The interrelationship between and the regulation of hepatic growth hormone receptors and circulating GH binding protein in the pig

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We evaluated the interrelationship between, and regulation of, the hepatic growth hormone receptor and serum GH binding protein (GH BP) in pigs treated with recombinant porcine growth hormone (rpGH). Infant and pubertal male pigs (N = 5 per group) received either rpGH 0.15 mg/kg daily or diluent intramuscularly for 12 days. Somatic growth, serum IGF-I and GH BP and [125I]bovine growth (bGH) binding to MgCl2-treated hepatic membrane homogenates were examined. Marked age-related increases were seen in serum GH BP (p < 0.001) and [125I]bGH binding to hepatic membranes (p < 0.001). GH BP was increased in rpGH treated animals (p = 0.03, mean = 1 x SEM) (controls) to 17.8 ± 2.0% in infants, and from 35.2 ± 2.6 (controls) to 41.8 ± 3.4% in pubertal animals. [125I]bGH binding to hepatic membranes was also increased by rpGH treatment (p < 0.05), from 7.0 ± 1.6 (controls) to 15.4 ± 3.6% in infants and from 53.7 ± 7.1 (controls) to 65.1 ± 11.8% in pubertal animals. No significant interaction between age and treatment was seen. Overall, serum GH BP correlated significantly with [125I]bGH membrane capacity (r = 0.82, p < 0.001), with a correlation of r = 0.83 in the infant animals but no significant correlation in the pubertal animals considered alone (r = 0.13). Serum IGF-I correlated significantly with serum GH BP (r = 0.93, p < 0.001) and [125I]bGH membrane binding capacity (r = 0.91, p < 0.001). These observations suggest that serum GH BP levels reflect changes in the population of the hepatic GH receptor. In addition, the present study demonstrates that the hepatic GH receptor can be induced by GH in the infant pig, despite a developmentally low GH receptor population at this age, suggesting potential efficacy of GH at earlier ages than generally considered.

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Considerable evidence exists that the hepatic growth hormone receptor is under active regulation. GH itself has a major influence on GH receptor density and chronic GH therapy has been shown to increase the number of GH receptors in both hepatic (1–3) and adipose (4) tissue. Indirect evidence suggests similar regulation by GH in growth plate (5–7).

Hepatic GH receptors are also under ontogenic regulation. Prenatal somatic growth is thought not to be significantly influenced by GH, with the GH receptor in the liver appearing either very late prenatally or early postnatally (8). In the developing pig we have demonstrated a 14-fold increase in the capacity of the hepatic GH receptor between 2 and 165 days of age, with significant increases in receptor number not occurring until after 35 days of age (9). However, the earliest age at which animals or man will demonstrate a biological response to GH administration is not clearly defined.

A specific GH binding protein (GH BP) has been recently demonstrated in the serum of a number of species (10–12). This GH BP appears to largely derive from the hepatic GH receptor protein either by enzymatic cleavage near the transmembrane domain or by alternate mRNA splicing mechanisms, and has structural and immunological identity with the extramembranous portion of the hepatic GH receptor (13–15). This has led to speculation whether serum GH BP measurement may be useful as a simple indicator of hepatic GH receptor status (16). Limited evidence in the rodent suggests a relationship between GH BP levels and hepatic somatogenic binding (17, 18).

We have used the effects of chronic GH treatment and developmental changes in receptor number to further evaluate the interrelationship between the hepatic GH receptor and serum GH BP. In particular, we aimed to assess the value of serum GH BP as an indirect measure of the hepatic GH receptor and examine for possible evidence of their independent regulation as has been
recently suggested (19). We therefore compared the acute effects of porcine GH (pGH) administration in infant and pubertal pigs, and examined the relationship between somatic growth, serum IGF-I, serum GH BP and hepatic GH receptors.

Materials and methods

Animals and experimental procedure

Ten male Large White × Landrace pigs were used at ages 19–20 days (infants) and 107–112 days (pubertal). Ethical approval was obtained from the Massey University Animal Ethics Committee. Recombinant pGH (rpGH) was a gift from Drs D. Burleigh and L. Gilbert (Batch 148-265/266, Pitman-Moore Inc, Terre Houte, IN). Within each age group, five animals received rpGH 0.15 mg/kg once daily intramuscularly into the left ham at 08.30–09.30 h, the remaining five receiving an equal volume of diluent. On the 12th day pigs were killed 9–11 h after the last injection with no preceding period of starvation. Blood was collected by cardiac puncture after halothane/oxygen anaesthesia and serum separated by centrifugation at 4°C and immediately frozen at −20°C. Liver was removed immediately, washed in distilled water, weighed and frozen in aliquots.

Plasma IGF-I and GH measurements

Plasma IGF-I was measured by radioimmunoassay using a rabbit antiserum to rh-metIGF-I (878/4) after extraction of plasma by acid ethanol cryoprecipitation (20). This extraction system has been extensively validated against gel chromatography under acid conditions. The correlation between values for pig plasma using this extraction technique and Sephadex G75 chromatography was \( r = 0.97 \) (\( p < 0.01 \)) and the slope of the linear regression was 0.86. The minimal detectable dose of IGF-I in this system is 0.013 pmol/tube and the cross-reactivity with IGF-II less than 0.5%. The assay has an interassay CV of 9.8% and a within assay CV of 5%. Plasma IGF-I concentrations are expressed against an internal standard (recombinant human IGF-I (rhIGF-I) batch CGP 35'126, Ciba Geigy, Basel, Switzerland).

Plasma growth hormone was measured using specific pGH radioimmunoassay by previously described methodology (21). In brief, serum samples were incubated with a polyclonal monkey anti-pGH antibody (final dilution 1:100 000) and 15 000 cpm \( ^{125}I \) pGH in a total incubation volume of 0.5 ml. Following incubation at room temperature for 20 h, 100 µl normal monkey serum (Sigma, St Louis, MO), secondary antibody (5% goat anti-monkey IgG) and 1000 µl of 6% polyethylene glycol were added in turn. Separation of bound and free ligand was achieved by centrifugation at 2000 \( \times g \) for 30 min at 4°C. Results were expressed against an internal recombinant pGH standard (AFP-10086C).

Serum growth hormone binding protein

Recombinant bovine GH (bGH) was a gift from Dr I. Hart (Batch AC 6086–109-1, American Cyanamid, Princeton, NJ). Iodination was by a modified lactoperoxidase method (22) to a specific activity of approximately 50 \( \mu \)Ci/µg. In brief, 0.5 mCi Na\(^{125}\)I is added to 0.45 nmol bGH dissolved in 10 µl 0.1 mol/l NaHCO\(_3\), Lactoperoxidase (10 µg dissolved in 10 µl of 0.4 mol/l acetate buffer, pH 5.6) and \( \text{H}_2\text{O}_2 \) (5 µl of 1.76 mmol/l) are added. After 1 h the reaction is terminated by the addition of 100 µl transfer buffer (0.47 mol/l sucrose, 0.06 mol/l KI, sodium azide 0.02%, pH 7.6). Radio labelled bGH is then separated by Sephadex G-100 chromatography. Radioligand was only used within four days of iodination for GH BP determination or radioreceptor assay.

Serum GH BP was measured using modification of the method described by Laron et al. (23). Serum (200 µl) with and without unlabelled bGH (0.09 nmol) was incubated with approximately 70 000 counts per minute (cpm) \( ^{125}I \) bGH for 18 h at room temperature. Preliminary validation experiments revealed that equilibrium conditions existed at 18 h (Fig. 1), and that full displacement of \( ^{125}I \) bGH from the GH BP was achieved with the concentration of unlabelled bGH used (Fig. 2). The mixture was separated over a Superose 12 column (FPLC system, \( 1 \times 30 \) cm, Catalog no. 17-0538-01, Pharmacia LKB, Uppsala, Sweden) and eluted with 0.025 mol/l TRIS, 0.01 mol/l CaCl\(_2\) (pH 7.6) at 0.5 ml/min at room temperature, with 0.5 min fractions collected. A typical elution profile is shown in Fig. 2. Four peaks of activity were seen as previously described (23), peak I a low affinity large molecular weight binding protein analogous to that described in human serum (24), peak II the high affinity \( ^{125}I \) bGH-BP complex, peak III unbound \( ^{125}I \) bGH and peak IV free \( ^{125}I \) iodine.

![Fig. 1. The effect of the incubation time on binding of \( ^{125}I \) bGH (42 000 cpm) to pig serum (100 µl) at room temperature. Open circles represent the elution peaks for \( ^{125}I \) bGH, closed circles that for the \( ^{125}I \) bGH-BP complex derived from chromatographic profiles. Equilibrium conditions are present by 18 h.](https://via.bridgebionet.com/10.1002/ajem.5311200103)
Hepatic cut

Hepatic GH levels absence concentrations binding displace excess minus iodine. Fig. 2. A typical Superose 12 elution profile of porcine serum incubated with [125I]bGH in the absence (unbroken line) and presence (broken line) of excess unlabelled bGH. Peak II represents the high affinity [125I]GH BP complex, peak III the unbound [125I]bGH and peak IV free iodine. Peak I corresponds to the lower affinity GH BP as described in human serum and only displaceable by very high concentrations of unlabelled ligand.

Specific binding of serum GH BP was expressed as cpm in peak II (expressed as a percentage of total counts eluted) minus the percent cpm in peak II in the presence of excess unlabelled bGH (representing the non-specific binding).

To assess for the possibility of interference of endogenous GH with GH BP measurement, serum pGH was measured in all animals and the ability of various concentrations of pGH in the incubation mixture to displace [125I]bGH was assessed using serum from one animal with a low serum pGH (0.18 nmol/l). Specific binding of [125I]bGH was reduced significantly only at concentrations of pGH exceeding 22 nmol/l in the incubation mixture. Porcine GH concentrations of 5.5, 11, 22, 44 and 440 nmol/l in the incubation mixture reduced the [125I] specific binding by 0. 2.9, 4.7, 39.2 and 70.9% respectively (compared to maximal binding in the absence of added pGH), consistent with our previous observations on the relative potencies of pGH and bGH binding to porcine hepatic membranes (9).

Serum pGH levels at the time of sacrifice were significantly higher in older animals (p < 0.05). A treatment effect was also evident with lower serum pGH levels in rpGH treated animals (p < 0.05), presumably reflecting negative feedback of administered rpGH on pituitary GH secretion. The serum pGH levels were 0.85 ± 0.20 nmol/l in infant controls, 0.36 ± 0.07 in GH-treated infants, 4.47 ± 1.81 in pubertal controls and 0.79 ± 0.23 in GH-treated pubertal animals, and thus were insufficient to cause appreciable interference with GH BP measurement in any of the groups.

Hepatic GH receptor measurement

Hepatic membrane preparations were produced as previously described (25). Liver tissue was thawed at 4°C, cut into small pieces (approximately 1 g) and washed in cold (4°C) buffer containing 0.025 mol/l TRIS and 0.01 mol/l CaCl2 (pH 7.6). The tissue was then weighed and homogenized (1:2 w/v) in the same buffer with the addition of aprotinin 10⁶ kallikrein inhibitor units (kiU)/l (Trasylol, Bayer Pharmaceuticals, Botany, NSW, Australia). Homogenization was performed at high speed for 4 min (Ultra-Turrax homogeniser, Janke & Kunkel, Staufen, FRG). The flask was kept in an ice bath during all steps. The resulting homogenate was centrifuged at 1000 × g for 60 min at 4°C. The supernatant obtained was incubated with 4 mol/l MgCl₂ (1:3 w/v ratio of initial liver weight) to remove endogenously bound growth hormone (9, 26). The preparation was then centrifuged at 30,000 × g for 90 min at 4°C, and the resulting pellet washed in buffer and centrifuged again at 30,000 × g for 60 min at 4°C. The final pellet was resuspended at a ratio of 1 ml per gram of initial liver weight in the same buffer used for the homogenization and immediately frozen.

Radioreceptor assays were performed in triplicate using 100 µl of membrane preparation per tube (equivalent to 100 mg wet weight of liver tissue). The assay buffer was 0.025 mol/l TRIS, 0.01 mol/l CaCl₂, 0.2% (w/v) bovine serum albumin, 0.02% (w/v) sodium azide and aprotinin 10⁶ kiU/l. The receptor preparations were incubated with various concentrations of unlabelled bGH (0–1000 ng/tube) and approximately 30,000 cpm [125I]bGH in an incubation volume of 0.5 ml for 20 h at room temperature. Equilibrium was achieved under these conditions. Non-specific binding was determined by excess unlabelled ligand (1000 ng/tube). Incubation was terminated by the addition of 3 ml of ice-cold 0.025 mol/l TRIS, 0.01 mol/l CaCl₂ buffer (pH 7.6), and bound and free hormone were separated by centrifugation at 3000 × g for 45 min at 4°C. The inter- and intra-assay cvs were 8.0% and 5.6% respectively.

Protein concentrations of the membrane preparations for individual animals were determined by a modified Lowry method (27) and specific binding and membrane binding capacity corrected to a common protein concentration. The mean protein concentration of the membrane preparations was 317 ± 100 µg per 100 mg initial wet weight of liver.

The ligand binding data were analysed using the LIGAND program (Version 3.0, Biosoft, Cambridge, UK). After Scatchard analysis (28), this uses weighted nonlinear iterative curve fitting procedures (29) and allows statistical comparison of a one- versus two-site model. Final estimates are obtained for membrane receptor capacity and affinity constants.

Data analysis

Data in this study were analysed using 2-way analysis of variance, with subsequent comparisons using Scheffe’s test. [125I]bGH membrane specific binding and binding capacity were also corrected to a common liver to body
weight ratio, since this better reflects the total hepatic growth hormone receptor pool available to influence somatic growth per unit of body weight. Logarithmic transformations were performed to normalize data for $[^{125}I]bGH$ binding to hepatic membranes and serum GH BP measurements. All data are expressed as mean $\pm 1 \times$ SEM.

Results

Growth and serum IGF-I (Table 1)

Older pGH-treated animals showed a tendency to lesser weight gain over the 12 days compared to their control group, presumably reflecting greater fat loss than protein deposition as previously shown with GH treatment (30, 31); however, this did not achieve statistical significance. Porcine GH treatment increased the weight of the liver relative to body weight (p = 0.02), and this ratio also increased with age (p = 0.001).

Age (p < 0.001) and pGH treatment (p = 0.03) related increases were seen in serum IGF-I levels, with no interaction between age and treatment. Covariance analysis suggested that the pGH treatment effect but not the age effect on serum IGF-I could be accounted for by the difference in liver to body weight ratio.

Growth hormone binding protein (Table 2)

A major increase of serum GH BP with age was noted (p < 0.001). pGH treated animals had higher serum GH BP than controls (p = 0.03), with no statistical interaction between age and treatment.

Hepatic growth hormone receptors (Table 2)

The specific binding of $[^{125}I]bGH$ to hepatic membranes was increased by pGH treatment (p < 0.02), with a more marked difference after correction to a common liver to body weight ratio (p < 0.01). Approximately 100% higher specific binding and membrane receptor capacities were seen in the infant pGH-treated pigs compared to controls, with only about 20% increase in the pubertal animals.

Analysis of the hepatic GH receptor competitive binding data revealed linear Scatchard plots in the infant animals. Plots of the binding data from 2 of the 10 pubertal animals had a tendency to curvilinearity, but non-linear curve fitting procedures (LIGAND) indicated statistical superiority of a one-site model. The calculated binding affinity ($K_a$) in the infant animals was 8.9 ± 4.1 and in the pubertal animals 5.5 ± 1.0 l/nmol (p = 0.02). No effect of GH treatment on $K_a$ was evident. These values are similar to the high affinity binding site in our previous studies in the pig (9). Mean values of hepatic membrane GH receptor capacity were higher in pGH treated animals. This was significant when corrected for liver to body weight ratio (p < 0.05), and was again most marked in the infant animals. A major age-related increase in binding capacity of the high affinity GH

Table 1. Body and liver weight data and serum IGF-I are indicated (± SEM) for the four experimental groups (N = 5 per group). The significance of differences between groups is indicated by p values obtained with analysis of variance (ANOVA).

<table>
<thead>
<tr>
<th>Group</th>
<th>Final weight (kg)</th>
<th>Weight gain (kg)</th>
<th>Liver weight (kg)</th>
<th>Liver weight (kg/kg body weight)</th>
<th>Serum IGF-I (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant controls</td>
<td>7.46 ± 0.67</td>
<td>2.76 ± 0.21</td>
<td>0.163 ± 0.013</td>
<td>0.022 ± 0.001</td>
<td>12.0 ± 1.6</td>
</tr>
<tr>
<td>Infant GH treated</td>
<td>7.10 ± 0.58</td>
<td>2.70 ± 0.31</td>
<td>0.174 ± 0.013</td>
<td>0.025 ± 0.001</td>
<td>19.4 ± 4.5</td>
</tr>
<tr>
<td>Pubertal controls</td>
<td>61.12 ± 1.34</td>
<td>15.02 ± 0.53</td>
<td>1.605 ± 0.054</td>
<td>0.26 ± 0.001</td>
<td>44.2 ± 4.9</td>
</tr>
<tr>
<td>Pubertal GH-treated</td>
<td>58.36 ± 0.89</td>
<td>10.76 ± 2.82</td>
<td>1.693 ± 0.027</td>
<td>0.029 ± 0.001</td>
<td>57.8 ± 5.8</td>
</tr>
<tr>
<td>Anova treatment effect</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p = 0.02</td>
<td>p = 0.03</td>
</tr>
<tr>
<td>Anova age effect</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p = 0.02</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Table 2. Hepatic growth hormone receptor and serum-binding protein data (± SEM) are shown for the four experimental groups (N = 5 per group). $[^{125}I]bGH$ specific binding and binding capacity (calculated from Scatchard analysis) are presented (1) corrected for membrane protein concentration (expressed per 300 μg membrane protein) and (2) corrected for membrane protein concentration and liver/body weight ratio. The significance of differences between groups is indicated by p values obtained with analysis of variance (ANOVA).

<table>
<thead>
<tr>
<th>Group</th>
<th>$[^{125}I]bGH$ specific binding (pM)$^1$</th>
<th>$[^{125}I]bGH$ specific binding (pM)$^2$</th>
<th>Affinity constant ($K_a$, l/nmol)$^1$</th>
<th>Binding capacity (pmol/l)$^1$</th>
<th>Binding capacity (pmol/l)$^2$</th>
<th>Serum GH BP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant controls</td>
<td>6.3 ± 0.6</td>
<td>4.5 ± 0.4</td>
<td>10.2 ± 2.5</td>
<td>9.9 ± 2.1</td>
<td>7.0 ± 1.6</td>
<td>13.8 ± 1.2</td>
</tr>
<tr>
<td>Infant GH treated</td>
<td>11.4 ± 1.8</td>
<td>9.1 ± 1.7</td>
<td>7.8 ± 0.5</td>
<td>19.3 ± 4.5</td>
<td>15.4 ± 3.6</td>
<td>17.8 ± 2.0</td>
</tr>
<tr>
<td>Pubertal controls</td>
<td>22.1 ± 1.2</td>
<td>18.8 ± 1.3</td>
<td>5.5 ± 0.6</td>
<td>63.2 ± 7.9</td>
<td>53.7 ± 7.1</td>
<td>35.2 ± 2.6</td>
</tr>
<tr>
<td>Pubertal GH-treated</td>
<td>25.5 ± 3.4</td>
<td>24.0 ± 1.5</td>
<td>5.5 ± 0.3</td>
<td>69.1 ± 11.5</td>
<td>65.1 ± 11.8</td>
<td>41.8 ± 3.4</td>
</tr>
<tr>
<td>Anova treatment effect</td>
<td>p &lt; 0.02</td>
<td>p = 0.006</td>
<td>NS</td>
<td>p = 0.1</td>
<td>p &lt; 0.05</td>
<td>p = 0.03</td>
</tr>
<tr>
<td>Anova age effect</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p = 0.02</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>
The speculation

Discussion

Interrelationships (Table 3, Fig. 3)

Table 3. Correlation coefficients between GH receptor specific binding and membrane capacity, serum GH BP and serum IGF-I are shown for all animals (All) and for the two age groups considered separately (Infants = 19–20-day pigs, Pubertal = 107–112-day-old pigs. N = 5 per group.

<table>
<thead>
<tr>
<th>Specified binding (%)</th>
<th>GH BP (%)</th>
<th>Serum IGF-I (nmol/l)</th>
<th>Membrane capacity (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0.84***</td>
<td>0.91***</td>
<td>0.98***</td>
</tr>
<tr>
<td>Infant</td>
<td>0.78**</td>
<td>0.73</td>
<td>0.92***</td>
</tr>
<tr>
<td>Pubertal</td>
<td>0.26</td>
<td>0.76*</td>
<td>0.95***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Membrane capacity (pmol/l)</th>
<th>All</th>
<th>Infant</th>
<th>Pubertal</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0.82***</td>
<td>0.83**</td>
<td>0.13</td>
</tr>
<tr>
<td>Infant</td>
<td>0.91**</td>
<td>0.82**</td>
<td>0.64*</td>
</tr>
<tr>
<td>Pubertal</td>
<td>0.13</td>
<td>0.64*</td>
<td></td>
</tr>
</tbody>
</table>

receptor was observed (p<0.001), confirming previous observations (9).

In both infant and pubertal animals [125I]BG hepatic membrane specific binding correlated highly with hepatic membrane GH receptor capacity as determined by Scatchard analysis (infants r = 0.92, p < 0.001; pubertal r = 0.95, p < 0.001; all animals r = 0.98, p < 0.001).

The relationship observed between serum GH BP and hepatic [125I]BG specific binding (r = 0.84, p < 0.001) or capacity (r = 0.82, p < 0.001) was strongest in the infant animals but was not significant in the pubertal animals (Fig. 3, Table 3). An age effect was seen in the ratio of serum GH BP to hepatic [125I]BG binding, this ratio being higher in the infant animals (p = 0.03), but no effect of pGH treatment on this ratio was evident.

Serum GH BP correlated significantly with serum IGF-I both in the overall study group (r = 0.93, p < 0.001) and in each age group considered separately (infant r = 0.89, p < 0.001; pubertal r = 0.65, p = 0.04) (Fig. 3, Table 3). Serum IGF-I also correlated significantly with membrane capacity (r = 0.91, p < 0.001; infant r = 0.82, p = 0.004; pubertal r = 0.64, p < 0.05) or membrane-specific binding (r = 0.91, p < 0.001; infant r = 0.73, p < 0.05; pubertal r = 0.76, p < 0.05) (Fig. 3, Table 3).

Discussion

The relationship of the serum GH BP to the hepatic membrane bound GH receptor is of considerable interest. The structural identity of the serum-binding protein to the extracellular hormone-binding domain of the membrane bound receptor (15) has led to investigation and speculation on possible mechanisms for its production. Proteolytic cleavage of the GH receptor near the transmembrane domain and release of the extramembranous portion into the circulation is one possible mechanism (12). Separate synthesis and secretion of the BP has also been suggested and could be explained by alternative mRNA splicing mechanisms for the BP and receptor. In the pregnant mouse (32) and the rat (33) two GH receptor related mRNA transcripts are described, one coding for the full length receptor, the other for a truncated receptor with a hydrophilic tail in place of the transmembrane domain.

Part of the interest in the relationship of the GH BP to the membrane bound GH receptor lies in the potential usefulness of having a serum-based test that could reflect tissue GH receptor status. One extreme example of co-regulation of the GH BP and hepatic GH receptor is in the Laron dwarf, where both are absent or non-functional (34, 35); however, this gives no information about normal physiological relationships. In the pregnant mouse, GH BP and liver receptors change in parallel, but not identically, during the course of gestation, and following hypophysectomy or GH administration (18, 36). Bick et al. (17) investigated the relationship between the hepatic receptor and GH BP in the hypophysectomized male rat treated with hGH and found a positive correlation, yet only 25% of the variance in the serum GH BP was accounted for by the total hepatic somatogenic binding. Our study demonstrates a very close relationship between levels of GH BP and the hepatic GH receptor when all animals are considered in the analysis and in the infant animals considered alone, but in the pubertal animals alone the correlation is not significant. The non-significant tendency to curvilinearity of Scatchard plots could contribute to a minor degree of uncertainty of estimation of hepatic growth hormone receptor capacity. Nonetheless, these observations suggest that the membrane bound hepatic GH receptor and GH BP do not vary in unity, and that they may be subject to individual regulation. We therefore suggest that measurement of serum GH BP does not always accurately represent differences in hepatic GH receptor status. Although major changes are generally reflected, some important variation in the number of hepatic GH receptors within the expected physiological range may not be detected by GH BP measurement alone.

Further, Carlsson et al. (19) have recently presented data suggesting that in rats the ratio of GH receptor...
mRNA to GH BP mRNA varies between tissues, supporting the concept of separate regulation. Therefore GH BP may have a functional role in the local regulation of GH action. If, as seems possible, separate regulation can also occur in the same tissue or if a considerable proportion of the serum GH BP is derived from extrahepatic sources, then variations in the ratio of serum binding protein to hepatic receptor are to be expected. It has been demonstrated that GH complexed to GH BP has a 10-fold lower metabolic clearance than free GH (37) and that the high affinity GH BP competes for GH binding with a similar affinity to the membrane bound receptor (13). The physiological variation of the GH BP may represent another significant control mechanism of somatic growth by regulating the availability of GH to the receptor, instead of merely reflecting changes in tissue GH receptors.

There is considerable evidence that the GH receptor can be actively regulated by GH, although data in young animals is scarce. In growing castrate lambs (age six months) we have demonstrated that GH treatment for nine weeks is associated with a major increase in the capacity of the high affinity GH receptor (1). Pigs treated with pGH for five weeks have also been shown to have a significant increase in hepatic growth hormone binding (38). Studies in hypophysectomized rabbits, sheep and rats (2, 3) have demonstrated reduced hepatic GH binding which is partially restored by GH administration. Ontogenic studies indicate that while the fetus has high circulating levels of serum GH, hepatic GH receptor capacity is low in the fetal or early neonatal animal (39, 40), suggesting that at this stage of development GH cannot induce the hepatic GH receptor. Our previous work on the ontogeny of the GH receptor in the pig demonstrates that a major ontologic increase in hepatic GH receptors is not seen at least until after 35 days of age (9). The current study demonstrates that the infant pig, despite showing a developmentally low population of hepatic GH receptors, exhibits a significant biological response to pGH administration and that the receptor can be induced by GH administration in this infantile period. This is reflected functionally by increases in the liver to body weight ratio, serum IGF-I and GH BP concentrations.

There has been little investigation of the biological or biochemical response of neonates or young animals to exogenous GH and no other studies have evaluated the effect of GH on the GH receptor at this age. Only indirect evidence exists in growth data on GH hypersecreting transgenic animals. An increased growth rate in transgenic mice is not evident until three weeks after birth (41). In limited studies in transgenic pigs, no differences in growth rate were evident in piglets up to 20 kg live weight (42). While the current study indicates that the hepatic GH receptor and IGF-I production can be induced by GH treatment at an early age, it is possible that other developmental factors impede growth responsiveness at this age. Further evaluation of this response may be relevant as wider indications for the therapeutic use of GH are considered, including short-stature syndromes identifiable from birth and intrauterine growth retardation in humans.

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