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Insulin-like Effects in the Rat of the Purified Growth Factor from *Spirometra mansonoides* Plerocercoids (42512)

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Abstract. The acute effects of injections of the human growth hormone-like factor purified from plerocercoids of the tapeworm *Spirometra mansonoides* on carbohydrate, lipid, and protein metabolisms were determined in intact rats. Male rats were injected ip with saline, insulin, or various doses of partially purified PGF. The rats injected with insulin had significantly reduced serum glucose concentrations but no dose of PGF caused a change in serum glucose levels. Insulin and PGF stimulated [^{14}C]glucose and [^{14}C]leucine oxidation to $^{14}CO_2$ in adipose tissue and muscle and increased incorporation of both [^{14}C]glucose carbons into lipids and [^{14}C]leucine into protein in fat and muscle. The responses to PGF were dose-dependent and persisted after 3 hr of incubation *in vitro*. Injections of naloxone prior to injecting PGF to block the stress response did not prevent the stimulation of insulin-like responses by PGF. Therefore, PGF has intrinsic insulin-like activities in normal male rats.

The curious phenomenon of body growth stimulation associated with infections of mice with the plerocercoid stage of the tapeworm, *Spirometra mansonoides,* was first described by Mueller (1). Plerocercoid growth factor (PGF) has subsequently been shown to mimic many of the actions of growth hormone (GH) (2–6). Similar to reported actions of GH, PGF-stimulated growth in rodents is associated with increased somatomedin activity (7, 8), suppressed GH levels of the blood and pituitary (3, 8, 9), stimulated liver ornithine decarboxylase (2, 10), and spleen thymidine kinase (11). Interestingly, PGF appears to be more like human GH (hGH) than other mammalian GHs as PGF has distinct lactogenic activity in the pigeon crop-sac assay (12) and displaces [^{125}I]hGH from its receptors in rabbit liver membranes (5). While PGF has some actions similar to those reported for hGH, in other ways it is distinctly different from any GH. A well-established effect of long-term GH treatment is its ability to stimulate body growth while reducing adipose tissue mass (13, 14). In contrast to the type of growth produced by GH, chronic exposure to PGF by plerocercoid infection stimulates body growth and increases adipose tissue (2, 7, 15). Therefore, it appears that adipose tissue is sensitive to both GH and PGF but the effects may be distinctly different.

Besides its distinct lipolytic activity, GH can produce transient insulin-like responses in GH-deficient animals (16, 17). Intact animals or their tissues are generally not sensitive to the insulin-like actions of GH, and this refractoriness is induced by GH itself (18).

Most of the work on the growth-promoting (2, 4, 7, 9, 19) and metabolic effects (2, 8, 9, 15, 20) of PGF has been accomplished with chronic infections with plerocercoids. The acute effects of purified PGF on intermediary metabolism in mammals has not been reported. Considering the fact that PGF-stimulated growth of experimental animals is associated with increased fattening it seemed reasonable to hypothesize that PGF might have direct insulin-like actions. This study was undertaken to see if injections of partially purified PGF would affect carbohydrate, lipid, or protein metabolism in tissues of intact male rats.

Materials and Methods. *Preparation of PGF for injection.* All stages of the life cycle of *S. mansonoides* are maintained in our laboratory with procedures similar to those described by Mueller (19). The procedures used for collection, solubilization, and quantitation of PGF were based on those previously described (21, 22). Plerocercoids were homogenized in 0.025 *M* Tris–HCl buffer, pH 7.6, which contained 0.3 *M* sucrose (5 ml of buffer for each gram of worms). A crude membrane fraction was collected after centrifugation of the homogenate at 5000*g* for 20 min. The membranes were incubated at room temperature for 30 min in 0.025 *M* Tris–HCl, pH 7.6, containing

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1% (v/v) Triton X-100 (Bio-Rad, Inc., Richmond, CA) and the mixture was then centrifuged at 105,000*g* for 2 hr. The solubilized membrane proteins (crude PGF) were subjected to chromatofocusing chromatography on PBE 94 (Pharmacia, Inc., Uppsala, Sweden) over a gradient from pH 6.3 to 4.0 formed by a 1:8 dilution of Polybuffer 74 (Pharmacia, Inc.) containing 0.01% Triton X-100. The pH of the eluted fractions was monitored and quickly adjusted to pH 7.4–7.8 with a few drops of a saturated solution of Trizma Base (Sigma, St. Louis, MO). The fractions were assayed for activity in a radioreceptor assay (RRA) as described below. Active PGF eluted from the chromatofocusing column between pH 4.7 and 4.3. Fractions containing active PGF were pooled, dialyzed (vs 0.01 *M* K_2HPO_4, pH 7.5), and chromatographed on aminohexyl (AH) Sepharose (Pharmacia, Inc.). Polybuffer 74 did not bind the AH-Sepharose and the bound PGF was subsequently eluted with a wash of 0.2 *M* K_2HPO_4, pH 7.5. Protein concentrations were determined using a Bio-Rad protein assay kit (Bio-Rad, Inc.) and bovine serum albumin (BSA) as the standard.

Quantitation of PGF. Due to the fact that PGF competes with hGH for receptors in membranes from late pregnant rabbits, a RRA using rabbit liver receptors, [^{125}I]hGH, and hGH as the standard for quantitation of PGF was used (5). The binding activities of duplicate samples of at least three dilutions of each preparation of PGF were determined and the quantity of PGF was expressed as the mean binding activity of the dilutions in nanogram equivalents (ng eq) of the hGH standard after correction for the dilution factors (21).

Animal treatment. The normal male Sprague–Dawley rats (SASCO, Inc., Omaha, NE) used in all studies weighed 150–200 g and had free access to food (Wayne Lab-Blox Rat Chow, Allied Mills, Inc., Chicago, IL) and water. They were kept on a 12-hr-light period. The rats were divided into groups (4–6 rats/group) and were injected ip with either 0.9% saline, insulin (4 U), or partially purified PGF (50 to 200 ng eq). Thirty minutes after their injection the animals were killed by decapitation and epididymal adipose tissue from each animal was quickly removed; only the thin distal sections of the fat pads were used after they had been dissected into 50- to 100-mg segments. The segments from each group were randomly distributed into four separate flasks prior to the metabolic assays. The muscular diaphragms were removed, pooled by group, and used intact.

As stress was shown to reverse refractoriness of tissues from normal animals to the insulin-like effects of GH (23), an experiment was conducted to investigate the possibility that injections of PGF might have induced stress in the normal rats. Injection of the opioid receptor inhibitor, naloxone, 15 min prior to laparotomy completely eliminated the ability of stress to relax refractoriness of normal animals to the insulin-like action of GH (23). Therefore, 20 normal male rats were divided into four groups: one group was injected ip with 250 μg naloxone 15 min before being injected with 200 ng eq of PGF; another group received 200 ng eq only of PGF. The control groups were treated in a similar manner except 0.9% saline was injected instead of PGF. The rats were killed 30 min after the PGF or saline injections and adipose tissue was removed and prepared for *in vitro* studies as described above.

Serum glucose measurement. Trunk blood was collected and allowed to clot at room temperature for 20 min and centrifuged at 1030*g* for 15 min, and the serum glucose level was determined using a glucose analyzer-2 (Beckman Instruments, Inc., Fullerton, CA).

Glucose metabolism in adipose tissue. Insulin-like activity was assessed by measuring the oxidation of [U-^{14}C]glucose (New England Nuclear, Boston, MA) to $^{14}CO_2$. The details of this procedure have been described (19, 21).

The incorporation of ^{14}C into total lipids of the adipose tissue was used to measure total lipid synthesis. For this assay adipose tissue was transferred into 5 ml Dole's extraction mixture: isopropanol:heptane:1 *N* H_2SO_4, 40:10:1 (24), and homogenized using an Omnimixer microhomogenizer (Sorvall, Inc., Norwald, CT). To the homogenate, 2 ml of heptane and 3 ml of water were added, vortexed thoroughly, and allowed to stand for a few minutes. The upper heptane phase containing total [^{14}C]lipids was collected and washed twice more with water, transferred into vials, and evaporated overnight in the hood.

Leucine metabolism in adipose tissue. Oxidation of [1-^{14}C]leucine (New England Nuclear) to $^{14}CO_2$ was studied using adipose tissue

segments as described by Goodman (25). After measuring the oxidation of leucine, the tissue was homogenized and [^{14}C]leucine which was incorporated into total protein was measured following extraction with Dole's mixture. To the lower aqueous phase, 0.1 ml of 1% BSA was added followed by 2 ml of 50% trichloroacetic acid (TCA). They were mixed well and centrifuged. The supernatant was discarded and the TCA-precipitated protein was dissolved in 90% formic acid. The protein was reprecipitated twice with 10% TCA and redissolved in a small amount of formic acid. The [^{14}C]protein was transferred into scintillation vials containing 15 ml of Aquasol (New England Nuclear) for counting.

Leucine metabolism in muscular diaphragm. To study the acute metabolic effects of PGF on skeletal muscle, the entire muscular diaphragm from each of the rats was removed, rinsed in Krebs–Ringer bicarbonate (KRB) buffer, and weighed after blotting off the buffer. Oxidation of [^{14}C]leucine to $^{14}CO_2$ was determined as described for [^{14}C]glucose in adipose tissue. The amount of [^{14}C]leucine incorporated into protein was determined as described for adipose tissue and was used as a measure of total protein synthesis in the muscular diaphragms.

All counting of ^{14}C was done using a liquid scintillation system (Tri-Carb 460 CD, Packard Instrumentation Co, Inc., Downers Grove, IL).

Statistical analysis. Data are expressed as the means ± SE. Differences between the means of the groups were compared using the Student's *t* test and *P* values less than 0.05 were considered to represent statistically significant differences. Dose response curves were analyzed by linear regression to determine the correlation coefficients (*r*). When more than two comparisons were made, the data were analyzed by one-way analysis of variance (ANOVA) followed by Scheffe's test for multiple comparisons (26).

Results. Solubilization of plerocercoid membranes in buffer containing 1% Triton X-100 followed by 2 hr of centrifugation at 105,000*g* yielded crude PGF which was chromatofocused through a pH-6.3 to -4.0 gradient and active PGF which was eluted from the column in fractions between pH 4.7 and 4.3. The three preparations of crude PGF used for these experiments had a mean total activity of 742 ± 70 ng eq/ml and a mean specific activity of 93 ± 7 ng eq/mg of protein. Chromatofocusing increased the specific activity to 2442 ± 692 ng eq/mg of protein. While the PGF was only partially purified, the 26-fold increase in specific activity represents a significant improvement in purity.

The results of the following experiments will clearly show that every dose of PGF used in the current experiments stimulated significant insulin-like responses in adipose tissue and muscle of normal male rats. Despite these results, none of the doses of PGF had any effect on serum glucose one-half hour after the injections. These results substantiate earlier work which showed that chronic exposure to PGF via plerocercoid infection did not alter serum glucose concentrations of intact (27), hypophysectomized (2), or diabetic rats (28). As expected, the insulin injections (4 U/rat) lowered serum glucose concentrations dramatically, from 155 ± 15 to 66 ± 4 mg/dl ($P < 0.005$).

Segments of epididymal adipose tissue removed 30 min after the rats were injected with

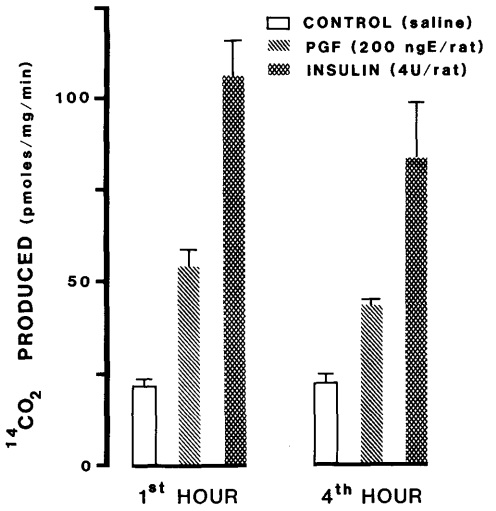

FIG. 1. Acute effects of injections of insulin and partially purified PGF on [U-^{14}C]glucose oxidation in adipose tissue from intact rats. PGF was administered ip at a dose of 200 ng eq/rat and insulin at 4 U/rat and the animals were sacrificed 30 min later. Adipose tissue was quickly removed, randomized, and transferred directly into medium containing [U-^{14}C]glucose and incubated for 1 hr at 37°C. Results are expressed as means ± SE of four observations. $P < 0.025$, control vs PGF; $P < 0.005$, PGF vs insulin.

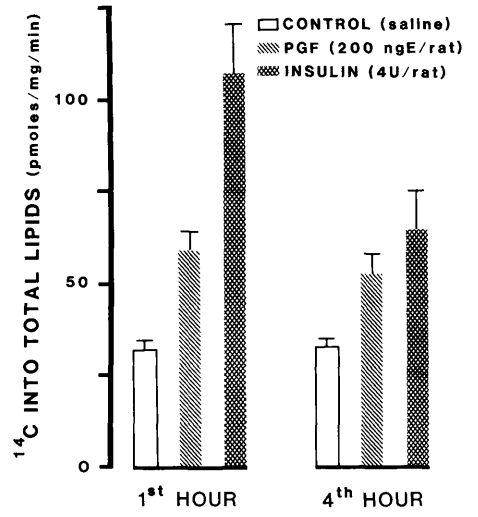

FIG. 2. Acute effects of injections of insulin and partially purified PGF on [U-^{14}C]glucose metabolism and incorporation of ^{14}C into lipids of adipose tissue from intact rats. Subsequent to measuring glucose oxidation total lipid was extracted and measured as described under Methods. Results are expressed as means ± SE of four observations. $P < 0.025$, control vs PGF; $P < 0.005$, PGF vs insulin.

insulin (4 U/rat) or PGF (200 ng eq/rat) had significantly higher rates of glucose oxidation after 1 hr of incubation than did tissue segments from the saline-injected controls (Fig. 1). The short time interval (30 min) between the injection of PGF and the removal of the tissues should preclude increased synthesis and release of somatomedins as a contributing factor to the observed results (17). Therefore, PGF had a direct insulin-like effect on the metabolism of adipose tissue. While the PGF injections stimulated glucose oxidation to a level more than twice that of the control tissue, this level was less than that produced by 4 U of insulin. The rate of glucose oxidation in adipose tissue from both PGF and insulin-injected rats had decreased after 4 hr of *in vitro* incubation but the rates of both were still significantly ($P < 0.05$) higher than those of tissue from saline-injected controls (Fig. 1).

An earlier report showed that the increase in $^{14}CO_2$ production associated with insulin treatment or the acute insulin-like action of GH paralleled ^{14}C incorporation into lipid (29). Consistent with that report, injections of both PGF and insulin caused significant increases in ^{14}C incorporation into total lipids in adipose tissue when measured immediately after removal or after the adipose tissue had been incubated *in vitro* for 3 hr (Fig. 2).

Amino acid metabolism in adipose tissue was very sensitive to the insulin-like action of PGF as the rate of $^{14}CO_2$ production from [^{14}C]leucine, as well as [^{14}C]leucine incorporation into protein in adipose tissue, was markedly increased compared to the saline-treated controls ($P < 0.005$). The response which resulted from the injection of 200 ng eq of PGF was not significantly different from the response produced by 4 U of insulin (Fig. 3).

In support of the insulin-like effects on adipose tissue metabolism, the muscular diaphragms from the PGF-injected rats had increased rates of [^{14}C]leucine oxidation and incorporation into protein ($P < 0.05$) when compared to the controls (Fig. 4).

We conducted a separate experiment to determine if the responses elicited in adipose tissue and skeletal muscle by PGF were dose dependent. The curve was limited to three doses

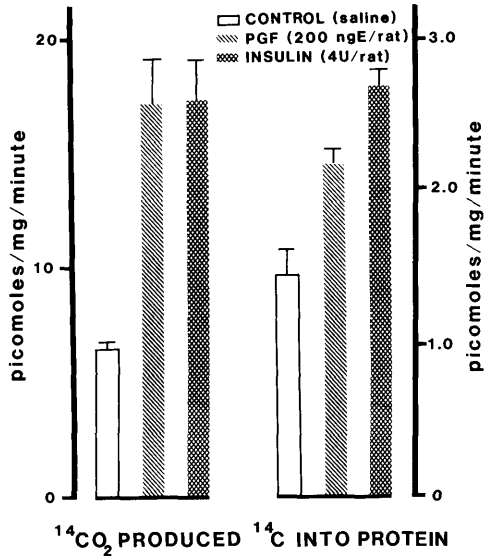

FIG. 3. Acute effects of injections of insulin and partially purified PGF on the rate of [1-^{14}C]leucine oxidation and incorporation into protein in adipose tissue. Intact male rats were injected ip with either 0.9% saline, insulin (4 U), or PGF (200 ng eq) and sacrificed 30 min later. The tissues were quickly removed, randomized, and assayed for [1-^{14}C]leucine oxidation and [^{14}C]leucine incorporation into protein. Results are expressed as means ± SE of four observations. $P < 0.025$, control vs PGF or insulin for $^{14}CO_2$; $P < 0.05$, control vs PGF or insulin for [^{14}C]protein.

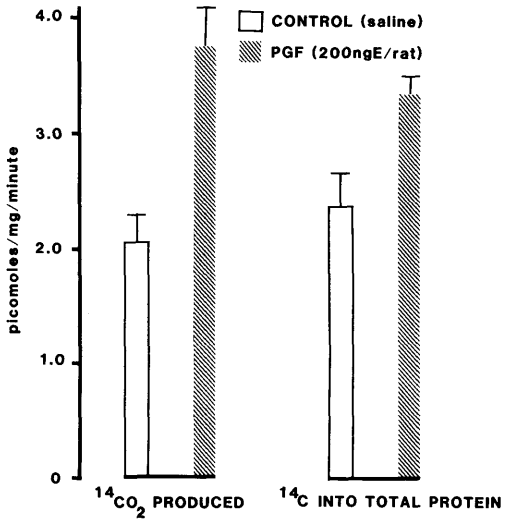

FIG. 4. Acute effects of injections of partially purified PGF on [1-^{14}C]leucine metabolism of intact rat muscular diaphragm. Male rats were injected ip with either 0.9% saline or PGF (200 ng eq/rat) and sacrificed 30 min later. The tissues were removed and assayed for [1-^{14}C]leucine oxidation and [^{14}C]leucine incorporation into protein. Results are expressed as means ± SE of six observations. $P < 0.05$, PGF vs control.

of PGF (50, 100, and 200 ng eq/rat). While these doses did not cover a broad range, it is obvious that the animals responded to PGF in a dose-dependent manner (Figs. 5–7). The smallest dose of PGF tested (50 ng eq) produced a statistically significant ($P < 0.05$) stimulation of all parameters measured in both adipose tissue and muscular diaphragm. Linear regression analysis of the dose response curves in Figs. 5–7 indicates a positive relationship between the doses of PGF and tissue responses with correlation coefficients (r) for each of the dose response curves greater than 0.90.

Insulin-like responses to GH are readily demonstrated in GH-deficient rats (30) but are usually not obtained in tissues from normal rats (29). Tissue refractoriness to the insulin-like actions of GH can be removed by preincubating adipose tissue from normal rats in media devoid of GH for a period of 2–3 hr prior to addition of GH *in vitro* (18). In addition, Coiro *et al.* (23) reported that physical or surgical stress rapidly reversed refractoriness to the insulin-like actions of injections of GH in normal rats. To investigate the possibility that the stress of the PGF injections was allowing the adipose tissue to be sensitive to the acute insulin-like effects of PGF, an experiment was conducted in which an inhibitor of the opioid receptor was included. Naloxone (250 μg/rat) given 15 min prior to injections of 200 ng eq of PGF did not affect the insulin-like responses. These results suggest that stress was not the cause of the lack of refractoriness to the insulin-like actions of PGF.

Discussion. From the foregoing results, it is apparent that injections of partially purified PGF in normal rats stimulated distinct insulin-like responses which resulted in altered metabolisms of carbohydrate, lipid, and protein similar to the responses observed in tissues from insulin-injected rats. However, there were very important differences between the actions produced by PGF injections in the current study and those previously reported for GH. Tissues in the normal rats were not refractory to the acute insulin-like activity of PGF and the stimulatory effect produced by PGF persisted even after the tissues had been incubated *in vitro* for 4 hr. This is in marked contrast to the insulin-like effects of GH which are demonstrated convincingly only in GH-

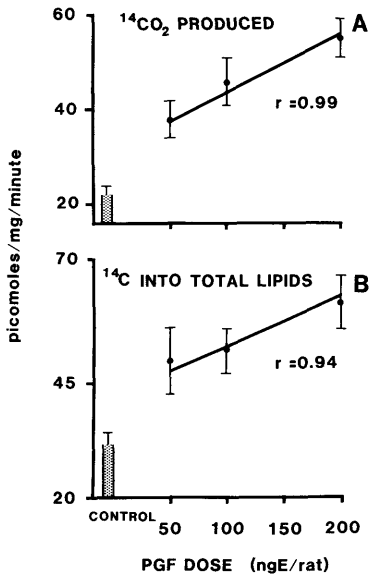

FIG. 5. Acute effects of injections of increasing doses of partially purified PGF on [U-^{14}C]glucose metabolism of intact rat adipose tissue. (A) [U-^{14}C]glucose oxidation to $^{14}CO_2$. (B) ^{14}C incorporation into lipids. Results are expressed as means ± SE of four observations. r = correlation coefficient. $P < 0.01$ for each dose of PGF vs the basal levels (by one-way ANOVA followed by Scheffe's test).

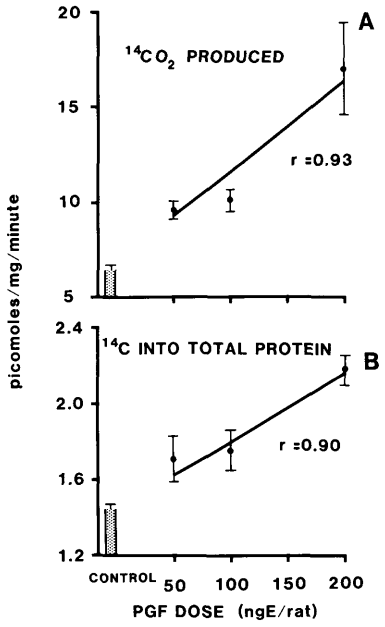

FIG. 6. Acute effects of injections of increasing doses of partially purified PGF on amino acid metabolism of intact rat adipose tissue. (A) [1-^{14}C]leucine oxidation to $^{14}CO_2$ and (B) [^{14}C]leucine incorporation into protein. Results are expressed as means ± SE of four observations. r = correlation coefficient. $P < 0.01$ for each dose of PGF vs the basal levels (by one-way ANOVA followed by Scheffe's test).

deficient animals (16, 17, 29) and during the 3-hr period after GH administration, as GH induces refractoriness to its own insulin-like actions (29, 31).

That GH has intrinsic insulin-like actions is well established and Cameron *et al.* (32) have recently reported that GHs from nonmammalian vertebrates also have both diabetogenic and insulin-like activities. It is well accepted that GH antagonizes the action of insulin, as insulin sensitivity is increased by GH deficiency and impaired by GH excess (33, 34). Recently, Schwartz and Eden (35) showed that endogenous GH suppresses basal glucose metabolism in adipocytes and that the effect was independent of changes in insulin binding. In addition to its physiologically important role in counteracting the actions of insulin, endogenous GH induces refractoriness to its own insulin-like actions. The insulin-like effects of GH can only be demonstrated in animals deficient in GH, as by hypophysectomy (29, 36), after treatment of normal animals with anti-GH antibodies (37) or in very young, fasted animals (38). Included among the insulin-like effects which GH produces in GH-deficient animals are accelerated transport of sugars with transient lowering of blood glucose, increased carbohydrate metabolism, and increased uptake of leucine and metabolism in fat and skeletal muscle (17, 25, 29, 36).

The lack of refractoriness to the insulin-like activities of PGF in the normal rats of the current work is very interesting. We considered the possibility that some component of the partially purified PGF or the injections themselves might have induced stress in the experimental rats which might have accounted for the absence of refractoriness (23). However, pretreatment of rats with naloxone to block the stress response did not abolish the stimulatory effect of PGF in adipose tissue. This indicates that the ability to evoke an acute insulin-like response in intact rats is an intrinsic

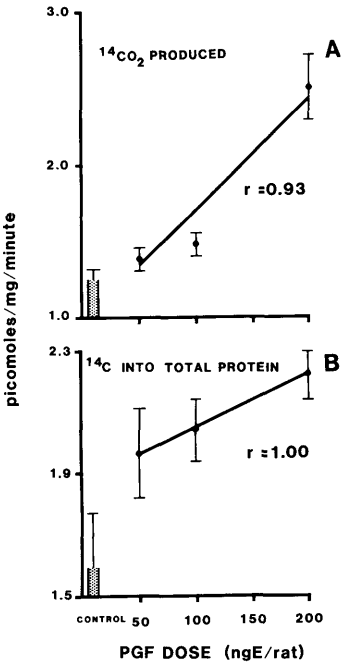

FIG. 7. Acute effects of injections of increasing doses of partially purified PGF on amino acid metabolism of intact rat muscular diaphragm. (A) [1-^{14}C]leucine oxidation to $^{14}CO_2$ and (B) [^{14}C]leucine incorporation into protein. Results are expressed as means ± SE of four observations. r = correlation coefficient. $P < 0.01$ for each dose of PGF vs the basal levels (by one-way ANOVA followed by Scheffe's test).

property of the PGF preparation and not due to the induction of stress. That PGF acts directly on tissue is shown by studies where purified PGF stimulated distinct insulin-like effects when added to adipose tissue from normal rats *in vitro* (39).

Another factor which could have influenced the ability of PGF to stimulate insulin-like responses in normal adult rats is associated with its ability to suppress endogenous GH (3, 8, 9). In the current experiments, it is possible that the injections of PGF caused a rapid reduction in secretion of pituitary GH and this was sufficient to remove or reduce tissue refractoriness to the insulin-like action of PGF. In argument against this, Goodman and Coiro (18) reported that tissues from rats with high blood levels of GH remained refractory to GH 4 hr after excision of the tissues. These authors further suggested that the period of refractoriness is longer than the interval between secretory episodes of GH and therefore normal rats remain insensitive to the insulin-like actions of GH. Therefore, it seems unlikely, even if PGF completely suppressed endogenous GH, that this could account for the lack of refractoriness in tissues removed one-half hour after administration of PGF.

Another important difference between the reported actions of GH and the results obtained with PGF in the present study was seen in the duration of the effects. Administration of GH to hypophysectomized rodents *in vivo* stimulates amino acid and sugar uptake of muscular diaphragm within the first hour of injection but this stimulatory effect disappears when the excised tissues are preincubated for 3 hr *in vitro* or removed from the animal 3 to 4 hr after the injection (31). In contrast to these transient insulin-like actions reported for GH the effects of PGF persisted even after the tissues were incubated for 4 hr *in vitro*.

At this point, it is unknown whether the complex actions of GH involve multiple receptors and/or multiple peptide sequences of the GH molecule with distinct activities. Others have concluded that induction of refractoriness by GH is independent of its ability to produce an insulin-like response (18). A GH-like molecule devoid of the peptide sequence responsible for induction of refractoriness could bind the GH receptor and stimulate insulin-like responses. Plerocercoid growth factor appears to be such a substance (39) as the current data support the hypothesis that the molecular sequence responsible for the induction of resistance to the insulin-like actions of GH is not found in the PGF molecule. While the structure of PGF has not been elucidated, support for this concept comes from the consistent observation that chronic treatment of animals with PGF produces growth with fattening (2, 7, 9, 15) and PGF does not stimulate lipolysis (2, 39).

In conclusion, our data suggest that PGF has intrinsic insulin-like activities but does not possess the ability to induce refractoriness to these activities.

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