Correspondence of Gonadotropin-Releasing Hormone and Luteinizing Hormone Secretion during Suckling in Postpartum Cows

A.S. Leaflet R1651

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Summary

The hypothalamus in the lower part of the brain contains neurons that produce a small peptide, gonadotropin-releasing hormone (GnRH, LHRH), that regulates luteinizing hormone (LH) secretion by the anterior pituitary gland. Important functions of LH include induction of ovulation in preovulatory follicles during estrus and the luteinization of granulosa cells lining those collapsed follicles to form corpora lutea that produce progesterone during the luteal phase of the estrous cycle or during pregnancy. The production of progesterone by the corpus luteum conveys a negative feed-back action at the central nervous system (CNS) for further episodic secretion of GnRH and in turn, LH secretion. Gonadal removal (i.e., ovariectomy) allows a greater amount of LH secretion to occur during a prolonged period. The objectives of this study were to characterize the pattern of GnRH secretion in the cerebrospinal fluid (CSF) of the bovine third ventricle region of the hypothalamus, determine its correspondence with the tonic and surge release of LH in ovariectomized cows, and examine the dynamics of GnRH pulse release activity in response to known modulators of LH release (suckling, neuropeptide-Y [NPY]). In ovariectomized cows, both tonic release patterns and estradiol-induced surges of GnRH and LH were highly correlated. A 500-microgram dose of NPY caused an immediate cessation of LH pulses and decreased plasma concentrations of LH for at least 4 hours. This corresponded with a decrease in both GnRH pulse amplitude and frequency. In anestrous cows, GnRH pulse frequency did not change before and 48 to 54 hours after weaning on day 18 postpartum, but GnRH concentration and amplitudes of GnRH pulses increased in association with weaning and heightened secretion of LH. It is clear that high-frequency, high-amplitude pulses of LH are accompanied by similar patterns of GnRH in CSF of adult cattle. Yet strong inhibitors of LH pulsatility, putatively acting at the level of the central nervous system (i.e., suckling) or at both the central nervous system and pituitary (NPY) levels, produced periods of discordance between GnRH and LH pulses.

Introduction

The hypothalamus is located at the base of the brain; the median eminence, a region joining the tuber cinereum and infundibulum, connects the pituitary gland by a stalk. A portal system of blood vessels between the median eminence and the adenohypophysis is the pathway for the hypothalamic regulation of pituitary function. Within the hypothalamus, cells with neuronal axons produce luteinizing hormone releasing hormone (LHRH or GnRH) that is secreted into the portal vascular system to regulate LH release from the adenohypophysis. Key events, including the onset of puberty, ovulation, and resumption of cyclic activity after parturition, are governed by the pattern of GnRH secretion and its electrophysiological correlates within the hypothalamus. The present study reports a technique for cannulating the third-ventricle within the hypothalamus and recovering cerebrospinal fluid (CSF) for the detection and quantitation of GnRH secretion (Gazal et al., 1998). The physiological objectives were to examine the correlation of GnRH and LH pulsatility using three experimental models: 1) ovariectomized cows; 2) ovariectomized cows implanted with estradiol and treated with a potent inhibitor of LH release, neuropeptide Y (NPY); and 3) intact, anestrous females before and after weaning-induced increase in LH.

Materials and Methods

Cannulation of third ventricle

Surgical cannulation of the third ventricle was achieved by stereotaxic positioning of the 16 gauge stainless steel cannula, based on radiographs. The cannula was set perpendicularly to the dorsal surface of the head along the midsagittal line at a position one-fourth of the distance from the orbital intersect to the poll. The orbital intersect was the intersection of the midsagittal line with a line connecting the caudal limits of the right and left orbits. A polyvinyl chloride ring was placed into the circumscribed area surrounding the cannula, and the ring and cannula were anchored to the frontal bone with acrylic cement. Using aseptic techniques, a blunt 22-gauge needle was attached to the proximal end of the cannula for CSF collection at 10-min intervals simultaneously with jugular blood samples for 6 h for radioimmunoassay (RIA) of GnRH and LH.

Experiment 1: Tonic and surge release of GnRH in ovariectomized cows

The hypothesis tested was that the pattern of GnRH secretion in third-ventricle CSF would be highly correlated with pulses of LH and with the preovulatory LH surge in peripheral blood. Brahman x Hereford (F_1) pluriparous cows were ovariectomized at least 1 month before cranial surgery using a standing paralumbar approach. Using aseptic procedures, a blunt 22-gauge needle was attached to the proximal end of the cannula for CSF collection during phase 1 (tonic secretion). CSF (400-600 μl) was collected at 10-min intervals simultaneously with jugular blood samples for 6 h.

Experiment 2: Effects of third ventricular infusion of NPY on GnRH and LH secretory dynamics in ovariectomized, estradiol-implanted cows

Mature cows (Brahman x Hereford, F_1) were ovariectomized, and a subcutaneous silastic implant containing crystalline estradiol was placed in one ear of each cow. Approximately 2 weeks later, cows were fitted surgically with third-ventricle cannulas and assigned randomly to receive 0, 50, and 500 μg porcine NPY. During each of three treatment periods, spaced at least 2 days apart, single blood (10 ml) and CSF (600 μl) samples were collected from each cow immediately before infusion of NPY or a saline vehicle into the third ventricle.

Experiment 3: GnRH secretion before and after weaning in intact, anestrous cows

Third-ventricle cannulae were surgically installed in seven crossbred (five Brahman x Hereford, F_1, and two 1/4 Brahman x 1/4 Hereford x 1/2 Angus) cows on day 270 of gestation. On day 18 postpartum, jugular blood and third-ventricle CSF were sampled at 10-min intervals for 6 h. Cows were weaned, and 48-54 h later (day 21) the sampling process was repeated.

RIAs

GnRH was measured in duplicate 75- to 150μl CSF samples as described previously. The sensitivity of the assay was 0.5 pg/ml, and average intra- and interassay coefficients of variation (CV) were 3% and 15%, respectively.

Plasma concentrations of LH were determined in duplicate 200-μl aliquots as previously described. The sensitivity of the assay averaged 0.1 ng/ml, and average intra- and interassay CV were 3% and 13%, respectively.

Results and Discussion

Experiment 1: Tonic and surge release of GnRH in ovariectomized cows

Tonic patterns of CSF GnRH and plasma LH secretion in four representative ovariectomized cows are shown in Figure 1. GnRH was secreted into the CSF of the third ventricle in a pulsatile pattern. A similar pattern of CSF GnRH and plasma LH secretion was observed. All LH peaks (100%) occurred within two sampling points after onset of a GnRH pulse.

Estradiol-induced surges of LH ranging from 14.7 to 97 ng/ml were observed in four of five cows between 18 and 21.25 h after estradiol injection. The duration of the surges varied considerably in these long-term ovariectomized females, ranging from 5 to 13.25 h (Table 1). Surges of GnRH occurred coincident with those of LH, and their magnitudes were proportional to those of corresponding LH surges.

Experiment 2: Effects of third ventricular injection of NPY on GnRH and LH secretory dynamics in ovariectomized, estradiol-implanted cows

Infusion of 50 μg NPY tended to cause a decrease ($P<0.10$) in mean LH concentrations compared with the control. At the higher NPY dose (500 μg), an immediate cessation of LH pulsatility was observed in all cows, and this was accompanied by lower mean concentrations of LH ($P<0.001$; Figure 2; Table 2).

Table 1. Characteristics of mean estradiol-induced surges of plasma LH and CSF GnRH in four long-term ovariectomized cows.

	Characteristics			
Hormone	Baseline concentration[a]	Onset of surge after injection (h)	Duration of surge (h)	Peak concentration[a]
LH	3.06	18.6	7.8	55.4
GnRH	4.7	18.6	N/A[b]	14.5

[a]LH (ng/ml) and GnRH (pg/ml), respectively, before estradioal injection.
[b]Sampling duration inadequate to characterize.

Experiment 3: GnRH secretion before and after weaning in intact, anestrous cows

Compared with the preweaning control period, mean baseline concentrations of GnRH (P<0.05), and overall mean concentrations of GnRH, (P>0.01) increased postweaning, but GnRH pulse frequency did not change (P>0.05). In all cows, the frequency of GnRH pulses was essentially identical before and after weaning, averaging 1.1-1.3 pulses/h.

Results from ovariectomized cows indicated that pulsatile secretion of GnRH in cattle can be detected by frequent sampling of CSF from the third ventricle. Recent studies have reported the detection of GnRH pulses in the CSF of rabbits, monkeys, sheep, and rats, but to our knowledge this is the first report of this phenomenon in cattle.

The general pattern of GnRH release during the estradiol-induced LH surge was similar to that reported previously for the ewe in either CSF or both CSF and portal blood. The sampling frequency of the current study was greater than in the sheep studies (15 min vs. 30 min); therefore, the acute pattern of GnRH pulsatility during the surge was more evident. The mean durations of LH and GnRH surges in this study were shorter than the mean duration of natural or estradiol-induced LH-surges reported previously in cattle.

Evidence suggests that the peptide NPY acts as a neuromodulatory link between nutritional status and the central reproductive axis. This peptide is highly expressed in hypothalamus, anterior pituitary, and adipose tissue; increases dramatically in CSF during undernutrition; and negatively modulates the secretion of LH when centrally infused. Effects of third ventricular infusion of NPY on LH secretion in this study were similar to those previously observed in the monkey, ewe, and rodent.

Suckling inhibits the pulsatile release of LH and delays first postpartum ovulation in cattle for an average of 45-60 days. Removal of calves as early as 2 weeks postpartum results in a rapid rise of LH pulse frequency within 2-6 days, with the majority of cows exhibiting this response within 48 hours. The current work indicates that suckling does not strongly inhibit the rate of GnRH neuronal activity in well-nourished cattle beyond the second to third week postpartum. Although weaning-induced increases in LH pulse frequency and plasma concentrations of LH occurred coincident with increases in basal and overall concentrations of CSF GnRH and GnRH pulse amplitude, no changes in GnRH pulse frequency were observed.

Implications

The technique of third-ventricle cannulation provides a more direct assessment of the activity of the hypothalamic GnRH pulse generator of adult cattle than deduced by monitoring only the dynamic changes in plasma LH concentration. It is clear that high-frequency, high-amplitude pulses of LH are accompanied by similar patterns of GnRH release in CSF. Yet strong inhibitors of LH pulsatility, putatively acting at the level of the central nervous system (suckling) or at both central nervous system and pituitary (NPY) levels, produced periods of discordance between GnRH and LH pulses.

Reference

Gazal, O.S., L.S. Leshin, R.L. Stanko, M.G. Thomas, D.H. Keisler, L.L. Anderson and G.L. Williams. 1998. Gonadotropin-releasing hormone secretion into third-ventricle cerebrospinal fluid of cattle: Correspondence with the tonic and surge release of luteinizing hormone and its tonic inhibition by suckling and neuropeptide Y. Biol. Reprod. 59:676-683.

Table 2. Effects of third ventricular infusion of NPY on mean secretion of plasma LH and CSF GnRH in three ovariectomized, estradiol-implanted cows treated with 0 (saline), 50, and 500 μg NPY in a Latin square arrangement.

	LH		GnRH	
Dose of NPY (μg)	Amplitude (ng/ml)	Frequency (pulses/h)	Amplitude (pg/ml)	Frequency (pulses/h)
0 (saline)	3.2	0.9	3.0	1.5
50	1.2[a]	1.0	2.6	1.4
500	0[b]	0[b]	2.0[a]	0.9[a]

[a]Differs from control (P<0.05).
bDiffers from control (P<0.001).

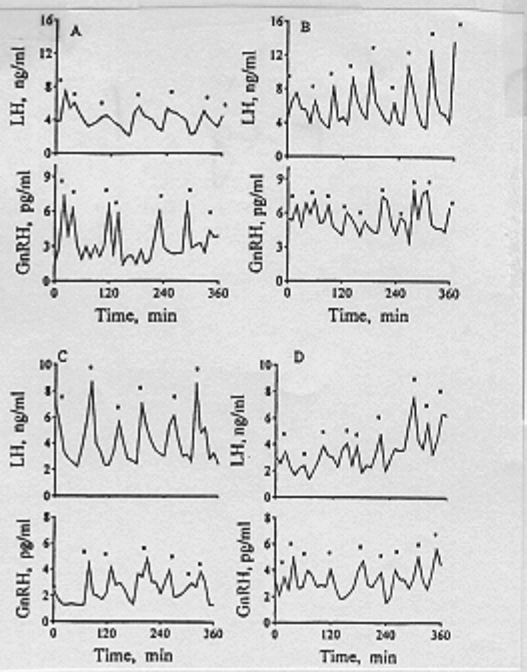

Figure 1. Patterns of CSF GnRH (bottom panels) and LH (top panels) secretion in four representative ovariectomized cows in experiment 1 (A-D). *Synchronous pulses of both hormones as detected by a pulse detection algorithm.

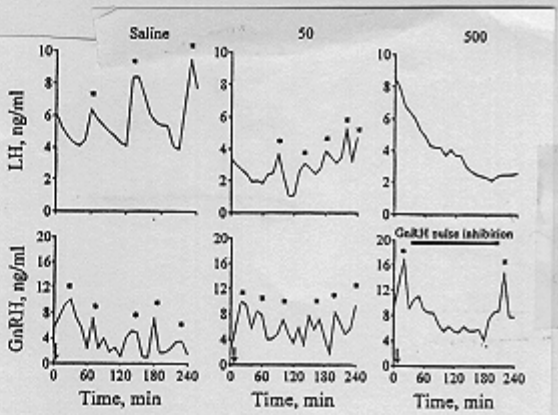

Figure 2. Patterns of CSF GnRH (bottom panels) and plasma LH (top panels) in two cows after third ventricular injection of 0, 50, and 500 μg NPY. Note 1) the complete absence of LH pulses after the 500-μg dose coincident with the cessation of GnRH pulses (denoted by solid horizontal bars) for 3 h, and 2) the rebound in CSF GnRH pulsatility coincident with a continued suppression of LH. *LH and GnRH pulses. Arrows, injection of NPY.