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Effect of α_2-Adrenergic Agonist Clonidine on Plasma Growth Hormone and Insulin-like Growth Factor-I Concentrations in Barrows

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Abstract. The effects of infusion of clonidine, an α_2-adrenergic agonist, on plasma insulin-like growth factor-I (IGF-I) concentrations were investigated with a single growth hormone (GH) injection in pigs. Four barrows were subjected to four treatments: saline infusion with a vehicle injection, clonidine infusion (0.5 nmol/kg/min for 8 h) with a vehicle injection, saline infusion with a bovine GH (bGH: 100 μg/kg) injection, and clonidine infusion with a bGH injection. Infusion was started 1 h before the injection. Plasma IGF-I, bGH, porcine GH (pGH), insulin, glucose, non-esterified fatty acid (NEFA), and blood urea nitrogen (BUN) concentrations were measured. Plasma IGF-I concentrations during saline infusion increased after a bGH injection ($P<0.05$). However, the IGF-1 concentrations during clonidine infusion did not increase after the bGH injection. Plasma endogenous GH (pGH) was not increased during clonidine infusion. The plasma glucose concentration was noticeably increased during clonidine infusion and moderately increased after the GH injection. Despite the extreme increase in plasma glucose during clonidine infusion, plasma insulin did not change. Neither plasma NEFA nor BUN was changed by these treatments. These results demonstrate that the α_2-adrenergic agonist clonidine altered the action of GH to increase the plasma IGF-I concentrations.

Key words: α_2-Adrenergic agonist, IGF-I, GH, Metabolites, Pig

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GROWTH hormone (GH) is one of the most important factors regulating the plasma insulin-like growth factor-I (IGF-I) concentration [1]. The nutritional status of animals also influences the plasma IGF-I level [2, 3]. Recently the effect of environmental temperature on plasma IGF-I has also been reported [3, 4]. Plasma IGF-I levels in pigs living at 4 °C were lower than in pigs at 20 °C, although plasma GH levels were similar in pigs in both environments. Plasma glucose and non-esterified fatty acid (NEFA) concentrations were significantly increased [4] in pigs at 4 °C. These increases in metabolites are caused by increased plasma noradrenaline and adrenaline levels in cold environments, as reported previously in pigs [5]. Additionally, thermogenesis also increases in cold environments, and this is assisted by the action of peripheral catecholamines, but the reason why plasma IGF-I concentrations decrease in a cold environment has not yet been elucidated.

Recently it was reported that the α_2-adrenergic agonist clonidine decreased plasma IGF-I concentrations in sheep [6]. Plasma IGF-I concentrations gradually decreased during the clonidine infusion, being significantly lower 3 h after infusion than the control, although the plasma GH concentration during infusion was increased. It is known that clonidine increases the secretion of GH in several species [6, 7, 8]. Despite its GH releasing activity, however, there are no reports of studies

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on the growth promoting effects of clonidine and other α_2-adrenergic agonists. Moreover, in mice, the administration of a long-acting α_2-adrenergic agonist, guanfacine, resulted in weight loss rather than growth stimulation [9], although plasma IGF-I concentrations were not determined in this study. According to these data, it is likely that the increase in plasma noradrenaline and adrenaline concentrations in cold environments may decrease the plasma IGF-I concentration.

In the present study, the effect of clonidine on plasma IGF-I concentrations was investigated in relation to the plasma GH concentration in pigs, which may have a different sensitivity to the α_2-adrenergic agonist from that in sheep. Plasma insulin, glucose, NEFA, and blood urea nitrogen (BUN) concentrations were also measured in order to compare with the response to acute cold exposure in pigs. The effect of GH administered during clonidine infusion on plasma IGF-I concentrations was also studied in order to clarify whether the action of GH was changed by clonidine or not.

Materials and Methods

Animals and experimental design

Four pigs (Landrace × Yorkshire × Duroc cross, barrows, 81 days old, 24.2 ± 1.6 kg body weight at the beginning of the experiments) were surgically fitted with a jugular vein catheter. These pigs were maintained at 20 °C and fed at 0900 h and 1700 h. The feeding level was set at 4% of body weight. The diet for fattening the pigs, which was formulated by our institute, was used throughout the experiments (TDN 70.1%, DCP 12.7%). The pigs were assigned randomly to one of four treatment sequences in 4 × 4 Latin squares. The four treatments were (1) saline infusion with a vehicle injection as control, (2) clonidine infusion with a vehicle injection, (3) saline infusion with a bovine GH (bGH) injection, and (4) clonidine infusion with a bGH injection.

Clonidine (Wako Pure Chemical Co.) was dissolved in saline, and the infusion rate was 0.5 nmol/kg BW/min. Infusions were started at 1000 h and continued for 8 h up to 1800 h. Ten milliliters of carbonate buffer (pH=9.5) was used as the vehicle for the bGH (100 μg/kg BW, provided by Eli Lilly Co.) and the vehicle and was injected subcutaneously at 1100 h. Blood samples were taken −2, −1, 0, 1, 2, 3, 4, 5, 6, and 7 h relative to the bGH injection. Samples were placed in tubes containing heparin and were chilled immediately on ice until centrifugation. Plasma samples were stored at −80 °C prior to hormone assays.

Assays for plasma hormones

To determine the basal level of plasma GH, a porcine GH (pGH) radioimmunoassay (RIA) was employed, which is described elsewhere [10].

In order to determine the plasma GH concentrations after the bGH injections, a bovine GH (bGH) RIA was employed, which has also been described before [11].

Plasma IGF-I was extracted with acid ethanol, and its concentration was determined by RIA as described before [4].

Plasma insulin concentrations were determined with a specific RIA kit (Eiken Chemical Co.).

Assays for plasma metabolites

Plasma glucose, NEFA, and BUN were determined with specific assay kits (Glucose B-test Wako, NEFA-test Wako, and BUN B-test Wako, respectively; Wako Pure Chemical Co.).

Statistical analyses

Statistical analyses were performed with the statistical software package, SAS (release 6.07.02, 1989, by SAS Institute). Plasma hormone and metabolite concentration means and standard errors (SEM) were calculated by the means procedure of SAS and expressed as the mean ± SEM. The paired *t*-test was used to assess the statistical significance of plasma hormone and metabolite concentrations between the saline infusion with a vehicle injection (control treatment) and the other treatments, using the means procedure. Data were also subjected to a repeated measures analysis of variance (ANOVA) by the GLM procedure of SAS to assess the effect of the treatments.

Results

Plasma pGH concentrations during the clonidine infusion with a vehicle injection did not signifi-

cantly increase (repeated measures ANOVA test) (Fig. 1, lower). Plasma bGH concentrations after the bGH injection, measured by bGH RIA, significantly increased within 1 h after the injection compared with the control ($P<0.05$) (Fig. 1, upper). Plasma bGH reached a peak 2 h after the injection. Peak concentrations of bGH following injection were 36.4 ± 5.4 ng/m*l* during the saline infusion and 39.1 ± 3.5 ng/m*l* during the clonidine infusion. The decline in bGH concentrations following peak values was then gradual. There were no significant differences in the bGH concentrations between the two treatments.

Plasma IGF-I concentrations during the saline infusion 4 h after the injection of bGH were significantly greater than for the control ($P<0.05$) (Fig. 2, upper). However, there was no such increase in plasma IGF-I concentrations following the bGH injection during the clonidine infusion when compared with either the control or the clonidine infusion with a vehicle injection. The ANOVA for repeated measures made possible a statistical comparison of the IGF-I profiles for the saline and clonidine infusions following the bGH injections. The plasma IGF-I concentration during the clonidine infusion with a vehicle injection did not significantly change compared with the control.

Plasma glucose concentrations were noticeably increased during the clonidine infusion and were moderately increased after the bGH injection (Fig. 3, upper). Both effects were also observed when clonidine was infused with the bGH injection. Despite the remarkable increase in plasma glucose levels during the clonidine infusion, plasma insulin concentrations did not change (Fig. 2, lower). Plasma NEFA and BUN concentrations did not change significantly in any treatment (Fig. 3, middle and lower).

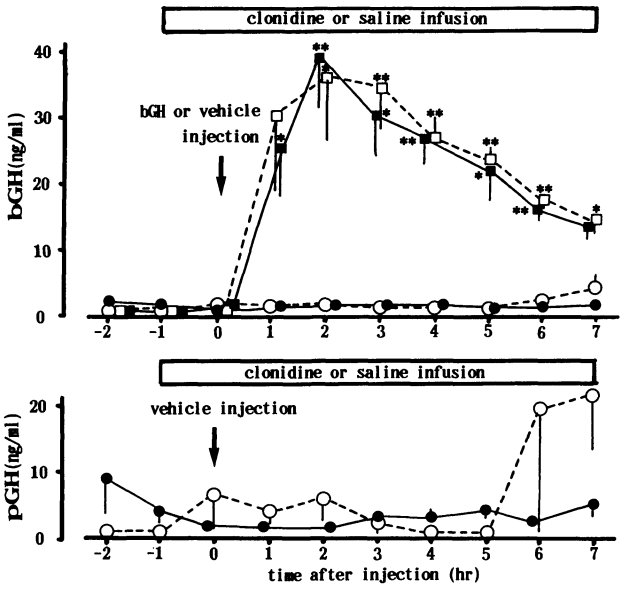

Fig. 1. Plasma bGH (upper) and pGH (lower) concentrations in pigs during saline infusion with a vehicle injection (control: broken line, ○), clonidine infusion (0.5 nmol/kg/min) with a vehicle injection (solid line, ●), saline infusion with a b GH injection (100 μg/kg, subcutaneously: broken line, □), and clonidine infusion with a bGH injection (solid line, ■). Arrows show the time of vehicle or bGH injection. Asterisks indicate the statistical significance; *$P<0.05$ and **$P<0.01$, compared with control. Each point with a vertical bar represents the mean ± SEM, n=4.

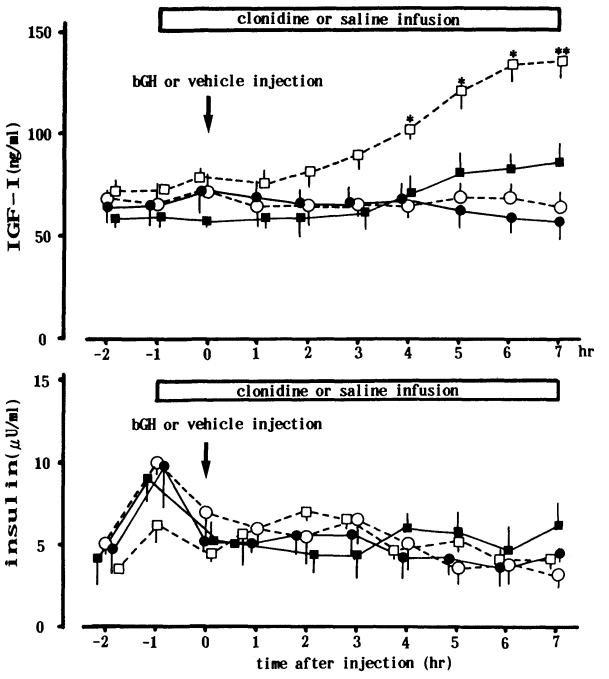

Fig. 2. Plasma IGF-I (upper) and insulin (lower) concentrations in pigs during saline infusion with a vehicle injection (control: broken line, ○), clonidine infusion (0.5 nmol/kg/min) with a vehicle injection (solid line, ●), saline infusion with a bGH injection (100 μg/kg, subcutaneously: broken line, □), and clonidine infusion with a bGH injection (solid line, ■). Arrows show the time of vehicle or bGH injection. Asterisks indicate the statistical significance; *$P<0.05$ and **$P<0.01$, compared with control. Each point with a vertical bar represents the mean ± SEM, n=4.

Discussion

Clonidine infusion suppressed the increase in plasma IGF-I caused by bGH injection in pigs. As far as we know, there is no report which mentions that the action of GH is modified by clonidine. There is only one report mentioning the relationship between clonidine and plasma IGF-I concentrations [6], in which plasma IGF-I gradually decreased during clonidine infusion in sheep. This decrease in plasma IGF-I concentrations during clonidine infusion was probably mediated by decreased sensitivity to GH in IGF-I producing tissues.

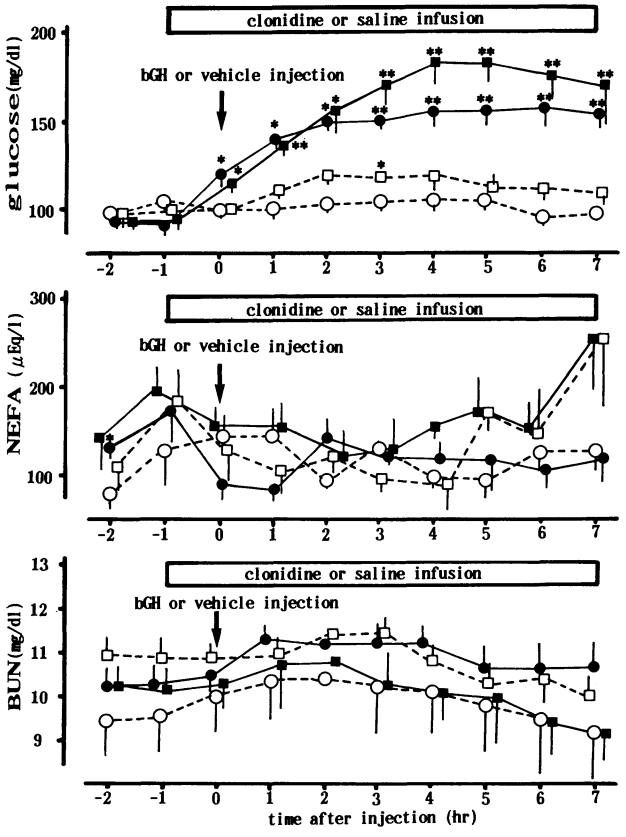

Fig. 3. Plasma glucose (upper), NEFA (middle), and BUN (lower) concentrations in pigs during saline infusion with a vehicle injection (control: broken line, ○), clonidine infusion (0.5 nmol/kg/min) with a vehicle injection (solid line, ●), saline infusion with a bGH injection (100 μg/kg, subcutaneously: broken line, □), and clonidine infusion with a bGH injection (solid line, ■). Arrows show the time of vehicle or bGH injection. Asterisks indicate the statistical significance; $^{*}P<0.05$ and $^{**}P<0.01$, compared with the control. Each point with a vertical bar represents the mean ± SEM, n=4.

In the present series of experiments, clonidine infusion alone did not significantly decrease plasma IGF-I, although plasma IGF-I was decreased in sheep administered clonidine at the same rate [6]. Plasma GH concentrations increased during clonidine infusion in the experiment on sheep, although plasma GH in pigs did not increase in our experiment. Increases in plasma GH caused by clonidine have also been reported before in other species [7, 8].

To explain the difference in the reactions of plasma IGF-I and GH to the infusion of clonidine between pigs and sheep, the sensitivity of the two species of animals to the adrenergic agonist should be considered. Clonidine has a sedative effect, which is antagonized by an α-adrenoceptor antagonist [12]. A related drug, xylazine, is a very popular sedative in veterinary medicine. The sedative action of xylazine, which is antagonized by an α_2-adrenoceptor antagonist [13], is different between pigs and sheep. Ruminants (cattle, sheep or goats) are much more sensitive to xylazine than pigs. Pigs require 20 to 30 times the ruminant dosage for equivalent sedation [14]. Plasma IGF-I and GH concentrations did not therefore change during clonidine infusion alone, because the sensitivity to the clonidine may be different between pigs and sheep.

The GH secreting and sedative actions of clonidine directly affect the central nervous system [12, 15]. The decrease in the ability of GH to increase plasma IGF-I concentrations during clonidine infusion is probably due to an effect on the liver, because the liver is a major site of plasma IGF-I production [16]. Both the central nervous system and the liver in pigs might be less sensitive to clonidine than in ruminants, but it is also possible that IGF-I production in the liver is partly controlled by the nervous system, for example, the sympathetic nervous system.

Plasma noradrenaline and adrenaline concentrations increase in cold environments [5], and this may decrease the plasma concentration of IGF-I. The sympathomimetic status in cold environments is not usually suitable for animals to grow. The decrease in the growth rate under these conditions is therefore probably caused by a decrease in plasma IGF-I levels, but it is unlikely that only the α_2-adrenergic effects of adrenaline or noradrenaline control the plasma concentrations of hormones

and metabolites in cold environments. The change in the metabolic status in cold environments probably influences other plasma hormones and metabolites. In this series of experiments, glucose concentrations during clonidine infusion were extremely high, whilst NEFA concentrations were not changed. Such NEFA and glucose concentrations are very different from glucose and NEFA concentrations caused by cold exposure [4]. The present experiments therefore demonstrate a different response between cold exposure and clonidine infusion. Further investigations are necessary to elucidate the decrease in plasma IGF-I in cold environments and the role of adrenergic hormones on plasma IGF-I levels and animal growth.

In conclusion, the α_2-adrenergic agonist clonidine altered the action of GH to increase the plasma IGF-I concentration, although clonidine infusion alone did not decrease plasma IGF-I concentrations significantly. These data indicate the possibility that a sympathomimetic status, such as cold exposure, may decrease the plasma IGF-I concentration in pigs.

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