

Single Versus Repeated Dose Human Chorionic Gonadotropin Stimulation in the Differential Diagnosis of Hypogonadotropic Hypogonadism*

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ABSTRACT. The responses of serum testosterone (T), 17α -hydroxyprogesterone, and 17β -estradiol (E_2) to four im injections of hCG (5000 IU/1.7 m²) given on days 0, 4, 7, and 10 were studied in 10 prepubertal and 10 pubertal boys with hypogonadotropic hypogonadism (groups O and P, respectively). Serum was obtained before each injection and on day 14. The results were compared with those of controls, 16 prepubertal boys with incomplete testicular descent and 6 pubertal boys with constitutional delay of puberty. Serum T levels increased significantly in groups O and P to 2.0 and 4.6 nmol/liter, respectively, after the first injection, then progressively to 5.8 and 11.2 nmol/liter. Basal T levels of group O did not differ from those of the controls, but were subnormal for group P ($P < 0.001$). Stimulated T levels were subnormal in both groups ($P < 0.01$ and $P <$

0.001), but repeated doses increased the difference from the control value only in group P. A difference in E_2 response between patients and controls appeared in puberty; only the pubertal control boys had substantial increases in E_2 ($P < 0.001$). Our results show that the optimal protocol for a diagnostic hCG test in prepubertal boys is a single dose of hCG, with determination of T levels 4 days later. In puberty, if the basal T levels are inconclusive, repeated doses of hCG should be given with determination of both T and E_2 . These findings also suggest that the full inhibitory effect of E_2 on T synthesis results from a pubertal maturation process, possibly induced by endogenous gonadotropins, which cannot be induced by two weeks of hCG stimulation in prepubertal boys or those with hypogonadotropic hypogonadism. (*J Clin Endocrinol Metab* 60: 333, 1985)

THE DIAGNOSIS of hypogonadotropic hypogonadism (HH) remains difficult despite the improvements brought about by GnRH and TRH tests (1). These tests give false negative results in some boys with HH (1). As serum testosterone (T) responses to hCG are often subnormal in these boys (2–6), combining the hCG test with the GnRH or TRH test might improve the diagnostic accuracy. Before exploring this possibility, however, the sensitivity of the hCG test in the diagnosis of HH needs to be established.

Several different protocols have been proposed for diagnostic hCG stimulation in boys (7–15). All but one (9) involve massive doses given repeatedly, and all use serum T levels as the only response indicator. While such stimulation is probably necessary for proving the absence of Leydig cells, it may not be ideal for differentiating HH from constitutional delay of puberty.

A single dose of hCG causes Leydig cell steroidogenic desensitization in adult rats and men (16–23). This is

linked with a decrease in 17α -hydroxylase and $17,20$ -lyase activity, which, in turn, leads to an accumulation of progesterone and 17α -hydroxyprogesterone (17-OHP) (17, 18). This process is thought to be mediated by estradiol (E_2) through the sequence of gonadotropin-induced aromatase stimulation, increase in E_2 and its occupation of estrogen receptors in Leydig cells (18, 20, 24, 25). An LH-priming effect appears necessary for this enzyme inhibition, as evidenced by the lack of E_2 and 17-OHP responses to a single dose of hCG or LH in prepubertal boys (26) and adult men with HH (27, 28). Prolonged hCG stimulation may, however, cause enzyme inhibition in HH, thus impairing the diagnostic value of the test.

The aim of this study was 1) to determine the kinetics of the T response to single and repeated doses of hCG in boys with HH, normal boys, and boys with constitutional delay of puberty to obtain the simplest, most reliable stimulation protocol for differentiating among them, and 2) to determine if serum E_2 and/or 17-OHP responses gave any further diagnostic information.

Materials and Methods

Subjects

Twenty boys with HH were studied (Table 1). Ten were prepubertal (group O), and 10 had entered puberty (group P)

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TABLE 1. Clinical data of the patients

Patient no.	Age (yr)	Testis vol (ml) ^a	Pubertal stage ^b		Etiology	Deficiency of			
			P	G		GH	TSH	ACTH	ADH
1	7.4		1	1	Prader-Willi syndrome	—	—	—	—
2	8.8		1	1	Prader-Willi syndrome	—	—	—	—
3	11.4		1	1	Breech delivery	+	+	—	—
4	12.0	2.2	1	1	CRF ^c	+	+	+	+
5	12.1	4.0	1	1	CRF	+	+	+	+
6	12.5	0.8	1	1	Histiocytosis-X	+	+	+	+
7	12.8	0.3	1	1	Idiopathic	+	+	+	+
8	13.9	1.4	1	1	Idiopathic	—	—	—	—
9	14.3	0.4	2	1	Kallmann syndrome	—	—	—	—
10	14.4	0.8	1	1	Idiopathic	+	+	+	+
11	15.6	2.8	5	4	Kallmann syndrome	—	—	—	—
12	17.5	2.5	4	4	Pituitary adenoma operated + radiated	+	+	+	—
13	18.5	3.3	5	4	CRF	+	+	+	+
14	19.3	16.7	3	3	CRF	+	+	+	+
15	19.4	3.0	4	4	CRF	+	+	+	+
16	19.4		4	4	CRF	+	+	+	+
17	19.9	3.6	3	3	CRF	+	+	—	—
18	20.5	2.8	3	5	CRF	+	+	+	+
19	22.2	2.4	3	4	CRF	+	+	+	+
20	27.7	9.8	3	4	CRF	+	+	+	+

—, Absent; +, present.

^a According to Hansen (29).^b Pubic hair (P) and genital (G) (excluding testis size) stage (30).^c Craniopharyngioma.

during androgen replacement therapy. Five had isolated gonadotropin deficiency; the rest had other pituitary deficiencies as well. Appropriate substitution therapy was given before and during the hCG tests (2 mg GH, 2 or 3 times weekly; T₄ to maintain normal serum T₄ levels; and 10–15 mg/m² cortisol in three daily doses). The diagnosis of gonadotropin deficiency was established earlier, and the present data were not used diagnostically. The diagnostic criteria of HH were a clearly prepubertal or subnormal testis size for bone age (29) and absence of pubertal penis growth at bone age 13 yr or older (before any androgen therapy). All patients were followed for several years to confirm deficient testicular growth. In 2 prepubertal boys (patients 1 and 2), the diagnosis was based on subnormal gonadotropin responses to GnRH. All patients with craniopharyngioma were investigated after surgery. The gonadotropin responses to GnRH and the steroidogenic response to hCG were similar in the patients with organic hypopituitarism and the other patients. Thus, the results of all patients with HH were pooled.

All of the pubertal boys had received T enanthate (Primoteston depot, Schering AG, Berlin, West Germany; 1–5 mg/kg (maximum, 250 mg), at 4-week intervals). This therapy was discontinued at least 3 months before the hCG tests.

Previous results from 16 prepubertal boys, aged 1.1–12.4 yr, with suspected incomplete testicular descent (true, or retractile testes) and 6 pubertal boys (genital stage 3) (30), aged 13.9–17.4 yr, with constitutional delay of puberty served as control values (31).

Protocol

Patients and control subjects were given four im injections of hCG (5000 IU/1.7 m² each; Pregnyl, Organon, Oss, The

Netherlands) on days 0, 4, 7, and 10. Blood for the determination of 17-OHP, T, and E₂ was obtained immediately before each injection and 4 days after the last one.

Methods

The sera were stored at –20 C until analyzed. They were quantified by RIA after chromatography on Lipidex-5000 (17-OHP and T) (32) or Sephadex L-20 (E₂) (33). Samples from an individual subject were analyzed at the same time.

Statistics

Group O was compared with the prepubertal controls, and group P was compared with the pubertal controls. The data were analyzed by BMDP computer programs (34). The means were compared by *t* test for independent and dependent samples (program 3D) and by general univariate and multivariate analysis of variance (program 4V). Because of positive skewness of the distributions, all calculations were made after logarithmic transformation. The specificity and sensitivity of each variable were calculated by applying Bayes' theorem, where sensitivity = (number of true positives)/(number of true positives + false negatives), and specificity = (number of true negatives)/(number of true negatives + false positives). In this study the sensitivity indicates the proportion of correct subnormal findings from all findings in the boys with HH and the specificity the proportion of normal findings from all findings in the controls.

Concentrations in nanomoles per liter (picomoles per liter)

can be converted to nanograms per dl (picograms per ml) by multiplying by 28.8 (T), 33.0 (17-OHP), or 0.27 (E_2).

Results

Steroidogenic response to hCG in boys with HH (Fig. 1)

The mean basal T levels were 0.2 and 0.7 nmol/liter ($P < 0.01$) for groups O and P, respectively. The levels rose to 2.0 and 4.6 nmol/liter after the first injection, then progressively after each injection to 5.8 and 11.2 nmol/liter at the end of the stimulation ($P < 0.001$ and $P < 0.01$ compared with day 4). There was no significant difference between the groups at any time.

E_2 levels did not increase in group O, but were slightly elevated in group P at the end of the stimulation. 17-OHP levels increased gradually, finally reaching a level significantly above the basal. There was no significant difference between the groups basally or after stimulation in either 17-OHP or E_2 levels.

Difference between patients and controls (Fig. 1)

The mean basal T concentration in group O did not differ from that in the controls, but the group P value

did. Mean stimulated T levels in both groups were markedly below those in controls. Mean basal E_2 levels in the patients were no different from those in the controls. However, neither group of patients had an E_2 response, whereas the pubertal controls did have an E_2 response. Thus, a difference between the patients and the controls appeared at puberty, and increased throughout the 2-week period of stimulation. The basal 17-OHP levels were markedly different between patients and controls in group P, but after stimulation, the difference was less or disappeared.

Discriminatory power of different test variables (Fig. 2)

In prepuberty, no HH patient could be identified by the basal T level. By contrast, a stimulated T level was a very specific discriminator and was equally sensitive on days 4 and 14. The E_2 levels had no discriminatory power.

At puberty, the basal T level was a very specific and sensitive discriminator. Stimulation increased the specificity of T on both day 4 and 14. However, for sensitivity, stimulated T exceeded basal T only on day 14. Almost as sensitive as the stimulated T levels on day 14 were the E_2 levels on days 7, 10, and 14.

Discussion

These results clearly demonstrate that the hCG test is a very sensitive discriminator between boys with HH and normal prepubertal boys and between boys with HH and boys with constitutional delay of puberty. For our

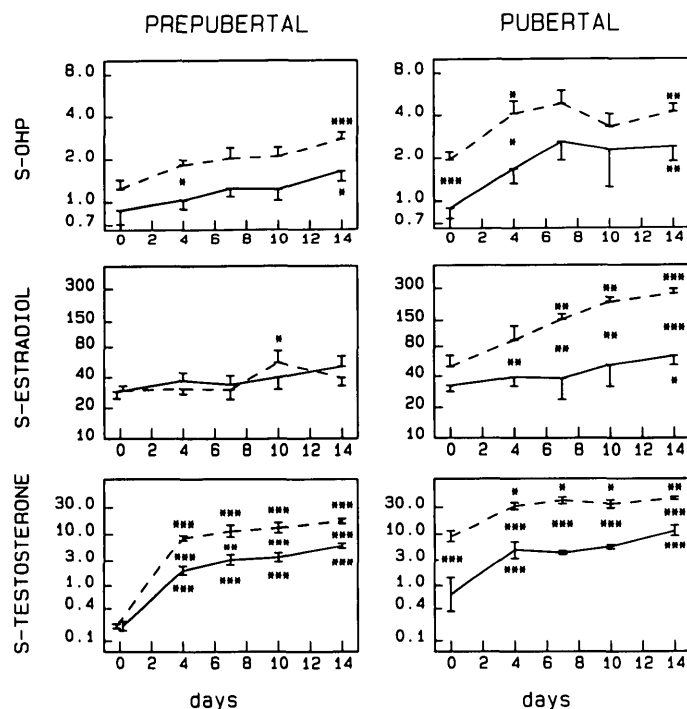


FIG. 1. Effect of four doses of hCG given on days 0, 4, 7, and 10 on serum (S-) concentrations (mean $\pm$ SEM) of T (nanomoles per liter), E_2 (picomoles per liter), and 17-OHP (OHP; nanomoles per liter) in prepubertal and pubertal boys with HH (—). The results are compared with those of 16 prepubertal and 6 pubertal (genital stage 3) control subjects (---) studied with the same protocol. Statistically significant differences are indicated, with asterisks between the curves for differences between patients and controls, above the curves for differences from the basal level in the controls, and below the curves for differences from the basal level in the patients. For converting nanomoles per liter (picomoles per liter) to nanograms per dl (picograms per ml), multiply by 28.8 (T), 33.0 (17-OHP), or 0.27 (E_2). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

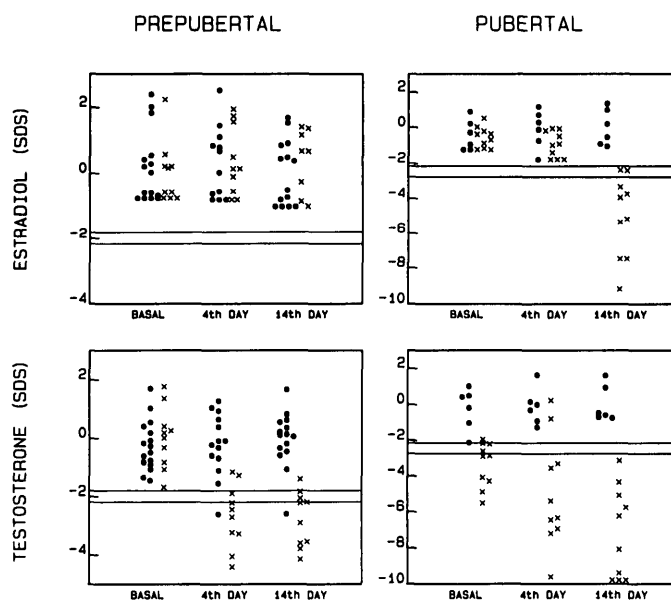


FIG. 2. Discrimination of hypogonadotropic boys (X) from the control subjects (●) by SD scores (SDS) of serum T and E_2 concentrations basally, after the first dose of hCG (4th day), and at the end of stimulation (14th day) in prepubertal (left panel) and pubertal (right panel) boys. The two horizontal lines represent the lower limit of the 90% and 95% confidence ranges, respectively.

prepubertal controls, we could only use boys with incomplete testicular descent, and some of them may have had partial LH deficiency (35). However, if the hCG test differentiated between this control group and boys with unequivocal HH, it should even better distinguish the normal state from HH. Previous studies of short hCG stimulation tests in boys with HH showed the T response to be blunted (2-6). In these studies, the stimulation protocol varied as to dose, number of injections, and interval between doses as well as timing of blood sampling. Thus, the diagnostic significance of the hCG test has not been well established. In the present study, we focused on the kinetics of the steroidogenic response to establish a protocol for this specific purpose. The hCG test is an indirect indicator of gonadotropin deficiency. Hence, for a diagnosis of HH, additional tests (GnRH or TRH) (1) as well as clinical criteria are necessary to exclude primary hypogonadism.

A subnormal T response and little or no 17-OHP and E₂ responses were characteristic of the boys with HH. Similar results were reported in adult men with HH after a short infusion of LH (28) or a single injection of hCG (27). It has been suggested that the acquisition of E₂-synthesizing capacity is a part of pubertal maturation (26), possibly induced by an increase in gonadotropin secretion. This suggestion is supported by our finding of identical E₂ responses in both the boys with HH and the prepubertal controls.

In the prepubertal patients and controls, serum T concentrations increased progressively after repeated hCG injections. This occurred despite an increase in serum 17-OHP levels, which is believed to reflect E₂-mediated inhibition of 17,20-lyase. Thus, it appears that the full inhibitory effect of E₂ on T synthesis only develops with puberty and cannot be induced in prepuberty even by 2 weeks of hCG stimulation. The pubertal patients had a somewhat greater E₂ response and a greater 17-OHP response, presumably as a result of the higher E₂ level.

In prepuberty, a single dose of hCG increased serum T concentrations significantly in both patients and controls. The difference between them was very significant after the first dose, but did not increase with further stimulation. Neither could the specificity or the sensitivity of the stimulated serum T level be increased by longer stimulation. Thus, for prepubertal boys, administration of a single im dose of 5000 IU/1.7 m² with determination of the serum T concentration 4 days after the injection gives information similar in value to that produced by a protocol including repeated doses. For pubertal boys, however, if the basal T measurement is inconclusive, longer stimulation with four injections at 3- to 4-day intervals, with determination of both T and E₂ concentrations 4 days after the last injection, appears best.

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