Endocrine, *vol. 18, no. 1, 27–32, June 2002* 0969–711X/02/18:27–32/$11.50

Effect of Prodynorphin-Derived Opioid Peptides on the Ovulatory Luteinizing Hormone Surge in the Proestrous Rat

Qiang Zhang and Robert V. Gallo

Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT

The objective of this study was to determine whether prodynorphin-derived opioid peptides could block the spontaneous luteinizing hormone (LH) surge and ovulation, and if so, whether this inhibitory action was mediated through κ-opioid receptors. Various doses of dynorphin peptides (dynorphin $A_{1–17}$, dynorphin $A_{1–8}$, dynorphin B, α- and β-neoendorphin) were infused into the brain through third-ventricle cannulae in rats between 1330–1800 h on proestrus. Each dynorphin peptide blocked the LH surge and ovulation in a dose-dependent manner. Dynorphin $A_{1–17}$ and $A_{1–8}$ were equally effective in producing these actions, and more potent than either dynorphin B or α- or β-neoendorphin. U50,488H, a specific κ-opioid receptor agonist, also blocked the LH surge and ovulation. When a mixture of five dynorphin peptides was infused intraventricularly, each at a dose that inhibited the LH surge, both the surge and ovulation were blocked. However, when norbinaltorphimine, a specific κ-opioid receptor antagonist, was coinfused with the mixture of dynorphin peptides, the LH surge and ovulation were fully restored. These results demonstrate that prodynorphin-derived opioid peptides, acting through κ-opioid receptors, can block the LH surge and ovulation. Dynorphin $A_{1–17}$ and $A_{1–8}$ are the most potent in this regard.

Key Words: Dynorphin; luteinizing hormone surge; κ-opioid receptors; norbinaltorphimine.

Introduction

Endogenous opioid peptides exert an inhibitory influence on luteinizing hormone (LH) secretion *(1)*. Evidence in the literature has shown a role for β-endorphin acting via μ-opioid receptors in mediating a suppressive effect on the ovulatory LH surge on proestrus *(2–6)*. However, work in our laboratory demonstrated that κ-opioid receptors in the medial preoptic area (MPOA) may also be involved in suppression of the proestrous LH surge *(7)*.

Dynorphin is a neuropeptide ligand with high binding affinity for the brain κ-opioid receptor *(8,9)*. Brain neuropeptides are first cleaved from large precursors and then processed to yield biologically active peptides. The prodynorphin precursor is proteolytically cleaved within neurons of the rat hypothalamus and other brain regions to yield a series of smaller opioid peptides, including dynorphin $A_{1–17}$, dynorphin $A_{1–8}$, dynorphin B, as well as α- and β-neoendorphin *(10–13)*. All of these peptides derived from prodynorphin are active at κ-opioid receptors *(14,15)*. Some studies have found no change in LH secretion in response to activation or blockade of κ-opioid receptors *(16–19)*. LH secretion has also been shown to be decreased by activation of κ-receptors *(16,20–23)*, and increased by blockade of these receptors *(7,16,24–29)*. With respect to dynorphin ligands, although intraventricular injection of dynorphin $A_{1–13}$ decreased LH secretion in gonadectomized rats *(20, 21)*, the effect of the end products of prodynorphin cleavage on LH release has been largely unexplored. The goal of the present study was to determine which of these dynorphin peptides could affect the proestrous LH surge, and whether this effect was mediated through κ-opioid receptors.

Results

Dynorphin Peptides

In control rats infused intraventricularly with artificial cerebrospinal fluid (aCSF) between 1330–1800 h on proestrus, 89% (eight of nine) exhibited LH surges (mean plasma LH level = 13.1 ± 2.9 ng/mL) and ovulated by the next morning (15.5 ± 0.9 ova/rat). By contrast, infusion of each of the five dynorphin peptides blocked the LH surge and ovulation (Fig. 1 and Table 1). On a molar basis, dynorphin $A_{1–17}$ and $A_{1–8}$ were equally effective in producing these actions, and more potent than either α-neoendorphin, β-neoendorphin, or dynorphin B. Significant decreases in mean plasma LH levels as well as the percentage of rats ovulating occurred in animals treated with dynorphin $A_{1–17}$, $A_{1–8}$, or α-neoendorphin. In rats treated with 10–125 ng/h of dynorphin B or β-neoendorphin, LH release was significantly decreased, but sufficient LH secretion occurred, causing ovulation in 71 and 54% of the rats, respectively. However, when the dose of each peptide was increased to 250–1000 ng/h, this

Received January 14, 2002; Revised April 1, 2002; Accepted April 12, 2002.

Author to whom all correspondence and reprint requests should be addressed: Robert V. Gallo, University of Connecticut, Department of Physiology and Neurobiology, 3107 Horsebarn Hill Road, Box U-156, Storrs, CT 06269-4156. E-mail: gallo@uconnvm.uconn.edu

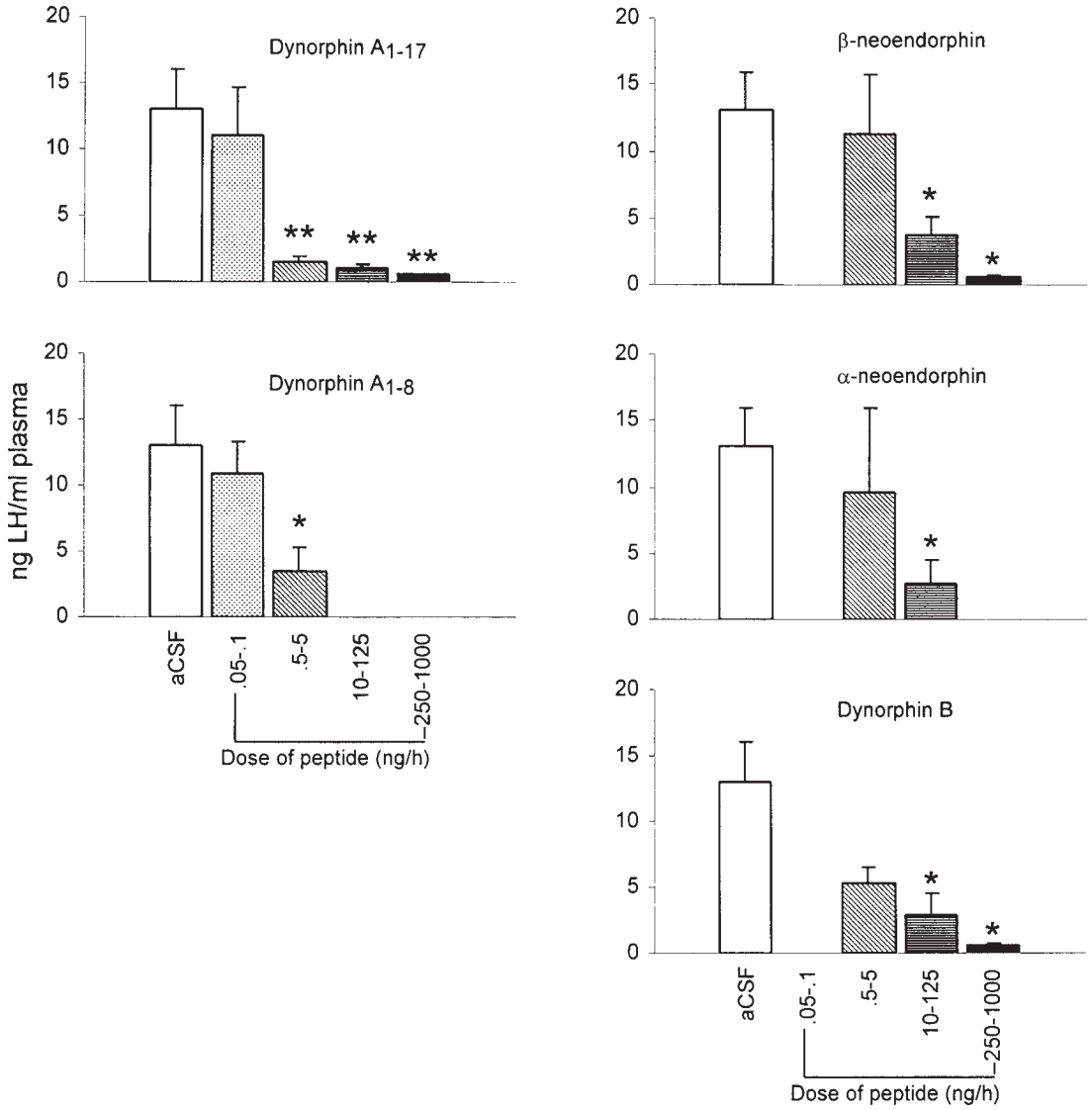

Fig. 1. Mean plasma LH concentrations in rats infused intraventricularly with aCSF or aCSF containing different dynorphin peptides at various doses between 1330–1800 h on the afternoon of proestrus. *$p < 0.05$; **$p < 0.01$ vs aCSF controls. For the number of rats per dose, see Table 1.

Table 1
Percentage of Rats Ovulating After Intraventricular Infusion of Various Doses of Dynorphin Peptides Between 1330–1800 h on the Afternoon of Proestrus[a]

Dose (ng/h)	Dynorphin $A_{1–17}$ (%)	Dynorphin $A_{1–8}$ (%)	α-Neoendorphin (%)	Dynorphin B (%)	β-Neoendorphin (%)
250–1000	0 (0/4)[b]			33 (2/6)[c]	0 (0/5)[b]
10–125	14 (1/7)[b]		40 (4/10)[c]	71 (5/7)	54 (7/13)
0.5–5	27 (4/15)[b]	23 (3/13)[b]	83 (5/6)	88 (7/8)	60 (3/5)
0.05–0.1	57 (4/7)	100 (5/5)			

[a]Numbers in parenthesis are number of rats per dose.
[b]$p < 0.01$ vs 89% ovulation (8/9) in rats treated with aCSF alone.
[c]$p < 0.05$.

resulted in a larger decrease in LH secretion and a blockade of ovulation. Third-ventricle infusion of U-50,488H, a specific κ-receptor agonist, also blocked the LH surge in six of six rats (mean plasma LH level = 0.9 ± 0.2 ng/mL) (Fig. 2), and none ovulated.

Dynorphin Peptides + Norbinaltorphimine

In four rats infused intraventricularly with a mixture of five dynorphin peptides, each at a dose that inhibited LH secretion (i.e., 0.5 ng/h of dynorphin $A_{1–8}$, 1 ng/h of dynorphin $A_{1–17}$, 50 ng/h of α-neoendorphin, 250 ng/h of β-neo-

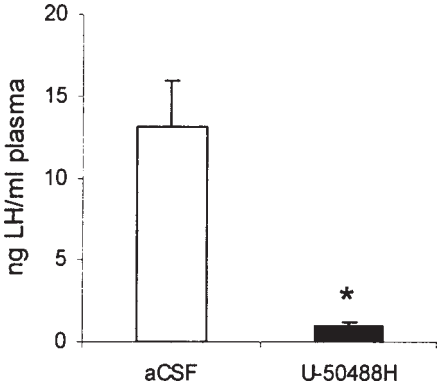

Fig. 2. Mean plasma LH concentrations in rats infused intraventricularly with aCSF ($n = 9$) or aCSF containing U50,488H ($n = 6$) between 1330–1800 h on the afternoon of proestrus. *$p <$ 0.01 vs aCSF controls.

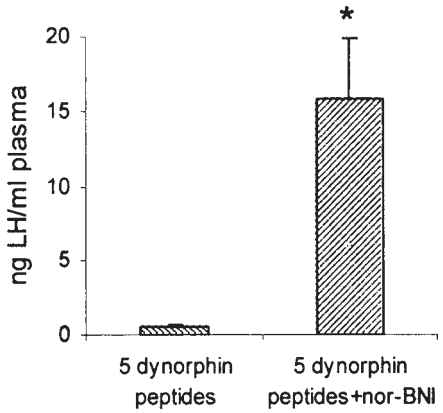

Fig. 3. Mean plasma LH concentrations in rats infused intraventricularly with aCSF containing five dynorphin peptides ($n = 4$) or five dynorphin peptides + nor-BNI ($n = 5$) between 1330–1800 h on the afternoon of proestrus. *$p < 0.05$ vs dynorphin peptides alone.

endorphin, and 1000 ng/h of dynorphin B), none exhibited an LH surge (mean plasma LH level = 0.6 ± 0.1ng/mL) (Fig. 3) or ovulated. However, when the κ-receptor antagonist norbinaltorphimine (nor-BNI) was coinfused with the mixture of peptides, this blocking action was prevented in five of five rats; all exhibited LH surges, with mean plasma LH levels (15.8 ± 4.1 ng/mL) greater than in rats infused with dynorphin peptides alone ($p < 0.05$) (Fig. 3), but the same as in rats infused with aCSF (13.1 ± 2.9 ng/mL). All five rats ovulated (12.8 ± 2.4 ova/rat).

Discussion

The present experiments demonstrate that prodynorphin-derived opioid peptides can block the LH surge and ovulation in a dose-dependent manner on proestrus. Moreover, this effect is mediated by κ-opioid receptors, since the LH surge and ovulation are fully restored in dynorphin peptide–treated rats by coadministration of nor-BNI, a specific antagonist of this receptor subtype *(30,31)*.

Previous results from our laboratory demonstrated that selective blockade of κ-opioid receptors in the MPOA prior to the critical period on the afternoon of proestrus prematurely evoked an ovulatory LH surge *(7)*. These findings were consistent with the hypothesis that disinhibition in existing inhibitory opioid tone on the afternoon of proestrus was part of the mechanism underlying generation of the LH surge *(1)*. This opioid inhibition of LH secretion had been shown to be mediated by β-endorphin originating in the arcuate nucleus and acting via μ-opioid receptors *(2–6)*. Our previous study demonstrated that κ-opioid receptors in the MPOA were also important in regulating the timing of the LH surge *(7)*. That study suggested that dynorphin, the endogenous ligand for the κ-opioid receptor *(8,9)*, was involved in this inhibitory response, and the present data support this hypothesis.

The large prodynorphin precursor peptide is proteolytically broken down within the hypothalamus and other brain regions into a series of five smaller opioid peptides *(10–13)*. Each of these peptides—dynorphin $A_{1–17}$, dynorphin $A_{1–8}$, dynorphin B, α-neoendorphin, and β-neoendorphin—effectively inhibited the LH surge. The inhibitory effect of these dynorphin peptides on the LH surge was eliminated by coinfusion of nor-BNI, a selective κ-receptor antagonist, even when all five peptides were combined and each was given at a dose that inhibited LH secretion. Moreover, the LH surge and ovulation were fully reinstituted. If any of the five dynorphin peptides was not suppressing LH secretion by activating κ-opioid receptors, then coinfusion with nor-BNI would not have prevented the blocking action of the peptide mixture on the LH surge. Thus, although dynorphin has also been shown to activate μ- and δ-opioid peptide receptors *(32–34)*, and NMDA receptors *(35,36)*, the inhibitory action of each dynorphin peptide on the LH surge must be mediated by activation of κ-opioid receptors.

When administered intraventricularly, opioid peptides could potentially influence LH secretion by acting at the level of the central nervous system (CNS) and/or anterior pituitary. Although opioids, or naloxone, an antagonist of all opioid receptor subtypes, have been shown to directly influence LH release from cultured anterior pituitary cells *(37–39)*, many studies have demonstrated no direct effect of either treatment on basal *(40,41)* or gonadotropin-releasing hormone (GnRH)–induced LH secretion *(38,40–43)*, and opioid receptors have not been found in the anterior pituitary *(44)*. Moreover, blockade of opioid receptors with naloxone has been shown to increase GnRH secretion from superfused rat *(45–47)* and human *(48)* hypothalami. Thus, although the site of action of the various dynorphin peptides was not specifically addressed in the present study, it is likely that their suppressive effect on the LH surge is mediated at the CNS level.

Two CNS areas that are important in the regulation of LH secretion are the MPOA and the medial basal hypothalamus (MBH). Both areas contain cell bodies and fibers of dynorphin $A_{1–8}$, $A_{1–17}$, and B neurons *(49–53)*, as well as κ-opioid receptors *(44,54,55)* and GnRH neurons *(56)*. We have reported that blockade of κ-receptors with nor-BNI in the MPOA or MBH during midpregnancy in the rat increased LH secretion *(28,29,57)*. The LH response to direct blockade of κ-opioid receptors at each site was different, with a large, prolonged increase in LH secretion occurring only in response to blockade of MPOA κ-opioid receptors *(28,57)*. Moreover, this large increase in LH release was similar to the premature LH surge occurring on the day of proestrus in response to blockade of MPOA κ-opioid receptors *(7)*. Since GnRH neurons do not have κ-opioid receptors *(58–60)*, blockade of such receptors in the MPOA could increase LH release by altering the activity of neurons regulating GnRH secretion, such as the noradrenergic system. However, while norepinephrine is involved in mediating the premature LH surge occurring in response to blockade of MPOA κ-receptors on proestrus *(7)*, as well as the large increase in LH release in response to blockade of MPOA κ-receptors in midpregnancy *(57)*, in neither instance is the importance of norepinephrine expressed as an increased release of norepinephrine in the MPOA. Taken together, these results suggest that dynorphin may block the LH surge by activating κ-opioid receptors in the MPOA.

An interesting observation in the present study was that dynorphin $A_{1–17}$ and $A_{1–8}$ were more potent than the other three dynorphin peptides in suppressing the LH surge. Both peptides were effective at doses as low as 0.5–1 ng/h, while doses at least 20-fold higher (10–125 ng/h) of dynorphin B or α- or β-neoendorphin were required to inhibit the LH surge on proestrus. The same order of potency was reported by Pugh et al. *(61)* in a study evaluating the enhancement of morphine-induced nociception by each dynorphin peptide at the spinal cord level. By contrast, dynorphin $A_{1–17}$ was more effective than dynorphin $A_{1–8}$ or α-neoendorphin with respect to inhibitory effects on contraction of the rabbit vas deferens *(62)*. Moreover, James et al. *(14)* reported a potency order of dynorphin $A_{1–17}$ >> dynorphin B ~ α-neoendorphin >> dynorphin $A_{1–8}$ and β-neoendorphin in evaluating the inhibitory action of each peptide on contraction of the guinea pig ileum. One observation consistent among all these studies and our own is that no dynorphin peptide was more effective than dynorphin $A_{1–17}$ in any physiological system examined. This may be due to the fact that dynorphin $A_{1–17}$ contains three amino acids at positions 7, 11, and 13 (arginine, lysine, and lysine, respectively) that are important in determining dynorphin peptide potency *(8,14)*, and that longer peptides are less easily degraded *(62,63)*. Relative binding affinity for the κ-opioid receptor could also influence the potency of a given dynorphin ligand. Although one report indicated that α-neoendorphin had a greater binding affinity for the κ-opioid receptor than dynorphin $A_{1–17}$ *(64)*, two other studies demonstrated that the affinity of dynorphin $A_{1–17}$ for the κ-opioid receptor was greater than that of other dynorphin ligands tested *(62,65)*.

In conclusion, prodynorphin-derived opioid peptides, acting through κ-opioid receptors, can block the ovulatory LH surge and ovulation. Dynorphin $A_{1–17}$ and $A_{1–8}$ are the most potent in this regard. Since these two peptides are more potent than dynorphin B or α- or β-neoendorphin in inhibiting the LH surge, this suggests that dynorphin $A_{1–17}$ and $A_{1–8}$ are the most likely candidates to act as endogenous ligands in the MPOA in suppressing the onset of the LH surge. In support of this hypothesis, we recently reported that selective neutralization of each of these peptides, by push-pull perfusion of the MPOA with antibodies specific for dynorphin $A_{1–17}$ or $A_{1–8}$ on the morning and early afternoon of proestrus, advanced the increase in plasma LH levels normally observed on this day of the rat estrous cycle *(66)*.

Materials and Methods

Animals

Adult female rats in our colony, derived from Charles River Sprague-Dawley CD rats, and weighing 270–350 g at the time of cannula implantation, were maintained on a 14:10 h light/dark schedule (lights on at 0500 h), and fed rat chow and water. Estrous cyclicity was determined by daily examination of vaginal smears. The experimental procedures were approved by the local University Animal Care and Use Committee.

Third-Ventricle Cannula Implantation

About 2–4 wk prior to the experiment, rats displaying 4-d estrous cycles were anesthetized with pentobarbital (3.5 mg/100 g body wt intraperitoneally), and a 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was stereotaxically implanted into the third ventricle at A 6.4 according to the atlas of deGroot *(67)*. A 5-mm piece of 18-gauge tubing cemented in place above the superior sagittal sinus served as a guide tube for cannula implantation. The cannula was cemented in place when a continuous flow of CSF was observed at its tip, and an inner stylette (28 gauge) was inserted. Cannula implantation was done on the day of estrus, resulting in minimal disruption of the estrous cycle. Daily vaginal smears were continued, and only rats displaying at least two consecutive 4-d estrous cycles were used in experiments.

Jugular Vein Cannulation

Between 0800–1000 h on the day before the experiment (diestrous d 2), rats were anesthetized briefly with ether, and a polyethylene (PE50) cannula was inserted into or near the right atrium via the external jugular vein. The next day rats displaying nucleated epithelial smears characteristic of proestrus were used in experiments.

Intraventricular Infusion

Thirty minutes before the onset of infusion, the inner stylette was removed and CSF flow was checked. Only those rats showing CSF flow were used. The infusion assembly consisted of an internal 28-gauge cannula attached to a spring-covered PE tubing containing the infusion solution. One end of the assembly was attached to a Hamilton microliter syringe set in place in an infusion pump. The inner cannula at the other end was inserted into the guide cannula and tightened. The infusion apparatus had been pre-filled with the appropriate solution immediately prior to setting it up. The infusion rate was 20 μL/h.

Experiment 1

The objective of this experiment was to determine whether dynorphin peptides or a κ-opioid receptor agonist could block the preovulatory LH surge on the afternoon of proestrus. Third-ventricle infusion of aCSF (140 m*M* NaCl, 4 m*M* KCl, 2.3 m*M* $CaCl_2$, 1 m*M* $MgSO_4$, 1.2 m*M* Na_2HPO_4, 0.3 m*M* NaH_2PO_4, 3.4 m*M* glucose, 7.4 pH), aCSF containing individual dynorphin peptides (dynorphin $A_{1–17}$, dynorphin $A_{1–8}$, dynorphin B, α-neoendorphin, or β-neoendorphin; Peninsula Laboratories, Belmont, CA; Sigma-Aldrich, St. Louis, MO) at doses indicated in Table 1, or aCSF containing the κ-opioid receptor agonist U-50,488H (100 μg/h) *(68)* (Upjohn, Kalamazoo, MI) was done in rats between 1330–1800 h on proestrus.

Experiment 2

The objective of this experiment was to determine whether blockade of the LH surge by dynorphin peptides (experiment 1) was mediated through κ-opioid receptors. Third-ventricle infusion of aCSF containing all five dynorphin peptides, each at a dose that inhibited the LH surge (as determined in experiment 1; i.e., 0.5 ng of dynorphin $A_{1–8}$ + 1 ng of dynorphin $A_{1–17}$ + 50 ng of α-neoendorphin + 250 ng of β-neoendorphin + 1000 ng of dynorphin B/h); or the five dynorphin peptides and nor-BNI, a selective κ-opioid receptor antagonist (40 μg/h) (*30,31)* (Sigma-Aldrich), was done between 1330–1800 h on proestrus. This dose of nor-BNI has been shown to induce a premature LH surge when applied to the MPOA on the morning of proestrus *(7)*.

Animals were injected with 500 U of heparin following the onset of infusion. Blood samples (300–400 μL each) were withdrawn with a 1-cc syringe at 60-min intervals starting at 1350 h. An equal volume of saline was given to the animal through the bleeding cannula following each blood sample. Blood samples were centrifuged, and the plasma was collected and stored at –70ºC until assayed for LH by radioimmunoassay (RIA). At the end of the third-ventricle infusion, rats were returned to the animal quarters overnight. The following morning (estrus) the ovaries were removed, the oviducts were separated from the ovaries, and ovulation was verified by counting the ova with the aid of a low-power microscope.

Radioimmunoassay

Plasma samples were analyzed for LH by the ovine:ovine rat LH double antibody RIA of Niswender et al. *(69)*, as previously described *(70)*. The sensitivity of the assay is 6–8 pg/tube. Inter- and intraassay variations determined at a mean plasma LH level of 3.1 ± 0.2 ng/mL (n = 11) were 18.3 and 14.1%, respectively. LH values (ng/mL of plasma) were expressed in terms of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) rat LH-RP-3 standard.

Data Analysis

Mean plasma LH levels for each experimental group were determined by first calculating the mean of all the blood samples collected from each rat during the infusion period, and then determining the average plasma LH for the experimental group from the individual means. Significant differences between groups in mean plasma LH levels were determined by student's unpaired t-test or analysis of variance followed by Dunnett's t-test. Differences between groups in the percentage of occurrence of ovulation were determined by Fisher's exact probability test. Results were considered significant at $p < 0.05$. All results are expressed as the mean ± SEM.

Acknowledgments

We wish to thank Dr. G. D. Niswender for antiovine LH#15, Dr. H. Papkoff for ovine LH for iodination, and Dr. A. F. Parlow and the NIDDK for the rat LH used as a reference preparation. This study was supported by National Science Foundation grant IBN-9807546.

References

1. Kalra, S. P. (1993). *Endocr. Rev.* **14,** 507–538.
2. Kalra, S. P. and Simpkins, J. W. (1981). *Endocrinology* **109,** 776–782.
3. Leadem, C. A. and Kalra, S. P. (1985). *Endocrinology* **117,** 684–689.
4. Maggi, R., Dondi, D., Rovati, G. E., Martini, L., Piva, F., and Limonta, P. (1993). *Neuroendocrinology* **58,** 366–372.
5. Sarkar, D. K. and Yen, S. S. (1985). *Endocrinology* **116,** 2075–2079.
6. Wise, P. M., Scarbrough, K., Weiland, N. G., and Larson, G. H. (1990). *Mol. Endocrinol.* **4,** 886–892.
7. Smith, M. J. and Gallo, R. V. (1997). *Brain Res.* **768,** 111–119.
8. Chavkin, C. and Goldstein, A. (1981). *Proc. Natl. Acad. Sci. USA* **78,** 6543–6547.
9. Chavkin, C., James, I. F., and Goldstein, A. (1982). *Science* **215,** 413–415.
10. Cone, R. I., Weber, E., Barchas, J. D., and Goldstein, A. (1983). *J. Neurosci.* **3,** 2146–2152.
11. Seizinger, B. R., Grimm, C., Hollt, V., and Herz, A. (1984). *J. Neurochem.* **42,** 447–457.
12. Weber, E., Evans, C. J., and Barchas, J. D. (1982). *Nature* **299,** 77–79.
13. Zamir, N., Weber, E., Palkovits, M., and Brownstein, M. (1984). *Proc. Natl. Acad. Sci. USA* **81,** 6886–6889.
14. James, I. F., Fischli, W., and Goldstein, A. (1984). *J. Pharmacol. Exp. Ther*. **228,** 88–93.

15. Hollt, V. (1986). *Annu. Rev. Pharmacol. Toxicol.* **26,** 59–77.
16. Cicero, T. J., Meyer, E. R., Miller, B. T., and Bell, R. D. (1988). *J. Pharmacol. Exp. Ther.* **246,** 14–20.
17. Gilbeau, P. M., Hosobuchi, Y., and Lee, N. M. (1987). *Neuroendocrinology* **45,** 284–289.
18. Pfeiffer, D. G., Pfeiffer, A., Shimohigashi, Y., Merriam, G. R., and Loriaux, D. L. (1983). *Peptides* **4,** 647–649.
19. Walsh, J. P. and Clarke, I. J. (1996). *Endocrinology* **137,** 3640–3648.
20. Kinoshita, F., Nakai, Y., Katakami, H., and Imura, H. (1982). *Life Sci.* **30,** 1915–1919.
21. Leadem, C. A. and Kalra, S. P. (1985). *Neuroendocrinology* **41,** 342–352.
22. Leadem, C. A. and Yagenova, S. V. (1987). *Neuroendocrinology* **45,** 109–117.
23. Marko, M. and Romer, D. (1983). *Life Sci*. **33,** 233–240.
24. Almeida, O. F., Nikolarakis, K. E., Webley, G. E., and Herz, A. (1988). *FASEB J.* **2,** 2874–2877.
25. Gallo, R. V. (1990). *J. Neuroendocrinol.* **2,** 685–691.
26. Panerai, A. E., Petraglia, F., Sacerdote, P., and Genazzani, A. R. (1985). *Endocrinology* **117,** 1096–1099.
27. Schulz, R., Wilhelm, A., Pirke, K. M., Gramsch, C., and Herz, A. (1981). *Nature* **294,** 757–759.
28. Zhen, S. and Gallo, R. V. (1992). *Endocrinology* **131,** 1650–1656.
29. Zhen, S. and Gallo, R. V. (1994). *Brain Res.* **642,** 178–184.
30. Portoghese, P. S., Lipkowski, A. W., and Takemori, A. E. (1987). *Life Sci.* **40,** 1287–1292.
31. Tortella, F. C., Echevarria, E., Lipkowski, A. W., Takemori, A. E., Portoghese, P. S., and Holaday, J. W. (1989). *Life Sci.* **44,** 661–665.
32. Hewlett, W. A. and Barchas, J. D. (1983). *Peptides* **4,** 853–858.
33. Quirion, R. and Pert, C. B. (1981). *Eur. J. Pharmacol.* **76,** 467, 468.
34. Zhang, S., Tong, Y., Tian, M., Dehaven, R. N., Cortesburgos, L., Mansson, E., Simonin, F., Kieffer, B., and Yu, L. (1998). *J. Pharmacol. Exp. Ther.* **286,** 136–141.
35. Massardier, D. and Hunt, P. F. (1989). *Eur. J. Pharmacol.* **170,** 125, 126.
36. Skilling, S. R., Sun, X., Kurtz, H. J., and Larson, A. A. (1992). *Brain Res.* **575,** 272–278.
37. Cacicedo, L., Sanchez Franco, F. (1986). *Life Sci.* **38,** 617–625.
38. Chao, C. C., Moss, G. E., and Malven, P. V. (1986). *Life Sci.* **39,** 527–534.
39. Blank, M. S., Fabbri, A., Catt, K. J., and Dufau, M. L. (1986). *Endocrinology* **118,** 2097–2101.
40. Cicero, T. J., Schainker, B. A., and Meyer, E. R. (1979). *Endocrinology* **104,** 1286–1291.
41. Wiesner, J. B., Koenig, J. I., Krulich, L., and Moss, R. L. (1984). *Life Sci*. **34,** 1463–1473.
42. Cicero, T. J., Wilcox, C. E., Bell, R. D., and Meyer, E. R. (1980). *J. Pharmacol. Exp. Ther.* **212,** 573–578.
43. Ferin, M., Wehrenberg, W. B., Lam, N. Y., Alston, E. J., and Vande Wiele, R. L. (1982). *Endocrinology* **111,** 1652–1656.
44. Mansour, A., Burke, S., Pavlic, R. J., Akil, H., and Watson, S. J. (1996). *Neuroscience* **71,** 671–690.
45. Kalra, P. S., Crowley, W. R., and Kalra, S. P. (1987). *Endocrinology* **120,** 178–185.
46. Leadem, C. A., Crowley, W. R., Simpkins, J. W., and Kalra, S. P. (1985). *Neuroendocrinology* **40,** 497–500.
47. Wilkes, M. M. and Yen, S. S. (1981). *Life Sci.* **28,** 2355–2359.
48. Rasmussen, D. D., Liu, J. H., Wolf, P. L., and Yen, S. S. (1983). *J. Clin. Endocrinol. Metab.* **57,** 881–884.
49. Fallon, J. H. and Leslie, F. M. (1986). *J. Comp. Neurol*. **249,** 293–336.
50. Khachaturian, H., Watson, S. J., Lewis, M. E., Coy, D., Goldstein, A., and Akil, H. (1982). *Peptides* **3,** 941–954.
51. Neal, C. R. Jr. and Newman, S. W. (1989). *J. Comp. Neurol.* **288,** 353–386.
52. Simerly, R. B., McCall, L. D., and Watson, S. J. (1988). *J. Comp. Neurol*. **276,** 442–459.
53. Vincent, S. R., Hokfelt, T., Christensson, I., and Terenius, L. (1982). *Neurosci. Lett.* **33,** 185–190.
54. Desjardins, G. C., Brawer, J. R., and Beaudet, A. (1990). *Brain Res.* **536,** 114–123.
55. Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H., and Watson, S. J. (1988). *Trends Neurosci.* **11,** 308–314.
56. Witkin, J. W., Paden, C. M., and Silverman, A. J. (1982). *Neuroendocrinology* **35,** 429–438.
57. Zhen, S. and Gallo, R. V. (1995). *Brain Res.* **698,** 121–129.
58. Maggi, R., Pimpinelli, F., Martini, L., and Piva, F. (1995). *Endocrinology* **136,** 289–295.
59. Mitchell, V., Prevot, V., Jennes, L., Aubert, J. P., Croix, D., and Beauvillain, J. C. (1997). *Neuroreport* **8,** 3167–3172.
60. Sannella, M. I. and Petersen, S. L. (1997). *Endocrinology* **138,** 1667–1672.
61. Pugh, G. Jr., Mason, D. J. Jr., Combs, V., and Welch, S. P. (1997). *J. Pharmacol. Exp. Ther.* **281,** 730–737.
62. Corbett, A. D., Paterson, S. J., McKnight, A. T., Magnan, J., and Kosterlitz, H. W. (1982). *Nature* **299,** 79–81.
63. Sandin, J., Tan-No, K., Kasakov, L., Nylander, I., Winter, A., Silberring, J., and Terenius, L. (1997). *Peptides* **18,** 949–956.
64. Mansour, A., Hoversten, M. T., Taylor, L. P., Watson, S. J., and Akil, H. (1995). *Brain Res.* **700,** 89–98.
65. Xue, J. C., Chen, C., Zhu, J., Kunapuli, S., DeRiel, J. K., Yu, L., and Liu-Chen, L. Y. (1994). *J. Biol. Chem*. **269,** 30,195–30,199.
66. Zhang, Q. and Gallo, R. V. (2001). *Soc. Neurosci. Abstr*. **630.5**.
67. deGroot, J. (1959). In: *The rat forebrain in stereotaxic coordinates*. North-Holland Publishers: Amsterdam.
68. Vonvoigtlander, P. F., Lahti, R. A., and Ludens, J. H. (1983). *J. Pharmacol. Exp. Ther.***224,** 7–12.
69. Niswender, G. D., Midgley, A. R. Jr., Monroe, S. E., and Reichert, L. E. Jr. (1968). *Proc. Soc. Exp. Biol. Med.* **128,** 807–811.
70. Gallo, R. V. (1981). *Biol. Reprod.* **24,** 771–777.