Changes in plasma concentrations of growth hormone and luteinizing hormone in ewes following central and peripheral treatment with kisspeptin

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Abstract

Kisspeptin (KP), a neuroendocrine regulator of gonadotropin releasing hormone, has been hypothesized as an integrator of nutrition and hormones critical to metabolism and regulation of reproduction. Recent evidence suggests growth hormone (GH) secretion may be influenced by KP. The objective of this study was to determine if the GH stimulatory effect of KP is due to actions on the hypothalamus or anterior pituitary gland in ewes. Adult ovariectomized ewes (n=8) were fitted with intracerebroventricular (ICV) cannula to facilitate central administration of experimental treatments. Ewes received one of eight treatments (four intravenously [IV] and four ICV). Peripheral treatments (0 [Veh], 100, 200, or 1000 pmol/kg body weight [BW] KP-10 [human KP 45-54; 4389-v, Peptide Institute, Osaka, Japan] in saline) were administered as a bolus via jugular cannula, and ICV treatments (Veh, 50, 100, or 200 pmol/kg BW KP-10) were administered via the ICV cannula. Blood samples were collected from a jugular cannula at -15, 0, 10, 20, 30, 45, 60, and 75 min relative to treatments. Experiments were repeated until all ewes received each treatment. Plasma GH and luteinizing hormone (LH) concentrations were determined by radioimmunoassay. Effects of treatment on plasma concentrations of LH and GH were tested using procedures for repeated measures (SAS Institute, Cary, NC). The 200 and 1000 pmol/kg IV KP-10 increased ($P < 0.05$) plasma concentrations of LH. However, there was no effect of IV KP-10 on plasma GH. Conversely, 100 and 200 pmol/kg KP-10 administered ICV increased ($P < 0.05$) plasma GH concentrations. Maximum GH responses occurred 30 min following ICV KP-10 injection and were greater ($P < 0.05$) than both Veh and the 50 pg/ml KP-10 ICV. In addition to activating the gonadotropic axis, KP can activate the somatotropic axis in ruminants, and present data support a central site of action.

KEYWORDS

Sheep, Kisspeptin, Growth Hormone

Introduction

The kisspeptin-G protein-coupled receptor 54 **(Kiss1r)** system is integral to central regulation of the gonadotropic-axis [5,8,22]. Kisspeptin immunoreactive fibers are found in the brains of rodents [24], sheep [7] and equids [6] in areas of the hypothalamus (arcuate nucleus, ARC) related to gonadotropin releasing hormone **(GnRH)** regulation. In addition, low but detectable amounts of kisspeptin have been found in hypophyseal portal blood from sheep [23]. The ovine hypothalamus expresses Kiss1r, and this expression is also seen in pituitary cells (specifically, lactotropes, gonadotropes and somatotropes). Although the role of the kisspeptin-Kiss1r system as gatekeeper of the hypothalamic-pituitary-gonadal axis is undisputed, it may control additional neuroendocrine processes such as the somatotropic axis. Interestingly, a functional somatotropic axis is important for proper reproductive development and function [11-12].

Recent evidence suggests that kisspeptin may have a role in regulating growth hormone (GH) secretion [10,14-15,28]. Pituitary cells from rats were activated (assessed by influx of ionized calcium) *in vitro* by kisspeptin to secrete not only luteinizing hormone (LH), but also GH [10]. Kisspeptin was also used to stimulate GH and prolactin release from cultured bovine anterior pituitary cells [15]. In an attempt to determine whether in vivo regulation of GH could be demonstrated, a very high dose of intravenous (IV) kisspeptin (3,653 pmol/kg BW) was administered to prepubertal bovine females [14] and found to cause a large, prolonged increase in plasma concentrations of GH. On the other hand, central and peripheral kisspeptin administration increased plasma LH concentrations in prepubertal female pigs, but failed to increase plasma GH concentrations [16]. Finally, a low dose of kisspeptin (100 pmol/kg BW) administered to ovariectomized (OVX) adult cows had no effect on plasma GH except in the presence of estrogen or progesterone [28]. Collectively, these studies suggest an effect of reproductive steroids to enhance GH response to kisspeptin. Failure of kisspeptin to stimulate GH release in certain species [16] and physiologic settings [28] suggests that there may be species and developmental differences in the ability of kisspeptin to increase GH secretion and that the site of kisspeptin action in the somatotropic axis is not understood. At present, the data on GH release in response to kisspeptin primarily indicates an action at the level of the pituitary gland [10,14-15,23]. Thus, the present experiments were designed to determine whether kisspeptin regulation of the somatotropic axis is mediated through interactions at the pituitary or whether kisspeptin actions occur within the hypothalamus.

Materials and Methods

Pre-experiment preparation

Adult mixed-breed, black face ewes weighing 70.8 ± 4.4 kg were kept indoors in individual pens with an environment consisting of 12 h light/dark photoperiod and ~24 °C. Ewes were bilaterally OVX at least 1 month before any experimental manipulations and fed a maintenance diet calculated to meet 100% of daily requirements for the duration of the experiment. Each sheep was fitted with an intracerebroventricular (ICV) cannula into a lateral ventricle with procedures modified from those previously described [27]. Following an overnight fast, sheep were anesthetized, placed in a sheep sterotaxic device *(David Kopf Instruments; Tujunga, CA)* and maintained under anesthesia with isoflurane. A 10-gauge Touhey needle with a luer closure stylette was placed into a lateral ventricle (typically the right). Stereotaxic coordinates for insertion of the Touhey needle were 15 mm posterior to the bregman, 6 mm lateral to the midline and 22 mm ventral to the skull surface, with the guide cannula angled 15° to vertical. The needle aperture was maintained in a rostral-dorsal orientation to facilitate ICV catheter placement in a lateral ventricle. A column of sterile physiologic saline was connected to the advancing Touhey needle at intervals during ventricular cannulation, and a significant drop and pulse of the saline indicated communication with the ventricle. A vascular access port and catheter (see ***Figure 1A***) were used to chronically catheterize a lateral ventricle. The cannulated ventricle was catheterized through the Touhey needle with a 4 French (0.6 mm X 1.2 mm [ID X OD]) silicone catheter having multiple fenestrations on the distal 15 mm. The catheter was advanced 20 mm into the ventricle, the Touhey needle was withdrawn and the proximal end of the catheter was secured to the port, which was placed subcutaneously near the external occipital protuberance. The catheter and port were both secured to the surrounding connective tissues with non-absorbable suture. Catheter placement was confirmed by taking a radiograph in the lateral-medial orientation immediately after injecting 1 ml of radiopaque dye ***(Omnipaque 300**; Sterling Drug, New York, NY)* into the catheterized ventricle (see ***Figure 1B***). Animals were given two weeks to recover from ICV cannulation surgery before experimentation began. During the recovery period animals received analgesic and antibiotics, as previously described [27].

Treatment protocols

Sheep received one of eight treatments (four administered IV and four ICV). ***Peripheral treatments*** (Veh and either 100, 200, or 1000 pmol/kg BW [130, 260 and 1300 ng/kg BW, respectively] KP-10) were administered in a 3 ml bolus via jugular cannula (placed the day before an experiment was initiated) to eight sheep. ***Central treatments*** (Veh and either 50, 100, or 200 pmol/kg BW [65, 130, and 260 ng/kg BW, respectively] KP-10) were administered in 500 µl via the vascular port previously implanted into a lateral ventricle to eight sheep. For ICV injections, the skin above the port site was aseptically prepared prior to central treatment, and treatments were administered through the skin and into the port via a 25-gauge Huber point needle *(Norfolk Vet Products, Skokie, IL)* followed with 250 µl of sterile 0.9% saline to flush the port and catheter. Blood samples (3 ml) were collected from the jugular cannula (placed the day before experiments were initiated) at -15, 0, 10, 20, 30, 45, 60, and 75 min relative to peripheral and central treatments and the blood volume replaced after each sampling with saline. Blood was collected into tubes containing 7.5 mg EDTA. Plasma was stored at -20 °C for radioimmunoassay of LH and GH. Experiments were repeated eight times with at least 4 days between experiments until all sheep received each treatment.

Hormone assays

Plasma GH and LH concentrations were assayed by double-antibody RIA using materials supplied by the National Hormone and Pituitary Program of NIDDK as previously described [2].

Intra- and interassay coefficients of variance for the assays were 9.9% and 17.5%, respectively, for LH, 14.9% and 15.8% for GH.

Statistics

To determine effects of treatment 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 [21]. The model included treatment, day, time, and all first- and second-order interactions, with a compound symmetric function used to model the covariance structure for the repeated measures. If a significant ($P<0.05$) treatment by time interaction was detected, effects of treatment by time were compared using the SLICE option of the LSMEANS statement of SAS. Mean concentration and incremental area under the curve (iAUC) of plasma LH and GH at fixed periods were subjected to generalized least squares ANOVA with repeated measures.

Results

There was no effect ($P > 0.05$) of IV KP-10 treatment on mean concentrations or iAUCs for plasma GH (see ***Figure 2A,C***). The 50 pmol/kg dose administered ICV did not affect GH concentrations (see ***Figure 2B***). However, the two highest doses of KP-10 (100 and 200 pmol/kg) administered centrally increased ($P < 0.05$) plasma GH concentrations (***Figure 2B***). Maximum GH responses occurred 30 min from the time of injection and were greater ($P < 0.05$) than both the Veh and the lowest ICV KP-10 dose (50 pmol/kg). Similarly, the magnitude of GH responses as assessed by iAUC from 0 to 75 min following treatment were greatest ($P < 0.05$) for the 100 and 200 pmol/kg doses of KP-10 (see ***Figure 2C***).

The effect of IV and ICV KP-10 on LH in OVX female sheep was also determined. When compared to controls, the highest IV doses of KP-10 (200 and 1000 pmol/kg) increased ($P < 0.05$) plasma concentrations of LH (see ***Figure 3A***). Onset of LH responses was similar for both doses (10 min), and plasma LH concentrations remained greater ($P < 0.05$) than those observed for controls through 30 and 45 minutes following the 200 and 1000 pmol/kg dose, respectively. Unlike the higher IV doses of KP-10, the lowest IV dose (100 pmol/kg) did not change circulating LH concentrations ($P > 0.05$). There was also an effect of IV KP-10 on iAUC for LH in the period from 0 to 75 min following treatment (see ***Figure 3C***; $P < 0.05$). When compared to controls, the iAUC for LH was not affected by the lowest IV KP-10 dose (100 pmol/kg). Similar to mean LH responses observed following IV KP-10, the highest IV doses increased ($P < 0.05$) the iAUC for LH during the period tested ($P < 0.05$). However, the iAUC for the middle IV KP-10 dose (200 pmol/kg) was not significantly different ($P > 0.05$) from that produced by the lowest dose (100 pmol/kg). When administered centrally (ICV), there was no effect ($P > 0.05$) of KP-10 on mean LH concentrations (***Figure 3B***). However, the highest ICV KP-10 dose (200 pmol/kg) increased ($P < 0.05$) the iAUC for LH as much as the highest IV KP-10 dose (1000 pmol/kg) (***Figure 2C***).

Conclusion

Effects of IV and ICV KP-10 on GH and LH plasma concentrations were tested in OVX sheep, where the ICV surgical and experimental procedures were well established and the stereotaxic apparatus available for this ruminant model [4,17,19-20,26-27]. While LH concentrations in OVX ewes were stimulated by the highest two doses of peripherally (IV)-injected KP-10 (***Figure 3A,C***; 200 and 1000 pmol/kg BW), no stimulatory effect was observed on concentrations of GH at any KP-10 doses that were administered IV (***Figure 2A,C***), similar to data in cattle. However, ICV-injected KP-10 in OVX ewes not only induced increases in circulating concentrations of LH (***Figure 3C***; 200 pmol/kg BW dose), but also produced a significant stimulus to GH (***Figure 2B,C***;100 and 200 pmol/kg BW). The stimulatory effect of ICV, but not IV, KP-10 on secretion of GH in OVX ewes, supports the concept that the hypothalamus is the primary target for the action of kisspeptin on the somatotropic axis. As mentioned previously, earlier studies [14,16,28] provided divergent results for effects of kisspeptin on plasma GH concentrations. Such differing effects may reflect disparities in species, developmental maturity, and route of kisspeptin administration. A physiological role for kisspeptin participating in GH regulation and its mechanism is unclear. However, a recent study by *Backholer (unpublished observations)* might explain the ability of kisspeptin to release GH. In that study *(Backholer)*, neuropeptide Y (NPY) gene expression was activated by ICV-injected kisspeptin. Since a subpopulation of growth hormone-releasing hormone (GHRH) neurons in the ARC also produce NPY [13], and NPY is a well known stimulus to GH in ruminants [9,17-18,25]; we hypothesize this pathway may provide a possible mechanism to explain the release of GH following ICV injection of KP-10. It is interesting to compare the different responses obtained between IV and ICV injections of KP-10. Both GHRH and GnRH neurons reside in the ARC. If IV doses of kisspeptin cross the blood-brain barrier to activate GnRH neurons, then GHRH neurons should also be activated. A possible explanation for the different effect of KP-10 on plasma LH and GH concentrations following IV or ICV administration could be site of action of kisspeptin. It is clear that peripheral injections of kisspeptin lead to a rapid increase in LH release [1,28], suggesting that one site of action of kisspeptin stimulation on GnRH release could be circumventricular organs such as the median eminence, with weak or no blood-brain barrier. A recent study in mice supports this concept by showing that KP-10 can stimulate GnRH release from nerve terminals in the median eminence in the absence of GnRH neuronal cell bodies [3]. However, regulation of kisspeptin-stimulated GH release may be exclusively within the ARC and not available with lower doses of kisspeptin, but may be accessed with very high doses of kisspeptin or when estrogen and progesterone are available in high concentrations.

In conclusion, the stimulatory effect of central, but not peripheral, KP-10 on secretion of GH in OVX ewes supports the hypothesis that effects of kisspeptin on GH release are mediated at the hypothalamic level and not at the level of the pituitary in ruminants. Further study is needed to determine the role for kisspeptin in gonadotropes and somatotropes. I n addition to the well developed actions of kisspeptin in the reproductive axis, kisspeptin may be a novel regulator of GH release and perhaps an important and novel link between the gonadotropic and somatotropic axes in ruminants.

Figure 1:

(A) Titanium vascular access port and catheter *(Norfolk Vet Products, Skokie, IL)* modified to chronically catheterize a lateral ventricle.
Courtesy of Access Technology and Norfolk Vet Products.

(B) Lateral ventriculogram in sheep immediately following injection of 1 ml of radiopaque dye ***(Omnipaque 300)*** through the subcutaneous port and catheter into the catheterized ventricle. Blue arrow indicates the subcutaneous port with a Huber-type needle inserted; open arrow indicate the catheter, and ellipse (- - - -) indicates the lateral ventricle (green arrow points to catheterization site of the ventricle).

Figure 2: Effects of IV and ICV KP-10 on plasma GH concentrations in OVX female sheep (n = 8).

(A) Response of circulating concentration of GH (mean minus SEM; pooled SEM = 0.8) to IV administration of Veh and KP-10 (100 KP-10 [100 pmol/kg]; 200 KP-10 [200 pmol/kg]; 1000 KP-10 [1000 pmol/kg]). No significant differences. Arrow indicates time of administration of treatment.

(B) Response of circulating concentration of GH (mean minus SEM; pooled SEM = 1.9) to ICV administration of Veh and KP-10 (50 KP-10 [50 pmol/kg]; 100 KP-10, 200 KP-10). * $P < 0.05$ vs Veh. # $P < 0.05$ vs 50 KP-10. Arrow indicates time of administration of treatment.

(C) Effect of IV and ICV bolus injection of Veh and KP-10 (50 KP-10, 100 KP-10, 200 KP-10, 1000 KP-10) on iAUC of GH concentrations from 0 to 75 minutes following treatment (mean ± pooled SEM). iAUCs with different superscripts differ significantly ($P < 0.05$).

Figure 3: Effects of IV and ICV KP-10 on plasma LH concentrations in OVX female sheep (n = 8).

(A) Response of circulating concentration of LH (mean minus SEM; pooled SEM = 0.54) to IV administration of Veh and KP-10 (100 KP-10 [100 pmol/kg]; 200 KP-10 [200 pmol/kg]; 1000 KP-10 [1000 pmol/kg]). * $P < 0.05$ vs Veh; # $P < 0.05$ 1000 KP-10 vs 100 KP-10. Arrow indicates time of administration of treatment.

(B) Response of circulating concentration of LH (mean minus SEM; pooled SEM = 0.54) to ICV administration of Veh and KP-10 (50 KP-10 [50 pmol/kg]; 100 KP-10, 200 KP-10). Arrow indicates time of administration of treatment.

(C) Effect of IV and ICV bolus injection of Veh and KP-10 (50 KP-10, 100 KP-10, 200 KP-10, 1000 KP-10) on iAUC of LH concentrations (mean ± pooled SEM). iAUCs with different superscripts differ significantly ($P < 0.05$).

FIGURE 1.

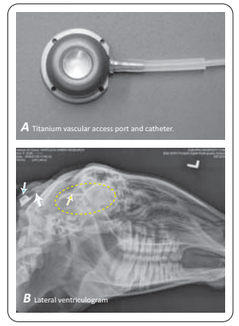


FIGURE 2. ***Plasma GH (IV, ICV)***

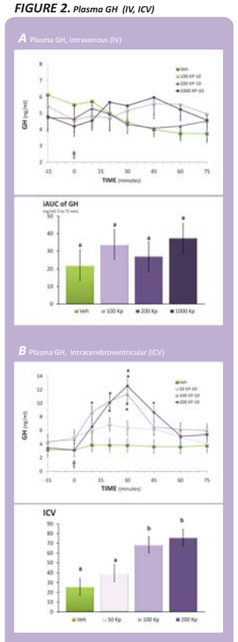
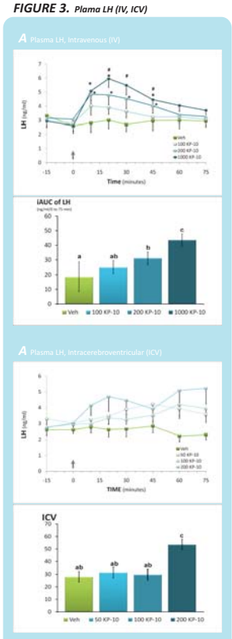


FIGURE 3. ***Plama LH (IV, ICV)***



References

1. Caraty A, Smith JT, Lomet D, et al. Kisspeptin synchronizes preovulatory surges in cyclical ewes and causes ovulation in seasonally acyclic ewes. Endocrinology 2007;148: 5258-5267.
2. Coleman ES, Eliassier TH, Kamppainen RJ, et al. Effect of endotoxin on pituitary hormone secretion in sheep. Neuroendocrinology 1993;58: 111-122.
3. d'Anglemont de Tassigny X, Fagg LA, Carlton MB, et al. Kisspeptin can stimulate gonadotropin-releasing hormone (GnRH) release by a direct action at GnRH nerve terminals. Endocrinology 2008;149: 3926-3932.
4. Daniel JA, Eliassier TH, Martinez A, et al. Interleukin-1beta and tumor necrosis factor-alpha mediation of endotoxin action on growth hormone. Am J Physiol Endocrinol Metab 2005;289: E650-657.
5. de Roux N, Genin E, Carel JC, et al. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A 2003;100: 10972-10976.
6. Decourt C, Tillet Y, Caraty A, et al. Kisspeptin immunoreactive neurons in the equine hypothalamus: Interactions with GnRH neuronal system. J Chem Neuroanat 2008;36: 131-137.
7. Franceschini I, Lomet D, Cateau M, et al. Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha. Neurosci Lett 2006;401: 225-230.
8. Funes S, Hedrick JA, Vassileva G, et al. The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. Biochem Biophys Res Commun 2003;312: 1357-1363.
9. Gladyui A, Krejci P, Simunek J, et al. Effects of central infusions of neuropeptide Y on the somatotropic axis in sheep fed on two levels of protein. Acta Neurobiol Exp (Wars) 2001;61: 255-266.
10. Gutierrez-Pascual E, Martinez-Fuentes AJ, Pinilla L, et al. Direct pituitary effects of kisspeptin: activation of gonadotrophs and somatotrophs and stimulation of luteinising hormone and growth hormone secretion. J Neuroendocrinol 2007;19: 521-530.
11. Hull KL, Harvey S. GH as a co-gonadotropin: the relevance of correlative changes in GH secretion and reproductive state. J Endocrinol 2002;172: 1-19.
12. Hull KL, Harvey S. Growth hormone: roles in female reproduction. J Endocrinol 2001;168: 1-23.
13. Iqbal J, Manley TR, Coff P, et al. Reduction in adiposity affects the extent of different projections to growth hormone-releasing hormone and somatostatin neurons and the degree of colocalisation of neuropeptides in growth-hormone-releasing hormone and somatostatin cells of the ovine hypothalamus. Endocrinology 2005;146: 4776-4785.
14. Kadokawa H, Matsui M, Hayashi F, et al. Peripheral administration of kisspeptin-10 increases plasma concentrations of GH as well as LH in prepubertal Holstein heifers. J Endocrinol 2008;196: 331-334.
15. Kadokawa H, Sunabi S, Hashizume T. Kisspeptin-10 stimulates the secretion of growth hormone and prolactin directly from cultured bovine anterior pituitary cells. Anim Reprod Sci 2008;105: 404-408.
16. Lents CA, Heidorn NL, Barb CR, et al. Central and peripheral administration of kisspeptin activates gonadotropin but not somatotropin secretion in prepubertal gilts. Reproduction 2008;135: 879-887.
17. McMahon CD, Buxton DF, Eliassier TH, et al. Neuropeptide Y restores appetite and alters concentrations of GH after central administration to endotoxic sheep. J Endocrinol 1999;161: 333-339.
18. Morrison CD, Daniel JA, Hampton JH, et al. Luteinizing hormone and growth hormone secretion in ewes infused intracerebroventricularly with neuropeptide Y. Domest Anim Endocrinol 2003;24: 69-80.
19. Sartin JL, Dyer C, Matteri R, et al. Effect of intracerebroventricular orexin-B on food intake in sheep. J Anim Sci 2001;79: 1573-1577.
20. Sartin JL, Marks DL, McMahon CD, et al. Central role of the melanocortin-4 receptors in appetite regulation after endotoxin. J Anim Sci 2008;86: 2557-2567.
21. SAS. SAS User's Guide (9.1.3). Cary, NC: Statistical Analysis Systems Institute, Inc., 2003.
22. Seminara SB, Messager S, Chatzidaki EE, et al. The GPR54 gene as a regulator of puberty. N Engl J Med 2003;349: 1614-1627.
23. Smith JT, Rao A, Pereira A, et al. Kisspeptin is present in ovine hypophysial portal blood but does not increase during the preovulatory luteinizing hormone surge: evidence that gonadotropes are not direct targets of kisspeptin in vivo. Endocrinology 2008;149: 1951-1959.
24. Sun Y, Tian Z, Zhao H, et al. Characteristic of hypothalamic kisspeptin expression in the pubertal development of precocious female rats. Neurosci Lett 2007;420: 12-17.
25. Thomas MG, Gazal OS, Williams GL, et al. Injection of neuropeptide Y into the third cerebroventricle differentially influences pituitary secretion of luteinizing hormone and growth hormone in ovariectomized cows. Domest Anim Endocrinol 1999;16: 159-169.
26. Wagner OS, McMahon CD, Marks DL, et al. A role for agouti-related protein in appetite regulation in a species with continuous nutrient delivery. Neuroendocrinology 2004;80: 210-218.
27. Whitlock BK, Daniel JA, McMahon CD, et al. Intracerebroventricular melanin-concentrating hormone stimulates food intake in sheep. Domest Anim Endocrinol 2005;28: 224-232.
28. Whitlock BK, Daniel JA, Wilborn RR, et al. Interaction of Estrogen and Progesterone on Kisspeptin-10-Stimulated Luteinizing Hormone and Growth Hormone in Ovariectomized Cows. Neuroendocrinology 2008;88: 212-215.