily contradictory to our findings. Indeed, shortly after the former finding it was demonstrated that elevated immunoreactive LH found in women with PCO is accompanied by a marked reduction in LH bioactivity (Meldrum et al. 1984). These qualitative changes in LH during GnRH-A treatment seem to be responsible for the suppression of ovarian steroid secretion reported.

Although our clinical experience in this syndrome is still limited, 2 hirsute PCO patients who have been treated for 5 months so far (data not shown) with a similar dose of analogue, have experienced a marked and persistent reduction of body hair growth and acne.

It can be concluded that inhibition of ovarian function can be accomplished with the use of Buserelin in patients with PCO; whether this may indicate a therapeutical use of this compound to inhibit the elevated circulating concentrations of androgens in PCO remains to be established by long-term studies.

At least, it is reasonable to conclude that this compound represents an important tool for the investigation of some aspects of the pathogenesis of PCO.

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References


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