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The inhibition of insulin action and glucose metabolism by porcine growth hormone in porcine adipocytes is not the result of any decrease in insulin binding or insulin receptor kinase activity

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The present study was undertaken to determine the effects of porcine growth hormone (pGH) on glucose transport, to establish which lipogenic enzymes were affected by pGH, and to determine if changes in insulin binding or insulin receptor kinase activity contributed to the diminished insulin responsiveness of adipocytes from pigs treated with pGH. Pigs were treated with pGH daily (70 μg/kg body wt.) for 7 days. pGH treatment reduced the basal (non-insulin-stimulated) glucose transport rate by 62% and the insulin-stimulated transport rate by 47%. The decline in glucose transport rate was paralleled by a 64% decrease in fatty acid synthesis. The reduction in the lipogenic rate was associated with a marked decline in the activity of several lipogenic enzymes: glucose-6-phosphate dehydrogenase (50% decrease), 6-phosphogluconate dehydrogenase (11% decrease), malic enzyme (62% decrease) and fatty acid synthase (activity not detectable after pGH treatment). The pGH-dependent decline in insulin responsiveness was not associated with any change in the binding of insulin to intact adipocytes or to plasma membrane preparations. The insulin-stimulated tyrosine kinase activity of the wheat-germ agglutinin-purified receptors from pGH-treated adipocytes was not different from that in control adipocytes, except when high concentrations of insulin were employed. These findings establish that pGH elicits a number of metabolic effects in porcine adipocytes which collectively diminish the rate of lipid synthesis, and thereby contribute to the decrease in lipid deposition observed in pGH-treated pigs. Furthermore, the pGH-dependent impairment in insulin action appears to be mediated at some location distal to the receptor kinase step or in other signal pathway(s) which mediate the biological effects of insulin that are not dependent on activation of insulin receptor tyrosine kinase activity.

INTRODUCTION

Administration of porcine growth hormone (pGH) daily to pigs for extended periods of time increases growth rate by 10–20%, improves feed efficiency (feed/gain) by as much as 35% and markedly alters the ratio of muscle to fat (Machlin, 1972; Chung *et al.*, 1985; Etherton *et al.*, 1986, 1987*b*; Evock *et al.*, 1988). Treatment of pigs with pGH can decrease adipose tissue accretion by as much as 70% (Evock *et al.*, 1988) and increase protein deposition by 60% (Campbell *et al.*, 1989). We have found that the decrease in lipid accretion is associated with a marked decline in lipogenesis (Walton *et al.*, 1987). Other work has established that insulin sensitivity (as determined by the concentration of insulin which half-maximally stimulates lipid synthesis and glucose oxidation) and responsiveness (maximal response to insulin) are decreased in adipose tissue from pigs treated chronically with pGH (Walton *et al.*, 1987), and in adipose tissue cultured for 48 h in the presence of pGH (Walton *et al.*, 1986). This latter response is not mimicked by prolactin, which suggests that the effects are specific to pGH.

Although it is apparent that pGH affects lipid deposition in pigs, there is little information about the mechanisms by which pGH alters adipocyte metabolism and insulin responsiveness. Therefore studies were undertaken to determine how pGH affects glucose transport, the activity of selected lipogenic enzymes and insulin binding. We also determined whether pGH affects insulin receptor tyrosine kinase activity to establish whether pGH alters this trans-membrane signalling system for insulin (Gammeltoft & Van Obberghen, 1986) in a manner independent of any putative change in insulin binding.

EXPERIMENTAL

Materials

Porcine pituitary growth hormone (USDA-B1) was donated by the United States Department of Agriculture, Beltsville, MD, U.S.A. This lot is equipotent to recombinant pGH in inhibiting binding of ^{125}I-labelled pituitary pGH (Chung & Etherton, 1986). Growth hormone was solubilized in a sterile bicarbonate buffer (25 mM-$NaHCO_3$/25 mM-Na_2CO_3/0.15 M-NaCl, pH 9.4;

Abbreviations used: pGH, porcine growth hormone; WGA, wheat-germ agglutinin; KRB, Krebs–Ringer buffer (composition given in text); IGF-I, insulin-like growth factor-I; IGFBP, IGF binding protein.

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Chung *et al.*, 1985). Solutions of pGH (5–7 mg/ml) were prepared every second day and stored at 4 °C. Porcine monocomponent insulin was donated by Dr. Ronald Chance, Eli Lilly & Co., Indianapolis, IN, U.S.A. Other reagents were obtained from the following sources: [2-^{14}C]malonyl-CoA (New England Nuclear, Boston, MA, U.S.A.); 3-*O*-[^{14}C]methylglucose and L-[^{14}C]glucose (Amersham Corp., Arlington Heights, IL, U.S.A.); collagenase (type CLS; Worthington Biochemical Corp., Freehold, NJ, U.S.A.); silicone oil (viscosity = 50 centistokes at 25 °C; Thomas Scientific, Philadelphia, PA, U.S.A.) osmium tetroxide (Electron Microscopy Sciences, Fort Washington, PA, U.S.A.); bovine serum albumin (CRG-7; Armour Pharmaceutical Co., Kankakee, IL, U.S.A.); Sephadex G-50 (Pharmacia, Piscataway, NJ, U.S.A.); wheat-germ agglutinin (WGA) agarose (Miles Laboratory, Elkhart, IN, U.S.A.) and the Bio-Rad standard protein assay kit (Bio-Rad, Richmond, CA, U.S.A.). Monoiodo-[A_{14}] porcine ^{125}I-insulin (radioreceptor grade, specific activity 250–370 μCi/μg) and (γ-^{32}P]ATP (specific activity ~ 3000 Ci/mMol) were obtained from Dupont–NEN (Boston, MA, U.S.A.). All other reagents were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Animals and collection of adipose tissue

Castrated male pigs (body wt. approx. 60–80 kg) were used for all experiments. Pigs were fed a corn/soybean-meal-based diet that contained 18% protein plus 0.5% added crystalline lysine (Evock *et al.*, 1988). For certain experiments, biopsies of subcutaneous adipose tissue were taken as described (Chung *et al.*, 1983). In other experiments, subcutaneous adipose tissue samples were taken after exsanguination at The Pennsylvania State University Meats Laboratory. Tissue samples were immediately placed in 37 °C Krebs–Ringer bicarbonate/Hepes buffer (KRB-Hepes; 118 mM-NaCl, 4.8 mM-KCl, 1.3 mM-$CaCl_2$, 1.2 mM-KH_2PO_4, 1.3 mM-$MgSO_4$ and 10 mg of BSA/ml, pH 7.4) for transport to the laboratory.

Treatment of pigs with pGH

Pigs were treated daily at 09:00 h with 70 μg of pGH/kg body wt. for 7 days by intramuscular injection into the extensor muscle of the neck (Etherton *et al.*, 1987*b*). This dose of pGH was selected because it causes a marked change in growth rate, carcass composition, insulin growth factor-I (IGF-I) concentration and lipogenic rates in pigs treated chronically (Etherton *et al.*, 1987*b*; Walton *et al.*, 1987; Evock *et al.*, 1988).

Lipogenic enzyme assays

Adipose tissue samples were homogenized in ice-cold homogenization buffer (1 g of tissue/3 ml of 0.15 M-KCl/50 mM-Tris/HCl, pH 7.4) as described (Anderson *et al.*, 1972). The homogenate was centrifuged at 3000 *g* for 15 min, after which the infranatant was removed and subsequently centrifuged at 27000 *g* for 30 min (Smith & Prior, 1981). The supernatant was kept on ice and used for the enzyme assays. Enzymes assayed (and in the order performed) were: fatty acid synthase (EC 2.3.1.85), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44) and malic enzyme (malate dehydrogenase; EC 1.1.1.40). Fatty acid synthase activity was measured using the procedure described by Roncari (1981), which is based on quantifying the rate of [2-^{14}C]malonyl-CoA incorporation into fatty acids. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were determined by using a modification of the double substrate assay of Glock & McLean (1953). Malic enzyme activity was assayed by the method of Ochoa (1955).

Quantification of lipogenesis

Conversion of [^{14}C]glucose into lipids in thin adipose tissue slices was determined as described (Walton & Etherton, 1986). Incubations were done in the presence of 0 or 10 ng of insulin/ml. This is a maximally effective dose of insulin for stimulating lipogenesis *in vitro* in porcine adipose tissue (Walton & Etherton, 1986).

Hexose transport by adipocytes

Hexose transport by isolated adipocytes was measured using the method described by Ciaraldi *et al.* (1979) with several modifications. Porcine adipocytes were isolated using collagenase (2.5 mg/ml) as described (Etherton & Chung, 1981). Transport assays were run at 37 °C in quadruplicate for each pig before and after treatment with pGH. A 100 μl portion of a packed cell suspension [approx. $(1–2) \times 10^5$ cells] was added to 20 μl of KRB/Hepes that contained 0.4 μCi of 3-*O*-[^{14}C]methylglucose in the bottom of a 17 mm × 100 mm polypropylene tube. After 15 s, the reaction was stopped by the addition of phloretin buffer (0.3 mM-phloretin/0.12% ethanol/0.05% dimethyl sulphoxide in KRB/Hepes). Silicone oil (1.5 ml) was added and tubes were centrifuged within 3 min at 8000 *g* for 30 s. After centrifugation, cells were harvested and added to scintillation fluid for counting. Diffusion-mediated uptake was estimated by incubating cells with L-[^{14}C]glucose (Whitesell & Gliemann, 1979). To determine insulin-stimulated glucose transport, adipocytes were incubated in the presence of different insulin concentrations for 45 min at 37 °C with gentle swirling.

Determination of adipocyte number

Adipocyte number was determined as described using osmium tetroxide (Etherton *et al.*, 1977; Etherton & Chung, 1981).

^{125}I-Insulin binding to porcine adipocytes

Insulin was iodinated with chloramine-T as described (Freychet *et al.*, 1971; Etherton & Walker, 1982) to a specific activity of 100–150 μCi/μg. Binding studies were conducted with 0.2 ng of iodinated insulin per tube at 30 °C as previously described (Etherton & Walker, 1982).

Preparation of adipocyte plasma membranes

Plasma membranes were prepared from subcutaneous adipose tissue using the procedure described by Czech & Lynn (1973). Plasma membranes were resuspended in 1 ml of 1 mM-EDTA before freezing in liquid N_2. The protein concentration of the membrane solution was determined by using a Bio-Rad micro protein assay kit. The yield of membranes was typically 3–4 mg per 150 g of adipose tissue. Before assay for ^{125}I-insulin binding and/or solubilization and WGA chromatography, membranes were sedimented at 30000 *g* for 30 min and resuspended either in Ca^{2+}-free Krebs–Ringer phosphate buffer (pH 7.8) for membrane binding or in 50 mM-Hepes (pH 7.6) for solubilization.

Binding of insulin to membranes

Insulin binding was carried out as described by Havrankova *et al.* (1978) with modifications. Binding studies were done at 4 °C for 18 h in 150 μl of Ca^{2+}-free Krebs–Ringer phosphate buffer, pH 7.8, containing 1% BSA and 1 mg of bacitracin/ml with 0.03 nM-monoiodo-[A_{14}] ^{125}I-insulin. Assays contained ~20 μg of membrane protein, determined according to Lowry *et al.* (1951). Unlabelled insulin concentrations included in the binding studies ranged from 0 to 1.33 μg/ml. Non-specific binding was determined by measuring the radioactivity in the pellet in the presence of 13.33 μg of insulin/ml.

Lectin purification of adipocyte membrane insulin receptors

Porcine adipocyte membrane insulin receptors were partially purified on WGA columns as described by Hedo *et al.* (1981). Membranes were suspended in 7 ml of 50 mM-Hepes, pH 7.6, containing 1% Triton X-100 and 1 mM phenylmethanesulphonyl fluoride. This solution was vortex-mixed vigorously and incubated overnight at 4 °C with shaking. The insoluble material was sedimented by centrifugation at 240000 *g* for 50 min at 4 °C. The supernatant was then recycled three times on a 2 ml WGA column. The column was washed with 40 ml of 50 mM-Hepes buffer, pH 7.6, containing 150 mM-NaCl and 0.1% Triton X-100. Glycoproteins bound to the WGA column were eluted with 50 mM-Hepes buffer, pH 7.6, containing 150 mM-NaCl, 0.1% Triton X-100 and 0.3 M-*N*-acetylglucosamine. A 3 ml pool consisting of fractions 2, 3 and 4 was retained for subsequent studies.

Insulin binding to WGA-purified receptors

Insulin binding to WGA-purified receptors was determined according to the method of Harrison & Itin (1980) with modifications. A 50 μl portion of 50 mM-Hepes/150 mM-NaCl/0.1% BSA, pH 7.8, without or with unlabelled insulin, was mixed with 125 μl of a solution containing 50 mM-Hepes, 150 mM-NaCl, 1.6 mg of bacitracin/ml, 0.16% BSA, pH 7.9, and monoiodo-[A_{14}] ^{125}I-insulin (0.03–0.04 nM). WGA-purified receptors (25 μl) were then added. Non-specific binding was determined using 10 μg of unlabelled insulin/ml, and competition/inhibition was determined using 1–3000 ng/ml. Tubes were incubated for 3 h at room temperature. The incubation was stopped by placing the tubes on ice, after which 100 μl of 0.3% γ-globulin (in water) was added to each tube. Poly(ethylene glycol) (300 μl; 25%) was then added to each tube and tubes were vortex-mixed and left undisturbed for 10 min. Tubes were centrifuged at 2500 *g* for 15 min at 4 °C. The pellet was washed with 300 μl of 12.5% poly(ethylene glycol) and recentrifuged. The supernatant was aspirated and the pellets counted for radioactivity in a γ counter.

Phosphorylation of artificial substrate

Phosphorylation of the artificial substrate poly-(Glu_4,Tyr_1) was conducted as described by Zick *et al.* (1985) with modifications. WGA-purified receptors were diluted to similar tracer ^{125}I-insulin bound/free ratios, and 20 μl was added to a mixture containing 20 μl of 75 mM-Hepes, pH 7.6, 20 μl of 50 mM-Hepes, pH 7.6, without or with 200 μg of poly(Glu_4, Tyr_1), and 10 μl of 50 mM-Hepes/150 mM-NaCl/0.1% BSA, pH 7.8, without or with insulin. After 45 min at room temperature for insulin binding, phosphorylation was initiated by adding (final concentrations) 50 μM-[γ-^{32}P]-ATP, 20 mM-$MgCl_2$ and 1 mM-CTP. After 20 min, 75 μl aliquots were spotted on Whatman 3MM filter papers which were then immersed in 10% trichloroacetic acid/10 mM-sodium pyrophosphate. After extensive washing in this solution and finally in 95% ethanol, filters were dried and counted for ^{32}P incorporation. In all cases, ^{32}P incorporation observed in the absence of poly(Glu_4,Tyr_1) was subtracted.

Statistics

Experiments conducted measuring the effects of pGH on lipogenesis were analysed by using a split-plot design with pGH as the whole plot factor and insulin as the subplot factor (SAS, 1979). The model included pig, pGH dose, insulin dose, pig × pGH, pGH × insulin and pig × pGH × insulin as factors. Data from the experiments in which enzyme activities and glucose transport were measured were analysed by one-way analysis of variance (SAS, 1979). The ALLFIT program (De Lean *et al.*, 1978) was used to fit regression curves and estimate binding parameters for data from experiments in which the effects of different concentrations of insulin on glucose transport, insulin binding and kinase activity were determined. Student's *t* test was then used to compare treatment means (Neter & Wasserman, 1974).

Table 1. Effects of pGH on lipogenesis, lipogenic enzyme activities, insulin binding and glucose transport

Adipose tissue explants were prepared from biopsies taken from pigs ($n = 7$) before and after treatment with 70 μg of pGH/d per kg body wt for 7 days. To determine lipogenic enzyme activity, adipose tissue samples were homogenized and the respective enzyme was assayed as described in the text. The procedures used to measure glucose transport and insulin binding, are described in the text. Units: [a]nmol of glucose converted to lipid/2 h per 10^6 cells; [b]nmol of malonyl-CoA converted to lipid/min per 10^6 cells; [c]nmol of NADPH produced/min per 10^6 cells; [d]pmol of glucose transported/s per 10^6 cells; [e]pg of ^{125}I-insulin bound/90 min per 10^6 cells. Values are means ± S.E.M. *Values significantly different ($P < 0.05$) from controls (non-pGH treated).

	−pGH	+pGH
Lipogenesis:		
Basal[a]	712±36	259±36*
Insulin-stimulated (10 ng/ml)[a]	977±36	405±36*
Lipogenic enzyme activity:		
Fatty acid synthase[b]	4.9±0.3	< 0.1*
Glucose-6-phosphate dehydrogenase[c]	156.9±9.9	80.2±9.9*
6-Phosphogluconate dehydrogenase[c]	117.2±3.1	104.7±3.1*
Malic enzyme[c]	172.7±33.2	65.6±32.3*
Glucose transport[d]	84.9±11.6	32.3±11.7*
Insulin bound[e]	7.8±0.5	7.4±0.6

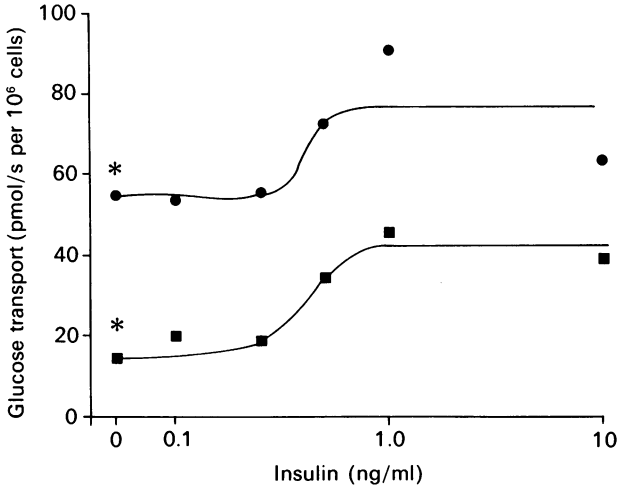

Fig. 1. Effect of pGH on insulin-stimulated glucose transport in porcine adipocytes

Adipocytes were isolated from adipose tissue obtained from pigs ($n = 2$) treated for seven days with 0 (●) or 70 μg of pGH/day per kg body wt. (■). Uptake of D-glucose, corrected for L-glucose uptake, was measured after preincubation of adipocytes with various concentrations of insulin. The pooled S.E.M. was 4.5.

RESULTS

As expected, treating pigs with pGH for 7 days decreased basal and maximally stimulated (10 ng of insulin/ml) lipogenic rates significantly (Table 1). The pGH-dependent decline in lipogenesis was paralleled by a decline in lipogenic enzyme activity (Table 1). Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and malic enzyme activities were depressed by 50%, 11% and 62% respectively by pGH. Treatment of pigs with pGH for 7 days abolished any detectable fatty acid synthase activity. In our hands, the limit of detection of the fatty acid synthase assay is approx. 30 pmol of malonyl-CoA incorporated into fatty acid/min per 10^6 cells.

The effects of pGH on basal hexose transport paralleled those observed for lipogenesis. pGH reduced basal hexose transport rates by approx. 62% (Table 1). This reduction agrees quite well with the observed decline in fatty acid synthesis. Responsiveness of adipocytes to the stimulatory effects of insulin was impaired by pGH (Fig. 1). Based on the ALLFIT analysis, maximally stimulated glucose transport was reduced by 47% by pGH.

In contrast with the decreases in glucose transport, lipogenic enzyme activities and glucose flux, insulin binding to intact adipocytes was not affected by pGH.

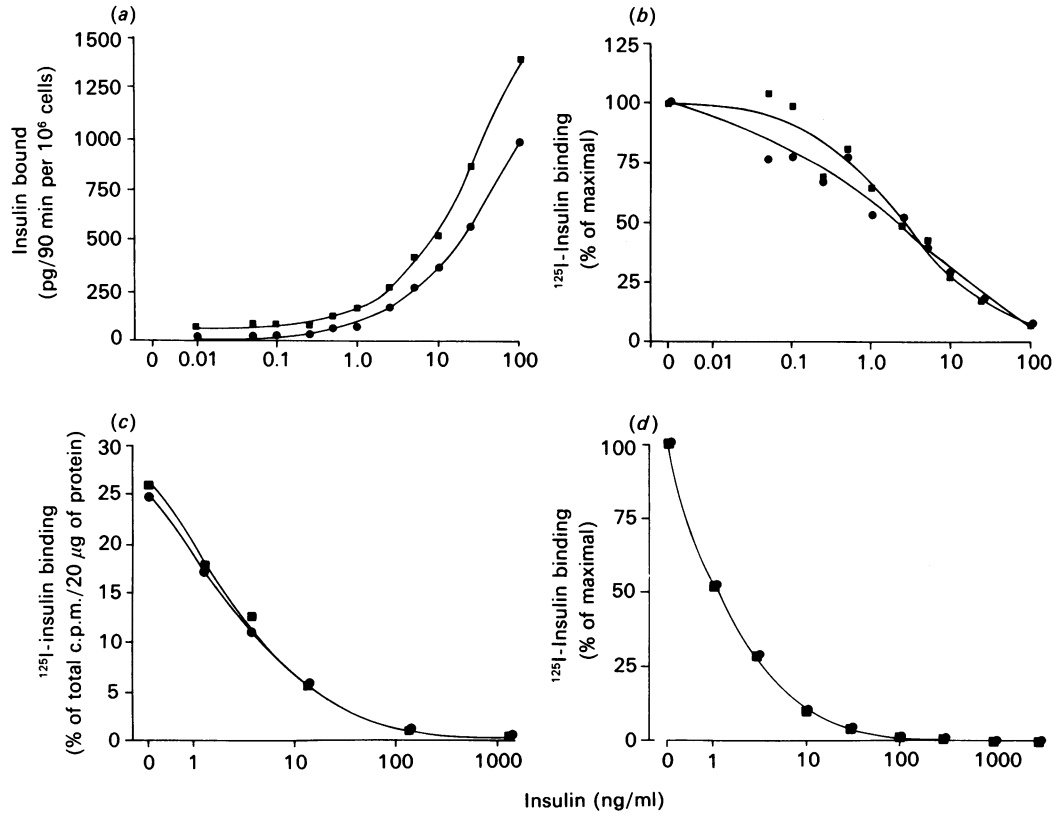

Fig. 2. Effect of pGH on insulin binding to isolated porcine adipocytes, membrane fractions and WGA-purified receptors

Insulin binding was determined before (●) and after (■) daily treatment of pigs ($n = 7$) with 70 μg of pGH/day per kg body wt. for 7 days. (*a*) Insulin binding to isolated porcine adipocytes (pooled S.E.M., 21.8); (*b*) inhibition of insulin binding to isolated adipocytes by insulin (pooled S.E.M., 4.2); (*c*) inhibition of insulin binding to adipocyte membrane fractions (pooled S.E.M., 0.6); (*d*) inhibition of insulin binding to WGA-purified porcine insulin receptors (pooled S.E.M., 1.2).

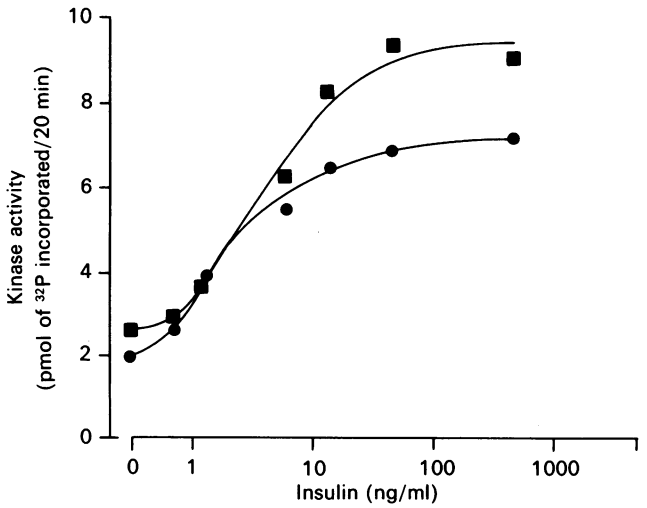

Fig. 3. Effect of pGH on tyrosine kinase activity of WGA-purified insulin receptors

Insulin receptors were purified from adipocyte membrane fractions prepared from adipocyte isolated from pigs treated with 0 (●) or 70 μg of pGH/day per kg body wt. (■) for 7 days. Details of the kinase assay are described in the text. The pooled S.E.M. was 0.3.

Binding of trace quantities of insulin did not differ (Table 1), nor were there any differences in the mass of insulin bound over a wide range of insulin concentrations (Fig. 2). In addition, there were no significant changes in insulin receptor affinity as estimated by the concentration of insulin (approx. 2.5 ng/ml) that half-maximally inhibited tracer binding (Fig. 2). Inhibition curves for insulin binding to plasma membrane preparations and purified insulin receptors also indicated that pGH did not affect binding capacity or affinity (Fig. 2). When the binding data were analysed by the method of Scatchard, there were no differences in receptor number or affinity for either membrane preparations or purified insulin receptors from control and pGH-treated pigs (results not shown).

Phosphorylation of the artificial substrate poly(Glu_4,Tyr_1) by the insulin receptor tyrosine kinase did not differ significantly between pGH-treated and normal animals at insulin concentrations up to 18 ng/ml (Fig. 3). At higher insulin concentrations, however, there was an increase in substrate phosphorylation by insulin receptors from pGH-treated pigs.

DISCUSSION

Our hypothesis has been that pGH decreases adipose tissue accretion in growing pigs primarily by decreasing both fatty acid synthesis and the stimulatory effects of insulin on glucose uptake and lipid synthesis, rather than by stimulating lipolysis (Walton *et al.*, 1986; Etherton, 1989). The pGH-dependent decreases in glucose transport, lipogenic enzyme activities and lipogenic rate observed in the present study are consistent with this speculation. Furthermore, the decrease in glucose flux agrees well with the decrease in adipose tissue accretion in pigs treated with the same dose of pGH for 11 weeks (54%; Evock *et al.*, 1988).

The studies conducted to date to determine whether pGH affects lipogenesis, lipolysis or both have been based predominantly on studies *in vitro*. Although these findings are substantive, in no instance has any information been reported about the effects of pGH on rates of fatty acid synthesis and lipolysis *in vivo*. Recently, Dunshea *et al.* (1989) reported that fatty acid synthesis was decreased by approx. 90% *in vivo* and that insulin-stimulated lipogenesis was also significantly reduced in pigs treated with pGH for 11 days. Moreover, they found that the rates of irreversible loss of plasma free fatty acids and glycerol were not altered by pGH. These results in conjunction with data from studies *in vitro* establish that pGH predominantly alters lipid deposition in adipose tissue by decreasing nutrient uptake (principally glucose), fatty acid synthesis and insulin sensitivity of these pathways, and that pGH does not reduce adipose tissue accretion by altering the lipolytic rate.

The decrease in lipogenic enzyme activity probably reflects a reduction in enzyme mass. This inference is based on studies we have conducted in which porcine adipose tissue is cultured for 48 h with pGH. These studies have revealed that pGH markedly inhibits the ability of insulin to maintain lipogenic capacity in a dose-responsive manner (Walton *et al.*, 1986; Evock *et al.*, 1988). This effect is not seen in short-term (i.e. 2 h) acute incubations, which suggests that pGH acts in a chronic manner to alter expression of important regulatory genes in the pathway of fatty acid synthesis (e.g. glucose transporters, fatty acid synthase, etc.) rather than affecting catalytic activity of lipogenic enzymes.

Previous studies with GH-deficient and normal mice have shown, however, that a single injection of ovine GH reduces hepatic acetyl-CoA carboxylase activity within 2 h (Adamafio & Ng, 1984). The differences between that study and the present one may reflect the different species used, the dose of ovine GH used (10 μg/mouse) and tissues used for the measurement of lipogenic enzyme activity. The dose of ovine GH used by Adamafio & Ng (1984) is a much larger dose than we used and may produce non-physiological effects, or alternatively may contain contaminants of other hormones.

The effects of pGH on adipose tissue are probably direct effects that are not mimicked by prolactin or IGF-I. Prolactin does not mimic the insulin-antagonistic effects of pGH when present in adipose tissue cultures (Walton *et al.*, 1986). Since pGH treatment elevates IGF-I concentrations in pigs (Etherton *et al.*, 1987*b*), it could be argued that IGF-I might mediate some of the effects of pGH on adipose tissue. The available evidence, however, suggests that this does not occur. *In vitro*, free IGF-I is an insulin mimic (Walton *et al.*, 1987, 1989) and not an insulin antagonist in porcine adipose tissue. However, the insulin-like effects of free IGF-I in porcine adipose tissue *in vitro* are blocked by the acid-stable IGF-I binding subunit of the pGH-dependent IGF-binding protein (IGFBP) (Walton *et al.*, 1989). Since there is no detectable free (unbound) IGF-I in pig blood (P. E. Walton & T. D. Etherton, unpublished work) and the IGF-I–IGFBP complex has no insulin-like effect in adipose tissue (Walton *et al.*, 1989), it does not seem likely that IGF-I acts in an insulin-like manner *in vivo* with respect to adipose tissue metabolism.

Our previous studies have established that pGH (both pituitary and recombinant) has the following effects: (1) it antagonizes insulin action in cultured porcine adipose tissue in a dose-responsive manner (Walton *et al.*, 1986; Evock *et al.*, 1988), and (2) it decreases insulin sensitivity

and responsiveness of adipose tissue from pigs treated with pGH for 7 days (Walton *et al.*, 1987). The observations of anti-insulin effects of pGH observed in cultured porcine adipose tissue have also recently been extended to 3T3-L1 adipocytes (Glenn *et al.*, 1988). The hypothesis that we tested in the present report was that pGH may impair tyrosine kinase activity of the receptor without any decrease in insulin binding. Previous studies have established that one important insulin signalling system is associated with binding of insulin to the α-subunit of the receptor followed by autophosphorylation of the β-subunit, which leads to an increase in the tyrosine kinase activity of the β-subunit (Kasuga *et al.*, 1982; Zick *et al.*, 1983*a*,*b*; Van Obberghen *et al.*, 1985). Our results show that neither insulin binding nor insulin receptor tyrosine kinase activity is impaired by pGH. This can be interpreted as suggesting that the pGH-dependent impairment in insulin action is due to (1) a defect in the receptor tyrosine kinase signalling pathway at some point beyond the kinase step or (2) inhibition of an insulin signal pathway(s) which is not dependent on stimulation of the tyrosine kinase activity of the receptor.

There is accumulating evidence that multiple intracellular signal pathways exist for insulin. A rabbit anti-(insulin receptor) antibody was found to stimulate glucose uptake, but unlike insulin it was unable to stimulate proliferation of rat Fao hepatoma cells (Ponzio *et al.*, 1988). Other studies have shown that monoclonal antibodies against the α-subunit of the human insulin receptor stimulate glucose transport in human adipocytes and α-aminoisobutyric acid uptake in rat HTC hepatoma cells, but do not stimulate the tyrosine kinase activity of the receptor (Forsayeth *et al.*, 1987; Hawley *et al.*, 1989). The observation that bovine GH decreases insulin-stimulated lipogenesis in bovine adipose tissue (Etherton *et al.*, 1987*a*) and yet enhances the anti-lipolytic effects of insulin *in vivo* (Sechen *et al.*, 1990) provides further support for the assertion that there are at least two insulin signal pathways. The most direct evidence that multiple signalling pathways exist for insulin, some of which are independent of receptor kinase activation, is the finding that certain insulin receptor kinase inhibitors can inhibit insulin-stimulated lipogenesis in rat adipocytes but do not block the anti-lipolytic effects of insulin (Schechter *et al.*, 1989).

In conclusion, the present study has shown that pGH inhibits insulin action in a manner that is not due to any changes in either binding to the insulin receptor or insulin receptor kinase activity. The precise site(s) at which pGH inhibits insulin action in adipocytes are not known. Furthermore, it is reasonable to speculate that pGH affects more than one insulin intracellular signal pathway in distinctly different ways.

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