ELSEVIER

Neuroendocrine mechanisms underlying the control of gonadotropin secretion by steroids

Virendra B. Mahesh and Darrell W. Brann

Neuroendocrine Research Laboratory, Department of Physiology and Endocrinology, Medical College of Georgia, Augusta, Georgia 30912-3000, USA

There is considerable evidence that although estradiol may trigger the preovulatory surge of gonadotropins, progesterone is required for its full magnitude and duration and that glucocorticoids bring about selective follicle-stimulating hormone release. The luteinizing hormone-releasing hormone (LHRH) neuron does not have steroid receptors and is regulated by excitatory amino acid neurotransmission. Steroids do not appear to modulate excitatory amino acid receptors directly but increase release of glutamate in the preoptic area. This may be due to the suppression by steroids of the enzyme glutamatic acid decarboxylase$_{67}$ that converts glutamate into GABA. NMDA receptors colocalize with nitric oxide synthase-containing neurons that surround the LHRH neurons in the preoptic area and intersect the LHRH fibers in the median eminence. Other potential novel pathways of LHRH release that are currently being explored include carbon monoxide generated by the action of heme oxygenase-2 on heme molecules and bradykinin acting via bradykinin B$_2$ receptors. (*Steroids* **63**:252–256, 1998) © *1998 by Elsevier Science Inc.*

Keywords: excitatory amino acids; LHRH; LH; bradykinin; carbon monoxide; nitric oxide

Introduction

A key event in mammalian reproduction is the preovulatory surge of gonadotropins that leads to ovulation. The regulation of this preovulatory gonadotropin surge has been under intensive investigation, and several excellent reviews have appeared on the subject.[1–5] Also of considerable interest is the finding that glucocorticoids may regulate follicule-stimulating hormone (FSH) secretion, which is essential for follicular development. Because luteinizing hormone-releasing hormone (LHRH) neurons do not appear to express steroid receptors, an extensive search has been made for interneuronal effects and neurotransmitters regulated by steroids, which in turn regulate the LHRH neuron. In addition to the well-recognized neurotransmitters such as catecholamines, neuropeptide Y (NPY), and galanin, recent work has focused on excitatory amino acids (EAAs), the gaseous neurotransmitters nitric oxide (NO) and carbon monoxide (CO), and bradykinin. This subject has also been extensively reviewed.[6–8] The purpose of this minireview is to examine how steroid hormones regulate EAA and NO systems at the level of the hypothalamus to influence LHRH release leading to the preovulatory luteinizing hormone (LH) surge. Possible roles for CO and bradykinin will also be discussed.

Address reprint requests to Dr. Virendra B. Mahesh. E-mail: vmahesh@mail.mcg.edu

Role of estradiol, progesterone, and glucocorticoids in gonadotropin release

The positive and negative feedback effects of estradiol on gonadotropin secretion are well established, and a landmark review on the subject by Everett[9] appeared as early as 1964. The evidence that estradiol secreted by the dominant follicle is an important ovarian signal for the preovulatory surge of gonadotropins is based on the abolition of the surge by ovariectomy, estrogen antagonists, or anti-estrogen antibodies, and reinstatement of at least a partial surge by estrogen administration.

Despite the strong evidence supporting the role of estradiol as the trigger for the preovulatory surge of gonadotropins, there is substantial evidence that it is not the only hormone involved. Hilliard et al.[10] showed that in the rabbit progesterone and progesterone metabolites were necessary for a full gonadotropin surge. Aiyer and Fink[11] showed that progesterone was required for full pituitary responsiveness to LHRH, and Mann and Barraclough[12] showed that if progesterone was eliminated by adrenalectomy and ovariectomy, estradiol could not induce a preovulatory gonadotropin surge. The facilitatory and inhibitory effects of progesterone on gonadotropin secretion are also well documented (see reviews 1–5, 9).

Mahesh and collaborators[13,14] showed that in the ovariectomized immature rat, low doses of estrogens were enough to induce progesterone receptors but not for an

Steroids 63:252–256, 1998

655 Avenue of the Americas, New York, NY 10010

0039-128X/98/$19.00
PII S0039-128X(98)00031-2

estrogen-induced gonadotropin surge and that administered progesterone was able to induce a proestrous LH surge accompanied by a prolonged FSH surge. In another animal model, the estrogen-induced surge was less than 10% the proestrous LH surge, and progesterone administration produced the full proestrous LH surge.[15] The physiological role of progesterone in the proestrous LH surge was also demonstrated by the effects of the progesterone antagonist RU486 and the progesterone synthesis inhibitor trilostane,[5,16] both of which attenuated the proestrous LH surge.

Glucocorticoids have been shown to regulate FSH secretion[17–19] and bring about an increase in pituitary FSH-β mRNA levels within an hour. Recently, acute glucocorticoid treatment in conjunction with clomid has been used to induce ovulation in women who had failed to respond to clomid alone.[20]

Role of excitatory amino acids in the proestrous and steroid-induced gonadotropin surge

Since the LHRH neuron does not have steroid receptors,[21,22] attention has focused on identifying neurotransmitters that are regulated by steroids, which can then in turn regulate the LHRH neuron.[6–8] Initial work showing that glutamate could stimulate LH release came from the laboratories of Olney[23] and Ondo.[24] *N*-Methyl-D-Aspartate (NMDA), a glutamate agonist, was subsequently shown to bring about both LHRH release from the hypothalamus and LH secretion.[25] The non-NMDA glutamate agonists, kainate and DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), when injected in the third ventricle also bring about LH release,[8,26–28] with kainate inducing a robust release only after the first injection followed by rapid desensitization of the effect.[26,27] The noncompetitive NMDA receptor antagonist MK801 blocked both estrogen/progesterone-induced and the proestrous LH surge, the latter suggesting a physiological role for NMDA neurotransmission in the preovulatory LH surge.[5,25] Similarly, the non-NMDA antagonist DNQX and the selective AMPA antagonist NBQX also blocked estrogen/progesterone-induced and the proestrous surge of LH.[28,29] Other investigators have also shown the blockade of the estrogen-induced LH surge by NMDA antagonists.[30–32]

Administration of NMDA for 5 h each day from 26 to 29 days of age advanced puberty in the immature rat,[30] and the normal onset of puberty was delayed by the administration of NMDA antagonist MK801.[31] A shorter period (90 min) of NMDA administration from 26 to 29 days of age also advanced puberty, while kainate administration had no effect on its onset.[33] The kainate/AMPA receptor antagonist DNQX similarly did not delay the onset of puberty,[33] perhaps related to the finding that DNQX does not block FSH release.[29]

Do estrogens and progesterone regulate hypothalamic EAA receptors?

In the adult rat, ovariectomy followed by replacement with estradiol or estradiol and progesterone to induce a preovulatory-type gonadotropin surge did not alter hypothalamic NMDA binding or NMDAR1 mRNA levels.[34] Similarly, NMDAR1 and kainate receptor binding did not change over the onset of puberty[33]; we and others have raised the possibility of a small increase in AMPA receptor numbers.[6] Dees and coworkers[35] found an increase in hypothalamic NMDAR1 mRNA levels at 20–25 days of age, confirmed by Zamorano et al.[36] and Gore et al.[37] However, the increase at first proestrous of NMDAR1 mRNA levels reported by Dees and coworkers[35] was confirmed neither by Zamorano et al.[36] nor Gore et al.[37]

No changes in NMDAR1, GluR1, and GluR6 mRNA levels were observed in the cycling rat[38] over the estrous cycle. However, the progesterone antagonist RU486 (which attenuates the proestrous LH surge) increased medial basal hypothalamus (MBH) GluR6 mRNA levels.[38] In the adult ovariectomized rat treated with estradiol or with estradiol followed by progesterone, progesterone induced an LH surge and decreased MBH GluR6 mRNA levels without altering NMDAR1 or GluR1 mRNA levels.[39] Thus, GluR6 may be important in the termination of the progesterone-induced LH surge.

Changes in glutamate secretion brought about by estrogen and progesterone treatment

Jarry et al.[40] demonstrated an increase in preoptic area (POA) glutamate and aspartate levels during the estrogen-induced LH surge. Goroll et al.[41] also showed an increase in glutamate and aspartate in the POA at the time of puberty in the female rat, in agreement with higher glutamate and aspartate levels in the hypothalamus of 30-day-old female rats compared with 16-day-old rats.[42] Microdialysis studies by Ping et al.[43] showed increased glutamate and aspartate levels in the POA of estrogen-treated ovariectomized rats after a single injection of progesterone to induce the proestrous type LH surge. The increase in glutamate and aspartate occurred at the time of the LH surge and appeared due to progesterone lowering POA glutamic acid decarboxylase$_{67}$ mRNA[44] and protein levels (unpublished data) at the time of the surge. The specificity of the action of progesterone on GAD$_{67}$ mRNA levels was confirmed by blocking this decrease and attenuation of the LH surge with the progesterone antagonist RU486.[44]

Nitric oxide as a mediator of EAA-induced LHRH release

Recent studies from several laboratories have implicated NO in the glutamate effect on the LHRH neuron.[45,46] Work from our and other laboratories has shown that LHRH neurons in the organum vasculosum of the lamina terminalis (OVLT) and POA do not express NMDAR1 or nitric oxide synthase (NOS).[47–49] However, LHRH neurons were often surrounded by NOS-containing neurons that colocalized with NMDAR1.[47] Furthermore, there was a significant overlap between en passant LHRH neuronal fibers and NO neuronal fibers in the median eminence (ME).[47] Moretto et al.[50] were the first to demonstrate that NO by itself stimulates LHRH release from hypothalamic fragments and immortalized LHRH neurons in vitro. A blockade of the steroid-induced and proestrous LH surge by NOS inhibitors

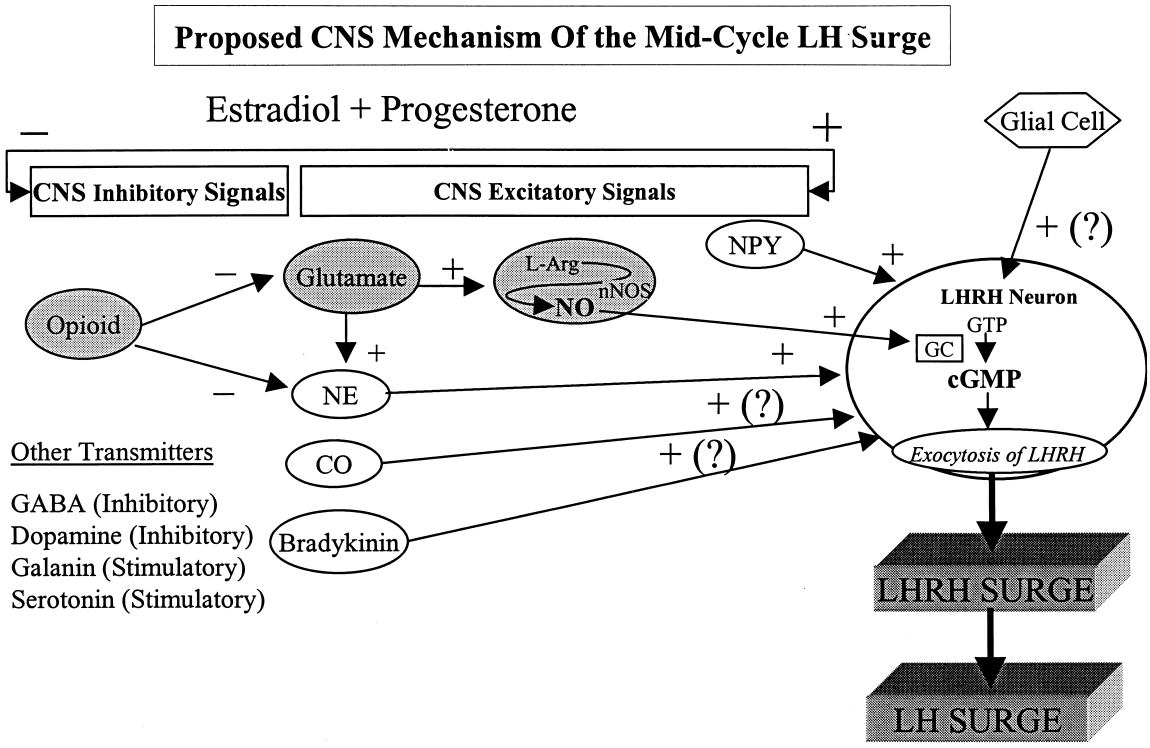

Figure 1 Central role of excitatory amino acids in the midcycle LH surge. The LHRH neuron has been shown to be under numerous excitatory signals for LHRH release, namely, excitatory amino acids norepinephrine (NE), neuropeptide Y (NPY), galanin, carbon monoxide (CO), bradykinin, serotonin, and peptides from glial cells. Major inhibitory signals for LHRH release are the opioids and gamma aminobutyric acid (GABA) and perhaps dopamine and neuropeptide K. The steroid-induced LHRH surge is initiated by an increase of the excitatory amino acids glutamate and aspartate that act by stimulating neuronal nitric oxide synthase (nNOS) to increase nitric oxide (NO) They may also activate the NE pathway. The inhibitory effects of opioids on LH release is exerted at least in part by their suppression of glutamate release. Progesterone also reduces the inhibitory neurotransmitter GABA by suppressing glutamic acid decarboxylase$_{67}$, which converts glutamate into GABA. CO and bradykinin are also potential novel modulators of LHRH secretion, and their integrative role in the complex mechanisms of LHRH neuronal regulation is under further study.

has been shown by Kalra et al.[51,52] and by us using b-NOS antisense oligonucleotides.[53] Glutamate-induced LHRH release from hypothalamic fragments is also blocked by NOS inhibitors,[45] as is NMDA-induced LH release in vivo.[47] Thus, NO appears to be an important mediator of EAA action in LHRH release.

Inhibitory effects of opioids on LH secretion are exerted by suppressing EAA neurotransmission

It is well known that the opioid antagonist naloxone brings about LH release. Naloxone enhances NOS activity in the POA and MBH[54] and increases POA glutamate levels; prior treatment with an NMDA antagonist MK801 blocks naloxone-induced LH release[54,55] and increase in hypothalamic NOS activity.[54] These data clearly indicate that opioid inhibition of LH release is at least in part through suppression of EAA neurotransmission.

Carbon monoxide as a mediator for LHRH release

Carbon monoxide (CO) is produced in the body by the metabolism of heme by the enzymes heme oxygenase-1 (HO-1), which is induced by brain injury or infection, and heme oxygenase-2 (HO-2), which is constitutive.[56,57] The hypothalamus has been shown to have one of the highest CO production rates in the brain.[58,59] HO-2 mRNA has been demonstrated to be highly expressed in the POA and MBH of the female rat (unpublished findings).

Hematin, a substrate for CO production, markedly stimulates LHRH release from female rat hypothalamic fragments and immortalized LHRH neurons (GT1–7 cells) in vitro.[60,61] Zinc protoporphyrin IX, a HO inhibitor, and the gas scavenger molecule, hemoglobin, were able to block the stimulatory effect of hematin on LHRH secretion, suggesting that CO mediates the effects of hematin. The CO effects do not appear to be mediated by iron or NO since iron-chelating agents and NOS inhibitors did not have any effect on hematin-induced LHRH release from GT1–7 cells.[62] The physiological role of CO in gonadotropin secretion and its regulation by steroids are currently under investigation.

Bradykinin as a mediator for LHRH release

The mRNAs for kallikrein, the enzyme responsible for bradykinin synthesis, for kininogen, the substrate for bradykinin production, and for the bradykinin B2 receptor are present in POA, OVLT, and arcuate nuclei (ARC) of the rat.[63] Bradykinin neurons were shown to be present in the

hypothalamus with the strongest staining in ARC and ME.[63] Bradykinin was able to stimulate a dose-dependent release of LHRH from rat MBH fragments in vitro, an effect blocked by the bradykinin B2 receptor antagonist HOE140.[63]

The bradykinin effect was not blocked by the EAA receptor antagonists MK801 and NBQX, indicating that its effects were downstream to EAA effects. Bradykinin B2 receptor antagonist HOE140 administered by the intracerebroventricular route blocked the steroid-induced gonadotropin surge, providing further evidence for an important role for bradykinin in LHRH release.[63]

Summary and conclusions

Since reproduction is such a critical event in the survival of the species, it is not surprising that there are multiple mechanisms to ensure reproductive success. Figure 1 summarizes the current concepts of the excitatory signals of steroids on the LHRH neuron being transmitted through the glutamate-NO pathway. The inhibitory effects of opioids on gonadotropin secretion are also exerted in part by their suppression of glutamate signals. Steroids increase the release of the excitatory transmitters glutamate and aspartate in the hypothalamus, while at the same time lower hypothalamic GAD_{67} levels, which may in turn lower levels of the inhibitory transmitter GABA. A potential role of CO and bradykinin on LHRH release is also proposed and further work of their integration in the LHRH regulatory network is in progress.

Acknowledgments

The research described in this paper was supported by research Grants HD16688 (to V. B. M.) and HD28964 (to D. W. B.) from the National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland.

References

1. Kalra SP (1993). Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge. *Endocr Rev* **14**:507–538.
2. Mahesh VB (1985). The dynamic interaction between steroids and gonadotropins in the mammalian ovulatory cycle. *Neurosci Biobehav Rev* **9**:245–260.
3. Fink G (1988). Gonadotropin secretion and its control. In: Knobil E, Neill J (eds), *The Physiology of Reproduction*, Vol I. Raven Press, New York, pp. 1349–1378.
4. Brann DW, Mahesh VB (1991). Regulation of gonadotropin secretion by steroid hormones. *Front Neuroendocr* **12:**165–207.
5. Mahesh VB, Brann DW (1992). Interaction between ovarian and adrenal steroids in the regulation of gonadotropin secretion. *J Steroid Biochem Mol Biol* **41**:495–513.
6. Brann DW, Mahesh VB (1994). Excitatory amino acids: Function and significance in reproduction and neuroendocrine regulation. *Front Neuroendocr* **15**:3–49.
7. Brann DW, Bhat GK, Lamar CA, Mahesh VB (1997). Gaseous transmitters and neuroendocrine regulation. *Neuroendocrinology* **65**:385–395.
8. Brann DW, Mahesh VB (1997). Excitatory amino acids: Evidence for a role in control of reproduction and anterior pituitary hormone secretion. *Endocr Rev* **18**:678–700.
9. Everett JW (1964). Central neural control of reproductive functions of the adenohypophysis. *Physiol Rev* **44**:373–431.
10. Hilliard J, Pernardi R, Sawyer C (1967). A functional role of 20α-hydroxy-pregn-4-en-3-one in the rabbit. *Endocrinology* **80**:901–909.
11. Aiyer MS, Fink G (1974). The role of sex steroid hormones in modulating the responsiveness of the anterior pituitary gland to LHRH in the female rat. *J Endocrinol* **62**:533–572.
12. Mann DR, Barraclough CA (1973). Role of estrogen and progesterone in facilitating LH release in 4-day cyclic rats. *Endocrinology* **93**:694–699.
13. McPherson JC, Costoff A, Mahesh VB (1975). Influence of estrogen-progesterone combinations on gonadotropins secretion in castrate female rats. *Endocrinology* **91**:771–779.
14. McPherson JC, Mahesh VB (1979). Dose-related effect of a single injection of progesterone on gonadotropin secretion and pituitary sensitivity to LHRH in estrogen-primed castrated female rats. *Biol Reprod* **20**:763–772.
15. Brann DW, O'Conner JL, Wade MF, Zamorano PL, Mahesh VB (1993). Regulation of anterior pituitary gonadotropin subunit mRNA levels during the preovulatory gonadotropins surge: A physiological role of progesterone in regulating LH-β and FSH-β mRNA levels. *J Steroid Biochem Mol Biol* **46**:427–437.
16. Rao IM, Mahesh VB (1986). Role of progesterone in the modulation of the preovulatory surge of gonadotropins and ovulation in the PMSG primed immature rat and the adult rat. *Biol Reprod* **35**:1154–1161.
17. Brann DW, Putnam CD, Mahesh VB (1991). Validation of he mechanisms proposed for the stimulatory and inhibitory effects of progesterone on gonadotropin secretion in the estrogen-primed ratA possible role for adrenal steroids. *Steroids* **56**:103–111.
18. Brann DW, O'Conner J, Wade M, Mahesh VB (1992). LH and FSH subunit mRNA concentrations during the progesterone-induced gonadotropin surge in ovariectomized estrogen-primed immature rats. *Mol Cell Neurosci* **3**:171–178.
19. Tebar M, Bellido C, Sanchez-Giado JE (1995). Luteinizing hormone and corticosterone in proestrus afternoon restore the follicle stimulating hormone secretion at early estrus in adrenalectomized LH-releasing hormone antagonist treated rats. *Biol Reprod* **52**:63–67.
20. Trott EA, Plouffe L, Jr, Hansen K, Hines R, Brann DW, Mahesh VB (1996). Ovulation induction in clomiphene-resistant anovulatory women with normal dehydroepiandrosterone sulfate levels: Beneficial effects of the addition of dexamethasone during the follicular phase. *Fertil Steril* **66**:484–486.
21. Fox SR, Harlan R, Shivers B, Pfaff DW (1990). Chemical characterization of neuroendocrine targets for progesterone in the female rat brain and pituitary. *Neuroendocrinology* **51**:276–283.
22. Leranth C, Shanabrough M, Naftolin F (1991). Estrogen induces ultrastructural changes in progesterone receptor-containing GABA neurons of the primate hypothalamus. *Neuroendocrinology* **54**:571–579.
23. Olney JW, Cicero TJ, Meyer E, De Gubareff T (1976). Acute glutamate-induced elevations in serum testosterone and luteinizing hormone. *Brain Res* **112**:420–424.
24. Ondo JG, Pass KA, Baldwin R (1976). The effects of neurally active amino acids on pituitary gonadotropin secretion. *Neuroendocrinology* **21**:79–87.
25. Brann DW, Mahesh VB (1991). Endogenous excitatory amino acid involvement in the preovulatory and steroid-induced surge of gonadotropins in the female rat. *Endocrinology* **128**:1541–1547.
26. Medhamurthy R, Gay VL, Plant TM (1992). Repetitive injections of L-glutamic acid, in contrast to those of *N*-methyl-D, L-aspartic acid, fail to elicit sustained hypothalamic GnRH release in the prepubertal male rhesus monkey (*Macaca mulatta*). *Neuroendocrinology* **55**:660–666.
27. Abbud R, Smith MS (1991). Differences in the luteinizing hormone and prolactin responses to multiple injections of kainate as compared to *N*-methyl-D, L-aspartate, in cycling rats. *Endocrinology* **129**:3254–3258.
28. Ping L, Mahesh VB, Bhat GK, Brann DW (1997). Regulation of gonadotropin-releasing hormone and luteinizing hormone secretion by AMPA receptors. *Neuroendocrinology* **66**:246–253.
29. Brann DW, Ping L, Mahesh VB (1993). Possible role of non-

NMDA receptor-mediated neurotransmission in steroid-induced and preovulatory gonadotropin surges in the rat. *Mol Cell Neurosci* **4**:292–297.
30. Urbanski HF, Ojeda SR (1987). Activation of luteinizing hormone-releasing hormone release advanced the onset of female puberty. *Neuroendocrinology* **46**:273–276.
31. Urbanski H, Ojeda S (1990). A role for *N*-methyl-D-aspartate (NMDA) receptors in the control of LH secretion and initiation of female puberty. *Endocrinology* **126**:1774–1776.
32. Lopez F, Donoso AO, Negro-Vilar A (1990). Endogenous excitatory amino acid neurotransmission regulates the estradiol-induced LH surge in ovariectomized rats. *Endocrinology* **126**:1771–1773.
33. Brann DW, Zamorano PL, Ping L, Mahesh VB (1993). Role of excitatory amino acid neurotransmission during puberty in the female rat. *Mol Cell Neurosci* **4**:107–112.
34. Brann DW, Zamorano PL, Chorich LP, Mahesh VB (1993). Steroid hormone effects on NMDA receptor binding and NMDA receptor mRNA levels in the hypothalamus and cerebral cortex of the adult rat. *Neuroendocrinology* **58**:666–672.
35. Nyberg C, Srivastava V, Hiney J, Lara F, Dees WL (1995). *N*-methyl-D-aspartic acid receptor messenger ribonucleic acid levels and luteinizing hormone release in immature female rats: Effects of stage of pubertal development and exposure to ethanol. *Endocrinology* **136**:2874–2880.
36. Zamorano PL, Mahesh VB, De Sevilla LM, Brann DW (1996). Glutamate receptor expression in the hypothalamus during the onset of puberty in the female rat. *Program of the 10th International Congress of Endocrinology, San Francisco, CA.* p. 220, Abstract P1–342.
37. Gore A, Wu TJ, Rosenberg JJ, Roberts JL (1996). Gonadotropin-releasing hormone and NMDA receptor gene expression and co-localization change during puberty in female rats. *J Neurosci* **16**: 5281–5289.
38. Zamorano P, Mahesh VB, De Sevilla LM, Chorich LP, Brann DW (1997). Expression of excitatory amino acid receptors in the hypothalamus during the estrus cycle of the female rat. *79th Annual Meeting of the Endocrine Society, Minneapolis, MN* p. 377, Abstract P2–372.
39. Zamorano P, Mahesh VB, De Sevilla L, Brann DW (1997). Excitatory amino acid receptors mRNA levels in the hypothalamus during the progesterone induced LH surge in estrogen primed female rats. *27th Annual Meeting of the Society for Neuroscience, New Orleans, LA* p. 25, Abstract 19.1.
40. Jarry H, Hirsch B, Leonhardt S, Wuttke W (1992). Amino acid neurotransmitter release in the preoptic area of rats during the positive feedback actions of estradiol on LH release. *Neuroendocrinology* **56**:133–140.
41. Goroll D, Arias P, Wuttke W (1993). Preoptic release of amino acid neurotransmitters evaluated in peripubertal and young adult female rats by push-pull perfusion. *Neuroendocrinology* **58**:11–15.
42. Carbone S, Szwarcfarb B, Otera Losada M, Moguilevsky JA (1992). Effects of ovarian steroids on the gonadotropin response to *N*-methyl-D-aspartate and on hypothalamic excitatory amino acid levels during sexual maturation in female rats. *Endocrinology* **130**: 1365–1370.
43. Ping L, Mahesh VB, Brann DW (1994). Release of glutamate and aspartate from the preoptic area during the progesterone-induced LH surge: *In vivo* microdialysis studies. *Neuroendocrinology* **59**:318–324.
44. Unda R, Brann DW, Mahesh VB (1995). Progesterone suppression of glutamic acid decarbosylase (GAD_{67}) mRNA levels in the preoptic area: Correlation to the luteinizing hormone surge. *Neuroendocrinology* **62**:562–570.
45. Rettori V, Kamat A, McCann SM (1994). Nitric oxide mediates the stimulation of luteinizing-hormone releasing hormone release induced by glutamic acid *in vitro. Brain Res Bull* **33**:501–503.
46. Mahachoklertwattana P, Black S, Kaplan S, Bristoe J, Grumbach M (1994). Nitric oxide synthesized by gonadotropin-releasing hormone neurons in a mediator of *N*-methyl-D-aspartate (NMDA)-induced GnRH secretion. *Endocrinology* **135**:1709–1712.
47. Bhat GK, Mahesh VB, Lamar CA, Ping L, Aguan K, Brann DW (1995). Histochemical localization of nitric oxide neurons in the hypothalamus: Association with gonadotropin-releasing hormone neurons and co-localization with *N*-methyl-D-aspartate. *Neuroendocrinology* **62**:187–197.
48. Grossman A, Rossmanith W, Kabigting E, Cadd G, Clifton D, Steiner R (1994). The distribution of hypothalamic nitric oxide synthase mRNA in relation to gonadotropin-releasing hormone neurons. *J Endocrinol* **140**:R5–R8.
49. Herbison AE, Simonian SX, Norris PJ, Emson PC (1996). Relationship of neuronal nitric oxide synthase immunoreactivity to GnRH neurons in the ovariectomized and intact female rat. *J Neuroendocrinol* **8**:73–82.
50. Moretto M, Lopez FJ, Negro-Vilar A (1993). Nitric oxide regulates luteinizing hormone-releasing hormone secretion. *Endocrinology* **133**:2399–2402.
51. Bonavera JJ, Sahu A, Kalra PS, Kalra SP (1994). Evidence in support of nitric oxide (NO) involvement in the cyclic release of prolactin and LH surges. *Brain Res* **660**:175–179.
52. Bonavera JJ, Sahu A, Kalra PS, Kalra SP (1993). Evidence that nitric oxide may mediate the ovarian steroid-induced luteinizing hormone surge: Involvement of excitatory amino acids. *Endocrinology* **133**:2482–2487.
53. Aguan K, Mahesh VB, Ping L, Bhat G, Brann DW (1997). Evidence for a physiological role for nitric oxide in the regulation of the LH surge: Effect of central administration of antisense oligonucleotides to nitric oxide synthase. *Neuroendocrinology* **64**:449–455.
54. Bhat GK, Mahesh VB, Ping L, Chorich L, Wiedmeier VT, Brann DW (1998). Opioid-glutamate-nitric oxide connection in the regulation of luteinizing hormone secretion in the rat. *Endocrinology* **139**:955–960.
55. Chiba A, Kema A, Nagami Y, Kimura F, Toyoda J (1997). Differential inhibition of NMDA- and naloxone-induced LH release by and NMDA receptor antagonist and CRH in ovariectomized estrogen-primed rats. *Neuroendocrinology* **65**:141–146.
56. Maines MD, Trakshel GM, Kutty RK (1986). Characterization of two constitutive forms of rat liver microsomal heme oxygenase, only one molecular form is inducible. *J Biol Chem* **261**:411–419.
57. Trakshel G, Maines M (1989). Multiplicity of heme oxygenase isozymes. *J Biol Chem* **264**:1323–1328.
58. Laitenen J, Juvonen R (1995). A sensitive microassay reveals marked regional differences in the capacity of rat brain to generate carbon monoxide. *Brain Res* **694**:246–252.
59. Mancuso C, Minotti G, Pozzoli G, Mordente A, Grossman A, Preziosi P, Navarra P (1995). Evidence that biliverdin and carbon monoxide are formed vie hemin catabolism through the heme oxygenase pathway in the rat hypothalamus in vitro. *77th Annual Meeting of the Endocrine Society* Abstract P3–697.
60. Lamar CA, Mahesh VB, Brann DW (1996). Regulation of gonadotropin-releasing hormone secretion by heme molecules: A regulatory role for carbon monoxide? *Endocrinology* **137**:790–793.
61. Lamar CA, Mahesh VB, Brann DW (1996). Heme molecules regulate GnRH secretion in GT1–7 cells. *10th International Congress of Endocrinology* Abstract P1–387.
62. Lamar CA, Bhat GK, Mahesh VB, Brann DW (1997). Stimulation of GnRH release by heme molecules - further studies on the mechanism of action. *27th Annual Meeting of the Society for Neuroscience, New Orleans, LA* p. 1504, Abstract 591.7.
63. Shi B, Mahesh VB, Bhat GK, Ping L, Brann DW (1998). Evidence for a role of bradykinin neurons in the control of luteinizing hormone-releasing hormone secretion. *Neuroendocrinology*, in press.