The effect of human growth hormone therapy on GH binding protein in GH-deficient children

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Abstract. Previous studies have described the close similarity of the GH binding protein to the liver membrane GH receptor. Since GH regulates its own liver receptors, we examined the effects of short- and long-term hGH therapy on GH binding protein in children with GH deficiency. Six GH-deficient children received their first hGH dose ever, and the pharmacodynamics of serum GH was followed for 12 h, along with measurements of GH binding protein activity. Over the first 6 h, serum GH and GH binding protein activity exhibited a parallel increase, followed by gradual decrease. At 8 h, some of the patients exhibited an apparent second peak in GH binding protein, despite the continuous decrease in serum hGH. During the period of hGH treatment, serum GH binding protein increased progressively over a period of 6 months. In a second uncontrolled group of 7 GH-deficient patients who had been treated with hGH for 30-36 months, GH binding protein activity was also significantly higher than pretreatment values. We suggest that the short-term pharmacodynamic changes probably represent the endogenous turnover of the GH receptor, whereas the elevated GH binding protein activity on hGH treatment may reflect up-regulation of the GH receptor.

The notion that the serum GH-binding protein (GHBP) might arise from the liver and be structurally related to the hepatic membrane GH receptor was entertained from the early report on rabbit serum BP (1). Two years later Leung et al. (2) purified the GH receptor from rabbit liver and the rabbit serum GHBP, and unravelled the identical N-terminal amino acid sequence of both molecules. Furthermore, GHBP was found to be absent in GH receptor-deficient patients with Laron-type dwarfism (3-5). The hepatic GH receptor and the GHBP have been shown to have similar immunochemical (6) and binding characteristics in terms of specificity and affinity (7,8). It has also been shown that IM-9 lymphocytes, a cell line which proliferates upon stimulation by somatogenic GH, can release GHBP to the culture medium (9). These indirect findings suggest that GHBP activity may provide an easily measured peripheral marker of GH-receptor activity. Since the responsiveness of a target organ is modulated not only by the concentration of circulating hormone, but also by the availability of hormone receptors, these findings could have far-reaching implications. Thus, the study of the hypothalamic-pituitary-target organ axis in hGH action, currently limited to serum levels studies of hGH secretion, could now be supplemented with the very important and, until now, elusive measure of a marker for target organ receptors.

The concept that GH may have a significant role in the regulation of its own receptors in non-primates is supported by previous studies (10-12). We have recently shown that the pulsatile secretion of GH is associated with concurrent fluctuations in
GH receptors in rat liver plasma membranes (13,14) and in GHBP (15,16). In the present report we describe the effects of short- and long-term modulation of GHBP by serum levels of GH in children treated with hGH for GH deficiency.

Patients and Methods

Recombinant authentic hGH for binding assays was a gift from BioTechnology General Ltd (Rehovot, Israel). Recombinant hGH for therapy, BioTropin, was obtained from BioTechnology General Ltd, and from Ely Lilly, Windlesham, England. Na\(^{125}\)I was purchased from the Nuclear Research Centre, Negev (Beer-Sheva, Israel). Sephadex G-100 and Dextran T-70 were the product of Pharmacia (Uppsala, Sweden). BSA (fraction V), polyethylene glycol (molW~6000) and Norit-A charcoal were purchased from Sigma Chemical Co (St. Louis, MO). Rabbit anti human IgA+G+M (H+L) were the product of Bio Makor (Rehovot, Israel).

Six GH-deficient patients, 7.5-13.2 years of age, were studied over the first 6 months of hGH therapy (Ely Lilly) (Table 1). GH-deficient patients had growth velocity smaller than 4.5 cm/year and GH response to pharmaceutical stimulations (arginine and insulin) of less than 10 \(\mu\)g/l. The protocol was approved by the Human Rights Committee of the Rambam Medical Centre and the parents signed an informed consent. The first sc injection of 1 or 2 IU of hGH (3 patients each) was followed by a pharmacodynamical study of serum hGH and measurements of GHBP activity over the next 12 h. Blood was collected before the hGH dose, 1 and 2 h later, and then 2-hourly. The data were analysed against those of 3 healthy early pubertal children, to account for normal variations of serum GHBP. hGH therapy was continued as a daily sc injection of 2 IU per m\(^2\) body area per day. Sera were analysed for serum GH and GHBP activity at 3 and 6 months after initiation of therapy.

A second group of GH-deficient patients, 9.6-16.3 years of age, who had already been on hGH therapy (BioTropin) for 30 to 36 months, was also studied (Table 1). They received 3 weekly sc doses of 0.1 mg/kg hGH for 2 years, and 0.043 mg · kg\(^{-1}\)·day\(^{-1}\) in the third year of therapy. In both groups of GH-deficient patients serum GH and GHBP activity were measured 24 to 48 h after the last dose of hGH. Results were compared with a control group of 3 prepubertal children with short stature and normal GH response to arginine or insulin.

**Table 1.** Characteristics of the GH-deficient patients.

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>Bone age</th>
<th>Pubertal stage</th>
<th>Treatment period (years)</th>
<th>Growth velocity cm/year</th>
<th>Pretreatment</th>
<th>*On treatment</th>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>1. 7.5/F</td>
<td>5</td>
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<td>0.5</td>
<td>4.1</td>
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<tr>
<td>2. 10.6/F</td>
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<td>1.5</td>
<td>7.6</td>
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<tr>
<td>3. 11.4/M</td>
<td>9.5</td>
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<td>0.5</td>
<td>2.5</td>
<td>12.8</td>
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</tr>
<tr>
<td>4. 12.9/F</td>
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<td>1</td>
<td>0.5</td>
<td>3.0</td>
<td>9.2</td>
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<tr>
<td>5. 13.0/M</td>
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<td>1</td>
<td>0.5</td>
<td>3.9</td>
<td>6.4</td>
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<tr>
<td>6. 13.2/M</td>
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<td>1.0</td>
<td>9.2</td>
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* Cumulative growth velocity for the treatment period.

Assay of GHBP

Human GH was iodinated by the chloramine-T method and chromatographed on a Sephadex G-100 column as previously described (17). The specific activity was ~80 Ci/g.

GHBP activity in human serum was measured by a GH-binding assay (17). The method does not separate the various binding peaks observed in gel chromatography. The method was validated against chromatographic methods and found comparable (17). \([^{125}\text{I}]\text{hGH}\) (1 ng) was incubated with human serum (50 \(\mu\)l) in the absence (total binding) or presence (non-specific binding) of excess unlabelled hGH (1 \(\mu\)g) in a final volume of 270 \(\mu\)l. Incubations were carried out at 4°C for 20 h. The incubations were terminated by addition of 1.0 ml cold dextran-coated charcoal (2% Norit-A charcoal – 0.2% dextran T-70) in 10 mmol/l phosphate buffer, pH 7.6, on ice for 15 min, followed by centrifugation at 3000 × g for 20 min at 4°C. The supernatant was collected and counted in an automatic gamma counter. The specific binding of \([^{125}\text{I}]\text{hGH}\) obtained with 50 \(\mu\)l serum was expressed as a percentage of the total cpm. Serum GH was measured by RIA and the specific binding to GHBP was then corrected for endogenous hGH concentration in the assayed serum by comparison to a previously determined displacement curve (16-18).

Inter- and intra-assay coefficients of variation for GHBP were calculated from the specific binding measured in 50 \(\mu\)l samples of normal human sera and were 4.6% (N=8) and 1.5% (N=10) of radioactivity, respectively. The coefficient of variation of repeated sampling of the same individual serum was 5.7% (N=9).
Anti-hGH antibodies
For assessment of anti-hGH antibodies, patient serum (50 μl) was incubated with [125I]hGH (1 ng) in the absence or presence of unlabelled hGH (1 μg) in 10 mmol/l phosphate buffer containing 1% BSA, pH 7.6, for 20 h at 4°C. Bound hormone was separated from free hormone by precipitation, after incubation for 2 h at 4°C with 0.1 ml rabbit anti-human IgA+G+M (H+L) (1:15) and 0.2 ml 20% PEG. Radioactivity in the pellets was determined after centrifugation at 3000 × g for 20 min at 4°C. Specific binding of more than 0.5% of the total counts incubated was regarded as positive evidence for antibodies.

Statistics
Statistical analysis of the results was based on the two-tailed Student's t-test, comparing experimental group means ±SEM for significance. The pharmacodynamical study was analysed by the two-tailed Friedman-test for non-parametric analysis of variance.

Results

Pharmacodynamics
Results of the pharmacodynamical study of serum GH and measurements of GHBP activity of the 6 GH-deficient patients, who received their very first hGH dose of 1 or 2 IU, are shown in Fig. 1 panels A and B. Serum hGH levels reached a peak at the second hour followed by a progressive decrease to basal levels at 12 h. The dynamic changes in GHBP followed those of serum GH. There was an initial rapid increase within the first 2 h followed by a subsequent decrease towards the 10-12th h. Friedman test for significance of GHBP levels following 1 or 2 IU GH revealed p<0.05 and p<0.001, respectively. Those results were compared with the 12 h profile of 3 healthy children (Fig. 1, panel C). The significance of GHBP natural fluctuation was p<0.001.

GH therapy
In the first group of GH-deficient patients, the pretreatment GHBP activity was comparable to that of short-statured children with normal GH secretion: 6.5±0.4 vs 7.4±0.2% of radioactivity, respectively, but significantly lower than that for adult subjects (11.3±0.45%, p<0.001). During the period of GH therapy, the GHBP activity increased progressively to 8.5±0.7% (p<0.05) and 9.5±0.9% of radioactivity (p<0.02) (Fig. 2) after 3 and 6 months of treatment, respectively. Neither the increase nor the absolute activity of GHBP correlated with the patients’ age, bone age, pretreatment growth velocity or treatment-induced growth velocity, probably reflecting the relatively small experimental group studied.

In the second group of 7 GH-deficient patients, GHBP activity measured after 30-36 months of hGH treatment was significantly higher compared with pretreatment values of the first group of GH-deficient patients (10.7±1.0%, p<0.01).

Fig. 1.
Pharmacodynamics of serum hGH (dashed line) and the concurrent serum GH-binding protein (GHBP) activity (solid line). One IU (panel A) or 2 IU (panel B) of hGH were given sc to 6 GH-deficient patients. Panel C represents a control group of 3 normal children with their normal GH and GHBP pulsatility. Mean ± SEM. GHBP activity is expressed as the percent of radioactivity obtained with 50 μl serum and corrected for endogenous hGH as determined by RIA.
Discussion

In the present study we documented the short and long-term effects of hGH on GHBP levels in GH-deficient patients. We found that in the short term GHBP follows the pattern of GH pharmacodynamics, whereas in the long term GH therapy upregulated GHBP levels.

The assay of GHBP used here was previously characterized and validated against other assays (17). The great precision of the assay with very small intra- and inter-assay variations enables us to document even small changes in GHBP levels. It is essential to correct the data in this and all other known assays for endogenous GH, as all these assays are based on binding by GHBP of a tracer-labelled hGH (18).

The results of the pharmacodynamical study of serum hGH and parallel measurements of GHBP levels in GH-deficient children suggest that GHBP activity followed the dynamic changes in serum GH levels. We have recently studied 24-h profiles of GHBP in normal individuals and found it to parallel those of the GH profile (16). The dynamics of GHBP changes in the GH-deficient patients are different and cannot be attributed to natural variation in GHBP levels. In view of a previously suggested association of serum GHBP with the hepatic GH receptor (1-4,6-8), the changes observed in GHBP activity may reflect similar endogenous fluctuations in the turnover-rate of liver GH receptor, causing the release of GHBP into the circulation in the course of receptor internalization (13,14), or that GHBP and the GH receptor are concomitantly upregulated. Such an effect was recently reported in the rat (15) and in the mouse (19). Further studies are required to verify such a hypothetical mechanism.

Determination of GHBP activity during hGH therapy to GH-deficient children demonstrates a mild up-regulation of GHBP by hGH treatment. This increase in GHBP cannot be attributed to the appearance of GH antibodies, since patient sera selected for studying GHBP activity included only those patients who did not develop GH antibodies in response to therapy. Whereas the increase in GHBP after the uncontrolled 3 years of therapy might be linked to age-dependent ontogeny (18,20), the changes over the 3-6 month period can hardly be expected to be so.

The up-regulation of GHBP following GH treatment is consistent with the concept established in the rat that GH may induce its own liver receptors (10-12,21), and further support the close association of GHBP with membrane-bound GH receptors. Since GH may oppositely affect the synthesis and degradation of its own receptors and GHBP, the regulation of GH receptors by GH appears to be considerably more complex and may prove to be dependent upon the pattern of GH treatment. Indeed, in GH-deficient rats, hepatic GH receptors were reported to be up-regulated by prolonged continuous GH treatment, unchanged after repeated GH injections, and down-regulated by a single GH surge (12,22). It is of interest that GH deficiency was not associated with low levels of GHBP. The increase observed during GH therapy was, therefore, not the effect of a replacement treatment. The sc route of administration, with its slow pharmacodynamics, resembles the continuous infusion experiments in the rat, and seems to induce receptor and GHBP up-regulation.

In summary, the present study demonstrated the effects of short- and long-term modulation of GHBP activity by GH in GH-deficient children treated with hGH. The short-term effects of GH on GHBP probably represent endogenous turnover of the GH receptor, whereas the elevated GHBP observed, following prolonged hGH therapy, may reflect the up-regulation of the GH receptor by the non-pulsatile nature of hGH administration.
References


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