Androgen Regulation of Growth Hormone Binding Protein

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Male puberty is associated with elevated plasma concentrations of growth hormone (GH) and insulin-like growth factor-I (IGF-I), as well as accelerated linear growth. These effects can be reproduced by administration of testosterone (T). To further elucidate the mechanisms underlying pubertal growth, we treated 14 boys with delayed puberty and short stature with either T (n = 7) or 5α-dihydrotestosterone (DHT) (n = 7) and compared the effect on plasma concentrations of GH, IGF-I, and GH binding protein (GHBP). Before treatment and after either three or four doses of T enanthate or DHT heptanoate, mean 12-hour GH concentration (8 AM to 8 PM) and plasma IGF-I, T, DHT, and GHBP levels were measured, and height velocity (HV) was measured over this interval. T treatment resulted in an increase of mean GH from 3.3 to 12.0 μg/L ($P < .005$) and of IGF-I from 22.3 to 45.4 nmol/L ($P < .01$). During treatment, HV was 11.0 ± 1.1 cm/yr, consistent with normal pubertal growth, and plasma T was 22.5 ± 5.3 nmol/L. GHBP decreased in this group from 937 to 521 pmol/L ($P < .025$). DHT treatment resulted in a small decrease of mean GH from 4.3 to 2.9 μg/L ($P < .025$) and of IGF-I from 29.4 to 27.2 nmol/L (nonsignificant [NS]). During treatment, HV was 9.3 ± 1.1, not significantly different from the HV obtained with T treatment, and plasma DHT was 24.2 nmol/L at 1 week and 29.2 at 2 weeks postinjection. Likewise, there was a decrease in GHBP from 928 to 698 pmol/L ($P < .025$). The decline in GHBP with T treatment was apparently due to an androgen receptor–dependent mechanism, since the same effect was seen during treatment with the nonaromatizable androgen, DHT. This effect is opposite to the normal chronological trend upward for GHBP, which occurs from infancy into midpuberty. Factors determining the upward trend are not known, but are evidently independent of the plasma concentration of sex hormones and GH. The increase in IGF-I in response to T treatment despite a moderate decline in GHBP (and possibly GH receptor) levels is most likely due to the large increase in GH, which may override a modest decrease in GHBP/GH receptor.

DURING PUBERTY IN MALES, there is an acceleration in the rate of linear growth[1] associated with increased secretion of growth hormone (GH)[2-4] and insulin-like growth factor-I (IGF-I).[5,6] Although the presence of circulating GH is considered necessary for the pubertal growth spurt,[7] the extent to which an increase may be necessary is not clear. In a previous study of androgen treatment of delayed puberty in boys,[8] we showed that both testosterone (T) and dihydrotestosterone (DHT) accelerated growth to about the same extent, although the two treatments had opposite effects on plasma GH levels, an increase with T and a decrease with DHT. The difference in the effect on GH was attributed to the fact that T, but not DHT, is aromatizable.

One possible effect of sex hormones on GH action is a change in bioavailability. The recent demonstration of a circulating GH binding protein (GHBP)[9,10] suggests a mechanism by which bioavailability could be regulated. Population studies suggest there is a steady increase in the plasma concentration of this glycoprotein[11-13] from birth into adulthood. A recent study by Martha et al[14] showed that mean GH concentration increases during male puberty while GHBP remains constant. Thus, the ratio of GH to GHBP increases during male puberty, with a possible increase in bioavailable GH.

Postel-Vinay et al[15] have shown that whereas GH treatment of hypopituitary subjects resulted in increased plasma GHBP, T treatment caused a reduction. In contrast, ethinyl estradiol treatment of postmenopausal women[16] and subjects with Turner's syndrome[17] resulted in elevation of plasma GHBP. The effect of T could in theory be the result of an androgen receptor–dependent action or, via aromatization, an estrogen receptor–dependent action. In the present study, we compare the effect of these two androgens, T and DHT, to further elucidate the control of plasma GHBP levels as a potential basis for their diverse activities.

SUBJECTS AND METHODS

The subjects of this study were 14 males aged 12.4 to 16.9 years with the clinical syndrome of constitutional delay in growth and adolescence (Table 1). They were short, with height below the 5th percentile for age, and pubertal development was Tanner stage 1 to 2 by physical examination, but otherwise they were in good general health. Bone age was delayed by 1 to 4 years. Studies were performed to rule out chronic diseases or hormonal deficiencies that could have contributed to the short stature or delayed puberty. The following normal results were obtained: complete blood cell count, erythrocyte sedimentation rate, and serum urea nitrogen, creatinine, electrolytes, calcium, phosphorus, AST, ALT, bilirubin, thyroxine, and thyrotropin. The GH response to stimulation by clonidine (150 μg/m^2) and arginine (0.5 mg/kg) was greater than 10 ng/mL in all subjects. Eight of the subjects were included in the report previously cited,[8] three from the T-treated group and five from the DHT-treated group.

Study Protocol

The studies were conducted at the Clinical Research Center of The University of Texas Medical Branch with informed consent

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Table 1. Characteristics of the Subjects With Delayed Puberty

Treatment Group	Age (yr)	Height (cm)	Height Age (yr)	Bone Age (yr)
T (n = 7)	14.6 ± 0.52	147.0 ± 1.8	11.6 ± 0.3	12.2 ± 0.7
DHT (n = 7)	14.5 ± 0.26	144.7 ± 2.8	11.3 ± 0.44	11.6 ± 0.3

NOTE. Data represent the mean ± SEM.

from the parents and assent from the subjects for this protocol, which was approved by the Institutional Review Board.

The subjects were studied on one of two slightly different protocols. Each was studied twice, once before treatment and once during treatment. In protocol A (eight subjects), three subjects received T enanthate and six DHT heptanoate intramuscularly, 100 mg initially and 200 mg after 1 and 2 months. Repeat studies were at 2.5 months (2 weeks after the third dose). The total androgen dose received by subjects in protocol A before the second study ranged from 360 to 500 mg/m^2 (Table 2). Previous studies[18,19] have shown that plasma levels of T or DHT remain elevated for at least 3 weeks after injection of these steroid esters. In protocol B (six subjects), the treatment regimen was 80 mg/m^2 per dose of either T (four subjects) or DHT (one subject), monthly for six doses. The follow-up study was at 3.25 months (1 week after the fourth dose). The total androgen dose received before the second study was 320 mg/m^2. The studies included a mean 12-hour GH concentration, which was determined as either an average of 12 hourly samples obtained by constant-withdrawal pump[3] in protocol A, or an average of 36 samples obtained every 20 minutes between 8 AM and 8 PM in protocol B. At 8 AM at each admission, a blood sample was obtained for plasma T and DHT and serum IGF-I and GHBP determination.

A luteinizing hormone (LH) releasing hormone (LHRH) stimulation test was performed the morning after GH sampling was completed. The dose of LHRH was 150 μg intravenously, and blood was obtained for LH at 0, 15, 30, 45, and 60 minutes. The peak level was used for analysis.

Since there was no appreciable difference among subjects in the two treatment protocols with regard to growth rate or change in GH or IGF-I, all T-treated subjects were considered a single group, as were all DHT-treated subjects.

Hormone Assays

GH level was measured in plasma by radioimmunoassay using reagents obtained from the National Pituitary Agency. ^{125}I-hGH was obtained from New England Nuclear (Boston, MA). IGF-I was determined by Endocrine Sciences (Oxnard, CA) using an acid ethanol extraction procedure. Plasma T and DHT concentrations were measured by radioimmunoassay using Celite (Johns-Manville Products, Manville, NJ) chromatography to purify the extract before the competitive binding step.[20] This method is 99.9% effective in separating T and DHT. Serum LH level was measured by radioimmunoassay.

GHBP was estimated by two methods: (1) total functional GHBP level was measured as the trimolecular complex ^{125}I-GH: GHBP:anti–GH receptor (monoclonal) at Endocrine Sciences,[21] and (2) ^{125}I-GH binding was measured by gel exclusion chromatography as previously described.[11]

Bone age was measured on radiographs of the wrist and hand by the method of Greulich and Pyle.[22]

Statistical Methods

Comparisons of the significance of differences in mean values for GH, IGF-I, and GHBP determined before and after the treatments were made using a paired *t* test. Comparison of GHBP levels during the two treatments was made using the *t* test for unpaired observations. When comparing results from the two methods of GHBP measurement, the method of least squares for linear regression was used.

Table 2. T Versus DHT Treatment

Group/ Patient No.	Dose (mg/m^2)	No. of Injections	GHBP		Mean GH		IGF-I	
			Pre	Post	Pre	Post	Pre	Post
T treatment								
Protocol A								
1	397	3	789	506	3.8	9.7	17.4	52.3
2	393	3	1,158	806	1.4	6.1	17.0	43.0
3	434	3	803	340	4.2	25.2	15.2	42.1
Protocol B								
4	320	4	699	350	3.0	10.7	10.7	29.3
5	320	4	601	304	5.8	12.2	29.2	49.9
6	320	4	1,506	743	1.8	7.8	22.1	53.1
7	320	4	1,006	601	4.1	10.7	24.8	43.1
Mean ± SEM			937 ± 127	521 ± 83*	3.5 ± 0.6	12.0 ± 2.5†	22.1 ± 2.0	45.4‡
DHT treatment								
Protocol A								
1	384	3	974	673	5.2	3.3	36.5	29.3
2	442	3	1,304	869	3.3	1.3	12.4	25.5
3	500	3	868	811	5.6	1.7	22.2	25.8
4	400	3	892	616	4.9	2.4	17.1	15.6
5	360	3	979	671	4.9	3.7	48.5	27.7
Protocol B								
6	320	4	676	765	4.3	4.3	31.9	33.2
7	320	4	804	548	4.4	4.3	34.5	35.2
Mean ± SEM			928 ± 80	698 ± 49*	4.3 ± 0.4	2.9 ± 0.4*	29.4 ± 4.1	27.4 ± 2.1

NOTE. Pre and post refer to before and after treatment, respectively.
*$P < .025$, †$P < .005$, ‡$P < .01$: treatment *v* control.

RESULTS

Growth Response to Androgen Administration

As we previously noted in a smaller number of patients,[8] T and DHT treatment resulted in similar rates of linear growth or height velocity (HV). Annualized HV during 10 to 14 weeks of treatment was 11.0 ± 1.1 cm/yr for T-treated and 9.3 ± 1.1 for DHT-treated subjects.

GH and IGF-I Response to Treatment With T and DHT

In the control period, the mean GH concentration measured from 8 AM to 8 PM was comparable in the two groups: 3.3 ± 0.6 (mean ± SEM) μg/L for T-treated subjects and 4.3 ± 0.4 for DHT-treated subjects. Likewise, plasma IGF-I concentrations were similar in the two groups: 22.1 ± 2.0 and 29.4 ± 4.1 nmol/L. However, the response to the two treatments was different (Table 2). T treatment resulted in a threefold increase in mean GH and a twofold increase in IGF-I. DHT treatment resulted in a modest decrease in GH and no change in IGF-I.

Plasma T level during T treatment increased from 1.06 ± 0.35 nmol/L (n = 5) to 22.5 ± 5.3 nmol/L (n = 7) at 2 weeks postinjection ($P < .01$). During DHT treatment, plasma T did not change significantly (2.0 ± 0.5 *v* 2.7 ± 0.8, control *v* treatment, respectively, NS). Plasma DHT concentrations in the T-treated group increased from 0.61 ± 0.13 to 2.82 ± 0.67 nmol/L at 1 week ($P < .05$) and 2.68 ± 1.48 at 2 weeks posttreatment ($P < .2$); in the DHT-treated group, plasma DHT levels at 1 week posttreatment were 24.2 ± 8.4 nmol/L ($P < .01$, n = 4), and at 2 weeks, 29.2 ± 7.7 ($P < .01$, n = 4). Thus, the T concentration during T treatment was similar to the DHT level during DHT treatment.

In both groups, there was suppression of the peak pituitary LH response to LHRH (T treatment, from 27.6 ± 5.9 to 5.9 ± 2.5 IU/L, $P < .10$; DHT treatment, from 45.7 ± 10.7 to 10.7 ± 5.8 IU/L, $P < .05$). This indicated that the effects seen were the result of treatment and were not due to endogenous T secretion.

Effect of Treatment on Plasma GHBP

Both T and DHT treatments resulted in a decrease of GHBP (Fig 1). Using the immunoprecipitation assay for GHBP, T treatment resulted in a decrease in seven of seven subjects, with values of 937 ± 127 pmol/L (mean ± SE) for controls and 521 ± 83 pmol/L for treated subjects ($P < .025$). With DHT, six of six subjects showed a decrease (one of the paired samples was lost for this series), with mean values of 928 ± 80 for controls and 698 ± 49 for treated groups ($P < .025$). When the effect of the two treatments was compared by *t* test, the effect of T was greater than that of DHT ($P < .025$). When initial or treatment values for GHBP were compared with HV, no significant correlation was observed.

The same plasma samples were analyzed for GHBP using the ^{125}I-GH-binding method of Baumann et al.[11] The results were qualitatively the same and quantitatively similar. With T treatment, seven of seven subjects showed a decrease, with mean values of 22.3% ± 3.8% for controls and 13.2% ± 2.0% for treated subjects ($P < .025$). With DHT treatment, the effect was less prominent. Nevertheless, seven of seven showed a decrease, with mean values of 18.7% ± 2.0% for controls and 14.2% ± 2.2% for treated subjects ($P < .025$). The correlation between the two GHBP assays was statistically significant ($r = .58$, $P < .01$).

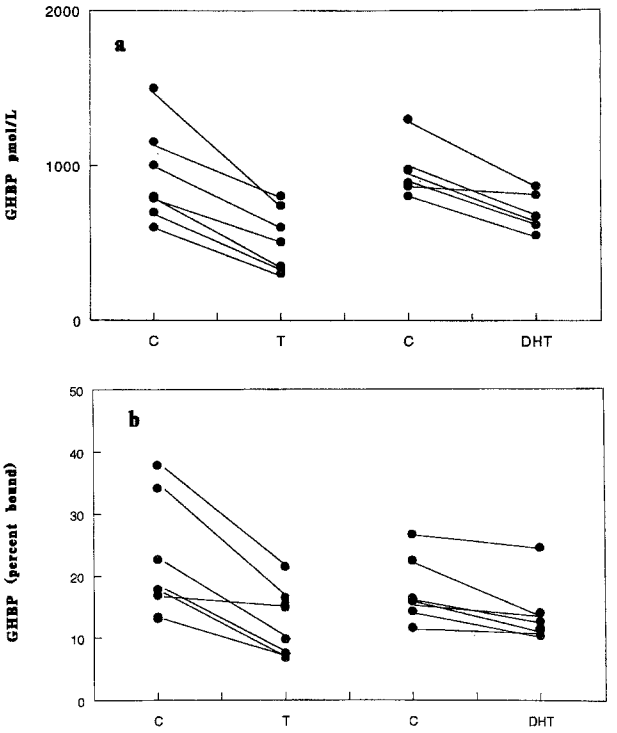

Fig 1. Effect of treatment of delayed puberty with T and DHT on GHBP levels as measured by (a) immunoprecipitation and (b) gel filtration. C, control.

DISCUSSION

The pubertal growth spurt in the male is associated with increased GH concentrations, as well as increased IGF-I levels in the blood. The increase in GH secretion in male puberty seems to be determined largely by T, acting through an estrogenic mechanism.[8,23] Thus, T treatment of boys with delayed puberty induced an increase in plasma GH, as well as IGF-I. However, treatment with DHT, a nonaromatizable androgen, resulted in a decrease in plasma GH. Since T could act either via conversion to DHT (androgenic mechanism) or estradiol (estrogenic mechanism), the lack of stimulation of GH by DHT suggested that the T effect on GH was via an estrogen-dependent mechanism. Subsequent studies by Metzger and Kerrigan[24] showed that the effect of T treatment on plasma GH was blocked by tamoxifen, further supporting this hypothesis. Acceleration of growth by DHT treatment to the same extent as with T treatment, and with no increase in either GH or IGF-I, was taken to indicate that androgens may act synergistically with locally produced IGF-I in this process.[8]

Since treatment with both T and DHT resulted in a decrease in plasma GHBP in all subjects, this decrease is

probably androgen receptor–dependent. The fact that DHT, a nonaromatizable metabolite of T, decreased GHBP levels indicates that the effect of T is not estrogen-dependent. Furthermore, studies in humans[16,17] and in rats[25] suggest that estrogens can increase the level of GHBP in blood, supporting the hypothesis. From these findings, it does not seem that the greater effect of T versus DHT treatment on plasma GHBP is explained by a combined androgen-estrogen effect. Therefore, since the effect of T treatment appears to be purely androgenic in nature, it may be that a greater intracellular concentration of DHT is achieved by T treatment than by DHT treatment.

In this study, we demonstrated a decrease in plasma GHBP with two different GHBP assays in response to two different androgens, T and DHT. The basis for specificity of the gel exclusion method of measuring GHBP is the stereospecificity of the binding of the ligand, GH, to GHBP. The specificity of the immunoprecipitation method is attributed to the interaction of the monoclonal antibody with other structural features of GHBP, since the antibody binds to GHBP in the presence of GH. The two assays showed good agreement in that there was a high degree of correlation and the direction of change was the same in the subjects studied. It should be noted that the GHBP assays have a relatively high coefficient of variation, which explains the less-than-perfect correlation between the two assays.[26]

There is much circumstantial evidence to suggest that plasma GHBP levels reflect the degree of expression of the GH receptor and thus of GH action.[27] However, in this study there was no evidence for a reduction in GH receptor–dependent activity in conjunction with the reduction in GHBP. During T treatment, GH concentration increased threefold, while GHBP decreased by 44.4%. The increase in GH was accompanied nevertheless by a twofold increase in IGF-I in these subjects, indicating a significant GH receptor–dependent response to GH in these subjects. This seems best explained by a large increase in GH compared with a relatively small decrease in GHBP (and possibly GH receptor). Although there was a small decline in GH in DHT-treated subjects, IGF-I levels did not decline, whereas GHBP decreased by 24.7%.

In principle, a decrease in GHBP level could reflect a decrease in GH activity in vivo as a manifestation of decreased GH receptor expression or by shortening the GH half-life; or a decrease in GHBP could indicate a positive effect, ie, less competition with GH receptor for the ligand. In the present investigation, it cannot be readily determined whether a subtle decrease in GH receptor activity occurred along with the decrease in GHBP, because of the indirect nature of the study.

The fact that acceleration of linear growth occurs in DHT-treated subjects, whose mean GH concentration decreased, supports the hypothesis that androgens enhance GH action either at the level of the GH receptor or distal to that point. Recent studies in hypophysectomized rats demonstrated a 30- to 40-fold increase in IGF-I mRNA in the liver and uterus with either T or DHT treatment.[28] Furthermore, in skin fibroblasts, DHT, but not T or estradiol, caused an increase in 5α-reductase that appeared to be IGF-I–dependent.[29] Thus, there is some experimental evidence to indicate that androgens can stimulate IGF-I synthesis independently of GH.

The changes in GHBP in this study were independent of changes in GH. The reduction in GHBP with T treatment occurred despite a threefold increase in the mean 12-hour plasma GH concentration. In contrast, DHT treatment decreased GHBP, while GH levels remained the same or decreased. In other studies, the effect of GH treatment on plasma GHBP has varied,[15,17,30] and Martha et al[31] showed a negative correlation between the GH secretion rate and GHBP in developing adolescents and children. There appear to be no significant differences in GHBP levels in subjects with GH deficiency or GH excess or normals.[27,32] Thus, there does not appear to be any consistent relationship between changes in GH and changes in GHBP.

Whereas GHBP levels decreased in androgen-treated boys, other studies have shown that plasma GHBP concentrations increase steadily with increasing age from birth into adulthood.[33] There was no apparent effect of puberty on this trend in either sex in the populations surveyed in cross-section. This suggests the possibility that the effect seen is pharmacologic rather than physiologic. However, in a longitudinal study of puberty in boys by Martha et al,[14] GHBP did not increase as a function of advancing pubertal stage, and the ratio of GH to GHBP increased in midpuberty. This suggests that androgens may exert a counterbalancing effect on steadily increasing levels observed before and after puberty.

Circulating levels of GHBP in the human seem to represent in some fashion the level of GH receptor in GH-responsive tissues. Thus, in Laron dwarfism, an inherited form of GH resistance, deletions in the GH receptor gene are associated with marked reduction in plasma GHBP activity.[34] In the pygmy, there is a failure of GHBP levels to increase with the age of the subject, with resultant short stature early in life and a particularly remarkable failure of the pubertal growth spurt.[33,35] Recent studies in short children indicate that there may be milder defects in the GH receptor that also are associated with decreased plasma GHBP concentration.[36]

GHBP is structurally identical to the extracellular portion of the GH receptor.[37] This protein can be produced by proteolysis of cells expressing the GH receptor gene.[38,39] These considerations and the clinical correlations mentioned herein suggest that reductions in GHBP indicate reduced expression of the GH receptor gene. However, in the case of androgen-induced changes this appeared not to be the case, since IGF-I levels increased with declining GHBP. It is likely that the large increase in GH could override a relatively small change in GH receptor levels. Other factors that might contribute to the response of target cells to GH, such as the rate of expression of the GH receptor gene, rate of cleavage of GHBP from the cell surface, and metabolic clearance of the protein in the circulation, are largely unexplored. Also, the possibility that plasma levels of GHBP may regulate GH bioactivity has not been fully explored. The present study does not elucidate these points, which remain for future investigations.

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