Gonadotropin and Prolactin Secretion following Intraventricular Administration of Morphine in Gilts (43007A)

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Abstract. **The effects of central nervous system administration of morphine on secretion of luteinizing hormone (LH), follicle-stimulating hormone, and prolactin were investigated in ovariectomized gilts stereotaxically implanted with lateral ventricular cannulas. In Experiment 1, mean serum LH and follicle-stimulating hormone concentrations and serum LH pulse frequency were unaffected by artificial cerebrospinal fluid administration ($P > 0.1$), but decreased ($P < 0.01$) in 8 of 11 gilts when 500 μg of morphine were given 3 hr later. Serum LH pulse amplitude was unaffected ($P > 0.1$) by cerebrospinal fluid or morphine injection. In Experiment 2, luteinizing hormone concentrations decreased ($P < 0.0001$) and prolactin concentrations increased ($P < 0.0001$), but follicle-stimulating hormone concentrations did not change ($P > 0.1$) after 500 μg of morphine. Gonadotropin responses to 10 μg of gonadotropin-releasing hormone, given 2 hr after intraventricular injection, were similar ($P > 0.1$) for morphine- and cerebrospinal fluid-treated gilts. These results indicate that morphine inhibits LH secretion at the level of the central nervous system, and are consistent with the concept that endogenous opioid peptides participate in the regulation of gonadotropin and prolactin release in pigs.**

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That opioid agonists inhibit luteinizing hormone (LH) secretion and stimulate prolactin release in rats (1–3), subhuman primates (4), and sheep (5, 6) is well documented. Few studies, however, have been conducted to determine the effects of opioids and their agonists on gonadotropin and prolactin secretion in swine. Injection of β-endorphin into the amygdala inhibited LH secretion in ovariectomized miniature pigs (7) and peripheral administration of morphine suppressed LH and prolactin release in postpartum sows (8). The following experiments were conducted to examine the effects of morphine on LH, follicle-stimulating hormone (FSH), and prolactin secretion in ovariectomized gilts. To directly access areas of the brain controlling anterior pituitary hormone secretion and to minimize confounding peripheral responses, morphine was injected into the central nervous system via a lateral cerebral ventricular cannula.

Materials and Methods

Surgery. Crossbred gilts, which had exhibited two consecutive estrous cycles of 18 to 21 days, were anesthetized and their heads placed in a stereotaxic instrument (Kopf, Inc., Tujunga, CA) with modifications patterned after a unit described by Poceta *et al.* (9). The head was oriented such that the forehead rose at a 30° angle to the horizontal plane. A circle of skin and underlying periosteum, 35 mm in diameter, centered 41 mm anterior to the ear bar tips of the stereotaxic unit and 3 mm lateral to midline, were excised from the frontal-parietal bone. Stereotaxic coordinates for approaching the lateral cerebral ventricles were derived from measurements of the heads of eight gilts (approximately 100 kg body wt) obtained from an abattoir. A 2-mm diameter hole was drilled through the skull perpendicular to the frontal-parietal bone and an 18-gauge, 29.5-mm long stainless steel guide tube inserted through the hole and anchored to the skull with stainless steel screws and acrylic cement. A 22-gauge stainless steel injection cannula, attached to a vertically held, 30 cm-long silicone tube filled with 0.9% saline, was slowly lowered stereotaxically through the guide tube until an influx of fluid was observed, indicating entrance into the ventricular space. Individual cannulas were then cut to a length, specific for each pig, to accommodate the distance from the surface of the skull to a lateral ventricle which averaged 44.6 ± 1.3 mm (mean ± SE;

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$n = 11$). Between experiments, the injection cannula was replaced with a 22-gauge, 29.5-mm long trocar.

Placement of the cannula into a lateral ventricle was confirmed roentgenographically (Toshiba x-ray Unit, model KCD-10M07A; of Toshiba, Tustin, CA) for each pig after intraventricular injection of 1 ml of radiopaque medium (metrizamide, 400 mg iodine/1 ml 0.9% saline; Sigma Chemical Co., St. Louis, MO).

Gilts were ovariectomized within 14 days after lateral ventricular cannulation. On the day before experiments, gilts were nonsurgically fitted with indwelling jugular vein cannulas (10) and the trocars were replaced with intraventricular injection cannulas.

Preliminary Experiment. Gilts, 60 days postovariectomy and weighing 110 ± 1.7 kg, received intraventricular injections of either 5 ($n = 2$), 50 ($n = 2$), or 500 μg ($n = 3$) of morphine sulfate (Eli Lilly Co., Indianapolis, IN) in 500 μl of artificial cerebrospinal fluid (CSF). The CSF consisted of 2.1190 g of $Na_2HPO_4 \cdot 7H_2O$, 0.5478 g of $NaH_2PO_4 \cdot H_2O$, 7.45 g of NaCl, 0.1864 g of KCl, 0.2 g of $CaCl_2 \cdot 2H_2O$, and 0.2465 g of $MgSO_4 \cdot 7H_2O$/liter of deionized water. The pH was adjusted to 7.2 with NaOH. Blood samples were collected from the jugular cannula every 15 min for 6 hr, beginning 3 hr before intraventricular injection. All samples were quantified for LH and prolactin.

Experiment 1. Experiment 1 was conducted 7 days after ovariectomy. At 1100 hr, 11 gilts, weighing 110 ± 2.5 kg, received 500 μl of CSF intraventricularly, followed 3 hr later by 500 μg of morphine in 500 μl of CSF. Blood was sampled from the venous cannula every 15 min for 12 hr, beginning 3 hr before CSF. Serum samples were analyzed for LH and FSH concentrations.

Experiment 2. Experiment 2 was conducted 47 days after ovariectomy. At 1100 hr, six gilts, weighing 113 ± 4.1 kg, received intraventricular injections of either 100 μl of CSF ($n = 3$) or 500 μg of morphine in 100 μl of CSF ($n = 3$). Two hours later, all gilts received an iv injection of 10 μg of gonadotropin-releasing hormone (GnRH) (Cystorelin; CEVA Laboratories, Inc., Overland Park, KS). Blood samples were collected from the jugular cannula every 15 min for 6 hr, beginning 3 hr before intraventricular injection. All samples were quantified for LH, FSH, and prolactin.

Hormone Assays. Concentrations of LH were determined by duplicate measurement of 300 μl of serum using a previously reported procedure (10) with modifications (11, 12). The first antibody was produced in rabbits against bovine LH (NIH-LH-B9). Purified porcine LH was used for iodination (USDA-pLH-I-1) and standards (USDA-pLH-B-1). Intra- and interassay coefficients of variation were 5.8 and 8.7%, respectively. Assay sensitivity, defined as the smallest amount of hormone significantly different from 0 at the 95% confidence level, was 0.05 ng/tube.

Serum FSH concentrations in duplicate 200-μl samples were quantified as previously reported (12). Rabbit anti-porcine FSH serum (USDA 10-1010) was used for the primary antiserum, purified porcine FSH (USDA FSH-PP1) for iodination, and porcine FSH (USDA P-FSH-B-1) as the standard. Intra- and interassay coefficients of variation and assay sensitivity were 2.2%, 10.8%, and 0.6 ng/tube, respectively.

Concentrations of prolactin were determined by duplicate measurement of 200 μl of serum using methods previously published (10) with modifications (11, 12). The primary antiserum, produced in goats, was purchased from Research Products, International Corp. (Elk Grove Village, IL). USDA-pPRL-I-1 and USDA-pPRL-B-1 were used for iodination and for standards, respectively. Intra- and interassay coefficients of variation were 10.8 and 19.8%, respectively. Assay sensitivity was 0.2 ng/tube.

Statistical Analyses. Hormone data from each experiment were analyzed using the general linear model procedure of the Statistical Analysis System (13). For the preliminary experiment and Experiment 2, serum hormone concentrations were subjected to a split-plot-in-time analysis of variance. The statistical model included treatment, time, and the treatment × time interaction. The effect of treatment was tested using animal within treatment as the error term. Time and treatment × time were tested against animal within treatment × time.

For analyzing Experiment 1, the 12-hr sampling period was divided into four periods corresponding to the 3 hr before CSF injection, the 3 hr after CSF injection, and the two consecutive 3-hr periods following morphine administration. Mean serum LH concentrations, LH pulse frequency and amplitude, and mean serum FSH concentrations were then calculated for each gilt within each period. Data were then subjected to analysis of variance using a model that included gilt and period as possible sources of variation. The gilt × period interaction was used as the error term. Differences between period means were determined by the following preplanned orthogonal contrasts: the 3 hr before CSF versus the 3 hr after CSF; the first 3 hr after morphine versus the second 3 hr after morphine; and the 6 hr before morphine versus the 6 hr after morphine. A LH pulse was defined as an increment exceeding twice the standard deviation associated with the mean LH concentration of a serum pool which was analyzed eight times in each assay (12). To be classified as a pulse, the increment had to occur within 15 min of the previous nadir (12). Pulse amplitude equalled the difference between the pulse peak and the preceding nadir (12). Pulse frequency was expressed as the number of pulses observed per 3 hr.

Results

Preliminary Experiment. Analysis of variance revealed an effect of time (before versus after morphine, $P < 0.05$), but no time by dose interaction ($P > 0.1$) for mean serum LH concentrations, suggesting that the three doses of morphine employed were equally effec-

tive in suppressing LH secretion (Table I). In contrast, morphine injection failed to alter ($P > 0.1$) mean prolactin concentrations (Table I).

Experiment 1. Mean serum LH and FSH concentrations, as well as serum LH pulse frequency, were unaffected ($P > 0.1$) by CSF injection, but were decreased ($P < 0.01$) by injection of 500 μg of morphine in 8 of 11 gilts. The data for these eight gilts are shown in Table II and will be discussed first. Serum LH pulse amplitude was unaffected by CSF injection ($P > 0.1$) and was similar ($P > 0.1$) before and after morphine administration (Table II). The profiles of serum LH concentrations for individual gilts are depicted in Figure 1. In five of the eight gilts in which serum LH pulse frequency was unaffected by CSF injection, no serum LH pulses occurred after administration of the opiate (Fig. 1A). In the other three gilts, LH pulse frequency was decreased, but not completely arrested (Fig. 1B). Serum FSH concentrations exhibited no clear pulsatile profile.

In the remaining 3 of 11 treated gilts, LH secretion appeared to be decreasing before morphine was given. Mean serum LH concentrations for these animals were 0.89 ± 0.09 and 0.53 ± 0.05 ng/ml for the 3-hr periods before and after CSF injection, respectively, and 0.22 ± 0.03 ng/ml for the 6-hr period following morphine.

Table I. Mean (±SE) Serum LH and Prolactin Concentrations in Ovariectomized Gilts before and after Intraventricular Injection of Various Doses of Morphine

Dose (μg)	*n*	LH (ng/ml)[a]		Prolactin (ng/ml)	
		Before	After	Before	After
5	2	0.97 ± 0.05	0.69 ± 0.11	4.92 ± 0.01	5.51 ± 0.09
50	2	0.87 ± 0.19	0.64 ± 0.01	4.75 ± 1.35	3.85 ± 0.05
500	3	1.13 ± 0.24	0.74 ± 0.30	4.60 ± 0.30	9.07 ± 2.80

[a] Effect of period (before versus after morphine), $P < 0.05$.

Table II. Mean (±SE) Serum LH and FSH Concentrations and LH Pulse Frequency and Amplitude in Ovariectomized Gilts before and after Intraventricular Injection of 500 μg of Morphine[a]

Item	Before morphine	After morphine
LH concentrations (ng/ml)	0.80 ± 0.05[b]	0.39 ± 0.07[c]
LH pulse frequency (pulses/3 hr)	3.7 ± 0.1[b]	0.9 ± 0.3[c]
LH pulse amplitude (peak-nadir; ng/ml)	0.37 ± 0.04[b]	0.52 ± 0.10[b]
FSH concentrations (ng/ml)	105 ± 0.9[b]	94 ± 2.2[c]

[a] Eleven gilts were given intraventricular injections of 500 μl of CSF followed 3 hr later by 500 μg of morphine. Blood samples were collected every 15 min for 12 hr, beginning 3 hr before CSF. Data shown represent eight gilts in which LH and FSH release was unaffected ($P > 0.1$) by CSF but decreased ($P < 0.01$) after morphine. Since CSF had no effect on hormone secretion, data were pooled to show means for the 6-hr periods before and after morphine injection.
[b,c] Means in a row with different superscripts differ ($P < 0.01$).

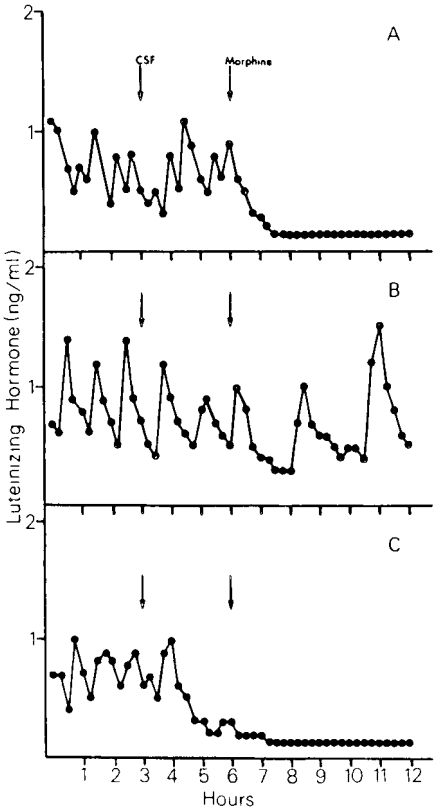

Figure 1. Serum LH concentrations for three ovariectomized gilts given intraventricular injections of 500 μl of CSF at Hour 3 and 500 μg of morphine in 500 μl of CSF at Hour 6. Gilt A is representative of five gilts in which no serum LH pulses occurred following morphine. Gilt B represents three gilts in which pulse frequency was decreased but not arrested following morphine. Gilt C represents three gilts that had decreasing serum LH concentrations following injection of 500 μl of CSF.

The serum LH concentrations for one of these three animals are depicted in Figure 1C. Serum FSH concentrations for these three gilts were 130 ± 4.2 ng/ml before CSF injection, 125 ± 3.6 ng/ml during the 3 hr following CSF injection ($P > 0.1$), but decreased ($P < 0.05$) to 112 ± 1.9 ng/ml following morphine.

Experiment 2. Serum LH and FSH concentrations were similar ($P > 0.1$) between groups prior to intraventricular injection, averaging 1.11 ± 0.05 ng/ml and 140 ± 4.3 ng/ml, respectively. Administration of CSF had no effect on the secretion of either gonadotropin, while morphine decreased serum LH ($P < 0.0001$; Fig. 2), but did not alter ($P > 0.1$) serum FSH concentrations. Serum LH and FSH concentrations in morphine-treated gilts were 0.33 ± 0.04 ng/ml and 137 ± 15 ng/ml, respectively, 2 hr after injection. The peak LH and FSH responses (4.7 ± 0.9 ng/ml and 191 ± 19 ng/ml, respectively) to an exogenous challenge of GnRH occurred within 30 min in all gilts and were similar ($P > 0.1$) between morphine- and CSF-treated gilts.

Serum prolactin concentrations were similar ($P > 0.1$) for CSF- and morphine-treated animals before intraventricular injection and averaged 6.0 ± 0.2 ng/ml. Morphine evoked an immediate increase ($P < 0.0001$) in prolactin release such that serum concentra-

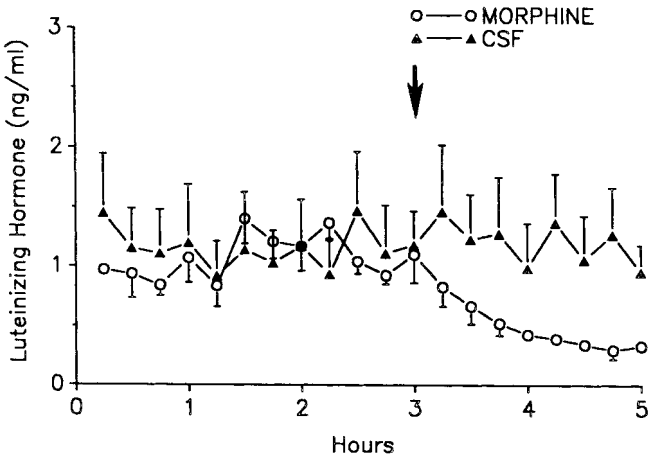

Figure 2. Serum LH concentrations in ovariectomized gilts treated intraventricularly with CSF (100 μl) or morphine (500 μg/100 μl of CSF). Drug or vehicle injection occurred at Hour 3 and is represented by the arrow. Values are mean ± SE for three gilts. Administration of morphine decreased ($P < 0.0001$) serum LH concentrations.

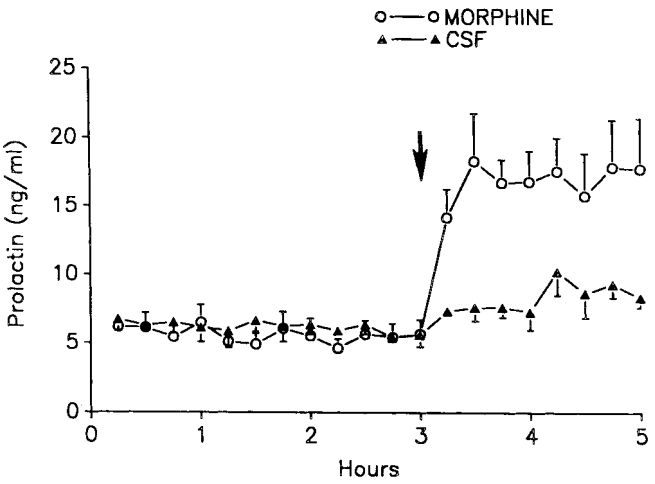

Figure 3. Serum prolactin concentrations in ovariectomized gilts treated intraventricularly with CSF (100 μl) or morphine (500 μg/100 μl of CSF). Drug or vehicle injection occurred at Hour 3 and is represented by the arrow. Values are mean ± SE for three gilts. Administration of morphine increased ($P < 0.0001$) serum prolactin concentrations.

tions averaged 16.8 ± 0.9 ng/ml for the 2-hr period following injection (Fig. 3).

Discussion

Morphine administration decreased serum LH concentrations in mature, ovariectomized gilts, which is consistent with earlier studies in rodents (1–3), subhuman primates (4), sheep (5, 6), and sows (8). The results of the present investigation, in conjunction with previous reports in which the opioid antagonist naloxone increased LH secretion (11, 14–17), provide evidence that the endogenous opioid peptides are involved in mechanisms which suppress LH secretion in the pig. This concept is supported by the recent demonstration of β-endorphin immunostaining in the gilt hypothalamus (18).

In three gilts from Experiment 1 (Fig. 1C), cessation of pulsatile LH secretion cannot be attributed to morphine since serum LH concentrations began to decrease before the opiate was administered. Conceivably, this inhibition could have been due to the larger volume of CSF injected during the initial studies. If so, the response was delayed (≥1 hr), in contrast to the immediate suppression following morphine (≤15 min; Fig. 1A and B) in the remaining eight gilts. In subsequent studies in which the vehicle volume was reduced from 500 to 100 μl (Experiment 2 [19]) serum LH concentrations were inhibited by morphine, but in no case did they decrease after vehicle alone.

Intraventricular morphine administration decreased not only mean serum LH concentrations, but also LH pulse frequency, suggesting that the hypothalamic discharge of GnRH was inhibited. While an abundance of evidence indicates that opioids inhibit LH secretion by a hypothalamic site of action (20–23), direct effects of opioids or their antagonists on the pituitary have also been demonstrated (24–26). Since substances introduced into the ventricular system reached the anterior pituitary gland via the hypophysial portal vasculature in rats (27), we determined whether morphine given centrally might directly compromise gonadotropin secretion by a similar route in pigs. Our finding that pituitary responsiveness to exogenous GnRH was not altered during a period when LH release was inhibited by morphine corroborates other reports (4, 5) and indicates that the principal inhibitory influence of the opiate on LH secretion was expressed at the level of the central nervous system.

Opioids and their agonists readily stimulated prolactin secretion in a variety of species including rats (2, 3), and sheep (6). This report extends these findings to the ovariectomized gilt. In postpartum sows, however, peripherally administered morphine inhibited prolactin secretion (8). The authors of that study suggested that the dose of morphine utilized and an assumed suckling-induced increase in endogenous opioid peptide neuronal activity may have caused desensitization or down-regulation of opiate receptors and thus decreased prolactin release. Indeed, the apparent opposite effects of morphine on prolactin secretion in the ovariectomized gilt and postpartum sow could be a result of the different doses of opiate utilized or routes of administration (intraventricular versus intravenous). Alternatively, the differential responses may be inherent to animals of varying reproductive status. The intimate association between reproductive status of swine and modulation of prolactin secretion by endogenous opioid peptides has been demonstrated in studies employing naloxone. Naloxone suppressed prolactin secretion in postpartum sows (16, 17, 28). Conversely, the opioid antagonist stimulated prolactin release in gilts during the luteal phase, but not during the follicular phase of the estrous cycle or after ovariectomy (14).

This is the first report of the effects of an opioid agonist on FSH secretion in the pig. In Experiment 1,

intraventricular morphine administration inhibited FSH secretion. The decrease, however, was not reproduced in Experiment 2. Perhaps the apparent refractoriness to morphine, demonstrated in Experiment 2, was related to time after gonadectomy. Experiments 1 and 2 were conducted at 7 and 47 days following ovariectomy, respectively. Similarly, in rats (29), the ability of naloxone or the opioid agonist FK 33-824, to alter FSH secretion was lost with time after castration. Our finding that FSH secretion was unaltered at a time when LH release was inhibited raises the possibility of dual neuroendocrine control of secretion of the gonadotropins in the pig. This work is in agreement with that in castrated rats (1) in which intraventricular morphine administration suppressed LH but not FSH secretion. Thus, a precise role for endogenous opioid peptides in regulating FSH secretion in swine remains to be determined. Analogous to their modulation of prolactin release, opioid control of FSH secretion may depend on the reproductive status of the pig. For example, naloxone stimulated FSH secretion in postpartum sows during suckling, but not after weaning (28).

The ventricular cannulation procedure described provides a reliable and effective method for administering substances into the central nervous system of the domestic pig. Intraventricular morphine administration inhibited LH and stimulated prolactin secretion. Furthermore, morphine likely suppressed LH secretion by acting at the level of the central nervous system.

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1. Piva F, Limonta P, Maggi R, Martini L. Stimulatory and inhibitory effects of the opioids on gonadotropin secretion. Neuroendocrinology **42**:504–512, 1986.
2. Bruni JF, Van Vugt D, Marshall S, Meites J. Effects of naloxone, morphine and methionine enkephalin on serum prolactin, luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and growth hormone. Life Sci **21**:461–466, 1977.
3. Ieiri T, Chen HT, Meites J. Effects of morphine and naloxone on serum levels of luteinizing hormone and prolactin in prepubertal male and female rats. Neuroendocrinology **29**:288–292, 1979.
4. Ferin M, Van Vugt D, Wardlaw S. The hypothalamic control of the menstrual cycle and the role of endogenous opioid peptides. Recent Horm Res **40**:441–485, 1984.
5. Schanbacher BD. Endogenous opiates and the hypothalamic-pituitary-gonadal axis in male sheep. Dom Anim Endocrinol **2**:67–75, 1985.
6. Schillo KK, Kuehl D, Jackson GL. Do endogenous opioid peptides mediate the effects of photoperiod on release of luteinizing hormone and prolactin in ovariectomized ewes? Biol Reprod **32**:779–787, 1985.
7. Parvizi N, Ellendorff F. β-Endorphin alters luteinizing hormone secretion via the amygdala but not the hypothalamus. Nature **286**:812–813, 1980.
8. Armstrong JD, Kraeling RR, Britt JH. Morphine suppresses luteinizing hormone concentrations in transiently weaned sows and delays onset of estrus after weaning. J Anim Sci **66**:2216–2223, 1988.
9. Poceta JS, Hamlin MN, Haack DW, Bohr DF. Stereotaxic placement of cannulae in cerebral ventricles of the pig. Anat Rec **200**:349–356, 1981.
10. Kraeling RR, Rampacek GB, Cox NM, Kiser TE. Prolactin and luteinizing hormone secretion after bromocryptine (CB-154) treatment in lactating sows and ovariectomized gilts. J Anim Sci 54:1212–1220, 1982.
11. Barb CR, Kraeling RR, Rampacek GB, Whisnant CS. Opioid inhibition stimulates luteinizing hormone and prolactin secretion in the gilt. Domestic Anim Endocr **2**:93–98, 1985.
12. Kesner JS, Kraeling RR, Rampacek GB, Johnson B. Absence of an estradiol-induced surge of luteinizing hormone in pigs receiving unvarying pulsatile gonadotropin releasing hormone stimulation. Endocrinology **121**:1862–1869, 1987.
13. SAS. SAS User's Guide. Cary, NC: Statistical Analysis System Institute, 1982.
14. Barb CR, Kraeling RR, Rampacek GB, Whisnant CS. Influence of stage of the estrous cycle on endogenous opioid modulation of luteinizing hormone, prolactin and cortisol secretion in the gilt. Biol Reprod **35**:1162–1167, 1986.
15. Barb CR, Kraeling RR, Rampacek GB, Whisnant CS. Opioid inhibition of luteinizing hormone secretion in the postpartum lactating sow. Biol Reprod **35**:368–371, 1986.
16. Mattioli M, Conte F, Galeati G, Seren E. Effect of naloxone on plasma concentrations of prolactin and LH in lactating sows. J Reprod Fertil **76**:167–173, 1986.
17. Armstrong JD, Kraeling RR, Britt JH. Effects of naloxone or transient weaning on secretion of LH and prolactin in lactating sows. J Reprod Fertil **83**:301–308, 1988.
18. Kineman RD, Kraeling RR, Crim JW, Leshin LS, Barb CR, Rampacek GB. Localization of proopiomelanocortin (POMC) immunoreactive neurons in the forebrain of the pig. Biol Reprod **40**:1119–1126, 1989.
19. Barb CR, Kesner JS, Rampacek GB, Kraeling RR, Kineman RD. Luteinizing hormone (LH) secretion following brain intraventricular (IVT) administration of morphine in the prepuberal gilt. J Anim Sci **66**(suppl 1):408, 1988.
20. Wilkes MM, Yen SSC. Augmentation by naloxone of efflux of LRF from superfused medial basal hypothalamus. Life Sci **28**:2355–2359, 1981.
21. Blank MS, Roberts DL. Antagonist of gonadotropin-releasing hormone blocks naloxone-induced elevations in serum luteinizing hormone. Neuroendocrinology **35**:309–312, 1982.
22. Ching M. Morphine suppresses the proestrous surge of GnRH in pituitary portal plasma of rats. Endocrinology **112**:2209–2211, 1983.
23. Kesner JS, Kaufman J-M, Wilson RC, Kuroda G, Knobil E. The effect of morphine on the electrophysiological activity of the hypothalamic luteinizing hormone-releasing hormone pulse generator in the rhesus monkey. Neuroendocrinology **43**:686–688, 1986.
24. Chao CC, Moss GE, Malven PV. Direct opioid regulation of pituitary release of bovine luteinizing hormone. Life Sci **39**:527–534, 1986.
25. Cacicedo L, Sanchez-Franco F. Direct action of opioid peptides

and naloxone on gonadotropin secretion by cultured rat anterior pituitary cells. Life Sci **38**:617–625, 1986.
26. Blank MS, Fabbri A, Catt KJ, Dufau ML. Inhibition of luteinizing hormone release by morphine and endogenous opiates in cultured pituitary cells. Endocrinology **118**:2097–2101, 1986.
27. Oliver C, Ben-Jonathan N, Mical RS, Porter JC. Transport of thyrotropin-releasing hormone from cerebrospinal fluid to hypophysial portal blood and the release of thyrotropin. Endocrinology **97**:1138–1143, 1975.
28. Barb CR, Kraeling RR, Rampacek GB, Leshin LS. Opioid modulation of follicle stimulating hormone (FSH) and prolactin (PRL) secretion in the postpartum sow. In: Mahesh VB, Dhindsa DS, Anderson E, Kalra SP, Eds. Advances in Experimental Medicine and Biology. New York: Plenum Press, vol **219**: pp 647–652, 1987.
29. Bhanot R, Wilkinson M. The inhibitory effect of opiates on gonadotropin secretion is dependent upon gonadal steroids. J Endocrinol **102**:133–141, 1984.