Peripheral administration of kisspeptin-10 increases plasma concentrations of GH as well as LH in prepubertal Holstein heifers

H Kadokawa[5,*], M Matsui[1,*], K Hayashi[2], N Matsunaga[3], C Kawashima[4], T Shimizu[2], K Kida[4] and A Miyamoto[2]

[1]Department of Clinical Veterinary Science, [2]Graduate School of Animal and Food Hygiene, [3]Department of Agricultural and Life Science and [4]Field Centre of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan

[5]Department of Veterinary Medicine, Faculty of Agricultural Science, Yamaguchi University, Yoshida 1677-1, Yamaguchi 753-8515, Japan

(Correspondence should be addressed to H Kadokawa; Email: hiroya@yamaguchi-u.ac.jp)

*(H Kadokawa and M Matsui contributed equally to this work)

Abstract

This study was conducted to estimate the effects of kisspeptin-10 on blood concentrations of LH and GH in prepubertal dairy heifers. Heifers received a single injection of 1 mg kisspeptin-10 ($n=5$) or saline ($n=5$) intravenously, and serial blood samples were collected at 15-min intervals to analyze the response curves of both LH and GH after injection. Peak-shaped responses were observed for concentrations of LH and GH, and the peaks were observed at 27 ± 3 and 75 ± 9 min, respectively, after injection, only in heifers injected with kisspeptin-10. These data suggest various possible important links among kisspeptin, the reproductive axis, and also the somatotropic axis in prepubertal Holstein heifers.

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Introduction

Kisspeptins are natural ligands encoded by the KiSS-1 gene for G protein-coupled receptor (GPR54) and have been thrust into the reproductive neuroendocrine spotlight as major regulators of the activity of gonadotropin-releasing hormone (GnRH) neurons. In particular, kisspeptin expressed in GnRH neurons in the arcuate nucleus (ARC) of the mediobasal hypothalamus may be a pivotal regulator of puberty in primates and laboratory animals (Smith & Clarke 2007). Central kisspeptin administration leads to a marked increase of luteinizing hormone (LH) in rodents, primates, and sheep (Gottsch *et al.* 2006), probably by stimulating GnRH secretion from GnRH neurons into portal blood (Plant 2006). Also, peripheral administration of kisspeptin-10 (the minimal sequence necessary for GPR54 activation) increases blood LH concentration in rats and monkeys (Plant *et al.* 2006), probably by both increasing GnRH secretion into portal blood and stimulating pituitary gonadotrophs to secret LH (Gutiérrez-Pascual *et al.* 2007, Suzuki *et al.* 2007). Kisspeptin may have other autocrine or paracrine, but undefined, roles in the pituitary, because rat pituitary expresses both kisspeptin and GPR54 (Gutiérrez-Pascual *et al.* 2007).

Puberty is the transition from immaturity to maturity, and it requires body growth. Close interactions between growth hormone (GH) secretion and puberty have been well studied in monogastric animals such as laboratory rodents and humans (Hull & Harvey 2002). However, the molecular and cellular mechanisms linking the reproductive axis and somatotropic axis at the levels of the brain and pituitary during puberty still remain incompletely understood. The fact that the kisspeptin–GPR54 system has an important role in puberty raises a question: is kisspeptin important only for the reproductive axis in prepubertal animals? A recent *in vitro* study suggests that kisspeptin may also have an important role in the somatotropic axis *in vivo*, because kisspeptin-10 stimulates the secretion of both LH and GH from cultured rat pituitary cells (Gutiérrez-Pascual *et al.* 2007).

The importance of GH for puberty has not been well established in ruminants. The average GH concentration decreased with age, but the GH pulse frequency increased from 1 to 12 weeks of age and remained constant thereafter in Holstein bull calves (McAndrews *et al.* 1993). Serum GH concentrations decrease before puberty also in sheep, but such GH decrease is not a requirement for puberty (Suttie *et al.* 1991). In contrast to Holstein bull calves and sheep, plasma LH and GH concentrations increased during the pubertal period in buffalo (Haldar & Prakash 2005), and administration of exogenous GH-releasing hormone (GHRH) advances their puberty (Haldar & Prakash 2006). Although these studies reported the relationship between changes in blood GH concentration and puberty, the molecular and cellular mechanisms linking the reproductive axis and somatotropic

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axis at the levels of the brain and pituitary during puberty remain to be determined in ruminants.

The importance of the kisspeptin–GPR54 system has not been evaluated in bovines *in vivo*. Furthermore, the effect of kisspeptin on GH secretion has not been evaluated in primates, rodents, and ruminants *in vivo*. Therefore, this study was conducted to estimate the effect of peripheral administration of kisspeptin-10 on the secretion of LH and GH in prepubertal Holstein heifers.

Materials and Methods

All procedures used in this experiment were approved by the Animal Care and Use Committee of the Obihiro University of Agriculture and Veterinary Medicine (Obihiro, Japan). A total of ten prepubertal Holstein heifers (210·2±4·6 kg, 7·0±0·3 months old; means±S.E.M. used for all data) were housed in a tie-stall barn in the Field Center of this university in Hokkaido, northern Japan. They were individually given *ad libitum* access to sufficient (about 2·6% dry matter (DM) of body weight/day) Timothy hay (89·4% DM, 2·92 Mcal/kg DM of digestible energy, 10·1% of digestible crude protein) to meet their growth requirements (Agriculture Forestry and Fisheries Research Council Secretariat 1999). Water and mineral blocks were provided *ad libitum*. Puberty in Holstein heifers in Hokkaido, Japan, occurs at older than 11 months of age, as described previously (Nakada *et al*. 2000). Prepuberty on the day of the kisspeptin-10 trial in all heifers was verified by confirming the absence of estrous cycles using both the two daily observations made with the aid of a Heat-Mount detector (Kamar Inc., Steamboat Springs, CO, USA) and progesterone measurement in blood sampled three or four times weekly from 4 weeks before to 2 weeks after the day of the trial.

On the day preceding the kisspeptin-10 injection, all heifers were fitted with an indwelling jugular vein catheter. On the day of the kisspeptin-10 trial, blood samples were collected to obtain plasma at 15-min intervals from the heifers for 6 h, from 1200 to 1800 h (depicted in this report as being from 120 to 240 min), for analysis of the concentrations of LH and GH. Heifers received an i.v. injection of 8 ml saline (n=5) or 1 mg kisspeptin-10 (human metastin 45–54 (YNWNSFGLRF-NH2), 4389-v, Peptide Institute Inc., Osaka, Japan) dissolved in 8 ml saline (n=5) at 1400 h (time=0 min). The dose was based on that which stimulates LH secretion after peripheral administration in rats (Tovar *et al*. 2006) and the ratio of the metabolic body weight of heifers to that of rats used in this study.

Plasma LH was measured by the time-resolved fluoroimmunoassay (TR-FIA) as described previously (Kaneko *et al*. 2002) using bovine LH (AFP11743B, National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), CA, USA) and anti-ovine LH antiserum (AFP192279, NIDDK). The limit of detection and the intra- and inter-assay coefficients of variation of LH TR-FIA were 0·20 ng/ml and 5·3 and 6·4% respectively. Plasma GH was assayed by enzyme immunoassays (EIA) as described previously (Kawashima *et al*. 2007) using bovine GH (AFP-9984C, NIDDK) and anti-ovine GH-antiserum (AFP-C0123080, NIDDK). The limit of detection and the intra- and interassay coefficients of variation of GH EIA were 0·50 ng/ml and 5·8 and 7·5% respectively.

Since peak-shaped responses were observed in the concentrations of both LH and GH, we calculated the maximum concentration observed among serial samples during the 360 min (peak concentration) and the timing of samples containing them. The area under the curve (AUC) of the LH or GH response curve, as linear trapezoidal summation between successive pairs of concentration and time, was calculated in the period either from −120 to 0 min, from 0 to 120 min, or from 120 to 240 min respectively. The AUCs of LH or GH were analyzed by repeated measure ANOVA and paired t-test. Significance was set at $P<0·05$.

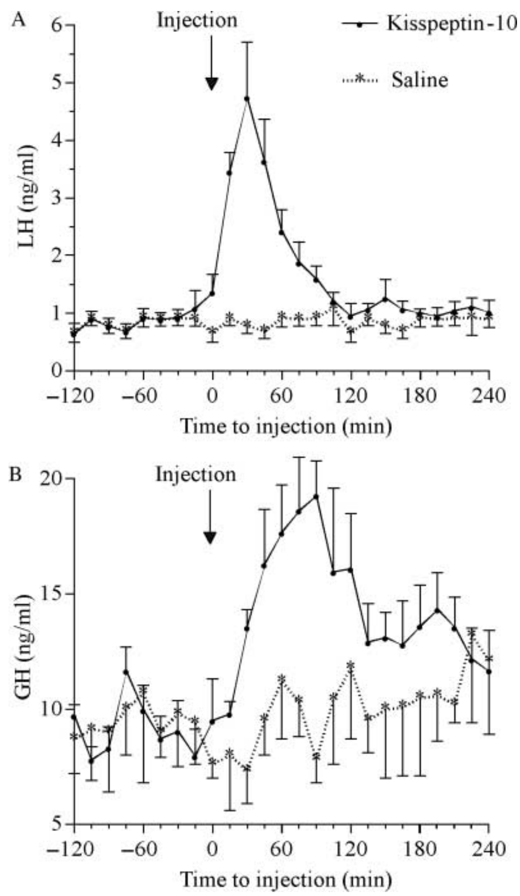

Figure 1 Changes (means±S.E.M.) in the blood concentrations of LH (A) and GH (B) over 6 h in prepubertal heifers that received an i.v. injection of saline (n=5) or 1 mg kisspeptin-10 dissolved in saline (n=5) at 0 min.

Results

Figure 1 shows the blood concentrations of LH and of GH around the time of the injection in prepubertal heifers. The kisspeptin-10 injection, but not the saline injection, increased blood LH concentration in the heifers (Fig. 1A). The peak LH concentrations were 5·0±0·9 ng/ml, and LH peaks were observed at 27±3 min after the injection. The AUC of LH in the period from 0 to 120 min was significantly ($P<0·05$) greater than in other periods (Fig. 2A).

The kisspeptin-10 injection, but not the saline injection, also increased blood GH concentration in the heifers (Fig. 1B). The GH peak (24·2±1·6 ng/ml) was observed at 75±9 min after the injection, thus, after the LH peak. The average duration from the LH peak to the GH peak was 48±10 min. The AUC of GH from 0 to 120 min was significantly greater ($P<0·01$) than those in other periods (Fig. 2B).

Discussion

The present study is the first demonstration *in vivo* that the injected kisspeptin-10 stimulates the secretion of GH as well as LH in prepubertal heifers. The colocalization of GnRH with kisspeptin is observed in cells in ARC, and also in nerve fibers of GnRH neurosecretory terminals of the median eminence in ovines (Pompolo *et al.* 2006). Thus, kisspeptin and GnRH might be cosecreted into the hypophyseal portal blood to act on GPR54 in the pituitary gonadotroph (Kotani *et al.* 2001). Kisspeptin is likely to have a synergistic effect with GnRH, rather than a sole effect, on LH secretion, because administration of GnRH antiserum completely prevented the increase in LH secretion induced by synthetic decapeptide of ovine kisspeptin1 in ewes (Arreguin-Arevalo *et al.* 2007). Furthermore, LH peaks after kisspeptin-10 injection observed in this study were smaller but earlier than those after the GnRH analog injection in similar prepubertal Holstein heifers (about 10 ng/ml at about 150 min) in another report (Kadokawa 2007). Therefore, the injected kisspeptin-10 may activate GnRH secretion from its neuron to hypophyseal portal blood and may work with GnRH to stimulate LH secretion from the pituitary.

Since the present study is the first to show that kisspeptin stimulates GH secretion in any species *in vivo*, there is little basis in the literature to support a particular mechanism of action. One possible mechanism is that injected kisspeptin-10 increased blood GH concentration by stimulating pituitary somatotrophs directly to secrete GH, because kisspeptin-10 stimulates the secretion of both LH and GH from cultured rat pituitary cells (Gutiérrez-Pascual *et al.* 2007). However, as GH peaks after kisspeptin-10 injection observed in this study were smaller and later than those after GHRH injection in similar prepubertal Holstein heifers (about 120 ng/ml at about 15 min) in another report (Taylor *et al.* 2006), we also need to consider an indirect mechanism of kisspeptin-10 to stimulate GH secretion. A possible mechanism is an indirect effect of kisspeptin on GH secretion at the levels of the hypothalamus and pituitary, although further work is required to determine whether kisspeptin-10 administered intravenously can influence bovine hypothalamus directly. Since neuronal somatostatin (SRIF) mRNA content in ARC decreases during early puberty in rats (Argente *et al.* 1991), SRIF may play an important role in the increase in GH secretion after the injection of kisspeptin-10. Pompolo *et al.* (2006) observed kisspeptin-immunoreactive and non-GnRH-immunoreactive neurons in ARC in ovines. Therefore, kisspeptin-immunoreactive and non-GnRH-immunoreactive neurons may have a pivotal role in the activities of GHRH neurons and SRIF neurons. Further study is required to confirm the hypothesis that GHRH neurons, SRIF neurons, or neighboring neurons contain either GPR54 or kisspeptin. Another possible mechanism is intercellular communication

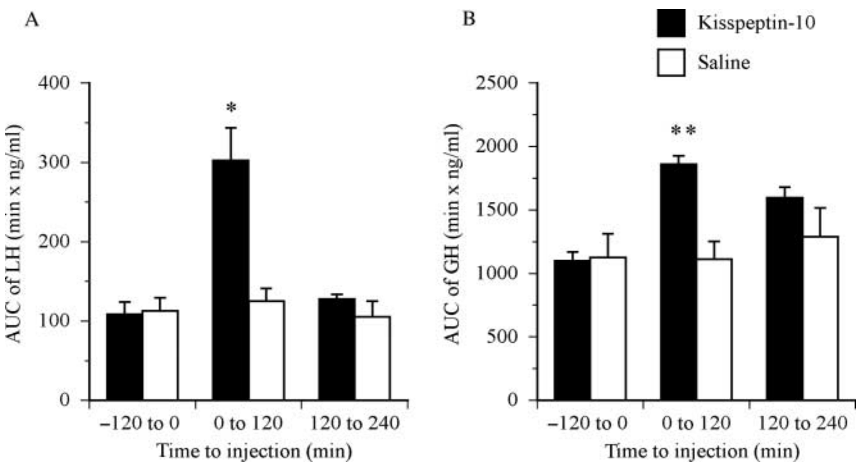

Figure 2 Areas under the curve (AUCs) of LH (A) and GH (B) concentrations during the period either from 120 to 0 min, from 0 to 120 min, or from 120 to 240 min. *$P<0·05$, **$P<0·01$ as compared with the period from 120 to 0 min.

between gonadotroph and somatotroph in the pituitary using various molecules (Schwartz 2000) induced by the pituitary kisspeptin–GPR54 system (Gutiérrez-Pascual *et al.* 2007).

In conclusion, injected kisspeptin-10 stimulates the secretion of LH and GH in prepubertal heifers. These data suggest various possible important links among kisspeptin, the reproductive axis, and also the somatotropic axis in prepubertal Holstein heifers.

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