SYNERGISTIC EFFECT OF PORCINE GROWTH HORMONE AND AN ADRENAL ENZYME INHIBITOR ON WEIGHT GAIN IN RATS

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SUMMARY

There is evidence to suggest that the anabolic effects of porcine growth hormone (pGH) might be constrained by a counteractive increase in the adrenal output of catabolic corticosteroid hormones. In the present study female rats were treated daily with pGH, with trilostane (an inhibitor of adrenal corticosteroid synthesis), or with a combination of pGH plus trilostane. In pGH-treated rats, liveweight gain was improved significantly (27–29%), however a significant increase (18%) in plasma concentrations of corticosterone was observed after 21 days. Treatment with trilostane alone had no significant effect on liveweight gain, but in combination with pGH, a synergistic effect was observed. Rats given the combined treatment gained 46% more weight than controls after 21 days of treatment, and this *response* was significantly greater than that to pGH alone. It is concluded that the anabolic effects of pGH are enhanced by concurrent administration of an adrenal inhibitor,

INTRODUCTION

There is considerable interest in the pharmaceutical industry in developing a porcine growth hormone (pGH) based growth promoter for *use* in swine production. We have shown that pGH markedly improves feed efficiency, increases muscle growth and decreases adipose tissue growth when given to pigs by daily injection (Chung *et al.* 1985; Etherton *et al.* 1987). However, in these studies it was observed that the effects of pGH on liveweight gain were less dramatic than expected and in contrast to the effects on carcass composition, growth rate was not improved in a dose-dependent fashion. This observation lead us *to* suspect that the growth *response* to pGH might be constrained by the action of a counteractive hormone, A substantial body of evidence suggests that the anabolic effects of GH can be antagonized by high concentrations of corticosteroid hormones. Corticosteroids are well known to be catabolic in muscle and can retard growth rate when given to young animals (McGrath *et al.* 1981; Young, 1970). In 1964, Soyka and Crawford observed that in children of short stature, the growth response to GH therapy could be arrested by cortisone injections. Furthermore, when chickens *or* rats are treated with high doses of GH, plasma concentrations of corticosterone are increased (Cheung *et al, 1988;* Cramer *et al.* 1977; Coyne *et al.* 1981). Trilostane, a selective inhibitor of adrenal corticosteroid synthesis, has been shown previously to lower plasma corticosterone concentrations and to improve growth rate in female rats (Sillence and Rodway, 1987). The aim of the present study was to determine whether the anabolic effect of pGH might be increased by concurrent administration of trilostane.

MATERIALS AND METHODS

Animals and treatments

Female Wistar rats (180 g) obtained from The Pennsylvania State University were housed in individual cages and allowed free access to a standard laboratory diet. A 12 h light: 12 h darkness cycle with lights on at 06:00 h was

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maintained. Animals were divided into blocks according to liveweight and were assigned randomly to treatment groups within each block.

In Experiment 1, ten animals *per* treatment were weighed and injected (s.c.) daily with trilostane (50 mg/kg); pGH (5 mg/kg); trilostane (50 mg/kg) plus pGH (5 mg/kg); *or* with vehicle (25 mM $NaHCO_3$, 25 mM Na_2CO_3, .154 M NaCl; pH 9.4) for ten days. Food intake, food conversion efficiency (g food eaten/g weight gain) and daily liveweight gain were recorded. To confirm the results *of* this experiment, the study was repeated over 21 days using a further 40 female rats (Experiment 2).

At the end of each experiment, trunk blood was collected and serum was assayed for corticosterone concentration. In experiment 2, the adrenal glands were removed, blotted dry and weighed soon after death. Carcass analysis was performed according to the method of Hartsook and Hershberger (1963).

Statistical analysis

Cumulative weight gain was calculated daily for each animal. A regression model was fitted to these data and the slopes estimated for each treatment group *were* contrasted. The method of Bonferroni was applied to adjust the estimated P value to the number of contrasts performed (Netter *et al.* 1985). Analysis of variance and Tukey's multiple comparison test were used to analyse all other parameters, except for plasma corticosterone concentrations which were compared using Student's t-test.

RESULTS

Changes in growth rate and food intake

The growth data are summarized in Table 1. Treatment with trilostane had no significant effect on growth rate. In experiment 1, animals treated with pGH alone gained 27% more body weight than controls after ten days ($P < 0.01$). This result was reproduced in experiment 2, with pGH-treated animals gaining 27% more body weight than controls over the first 10 days of treatment, and 29% more body weight than controls over 21 days of treatment ($P < 0.05$). In both experiments, rats treated with pGH plus trilostane gained significantly ($P < 0.05$) more body weight than rats in either the control group or in the pGH-treated group. After 21 days, rats given the combined treatment had gained 46% more weight than controls.

Table 1 Effects of trilostane, pGH, or pGH plus trilostane on weight gain (g/day), food intake (g/day) and food conversion efficiency (FCE: g food eaten/g weight gain) in female rats

	Control	Trilostane	pGH	Trilostane plus pGH
Experiment 1 (10 days)				
Liveweight gain[a]	3.33 ± 0.17^{b}	3.50 ± 0.17^{b}	4.22 ± 0.16^{c}	4.48 ± 0.16^{d}
Food intake	18.8 ± 0.57^{b}	20.0 ± 0.39^{b}	19.2 ± 0.35^{b}	19.6 ± 0.45^{b}
FCE	6.53 ± 0.60^{b}	6.60 ± 0.54^{b}	5.14 ± 0.36^{bc}	4.62 ± 0.13^{c}
Experiment 2 (21 days)				
Liveweight gain[a]	3.41 ± 0.16^{b}	3.47 ± 0.16^{b}	4.40 ± 0.09^{c}	4.66 ± 0.16^{d}
Food intake	20.2 ± 0.39^{bc}	19.0 ± 1.02^{b}	21.30 ± 0.45^{bc}	21.93 ± 0.17^{c}
FCE	7.67 ± 0.67^{b}	6.96 ± 0.43^{b}	5.95 ± 0.25^{c}	5.55 ± 0.21^{c}

[a] Estimated from the regression of weight gain v. time. r^2 = 0.93 Experiment 1; r^2 = 0.97, Experiment 2. ^{bcd} Means with diferent superscripts differ ($P<0.05$). Differences obtained by contrasts. All other comparisons made using Tukey's test. Results are expressed as means ± s.e.m.

Treatments caused no change in food intake over 10 days in experiment 1. In experiment 2, over 21 days, there were no marked changes in food intake, other than a slight reduction caused by trilostane treatment. However, this was counteracted when trilostane was given in combination with pGH. Treatment with pGH caused a significant ($P<0.05$) improvement in feed efficiency in experiment 2 only. The combined treatment improved feed efficiency in both experiments ($P<0.05$).

Changes in body composition and adrenal weight

Body composition was determined in experiment 2 (Table 2). All treatments tended to increase adenal weight, with significant changes ($P<0.05$) being observed in animals treated with trilostane, alone or in combination with pGH. Trilostane alone had no effect on body composition, whereas treatment with pGH, alone or in combination with trilostane, increased both carcass protein and water. Carcass lipid was not significantly affected by treatment.

Table 2 Effects of trilostane, pGH, or pGH plus trilostane on body composition (g) and adrenal weight (% body wt. x 10^{-2}) in female rats after 21 days of treatment.

	Control	Trilostane	pGH	Trilostane plus pGH
Adrenal wt.	2.49 ± 0.15^{a}	3.78 ± 0.20^{b}	3.31 ± 0.14^{ab}	3.92 ± 0.38^{b}
Gut content	17.33 ± 0.47^{a}	15.07 ± 1.14^{a}	18.89 ± 1.22^{a}	18.58 ± 1.11^{a}
Crude protein	41.34 ± 0.60^{a}	42.01 ± 1.08^{a}	45.44 ± 0.89^{b}	45.53 ± 0.70^{b}
Carcass lipid	22.86 ± 1.60^{a}	19.60 ± 1.00^{a}	18.73 ± 1.04^{a}	20.54 ± 1.52^{a}
Carcass water	144.5 ± 3.65^{a}	146.8 ± 2.80^{a}	160.6 ± 3.23^{b}	165.6 ± 2.04^{b}
Live weight	239.1 ± 5.11^{a}	235.8 ± 4.07^{a}	254.1 ± 5.79^{ab}	263.3 ± 3.67^{b}

Means in a row with different superscripts differ ($P<0.05$; Tukey's test). Results are expressed as means $\pm$ s.e.m.

Changes in plasma concentrations of corticosterone

Unfortunately, trilostane in the plasma of treated animals interfered with the radioimmunoassay used to measure plasma concentrations of corticosterone. Thus,meaningful results were obtained only for rats in the control and pGH-treated groups (Table 3). Treatment with pGH caused an apparent increase in plasma concentrations of corticosterone after 10 days (7%), and a significant ($P<0.05$) increase after 21 days (18%).

Table 3 Plasma corticosterone concentrations (nmol/l) in control and pGH-treated female rats after 10 days (Experiment 1) and 21 days (Experiment 2) of treatment

	Experiment 1		Experiment 2	
	Control	pGH	Control	pGH
Plasma corticosterone	297 ± 20	317 ± 18	364 ± 16	413 ± 22*

* Significantly different from control ($P<0.05$; Student's t-test). Results are expressed as means $\pm$ s.e.m.

DISCUSSION

The growth response to pGH is in agreement with previously published studies (Groesbeck *et al.* 1987). As in previous experiments using the same dose of pGH (Sillence and Etherton 1989), a small increase in plasma concentrations of corticosterone and a trend towards increased adrenal size were observed. However, when rats are implanted with GH-secreting tumors, adrenal size is tripled and basal plasma concentrations of corticosterone are increased markedly, as is the corticosteroid response to stress (Coyne *et al.* 1981). Thus, the trends observed in the present study most likely reflect weak inductive action of pGH on the adrenal. This effect seems to be amplified by a longer duration of treatment and might be more apparent at higher doses of pGH, or when treated animals are subjected to stress. The lack of growth response to trilostane is in contrast to the effects observed previously in female rats of a similar age (Sillence and Rodway, 1987). Previous studies have shown that male rats and young females are not responsive to the anabolic effects of trilostane, but that in older females growth may be stimulated by up to 30%. In the latter group of rats, corticosterone concentrations were not increased, despite marked adrenal hypertrophy (Sillence and Rodway, 1987). It has been noted in studies using the growth promoter trenbolone acetate, that anabolic responsiveness and adrenal sensitivity vary among strains of rats (Sillence *et al.* 1985). We have not investigated whether the degree of sensitivity of older female rats to trilostane varies among strains, but the results of the present study suggest this possibility. Trilostane and pGH in combination, acted synergistically to improve growth rate and this observation supports the hypothesis that growth can be held back by physiological concentrations of corticosteroid hormones. In rats, humans, and in many domestic species, females have higher plasma concentrations of corticosteroids than males. Sex differences in plasma cortisol concentrations have been given to explain the slower growth rate of young female cattle (Henricks *et al.* 1984) and sheep (Sillence *et al.* 1987). Furthermore, when cortisol concentrations in female sheep are lowered by the anabolic steroid trenbolone acetate, no adverse effects are observed (Sillence *et al.* 1987). Therefore, we conclude that inhibitors of adrenal activity may be of some benefit to the livestock industry by enhancing the growth promoting effects of GH, particularly in females.

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