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Glutamate: a Major Neuroendocrine Excitatory Signal Mediating Steroid Effects on Gonadotropin Secretion

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The preovulatory gonadotropin surge is induced by progesterone in the cycling female rat or in the ovariectomized estrogen-treated female rat after adequate estrogen-priming activity is present. The source of progesterone under physiological conditions could be the ovary and/or the adrenal. Since the GnRH neuron does not possess estrogen and progesterone receptors, its function is modulated by other CNS neurotransmitters and neurosecretory products. Among these, excitatory amino acids (EAAs) have now been shown to play an important role in the regulation of pulsatile gonadotropin release, induction of puberty and preovulatory and steroid-induced gonadotropin surges. Glutamate, the major endogenous EAA exerts its action through ionotropic and metabotropic receptors. The ionotropic receptors consist of two major classes, the NMDA (*N*-methyl-*D*-aspartate) and non-NMDA: kainate and AMPA (DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors. EAA receptors are found in hypothalamic areas involved with reproduction. While both NMDA and non-NMDA receptors are involved in the regulation of LH secretion, the NMDA receptors appear to be involved with the regulation of puberty and FSH secretion as well. Steroids increase the release rates of glutamate and aspartate in the preoptic area during the gonadotropin surge. Steroids may also regulate the hypothalamic AMPA receptors.

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INTRODUCTION

The regulation of the preovulatory surge of gonadotropins involves a complex interaction of steroids secreted by the ovary and the adrenal cortex and other CNS neurosecretory products ([1–6] for reviews). The classical concept that estradiol secreted by the maturing ovarian follicle(s) is the neural trigger for the preovulatory surge of gonadotropins was based on the evidence that ovariectomy or administration of estrogen antagonists or antibodies abolished the preovulatory surge of gonadotropins and estrogen administration reinstated at least a partial surge. That estradiol was the only steroid hormone involved has been questioned based on the fact that progesterone is needed to restore the full gonadotropin surge and GnRH sensitivity in the ovariectomized estrogen-primed animal [7–9]. Furthermore, estradiol does not induce a gonadotropin surge in the ovariectomized animal if the other source of progesterone namely the adrenal was removed by adrenalectomy [7]. The administration of progesterone to ovariectomized rats treated with estrogens in a manner in which estrogens by themselves do not induce surge changes in serum LH and FSH levels, has been shown to induce a preovulatory type gonadotropin surge [10–15]. The gonadotropin surge at proestrus is also attenuated by progesterone antagonists and progesterone synthesis inhibitors [4, 5, 16]. These data indicate an important physiological role of progesterone in modulating the gonadotropin surge. In the estrogen-primed rat, increasing progesterone levels by the administration of ACTH have been shown to induce a gonadotropin surge [17].

The GnRH neuron reportedly does not contain estrogen and progesterone receptors and is largely regulated by sex steroid modulated neurotransmitters [18, 19]. Prominent among the stimulatory regulators are the catecholamines while GABA and opioids are the inhibitory regulators. This area is reviewed by Kalra and Kalra [1]. Neuropeptide Y (see [20] for review) and galanin [21, 22] have also been shown to be (1) present

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in the hypothalamus, (2) cosecreted in the portal vein blood with GnRH and (3) to enhance pituitary sensitivity to GnRH in the release of gonadotropins. Evidence has also accumulated that glucocorticoids may bring about selective secretion of FSH [12–14]. Gonadal peptides such as inhibin have also been implicated in the regulation of FSH secretion [23].

Added to the above complexity in the regulation of gonadotropin secretion are the recent observations of the role of excitatory amino acids (EAAs) in such regulation (see [6] for review). Endogenous excitatory amino acids are primarily glutamate and aspartate with glutamate being the most abundant in the brain. EAA receptors are considered to be the main transmitter receptors mediating synaptic excitation in the CNS. EAA receptor mediated neurotransmission has now been shown to be of considerable importance in proestrus and estrogen–progesterone-induced preovulatory gonadotropin surge, the induction of puberty and the regulation of the pulsatility in gonadotropin secretion. This will be the major focus of this paper.

ENDOGENOUS EAAs AND THEIR RECEPTORS

Glutamate and aspartate are the primary EAA neurotransmitters in the CNS, with glutamate being the most abundant amino acid found in the brain. Strong immunoreactivity for glutamate is found in suprachiasmatic (SCN), ventromedial (VMN), arcuate (ARC) and parvocellular and magnocellular paraventricular (PVN) nuclei in the rat hypothalamus [24, 25]. Immunoreactive glutamate axons are in synaptic contact with dendrites and cell bodies in the medial basal hypothalamus (MBH), supraoptic nuclei (SON), ARC, SCN and PVN [24, 25].

EAA receptors can be divided into two major subgroups: the ionotropic receptors that regulate cation-specific ion channels and the metabotropic receptors that are coupled to G-proteins and modulate the production of second messengers. Thus far, only a limited amount of work has been done on metabotropic EAA receptors. The ionotropic receptors can be further subdivided into two classes: the *N*-methyl-D-aspartate (NMDA) receptors and the non-NMDA receptors consisting of kainate and DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. These classifications have been made based on their selective agonists.

NMDA R1 receptors are found in the organum vasculosa of the lamina terminalis (OVLT), preoptic area (POA), ARC, median eminence (ME), SON, SCN and PVN in the hypothalamus. Kainate receptors are found in higher concentrations in the ARC, ME and SCN while other areas of the hypothalamus have lower concentrations. The distribution of AMPA receptors are similar to that of the NMDA R1 receptors (see [6] for review).

REGULATION OF GONADOTROPIN SECRETION BY EAAs

The administration of either NMDA, kainate or AMPA in male and female animals causes a rapid release of LH within 10–15 min in a variety of animal species. This topic has been reviewed by Brann and Mahesh [6]. NMDA has been shown to be able to release LH with every pulse administered whereas kainate releases LH only after the first injection [26–28]. The major site of action appears to be the release of GnRH in the hypothalamus. This is based on the stimulation of GnRH release by NMDA and kainate in hypothalamic fragments *in vitro* [29, 30]. The NMDA effect on GnRH release can be blocked by a specific NMDA receptor antagonist AP-5. The NMDA agonists are more potent in OVLT/POA release of GnRH as compared to non-NMDA agonists, which appear to be more potent in the ARC–ME region [31, 32]. This conclusion is further supported by the observation that GnRH release from ARC–ME fragments induced by glutamate *in vitro* is blocked by the AMPA/kainate receptor antagonist, DNQX but not by the NMDA receptor antagonist AP-7 [31].

PHYSIOLOGICAL ROLE OF EAAs IN THE PREOVULATORY GONADOTROPIN SURGE

The physiological role of EAAs in the regulation of the preovulatory surge of gonadotropins in the cycling adult rat was first demonstrated by Brann and Mahesh [33] who showed that the administration of the NMDA antagonist MK801 completely blocked the proestrus LH surge and lowered but not blocked mean serum FSH levels. In the PMSG-primed immature rat in which PMSG was used to induce the first preovulatory surge of gonadotrophins, the NMDA antagonist MK801 attenuated both the LH and FSH surge [33]. The third ventricle injection of kainate/AMPA receptor antagonist DNQX in PMSG-primed immature rats also attenuated the LH surge with no effects on the FSH surge [34]. Thus both NMDA and non-NMDA neurotransmission is important for the preovulatory surge of LH with NMDA neurotransmission having a role in FSH secretion as well.

ROLE OF EAA NEUROTRANSMISSION IN STEROID-INDUCED GONADOTROPIN SURGE

Since progesterone exerts a pivotal role in the induction of the preovulatory type gonadotropin surge, in the ovariectomized animal primed with estrogens, in a manner that estrogens by themselves do not induce the surge, the role of NMDA and non-NMDA neurotransmission in the progesterone-induced surge was examined in detail. The NMDA antagonist MK801 administered 1 h before the administration of progesterone completely blocked the LH and FSH surge

[35] whereas the non-NMDA antagonist DNQX only blocked the LH but not the FSH surge [34]. The estrogen-induced LH surge (perhaps with participation of progesterone from the adrenal) can also be blocked in immature and adult ovariectomized rats by NMDA and non-NMDA antagonist [36, 37]. Progesterone-induced GnRH mRNA is also attenuated by MK801 [38].

REGULATION OF EAA RECEPTORS AND LIGAND CONCENTRATIONS BY ESTROGENS AND PROGESTERONE

The steroid milieu appears to be very important for the LH-releasing ability of NMDA, as NMDA has either no effect or is inhibitory to LH secretion in the ovariectomized animal not treated with estrogens [39–41]. In appropriately estrogen-primed animals, progesterone appears to significantly enhance the effects of NMDA on stimulating LH release [42, 43]. These observations raise the question of whether steroid treatment results in an increase in EAA receptors or the ligand itself or a combination of the two.

NMDA receptor binding and NMDA R1 mRNA levels were not altered in male or female rats after castration or after castration and testosterone replacement in the male rat and estrogen replacement with or without progesterone in the female rat [44]. NMDA and kainate receptor binding also did not change in the hypothalamus during the onset of puberty [45]. These findings are supported by the work of Kus *et al.* who found no effects of castration or dihydrotestosterone treatment on NMDA R1 mRNA levels in the ARC and POA regions of the hypothalamus in the adult male rat [46]. Weiland reported an increase in [^{3}H]glutamate binding in the POA area of ovariectomized rats treated with estrogens only when they were administered progesterone [47]. The increase in [^{3}H]glutamate binding was not displaced by NMDA and hence represented an increase in non-NMDA binding sites. Immunohistochemical studies from our laboratory suggest that this increase may be due to an increase in GluR1 subunit immunoreactivity representing AMPA receptor binding sites [6].

In the absence of estrogen and progesterone-induced changes in NMDA and kainate receptors, the possibility that progesterone increased glutamate and aspartate levels in the POA resulting in progesterone-induced activation of EAA neurotransmission was next considered. Microdialysis studies by Ping *et al.* [48] in the estrogen-primed ovariectomized rat treated with progesterone showed that the release rates of glutamate and aspartate were significantly increased immediately preceding the progesterone-induced LH surge. Similar results were obtained by Jarry *et al.* [49] during the estrogen-induced LH surge, while Goroll *et al.* [50] reported that the release rates of glutamate and aspartate are increased during puberty in the POA in female rats. Thus, estrogen and progesterone-induced EAA neurotransmission in regulating the gonadotropin surge appears to be mediated by increase EAA levels in the POA as well as an increase in AMPA receptors.

ROLE OF EAAs IN THE INDUCTION OF PUBERTY

NMDA treatment on postnatal days 26–29 has been shown to advance puberty by a number of investigators and the NMDA antagonist MK801 delays the onset of puberty [51–54]. This may be due to the synchronization of GnRH pulses on a particular day. However, the non-NMDA agonist kainate administered in a similar way did not advance puberty and the non-NMDA antagonist DNQX did not delay the onset of puberty [30]. It may be of interest to note that the inability of DNQX to delay puberty may be due to its inability to block FSH release [34]. Thus, FSH required for follicular maturation can be secreted in spite of DNQX treatment. Thus, EAAs acting through at least the NMDA receptors appear to be involved in sexual maturation during puberty.

ROLE OF EAAs IN PULSATILE LH AND FSH SECRETION

In ovariectomized female rats both the NMDA receptor antagonist AP-5 and the non-NMDA receptor antagonist DNQX significantly suppressed the LH pulse frequency, LH pulse amplitude and mean and trough LH levels [55]. AP-5 suppressed LH pulse amplitude and mean and trough LH levels more effectively than DNQX. The FSH pulse amplitude and mean and trough FSH levels were suppressed by AP-5 whereas FSH pulse frequency was not altered. On the other hand DNQX did not alter any parameter of FSH secretion. Single injections of AP-5, administered in doses and a manner similar to the female rat, to the castrated male rat resulted in suppression of pulse amplitude but not frequency and mean and trough levels of LH and FSH [56]. DNQX did not alter any parameter of LH secretion and only the mean levels of FSH were slightly reduced. Prolonged administration of DNQX reduced LH pulse amplitude and mean and trough levels of LH similar to AP-5. Similar results were reported in the male rat after systemic administration of AP-5 [57]. Thus, EAAs appear to drive the GnRH pulse generator or modulate its activity with females showing greater sensitivity than males. Furthermore, the pulsatile discharge of LH and FSH is more sensitive to NMDA neurotransmission as compared to non-NMDA neurotransmission.

CONCLUSIONS

It is now well established that EAA neurotransmission is an important mechanism involved in pulsatile LH and FSH secretion, the induction of puberty,

the preovulatory gonadotropin surge in the cycling rat and steroid-induced preovulatory type surges in the ovariectomized rat. The major site of action is the hypothalamic secretion of GnRH. Although both NMDA and non-NMDA receptors are involved, the NMDA receptor neurotransmission appears to play a more prominent role. The steroid-induced EAA neurotransmission is activated primarily by an increase in the ligands in the POA and possibly by an increase in AMPA receptors. The question of whether EAAs stimulate GnRH through a direct effect of GnRH neurons or indirectly through the regulation of other neurotransmitter neurons which synapse on GnRH neurons is unresolved. GnRH neurons have been reported not to express c-Fos after NMDA stimulation, while neurons surrounding GnRH neurons express c-Fos [58, 59]. Less than 5% of GnRH neurons express NMDA R1 mRNA levels. EAA receptor and action has been reported in immortalized GnRH neuronal cell lines in culture (GT1-1 cells) [60]. However, whether these cell lines represent the function of the endogenous GnRH neuron *in vivo* has to be resolved by further studies.

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REFERENCES

1. Kalra S. P. and Kalra P. S.: Neural regulation of LH secretion in the rat. *Endocrine Rev.* **4** (1983) 311–315.
2. Mahesh V. B.: The dynamic interaction between steroids and gonadotropins in the mammalian ovulatory cycle. *Neurosci. Biobehav. Rev.* **9** (1985) 245–260.
3. Fink G.: Gonadotropin secretion and its control. In *The Physiology of Reproduction* (Edited by E. Knobil and J. Neill). Raven Press, NY (1988) **Vol. I,** pp. 1349–1378.
4. Brann D. W. and Mahesh V. B.: Regulation of gonadotropin secretion by steroid hormones. *Front. Neuroendocr.* **12** (1991) 165–207.
5. Mahesh V. B. and Brann D. W.: Interaction between ovarian and adrenal steroids in the regulation of gonadotropin secretion. *J. Steroid Biochem. Molec. Biol.* **41** (1992) 495–513.
6. Brann D. W. and Mahesh V. B.: Excitatory amino acids: function and significance in reproduction and neuroendocrine regulation. *Front. Neuroendocr.* **15** (1994) 3–49.
7. Mann D. R. and Barraclough C. A.: Role of estrogen and progesterone in facilitating LH release in 4-day cyclic rats. *Endocrinology* **93** (1973) 694–699.
8. Krey L. C., Tyrey L. and Everett J. W.: The estrogen-induced advance in the cyclic LH surge in the rat: dependency on ovarian progesterone secretion. *Endocrinology* **93** (1973) 385–390.
9. Aiyer M. S. and Fink G.: The role of sex steroid hormones in modulating the responsiveness of the anterior pituitary gland to LHRH in the female rat. *J. Endocr.* **62** (1974) 533–572.
10. McPherson J. C., Costoff A. and Mahesh V. B.: Influence of estrogen–progesterone combinations on gonadotropin secretion in castrate female rats. *Endocrinology* **91** (1975) 771–779.
11. McPherson J. C. and Mahesh V. B.: 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** (1979) 763–772.
12. Brann D. W., Putnam C. D. and Mahesh V. B.: Corticosteroid regulation of gonadotropin and prolactin secretion in the rat. *Endocrinology* **126** (1990) 159–166.
13. Brann D. W., Putnam C. D. and Mahesh V. B.: Validation of the mechanisms proposed for the stimulatory and inhibitory effects of progesterone on gonadotropin secretion in the estrogen-primed rat. A possible role for adrenal steroids. *Steroids* **56** (1991a) 103–111.
14. Brann D. W., O'Connor J., Wade M. and Mahesh V. B.: LH and FSH subunit mRNA concentrations during the progesterone-induced gonadotropin surge in ovariectomized estrogen-primed immature rats. *Molec. Cell. Neurosci.* **3** (1992) 171–178.
15. Brann D. W., O'Connor J. L., Wade M. F., Zamorano P. L. and Mahesh V. B.: 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. Molec. Biol.* **46** (1993) 427–437.
16. Rao I. M. and Mahesh V. B.: 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** (1986) 1154–1161.
17. Putnam C. D., Brann D. W. and Mahesh V. B.: Acute activation of the ACTH-adrenal axis: effect upon gonadotropin secretion in the female rat. *Endocrinology* **128** (1991) 2558–2566.
18. Leranth C., Shanabrough M. and Naftolin F.: Estrogen induced ultrastructural changes in progesterone receptor-containing GABA neurons of the primate hypothalamus. *Neuroendocrinology* **54** (1991) 571–579.
19. Flugge G., Oertel W. H. and Wuttke W.: Evidence for estrogen-receptive GABAergic neurons in preoptic area of the rat brain. *Neuroendocrinology* **43** (1988) 1–5.
20. Kalra S. and Crowley W.: Neuropeptide Y: a novel neuroendocrine peptide in the control of pituitary hormone secretion and its relationship to LH. *Front. Neuroendocr.* **13** (1992) 1–46.
21. Lopez F. J., Maede E. V. and Negro-Vilar A.: Endogenous galanin modulates the gonadotropin and prolactin proestrus surges in the rat. *Endocrinology* **132** (1993) 795–800.
22. Brann D. W., Chorich L. P. and Mahesh V. B.: Effect of progesterone on galanin mRNA levels in the hypothalamus and the pituitary: correlation with the gonadotropin surge. *Neuroendocrinology* **58** (1993) 531–538.
23. Ying S.: Inhibins, activins and follistatins: gonadal proteins modulating the secretion of FSH. *Endocrine Rev.* **9** (1988) 267–293.
24. van den Pol A.: Glutamate and aspartate immunoreactivity in hypothalamic presynaptic axons. *J. Neurosci.* **11** (1991) 2087–2101.
25. van den Pol A., Waurin J. and Dudek F.: Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. *Science* **250** (1990) 1276–1278.
26. Abbud R. and Smith M. S.: 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** (1991) 3254–3258.
27. McGeer E. G. and McGeer P. L.: Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamate and kainic acids. *Nature* **263** (1976) 517–519.
28. Medhamurthy R., Gay V. L. and Plant T. M.: Repetitive injections of L-glutamatic 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. *Neuroendocrinology* **55** (1992) 660–666.
29. Bourguignon J., Gerard A. and Franchimont P.: Direct activation of gonadotropin-releasing hormone secretion through different receptors to neuroexcitatory amino acids. *Neuroendocrinology* **49** (1989) 402–408.
30. Bourguignon J., Gerard A., Mathieu J., Simons J. and Franchimont P.: Pulsatile release of gonadotropin-releasing hormone from hypothalamic explants is restrained by blockade of *N*-methyl-D,L-aspartate receptors. *Endocrinology* **125** (1989) 1090–1096.
31. Donose A., Lopez F. and Negro-Vilar A.: Glutamate receptors of the non-*N*-methyl-D-aspartic acid type mediate the increase in luteinizing hormone-releasing hormone release by excitatory amino acids. *Endocrinology* **126** (1990) 414–420.
32. Lopez F., Donozo A. and Negro-Vilar A.: Endogenous excitatory amino acids and glutamate receptor subtypes involved in the control of hypothalamic luteinizing hormone-releasing hormone secretion. *Endocrinology* **130** (1992) 1986–1992.

33. Brann D. W. and Mahesh V. B.: Endogenous excitatory amino acid involvement in the preovulatory and steroid-induced surge of gonadotropins in the female rat. *Endocrinology* **128** (1991) 1541–1547.
34. Brann D. W., Ping L and Mahesh V. B.: Role of non-NMDA receptor neurotransmission in steroid and preovulatory gonadotropin surge expression in the female rat. *Molec. Cell. Neurosci.* **4** (1993) 292–297.
35. Brann D. W. and Mahesh V. B.: Endogenous excitatory amino acid regulation of the progesterone-induced LH and FSH surge in estrogen-primed ovariectomized rats. *Neuroendocrinology* **53** (1991) 107–110.
36. Urbanski H. D. and Ojeda S. R.: Activation of luteinizing hormone-releasing hormone release advances the onset of female puberty. *Neuroendocrinology* **46** (1987) 273–276.
37. Lopez F., Donoso A. and Negro-Vilar A.: Endogenous excitatory amino regulates the estradiol-induced LH surge in ovariectomized rats. *Endocrinology* **126** (1990) 1771–1773.
38. Seong J. Y., Lee Y. K., Lee C. C. and Kim K.: NMDA receptor antagonist decreases the progesterone-induced increase in GnRH gene expression in the rat hypothalamus. *Neuroendocrinology* 58 (1993) 234–239.
39. Brann D. W. and Mahesh V. B.: Excitatory amino acid regulation of gonadotropin secretion: modulation by steroid hormones. *J. Steroid Biochem. Molec. Biol.* **41** (1992) 847–850.
40. Luderer U., Strobl F., Levine J. and Schwartz N.: Differential gonadotropin responses to *N*-methyl-D-aspartate (NMDA) in metestrous, proestrus, and ovariectomized rats. *Biol. Reprod.* **48** (1993) 857–866.
41. Reyes A., Luckhaus J. and Ferin M.: Unexpected inhibitory action of *N*-methyl-D,L-aspartate on luteinizing hormone release in adult ovariectomized rhesus monkeys: a role of the hypothalamic-adrenal axis. *Endocrinology* **127** (1990) 724–729.
42. Carbone S., Szwarcfarb B.. Losada M. and Moguilevsky J.: Effects of ovarian steroids on the gonadotropin response to *N*-methyl-D-aspartate and on hypothalamic excitatory amino acid levels during dexual maturation in female rats. *Endocrinology* **130** (1992) 1365–1370.
43. Reyes A., Xia L. and Ferin M.: Modulation of the effects of *N*-methyl-D,L-aspartate on luteinizing hormone by the ovarian steroids in the adult rhesus monkey. *Neuroendocrinology* **54** (1991) 405–411.
44. Brann D. W., Zamorano P. L., Chorich L. P. and Mahesh V. B.: Steroid hormone effects on NMDA receptor binding and NMDA receptor mRNA levels in the hypothalamus and cerebral cortex of the adult rat. *Neuroendocrinology* **58** (1993) 666–672.
45. Brann D. W., Zamorano P. L., Ping L. and Mahesh V. B.: Role of excitatory amino acid neurotransmission during puberty in the female rat. *Molec. Cell. Neurosci.* 4 (1993) 107–112.
46. Kus L., Beitz A. J., Kerr J. E. and Handa R. J.: NMDA R1 receptor mRNA expression in the hypothalamus of intact, castrate and DHT-treated male rats. *Soc. Neurosci.* **19** (1993) 919 (Abstract 379.5).
47. Weiland N. G.: Estradiol selectively regulates agonist binding sites on the *N*-methyl-D-aspartate receptor complex in the CA1 region of the hypothalamus. *Endocrinology* **131** (1992) 662–668.
48. Ping L., Mahesh V. B. and Brann D. W.: Release of glutamate and aspartate from the preoptic area during the progesterone-induced LH surge: *in vivo* microdialysis studies. *Neuroendocrinology* **59** (1994) 318–324.
49. Jarry H., Hirsch B., Leonhardt S. and Wuttke W.: Amino acid neurotransmitter release in the preoptic area of rats during the positive feedback actions of estradiol on LH release. *Neuroendocrinology* **56** (1992) 133–140.
50. Goroll D., Arias P. and Wuttke W.: Preoptic release of amino acid neurotransmitters evaluated in peripubertal and young adult female rats by push-pull perfusion. *Neuroendocrinology* **58** (1993) 11–15.
51. MacDonald M. C., Robertson H. A. and Wilkinson M.: Expression of c-Fos protein by *N*-methyl-D-aspartic acid in hypothalamus of immature female rats: blockade by MK-801 or neonatal treatment with monosodium glutamate. *Dev. Brain. Res.* **56** (1990) 294–297.
52. Meijs-Roelofs H., Kramer P. and van Leeuwen E.: The *N*-methyl-D-aspartate receptor antagonist MK-801 delays the onset of puberty and may acutely block the first spontaneous LH surge and ovulation in the rat. *J. Endocr.* **131** (1991) 435–441.
53. Urbanski H. and Ojeda S.: A role for *N*-methyl-D-aspartate (NMDA) receptors in the control of LH secretion and initiation of female puberty. *Endocrinology* **126** (1990) 1774–1776.
54. Veneroni O., Cocilovo, Muller E. and Cocchi D.: Delay of puberty and impairment of growth in female rats given a non competitive antagonist of NMDA receptors. *Life Sci.* **47** (1990) 1253–1260.
55. Ping L., Mahesh V. B. and Brann D. W.: A physiological role for NMDA and non-NMDA receptors in pulsatile gonadotropin secretion in the adult female rat. *Endocrinology* **135** (1994) 113–118.
56. Ping L., Mahesh V. B. and Brann D. W.: Effect of NMDA and non-NMDA receptor antagonists on pulsatile luteinizing hormone secretion in the adult male rat. *Neuroendocrinology* **61** (1995) 226–234.
57. Arsian M., Pohl C. and Plant T.: DL-2-amino-5-phosphonopentanoic acid, a specific *N*-methyl-D-aspartic acid receptor antagonist, suppresses pulsatile LH release in the rat. *Neuroendocrinology* **47** (1988) 465–468.
58. Lee W., Abbud R., Hoffman G. E. and Smith M. S.: Effects of *N*-methyl-D-aspartate receptor activation on c-Fos expression in luteinizing hormone-releasing hormone neurons in female rats. *Endocrinology* **133** (1993) 2248–2254.
59. Saitoh Y., Silverman A. and Gibson M.: Norepinephrine neurons in mouse locus coeruleus express c-Fos protein after *N*-methyl-D,L-aspartic acid (NMDA) treatment: relation to LH release. *Brain Res.* **561** (1991) 11–19.
60. Abbud R. and Smith M. S.: Do GnRH neurons express the gene for the NMDA receptor? *75th Ann. Meeting Endocr. Soc.* (1993) (Abstract 1181).
61. Mahachoklertwattana P., Sanchez J., Kaplan S. L. and Grumbach N. M.: NMDA receptors mediate the release of GnRH by NMDA in a hypothalamic GnRH neuronal cell line (GT-1). *Endocrinology* **134** (1994) 1023–1030.