Changes in plasma ghrelin and growth hormone concentrations in mature Holstein cows and three-month-old calves

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ABSTRACT: We measured changes in plasma ghrelin and GH concentrations in mature Holstein cows and 3-mo-old female Holstein calves fed at scheduled times. Our objective was to determine the characteristics of ghrelin secretion in dairy cattle and its influence on GH. Animals were fed at 0800 and 1600 for 2 wk before and during experiments. Plasma was sampled for 24 h at 2-h intervals in Exp. 1. In mature cows, plasma ghrelin concentrations decreased (P < 0.01) just after 0800 but not at the 1600 feeding. Ghrelin concentrations were lower (P < 0.01) in calves than in mature cows and they did not decrease after feeding in calves.

Key Words: Calves, Cows, Feeding, Ghrelin, Growth Hormone

Introduction

In 1999, an endogenous growth hormone secretagogue ligand called ghrelin was purified from the stomach of rats (Kojima et al., 1999). Ghrelin is a unique 28-AA peptide hormone with an n-octanoyl ester at the third serine residue that is essential for its potent stimulation of somatotropin secretion (Bednarek et al., 2000; Tolle et al., 2001). Ghrelin also stimulates GH secretion (Arvat et al., 2000; Date et al., 2000; Peino et al., 2000), increases feed intake (Kamegai et al., 2000), induces adiposity (Tschop et al., 2000), and influences feeding and gastric acid secretion (Dieguez and Casanueva, 2000; Masuda et al., 2000). Ghrelin is mainly produced by the oxyntic cells of the stomach, and gastrectomy decreases plasma ghrelin concentrations (Ariyasu et al., 2001). Ghrelin also may mediate transmission of signals from the stomach to the pituitary gland and influence GH secretion (Dieguez and Casanueva, 2000).

Because ghrelin has been identified in bovine oxyntic glands of the abomasum and in the stomach of nonruminant animals, this peptide may also function in the regulation of feeding or energy balance in ruminants (Hayashida et al., 2001). However, the digestive organs of ruminants and nonruminants, such as rats and humans, are quite different. Therefore, it remains to be determined whether the function and secretion of ghrelin in ruminants is the same as that in nonruminants. Thus far, reports on circulating concentrations of ghrelin in ruminants are limited. Sugino et al. (2002a,b) demonstrated a prefeeding surge and a decrease in ghrelin concentration after feeding in ewes, and suggested that the ghrelin released from the abomasum stimulates GH secretion.

We measured plasma ghrelin and GH concentrations to determine the pattern of ghrelin secretion in mature cows and 3-mo-old calves on a controlled feeding regimen.
Materials and Methods

Animals

All animal use conformed to the Guidelines Governing Animal Experimentation published by Kitasato University. In Exp. 1, three mature Holstein cows with normal ovulatory cycles and three Holstein calves were used. In Exp. 2, animals used in Exp. 1 plus another mature normal Holstein cows were used. Thus, in all, four mature Holstein cows of unknown age (BW 635 to 752 kg) that had normal ovulatory cycles, and three Holstein calves, aged 3 mo (BW 100 to 132 kg), were used. Animals were housed individually, indoors at Kitasato University, and were fed at 0800 and 1600, with free access to water. The amount of feed was based on the Japanese Feeding Standard for Dairy Cattle (1994). Mature cows were fed 6 kg (DM basis) of long-stemmed Italian ryegrass hay and 3 kg (DM basis) of long-stemmed alfalfa hay daily. Calves were fed 3 kg of long-stemmed Italian ryegrass hay and 1 kg of commercially available combination feed (52% of corn, 30% of oil cake, 5% of bran, and 13% others). This combination feed provided 20% CP and 70% TDN on a DM basis. All animals ate all feed within 1 h.

Experiment 1: Diurnal Rhythm of Plasma Ghrelin Concentrations

A jugular venous catheter was implanted into three mature female Holsteins and three female Holstein calves at 1000. Blood sampling from the catheters started at 1200 and continued for 48 h at 2-h intervals. Blood samples were immediately placed into heparinized tubes containing aprotinin (10 U/mL of blood; Trasylol, Bayer Yakuhin, Osaka, Japan) and were centrifuged at 1,600 × g for 10 min (4°C). Plasma was stored at −80°C.

Experiment 2: Feeding and Changes in Ghrelin Concentrations

Experiment 2 began a few days after Exp. 1 and used the three mature cows and three calves from Exp. 1 and another mature cow fed under the same conditions. Blood was sampled from 0600 to 1000 and from 1400 to 1800 at 20-min intervals for 2 d, and was processed as described for Exp. 1.

Time-Resolved Fluoro-Immonoassay of Plasma Ghrelin and GH

Blood sampling and extraction and measurement of plasma ghrelin and GH were as described by Sugino et al. (2002a). The antibody and ghrelin standard was a gift from M. Kojima and K. Kangawa of the National Cardiovascular Center Research Institute (Osaka, Japan). The polyclonal anti-rat ghrelin was raised against the N-terminal fragment of ghrelin, which is almost identical among species (Hayashida, 2001).

Ghrelin Concentrations

Ghrelin concentrations were measured by competitive solid-phase immunoassay using synthetic rat ghrelin labeled with Europium (Eu), according to the instructions of the manufacturer (Wallac Oy, Mustionkatu, Finland), and with polystyrene microtiter strips (Nunc-Immuno Modules, Nalge Nunc Int., Rochester, NY) coated with anti-rabbit γ-globulin. Ghrelin was extracted using acetone, evaporated, and resuspended in Tris buffer containing 10 U/mL of aprotinin. Anti-ghrelin rabbit serum (Kojima et al., 1999; Date et al., 2000; Hayashida et al., 2001), diluted 1:2,000,000, was added to each well and incubated overnight. After washing, serially diluted ghrelin standard (0.01 to 10 ng/mL) and extracted ghrelin from plasma were dissolved in assay buffer (100 µL/well) and incubated overnight. The Eu-labeled ghrelin (approximately 50 pg/100 µL) was added to the wells and incubated for 3 h. After washing, 100 µL of enhancement solution (Wallac Oy) was added to each well and fluorescence was measured using a time-resolved fluorimeter (multilabel counter, 1420 ALVCO, Wallac Oy). The intra- and interassay CV were 8 and 10%, respectively.

Growth Hormone Concentration

An antibody to ovine GH (NIDDK-anti-oGH-2, diluted 1:100,000) was added to wells that were coated with anti-rabbit γ-globulin antiserum, and incubated overnight. After removing GH antibody, serially diluted GH standard (0.1 to 100 ng/mL, dissolved in assay buffer) was added, and plasma samples (100 µL/well) were incubated in the wells overnight. After washing the wells, Eu-labeled GH (NIDDK-oGH-I-5; approximately 250 pg/100 @mL) was distributed in all wells and incubated for 3 h. After washing, enhancement solution was added to the well and fluorescence was measured using the time-resolved fluorimeter. The intra- and interassay CV were 10 and 14%, respectively.

Statistical Analysis

Data from the first 2 d were averaged in both experiments. The significance of differences in sequential changes of ghrelin and GH concentration at specific times of the day, age groups (mature cow vs. calf), and time × age group interaction were assessed using two-way, repeated-measures ANOVA (StatView Japanese version 4.51.1; Hulinks, Tokyo, Japan). A paired t-test identified time points that differed significantly. Correlations between ghrelin and GH were tested using Pearson’s correlation coefficient. Test statistics from two-tailed tests that yielded P-values <0.05 were considered significant.

Results

Experiment 1

Changes in plasma ghrelin and GH concentrations in mature cows and 3-mo-old calves fed twice daily are...
Figure 1. Changes in plasma ghrelin concentrations (Panel A) and GH concentrations (Panel B) in three mature cows (closed symbols) and three calves (open symbols) fed twice daily. Arrows show time of feeding. Effects of time of day \((P < 0.01)\), age group \((P < 0.01)\), and time \(\times\) age interaction \((P < 0.01)\) were detected for ghrelin concentrations. The feeding and next sampling time was tested to assess influence of feeding. In mature cows, there was a change in ghrelin concentrations after feeding between 0800 and 1000 \((P < 0.01)\), but not between 1600 and 1800. In calves, no significant changes in ghrelin concentrations were detected after either feeding time. Effects of time of day and age group, and time \(\times\) age interaction were not detected in GH.

Figure 2. The scatter graph between ghrelin and GH in Exp. 1. The correlation coefficient between ghrelin and GH was \(r = -0.095 \ (P = 0.408)\).

1600 and 1800. Neither feeding times (0800 to 1000 and 1600 to 1800) differed significantly in calves.

The scatter plot of ghrelin and GH concentrations presented in Figure 2 shows an absence of a correlation between ghrelin and GH \((r = -0.095; \ P = 0.408)\).

Experiment 2

Changes in the ghrelin concentrations in mature cows and calves from 1400 to 1800 and from 0600 to 1000 are shown in Figure 3. Figure 3A shows that the time of day \((P < 0.05)\) and time \(\times\) age group \((P < 0.05)\) were significant, and that the time \(\times\) age interaction was not. We confirmed the time of the decrease using a paired \(t\)-test for samples collected from 1600 to 1640 \((P < 0.05)\) and from 1600 to 1700 \((P < 0.05)\) in mature cows. No effects were significant in calves from 1600 to 1640 and from 1600 to 1700.

Figure 3B shows that the time of day \((P < 0.001)\) was significant, whereas age group and the time \(\times\) age interaction were not. Values differed significantly from 0800 to 0900 \((P < 0.01)\) but not from 0800 to 0840 in mature cows, and no values differed significantly in calves.

Changes in GH concentrations in mature cows and calves from 1400 to 1800 and from 0600 to 1000 are shown in Figure 4. Time of day \((P < 0.05)\), age group \((P < 0.05)\), and the time \(\times\) age interaction \((P < 0.05)\) were significant. We confirmed the time of the decrease using a paired \(t\)-test for samples collected from 1600 to 1700 \((P < 0.01)\) in mature cows. In calves, no significant changes were detected between 1600 and 1700. In Figure 4B, time of day, time \(\times\) age group, and time \(\times\) age interaction were not significant.

Discussion

Experiment 1 showed that all ghrelin concentrations in cows were higher than those of calves at all sampling times. The significant time \(\times\) age interaction indicates
that the ghrelin concentrations in mature cows change in a manner different from that in calves. Ghrelin concentration decreased before the 0800 feeding in mature cows, indicating a diurnal effect based on feeding. Plasma ghrelin concentration in mature cows decreased after feeding, then gradually recovered until the next feeding. Such diurnal rhythms were not found in calves. The decrease in ghrelin concentration in cows after feeding at 1600 was not significant, probably because ghrelin had not yet returned to prefeeding values. The interval between 1600 and 0800 is 16 h, whereas that between 0800 and 1600 is 8 h. A change in GH concentrations related to ghrelin was not evident.

Experiment 2 showed that the decrease in ghrelin concentrations in mature cows occurred 40 to 60 min after feeding, but the decrease in GH concentration occurred only after the afternoon feeding. Thus, a relationship between GH and ghrelin was still unclear. Furthermore, though ghrelin concentrations in mature cows were higher than calves, GH concentrations in mature cows were not so different or relatively lower than calves.

Hayashida et al. (2001) found that plasma ghrelin concentrations decreased 1 h after feeding in Japanese black cattle, and the present study supports this finding. Sugino et al. (2002a,b) presented evidence that the ghrelin surge related to feeding in goats was regulated by sensory signals transmitted via the vagus nerve. If so, the ghrelin profile should be similar between ruminants and nonruminants. Plasma ghrelin concentrations in calves were lower and no response to feeding was seen, as was the case in mature cows. Thus, the ghrelin secretory system may be immature in 3-month-old calves.

Ghrelin and GH concentrations do not seem to be related to each other in mature cows and calves. Espe-
cially in calves, ghrelin concentrations were lower than in the mature cow, whereas GH concentrations were not. One possibility is that the effect of the GHRH system on GH secretion is relatively strong in calves. Furthermore, blood ghrelin concentrations increase just before feeding in humans (Cummings et al., 2001), goats, and sheep (Sugino et al., 2002a,b), and this causes a transient GH surge in these animals. An increase in ghrelin concentrations and a related GH surge was not detected for Holstein cows in the present study, probably because of differences among species, feeding regimens, or experimental conditions. More detailed experiments are required to clarify the relationship between plasma ghrelin and GH concentrations in cows, although ghrelin is known to be a strong initiator of GH release (Kojima et al., 1999).

In conclusion, plasma ghrelin concentrations were affected by diurnal rhythms based on feeding schedules. Concentrations of this hormone decreased 40 to 60 min after feeding, and gradually recovered until the next feeding in mature dairy cows. Three-month-old calves have lower plasma ghrelin concentrations and these were not subject to diurnal rhythms. The present study found that plasma GH concentrations were not associated with changes in plasma ghrelin concentrations.

Implications

When mature animals were fed twice per day, plasma ghrelin concentration decreased 40 to 60 min after feeding. In calves, ghrelin concentrations and changes tended to be lower than in mature cows. These results agree with reports for other ruminant and nonruminants animals. Although ghrelin is known to be a strong secretagogue of growth hormone, a relationship between ghrelin and growth hormone in plasma was not observed. Therefore, further study is warranted to assess endogenous ghrelin as a growth hormone secretagogue in cows and calves.

Literature Cited


