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TESTICULAR RESPONSIVENESS TO hCG BEFORE AND AFTER LONG-TERM ANTIESTROGEN TREATMENT IN OLIGOZOOSPERMIC MEN

H. MARTIKAINEN,* L. RÖNNBERG, A. RUOKONEN and R. VIHKO
Departments of Clinical Chemistry and Obstetrics and Gynecology, University of Oulu, Oulu, Finland

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Summary—In order to further investigate the role of endogenous estradiol in the regulation of testicular steroidogenesis an hCG-stimulation test (a single dose of 5000 i.u. i.m.) was performed in 5 normogonadotropic oligozoospermic men before and after 3 months of antiestrogen (clomiphene citrate = CC) treatment (50 mg p.o. daily). Peripheral blood samples were collected immediately before hCG administration and thereafter at 1, 4 and 7 days, and were analyzed for testosterone (T), estradiol (E2), 17-hydroxyprogesterone (17-OHP4), 17-hydroxypregnenolone (17-OHP5), 11 other free and sulfate-conjugated steroids, and for sex hormone-binding globulin (SHBG). The results demonstrated that CC-treatment caused a significant rise in peripheral serum concentrations of SHBG, T, 5α-dihydrotestosterone (DHT), E2 and the sulfate conjugates of pregnenolone, 17-OHP5, 5-androstene-3β, 17β-diol and T. In the basal state, before the CC-treatment, apparently normal responses to hCG were seen in unconjugated steroids: 17-OHP4 and E2 concentrations were significantly elevated at 1 day, and those of T at 4 days. After CC, only the concentrations of 17-OHP4 rose significantly following hCG administration. The peripheral serum concentrations of the 5-ene- and sulfate-conjugated precursors of T were not influenced by hCG in the basal state or after CC.

These results suggest that the apparently E2-mediated inhibition of 17,20-lyase activity in the 4-ene-pathway of T synthesis could not be totally prevented by long-term antiestrogen treatment, and that in the 5-ene-pathway no sign of 17,20-lyase inhibition was demonstrated either before or following CC-treatment. The significant rise in the circulating concentrations of sulfate-conjugated steroids following long-term CC administration, apparently due to increased synthesis of T and its precursors in the 5-ene-pathway, strengthens the concept of their importance in testicular steroidogenesis.

INTRODUCTION

A single intramuscular injection of hCG stimulates human testicular testosterone (T) secretion, with the maximum seen at approx 4 days following hCG administration [1–3]. This T peak is preceded by maximal estradiol (E2) production at 24–48 h associated with increased serum 17-hydroxyprogesterone (17-OHP4), suggesting inhibition of 17,20-lyase in the steroidogenic pathway [2–5]. This rise in E2 production is thought to be an important part of the mechanism leading to desensitization of steroidogenesis following a large single dose of hCG.

In previous studies it has been shown that accumulation of 17-OHP4 can be partially prevented by short term antiestrogen treatment in man [6–7], strongly suggesting that inhibition of the lyase enzyme is an E2-mediated phenomenon. However, the maximal response in serum T following hCG was not increased during short-term antiestrogen treatment [6–7], which might have been due to an initial estrogen-like effect of CC. To exclude this possibility we designed a new experiment, in which the CC-treatment period was long enough to permit manifestigation of all possible antiestrogenic effects of CC. For this purpose, an hCG stimulation test was performed in 5 normogonadotropic oligozoospermic men in the basal state, and the response curves of sex hormone-binding globulin (SHBG), T, E2, 17-OHP4, 17-hydroxypregnenolone (17-OHP5) and several other steroids were compared to those obtained following hCG stimulation performed after 3 months of CC-treatment.

*Address correspondence to: Dr Hannu Martikainen, Department of Clinical Chemistry, University of Oulu, Kajaanintie 52, SF-90220 Oulu, Finland.

EXPERIMENTAL

Subjects and experimental approach

Five normogonadotropic oligozoospermic men (mean age 30 ± 6.6, SD, age range 23–40 years) with infertility problems were selected for this study. They were injected at 08.00 h with 5000 i.u. of hCG (Pregnyl, Organon, Oss, The Netherlands) and peripheral blood samples were collected immediately before the injection and thereafter at 1, 4 and 7 days following hCG administration. After this stimulation test they were treated with clomiphene for 3 months (Clomifen, Star, Finland, 50 mg once a day p.o.). The second hCG stimulation test was then performed as described above. Sperm count was 3.1 ± 0.9 mill./ml (mean ± SEM) before and 11.7 ± 5.6 mill./ml after clomiphene treatment.

Determination of hormone and SHBG concentrations

The sera were stored at −20°C until the hormone analyses. The concentrations of free and sulfate-

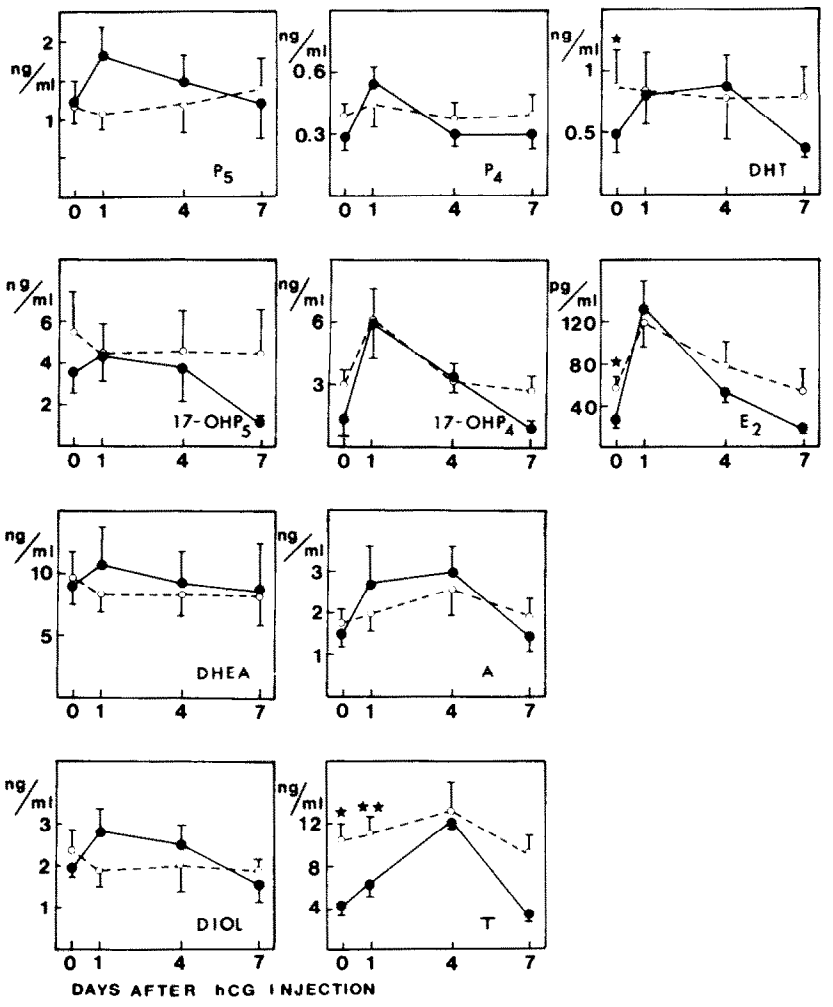

Fig. 1. Mean (±SEM) serum concentrations of pregnenolone (P5), 17-hydroxypregnenolone (17-OHP5), dehydroepiandrosterone (DHEA), 5-androstene-3β, 17β-diol (DIOL), progesterone (P4), 17-hydroxyprogesterone (17-OHP4), androstenedione (A), testosterone (T), 5α-dihydrotesterone (DHT) and estradiol (E2) after a single intramuscular dose of 5000 i.u. of hCG in 5 oligozoospermic men in the basal state (continuous line) and after 3 months of clomiphene treatment (dotted line). The concentrations at each time point were compared by paired t-test, one asterisk: $P < 0.05$, two asterisks: $P < 0.02$.

conjugated steroids were measured by specific radioimmunoassays following fractionation by Lipidex-5000 ™ chromatography as previously in detail described from this laboratory [8–11]. Serum E2 was measured by direct radioimmunoassay without chromatography using a radioimmunoassay kit provided by Farmos Diagnostica (Oulunsalo, Finland) and SHBG by a liquid-phase immunoradiometric assay (Farmos Diagnostica, Oulunsalo, Finland).

Statistics

The hormone levels at the different time points after hCG injection were compared with the 0-h levels using Duncan's new multiple range test of least significant differences [12]. The values during hCG stimulation in the basal state and after CC-treatment were compared at each time point by using paired t-tests. $P < 0.05$ was chosen as the limit of statistical significance.

RESULTS

Peripheral serum steroid and SHBG concentrations during hCG stimulation are depicted in Figs 1–2. Long-term CC administration induced significant increases in T, 5α-dihydrotestosterone (DHT), E2 and in all steroid sulfate (except DHEA-S) concentrations (see 0-points in Figs 1–2). Serum SHBG concentrations were significantly elevated after CC-treatment at all the time points studied.

The response patterns of the 4-ene-precursors of T and its metabolites to hCG before CC were similar to those found previously in normal men [3]: T reached its maximum at 4 days ($P < 0.01$, compared to concentrations at the 0-point) and then it significantly decreased by 7 days ($P < 0.01$). Levels of E2 and 17-OHP4 showed peak values at day 1 ($P < 0.01$), and significantly lower values were observed at 4 (E2, $P < 0.01$) and at 7 days (17-OHP4, $P < 0.01$). In the

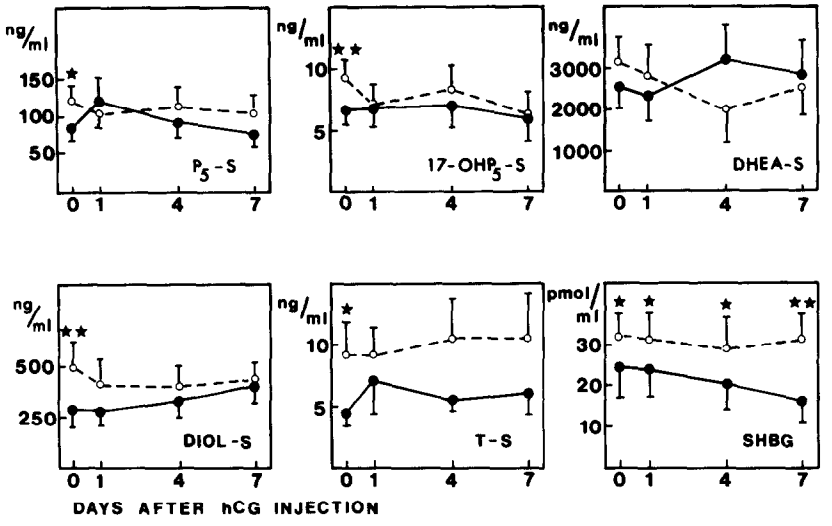

Fig. 2. Mean serum concentrations of the sulfate conjugates of pregnenolone (P5-S), 17-hydroxypregnenolone (17-OHP5-S), dehydroepiandrosterone (DHEA-S), 5-androstene-3β, 17β-diol (DIOL-S) and testosterone (T-S), and sex hormone-binding globulin (SHBG), after a single dose of hCG in the basal state (continuous line) and after 3 months of clomiphene treatment (dotted line). For further details see the legend to Fig. 1.

unconjugated and sulfated 5-ene-precursors of T, including 17-OHP5, no major changes were found following hCG administration.

The overall response pattern to hCG after 3-month treatment with CC had certain similarities and certain differences compared to that before CC. A significant increase in 17-OHP4 at day 1 ($P < 0.05$) was observed and the concentrations of serum E2 also displayed an apparent approximate 100% increase from the 0-point to day 1, but this increase was not statistically significant. No significant changes were observed after hCG injection in the concentrations of the other steroids measured.

DISCUSSION

Intratesticularly-formed E2 following gonadotropin stimulation has been proposed to be an important regulator of T synthesis, and it is thought to be responsible, at least partly, for the desensitization phenomenon by causing inhibition of 17,20-lyase and 17-hydroxylase activities (steroidogenic lesions) in the steroidogenic pathway, both in experimental animals [13–15] and man [2, 3, 5]. This suggestion has been strengthened by the observation that the accumulation of 17-OHP4 following hCG could be partly inhibited by short term antiestrogen treatment with tamoxifen [6] or CC [7]. However, this alleviation of the steroidogenic lesion was not associated with an augmented T-response to hCG, which we thought to be possibly caused by the short duration of the antiestrogen treatment and the domination of the intrinsic estrogenic properties of CC at that time point. Hence, long-term antiestrogenic treatment was employed in order to obtain further information concerning the role of endogenous E2 in human testicular steroidogenesis.

Previously it has been observed that long-term CC-treatment leads to increased circulating concentrations of gonadotropins, T and E2 [see 16 and references therein]. It is therefore very likely that under these conditions, sustained elevation of serum LH concentrations does not lead to steroidogenic lesions, which is compatible with the recent finding that multiple small-dose hCG administration does not desensitize but rather enhances testicular steroidogenesis [17]. It is also possible that the elevated concentrations of circulating SHBG, a typical response to antiestrogen treatment [18, 19], may have been involved in the increased concentrations of T, DHT and E2, all of which bind to this protein [20]. The concentrations of all other unconjugated steroids measured were not significantly elevated. The increases in the circulating concentrations of the sulfates of pregnenolone (P5), 17-OHP5, 5-androstene-3β, 17β-diol (DIOL) and T, the steroid moiety of which is at least partly, and in the case of T, almost exclusively, of testicular origin, indicate that the latter hCG stimulation test was performed at a time of stimulated steroidogenesis. It is also very likely that at that time the rate of steroidogenesis was close to maximal, because only very insignificant increases were seen in circulating androstenedione (A) and T concentrations during the latter stimulation test. However, the concentrations of 17-OHP4 were significantly elevated at day 1, indicating transient blockade of C21-steroid side-chain cleavage in the 4-ene-pathway. Accumulation of 17-OHP4 was associated with an increase in circulating E2, and the peak values at day 1 were very similar to those found in the basal state. However, due to the CC-induced

increase in E2 output before the second hCG stimulation test, this change was not statistically significant.

The decrease in the ratio of 17-OHP4 to T at 1 day (from 1.27 ± 0.83 to 0.58 ± 0.33, mean $\pm$ SD, N.S.) during long-term CC treatment suggests alleviation of the possibly E2-mediated effect on the 17,20-lyase enzyme, but this influence has been more obvious after short-term CC [7] or tamoxifen treatment [6]. The inability of the long-term antiestrogen treatment to prevent the appearance of the steroidogenic lesion at the 17,20-lyase step cannot at present be explained. It is possible that the significant increase in E2 levels (from 26.9 ± 5.1 to 60.9 ± 27.5 pg/ml, $P < 0.05$) was sufficient to allow competition with CC at the estradiol receptor site in Leydig cells, leading to expression of estrogen effects.

In contrast to the behavior of 17-OHP4 after a single high dose of hCG, 17-OHP5 concentrations did not show any significant response to hCG in the basal state or after antiestrogen treatment. This strongly suggests that in the 5-ene-pathway no inhibition of side-chain cleavage had occurred. This observation is in accordance with the results obtained in normal men after hCG administration, by ourselves [21] and others [22, 23]. Evidence for this divergent vulnerability of lyase activity in these two steroidogenic pathways has been received also in *in vitro* studies [24, 25]. These results cast some doubt on the quantitative importance of the E2-induced inhibition of 17,20-lyase activity in the 4-ene-pathway in the regulation of T-synthesis in human testis, in which the 5-ene-pathway is known to be the preferred route in androgen formation [26].

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