Assessment of Factors Regulating Serum Growth Hormone Binding Protein in Pigs

T. M. Mullins and S. L. Davis

Department of Animal Sciences, Oregon State University, Corvallis 97330

ABSTRACT: These studies were conducted to examine the influence of several variables on the growth hormone binding protein (GHBP) activity in serum of pigs. Continuous long-term porcine somatotropin (pST) injections (daily for 6 to 7 wk) increased GHBP activity (P < .05). However, periodic short-term pST injections (daily, every 2nd d, or every 4th d for 2 wk) did not cause a significant change in GHBP levels (P > .40). Although fasting seems to reduce liver GH receptors, no difference was observed between fed animals and animals fasted for 5 d (P > .30). Between 0 and 6 mo of age, boar and gilt serum GHBP activity were not significantly different from each other but increased with age in both sexes (P < .0001). There was no significant correlation between serum GHBP and BW at 6 mo of age in this study (P > .30). In pregnant sows, GHBP concentrations were highest at the beginning (d 72) of the third trimester (P < .05). Growth hormone receptor activity reported by other researchers and GHBP activity in this study seem to vary similarly except during fasting, which may indicate alternate regulation of either the GHBP or the GH receptor.

Key Words: Pigs, Somatotropin, Binding Proteins, Fasting, Growth, Pregnancy

Introduction

A soluble serum growth hormone binding protein (GHBP) has been reported from several species (Baumann, 1991) including sheep, pig, and chicken (Davis et al., 1992). The amino acid sequence of the GHBP seems to be identical to that of the extracellular domain of the GH receptor (Spencer et al., 1988).

Recent studies have suggested that the serum GHBP is influenced by physiologic and metabolic status. For example, the amount of GHBP activity is reportedly influenced by level of nutrition (Hizuka et al., 1990), sex (Bick et al., 1990; Heinze et al., 1990; Hochberg et al., 1990; Sanchez-Jimenez et al., 1990), pregnancy (Amber et al., 1990; Hizuka et al., 1990; Sanchez-Jimenez et al., 1990), and age (Daughaday et al, 1987; Silbergeld et al., 1989; Ambler et al., 1990; Amit et al., 1990). Furthermore, these reported changes in GHBP activity seem to be positively correlated with changes in liver cell growth hormone (CH) receptors (Bick et al., 1990; Hochberg et al., 1990; Massa et al., 1990), suggesting that serum GHBP may be functionally related to the interaction of GH with its liver cell receptors.

Although considerable work has been published on the relative GHBP activity in humans and laboratory animals, little is known about factors affecting the GHBP in serum of domestic animals. Davis et al. (1992) have reported the partial purification of GHBP from the serum of chickens, pigs, and sheep, as well as a significant increase in GHBP activity associated with age in sheep. The purpose of the present studies was to examine the effect of several physiological and nutritional variables on the amount of GHBP activity in the serum of pigs.

Materials and Methods

Continuous Long-Term Porcine Somatotropin Treatment. Fourteen crossbred barrows with an average weight of 50 kg were randomly assigned to control and treated groups. Animals were housed four to a
pen and allowed ad libitum access to food and water. Experimental animals were given intramuscular injections of 60 μg·kg⁻¹·d⁻¹ of recombinant porcine somatotropin (rpST) (provided by Pitman-Moore, Terre Haute, IN) for 7 wk. Control animals were similarly injected with the buffer used to dilute the pST. Blood was collected at slaughter on the last day of treatment. Serum kindly provided by C. Y. Hu and R. Wander, Oregon State Univ., Corvallis) was collected and frozen until it was assayed for GHBP using a dextran-charcoal assay described by Davis et al. (1992). Results were analyzed with a one-tailed t-test (Statgraphics, 1991).

Periodic Short-Term Porcine Somatotropin Treatment. Fifteen crossbred barrows between 5 and 8 mo of age were penned individually and limited to 85% of normal ad libitum consumption. Animals were randomly assigned to one of four experimental groups. Four pigs in Group 1 served as controls. Three pigs in Group 2 received daily i.m. injections of rpST (provided by Pitman-Moore) 60 μg/kg. Four pigs in Group 3 received injections of 120 μg/kg of rpST every 2nd d, and four pigs in Group 4 received injections of 240 μg/kg of rpST every 4th d. Injections were given for 2 wk. Blood was collected from the vena cava on the last day of treatment and centrifuged to collect serum. Serum (kindly provided by C. Evock and N. Steel, USDA-ARS, Beltsville, MD) was frozen until it was assayed for GHBP (Davis et al., 1992). Results were analyzed by one-way ANOVA (Statgraphics, 1991).

Fasting. Twenty Landrace × Yorkshire pigs weighing 80 to 110 kg were assigned randomly to control and treatment groups. Each group consisted of five gilts and five barrows and each group was housed in a separate pen. All animals had ad libitum access to feed and water for 10 d before the experiment. On d 0 feed was removed from the experimental group but access to water was continued. Blood samples were drawn from each pig via the ear veins on d 0, 1, 3, and 5. Immediately after the final sample was obtained, feed was returned to the experimental group. Serum was collected by centrifugation of blood and frozen until it was assayed. Results were analyzed by split-plot repeated measures ANOVA with day, treatment, and day × treatment as the main effects. Log transformation allowed the univariate hypothesis of equal variation to be accepted (SAS, 1988).

Age and Sex. Three gilts and three boars (1.5 to 2.5 kg) were assigned randomly from each of five litters of Landrace × Yorkshire pigs, for a total of 30 pigs. Pigs stayed in their litters until weaning and were then allotted randomly by sex to six pens, three pens of five gilts and three pens of five boars. One milliliter of blood was collected at 5 d of age from each pig by tail clipping. At 1, 3, and 6 mo, 5 mL of blood was collected from the jugular vein. Animals were allowed ad libitum access to feed and water. Collected serum was frozen until it was assayed. Specific binding of GH in sera from males, and females was analyzed by split-plot repeated measures ANOVA with age, sex, and age × sex as the main effects. A transformation was not required for the univariate hypothesis to be acceptable (SAS, 1988).

Weight. Twenty randomly allotted Landrace × Yorkshire gilts and boars at 6 mo of age between 72 and 125 kg were weighed, and 5 mL of blood was drawn from the jugular vein. Serum was frozen until it was assayed. Simple linear regression was performed with weight dependent on the specific binding of GH (Statgraphics, 1991).

Gestation. Blood was collected from the ear veins of Landrace × Yorkshire sows on d 0, 36, 72 and 112 of gestation. Sera were frozen until they were assayed for GHBP. Ten samples were chosen randomly for each of the four time periods (kindly provided by W. Kwansa, PIC Inc., Franklin, KY). Results were analyzed by ANOVA. Log transformation was required to reduce unequal variability (Statgraphics, 1991).

Assay. Human (h) GH (NIDDK-hGH-1-1, 2.2 IU/mg) was radioiodinated (¹²⁵I; Amersham, Arlington Heights, IL) using a modification of the chloramine-T method originally described by Hunter and Greenwood (1982). We used hGH as a radioligand because it binds to porcine GHBP with higher affinity than does porcine GH (Davis et al., 1992). Specific activity ranged from 85 to 180 μCi/μg. To eliminate the possible influence of specific activity on binding activity, sera from any given experiment were assayed with the same preparation of [¹²⁵I]hGH. The [¹²⁵I]hGH was purified twice on a Biogel P100 (Biorad, Hercules, CA) column (1 cm × 50 cm). The assay described by Amit et al. (1990) was modified as follows: total-binding tubes for each sample were prepared by incubating them for 2 h at room temperature with 50 μL of serum, 350 μL of PBS (14 M NaCl, .01 M NaPO₄, pH 7.0), and 100 μL of [¹²⁵I]hGH containing 20,000 cpm. Then 500 μL of ice-cold dextran-coated charcoal (2% activated charcoal and .2% dextran in PBS. Sigma Chemical, St. Louis, MO) was added and vigorously vortexed for 1 s. This mixture was then centrifuged at 1,700 × g for 20 min at 4°C. The supernatant was counted in a Beckman gamma counter (Model 5500, Beckman Instruments, Palo Alto, CA). Non-specific binding (NSB) was determined similarly except that 1 μg of unlabeled hGH was added to the incubation mixture. Specifically bound hormone was calculated as total binding minus NSB. The standard measurement of GHBP in samples was recorded as percentage specifically bound (%SB), determined as counts per minute of [¹²⁵I]hGH specifically bound divided by counts per minute of
Table 1. Effects of continuous long-term porcine somatotropin treatment on growth hormone binding protein activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Daily rpST&lt;sup&gt;a&lt;/sup&gt; dose, per kg BW</th>
<th>%SB&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>10.8 ± 0.5</td>
</tr>
<tr>
<td>Experimental</td>
<td>7</td>
<td>12.3 ± 0.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>rpST = recombinant porcine somatotropin.
<sup>b</sup>Percentage specifically bound, values represent mean ± SE.
<sup>c</sup>Significantly different from control (P < .05).

Total [<sup>125</sup>I]GH added. Nonspecific binding ranged from 800 to 6,000 cpm. Specific binding ranged from 1,000 to 3,000 cpm. Intraassay and interassay CV were 8 to 15%. A standard pig serum pool was included in all assays as an internal check on the system.

Results and Discussion

Continuous Long-Term Porcine Somatotropin Treatment. Animals treated with rpST had a significantly higher (P < .05) %SB than did the control animals (Table 1). This response confirms the previous report that GH treatment increased GHBP activity in pigs (Ambler et al., 1990) and coincides with a reported increase in pig liver GH receptors (Chung and Etherton, 1988) and rat skeletal muscle GH receptors (Zanelli et al., 1989) under long-term GH treatment. The growth-stimulating effects of exogenous GH treatment may be due, in part, to increased cellular stimulation associated with increased receptor numbers. If the serum GHBP is indeed derived from proteolytic cleavage of the cell membrane GH receptor (Spencer et al., 1988), then up-regulation of the receptors may in turn yield increased GHBP activity. Exogenous GH increases systemic levels of GH but decreases GH secretion (Abe et al., 1983).

Periodic Short-Term Porcine Somatotropin Treatment. Treated groups were not different (P = .43) from each other or from the control group (Table 2), even though treatments did increase growth rate and longissimus muscle area (Evock and Steele, 1991). Ambler et al. (1990) reported an increase in GH liver receptors and GHBP for pigs treated with rpST for 12 d at 3 mo of age. The lack of short-term response in this study may have resulted from age differences between these pigs and those of Ambler et al. (1990) or from low sample numbers. Standard errors were > 10% of the mean. The effect of periodic GH treatment on receptor levels has not been reported.

Fasting. No difference (P > .30) in GHBP activity was observed between fed and fasted pigs. Percentage specifically bound on each sampling day showed no difference (P > .31) between control and experimental groups (Figure 1). Machlin et al. (1968) and Buonomo and Baile (1991) reported that fasting caused an increase in serum GH levels in pigs after 48 h, whereas fasted rats had a decrease in liver GH receptor levels (Baxter et al., 1981) and decreased serum insulin-like growth factor-I (Clemmons and Underwood, 1991). In the current study it seems the fasted pigs either did not experience a decrease in GH receptor levels or the GHBP did not reflect that decrease.

Figure 1. Serum growth hormone binding protein activity (percentage of specific binding of [<sup>125</sup>I]hGH) in pigs fed daily (○) and pigs fasted for 5 d (△). Bars indicate SE.

Table 2. Effects of periodic short-term porcine somatotropin treatment on growth hormone binding protein (GHBP) activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose, μg/kg</th>
<th>%SB&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>6.9 ± .7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Daily</td>
<td>3</td>
<td>7.8 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Every 2nd d</td>
<td>4</td>
<td>8.3 ± .9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Every 4th d</td>
<td>4</td>
<td>7.9 ± .7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percentage specifically bound.
<sup>b</sup>Means ± SE with similar superscripts do not differ (P > .40).
Age and Sex. Newborn pigs had an average %SB of 6.2, which increased (P < .0001) with age to an average of 10.3 at 6 mo of age (Figure 2). Binding observed in males and females was not different (P > .05) at any age. Klindt and Stone (1984) and Scanes et al. (1987) reported a decrease in serum GH concentration in pigs during the first 2 mo of life. Dubreuil et al. (1987) reported a further decrease between 7 and 23 wk of age with no difference between male and female pigs. A difference in GHBP activity was reported between male and female rats (Massa et al., 1990). However, no difference was reported between male and female humans (Baumann et al., 1989; Silbergeld et al., 1989). Both groups reported an increase in GHBP activity with age in humans. Similarly, Ambler et al. (1990) observed higher concentrations of serum GHBP in pigs older than 100 d than in 20-d-old pigs.

Weight. There was no significant (P = .34) regression of BW on percentage of specific binding for either boars or gilts at 6 mo of age. Decreases in serum GH concentrations of gilts and barrows were reported to be more closely related to weight than to age (Siers and Swiger, 1971). In our study, GHBP levels did not seem to be related to individual animal BW.

Gestation. Serum of pregnant sows displayed highly variable binding (SE > 10% of the mean). Log transformation of the data was required to remove a funnel-shaped pattern (indicative of unequal variance) from the residuals. Samples from sows collected on d 72 of gestation displayed significantly (P < .05) higher binding activity than samples collected on other days of gestation (Figure 3). Increases in GHBP levels have also been noted for mice during mid-pregnancy (Smith and Talamantes, 1988) and in humans during the first trimester (Maheshwari and Norman, 1990). Mathews et al. (1989) reported an increase in GH liver receptors of pregnant rats. No increase in GH levels was reported for pregnant sows under normal feeding conditions (Antinmo et al., 1976).

In conclusion, we have demonstrated that the serum GHBP activity in pigs is influenced by a number of factors. Furthermore, it would seem that these changes were in general accord with the expected changes in liver GH receptor activity.

Implications

Pig growth hormone binding protein seems to be regulated by factors that similarly regulate the GH receptor activity, suggesting a common origin of these proteins. This lends further support to a previous suggestion that measurement of growth hormone binding protein concentrations may be a noninvasive way to assess growth hormone receptor activity under normal growing conditions.

Figure 2. Serum growth hormone binding protein activity in male (●) and female (Δ) pigs from birth through 6 mo of age. Bars indicate SE.

Figure 3. Serum growth hormone binding protein activity throughout gestation in the sow. Bars indicate SE.
Literature Cited


