The effects of lactation and negative energy balance on kisspeptin-stimulated luteinizing hormone and growth hormone in dairy cows.

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Abstract

Kisspeptin is hypothesized to integrate nutrition and hormones critical to metabolism and the regulation of reproduction. Since the negative energy balance of early lactation is associated with reduced fertility via suppression of gonadotropin secretion and enhanced growth hormone (GH) responsiveness, this experiment was designed to determine the effects of stage of lactation and negative energy balance on kisspeptin–10 (Kp–10) stimulated luteinizing hormone (LH) and GH concentrations. Five nonlactating [5.1 ± 0.8 (SEM) years; 577 ± 19 kg body weight (BW)] and five lactating [4.1 ± 0.6 years; 608 ± 11 kg BW] multiparous Holstein cows were utilized. Experiments were conducted on the lactating cows at weeks 1, 5 and 11 after parturition and on the nonlactating cows over the same six month period. Except for lactating cows in the first week of lactation (prior to resumed cyclicity and ovarian activity) all other experiments were conducted on cows in the luteal phase of the estrous cycle. The experimental treatments [physiologic saline (control) and Kp–10 (100 and 400 pmoles/kg BW)] were administered as a bolus via jugular cannula. Treatments were successive with 48 hrs between each Kp–10 treatment. Lactating cows were given all treatments for each experimental week of lactation (1, 5 and 11) and each nonlactating cow received all treatments during only one week. Plasma was collected at –30, –15, 0, 5, 10, 15, 20, 30, 45, 60, 75 and 90 min relative to treatment and stored until assayed for LH, GH and non-esterified fatty acids (NEFA). Lactation (nonlactating and week of lactation) affected the energy balance of the cows as indicated by peripheral concentration of NEFA. Peripheral NEFA concentrations were highest during week one and five of lactation. Neither dose of Kp–10 stimulated an increase in GH concentration in lactating or nonlactating cows. The low Kp–10 dose significantly increased LH concentrations in the lactating cows only. However, the higher dose of Kp–10 elicited an increase in LH concentrations in all treatment groups and stages of lactation. The incremental area under the curve (iAUC) of LH from 0 to 90 min after treatment with the lower Kp–10 dose was significantly greater than the saline treatment only during week 5 of lactation and the iAUC of LH following the highest dose of Kp–10 was significantly greater in cows in week 5 of lactation than all other lactation and nonlactating groups. These data demonstrate impact of energy balance and lactation on kisspeptin–stimulated gonadotropin increase the opposite response seen in lactating rats. The study of the kisspeptin system during lactation in high producing dairy cows may yield critical insights into the mechanisms for lactation associated infertility.

Introduction

Lactation is energetically demanding and in most female mammals, ovulation, mating, and pregnancy are suppressed if not blocked during lactation. Since high producing dairy cows enter the early postpartum period of negative energy balance and are expected to become pregnant soon thereafter, the hormones regulating the interplay between metabolism and reproduction have received intense scrutiny. The molecular mechanisms underlying disruption of reproductive function during energy insufficiency remain to be fully understood. The hypothalamus plays a crucial role in maintaining fertility in all mammals and is the focus of research in the integration of metabolism and reproduction. A possible link between metabolism and reproduction is the neuropeptide, kisspeptin [1]. The kisspeptin-GPR54 signaling system is necessary for normal reproduction and kisspeptin stimulates gonadotropins in rodents, sheep, swine, cattle, and primates [2-9]. The kisspeptin neurons have direct links to leptin, which integrates signaling of the magnitude of body energy reserves to multiple neuroendocrine axes [10]. Kisspeptin may also have a role in regulating GH secretion and systemic administration to cattle stimulates GH [2,8]. Theses data suggest that kisspeptin may serve as an integrator between reproduction, and metabolism in cattle. Lactating rats have lower levels of KiSS-1 mRNA and kisspeptin in the hypothalamus compared to nonlactating controls [11]. The hypothalamic-pituitary axis may be less sensitive to kisspeptin during lactation [12] and this may be explained by changes in expression of GPR54 mRNA in the hypothalamus of lactating rats. However, the effects of negative energy balance and lactation on kisspeptin mediated GH and LH release in large domestic species, in particular cattle, remains to be fully elucidated. The present study therefore aimed to test whether stage of lactation and degree of negative energy balance affected kisspeptin (Kp10, human Metastin 45-54, 4389-v, Peptide Institute, Inc., Osaka, Japan) stimulated increase in LH or GH in cattle.

Materials and Methods

All procedures were approved by the *Auburn University Institutional Animal Care and Use Committee [AU-IACU]*. Five nonlactating and five lactating multiparous Holstein cows were used in the study. Cows were housed at the AU Veterinary Teaching Dairy and experiments were conducted over a six month period (November – April). Lactating cows were milked twice daily and individually fed grain and alfalfa hay and *ad libitum* Coastal Bermuda Grass hay following each milking. The diet consisted of approximately 1.80 Mcal NEL/kg, 22% crude protein and 29% NDF. The nonlactating cows were fed the same grain as the lactating cows and also given *ad libitum* access to Coastal Bermuda Grass hay. Both groups were fed diets balanced to meet 100% of daily requirements [13]. Experiments were conducted on the lactating cows serially at weeks 1, 5 and 11 after parturition and on the nonlactating cows over the six month experimental period. When possible (except week one of lactation) experiments were conducted on cows in the luteal phase of the estrous cycle. The experimental treatments [physiologic saline *(control)* and Kp10 *(100 and 400 pmoles/kg BW)*] were administered as a bolus via jugular cannula. Treatments were administered successive with 48 hours between each treatment. Lactating cows were given all treatments for each experimental week of lactation and each nonlactating cows received all treatments during only one week. Plasma was collected at -30, -15, 0, 5, 10, 15, 20, 30, 45, 60, 75 and 90 minutes relative to treatment. Plasma was stored until assayed to determine LH, GH, progesterone, NEFA and glucose concentration [14-16]. To determine the effect of lactation and Kp10 on plasma concentrations of LH and GH, data were subjected to least-squares analysis of variance with repeated measures using the MIXED procedures of SAS. Incremental area under the curve (iAUC) of plasma LH and GH at fixed periods were subjected to generalized least squares ANOVA with repeated measures [17].

Conclusions

These data ...

... demonstrate the impact of energy balance and lactation on kisspeptin-stimulated gonadotropin increase in cattle.

... suggest the effect of energy balance and lactation on kisspeptin-stimulated gonadotropin in lactating cows is opposite that of lactating rodents.

A greater understanding of the mechanism where kisspeptin signaling may participate in the regulation of gonadotropin secretion in cows during certain physiological conditions may yield novel information into the mechanisms for lactation associated infertility.

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Table 1:

Least squared means of production, metabolic and hormone parameters for nonlactating (NL) and lactating (WK 1, 5 and 11) cows (n = 5).

	NL	WEEK #			Pooled SEM
		1	5	11	
In Milk *(days)*	na	8.0[a]	35.0[b]	74.4[c]	0.8
Milk *(kg/d)*	na	28.71[a]	35.79[b]	37.66[b]	1.67
Non-esterfied Fatty Acids *(mEq/L)*	0.093[a]	0.491[b]	0.348[c]	0.183[a]	0.047
Glucose *(mg/dL)*	73.8[a]	53.0	55.2	57.9	1.9
Progesterone *(ng/ml)*	3.18[a]	0.12[b]	2.78[a,b]	7.24[c]	0.94

a,b,c Least squared means in rows with different superscripts differ (P<0.05).

Figure 1:

Effect of lactation and Kp10 on LH (A) and GH (B) concentrations in nonlactating (NL) and lactating (WK 1, 5 and 11) cows (n = 5; mean ± SEM).
* P < 0.05 vs. control. # P < 0.05 vs. 100 Kp10.

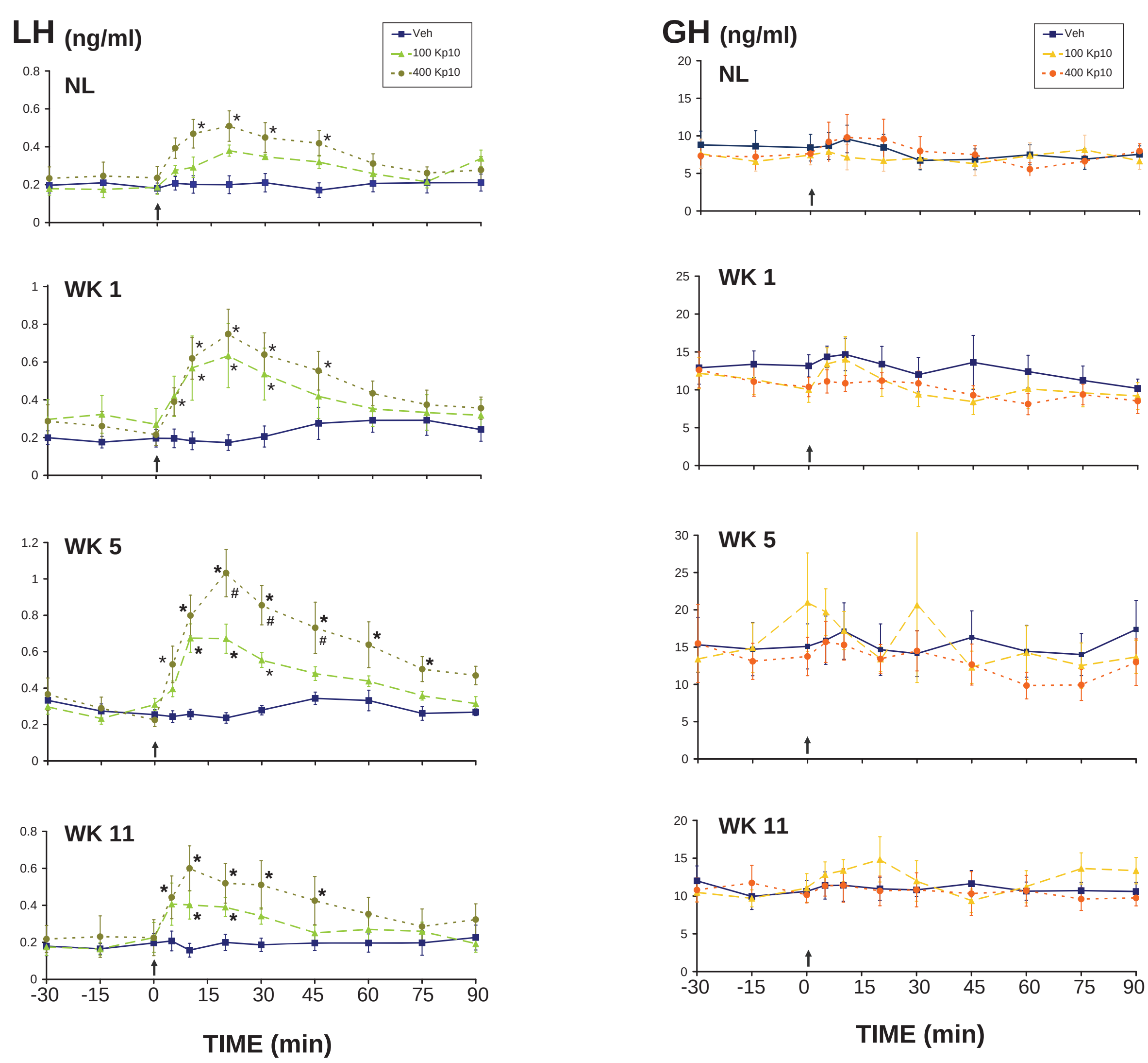
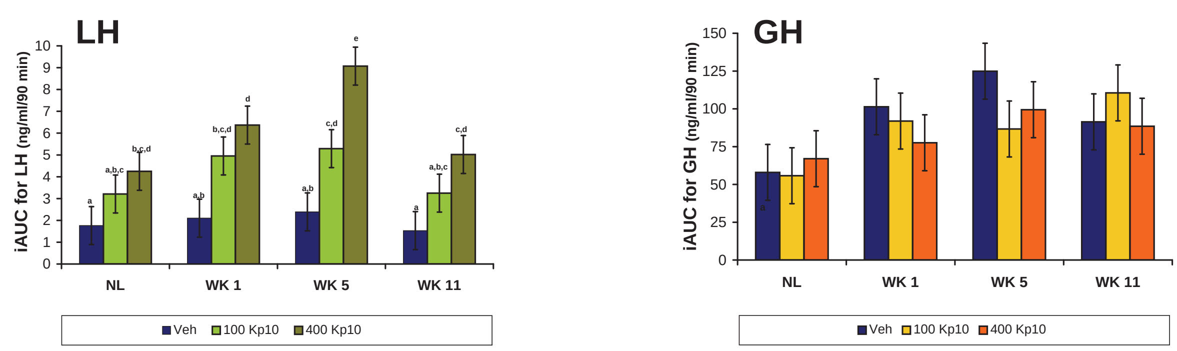


Figure 2:

Incremental areas under the curve (iAUCs) of LH and GH concentrations in nonlactating (NL) and lactating (WK 1, 5 and 11) cows (n = 5; mean ± SEM) from 0 to 90 minutes post-treatment. iAUCs with different superscripts differ (P < 0.05).



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