A pilot study of the human chorionic gonadotrophin test for ovarian hyperandrogenism

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A controlled clinical study was designed to investigate the value of human chorionic gonadotrophin (HCG) challenge as a test for functional ovarian hyperandrogenism. Dexamethasone administration was followed by 5000 IU HCG and blood samples for steroid hormone assay were obtained 0, 8, 16, and 24 h thereafter. Study subjects were normal women (n = 13); women with functional ovarian hyperandrogenism, defined by androgen excess, amenorrhoea and an increased 17-hydroxyprogesterone response to nafarelin (n = 6); and normal men (n = 4). The responses of 17-hydroxyprogesterone, androstenedione and testosterone to HCG in women with functional ovarian hyperandrogenism were significantly greater than in normal women. However, the 17-hydroxyprogesterone response to HCG in functional ovarian hyperandrogenism was significantly lower after HCG than after nafarelin. The oestradiol response was also significantly lower after HCG than nafarelin, although oestradiol concentration more than doubled in normal women as well as in women with functional ovarian hyperandrogenism. The responses to HCG confirm that functional ovarian hyperandrogenism abnormalities are luteinizing hormone (LH)-dependent. Therefore, the 17-hydroxyprogesterone response to HCG could represent a useful test for the diagnosis of ovarian hyperandrogenism. The lower 17-hydroxyprogesterone response to HCG than to nafarelin in functional ovarian hyperandrogenism suggests that a follicle-stimulating hormone (FSH)-responsive factor modulates thecal 17-hydroxyprogesterone secretion. The oestradiol response to HCG is consistent with HCG directly stimulating the oestriadiol secretion by thecal cells.

Key words: PCOS/hyperandrogenism/HCG/GnRHa

Introduction

Patients with polycystic ovary syndrome (PCOS) and other forms of functional ovarian hyperandrogenism (FOH) respond to the endogenous luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion induced by a single dose of the gonadotrophin-releasing hormone (GnRH) agonist (GnRHa) nafarelin with a markedly increased response of 17-hydroxyprogesterone (17-Prog) (Barnes et al., 1989; Ehrmann et al., 1992). Responses of androstenedione (AD) and testosterone (T) are also increased, but not to the same degree as 17-Prog. The 17-Prog response is similar to that seen in normal men, and the T response is intermediate between those of normal men and normal women. We have found that an exaggerated 17-Prog response to GnRHa correlates closely with a failure of suppression of free T by dexamethasone and is a reliable diagnostic test for FOH. This abnormality appears to result from a failure in the modulation of LH action within thecal cells (Rosenfield et al., 1994).

One might expect to demonstrate an exaggerated 17-Prog response in FOH by stimulation with human chorionic gonadotrophin (HCG), which is essentially an LH analogue (Albert, 1969). However, HCG has seldom been used as a test of ovarian androgenic function since Abraham et al. (1975) concluded that ‘the HCG stimulation test was of no practical value in evaluating the source of excess androgens in hirsutism’. In spite of our finding of a rise in urinary pregnanetriol, the unique urinary metabolite of 17-Prog, significantly greater than normal, in hyperandrogenic anovulatory women (Rosenfield et al., 1972), the plasma 17-Prog responses to HCG were not determined, nor, for that matter, were oestradiol responses. At the time we considered that HCG test results could not be interpreted without relating them to the coordinated function of the specific follicle that secretes oestradiol and that this would not be possible. Having determined that GnRHa stimulation provides a practical method to evaluate the combined effect of LH and FSH on ovarian follicular function, it is now, however, once again of interest to reevaluate the HCG stimulation test for the diagnosis of ovarian hyperandrogenism.

Materials and methods

Experimental subjects

This protocol was approved by the University of Chicago Institutional Review Board. After giving informed, written consent, three groups aged 18–35 years underwent HCG testing. Group 1 consisted of 13 normal female volunteers (age 22 ± 4.3; range 18–35 years) without hirsutism or acne who had regular menses every 26–35 days. They were studied in the mid-follicular phase (days 5–9) of the menstrual cycle. Group 2 consisted of six oligoamenorrhoic, hyperandrogenic women with FOH, identified by nafarelin testing (age 28 ± 4.4; range 22–33 years). Their peak plasma 17-Prog concentration post-nafarelin was greater than 7.8 nmol/l, 2 SD above the mean values of normal women with FOH, identified by nafarelin testing (age 28 ± 4.4; range 22–33 years).

Group 3 consisted of six oligoamenorrhoic, hyperandrogenic women with FOH, identified by nafarelin testing (age 28 ± 4.4; range 22–33 years). Their peak plasma 17-Prog concentration post-nafarelin was greater than 7.8 nmol/l, 2 SD above the mean values of normal women with FOH, identified by nafarelin testing (age 28 ± 4.4; range 22–33 years).
in controls (Ehrmann et al., 1992). They were studied after at least 2 months of amenorrhoea and when low serum progesterone values were indicative of anovulation. Group 3 consisted of four normal male volunteers of normal virility (age 27 ± 5.4; range 21–34 years) who had previously undergone nafarelin testing. Nafarelin and HCG testing were performed at least 30 days apart.

**HCG test**

Dexamethasone (Roxane Laboratories, Colombus, Ohio, USA), 0.5 mg, was administered four times a day, beginning 4 days before and continuing through the day of HCG (Profasi; Serono Laboratories, Randolph, MA, USA) administration to suppress adrenal secretion throughout this study of gonadal function. Subjects were admitted to the University of Chicago Clinical Research Center on the morning of the HCG test. An i.v. catheter was inserted and a baseline blood sample obtained. HCG (5000 IU) was then given i.m., and blood samples were obtained 0, 8, 16, and 24 h thereafter. Assays of 17-Prog, T, oestradiol and AD were performed using previously published methods (Barnes et al., 1989; Rosenfeld et al., 1994). Prolactin values were within the normal range. Basal gonadotrophin values have been published previously (Ehrmann et al., 1992). All subjects received a pelvic examination to exclude ovarian enlargement.

**Nafarelin test**

The nafarelin test was performed after dexamethasone administration in similar fashion to the HCG test, except that nafarelin (100 µg) was given subcutaneously.

**Statistical analysis**

Comparison between study groups (FOH, normal women and men) was carried out by unpaired t-test, one-way ANOVA, or Mann–Whitney U test as appropriate. In the normal women, HCG test parameters were secondarily compared to nafarelin test parameters in 17 normal women (age 25 ± 3.9; range 20–34 years) previously reported (Ehrmann et al., 1992) using the unpaired t-test. In the FOH women and normal men, HCG and nafarelin test parameters were secondarily compared using the paired t-test. In situations where data were not normally distributed, they were analysed after logarithmic transformation.

**Results**

**Women**

**Baseline**

Baseline AD, T, and 17-Prog were higher in the FOH group compared to normal women (P < 0.01), while baseline plasma oestradiol concentrations were similar (Table I). No significant differences in baseline steroids were present between the HCG and nafarelin tests in either group.

**Response to HCG**

HCG administration resulted in a peak steroid response between 16 and 24 h, as also occurs with nafarelin (Figure 1). The peak 17-Prog response to HCG was significantly higher in FOH (P < 0.01) than in normal women, as was the incremental rise (P < 0.01; Figure 2; Table I). Although the 17-Prog response was significantly greater in FOH than in normal women, there was an overlap in the distribution of 17-Prog responses between FOH and normal women after HCG testing that did not occur with the nafarelin test as shown in Figure 2. In FOH, both peak and incremental 17-Prog were significantly lower after HCG stimulation compared with nafarelin (Table I). HCG stimulation resulted in significantly greater peak responses of AD and T, but not of oestradiol, in FOH compared to normal women (Figure 2). In FOH, oestradiol responses were significantly lower after HCG stimulation compared with nafarelin (Table I). Androgen responses to HCG and nafarelin were similar in women with FOH.

**Men**

**Baseline**

Baseline AD and oestradiol were lower, and 17-Prog and testosterone higher in the men compared to FOH group. No significant differences in baseline steroids were present between HCG and nafarelin tests.

**Response to HCG**

The 17-Prog responses in men and FOH were not significantly different after HCG (Figure 1; Table I). Baseline 17-Prog and 17-Prog responses to HCG were significantly greater in normal men than normal women (P < 0.005). In FOH, the total androgen response, AD plus T, was intermediate between normal women and men after HCG. Baseline and peak AD and T were significantly greater in men than in both groups of women after HCG (P < 0.05). Baseline and peak oestradiol during HCG testing were significantly higher (P < 0.05) in FOH than men. Baseline oestradiol during the HCG test was significantly higher in normal women than men (P < 0.04), but the peaks were similar.

The peak T response to HCG was significantly greater than to nafarelin, although baseline values were similar (P < 0.05; Table I). Oestradiol, AD and 17-Prog values did not differ between stimulation tests (Table I). However, there was a significant rise in the oestradiol to T ratio with both stimulation tests (HCG: 0.0039–0.0129; nafarelin: 0.0077–0.0183; P < 0.05), suggesting that both tests resulted in an increase in aromatase activity.

**HCG versus nafarelin tests**

The findings resembled our previous observations in men, normal women, and FOH challenged with GnRHa without dexamethasone pretreatment (Barnes et al., 1989). The 17-Prog responses in women and men were not significantly different comparing HCG to nafarelin. The peak and incremental 17-Prog response in FOH was significantly lower after HCG compared to nafarelin (Figure 1; Table I). The distribution of 17-Prog responses overlapped between FOH and normal women with HCG, but not nafarelin, testing. Baseline 17-Prog and 17-Prog responses to both HCG and nafarelin were significantly greater in normal men than normal women (P < 0.005), but neither differed significantly compared to FOH. In FOH the total androgen response, AD plus T, was intermediate between normal women and men after both HCG and nafarelin. Baseline and peak AD and T were significantly greater in men than in both groups of women after either HCG or nafarelin (P < 0.05). Baseline and peak oestradiol during HCG testing, and the oestradiol peak and incremental rise upon nafarelin testing were significantly higher (P < 0.05) in FOH than
Table I. Hormones during dexamethasone-suppressed human chorionic gonadotrophin (HCG) and nafarelin tests\(^{a,b}\)

<table>
<thead>
<tr>
<th></th>
<th>17-hydroxyprogesterone (nmol/l ± SEM)</th>
<th>Androstenedione (nmol/l ± SEM)</th>
<th>Testosterone (nmol/l ± SEM)</th>
<th>Oestradiol (pmol/l ± SEM)</th>
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<tr>
<td></td>
<td>basal</td>
<td>peak</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<tr>
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<tr>
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<td>9.24</td>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>±1.33</td>
<td>±0.88</td>
<td>±0.39</td>
<td>±0.32</td>
</tr>
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</table>

\(^{a}\)Comparisons between functional ovarian hyperandrogenism (FOH) and normal responses to the HCG or nafarelin test are shown in Figure 2 and given in the text. Basal 17-hydroxyprogesterone (17-Prog), androstenedione (AD) and testosterone (T) values are significantly higher in FOH compared to normal women (\(P < 0.01\)). HCG and nafarelin stimulation cause significant differences between basal and peak values (\(P \leq 0.03\)) for all test groups with the exception of androstenedione response to nafarelin in men. Refer to text for significance testing between men and FOH or normal women.

\(^{b}\)Conversion factors (ng/dl): 17-hydroxyprogesterone, 33.0; androstenedione, 28.6; testosterone, 28.8; oestradiol, 0.272 (pg/ml).

Significant differences between responses to HCG and nafarelin within each group are designated by a common letter:

\(^{c,d,e,f} P < 0.01\)

\(^{P} P < 0.05\)

**Discussion**

These studies demonstrate abnormalities of 17-Prog and androgen secretion in response to HCG in FOH which are similar in many ways to those after GnRHa administration and which, therefore, indicate that these abnormalities are LH-dependent. 17-Prog rose two-fold more than AD or T in response to HCG stimulation. The response of all these steroids is significantly greater in FOH than in normal women, and the magnitude of the androgen responses to HCG is similar to that in response to nafarelin.

However, in FOH the peak and absolute rise in 17-Prog were significantly lower after HCG than after nafarelin. If confirmed in a prospective study, this would have diagnostic and physiological implications. Diagnostically it would suggest the superiority of one test over the other. Physiologically it would imply that FSH plays a role in modulating androgen secretion in response to HCG in FOH which are similar in many ways to those after GnRHa administration and which, therefore, indicate that these abnormalities are LH-dependent.
HCG test in hyperandrogenism

they and the oestradiol/T ratio were less than half of that in response to nafarelin. Considerable data indicate that human thecal cells are capable of forming oestradiol throughout the life span of the antral follicle (Ryan and Petro, 1966; Channing, 1969; McNatty et al., 1979a; Gilling-Smith et al., 1994). Additional data indicate that this oestradiol synthesis is under LH control rather than FSH control (McNatty et al., 1979b; Gilling-Smith et al., 1994). The only evidence that human thecal cells cannot form oestradiol was obtained in short-term cultures in the absence of supplemental serum (Moon et al., 1978; Tsang et al., 1980), which is compatible with a dependency of aromatase activity upon growth factors similar to that shown for other steroidogenic steps (Adashi et al., 1985; Ehrmann et al., 1995). The molecular confirmation of thecal localization of aromatase activity is unsettled. Thecal immunostaining for cytochrome P450 aromatase has been variously reported as strong (Inkster and Brodie, 1991), inconsistent (Suzuki et al., 1993) or non-existent (Tamura et al., 1992). A conclusion that the oestradiol rise after HCG is due to thecal cell aromatase activity should, however, be reached with caution.

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Oestrogen secretion in vitro by thecal cells from normal and polycystic ovaries is considerably lower than that by granulosa cells (McNatty et al., 1979a,b; Hillier et al., 1981). An alternate explanation for these findings could be that androgens exert a rapid paracrine effect on granulosa cells, upregulating their aromatase activity (Haning et al., 1993). If so, in view of the time course, this inductive process would have to be exceedingly rapid. Yet another possibility is that endogenous FSH stimulates aromatase sufficiently so that HCG-induced androgens are efficiently converted to oestrogens in the granulosa cell compartment.

In conclusion, the responses to HCG confirm that the secretory abnormalities in FOH are LH-dependent and suggest that HCG may be useful as a diagnostic test for FOH. Whether HCG testing will have the same diagnostic efficiency as GnRHa stimulation remains to be determined.

The greater 17-Prog response to GnRHa than to HCG is consistent with the hypothesis that an FSH responsive factor exerts a modulating effect on thecal 17-Prog secretion. Furthermore, our data are consistent with in-vitro evidence that thecal cells secrete oestrogen in response to LH in addition to their well-established indirect effect of supplying androgens as substrates for oestradiol formation. With further investigation it may become necessary to modify the standard 2-gonadotrophin, 2-cell model of ovarian steroid production to include the lack of rigid compartmentalization of oestrogen synthesis and to include paracrine regulation of thecal androgen production (Channing, 1969; Suzuki et al., 1993). Dose–response studies utilizing recombinant FSH and LH should further delineate the FSH responsive factor effect on 17-Prog secretion in FOH.

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References


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