

Testicular Responsiveness to Chronic Human Chorionic Gonadotropin Administration in Hypogonadotropic Hypogonadism

ROSARIO D'AGATA, ENZO VICARI, ANTONIA ALIFFI, GRAZIA MAUGERI,
ALESSANDRO MONGIOÌ, AND SALVATORE GULIZIA

*Endocrinology Unit, Andrology Center, Department of Internal Medicine, Catania Medical School,
University of Catania, Catania, Italy*

ABSTRACT. Steroidogenic responsiveness to long term hCG administration (1500 U three times a week for 23 months) was characterized in 8 males with hypogonadotropic hypogonadism (HH). During hCG treatment, testosterone (T), which was in the prepubertal range under basal conditions, rose considerably to the upper end of the normal range and remained at that level during the 23 months of observation. A 2.5-fold increase was observed in serum levels of 17β -estradiol (E_2) an increment less than seen with T. The increment in 17α -hydroxyprogesterone was also lower than that in T throughout the study; thus, the 17α -hydroxyprogesterone to T ratio, despite continuous hCG administration, remained low. Serum androstenedione was

slightly increased during hCG therapy. No significant changes were observed in serum levels of dehydroepiandrosterone. These data indicate that continuous long term hCG administration stimulated T levels in HH, with a relatively small change in E_2 . The kinetics of the T and E_2 responses to 2000 U hCG, evaluated after 23 months of therapy, indicated that the testicular response was markedly reduced. No increment in T levels was observed at 24 h; the maximal response occurred at 48 h. This pattern of T response supports the idea that partial testicular desensitization occurs in HH patients receiving chronic treatment with hCG. (*J Clin Endocrinol Metab* 55: 76, 1982)

hCG PREPARATIONS have been used extensively in patients with hypogonadotropic hypogonadism (HH) as exogenous replacement for gonadotropin deficiency. The clinical improvement which accompanies such treatment indicates that testicular steroidogenesis in HH is well preserved and responds normally to hCG stimulation. However, steroidogenic responsiveness to exogenous hCG administration in HH has not been extensively evaluated. Two recent reports postulate a qualitative and quantitative difference in the acute steroidogenic response to hCG compared to normals (1, 2).

Besides increasing androgens, the administration of pharmacological doses of hCG resulted in paradoxically large increases in estrogens and C-21 androgen precursors in men [progesterone and 17α -hydroxyprogesterone (17OHP)] (3, 4). In animals, hCG administration reduced the specific receptors in the membranes of Leydig cells (5, 6). Estrogen increases and decreases in specific receptors in Leydig cells are well established as side effects of hCG administration. The practical implication of such recent findings during this therapy in humans is still

unknown, since few studies have been made on the testicular effects of prolonged use.

We undertook our study with the aim of defining the effects of chronic hCG treatment on C-21, C-19, and C-18 steroids and on the dynamics of the testosterone (T) and 17β -estradiol (E_2) responses to hCG challenge in HH.

Materials and Methods

Eight patients with selective HH, aged 18–31 yr, were studied. All subjects had eunuchoid habitus, prepubertal testicular volume (Table 1), and T blood concentrations within the prepubertal range (Fig. 1). None had hyposmia or cryptorchidism. Family history revealed that their diseases represented isolated sporadic occurrences. The patients were given hCG (Profasi, Serono, Rome, Italy) for correction of androgen deficiency symptoms (1500 U three times a week) for almost 2 yr. Hormonal levels were measured before (basal) and at 4, 8, 16, and 23 months during hCG administration. During therapy, blood was sampled 48 h after drug injection. Previous medication, as reported in Table 1, had been omitted for at least 3 months before this study began (Table 1).

To evaluate the dynamics of T and E_2 responses during chronic hCG administration, 2000 U hCG were given to five patients 48 h after their last injection of 1500 U hCG at the end of replacement therapy. Blood samples were collected daily

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Address requests for reprints to: Dr. R. D'Agata, Unità Endocrina, Istituto di Patologia Medica I, Ospedale Garibaldi, 95124 Catania, Italy.

TABLE 1. Clinical details and basal LH and FSH concentrations (mean $\pm$ SE) in our patients

Case No.	Age (yr)	Duration of previous therapy (months)	Duration of hormone therapy withdrawal before study (months)	Testis size (ml)		LH (mUI/ml) ^a	FSH (mUI/ml) ^b
				Right	Left		
1	20	6 (hCG)	9	4	3	1.2 $\pm$ 0.2	1.2 $\pm$ 0.1
2	31	7 (hCG + hMG)	3	2	2	1.9 $\pm$ 0.1	1.12 $\pm$ 0.08
3	26	24 (hCG)	3	3	3	0.7 $\pm$ 0.3	2.5 $\pm$ 0.15
4	19	^c	^c	3	3-4	1.7 $\pm$ 0.1	0.9 $\pm$ 0.2
5	18	^c	^c	6	6-8	1 $\pm$ 0.4	0.62 $\pm$ 0.2
6	18	^c	^c	2	2-3	1 $\pm$ 0.5	1.57 $\pm$ 0.16
7	18	19 (hCG + hMG)	3	6	5	2.5 $\pm$ 0.1	1 $\pm$ 0.09
8	18	24 (hCG + hMG)	12	5-6	5-6	3.1 $\pm$ 0.2	0.8 $\pm$ 0.13

hMG, Human menopausal gonadotropin.

^a Normal value in healthy adults, 2.8 $\pm$ 0.2.

^b Normal value in healthy adults, 3.6 $\pm$ 0.2.

^c No therapy.

during the next 6 days. In these subjects, due to the limited serum available, other hormones were not measured.

Plasma T, 17OHP, and androstenedione (A) were measured by RIA using paper chromatography (7, 8) (intraassay coefficients of variations were 11% or less for each of these assays, and interassay variation was 16% or less). Plasma E₂ was evaluated by RIA using specific antiserum after purification on a Sephadex LH-20 column. Coefficients for intra- and interassay variations were 8% and 16%, respectively. Dehydroepiandrosterone (DHEA) was measured in ether-extracted samples using a specific antiserum. Intra- and interassay coefficients were 11.1% and 12.5%, respectively. In both the chronic and acute studies, all samples from each subject were measured in the same assay. Differences were statistically evaluated by analysis of variance. Results in the text and figures are expressed as the mean $\pm$ SE.

Results

The mean ($\pm$ SE) basal T concentration in HH subjects was in the prepubertal range (45 $\pm$ 8 ng/dl); E₂ levels were also below normal (4.4 $\pm$ 0.4 *vs.* 12.8 $\pm$ 1 pg/ml in controls; $P < 0.01$; Fig. 1). The mean basal 17OHP value was 52.6 $\pm$ 18.4 (SE) ng/dl, not statistically different from that of eugonadal men (107 $\pm$ 11 ng/dl). In four of eight patients, basal 17OHP values were, as expected, very low (28.4 $\pm$ 6.7 ng/dl). Basal A was 83.9 $\pm$ 13 (SE) *vs.* 121 $\pm$ 95 ng/dl in normals, and DHEA levels were within the normal range (Fig. 1).

Effect of chronic hCG administration on plasma T and E₂ levels

After 4 months of hCG treatment, blood concentrations of T had increased considerably (11-fold; $P < 0.01$) and were already within the normal range (Fig. 1). A further consistent increase was noted after 8 months of therapy (16-fold above the basal value). Subsequent

blood concentrations remained similar to those observed after 8 months of treatment. After an initial 2.5-fold rise in E₂ levels ($P < 0.01$) during the first 4 months, no further marked changes were noted (Fig. 1) in plasma E₂. The magnitude of the rise was thus much greater for T than for E₂.

Effect on DHEA, A, and 17OHP levels

No significant variations above basal values were observed in DHEA levels (Fig. 1). The levels of A were slightly increased, achieving mean values 1.2-, 1.3-, and 1.6-fold above basal levels at 8, 16, and 23 months, respectively. The mean 17OHP levels after hCG administration were higher than the basal value (Fig. 1), but the differences became significant from 8 months on ($P < 0.02$). Since the 17OHP increase was much lower than the T rise, the 17OHP to T ratios 4, 8, 16, and 23 months after hCG treatment were very low (mean $\pm$ SE, 0.13 $\pm$ 0.02, 0.14 $\pm$ 0.02, 0.21 $\pm$ 0.01, and 0.16 $\pm$ 0.01, respectively), which suggests that no accumulation of C-21 precursors occurred. Interestingly, the relative steroid ratios did not change during the long period of study, hence demonstrating that no steroidogenic variations took place with increased time of treatment.

Acute responses of T and E₂ to hCG during chronic hCG therapy

The dynamics of T and E₂ responsiveness were best seen during the period of frequent sampling after an acute administration of 2000 U hCG, 48 h after the last chronic dose of hCG. Figure 2 shows clearly that T levels remained virtually unchanged after 24 h and rose at 48 h, when they were slightly higher than those observed during chronic therapy (1.1 times the basal value). In two subjects, no changes were observed in serum T after the

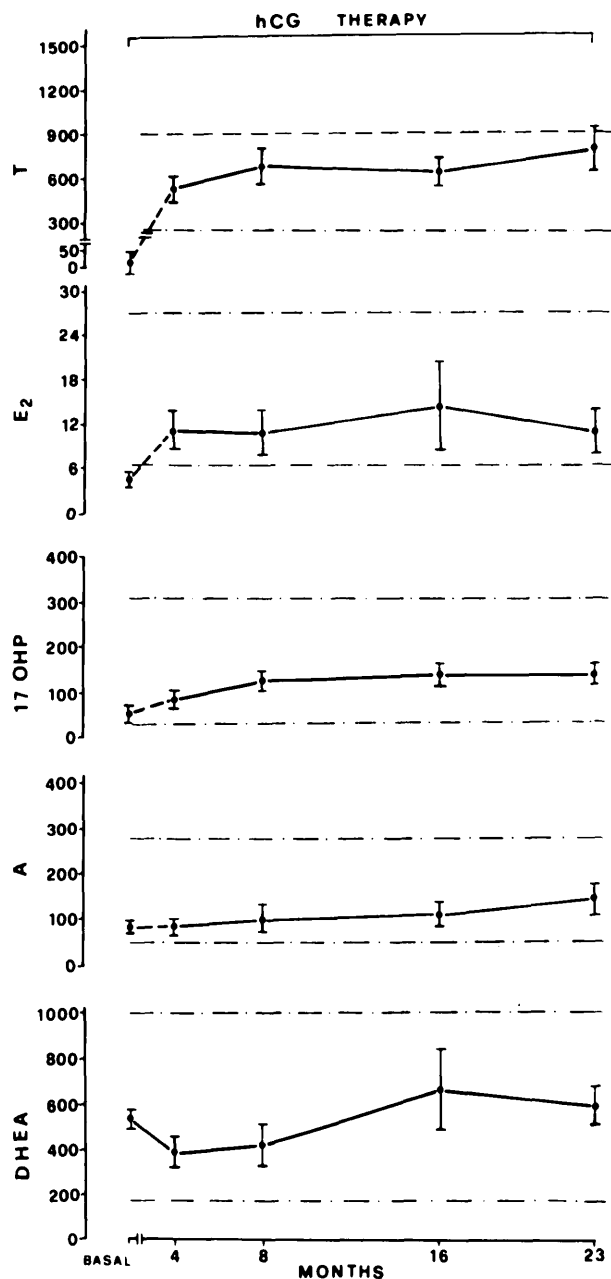


FIG. 1. Serum blood concentrations of T (nanograms per dl), E₂ (picograms per ml), 17OHP (nanograms per dl), A (nanograms per dl), and DHEA (nanograms per dl) before (basal) and during long term hCG therapy in our patients. All values are the mean $\pm$ SEM. The dashed lines encompass the normal range.

2000-U dose of hCG. After 48 h, T levels gradually decreased to a nadir of 300 ± 204 (SE) ng/100 ml at 144 h, the lower limit of our normal values. Hence, the pattern of testicular responsiveness to 2000 U hCG indicates a reduced response of the steroid to the trophic stimulus, with a delayed T rise.

The response peak for E₂ was observed within 24 h, and the E₂ levels tended to plateau for the next 2 days, followed by a fall in blood levels, with a nadir at 144 h (mean $\pm$ SE, 5.4 ± 1.9 pg/ml).

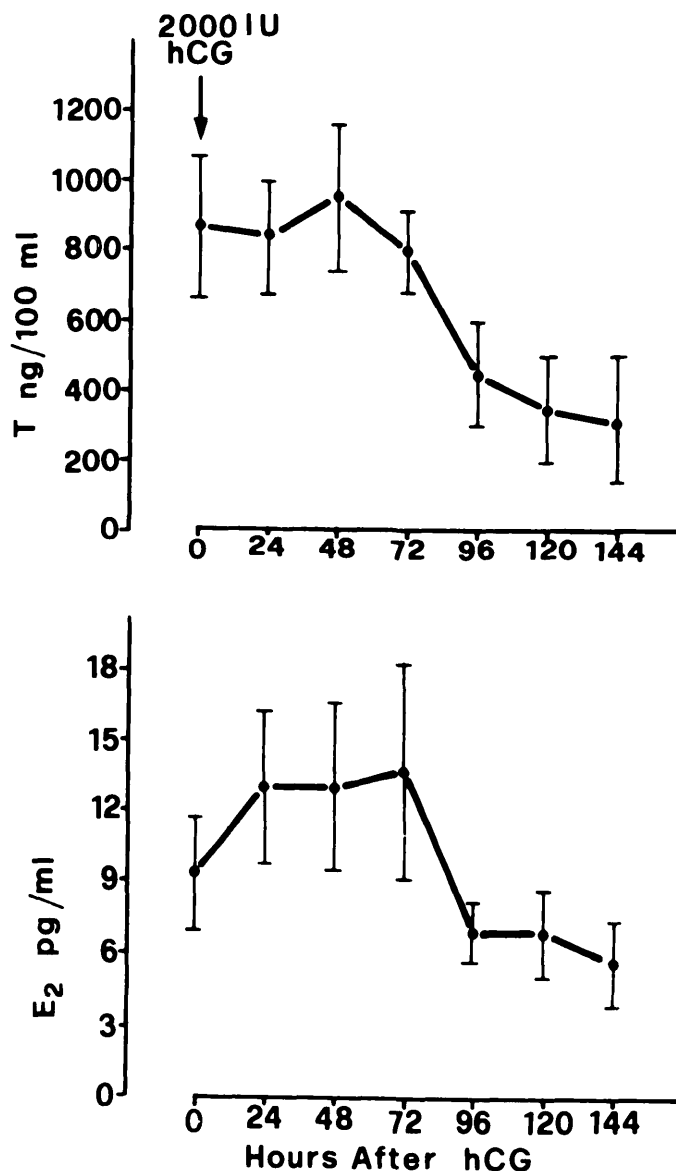


FIG. 2. Kinetics of T and E₂ responses to 2000 U hCG in five hypogonadotropic patients. All values are the mean $\pm$ SE.

Discussion

This report shows that hCG chronically administered to HH patients brought about a predominant increase in T and minor increases in E₂ levels. It is noteworthy that after the initial rises observed at 4 and 8 months of therapy with hCG, serum T remained at a plateau despite continuous hCG administration. The hCG course also augmented the serum 17OHP and A concentrations. Compared to the T increment, the rises of Δ^4 precursors (17OHP and A) were less significant, resulting in low precursor to T ratios. This steroidogenic responsiveness pattern is quite different from that found in normal males.

In normal subjects, an acute load with hCG/human LH resulted in an exaggerated increase in E₂, with a

relatively smaller rise in T (3, 4). The stimulation of aromatase enzyme activity by hCG (9, 10) may account for this marked increase in estrogen. This is commonly explained as resulting in a relative temporary defect in T biosynthesis, on the one hand, and an accumulation of precursors via suppression of some enzymes involved in its biosynthesis, mainly 17 α -hydroxylase, 17,20 desmolase, or 17 β -dehydrogenase activities, on the other (desensitization) (11–14). Interestingly, a recent report shows the persistence of this block for 10 days after acute hCG administration (15).

Therapy with hCG increased E₂ levels from below normal to normal levels. hCG stimulates estrogen production not only by directly increasing the aromatase activity in the testis (9, 10), but also by raising the level of T, which is then converted to E₂. This latter effect appears more likely to account for the hCG stimulation of E₂ production seen in HH subjects and suggests that long term hCG administration in HH does not inappropriately enhance aromatase activity. This either implies that hCG does not stimulate aromatase activity as it does in normal subjects or that much higher amounts of hormone are necessary in HH.

The present data referring to a chronic hCG load are in close agreement with those recently presented by Wang *et al.* (1). These investigators reported a prevalent increase in T levels, with a small increase in E₂, in response to human LH infusion, and no accumulation in C-21 and C-19 precursors. Unfortunately, their study was not carried out while patients were on replacement therapy. Since the testicular response to hCG in HH patients appears to reflect the degree and duration of previous exposure to gonadotropin (16, 17), the testicular steroidogenic responsiveness pattern in HH subjects would be expected to be similar to that in controls after long term gonadotropin replacement. The data presented here show that this was not so, as the pattern remained unchanged even while the patients were receiving hCG replacement. Hence, our data after chronic stimulation with hCG complete those of both Wang *et al.* (1) and Smals *et al.* (2), who, by acute hCG stimulation, demonstrated qualitative deviations in the testicular response in HH subjects.

In man, the injection of a pharmacological dose of hCG induces an initial acute rise in serum T, followed by a plateau for 24–48 h despite high serum hCG; this is evidence of a temporary and partial inability of Leydig cells to respond to hCG with an increase in T production (desensitization). It is widely accepted that such desensitization of Leydig cells accounts for their subsequent inability to respond to additional repeated hCG injections with an increase in T (15, 18, 19). Accordingly, the lack of a further increase in serum T after 8 months of therapy, despite continuous hCG administration, might

then be explained by the occurrence of partial testicular desensitization to hCG in HH subjects exposed to chronic treatment with hCG. Indirect evidence of self-regulation of testicular responsiveness to gonadotropin has been suggested by the fact that male patients with gonadotropin-producing tumor have normal plasma T levels in spite of the large amounts of hCG produced by these tumours (20, 21). This seems to suggest that there exists a negative regulation of Leydig cell responsiveness by high concentrations of circulating hCG.

Another plausible explanation for the lack of further T increment during hCG therapy might be that the testes of HH subjects have limited steroidogenic capability due to the presence of an inadequate enzyme system.

The pattern of T response to the injection of 2000 U hCG is not easy to explain. Hypothetically, it could be ascribed to a temporary mechanism of refractoriness of the testicular response, most likely induced by chronic hCG administration. One might postulate that the T increase observed 48 h after the administration of 2000 U hCG may represent resensitization of the testicular responsiveness to the last injection of 1500 U hCG, with a peak occurring 72–96 h after injection. In fact, Glass and Vigersky (15) found that the resensitization phenomenon, with normal rises in 170HP and major T release, takes place 72 h after the injection of a desensitizing dose of hCG.

Hence, the finding of a T response to 2000 U hCG would further favor the idea that desensitization did indeed occur in HH subjects receiving chronic treatment with hCG.

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