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NOTE

Plasma Insulin-like Growth Factor-I Response to Cold Exposure in Barrows

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Abstract. Information about the plasma IGF-I concentrations in domestic animals in a cold environment is still limited. And mechanisms to change plasma IGF-I concentrations in cold environments are not fully elucidated. In this study, plasma insulin-like growth factor-I (IGF-I) in relation to plasma growth hormone (GH) and metabolite concentrations was investigated in pigs living at 20°C and at 4°C. Six pigs (Landrase breed; barrows, 118 days old, 51.0 ± 3.5 kg body weight) were maintained for 2 weeks at 20°C in a climatic room. Then a placebo or recombinant bovine GH (100 μg/kg body weight) was injected subcutaneously. Blood samples were taken through a catheter at −2, 0, 1, 2, 3, 4, 5, 6, 9, 12, 22, and 24 h after the injections. The same experiments were conducted on days 5 and 6 after the room temperature was changed to 4°C. Mean (± SD) basal plasma GH concentrations in pigs without bovine GH administration living at 20°C and at 4°C were 4.8 ± 1.7 ng/ml and 4.6±2.8 ng/ml, respectively. There were no significant differences between GH concentrations. On the other hand, the mean plasma IGF-I concentrations were 80.8 ± 25.1 ng/ml and 57. 3 ± 14.3 ng/ml respectively. Plasma IGF-I concentrations in pigs living at 4°C were significantly lower than in pigs living at 20°C ($P<0.05$). Plasma glucose and non-esterified fatty acid (NEFA) concentrations in pigs at 4°C were significantly higher than in pigs at 20°C ($P<0.05$). Blood urea nitrogen (BUN) concentrations in pigs at 4°C were also higher than in pigs at 20°C. In an experiment on GH administration, the plasma GH concentrations in pigs at both 20°C and 4°C were increased to the peak (49.1 ± 2.5 ng/ml and 43.0 ± 23.6 ng/ml, respectively) 2 h after the GH injection. They then gradually decreased to the basal level within 22 h. Plasma IGF-I concentrations were significantly increased 3 or 4 h after the GH injection, and they reached to the maximum 9 or 12 h after GH injection. No statistical significance was observed in the increase in the plasma IGF-I concentrations between pigs living at 20°C and at 4°C after GH injection. These results indicate that basal plasma IGF-I concentrations in pigs living at 4°C were lower than at 20°C. And the increase in the plasma IGF-I after the bovine GH injection were not different at the two environmental temperatures.

Key words: IGF-I, GH, Metabolite, Cold exposure, Pig

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GROWTH Hormone (GH) is essential to normal growth in young mammals. The growth promoting effect of GH is believed to be mediated mainly through insulin-like growth factor-I (IGF-I). Although GH is one of the important factors regulating the plasma IGF-I concentrations, other regulatory factors are known to influence these concentrations [1]. The nutritional status or energy intake of animals influences plasma IGF-I concentrations [2, 3, 4]. Recently the influences of the environmental temperature to plasma IGF-I concentrations were reported. Plasma IGF-I concentrations in pigs living at 10°C were lower than in pigs at 35°C [4]. On the other hand, plasma IGF-I concentrations of heifers were not affected

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in the cold environment [5]. Information about the plasma IGF-I concentrations in domestic animals in a cold environment is still limited and confused. Furthermore, the response of plasma IGF-I to the exogenous GH in cold environments is not studied yet to elucidate mechanisms to change plasma IGF-I concentrations in cold environments.

In this study, plasma IGF-I concentrations in relation to plasma GH, glucose, non-esterified fatty acid (NEFA), and blood urea nitrogen (BUN) concentrations were investigated in pigs living at 20°C and at 4°C. And the plasma IGF-I concentrations after a single GH injection were measured at two different environmental temperatures (20°C or 4°C).

Materials and Methods

Experimental animals

Six pigs (Landrase breed: barrows, 118 days old, 51.0 ± 3.5 kg body weight at the beginning of the experiments) were surgically fitted with jugular vein catheters. These pigs were kept in the climatic room and fed at 0900 h and 1700 h. The feeding level was set at 4% of body weight and the diet for fattening pigs, which was formulated at the direction of our institute, was used through the series of experiments (TDN 70.1%, DCP 12.7%).

Experiment at 20°C

Six pigs were kept at 20°C for 2 weeks before the experiments. At 1100 h, 10 m*l* of carbonate buffer (pH 9.5) as the placebo was injected subcutaneously and blood samples were taken −2, 0, 1, 2, 3, 4, 5, 6, 9, 12, 22, 24 h after the injection. On the next day, 100 μg/kg body weight of recombinant bovine GH (rbGH: provided by Eli Lilly Co.) was injected subcutaneously at 1100 h and blood samples were taken at the same time in the day. Samples were taken into tubes containing heparin and chilled immediately in ice until centrifuged. Plasma samples were stored at −80°C until hormone assays.

Experiment at 4°C

After the experiment at 20°C, the environmental temperature was lowered to 4°C. On the 5th and 6th days after the temperature was changed, the same experiments as described above were conducted.

Assay procedure

To determine the basal plasma GH concentrations, porcine GH (pGH) radioimmunoassay (RIA) was employed. This has been described elsewhere [6].

To determine the plasma GH concentrations after rbGH injections, bovine GH (bGH) RIA was employed. This also has been described before [7].

Plasma IGF-I was extracted with acid ethanol and its concentration was determined by the RIA described before [8]. Standard curves were constructed with the International Reference Reagent of Insulin-like Growth Factor-I distributed by the National Biological Standard Board [9]. The amino-acid sequence of porcine IGF-I deduced from cloned complementary DNAs is identical to that of human and bovine IGF-I [10]. Porcine plasma and its dilutions indicated a parallel with the IGF-I standard curve.

Plasma glucose, NEFA and BUN concentrations were measured with assay kits (Glucose B-test Wako, NEFA-test Wako, and BUN B-test Wako, respectively).

Statistical analyses

Statistical analyses were performed with the statistical software package: SAS (release 6.07.02, 1989, by SAS Institute). Means and standard deviations (SD) for plasma GH, IGF-I and metabolite concentrations in 6 pigs were calculated and expressed as the means ± SD. Paired *t*-test was used to assess the statistical significance of hormone and metabolite levels. Data were subjected to a repeated measures analysis of variance (ANOVA) to assess the basal hormonal levels at the two temperatures.

The basal plasma IGF-I concentration at each environmental temperature was subtracted from the plasma IGF-I concentration at the corresponding time after the rbGH injection, to analyse the increase in the plasma IGF-I concentration after the rbGH injection. Then a repeated measures ANOVA was used to assess the difference in the increase in the plasma IGF-I concentration after rbGH injection at the two different environmental temperatures.

Results

Basal plasma GH concentrations (without bovine GH injection), measured by porcine GH RIA, were not changed by temperature (Fig. 1). Mean plasma GH concentrations were 4.8 ± 1.7 ng/ml in pigs living at 20°C and 4.6 ± 2.8 ng/ml at 4°C. There was no significant difference at the two different temperatures.

On the other hand, plasma IGF-I concentrations in pigs living at 4°C were lower than those at 20°C (Fig. 1). The mean plasma IGF-I concentrations were 80.8 ± 25.1 ng/ml at 20°C and 57.3 ± 14.3 ng/ml at 4°C. ANOVA test showed statistical significance ($P<0.05$).

Plasma glucose and NEFA concentrations in pigs living at 4°C were significantly higher than those at 20°C ($P<0.05$: ANOVA test) (Fig. 2). BUN concentrations in pigs living at 4°C were not greatly increased, compared with those at 20°C, although the BUN concentrations in pigs living at 4°C were significantly higher than those at 20°C at several times in the day ($P<0.05$: paired-t test) (Fig. 2).

Plasma GH concentrations after rbGH injection, measured by bovine GH RIA, significantly increased within one hour after GH injection. Plasma GH concentrations at the peak were 49.1 ± 2.5 ng/ml at 20°C and 43.0 ± 23.6 ng/ml at 4°C 2 h after rbGH injection. They then gradually decreased and returned to the basal level within 22 h (Fig. 3).

Plasma IGF-I concentrations after rbGH injection increased slowly (Fig. 4). They reached significantly higher levels 3 (4°C) or 4 h (20°C) after rbGH injection, compared with control levels (placebo injection). These significantly higher levels were maintained to the end of the experiment. The peak IGF-I concentrations were 162.8 ± 12.4 ng/ml at 20°C 12 h after GH injection and 131.2 ± 29.5 ng/ml at 4°C 9 h after GH injection. Plasma IGF-I concentrations at 4°C were lower than those at 20°C throughout the experiment, and significantly lower at the four points indicated by small arrow heads. The increases in the plasma IGF-I concen-

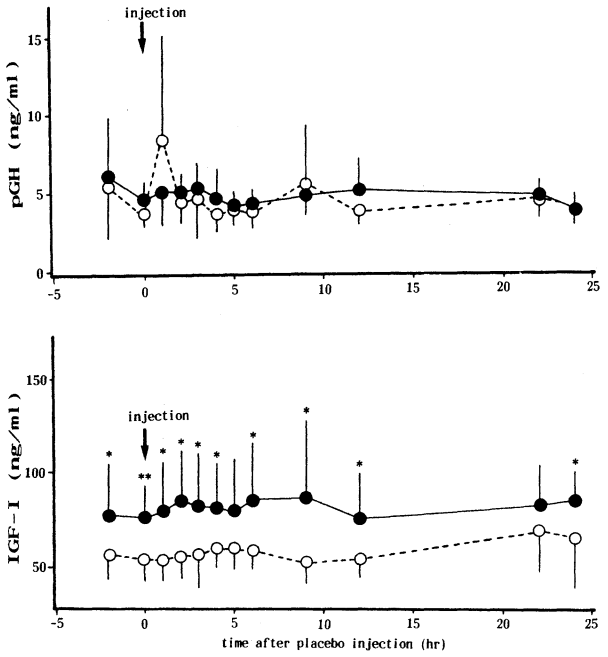

Fig. 1. Basal plasma pGH (upper) and IGF-I (lower) concentrations (without rbGH injection) for 24 h in pigs living at 20°C (solid line, ●) and in pigs living at 4°C (broken line, ○). Arrows show the time of placebo injection. Asterisks indicate the statistical differences; * $P<0.05$ and ** $P<0.01$, at 20°C and 4°C. Each point with a vertical bar represents the mean ± SD, n=6.

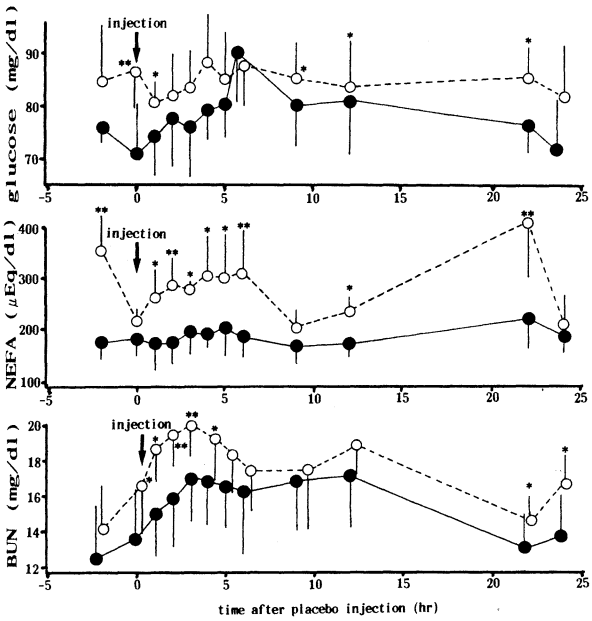

Fig. 2. Basal plasma glucose (upper), NEFA (middle), and BUN (lower) concentrations (without rbGH injection) for 24 h in pigs living at 20°C (solid line, ●) and in pigs living at 4°C (broken line, ○). Arrows show the time of placebo injection. Asterisks indicate the statistical differences; * $P<0.05$ and ** $P<0.01$, at 20°C and 4°C. Each point with a vertical bar represents the mean ± SD, n=6.

tration after GH injection was similar at both temperatures. ANOVA test did not show a statistical significance between temperatures.

Discussion

Our results demonstrated that plasma IGF-I concentrations in pigs living at 4°C for 5 days were lower than those in pigs living at 20°C. It was reported previously that plasma IGF-I concentrations in pigs living at 10°C for 2–3 weeks were lower than those at 35°C [4]. Our experimental conditions were severer (4°C) and more acute (for 5 days). For this reason, it is difficult to conclude that the same mechanisms reduced the plasma IGF-I concentrations.

GH is known to be one of the important factors increasing the plasma IGF-I concentrations, but it is difficult to explain the difference between the basal plasma IGF-I levels at 20°C and 4°C by the difference in the plasma GH levels, because no significant difference in the plasma GH concentration was found between pigs living at 20°C and at 4°C.

The nutritional conditions of animals is also known to be one of the important factors modulating the plasma IGF-I concentration. In pigs living in a cold environment, the heat production is increased and the energy demand is also increased [4]. The amount of energy available for growth is also decreased in animals living in a cold environment and the plasma IGF-I concentration is decreased, even though the same amount of energy is provided in feeding. The reduced responsiveness to exogenous GH during fasting or low energy intake was reported earlier [3]. If the reduced plasma IGF-I concentration in pigs living at 4°C is due to the low energy intake compared with their energy demand, the response of IGF-I to the exogenous GH may be reduced. But our data showed

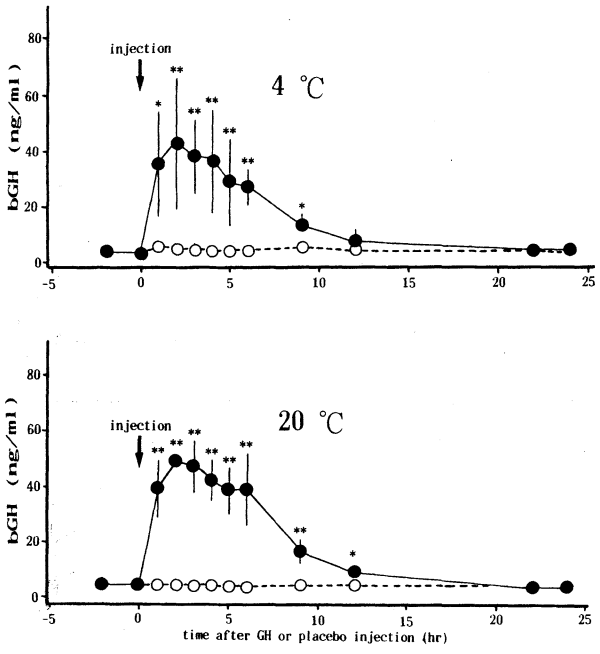

Fig. 3. Plasma bGH concentrations for 24 h after rbGH (solid line, ●) or placebo injection (broken line, ○) in pigs living at 4°C (upper) and in pigs living at 20°C (lower). Arrows show the time of injection. Asterisks indicate the statistical differences; * $P<0.05$ and ** $P<0.01$, for GH and placebo injection. Each point with a vertical bar represents the mean ± SD, n=6.

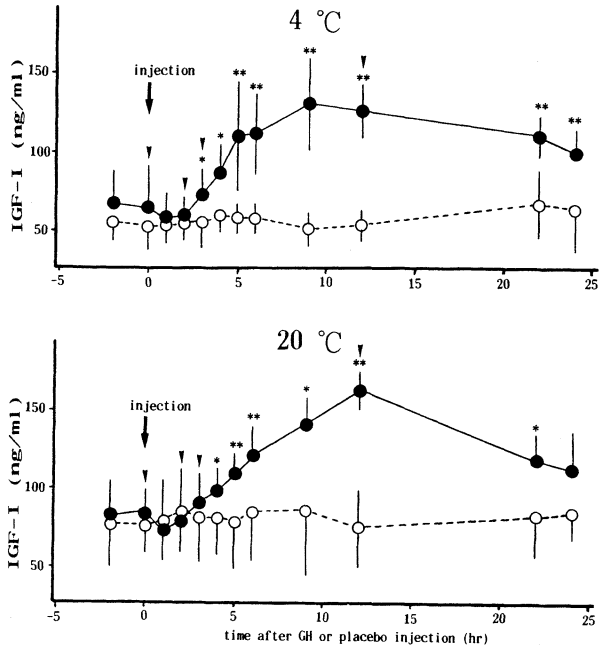

Fig. 4. Plasma IGF-I concentrations for 24 h after rbGH (solid line, ●) or placebo injection (broken line, ○) in pigs living at 4°C (upper) and in pigs living at 20°C (lower). Arrows show the time of injection. Asterisks indicate the statistical differences; * $P<0.05$ and ** $P<0.01$, as compared for GH and placebo injection. Small arrow heads indicate the statistical differences; † $P<0.05$, as compared at 4°C and 20°C for pigs which received an rbGH injection. Each point with a vertical bar represents the mean ± SD, n=6.

that the increase in the plasma IGF-I concentration after exogenous GH injection in pigs living at 4°C was not decreased, in comparison with the elevation in pigs at 20°C. These data showed that the energy status of the experimental animals was similar under both conditions. It may therefore be difficult to simply attribute the low plasma IGF-I concentration in the cold environment to increased energy demand.

Plasma glucose and NEFA concentrations were different in the pigs living at 20°C and at 4°C (Fig. 2). The BUN concentrations also changed at certain times in the day (Fig. 2). These changes in metabolites showed the changes of energy metabolism in the pigs when living at 20°C and at 4°C. The changes in metabolites or hormone balance causing these changes might influence the plasma IGF-I concentration.

Increased plasma noradrenaline and adrenaline concentrations were reported in pigs in the cold environment [11]. The plasma noradrenaline concentration increased within 4 h after the environmental temperature was lowered. It is well-known that the increased plasma noradrenaline and adrenaline concentrations increase the plasma glucose and NEFA concentrations in animals in the cold environment. Plasma glucose and NEFA concentrations in pigs living at 4°C were higher than at 20°C in our data. Recently it was reported that α_2-adrenergic agonist affected the plasma IGF-I concentration in sheep [12]. Plasma IGF-I concentrations were gradually decreased during the infusion of α_2-adrenergic agonist, although plasma GH concentrations during the infusion were increased. An increase in plasma catecholamines may therefore decrease the plasma IGF-I concentrations in pigs living at 4°C, although further studies are necessary to clarify the role of catecholamine in IGF-I production.

In conclusion, plasma IGF-I concentrations in pigs living at 4°C for 5 days were lower than in pigs living at 20°C, even though plasma GH concentrations were similar at both the environmental temperatures. And it may be difficult to satisfactorily explain the low plasma IGF-I concentrations in the cold environment by increased energy demand, because the increase in the plasma IGF-I concentration after the bovine GH administration was similar at both environmental temperatures.

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