Effect of Exogenous Porcine GH Administration on GH Responses to GH-releasing Peptide-2 and GH-releasing Hormone in Swine

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ABSTRACT : Negative feedback on GH responses to GH-releasing hormone (GHRH) and GH-releasing peptides (GHRPs) has been reported and this action has been suggested to act through an increase in somatostatin. To determine whether the acute administration of porcine GH (pGH) inhibits GH responsiveness to GHRP-2 and GHRH in swine, swine were intravenously administered with pGH (5 μg/kg BW) or placebo followed 180 min later by a second intravenous administration of saline, GHRP-2 (30 μg/kg BW), GHRH (1 μg/kg BW) and a combination of GHRP-2 and GHRH. Plasma GH concentration was measured by radioimmunoassay. Administration of pGH caused a significant increase in GH area under curve and GH peak concentrations ($p<0.001$) over placebo-treated group. Plasma GH concentrations peaked at 15 min and returned to baseline level within 90 min. Pretreatment of pGH abolished ($p<0.01$) GH response to GHRH and attenuated ($p<0.05$) GH response to GHRP-2 and GHRH combined, without affecting GH response to GHRP-2. These results demonstrate that negative feedback action on GH releasing effect of GHRH occurs in swine, and that GHRP-2 has ability to interact in this action. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 8 : 1188-1192)***

Key Words : GH-releasing peptide-2 (GHRP-2), GH-releasing hormone (GHRH), GH Feedback

INTRODUCTION

Growth hormone regulates its own secretion by a feedback mechanism operating both on the central nervous system and the pituitary (Muller et al., 1991). Direct evidence indicated that *in vivo* or *in vitro* administration of exogenous GH decreased pituitary GH-releasing hormone (GHRH) mRNA, and increased somatostatin (SRIF) secretion from the hypothalamus in rats (Chihara et al., 1981). In humans and rats, administration of exogenous GH blunted GH response to GHRH (Ross et al., 1987; Lanzi and Tannenbaum, 1992). Several data indicated that SRIF-mediated mechanism accounted for the short-term negative GH autofeedback (Lanzi and Tannebaum, 1992).

A series of small peptides, known as GH-releasing peptides (GHRPs), have been synthesized that selectively stimulate GH secretion (Bowers, 1993). One of these, GHRP-2 (known as KP102) is well known to markedly increase GH secretion both *in vivo* and *in vitro* (Sawada et al., 1994; Roh et al., 1997a). The GH-releasing effect of the peptide is two to three folds more effective than GHRP-6 and GHRP-1 in rats and humans (Bowers, 1993). In domestic animals, GHRP-2 has been reported to release GH secretion, including swine (Hashizume et al., 1997; Phung et al., 2000). GHRP-2 appeared to interact SRIF effect in men (Meacham et al., 1999), potently stimulated GH release by direct or indirect antagonism of SRIF, by releasing GHRH, and by a direct action on pituitary *in vivo* in rats (Sawada et al., 1994; Nakagawa et al., 1996). The peptide acted synergistically with GHRH in releasing GH in swine (Phung et al., 2001) and the synergistic effect on GH release appeared to be depending on the antagonism of SRIF by GHRP-2 in urethan-anesthetized rats (Sawada et al., 1994).

In the present study, we have investigated the effect of acute exogenous porcine GH (pGH) administration on GH responses to GHRP-2, GHRH, and a combination of both peptides.

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METERIALS AND METHODS

Animals

Eight cross-bred (Landrace×Large white×Duroc) barrows, 160 days of age, and weighing 86.2±1.6 kg (mean±SE) were used in this study. The swine were housed in individual pens for the entire study and fed *ad libitum* twice daily at 09:00h and 17:00h on a commercial diet for growing swine (16% crude protein, 2.5% crude fat, 5% crude fiber and 7% crude ash). Animals had free access to water at all times. Two days before initiation of treatment, all swine were anesthetized and indwelling catheters were inserted (Smith and Ficken, 1991; Phung et al., 2000). Experimental protocol and animal care procedures were approved by the Animal Care and Use Committee of Obihiro University.

GHRP-2, GHRH, pGH and method of administration : GHRP-2, D-Ala-D-βNal-Ala-Trp-D-Phe-Lys-NH_2, was generously provided by Kaken Pharmaceutical Co. Ltd. Japan. hGHRH (amide, Ares-Sereono, lot: 01) and recombinant pGH (Monsanto, lot: FGHRH1299701)

obtained from Dr. A. F. Parlow (National Hormone and Pituitary program, Harbor-UCLA Medicine Center, Torrance, CA). GHRP-2 and GHRH were dissolved in 0.9% sterile saline. Porcine GH was dissolved in placebo (25 mM $NaHCO_3$, 25 mM Na_2CO_3, 0.154 M NaCl, pH 9.4) (Sillence and Etherton, 1987). These preparations were made a day before treatment and stored at 4°C for intravenous (i.v.) administration via indwelling catheter.

Experimental Design : Animals were allocated into two groups, control and treatment, of 4 swine each. Control and treatment of swine received single i.v administration of placebo and pGH (5 μg/kg BW), respectively, at 09:00h in equal volume of 5 ml. Three hours after the administration of placebo and pGH, all swine were under i.v. administration of saline, GHRP-2 (30 μg/kg BW) (Phung et al., 2000), GHRH (1 μg/kg BW) (Phung et al., 2001), or a combination of GHRP-2 and GHRH. The experimental animals were not given feed for 16 h before and during the experiment, and were under treatment administration at 2-d intervals. A dose of 5 μg/kg BW of pGH was assumed to increase plasma porcine GH by returning to baseline level within 3 hours post-administration (Sillence and Etherton, 1987). Blood samples were taken at -195 min and -180 min, and then every 15 min for 3 h after i.v. administration of placebo or pGH at -180 min. After administration of saline, GHRP-2, GHRH, and GHRP-2 and GHRH combined at 0 min, blood samples were taken at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120 min. Blood samples were collected via indwelling jugular catheters into centrifuge tubes containing heparin (10 IU/ml) and chilled with ice. Individual plasma samples were obtained after centrifugation at 3,000 rpm at 4°C and stored at -20°C until being assayed for GH concentrations.

GH radioimmunoassay (RIA) : Plasma pGH concentration was measured in duplicate by double-antibody RIA procedure. Normal monkey serum was kindly provided by Primate Research Institute, Kyoto University, Japan. Goat anti-monkey IgG serum (HAC-MKA2-02GTP88) was supplied by Biosignal Research Center Institute for Molecular and Cellular Regulation, Gunma University, Japan. pGH antiserum (Lot AFP-10318545) and pGH (Lot AFP-10864), were obtained from Dr. A. F. Parlow (National Hormone and Pituitary program, Harbor-UCLA Medicine Center, Torrance, CA). Na [^{125}I] was purchased from Amersham (code IMS30). pGH was used as a reference standard and radioiodinated by the chloramine-T method. The process of measuring plasma pGH concentration was conducted as previously described (Phung et al., 2000). The assay of plasma samples obtained from control and treatment groups of swine of each treatment administration were run at the same time. Sensitivity was 0.1 ng/ml and intra-assay and inter-assay coefficients of variation were 5.74% and 11.13%, respectively.

Statistical analysis : GH AUC is the area under the curve of the plasma GH concentration responded after saline or peptides administration. The GH AUC was calculated using the trapezoidal method. Difference in means of GH peak concentrations and GH AUCs after administration of saline, GHRH, GHRP-2, and GHRP-2 and GHRH combined within and between groups of swine were analyzed by student's *t-test* and paired *t-test*, respectively. Values of $p<0.05$ were considered statistically significant. All values are expressed as mean±SE.

RESULTS

Effect of acute administration of pGH or placebo on GH

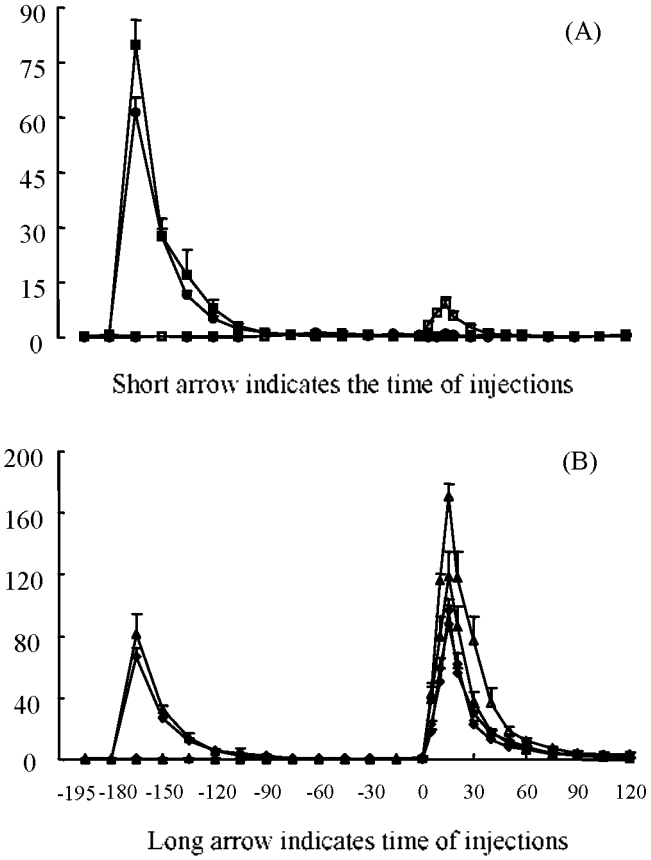

Figure 1. Effect of pGH administration on plasma GH response to GHRH, GHRP-2, and a combination of GHRH and GHRP-2. Animals were injected with placebo and pGH (5 μg/kg BW) at −180 min in control and treatment groups, respectively (short arrow indicates the time of injections), and then both groups of swine were bolus i.v. administered with saline or peptides at time 0 min (Long arrow indicates time of injections). (A): saline (○ Control, ● Treatment) or 1 μg/kg BW of GHRH (□ Control, ■ Treatment). (B): 30 μg/kg BW of GHRP-2 (◇ Control, ◆ Treatment) or a combination of 30 μg/kg BW of GHRP-2 and 1 μg/kg BW of GHRH (△ Control, ▲ Treatment). Values are expressed as mean±SE.

Table. GH response to the i.v. administration of saline, GHRH, GHRP-2, and a combination of GHRH and GHRP-2 with pretreatment with placebo and pGH in control and treatment group, respectively in swine

	Item	Control	Treatment
Pretreatment GH AUC	Saline	$69.12^{b}\pm3.86$	$1687.70^{b}\pm125.64^{***}$
(-180 to 0min, ng/ml/min)	GHRP-2	$71.79^{b}\pm10.25$	$1809.99^{b}\pm170.84^{***}$
	GHRH	$73.06^{b}\pm5.47$	$2109.92^{b}\pm320.75^{***}$
	GHRH+GHRP-2	$78.17^{b}\pm6.80$	$2089.80^{b}\pm295.16^{***}$
Pretreatment GH peak	Saline	$1.25^{b}\pm0.32$	$61.22^{b}\pm4.23^{***}$
(ng/ml)	GHRP-2	$1.37^{b}\pm1.15$	$66.86^{b}\pm5.52^{***}$
	GHRH	$0.81^{b}\pm0.22$	$79.91^{b}\pm6.60^{***}$
	GHRH+GHRP-2	$0.59^{b}\pm0.11$	$81.45^{b}\pm13.08^{***}$
Post treatment GH peak	Saline	$0.42^{b}\pm0.30$	$0.92^{b}\pm0.65$
(0 to 120min; ng/ml)	GHRP-2	$97.03^{c}\pm7.20$	$87.69^{c}\pm12.41$
	GHRH	$9.21^{d}\pm1.70$	$0.80^{b}\pm0.41^{**}$
	GHRH+GHRP-2	$170.34^{e}\pm8.46$	$118.95^{c}\pm15.63^{*}$
Post treatment GH AUC	Saline	$23.10^{b}\pm3.06$	$39.68^{b}\pm10.58$
(0 to 120min; ng/ml/min)	GHRP-2	$2078.54^{c}\pm278.54$	$1978.73^{c}\pm204.21$
	GHRH	$214.96^{d}\pm29.42$	$52.62^{b}\pm11.03^{**}$
	GHRH+GHRP-2	$4207.55^{e}\pm467.69$	$2723.27^{c}\pm272.80^{*}$

* Means in the same row differ (*$p<0.05$, **$p<0.01$, ***$p<0.001$,).

[b,c,d,e] Means in the same column of each category with different letters differ ($p<0.01$).

Values are expressed as mean = SE for 4 swine.

responses to administration of saline, GHRP-2, GHRH, and a combination of GHRP-2 and GHRH were shown in Figure 1 (A, B) and Table. pGH administration significantly increased plasma GH peak concentration and GH AUC for 180 min ($p<0.001$) compared to placebo administration. Plasma GH concentration peaked at 15 min and returned to baseline level within 90 min after the administration. In control group of swine, administration of GHRP-2, GHRH, and GHRP-2 and GHRH combined followed 3 hours after administration of placebo, significantly increased ($p<0.01$) GH peak concentrations and GH AUCs (0-120 min) over those of saline administration. GH AUC response to a combination of GHRP-2 and GHRH was significantly higher than arithmetic sum of those following their separate administration ($p<0.05$), suggesting a synergistic effect of GHRP-2 and GHRH. Pretreatment of pGH (5 μg/kg BW), completely abolished GH response to GHRH and attenuated ($p<0.05$) GH response to GHRP-2 and GHRH combined, respectively, without affecting GH response to GHRP-2 only. GH AUC response to GHRP-2 and GHRH combined was not significantly different from the sum of those when administered separately, although numerically higher value ($p=0.08$) was observed.

DISCUSSION

In the control group of swine pretreated with placebo, administration of GHRP-2, GHRH, and a combination of both peptide significantly increased plasma GH concentrations as well as GH AUCs compared to saline administration. Moreover, GHRP-2 acts synergistically with GHRH to release GH. The synergistic effect of GHRP-2 and GHRH was also reported in swine (Phung et al., 2000), rats (Sawada et al., 1994) and humans (Pihoker et al., 1995). Magnitude of GH responses to GHRP-2 and GHRH in our present study was similar to that reported in previous studies in swine (Phung et al., 2000, 2001). However, our result is different from the report in goats that combined administration of GHRP-2 and GHRH caused only an additive effect on GH response (Hashizume et al., 1997). This discrepancy could be due in part to difference in mechanism of GHRP-2 among species (Chen et al., 1996).

Acute administration of GH or GHRH has been reported to inhibit GH responsiveness to GHRH (Ross et al., 1987; Rosenbaum et al., 1989; Rosenthal et al., 1989). The inhibition effect has been suggested to act through the SRIF-mediated mechanism (Chihara et al., 1981; Lanzi and Tannenbaum, 1992). Thus, it could be explained by the abolishment of GH response to GHRH stimulation after acute administration of GH in our present study.

Although mechanism of GHRPs is not yet completely understood, the available data supported the hypothesis that GHRPs action is mediated by non-GHRH receptor (Roh et al., 1997b; Gondo et al., 2001; Phung et al., 2001). Specific binding sites for GHRPs have been found in hypothalamus and pituitary (Howard et al., 1996). Interestingly, GHRPs could act as functional SRIF antagonists (Sawada et al., 1994; Nakagawa et al., 1996; Meacham et al., 1999). *In vivo* study, GHRP-2 has been suggested to act via different mechanism from GHRH (Sawada et al., 1994; Roh et al., 1997b; Gondo et al., 2001) and to have antagonist action on SRIF effect by the reports, in which GHRP-2 had ability to release GH during treatment of prednisone, known as SRIF releaser, to the same level before the treatment in men (Meacham et al., 1999). Consistently, injection of the peptide into intra-third cerebral ventricle (i.c.v.), the SRIF

releasing portion of hypothalamus, increased GH secretion with same level as it did in arcuate nucleus, the GHRH releasing portion in pentobarbital-anesthetized rats (Nakagawa et al., 1996). Moreover, the synergistic effect of GHRP-2 and GHRH was observed in rat pretreated with saline but only an additive effect was seen in rat pretreated with antiserum to SRIF (Sawada et al., 1994). Therefore, the similar GH response to GHRP-2 in swine pretreated with pGH and placebo in our study could be explained by the ability of GHRP-2 to directly or indirectly inhibit the SRIF effect. In contrast to our result, Anderson et al. (2001) reported in postmenopausal women that infusion of hGH (10 μg/kg BW) over 6 min suppressed GH response to bolus administration of GHRP-2 (1 μg/kg BW) when administered 2 hours later. This discrepancy could be due partly to the difference in mode of administration, or dose of GHRP-2 (30 μg/kg BW) and pGH (5 μg/kg BW) used in our study; hence the SRIF-interacting ability of GHRP-2 might be stronger to a level that it could completely overcome the inhibitory effect of SRIF caused by lower dose of exogenous pGH administration. However, it has been previously demonstrated that exogenous GH inhibition of its own secretion is a time-dependent effect (Rosenbaum et al., 1989; Rosenthal et al., 1989). Moreover, whether prolonged administration or high dose of exogenous GH would inhibit the GH response to GHRP-2 cannot be ruled out from our present study, and requires further investigation.

In our present results, pretreatment with exogenous pGH caused a significant attenuation in GH peak and GH AUC response to combined administration of GHRP-2 and GHRH. Interestingly, although the GH response to GHRH was completely abolished, the GH AUC response to GHRP-2 and GHRH combined was markedly higher ($p=0.08$) than the sum of GH AUC responses to GHRP-2 and GHRH when administered separately. It is suggested an ability of GHRP-2 to partly counteract SRIF effect, thereby increasing GH response to GHRH. Attenuation of GH response to GHRP-2 and GHRH combined in our present results was similar to the report, in which pretreatment with atropine, known as SRIF releaser, caused a significant attenuation of GH response to GHRP-6 and GHRH combined in men (Peñalva et al., 1993). However, although atropine or somatostatin completely abolished GH responses to GHRP-6 and GHRH when administered separately, there was an increase of GH response to the combined administration of the both peptides (Casanueva et al., 1986; Massara et al., 1984; Peñalva et al., 1993). Therefore, the mechanism underlying the combined administration of GHRP and GHRH on GH release needs to be investigated.

In summary, acute administration of exogenous pGH completely abolished response to GHRH and blunted GH response to GHRP-2 and GHRH combined, without affecting GH response to GHRP-2. These results suggested that GH negative feedback on the GH response to GHRH occurred in swine, and that GHRP-2 has ability to interact in this action. However, whether the time dependent effect, higher dose or prolonged administration of exogenous GH would inhibit the GH response to GHRP-2 cannot be ruled out from the present study, and requires further investigation.

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