Assay Systems for the Growth Hormone-Binding Protein (43766)

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Abstract. **The first method used for detection of growth hormone-binding protein (GHBP) in biological fluids was based on the incubation of the sample with radiolabeled GH followed by separation of bound and free GH by gel exclusion chromatography. Recently, other methods have been developed which are faster and easier to use. These methods include variants of the original binding/column assay (e.g., separation of bound and free GH is obtained by immunoprecipitation, charcoal adsorption, ion exchange chromatography, or HPLC), and a ligand-mediated immunofunctional assay (LIFA), in which a monoclonal antibody is used to capture the GHBP on a microtiter plate; all binding sites are saturated with GH and an anti-GH antibody is used to detect the amount of GH (endogenous and exogenous) bound to the GHBP. To permit comparison of results obtained by different methods we have cross-validated the LIFA with two different binding assays: (i) the original long column assay (column assay), and (ii) an assay based on immunoprecipitation (RIPA) of the GH/GHBP complex with an anti-GHBP antibody.**

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Human blood contains a high-affinity growth hormone-binding protein (GHBP) (1, 2) which shares sequence homology with the extracellular domain of the GH-receptor (3). It has been suggested that GHBP may reflect the number and function of GH-receptors, and that it can be used to estimate GH-sensitivity. Several different methods have been used to estimate the amount of GHBP in blood (4–9). In patients with Laron-type dwarfism, who are completely insensitive to GH due to severe GH receptor defects, GHBP levels measured by different methods are, with a few exceptions, low or undetectable (10–12). Since it is possible that GHBP measurements also can be used to estimate GH sensitivity in other groups of patients, it is important to cross-evaluate different methods in samples representing a wide range of GHBP concentrations. We summarize here the results of comparisons of GHBP concentrations measured by ligand-mediated immunofunctional assay (LIFA) (6), GH binding with separation on a long column (column assay) (4), and a radioimmunoprecipitation method (RIPA, Endocrine Sciences) (8). Some of the results have also been reported elsewhere (13).

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Methods

Column Assay. The assay was performed essentially as described (4). Briefly, plasma (400 μl) was incubated for 45 min with [^{125}I]human GH (hGH). Separation of bound GH from free GH was performed on a 1.5 × 100-cm Sephadex G-100 column at 4°C. Peaks corresponding to the high-affinity GHBP-GH complex and the free GH were integrated. When necessary, corrections for GHBP saturation by endogenous GH were made. Results obtained by this assay represent a single determination. The functional range in this assay was 1%–40% GH bound. The interassay coefficient of variation was 12%.

RIPA. Serum was incubated overnight with excess [^{125}I]hGH and a monoclonal antibody directed against the extracellular domain of the GH receptor (Mab #263) (14). The trimolecular complex (Mab #263<> GHBP<>[^{125}I]hGH) was separated from free [^{125}I]hGH by precipitation with goat–anti-mouse immunoglobulin and polyethylene glycol. Unknown sample data were compared with standard data. The serum standard containing 1270 pmol/l of GHBP, as

determined by Scatchard analysis of GH binding to GHBP in the presence of Mab #263, was derived from 10 normal adults. The specific binding of [^{125}I]hGH in the assay ranged from 0.75% to 26%. The interassay coefficient of variation was 12.2% at 700 pmol/l, 12.4% at 1200 pmol/l, and 7.6% at 1900 pmol/l; intraassay coefficient of variation at 1900 pmol/l was 4.7%. Endogenous GH can interfere in the RIPA by competing with [^{125}I]hGH for binding to the GHBP and the effect of endogenous GH in the samples is therefore monitored in each assay by including GH interference controls. The interference by GH can be reduced by running the assay under modified conditions.

LIFA. The assay was performed as reported (6). Briefly, microtiter plates were coated overnight with a monoclonal antibody (Mab #263) directed against the extracellular domain of the GH-receptor, blocked for 2 hr and washed. The sample (50 μl) or standard was added together with 200 μg/l of rhGH. The plates were washed and an HRPO-conjugated Mab (MCB, Genentech Inc., San Francisco, CA) directed against hGH was added to detect the bound GH. The plates were washed again and substrate was added. The reaction was stopped after 15 min and optical density at 490 nm was measured. Recombinant hGHBP purified from a mammalian cell line is used as standard in the LIFA. The concentration of the standard preparation was determined by Scatchard analysis and quantitative amino acid analysis. All samples were measured in duplicate. Assay range was 16–1000 pmol/l, samples with GHBP concentrations above 1000 pmol/l were diluted. The inter- and intra-assay coefficients of variation were 7.4% and 2.7%, respectively.

Results and Discussion

Comparison between LIFA and Column Assay. Plasma GHBP concentrations determined by LIFA and the column assay were compared in 61 normal adults (19–69 years old), 10 patients with acromegaly and two patients with Laron dwarfism (Fig. 1) (13). In addition, a pool of normal adult plasma was measured. Corrections for GHBP saturation by high endogenous GH in the column assay were made for 16 of the samples. The GHBP concentrations obtained in the two assays correlated significantly (r = 0.669, P < 10–16), but there was a considerable spread of individual data points around the regression line. Thus, the column assay and the LIFA gave similar results when applied to a group of samples, while the agreement is considerably lower for a single sample.

Comparison between LIFA and RIPA. Comparison of GHBP values obtained in the LIFA and in the RIPA is shown in Fig. 2. GHBP concentrations were measured in 35 samples (17 normal volunteers and 18 short children). For some of these samples values could not be obtained in the RIPA due to high endogenous GH or high titers of anti-GH antibodies. In the remaining 25 samples, negative for GH-antibodies and with GH levels less than 25 ng/ml, there was an excellent correlation between the values obtained in the two assays (r = 0.926). The good agreement between the two assays is probably due to the fact that both assays use the same anti-GHBP Mab.

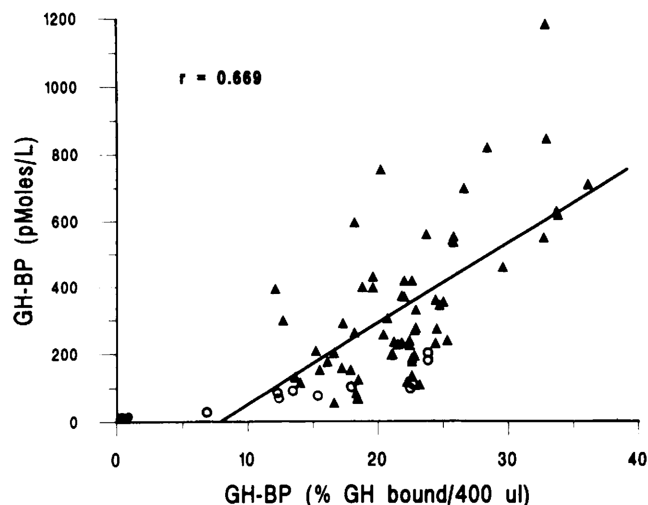

Figure 1. Plasma GHBP concentrations in 61 normal adults (triangles), 10 patients with acromegaly (open circles), and two patients with Laron dwarfism (filled circles), determined by LIFA and the column assay. There was a significant correlation between the GHBP concentrations obtained in the two assays (r = 0.669, p < 10–16) (from 13, with permission).

Diurnal Pattern of GHBP. Figure 3a shows GHBP concentrations measured by LIFA in blood samples taken every 20 min in a 13-year-old boy. In this boy, the coefficient of variation for GHBP was 7.7%, and, in a group of 12 healthy children, the mean coefficient of variation was 10% (15). In five subjects

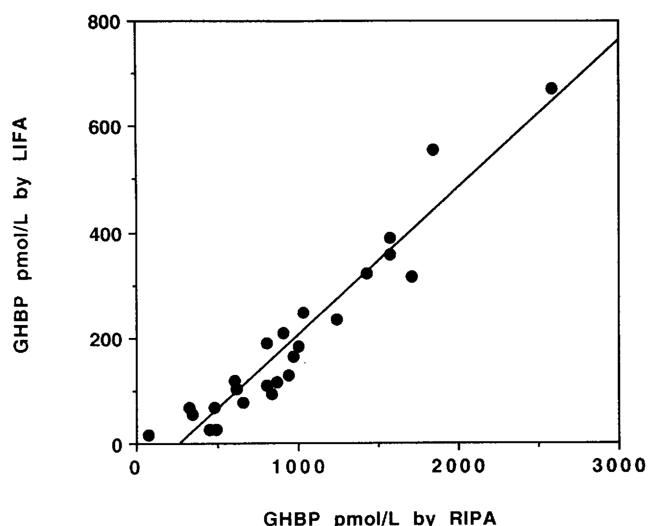

Figure 2. Plasma GHBP concentrations in 17 normal volunteers and 18 short children measured by LIFA and RIPA. There was an excellent correlation between the values obtained in the two assays (r = 0.926).

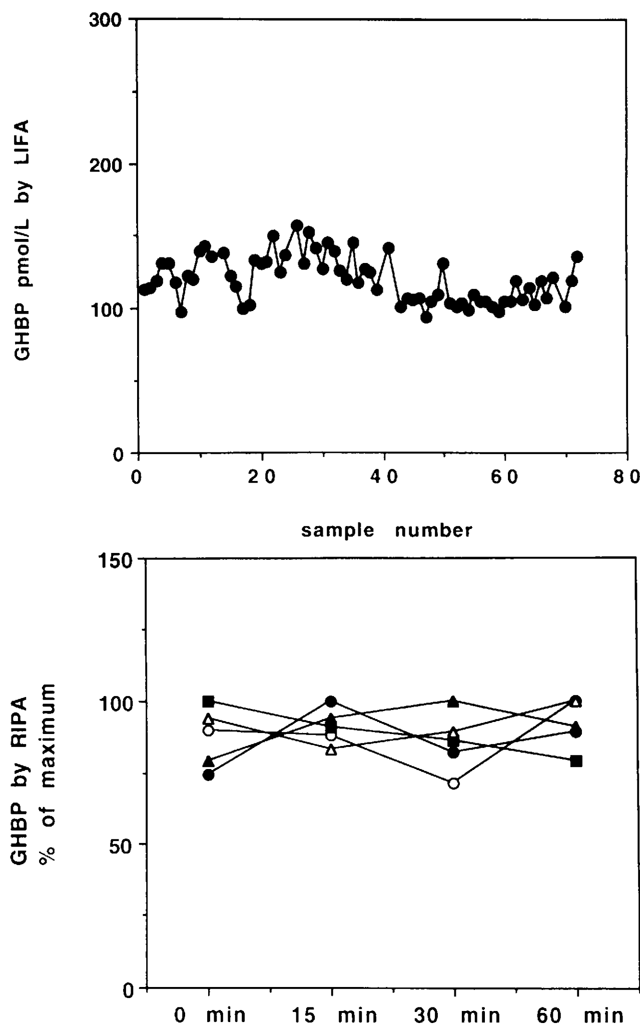

Figure 3. Diurnal pattern of GHBP in blood. GHBP concentrations were measured by LIFA in samples taken at 20-min intervals for 24 hr from a 13-year-old boy (a) and by RIPA in 5 subjects sampled every 15 min for 1 hr (b).

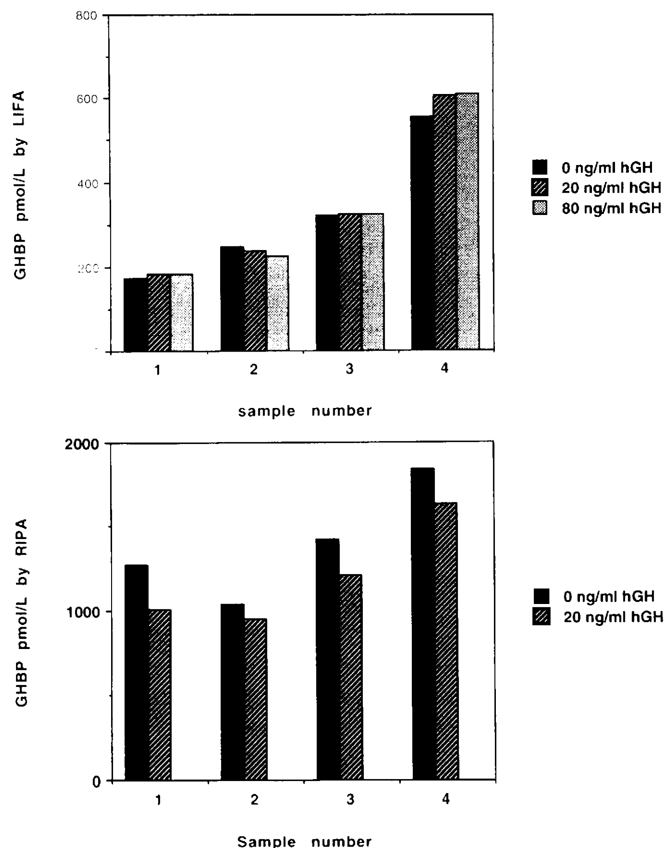

Figure 4. Effects of GH on GHBP measurements by LIFA and RIPA. Plasma samples were spiked with rhGH at 0 ng/ml, 20 ng/ml, and 80 ng/ml for GHBP determination by LIFA (a), and spiked with rhGH at 0 ng/ml and 20 ng/ml for GHBP determination by RIPA (b).

sampled every 15 min for 1 hr, GHBP levels, measured by RIPA, varied an average of 10% (Fig. 3b). The absence of a significant diurnal pattern of GHBP in plasma has also been reported for the column assay (16). Thus, in contrast to GHBP levels measured by a charcoal adsorption assay (17), we find that GHBP concentrations in blood, measured by LIFA, RIPA, or the column assay are relatively stable and that the concentration can be estimated from a single random blood sample.

Interference by Endogenous GH and Anti-GH Antibodies. We have tested the interference by GH in the LIFA and the RIPA by spiking in rhGH at different concentrations (Fig. 4). Incubating the samples with rhGH at 20 ng/ml or 80 ng/ml did not change the GHBP levels measured by LIFA (Fig. 4a). This is in agreement with our previous report (6), and the lack of interference is also supported by the fact that GHBP levels measured by LIFA show very small diurnal variations in spite of very marked variations in endogenous GH concentrations (15). In contrast, high levels of rhGH reduced the GHBP concentrations measured by RIPA (Fig. 4b). When hGH was spiked to 20 ng/ml, GHBP levels by RIPA were reduced 14% ± 4.6%. High endogenous GH concentrations also interfere in the column assay, and the GHBP levels must be adjusted (4). GH is secreted in a highly pulsatile fashion and, with few exceptions (e.g., acromegaly, pregnancy, diabetes, during stress), very low or undetectable GH concentrations are expected between the pulses. If a series of samples is taken over several hours (e.g., when GH secretion is evaluated in a short child), it should therefore be possible to avoid the interference by GH, by measuring the GHBP levels in samples with low GH concentrations.

Antibodies against hGH, which are sometimes present in samples from patients treated with hGH, can, in theory, interfere with GHBP measurements in all the assays. In the LIFA, anti-GH antibodies could interfere with the measurements by blocking the binding of GH to the GHBP, or by blocking the binding of the anti-GH monoclonal antibody to the GH, and the expected effect would be a decrease in the measured GHBP. In the RIPA, auto antibodies to GH could ei-

ther increase the apparent GHBP level (if the goat–anti-mouse IgG cross-reacts significantly with human IgG), or reduce the value by retaining the labeled GH in the supernatant.

Conclusions

Comparison of the three different assays for GHBP in human blood showed that there was a good correlation among the results from the different assays when applied to groups of samples. We have previously observed that GHBP levels measured by LIFA are lower than those derived from saturation analysis and the discrepancy between the LIFA and the RIPA is even more pronounced (Fig. 2). The reason for the differences in the absolute levels of GHBP in different assays is unknown.

Using all three methods we have shown that the GHBP levels are relatively stable during the day and GHBP activity in blood can therefore be estimated in a single sample from each subject. Endogenous GH does not interfere in the LIFA. High endogenous GH levels reduce the measured GHBP activity in the column assay and in the RIPA. When endogenous GH concentrations are high, GHBP levels have to be adjusted in the column assay and modified conditions should be used in the RIPA. When serial samples are taken, samples with low GH concentrations should be used for the GHBP determinations in the column assay and in the RIPA to avoid the interference by GH. Anti-GH antibodies may interfere with GHBP measurements in all three assays, and results from samples with high titers of antibodies should be interpreted with caution.

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