Stoichiometry of the Pulsating Growth Hormone (GH) Binding to the GH-Binding Protein and the Turnover GH-Receptor (43753)

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Abstract. **The pulsatile pattern of growth hormone (GH) secretion is synchronized with the GH-receptor (GHR) turnover and the ensuing GH-binding protein (GHBP). We investigated the effect of GH pulse frequency on the turnover of GHR, and tested the theoretical reciprocal impacts of GH, GHR, and GHBP in different species and clinical conditions. Male Sprague–Dawley rats were hypophysectomized (hypox) at 35 days of age. Two groups of 16 rats received a single or two iv injections of 10 μg human GH (hGH) at an interval of 45 min. They were killed 45, 90, or 135 min after the single or second injection. A third group of 25 hypox rats were given continuous sc infusion of hGH for 6 days. Liver membranes were prepared for hGH somatogenic binding. A bolus of GH at 45-min intervals expedited GHR turnover, and continuous GH resulted in faster turnover cycles of 90 min. The impact of GHBP on GH bioactivity was then calculated in human serum by a rabbit liver membrane displacement assay. Bioactivity was diminished by GHBP with increasing GH levels up to a point, within the physiological range, where GH bioactivity is gradually restored. Finally, simulation calculation of the bound and free fraction of GH over a typical pulse in man and male rat showed the changing relations of free/bound GH. In man free hormone predominates most of the pulse, whereas in rat free GH comprised a smaller fraction. Thus, the reciprocal effects of GH on GHR turnover and GHBP generation, and that of GHBP on GH $t_{1/2}$ lead to self-perpetuation of high (mostly free) GH in downregulation of GHR and its turnover with resultant lower GHBP.** [P.S.E.B.M. 1994, Vol 206]

The pulsatile pattern of growth hormone (GH) secretion is synchronized with the GH-receptor (GHR) turnover (1, 2). The latter gives rise to the circulating GH-binding protein (GHBP) by either an alternatively spliced GHBP transcript of the GHR gene, in rat and mouse (3, 4), or by proteolytic cleavage of the membrane GHR (5, 6). Serum GHBP levels oscillate in imperfect but statistically significant correlation with serum GH levels in both man (7) and rat (8). Over a wide range of mammalian species and clinical conditions the GHR and GHBP levels correlate negatively with the pulsatility of serum GH levels, as recently reviewed (9). This correlation seems to result from a reciprocal influence of GH pulsatility on GHR and GHBP (8, 10), and of GHBP on the profile of free and bound GH, the half-life of GH (11), and its biological effect (12).

In the present study we have investigated the effect of GH pulse frequency on the turnover of the GHR, and tested the theoretical reciprocal impacts of GH, GHR, and GHBP in different species and clinical conditions.

Materials and Methods

Animals and Protocol. Male Sprague–Dawley rats were hypophysectomized (hypox) at 35 days of age. Complete hypophysectomy was assured by growth arrest for 10 days and by postmortem visualization. Postoperatively the rats received subcutane-

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ous replacement therapy with L-thyroxine, 1 μg/100 g BW/day, and dexamethasone 2.5 μg/100 g BW/day. Experiments were performed 10 days after surgery. Two groups of 16 rats received a single or two intravenous injections of 10 μg human GH (hGH) at an interval of 45 min into the tail vein. The rats were killed 45, 90, or 135 min after the single or second injection. A third group of 25 hypox rats was treated with continuous subcutaneous infusion of hGH for 6 days via osmotic minipumps (Alza model 1001, Palo Alto, CA) implanted in their flanks. Each pump was loaded with 1 mg hGH in 0.2 ml saline, to deliver 1 μg/hr. On Day 6, groups of five rats were decapitated every 45 min, from 09:45 hr through 12:45 hr. Cervical blood was collected for hGH determination and for determination of anti-hGH antibodies. Six rats that developed antibodies against hGH were excluded from the study. The livers of all rats were removed and rapidly frozen in liquid N_2 for preparation of enriched plasma membranes.

Effect of Serum GHBP on GH Binding to GH Receptor. Sera (0.2 ml) of human subjects with high or low GHBP were preincubated for 20 hr at 4°C in the absence or presence of increasing amounts of unlabeled hGH (0.5–100 ng). Incubation was continued for a further 20 hr at 4°C with a membrane preparation of pregnant rabbit liver (0.5 mg protein), and with [^{125}I]hGH (1 ng) (13). The decline in receptor availability for each hGH value was calculated by subtraction of the percent displacement in the absence of GHBP from the percent displacement in the presence of a given GHBP value, as previously reported (12).

Liver Membrane Preparation and Binding Assay. Rat or pregnant rabbit liver membranes and [^{125}I]hGH for the binding studies were prepared as previously described (1, 13). Somatogenic and lactogenic binding after dissociation of endogenous ligands by 3 *M* $MgCl_2$ were determined on an enriched liver plasma membrane fraction (200 μg/tube), using as ligand [^{125}I]hGH ng/100 μl, as previously described and validated (1). Somatogenic binding of [^{125}I]hGH was determined in the presence of excess ovine prolactin (1 μg/tube).

Simulation of GH Dynamics. The association and dissociation of GH with GHBP has been previously reported to be short, reaching in man 65% of saturation within 10 min and 75% within 20 min (14), and in the rat 90% within 10 min (15). The *in vitro* dissociation of serum-bound [^{125}I]hGH is slow in man, with a $t_{1/2}$ of 4 hr (11) and faster in rat, a $t_{1/2}$ of 20 min (15). The *in vivo* dissociation may be faster, and follow the GH clearance rate, as the RIA used measured both free and GHBP-bound GH (13). The following assumptions were made: (i) a single high-affinity GHBP binds GH with the species characteristic affinity (16); (ii) the homodimerization of GHBP in its binding to GH (17); (iii) that 22-kDa variant was the sole effective GH; (iv) that the distribution volume of free GH is the entire extracellular volume, and that of BP-complexed GH is 5 l; and (v) that the $t_{1/2}$ of free GH is 7 min, and that of bound GH is 27 min. Using these parameters and assumptions we calculated the free and GHBP-bound GH fractions from the association curves and the Scatchard analyses capacities of man (14), rat (15), and rabbit (16). The percent binding for each GH level was multiplied by 50% (dimerization) of GHBP saturation molarity to give the bound GH.

Results

GH Administration Frequency. In untreated male rats, GH pulse frequency is 0.3/hr, GHR $t_{1/2}$ is 45–100 min (18), and it completes a sequence of down-regulation, recycling, and up-regulation within 180 min (1, 2). Administration of GH at 45-min intervals to hypox rats expedite this sequence to 135 min, and continuous GH results in GHR turnover cycles of 90 min (Fig. 1).

Competitive Experiments of GHBP and GHR. The inset of Figure 2 shows the effect of serum GHBP on the receptor availability of increasing amounts of hGH, added to the serum, as measured by the capacity of hGH to displace [^{125}I]hGH from rabbit liver membranes. A high GHBP level of 22% shifted the displacement curve to the right more than did a low level of 5.6%, as previously reported (13). By subtraction of the percent displacement in the absence of GHBP (undisturbed hGH displacement capacity) from the percent displacement in the presence of a given GHBP value, the activity factor was calculated as previously reported (12). The shape of the activity dependence on GH level is contrived by an increase in activity with low GH, a decrease by GH levels to a minimum at 25 ng/tube, and an increase by higher levels.

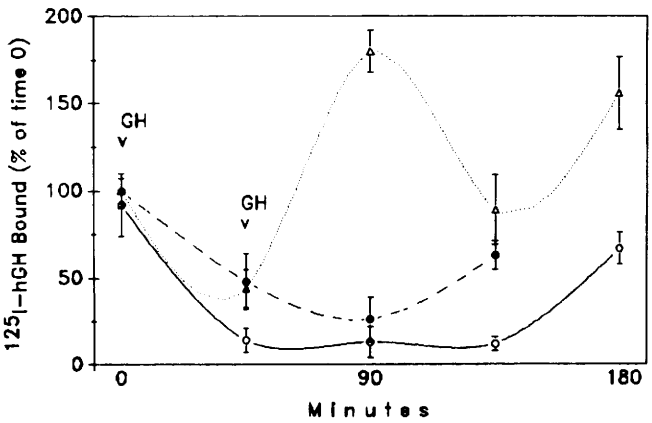

Figure 1. The effect of the pattern of hGH administration of somatogenic GH-receptor turnover. Hypophysectomized male rats were injected once (solid line), twice, at 45-min interval (dashed) or continuously for 6 days (dotted). Rats were killed at 45-min intervals and liver somatogenic GH-receptors were measured. Mean ± SEM, n = 3–5.

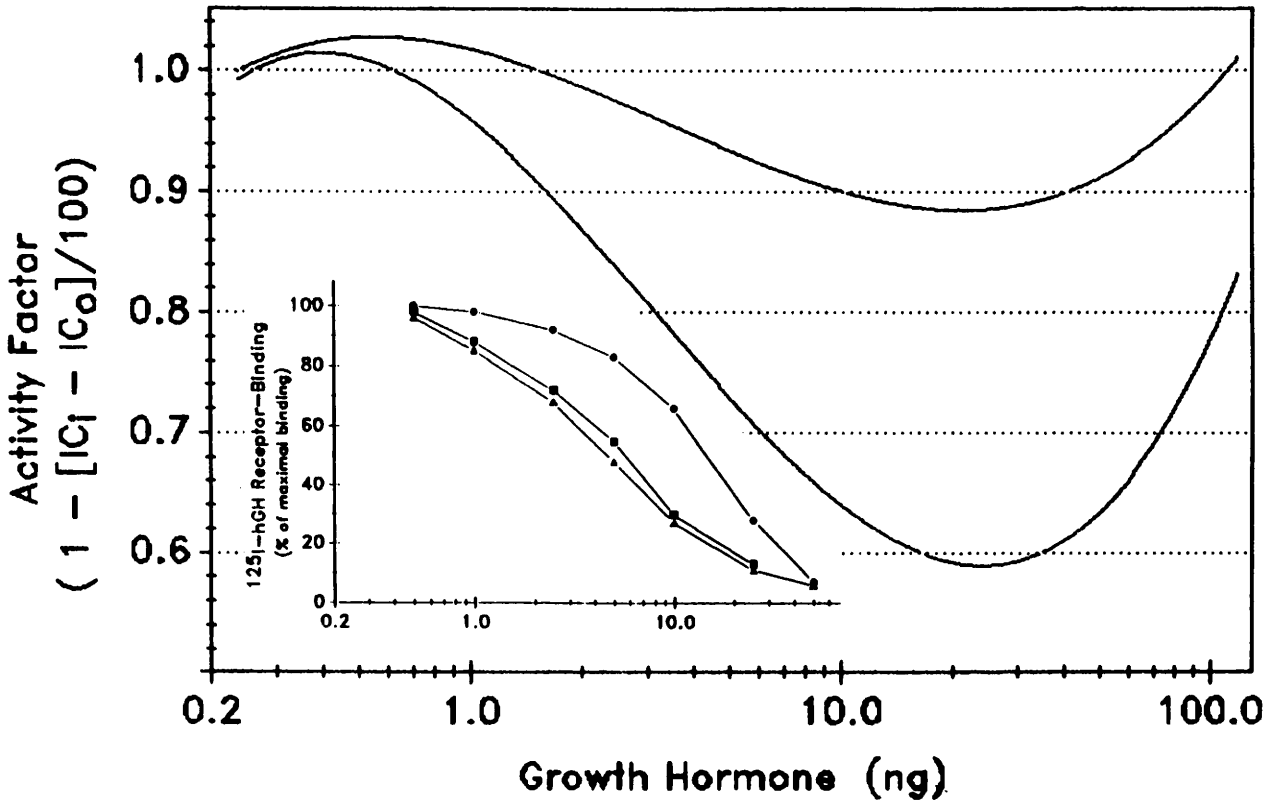

Figure 2. The "activity factor" for GH bioactivity, as measured by the change in GH displacement from rabbit liver membranes (inset) was calculated for two patients with GHBP levels of 21.6% (circles in inset and upper line in frame) and 6% (squares in inset and lower line in frame), adding rhGH, 0.2–100 ng/tube. Triangles indicate control tube with no GHBP (12).

Simulation of GH Dynamics. Control human GHBP binding capacity is 700 nmol/l. Assuming homodimerization of GHBP, theoretical saturation will require 350 nmol/l (7.5 μg/l) GH. From the GHBP/GH association curve, with an affinity $K_a = 10^9\ M^{-1}$, it was calculated that GH will be 50% bound at 4 μg/l, decreasing to 24% at a physiological GH peak level of 24 μg/l (Fig. 3). In the male rat GHBP binding capacity is 25 pmol/ml. Assuming homodimerization of GHBP, theoretical saturation will require 12.5 pmol/ml (270 μg/l) GH. From the rat GHBP/GH association curve, with an affinity $K_a = 10^8\ M^{-1}$, it was calculated that GH will be 74% bound at physiological serum levels of 240 μg/l. In the rabbit, with an affinity $K_a = 10^{10}\ M^{-1}$ GH will be 91% bound at high-physiological serum levels of 12 μg/l (data not shown). Analysis of the bound and free fraction of GH over a typical pulse in a man and a male rat (Fig. 3) shows the changing relations of free/bound GH. In man free hormone predominates most of the pulse, whereas in rat free GH comprises a smaller fraction.

Discussion

A recent review from this laboratory postulated that both GHR and GHBP are regulated by the degree of GH-pulsatility (9). The first part of the present study shows that indeed, the turnover is prone to modulation by the frequency of GH pulses, as well as by GH level (18, 19).

Association and dissociation of GH from the receptor and GHBP are rapid and occur within minutes (14, 15). Thus, the span of a GH pulse is certainly long enough to allow for substantial association and dissociation to steady states. Indeed, rapid and complete dissociation is evident from nullification of serum GH level after a pulse, while most RIAs are unable to sort out bound from free GH (13, 20).

During a GH pulse, serum GH advances through a spectrum of levels with diverse impacts on GH availability to GHR. We previously reported on the biphasic effect of GHBP on GH displacement from GHR (12). GH bioactivity is diminished by GHBP with increasing GH levels up to a point, within the physiological range, where GH bioactivity is gradually restored. This seems to be due to the homodimerization of GHR and eventually GHBP as well. We now show that in the face of very low GH levels (<1 μg/l), GHBP slightly enhances GH bioactivity. It is speculated that this may be due to a more efficient "presentation" of GH to GHR by the largely free GHBP.

The fractions of free and bound GH certainly bear on the bioactivity of GH; they change during a GH pulse in concert with the GH level, mild but synchronous GHBP fluctuation, and GHR turnover. The re-

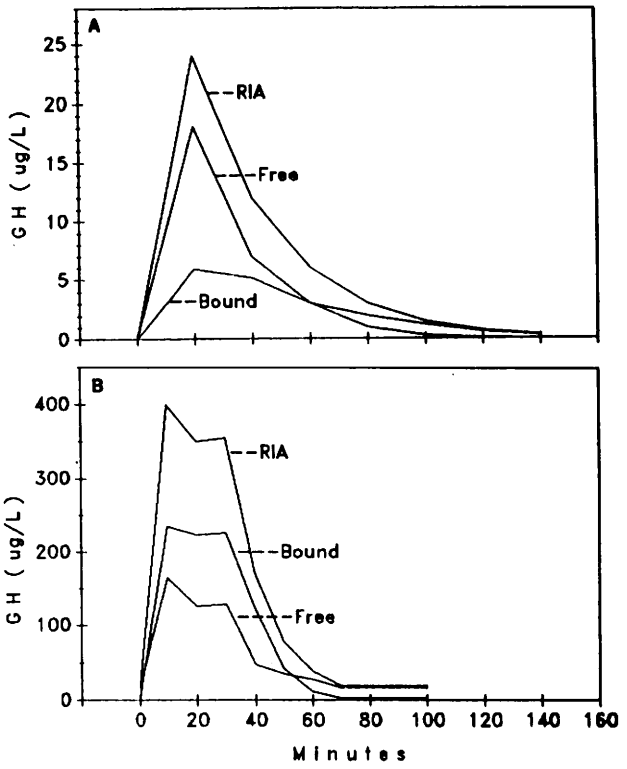

Figure 3. The levels of total, free, and bound GH in a typical pulse of a pubertal boy (A) and of a 45-day-old male rat (B).

sults shown here are in conceptual agreement with a recent report by Carlsson *et al.* (21), although the percents of free and bound GH are somewhat disparate. The ratios of bound/free GH vary between species and clinical conditions, as expected from the variations in GHBP affinity and capacity, and with variations in GH pulsatility. Thus, in a normal individual with GHBP capacity of 700 pmol/l, and assuming homodimerization of GHBP, theoretical saturation will require 350 pmol/l (7.5 μg/l) GH. From the GHBP/GH association curve, with an affinity $K_a = 10^9\ M^{-1}$, it was calculated that GH will be 50% bound at a low physiological 4 μg/l, decreasing to 24% at a high physiological GH peak level of 24 μg/l. This fits nicely with IC_{50} of 6 ng hGH, which also produces maximal interference by GHBP with GH effect, as measured by the effect of GHBP on GH binding to rabbit hepatic membranes.

In the male rat, with low GH specific serum binding, GHBP binding capacity is 25 nmol/l. Assuming homodimerization of GHBP, theoretical saturation will require 12.5 nmol/l (270 μg/l) GH. From the rat GHBP/GH association curve, with an affinity $K_a = 10^8\ M^{-1}$, it was calculated that GH will be 74% bound at physiological serum levels of 240 μg/l. In the rabbit, with high GH specific serum binding, with an affinity $K_a = 10^{10}\ M^{-1}$, GH will be 91% bound at high physiological serum levels of 12 μg/l. In experimental animals the corresponding changes in GHR have been documented, whereas in humans it can currently be speculated.

In species (rabbit) and clinical conditions (obesity) with low serum GH and high GHBP levels, GHBP-bound GH fraction increases, with resultant prolonged GH $t_{1/2}$, contraction of the interpulse interval, which may contribute to lower GH bioactivity. This resembles continuous GH administration, with resultant up-regulation of GHR and GHBP, and further amplification of this sequence. In species (male rat, guinea pig) and clinical conditions (newborn, malnutrition, diabetes) with high GH and low GHBP levels, the higher free GH fraction has shorter $t_{1/2}$, with resultant longer GHR turnover, leading to lower GHR and GHBP. Thus, the reciprocal effects of GH on GHR turnover and GHBP generation, and GHBP on GH $t_{1/2}$ lead to self-perpetuation of high (mostly free) GH in down-regulating GHR and its turnover with resultant lower GHBP.

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