EFFECTS OF PORCINE GROWTH HORMONE ON GLUCOSE METABOLISM OF PIGS: I. ACUTE AND CHRONIC EFFECTS ON PLASMA GLUCOSE AND INSULIN STATUS

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ABSTRACT

The acute and chronic effects of porcine growth hormone (pGH) administration on glucose homeostasis of pigs were investigated in the present study. Twelve Yorkshire barrows (average BW = 65 kg) fitted with femoral artery catheters were allotted to three groups. Pigs received acute, intra-arterial injections of either pituitary pGH, a recombinantly derived pGH analog (ppGH or rpGH, 100 μg/kg BW) or saline. Acute injection of pGH did not affect fasting plasma glucose or insulin status. Pigs then were treated daily by i.m. injection for 24 d with 70 μg ppGH/kg BW. Serum glucose and insulin concentrations during the fed and fasted states were higher in pGH-treated than in control pigs. On d 25, an acute intra-arterial injection of ppGH (100 μg/kg BW) elicited increases in plasma glucose and insulin in pigs chronically treated with pGH. The area circumscribed by the glucose and insulin response curves 5 min to 7 h postinjection was 40% (P < .005) and 177% (P < .001), respectively, higher in ppGH-treated than in control pigs. These data indicate that pGH does increase plasma glucose and insulin in the fed and fasted states; however, this response is only observed after chronic pGH administration. In addition, pGH is capable of increasing plasma glucose and insulin acutely in the pig. This effect, however, only is observed in pigs treated chronically with pGH. The mechanisms by which pGH elicit these effects on glucose homeostasis are not known.

(Key Words: Growth Hormone, Pigs, Insulin, Glucose.)


Introduction

Exogenous administration of porcine growth hormone (pGH) increases growth rate and markedly changes carcass composition of pigs (Machlin, 1972; Chung et al., 1985; Etherton et al., 1986, 1987; Evock et al., 1988). These changes include an increase in carcass protein and a decrease in carcass lipid. The mechanisms by which pGH alters growth and metabolism of pigs, however, are not clear. We have found that pGH antagonizes insulin action and causes insulin resistance in adipose tissue (Walton and Etherton, 1986; Walton et al., 1987). These effects appear to be intrinsic properties of the pGH molecule because recombinately derived pGH (rpGH) mimics the effects of pituitary pGH (ppGH) (Walton et al., 1987; Evock et al., 1988). Furthermore, we have found that serum glucose and insulin concentrations are increased in pGH-treated pigs (Chung et al., 1985; Etherton et al., 1986, 1987; Evock et al., 1988). None of the previous studies has established the temporal nature of the changes in plasma glucose and insulin that occur during the time immediately after injection of pGH.
Hence, we conducted experiments to determine the effects of acutely administering pGH on plasma glucose and insulin response in pigs that had been treated either acutely or chronically with pGH.

Materials and Methods
Yorkshire barrows were fitted with femoral artery catheters (Wangsness et al., 1977) and maintained as described (Etherton et al., 1986). Pigs were housed in individual pens with access to water at all times. All pigs were fed ad libitum a corn-soybean meal ration formulated to contain 18% crude protein (Etherton et al., 1986). Dietary protein content was increased above NRC (1979) recommendations and lysine was added (.5%) to ensure adequate amino acid availability if pGH decreased feed intake.

Experiment 1: Acute Effect of pGH. The acute effect of pGH on plasma glucose and insulin concentration was examined in barrows weighing approximately 60 kg (50 to 75 kg). Barrows were randomly assigned (n = 4 per treatment) to three groups and received saline, ppGH4 (100 µg/kg BW) or rpGH5 (100 µg/kg BW) infusions. Pigs were fasted for 12 to 14 h and injected intra-arterially with saline or the pGH solutions. The pGH was dissolved in sterile bicarbonate buffer as described by Chung et al. (1985) and injected in a total volume of 5.0 ml. The catheter was flushed immediately with 5 ml of saline. Blood samples (5 to 10 ml) were obtained at --60, -30 and 0 min before and at 15, 30, 45 and 60 min and at hourly intervals for up to 7 h following saline or pGH injections. The catheters were filled with .04 M sodium citrate between sampling periods. Blood samples were collected in tubes containing heparin as an anticoagulant and were centrifuged immediately to obtain plasma, which was stored frozen at -20°C until analysis.

Experiment 2: Chronic Effect of pGH. The effect of chronic administration of pGH on glucose and insulin status was investigated in barrows (65 kg) fitted with femoral artery catheters. Eight barrows were randomly assigned to two groups, control and pGH (ppGH, 70 µg/kg BW), with four pigs per treatment. Pituitary pGH was dissolved in bicarbonate buffer and injected i.m. daily for 24 d between 1000 and 1200 (Chung et al., 1985; Etherton et al., 1987). The control pigs received an equal volume of bicarbonate buffer. Blood samples were collected in tubes containing heparin from the femoral artery catheters 3 to 6 h following pGH administration on different days of the experiment. It should be noted that for this experiment pigs were not fasted. Plasma samples were stored at -20°C until assays were conducted.

Experiment 3: Acute Effect of pGH in Pigs Chronically Exposed to pGH. In Exp. 1 we observed that acute injection of pGH (ppGH and rpGH) did not increase plasma glucose or insulin concentrations. Because of this, we wanted to establish whether an acute intra-arterial injection of pGH to pigs treated for 24 d with pGH would alter plasma glucose and insulin concentrations. The same group of pigs used in Exp. 2 was used. Pigs were treated with pGH for 24 d by daily i.m. injections (70 µg/kg BW). Control pigs were injected with the vehicle. On d 24 of the experiment, pigs were fasted overnight for 12 to 14 h. The following morning, pigs treated with pGH were given an intra-arterial injection of ppGH (100 µg/kg BW) through the femoral catheter. Control pigs received an equal volume (5 ml) of sterile bicarbonate buffer. Blood samples were obtained and processed as described in Exp. 1 and the plasma was stored frozen at -20°C.

Plasma insulin and glucose concentrations were determined as described previously (Chung et al., 1985; Etherton et al., 1986, 1987). Statistical analysis of glucose and insulin data from different periods was carried out using a split-plot design with repeated measures (SAS, 1979). The area under the glucose and insulin response curves was calculated by Simpson’s rule of integration using an IBM personal computer; the treatment differences were tested by Student’s t-test (Ryan et al., 1976).

Results

Experiment 1: Acute Effect. Acute injection of either ppGH or rpGH did not affect fasting plasma glucose or insulin concentrations (Fig-
Figure 1. Acute effects of pGH on fasting plasma glucose and insulin in pigs (Exp. 1). Pigs were fasted for 12 to 14 h and pituitary pGH (ppGH), recombinately derived pGH (rpGH) (100 μg/kg) or saline were injected (time indicated by the arrow) through a femoral artery catheter. Each point represents the mean ± standard error (vertical bar) of values obtained from four pigs. There were no differences observed between ppGH and rpGH with respect to their effects on plasma insulin concentration (data not shown).

The areas under the glucose response curves from 5 min to 7 h postinjection were: saline = 40,416 ± 4,357; ppGH = 43,065 ± 3,648; rpGH = 40,419 ± 4,103 mg·min⁻¹·100 ml⁻¹. Similarly, there were no differences in the insulin response curves (Figure 1) 5 min to 7 h postinfusion of saline or pGH (the effects of ppGH did not differ from rpGH, data not shown).

Experiment 2: Chronic Effect. Chronic administration of pGH markedly increased plasma glucose and insulin concentrations (Table 1). Glucose concentration of pGH-treated pigs was 10 to 60% higher than that of control pigs at the various time periods studied. The pGH-dependent difference in glucose concentration became greater as the experiment progressed (10% on d 6 vs 60% increase on d 21). However, this was due to a decrease in plasma glucose in control pigs over the sampling periods rather than to any additional increase in pGH-treated pigs. In contrast, plasma insulin increased over the sampling period in response to pGH treatment. Treatment of pigs with pGH resulted in a 25 to 330% increase in insulin concentrations. The greatest increase in plasma insulin concentration was observed on d 21, when plasma
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TABLE 1. EFFECT OF DAILY ADMINISTRATION OF pGH ON SERUM GLUCOSE AND INSULIN CONCENTRATIONS IN PIGS (EXP. 2)*

<table>
<thead>
<tr>
<th>Day</th>
<th>Glucose, mg/100 ml</th>
<th>Insulin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>pGH</td>
</tr>
<tr>
<td>6</td>
<td>119</td>
<td>133</td>
</tr>
<tr>
<td>8</td>
<td>127</td>
<td>142</td>
</tr>
<tr>
<td>10</td>
<td>129</td>
<td>141</td>
</tr>
<tr>
<td>15</td>
<td>83</td>
<td>122</td>
</tr>
<tr>
<td>21</td>
<td>90</td>
<td>145</td>
</tr>
</tbody>
</table>

*Pigs received daily i.m. injections of pituitary pGH (ppGH; 70 µg/kg) or vehicle. Blood samples were collected 3 to 6 h following pGH injections on the day noted, n = 4.

**NS = not significant.

glucose concentration peaked. The concurrent increase in plasma glucose and insulin concentration suggests that insulin sensitivity is impaired in vivo by pGH.

Because we did not observe an acute effect of pGH on plasma glucose and insulin, at least with the dose of pGH used, we next determined whether an acute injection of pGH would affect plasma insulin and glucose status in pigs that have been treated with pGH for 24 d. As depicted in Figure 2, chronic administration of ppGH was associated with a marked fasting hyperglycemia and hyperinsulinemia (indicated by difference in basal values of control and pGH-treated pigs before hormone infusion in Figure 2). Furthermore, an acute injection of ppGH to pigs that had been treated previously with ppGH for 24 d significantly elevated plasma glucose and insulin concentrations. There was a 2- to 3-h lag before glucose and insulin concentrations increased (Figure 2). The areas under the glucose and insulin response curves (5 min to 7 h postinjection) were 40% (P < .01) and 177% (P < .001) higher in ppGH-treated pigs that had received an acute injection of ppGH than in control pigs that had received saline (Table 2). The maximum glucose and insulin response to pGH infusion in ppGH-treated pigs occurred 4 to 7 h postinjection. In contrast, insulin and glucose concentrations were unaffected in control pigs during the sampling period. Administration of saline rather than of ppGH to ppGH-treated pigs had no effect on glucose or insulin concentrations for up to 7 h (data not shown).

Discussion

Growth hormone elicits profound effects on carbohydrate metabolism of both man and experimental animals (Bratusch-Marrain et al., 1984; Davidson, 1987; Sherwin et al., 1983). Numerous studies have addressed the acute or chronic effects of GH on glucose metabolism in man (Rosenfeld et al., 1982), in dogs (Altszuler et al., 1968; Vaitkus et al., 1984) and in sheep (Manns and Boda, 1965; Hart et al., 1984). These studies have established the diabetogenic effects of GH.

Although we have found that chronic administration of pGH elevates serum glucose and insulin concentrations (Chung et al., 1985; Etherton et al., 1986, 1987; Evock et al., 1988), none of these studies addressed the acute and chronic effects of pGH on glucose homeostasis. In the present study it was evident that pGH did not elicit an effect on fasting plasma glucose and insulin status of
pigs that had not been treated chronically with pGH. These observations are in accord with the results of Manns and Boda (1965), who reported that ovine GH, injected at a dose sufficient to produce anabolic effects, was devoid of an acute effect on fasting plasma glucose or insulin concentrations in sheep. Our finding that pGH did not have an acute effect in pigs not treated with pGH contradicts the findings of Hart et al. (1984) who found that an acute injection of pituitary or recombinant bovine GH increased fasting plasma glucose and free fatty acid concentrations in sheep. Similar acute effects of GH on glucose turnover in dogs (Altszuler et al., 1987; Vaitkus et al., 1984) and on glucose concentration in sheep have been reported (Bassett and Wallace, 1966). It is possible that GH does not have any acute effect on glucose homeostasis and that the acute effects observed in some of the earlier reports could be due to contaminations in the GH preparations or the chemical and(or) conformational artifacts introduced by the solubilization procedure used (see review by Bauman and McCutcheon, 1986).

Although pGH did not acutely elevate glucose and insulin concentrations, chronic administration of pGH significantly elevated

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Figure 2. Acute effect of pGH on fasting plasma glucose and insulin response in pigs that had been treated with pGH. Pigs received i.m. injections of pituitary pGH (ppGH; 100 μg·kg⁻¹·d⁻¹) or vehicle for 24 d. On d 25, pGH-treated pigs received an injection (time indicated by the arrow) of ppGH (100 μg/kg); the control pigs received an equal volume of saline. Each point represents the mean ± standard error (vertical bars) of values obtained from four pigs.
both plasma glucose and insulin concentrations in both the fed and fasted states. This latter finding agrees with previous observations from our laboratory and others (Chung et al., 1985; Etherton et al., 1986, 1987; Wray-Cahen et al., 1987; Evock et al., 1988). Kostyo et al. (1984) found that chronic, rather than acute, treatment was necessary in order to observe changes in fasting glucose concentration and glucose tolerance in obese mice. Other evidence to suggest that chronic elevations of plasma GH affect glucose metabolism are the well established increases in fasting glucose, insulin and insulin resistance observed in acromegalic subjects and in man and rodents treated with GH (Beck et al., 1965; Fineberg and Merimee, 1974; Kostyo et al., 1984; Hansen et al., 1986). The findings presented in the current study do not show directly that insulin action in vivo is impaired; however, we have carried out insulin tolerance tests in pigs chronically treated with pGH and found that insulin sensitivity was decreased significantly (Gopinath and Etherton, 1989). The results from the insulin tolerance tests suggest that insulin sensitivity of the major insulin-dependent, glucose-utilizing tissues (muscle and liver) is blunted by pGH. Whether there are differential effects among the target tissues (including adipose tissue) is not yet clear.

The observation that acute administration of pGH increased plasma glucose and insulin in pigs that had undergone some physiological adaptation(s) induced by chronic treatment with pGH is intriguing. This increase in fasting glucose and insulin occurred in spite of a pre-existing hyperinsulinemia and hyperglycemia. This change in plasma glucose concentration is either the result of a change in glucose removal from blood and(or) a change in glucose production rates. Based on studies we have done, it appears that both occur. We have found that chronic pGH treatment impairs glucose clearance (as measured by glucose tolerance tests), induces an insulin-resistant state in vivo and increases hepatic glucose production rates (Gopinath and Etherton, 1989). It appears that the increased hepatic glucose production in the face of an elevation in circulating insulin concentration in pGH-treated pigs is the result of an impairment in hepatic sensitivity to insulin. Thus, the inhibitory effects of insulin on hepatic glucose production would be blunted. Hepatic insulin resistance has been reported in acromegalic patients and in nondiabetic humans after short-term GH infusion (Rizza et al., 1982; Hansen et al., 1986).

The change in circulating insulin concentrations in pigs treated chronically with pGH likely is the result of changes in insulin secretion. Although it is possible that pGH may have direct effects on the pancreas independent of changes in blood glucose, we have no evidence to support this idea. The fact that the elevation in insulin coincides with the increase in glucose concentration (Figure 2) coupled with an increased insulin response to glucose infusion in pGH-treated pigs (Gopinath and Etherton, 1989) suggests that the increased insulin response in pGH-treated pigs is due to the insulin secretogogue effects of hyperglycemia.

In summary, we have demonstrated that pGH does not elicit an acute effect on fasting plasma glucose and insulin concentrations, at least not with the dose used in this study. An acute effect, however, was observed when pGH was injected into pigs that had been treated with pGH for 24 d. When administered chronically, pGH causes significant hyperglycemia and hyperinsulinemia under the fed and fasted states. These data support the contention that pGH is diabetogenic in pigs. It is our speculation that the hyperglycemia of pGH-treated pigs is due to increased hepatic glucose production and a concurrent impairment in glucose clearance (see Gopinath and Etherton, 1989). The associated hyperinsulinemia appears to be the result of metabolic adaptations caused by hyperglycemia.

**Literature Cited**


