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Regulatory Role of Excitatory Amino Acids in Reproduction

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Glutamate, the major excitatory amino acid (EAA) transmitter in the central nervous system, has been implicated as a critical mediator in brain function. Glutamate and its receptors are found in all key hypothalamic areas critically involved in reproduction. Administration of glutamate and its agonists can bring about LH release in animals with a steroid background. Antagonists of the ionotropic glutamate receptors inhibited LH release and abolished the steroid-induced and the preovulatory LH surge. Both NMDA and non-NMDA receptor antagonists can also inhibit pulsatile LH release in castrated animals. The preoptic area has been implicated as a primary site of action of NMDA, while non-NMDA agonists have been suggested to act primarily at the arcuate/median eminence level. While EAAs may act directly on GnRH neurons to enhance GnRH release, the majority of evidence suggests that an indirect mechanism, involving EAA activation of nitric oxide and/or catecholamines, plays a major role in the GnRH-releasing effects of EAAs. Furthermore, there is also some evidence that the tonic inhibitory effect of opioids on GnRH may also involve, at least in part, a suppression of glutamate. Finally, EAA stimulation of GnRH/LH release is markedly attenuated in middle-aged rats, suggesting that a defect in glutamate neurotransmission may underlie the attenuated LH surge observed in aging.

Key Words: Glutamate; NMDA; kainate; AMPA; LH; GnRH; nitric oxide; opioids; hypothalamus; reproduction.

Introduction

Glutamate, the major excitatory amino acid (EAA) in the central nervous system, is an important neurotransmitter in the control of brain function. It is widely localized in synapses in the brain and a large number of glutamate receptor subtypes are found in the central nervous system. Thus, it is recognized as a central regulator of a large number of physiological processes and has also been implicated in a number of pathophysiological syndromes and diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, epilepsy, stroke, and brain injury. A number of excellent reviews have appeared in the literature regarding glutamate's role in brain function *(1–11)*. Glutamate receptors are also localized in a variety of hypothalamic nuclei critical for the regulation of various reproductive functions such as puberty, LH pulsatility, the midcycle surge of gonadotropin, and reproductive behavior. The role of glutamate in these important reproductive and neuroendocrine events will be reviewed in this article.

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Excitatory Amino Acid Receptors and Their Localization in the Hypothalamus

Types of EAA Receptors

The more than 23 receptor proteins along with their splice variants form two major classes of EAA receptors, namely, ionotropic and metabotropic receptors. The ionotropic receptors contain integral, cation-specific ion-channels, whereas metabotropic receptors are coupled to G proteins and modulate the production of second messengers such as inositol phosphates and/or adenylate cyclase. To our knowledge the role of metabotropic receptors on reproduction is currently unclear and requires extensive additional work. Thus, this review will deal with only ionotropic EAA receptors. The ionotropic receptors can be subdivided into *N*-methyl-D-aspartate (NMDA), kainate, and DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors according to their selective agonists. Multiple subunits exist for each class of EAA receptors, which combine together to form a functional EAA receptor. Activation of the ionotropic EAA receptors leads directly to the opening of a group of ion channels that have different permeabilities to Na^+, K^+, and Ca^+ ions. The ionotropic receptors are large molecules with molecular masses of 100,000 or more *(1,12)*. NMDA receptor channels are regulated by Mg^+ and glycine. Mg^+ binds to a site inside the NMDA receptor–coupled ion channel resulting in a block of the channel *(13,14)*. Glycine, on the other hand, is a steric enhancer of the NMDA receptor *(15,16)*. In AMPA receptors, the Glu R2 subunit does not allow calcium to pass through its channels and, when expressed with other subunits to form the receptor, is impermeable to calcium *(17,18)*.

EAA Receptor Localization in the Hypothalamus and Pituitary

A significant number of EAA receptor subunits have been localized in the hypothalamus and the pituitary. Moderate to dense immunostaining for the NMDA-R1 receptor was demonstrated by Bhat et al. *(19)* in the female rat in the organum vasculosum of the lamina terminalis (OVLT), ventromedial nucleus (VMN), supraoptic nucleus (SON), arcuate nucleus (ARC), median eminence (ME), medial preoptic area (MPOA), and parvocellular paraventricular nucleus (PVN). Moderate to dense immunostaining for NMDA-R1 receptor mRNA was also reported by Kus et al. *(20)* and Petralia et al. *(21)* in almost all hypothalamic nuclei of the male rat. Petralia et al. *(21)* have reported moderate NMDA-R1 immunostaining in the male rat anterior pituitary and Bhat et al. *(22)* found that approx 9–11% of LH and FSH cells co-localize the NMDA-R1 subunit. GH- and prolactin-containing cells also co-localize the NMDA-R1 receptor subunit *(22)*.

Using polyclonal antibodies to AMPA-type glutamate receptor subunits, Brann et al. *(23)* found that AMPA GluR1 subunit staining was moderately dense in OVLT, ARC, SON, PVN, and suprachiasmatic nucleus (SCN) with lighter staining in MPOA in the female rat. GluR2-3 antibody showed a similar pattern of staining and GluR4 antibody showed a very light staining in ARC and MPOA. AMPA GluR subunits are also widely distributed in the male rat hypothalamus *(24)*. Kainate binding has been reported to be high in ARC and ME of the monkey *(25)*, and GluR5 subunit and GluR6 subunit mRNA distribution in the rat is similar to that described above *(26,27)*. The distribution of each class of ionotropic receptors was confirmed by Meeker et al. *(28)* by receptor-binding studies. The approximate relative densities of ionotropic glutamate receptors was NMDA >kainate>AMPA.

In addition to the presence of EAA receptors in the majority of hypothalamic nuclei in strategic positions that control reproduction, studies have revealed that in the hypothalamus of the rat and the monkey, dense glutamate immunostaining was found in the PVN, SON, lateral hypothalamic area, SCN, ARC, infundibular stalk and ME (reviewed in ref. *3*). The highest level of glutamate activity was observed in the presynaptic axon terminals approaching 100 m*M*, with much lower concentrations in astrocytic processes (approx 4–5 m*M*) *(29–32)*.

Role of Excitatory Amino Acids on LH Secretion and LH Pulsatility

Effect of EAA Agonists on LH Secretion

The first report that glutamate can stimulate LH secretion came from the laboratories of Olney et al., using subcutaneous and intracerebroventricular (ICV) administration *(33)*, and Ondo et al. *(34)* in the male rat. Glutamate did not alter FSH secretion. The effect of glutamate was considered to be at a hypothalamic site because direct pituitary injections failed to alter LH levels *(35)*. Subsequent studies demonstrated that glutamate was also able to stimulate LH secretion in the prepubertal male monkey *(36)* and the immature female rat *(37)*.

The first report that NMDA, the selective agonist for the NMDA receptor, could stimulate LH secretion in the male rat came from the laboratory of Cierco in 1978 *(38)*. That NMDA brought about an increase in LH in male animals was confirmed in rats *(35,39–43)*, monkeys *(36,44)*, and hamsters *(45,46)*. In female animals it has been demonstrated that NMDA stimulates LH secretion and less consistently FSH secretion in the intact immature female rat *(47–49)*, the estrogen-primed ovariectomized rat and ewe *(37,47,50–52)*, in the cycling female rat *(53,54)* and the monkey *(55)*. A comparison of the effects of NMDA with other EAA ionotropic receptor agonists after ICV injections in ovariectomized estrogen-primed adult female rat is shown in Fig. 1. Peak levels of LH occur 10 min after the ICV injection, and the effect is transient as LH levels return to the baseline within 30–60 min postinjection.

Kainate administration has been shown to bring about LH elevation in male rats *(35,38)*, male monkeys *(36)*, and the female rat *(53)*. The effect of AMPA on LH release has not been studied extensively, although there are reports that AMPA increases LH release in the hamster *(56)*, the ram *(57)*, and the adult ovariectomized estrogen-primed female rat as shown in Fig. 1. Thus, the major excitatory amino acid glutamate and the three selective ionotropic receptor agonists NMDA, kainate and AMPA are able to bring about LH release in a variety of species of animals.

Steroid Dependency of EAA Effects on LH Secretion

Based on the observation that the administration of NMDA to intact and steroid-primed ovariectomized animals brings about LH release while it has either no effect or is actually inhibitory in non-steroid primed ovariectomized animals, it has been widely postulated that EAA effects are steroid dependent *(37,40,52,54,58–60)*.

The first question that arose from these findings was whether steroid hormones up regulate EAA receptor concentrations in the hypothalamus as this would explain the steroid effects on LH release. This issue was investigated by Brann et al. *(61,62)* who found that neither acute nor chronic treatment with estradiol or progesterone altered NMDA receptor binding or NMDA-R1 mRNA levels in the hypothalamus of female rats. Likewise, NMDA receptor binding or NMDR-R1 mRNA levels were not changed in the hypothalamus of male rats treated with testosterone. Similarly Kus et al. *(20)* using *in situ* hybridization techniques found that castration or dihydrotestosterone treatment had no effect on NMDA-R1 mRNA levels in the ARC and MPOA of male rats. On the other hand, Wieland *(63)* found that estradiol plus progesterone caused a significant increase in [^{3}H] glutamate binding in the POA of the ovariectomized

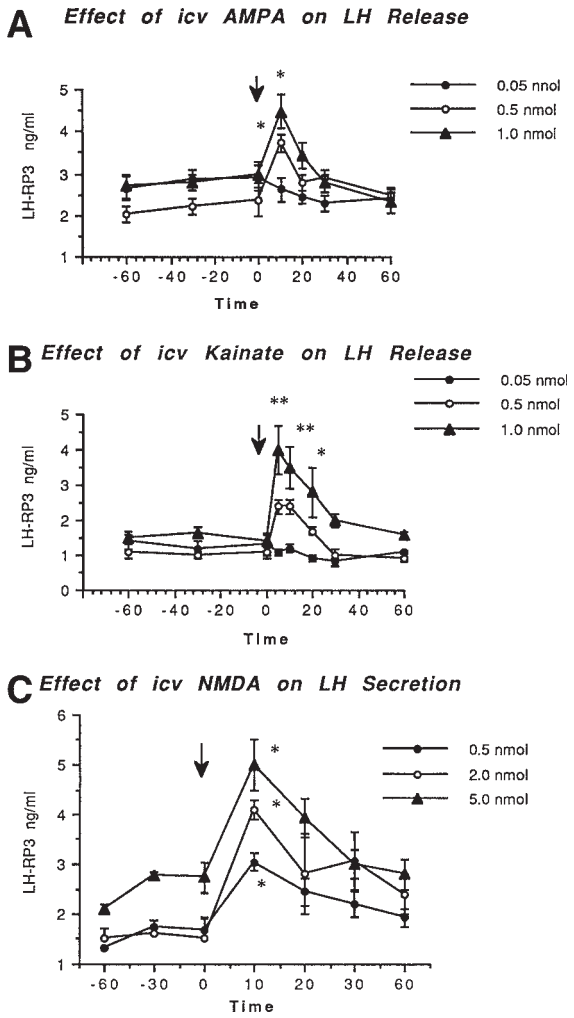

Fig. 1. Temporal and dose characteristics of the stimulation of LH secretion after the cerebroventricular administration of either AMPA (**A**), kainite (**B**), or NMDA (**C**) in ovariectomized estrogen-primed adult female rats. All three EAAs stimulated LH release rapidly with peak values generally observed 10 min after injection. $n = 6$ animals per group. $*p < 0.05$, $**p < 0.01$. [From Brann and Mahesh (1997). *Endocr. Rev.* **18,** 678–700.]

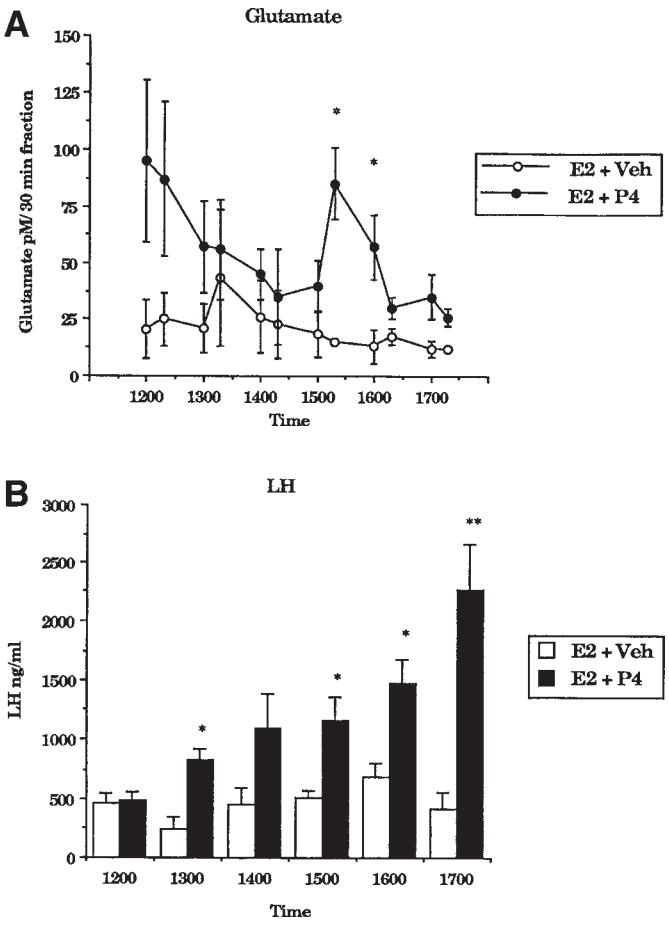

Fig. 2. Effect of progesterone on POA release rates of glutamate (**A**) and serum LH (**B**) in estrogen-primed ovariectomized adult rat. The animals were ovariectomized 2 wk before use. Estradiol (E2; 5 μg) was injected for 2 d followed by vehicle (Veh) or progesterone (P4; 1 mg/rat) at 09:00 h on the third day. Preoptic release rates of glutamate were determined by microdialysis. Perfusate samples were collected from the POA in 30-min fractions from 12:00 h to 17:30 h and analyzed by HPLC. Blood was collected hourly from a jugular cannula and LH levels were determined by RIA. $*p < 0.05$ as compared to E2. [From Ping et al. (1994). *Neuroendocrinology* **59,** 318–324.]

rat. The increase in [^{3}H]glutamate binding was not displaced by NMDA, indicating that the increased binding was probably due to an elevation of non-NMDA receptor sites. The findings of Brann and Mahesh *(64)* that estradiol plus progesterone treatment increased AMPA receptor Glu R1 subunit immunoreactive levels in the POA and ARC of immature female rats, and Diano et al. *(65)* that estradiol upregulated Glu R1 and Glu R2-3 immunoreactive levels in the adult rat further support the above-mentioned results. Changes in the NMDA-R1 mRNA levels during development have been reported by several investigators *(66–68)*.

The absence of a change in NMDA-R1 receptor with steroid treatment and an enhanced response to NMDA on LH secretion was partially explained by Arias et al. *(59)* by demonstrating that NMDA stimulated GnRH release equally well from hypothalamic fragments obtained either from steroid-primed or non-steroid-primed ovariectomized rats, indicating that increased sensitivity of the pituitary to GnRH in steroid primed rats may be the major reason. However, one cannot rule out the influence of other hypothalamic factors that may augment or interfere with LH secretion.

Role of EAAs in the Steroid-Induced and Preovulatory LH Surge

It is well established that estrogens and progesterone play an important role in triggering the preovulatory surge of LH and estradiol treatment followed by progesterone treatment in ovariectomized female rats can induce a proestrous-type LH surge *(69,70)*. To examine the regulatory role of excitatory amino acids in inducing this preovulatory-type LH surge, Ping et al. *(71)* carried out microdialysis studies of the hypothalamic area before and during the LH surge induced by estradiol and progesterone treatment in the adult ovariectomized rat (Fig. 2). The release rate of

glutamate was significantly enhanced in the preoptic area of the hypothalamus 3–5 h after progesterone administration that preceded the preovulatory-type LH surge. Aspartate release rates were also enhanced by the steroid treatment in the preoptic area, whereas the neurally inactive amino acid serine did not change significantly. Thus, it appeared that the mechanism involved in the steroid-stimulated LH release was mediated through the release of EAAs in the preoptic area. Work by Jarry et al. *(72)* also shows that POA release rates of glutamate and aspartate are increased during the estrogen-induced LH surge. They further demonstrated that the increase in glutamate and aspartate release rates occurred only in the POA and not in the medial basal hypothalamus during the estrogen-induced LH surge *(73)*. Goroll et al. *(74)* have also demonstrated increased rates of aspartate and glutamate in the POA at the time of puberty. Similarly, Carbone et al. found higher concentrations of glutamate and aspartate in the POA/MBH of 30-d-old female rats as compared to 16-d-old rats *(75)*.

It is relevant at this point to comment on the possible mechanisms involved in the increased release of glutamate at the time of preovulatory surge. The work of Jarry et al. *(72)* showed that the increase in glutamate release was preceded by a decrease in the levels of γ-aminobutyric acid (GABA). Because, in the hypothalamus, the enzyme glutamic acid decarboxylase (GAD_{67}) converts glutamate into GABA, Unda et al. *(76)* examined the expression of GAD_{67} in the POA of immature ovariectomized female rats treated with estradiol followed by progesterone to induce the preovulatory LH-type surge. Progesterone administration resulted in a significant decrease in the GAD_{67} mRNA levels and protein (unpublished results) in the POA just preceding the LH surge. The administration of the progesterone receptor antagonist RU486 resulted in the attenuation of the LH surge and prevented the decrease in GAD_{67} mRNA. Subsequent studies in the adult cycling rat showed that RU486 administration blocked the LH surge and increased GAD_{67} mRNA levels in the POA *(77)*. In addition, GAD_{67} mRNA levels showed a significant increase in the non-RU486-treated cycling rat at 1800 h, the start of the termination of the LH surge *(77)*. The importance of GAD_{67} in reducing the levels of GABA and increasing the release of glutamate was further shown in the monkey in which antisense to GAD_{67} brought about LH release, which was attenuated by the NMDA receptor antagonist MK801 *(78)*. However, Leonhardt et al. *(79)* has also reported an increase in GAD_{67} mRNA as well as a GABA transporter during the preovulatory LH surge.

In order to establish a physiological role for EAA neurotransmission in endocrine signals involved in the preovulatory-type LH surge, several investigators have employed specific EAA receptor antagonists to block EAA neurotransmission and studied the effect on the steroid-induced LH surge. A role for EAA neurotransmission in the estradiol-induced LH surge was demonstrated by Urbanski and Ojeda *(48,80)* and Lopez et al. *(81)*, who showed that the administration of NMDA and non-NMDA antagonists blocked the estradiol-induced LH surge in immature and adult ovariectomized rats. Likewise, work by our laboratory *(50,82)* demonstrated that the administration of an NMDA receptor antagonist completely blocked the progesterone-induced LH and FSH surge in the estrogen-primed ovariectomized immature rat. The kainate/AMPA receptor antagonist DNQX was also demonstrated to attenuate the progesterone-induced LH surge *(83)*. In further work from our laboratory, the selective AMPA antagonist NBQX was also found to attenuate the steroid-induced LH surge in the adult ovariectomized rat *(84)*.

Because there could be questions of whether models of steroid-induced LH surge can be regarded as physiological, our laboratory used the NMDA antagonist MK-801 in the adult cycling rat. The blockade of NMDA receptor-mediated neurotransmission by MK-801 treatment resulted in the abolishment of the proestrous LH surge, whereas mean levels of FSH did not change significantly *(50)*. Non-NMDA receptors also appeared to be important for the LH surge as DNQX administration to PMSG-primed immature rats significantly attenuated the preovulatory LH surge without modifying the FSH surge *(83)*. Schwartz and co-workers *(54)* also found that the NMDA receptor antagonist MK-801 completely blocked the proestrous LH surge in the adult female rat.

Thus, based on the several studies mentioned above and a variety of experimental models and the adult cycling rat, it is reasonable to conclude that during the ovulatory cycle, ovarian steroids trigger the release of EAAs, which, in turn, stimulate GnRH secretion and LH release.

Role of EAAs on Pulsatile LH Secretion

Repetitive NMDA stimulation has been demonstrated to produce GnRH pulses in vitro and LH pulses in vivo with each stimulation *(36,53)*. The competitive NMDA receptor antagonist AP5 suppressed pulsatile LH release in the castrated male rat *(85)* and MK-801 abolished pulsatile GnRH release in the male hypothalamus in vitro *(86)*. Work from our laboratory showed that a single ICV administration of AP5 decreased mean and trough levels of LH and FSH and their pulse frequency in the adult castrated male rat, while it took multiple injections of the non-NMDA antagonist DNQX to suppress LH pulses *(87)*. In the ovariectomized female rat, AP5 and DNQX were both able to suppress mean LH levels and LH pulse frequency *(88)*. Because DNQX suppresses both kainate and AMPA receptors, the individual role of kainate and AMPA needs to be further investigated. Thus, in addition to regulating the preovulatory LH surge, EAAs also regulate pulsatile LH release.

Zuo et al. *(89)* recently reported that middle-aged rats display an attenuated GnRH response to glutamate agonists on proestrous afternoon as compared to young proestrous rats. Quantitative RT-PCR also revealed significantly lower

NMDA-R1 mRNA levels in the POA and ARC/ME of middle-aged rats as compared to young proestrous rats *(89)*. Thus, the decreased hypothalamic glutamate response may be due to a decrease in hypothalamic glutamate receptors.

Site of Action of EAAs in the Regulation of LH Secretion

Evidence showing that the direct injection of glutamate in the hypothalamus or into the third cerebroventricle induced LH release *(19,34,51)* seemed to indicate that the hypothalamus was the primary site for EAA action for LH release. This concept was further supported by the fact that GnRH antagonists blocked the ability of EAAs to stimulate LH release *(41,90,91)*. Furthermore, direct injection of glutamate into the anterior pituitary *(34)* or treatment of pituitary cell cultures with NMDA in static culture *(92)* did not bring about LH release. However, ionotropic glutamate receptors have been reported to be present in the pituitary by several investigators *(17,21,22,24,93)*, and a few studies have shown enhancement of LH and other pituitary hormones with glutamate agonists in perifused pituitary cell cultures *(94–96)*. Nevertheless, the hypothalamus is regarded to be the primary site of action of EAAs in the release of LH.

To identify the discrete site of action of EAAs in the hypothalamus, several investigators have used c-Fos immunoreactivity as a neuronal marker of activation after EAA treatment. Results from our laboratory showed that the administration of AMPA or kainate in the third ventricle of an estrogen-primed ovariectomized adult rat resulted in the activation of c-Fos in the ARC *(93)*. In addition, AMPA and kainate are more potent than NMDA in stimulating GnRH release in vitro from proestrous rat ARC/ME fragments *(89)*. Similarly, Negro-Vilar and co-workers found that AMPA and kainate were much more potent than NMDA in stimulating GnRH release from male rat ARC/ME fragments *(97,98)*. Furthermore, the glutamate-induced GnRH release from ARC/ME fragments from male rats was blocked by the AMPA/kainate receptor antagonist DNQX but not the NMDA receptor antagonist AP-7. The above evidence points to the ARC/ME as the primary site for non-NMDA glutamate receptors, although kainate has also been shown to stimulate LH release when injected in POA *(35)*.

The injection of NMDA results in the activation of c-Fos immunoreactivity in the ARC, ME, OVLT, PVN, and the cerebral cortex, and in the noradrenergic cells of the locus coeruleus in immature and adult rats *(93,99–101)*. This indicates that NMDA can act at multiple sites inside and outside the hypothalamus. Considerable evidence in the literature points to POA as the major site of NMDA action in the release of LH. This evidence consists of LH stimulation after NMDA is injected in the MPOA *(35)* and the ability of NMDA to stimulate LH secretion even after the destruction of ARC by monosodium glutamate treatment *(49,102)*. In addition, NMDA has been demonstrated to stimulate POA GnRH synthesis and secretion *(74,103)*. Further confirmation was obtained by Liaw and Barraclough *(104)* by demonstrating a rise in OVLT/rostral POA GnRH mRNA levels 1 h after the administration of NMDA. Antisense oligonucleotide to NMDA-R2A subunit was also found to inhibit pulsatile GnRH release from the complete POA/medial basal hypothalamus fragment but not when the medial basal hypothalamus fragment was used alone *(105)*.

In summary, the POA appears to be a major site of action of NMDA in the regulation of GnRH-induced LH release, whereas the ARC/ME appears to be an important site for non-NMDA receptor agonist regulation of GnRH release.

Mechanism of Action on Excitatory Amino Acids in the Regulation of LH Secretion

Is the Regulation of GnRH Neurons Direct or Indirect?

The majority of evidence supporting a direct effect of EAAs on GnRH neurons has come from the immortalized GnRH neuron (GT1-1 and GT1-7) cell lines. This was based on the ability of glutamate, NMDA, AMPA, and kainate to stimulate GnRH release from GT1-7 cells in vitro, an effect blocked by specific EAA ionotropic receptor antagonists *(106,107)*. GT1-7 cells have also been shown to express the NMDA-R1 mRNA transcript *(107–109)*. However, *in situ* hybridization studies have revealed that less than 5% of the GnRH neurons in the adult male and female rats express NMDA-R1 mRNA *(110)*. GnRH neurons in adult male Syrian hamsters also did not colocalize NMDA-R1 receptor mRNA or protein or AMPA subunit mRNA (*111*, for review). It was also reported by two separate groups that GnRH neurons do not express c-Fos after treatment with NMDA but neurons that surround the GnRH neurons did *(100,101)*. It has also been demonstrated that GnRH neurons in the hypothalamus are immune to the neurotoxic effects of glutamate, kainite, and NMDA *(112)*. These results suggested that NMDA may not stimulate the GnRH neuron directly; rather, its actions may be mediated through other interneurons. However, a direct effect of EAAs on the GnRH neuron cannot be ruled out. In fact, studies with GnRH–green fluorescence protein (GFP) transgenic mice have shown that glutamate agonists can depolarize GnRH neurons upon direct application of glutamate on GnRH–GFP labeled neurons *(113)*.

More recent work has shown that female rat hypothalamic neurons in the ventromedial nuclei, periventricular nuclei, preoptic nuclei, and the arcuate nuclei colocalize estrogen receptor-α and the vesicular glutamate transporter-2 (VGLUT-2) mRNA *(114)*. VGLUT has been used extensively as a marker of glutamate neurons. These results suggest that glutamate neurons are targets of estrogen action. Examination of synaptic input to intermittent firing of GnRH neurons appeared to be stimulated by AMPA inputs and NMDA *(115,116)*. Estrogens were also shown to enhance glutamate and L-arginine–evoked GnRH release in male rat hypothalamic explants as well as the effects of AMPA/kainate agonists *(117)*. The effects of glutamate appeared to be

attenuated by AMPA/kainate antagonists *(117)*. Studies of Ottem et al. *(118)* showed that 80% of the GnRH neurons in the MPOA and less than 40% in the lateral preoptic area contained NMDA mRNA. Administration of NMDA doubled the GnRH mRNA levels in the medial preoptic neurons but increased GnRH mRNA less than 30% of the cells in the lateral preoptic nuclei. In contrast, estrogen treatment increased GnRH mRNA levels to the same extent in both areas and this increase was not blocked by the NMDA antagonist MK801. Thus, NMDA receptors appear to directly influence preferential neurons in the MPOA and it is unlikely that this activation is involved in the estrogen-induced increase in GnRH mRNA *(118)*. Further studies with the vesicular glutamate transporter-1 (VGLUT-1) and vesicular glutamate transporter-2 (VGLUT-2) showed weak to moderate intensity of staining of VGLUT-1 and moderate to intense density of staining of VGLUT-2 in the MPOA in the female rat *(119)*. VGLUT-2–but not VGLUT-1–labeled axon terminals were found to establish asymmetric synaptic contacts with GnRH neurons mainly on their dendrites. In the male rat, VGLUT-2 mRNA was expressed in the GnRH neuron axon terminals in the POA, OVLT, and ME *(120)*. Further studies have shown that all neurons in the anteroventral periventricular nucleus of the female rat express VGLUT-2 as well as the glutamic acid decarboxylase and vesicular GABA transporter (VGAT) *(121)*. These dual-phenotype synaptic terminals contact GnRH neurons and at the time of the gonadotropin surge the VGAT-containing vesicles decrease and VGLUT-2–containing vesicles increase in those terminals *(121)*. Further work in the area of GABA and glutamate transporters is expected to clarify the potential direct effects of glutamate on the GnRH neuron.

Catecholamines as Potential Mediators of EAA Actions on the GnRH Neuron

NMDA and non-NMDA receptors have been demonstrated in the locus coeruleus where many hypothalamic-projecting catecholamine cell bodies are located *(122)*, and ICV injection of NMDA induces c-Fos in noradrenergic neurons of the locus coeruleus and dopaminergic neurons in the medial basal hypothalamus *(101)*. EAAs have also been shown to stimulate hypothalamic norepinephrine release in the rat *(123–125)*. Thus, dopaminergic or noradrenergic neurons could play a role in mediating the EAA effects on GnRH release. Liaw and Barraclough *(104)* reported somewhat contradictory results with pretreatment with the α-1 adrenergic antagonist, prazosin, which blocked NMDA-induced LH release in four out of seven rats, while the remaining three showed a significant stimulation of LH after NMDA. The use of norepinephrine synthesis inhibitors also produced contradictory results with the block of NMDA-induced LH release in one study *(49)* and no effect found in another study *(126)*. In vitro studies of McCann and coworkers *(127)* appeared to be more conclusive as phentolamine, a noradrenergic α-receptor antagonist, clearly blocked the ability of glutamate to stimulate GnRH release from ARC/ME fragments from male rats. Dopamine appears to be less important than norepinephrine as Price et al. *(43)* found that pretreatment with dopamine antagonist had no effect on *N*-methyl-aspartate–induced LH release in the male rat.

Interaction Between Glutamate Neurons with Nitric Oxide in the Hypothalamus

Studies from a number of laboratories have shown that the novel gaseous neurotransmitter nitric oxide plays an important role in mediating glutamate effects on the GnRH neuron *(128,129)*. Work from our laboratory and others have shown that nitric oxide–containing neurons are present in key reproductive hypothalamic nuclei such as OVLT, POA, and ME *(19,130,131)*. However, GnRH neurons in the OVLT and POA from male and female rats did not contain nitric oxide synthase (NOS); rather, GnRH neurons were often surrounded by nitric oxide–containing neurons, and potential contacts between the two neuronal types in the OVLT and POA were observed *(19,130,131)*. Furthermore, there was a significant overlap between *en passant* GnRH neuronal fibers and nitric oxide neuronal fibers in the ME *(19)*. The major form of NOS in the hypothalamus was found to be b-NOS (brain NOS or neuronal NOS), while e-NOS (endothelial NOS) and m-NOS (macrophage or inducible-NOS) expression was low *(132)*.

That nitric oxide neurons could be a target for glutamate action in the hypothalamus was demonstrated by Bhat et al. *(19)*, who found that nitric oxide neurons in many hypothalamic nuclei colocalize with NMDA-R1 receptor. Functional evidence for such a role of nitric oxide came from the studies of Rettori et al. *(128)*, who showed that the glutamate-induced GnRH release from ARC/ME fragments in vitro was blocked by a competitive inhibitor of nitric oxide NG-monomethyl-L-arginine (NMMA) as well as the nitric oxide scavenger hemoglobin. Similarly Grumbach and coworkers *(129)* showed that GT1-1 cell line of immortalized GnRH neurons contained NOS activity and NO agonists brought about GnRH release in vitro and NO antagonists blocked the NMDA-induced GnRH secretion. Moretto et al. *(133)* were the first to demonstrate that nitric oxide itself stimulated GnRH release from hypothalamic fragments and from immortalized GnRH neurons in vitro. This finding was confirmed by Rettori et al. *(128)* and Grumbach and coworkers *(129)*. A functional role of nitric oxide in the induction of the steroid-induced and preovulatory LH surge was first demonstrated by Kalra and co-workers *(134,135)*, who showed that the surge was blocked in the rat by NO inhibitors. Aguan et al. *(136)* also demonstrated that the steroid-induced LH surge in the adult ovariectomized rat was attenuated by the administration of b-NOS antisense oligonucleotides. e-NOS antisense oligonucleotide administration also attenuated the LH surge, suggesting that e-NOS, while lightly expressed in the hypothalamus, still may have an important role in control of LH secretion.

In summary, the evidence that GnRH neurons are surrounded by NOS-containing neurons that colocalize glutamate receptors and that NO antagonists block the glutamate-induced GnRH release clearly points to NO being a potential mediator of EAA-induced GnRH release.

While the mechanism of how nitric oxide mediates glutamate signals to induce GnRH release remains to be elucidated, one of the suggested pathways is by binding to and modifying the function of heme-containing signaling enzymes. Nitric oxide donors have been shown to stimulate cGMP levels in the POA of female rats *(132)* and cGMP analogs have been shown to stimulate GnRH release. Conversely, a cGMP-dependent protein kinase inhibitor has been also shown to block nitric oxide–stimulated GnRH release *(133)*.

Glutamate–Opioid Interaction

It is well recognized that opioids tonically inhibit GnRH release and LH secretion in the rat ovarian cycle at all times except the time of the LH surge *(137–139)*. The tonic inhibition by opioids can be counteracted by the administration of the opioid antagonist naloxone, which brings about an elevation in LH secretion. The opioid antagonist naloxone was found to increase NOS activity in the POA and MBH preceding the naloxone-induced elevation of serum LH *(140)*. Prior treatment with a glutamate receptor antagonist was found to block both the naloxone-induced LH surge *(140,141)* and the naloxone-induced increase of hypothalamic NOS activity *(140)*. These findings suggest that the inhibitory tone exerted by opioids was by suppressing endogenous glutamate secretion, which was reversed by naloxone treatment. This was found to be the case as Bhat et al. *(140)* demonstrated a significant increase in glutamate release in the POA within 15 min of naloxone treatment using the microdialysis technique. Evidence that the glutamate transporter VGLUT-2 synapsing terminals establish asymmetric synapses with beta-endorphin–positive neurons has just been provided by Kiss et al. *(142)*.

Potential Direct Effects of Excitatory Amino Acids on the Ovary, Testes, and Reproductive Behavior

Effects of EAAs on the Ovary and Testes

Relatively little work has been done on the presence of EAAs in the ovary, but more extensive work has been done on the testes. The earliest report was the occurrence of D-aspartic acid in the male and female octopus *(143)*. In the male octopus high concentrations of D-aspartic acid were found in the prostate, Needham's sac, vas deferens, and the testis. In the female octopus, high concentrations of D-aspartic acid were found in the oviduct, accessory nidamental gland, and the ovary. In the ovary of the female green frog, testosterone was found to be high associated with low levels of D-aspartic acid and low in association with high levels of D-aspartic acid *(144)*. However, in the testes of the green frog, high levels of D-aspartic acid are associated with high testosterone levels *(145)*. In the lizard, high levels of D-aspartic acid were associated with high levels of ovarian estradiol, and D-aspartic acid was found to stimulate ovarian aromatase *(146)*.

In the male rat, D-aspartic acid was synthesized in the testes along with testosterone about 17 d after fertilization before birth and at sexual maturity *(147)*. Injections of D-aspartic acid in the male rat resulted in accumulation in the pituitary and testes and D-aspartic acid stimulated testosterone synthesis in the rat testes in vitro *(148)*. D-aspartate was also found to stimulate further testosterone synthesis in the rat Leydig cell stimulated with human chorinic gonadotropin and this synthesis was inhibited by L-cysteine sulfinic acid, an inhibitor of D-aspartate uptake in Leydig cells *(149)*. The effect of D-aspartate appeared to increase steroidogenic acute regulatory protein (STAR) mRNA and protein levels *(150)*. Ionotropic glutamate receptors have also been reported in the mouse testes along with glutamate transporters *(151)*.

Role of EAAs in Reproductive Behavior

Work by several groups of investigators have indicated that EAAs may regulate reproductive behavior in male and female animals. The work of Hsu et al. *(102)* showed that systemic injection of NMDA induced lordosis behavior in female rats. This NMDA injection also resulted in GnRH release, and the authors concluded that the GnRH release may be responsible for the lordosis behavior. In a subsequent study, Gargiulo and Donoso *(151)* demonstrated that an intraventricular injection of NMDA to estrogen-primed ovariectomized rats induced lordosis behavior and LH release. Prior treatment with a GnRH antagonist blocked the NMDA-induced LH release but had no effect on NMDA-induced lordosis behavior. The stimulatory effect of NMDA on lordosis behavior required an estrogen background.

There have been few reports suggesting that glutamate inhibits lordosis behavior *(152–154)*. However, the results from another study *(155)* show that NMDA antagonists AP-5 or MK-801 inhibit lordosis behavior in estrogen-treated–progesterone-primed ovariectomized rats, whereas non-NMDA receptor antagonists did not have any effect. Collectively, these data suggest a regulatory role of glutamate neurotransmission in lordosis behavior.

In the male rat, the administration of an NMDA receptor antagonist MK801 reduced experience-based facilitation of mounting such that experienced animals treated with MK801 behaved similar to inexperienced animals. Subsequently, Melis et al. *(156)* showed glutamate and aspartate levels increased significantly in the PVN of male rats in which erection and copulatory behavior was induced when exposed to estrogen–progesterone-treated ovariectomized rats. No erection or copulatory behavior occurred if the rats were exposed to non-receptive (untreated ovariectomized) female

rats, nor was there any change in the paraventricular glutamate or aspartate. The administration of NMDA in the paraventricular region strongly inhibited erection and copulatory behavior whereas an AMPA antagonist was less effective. Thus, the data obtained in male and female rats suggest that NMDA signals may mediate steroid signals in the CNS to facilitate reproductive behavior.

Conclusions

Extensive work supports a regulatory role for glutamate neurotransmission in the control of GnRH and LH secretion. Both NMDA and non-NMDA receptors have been implicated in control of pulse and surge GnRH/LH secretion. NMDA receptors in the preoptic area may be particularly important to glutamate regulatory control of GnRH/LH secretion, whereas non-NMDA receptors in the ARC/ME have also been implicated in the control of GnRH/LH secretion. Whether glutamate acts directly on GnRH neurons or indirectly via interneurons to control GnRH secretion is a subject of continued debate. Evidence for a direct effect have come from electrophysiological studies in GnRH–GFP-tagged transgenic mice and from immortalized GnRH neuronal cell lines. Evidence for an indirect action involving nitric oxide, catecholamines, and other molecules has come from both in vivo and in vitro studies using pharmacological approaches such as inhibitors and antisense oligonucleotides. The discovery of vesicular glutamate transporters and their use as markers of glutamate neurons has added a new tool in the arsenal of neuroendocrinologists. This tool should be very useful in studying direct versus indirect glutamate control of GnRH neurons and in identifying populations of glutamatergic neurons that are controlled by steroid feedback, and which play an important role in GnRH/LH secretion. Thus, while much knowledge has been acquired in the last decade or so on EAAs and reproduction, there is still considerable work ahead to gain a full understanding of the complex milieu of factors that control GnRH/LH secretion, and how precisely EAAs interact with these other factors and steroids to exert control of pulsatile and surge gonadotropin secretion.

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References

1. Monaghan, D. T., Bridges, R. J., and Cotman, C. W. (1989). *Annu. Rev. Pharmacol. Toxicol.* **29,** 365–402.
2. Brann, D. W. (1995). *Neuroendocrinology* **61,** 213–225.
3. van den Pol, A. N., Wuarin, J. P., and Dudek, F. E. (1990). *Science* **250,** 1276–1278.
4. Collingridge, G. L. and Singer, W. (1990). *Trends Pharmacol. Sci.* **11,** 290–296.
5. Baron, S. P., Auta, J., and Moerschbaecher, J. M. (1996). In: *Excitatory amino acids: their role in neuroendocrine function.* Brann, D. W. and Mahesh, V. B. (eds.). CRC Press: Florida.
6. Nakanishi, S. (1992). *Science* **258,** 597–603.
7. Greenamyre, J. T. and Young, A. B. (1989). *Neurobiol. Aging* **10,** 593–602.
8. McDonald, J. W. and Johnston, M. V. (1990). *Brain Res. Brain Res. Rev.* **15,** 41–70.
9. Dingledine, R., McBain, C. J., and McNamara, J. O. (1990). *Trends Pharmacol. Sci.* **11,** 334–338.
10. Ikonomidou, C. and Turski, L. (1995). In: *CNS neurotransmitters and neuromodulators: glutamate.* Stone, T. W. (ed.). CRC Press: Florida.
11. Brann, D. W. and Mahesh, V. B. (1997). *Endocr. Rev.* **18,** 678–700.
12. Petralia, R. S. and Wenthold, R. J. (1996). In: *Excitatory amino acids: their role in neuroendocrine function.* Brann, D. W. and Mahesh, V. B. (eds.). CRC Press: Florida.
13. Mayer, M. L. and Westbrook, G. L. (1987). *Prog. Neurobiol.* **28,** 197–276.
14. Nowak, L., Bregestoviski, P., Ascher, P., Herbet, A., and Prochiantz, A. (1984). *Nature* **307,** 462–465.
15. Hansen, J. J. and Krogsgaard-Larsen, P. (1990). *Med. Res. Rev.* **10,** 55–94.
16. Reynolds, I. J., Murphy, S. N., and Miller, R. J. (1987). *Proc. Natl. Acad. Sci. USA* **84,** 7744–7748.
17. Hollmann, M., Hartley, M., and Heinemann, S. (1991). *Science* **252,** 851–853.
18. Verdoorn, T. A., Burnashev, N., Monyer, H., Seeburg, P. H., and Sakmann, B. (1991). *Science* **252,** 1715–1718.
19. Bhat, G. K., Mahesh, V. B., Lamar, C. A., Ping, L., Aguan, K., and Brann, D. W. (1995). *Neuroendocrinology* **62,** 187–197.
20. Kus, L., Handa, R., Sanderson, J., Kerr, J., and Beitz, A. (1995). *Mol. Brain Res.* **28,** 55–60.
21. Petralia, R. S., Yokotani, N., and Wenthold, R. J. (1994). *J. Neurosci.* **14,** 667–696.
22. Bhat, G. K., Mahesh, V. B., Chu, Z. W., Chorich, L. P., Zamorano, P. L., and Brann, D. W. (1995). *Neuroendocrinology* **62,** 178–186.
23. Brann, D. W., Kumaresan, K., and Mahesh, V. B. (1994). *Program of the 76th Annual Meeting of The Endocrine Society,* Abstract 449, p. 313.
24. Petralia, R. S. and Wenthold, R. J. (1992). *J. Comp. Neurol.* **318,** 329–354.
25. Billy, A. J., Medhamurthy, R., Shaw, C., and Plant, T. M. (1990). *Soc. Neurosci.* **16,** 284 (Abstract 126.5).
26. Gregor, P., O'Hara, B. F., Yang, X., and Uhl, G. R. (1993). *NeuroReport* **4,** 1343–1346.
27. van den Pol, A. N., Hermans-Borgmeyer, I., Hofer, M., Ghost, P., and Heinemann, S. (1994). *J. Comp. Neurol.* **343,** 428–444.
28. Meeker, R. B., Greenwood, R. S., and Hayward, J. N. (1994). *Endocrinology* **134,** 621–629.
29. van den Pol, A. N. (1991). *J. Neurosci.* **11,** 2087–2101.
30. Fonnum, F. and Hassel, B. (1995). In: *CNS neurotransmitters and neuromodulators: glutamate*. Stone, T. W. (ed.). CRC Press: Florida.
31. Fonnum, F. (1985). *Neuromethods* **3,** 201–215.
32. Fonnum, F. (1991). In: *Excitatory amino acids.* Meldrum, B. S., Meroni, R., Simen, R., and Woods, J. (eds.). Raven Press: New York.
33. Olney, J. W., Cicero, T. J., Meyer, E., and De Gubareff, T. (1976). *Brain Res.* **112,** 420–424.
34. Ondo, J. G., Pass, K. A., and Baldwin, R. (1976). *Neuroendocrinology* **21,** 79–87.
35. Ondo, J. G., Wheeler, D. D., and Dom, R. M. (1988). *Life Sci.* **43,** 2283–2286.

36. Medhamurthy, R., Gay, V. L., and Plant, T. M. (1992). *Neuroendocrinology* **55,** 660–666.
37. Brann, D. W. and Mahesh, V. B. (1992). *J. Steroid Biochem. Molec. Biol.* **41,** 847–850.
38. Price, M., Olney, J., and Cicero, T. (1978). *Neuroendocrinology* **26,** 352–358.
39. Schainker, B. A. and Cicero, T. J. (1980). *Brain Res.* **184,** 425–437.
40. Bonavera, J. J., Kalra, S. P., and Kalra, P. S. (1993). *Endocrinology* **133,** 178–182.
41. Strobl, F. J., Luderer, U., Besecke, L., Wolfe, A., Schwartz, N. B., and Levine, J. E. (1993). *Biol. Reprod.* **48,** 867–873.
42. Farah, J. M., Rao, T. S., Mick, S. J., Coyne, K. E., and Na Iyengar, S. (1991). *Endocrinology* **128,** 1875–1880.
43. Price, M. T., Olney, J. W., Mitchell, M. V., Fuller, T., and Cicero, T. J. (1978). *Brain Res.* **158,** 461–465.
44. Plant, T. M., Gay, V. L., Marshall, G. R., and Arslan, M. (1989). *Proc. Natl. Acad. Sci. USA* **86,** 2506–2510.
45. Hui, Y., Hastings Maywood, E. S., and Ebling, F. (1992). *J. Reprod. Fertil.* **95,** 935–946.
46. Urbanski, H. F. (1990). *Endocrinology* **127,** 2223–2228.
47. Carbone, S., Szwarcfarb, B., Otera Losada, M., and Moguilevski, J. A. (1992). *Endocrinology* **130,** 1365–1370.
48. Urbanski, H. F. and Ojeda, S. R. (1987). *Neuroendocrinology* **46,** 273–276.
49. MacDonald, M. C. and Wilkinson, M. (1992). *J. Neuroendocrinol.* **4,** 223–229.
50. Brann, D. W. and Mahesh, V. B. (1991). *Endocrinology* **128,** 1541–1547.
51. Akema, T. and Kimura, F. (1993). *Neuroendocrinology* **57,** 28–33.
52. Estienne, M. J., Schillo, K. K., Hileman, S. M., Green, M. A., and Hayes, S. H. (1990). *Biol. Reprod.* **42,** 126–130.
53. Abbud, R. and Smith, M. S. (1991). *Endocrinology* **129,** 3254–3258.
54. Luderer, U., Strobl, F. J., Levine, J. E., and Schwartz, N. B. (1993). *Biol. Reprod.* **48,** 857–866.
55. Wilson, R. and Knobil, E. (1983). *Brain Res.* **248,** 177–179.
56. Ebling, F. J., Hui, Y., Mirakhur, A., Maywood, E. S., and Hastings, M. H. (1993). *J. Neuroendocrinol.* **5,** 609–618.
57. Kumar, V., Lincoln, A., and Tortonese, D. (1993). *J. Neuroendocrinol.* **5,** 649–654.
58. Reyes, A., Xia, L., and Ferin, M. (1991). *Neuroendocrinology* **54,** 405–411.
59. Arias, P., Jarry, H., Leonhardt, S., Moguilevsky, J., and Wuttke, W. (1993). *Neuroendocrinology* **57,** 710–715.
60. Reyes, A., Luckhaus, J., and Ferin, M. (1990). *Endocrinology* **127,** 724–729.
61. Brann, D. W., Zamorano, P. L., Chorich, L. P., and Mahesh, V. B. (1993). *Neuroendocrinology* **58,** 666–672.
62. Brann, D. W., Zamorano, P. L., Ping, L., and Mahesh, V. B. (1993). *Mol. Cell Neurosci.* **4,** 107–112.
63. Weiland, N. G. (1992). *Endocrinology* **131,** 662–668.
64. Brann, D. W. and Mahesh, V. B. (1994). *Front. Neuroendocrinol.* **15,** 3049.
65. Diano, S., Naftolin, F., and Horvath, T. L. (1997). *Endocrinology* **138,** 778–789.
66. Nyberg, C., Srivastava, V., Hiney, J., Laraa, F., and Dees, W. L. (1995). *Endocrinology* **136,** 2874–2880.
67. Zamorano, P. L., Mahesh, V. B., DeSevilla, L. M., and Brann, D. W. (1996). *Program of the 10th International Congress of Endocrinology* Abstract P1-342, p. 220.
68. Gore, A., Wu, T. J., Rosenberg, J. J., and Roberts, J. L. (1996). *J. Neurosci.* **16,** 5281–5289.
69. Mahesh, V. B. and Brann, D. W. (1992). *J. Steroid Biochem. Mol. Biol.* **41,** 495–513.
70. Mahesh, V. B. and Brann, D. W. (1998). *Steroids* **63,** 616–629.
71. Ping, L., Mahesh, V. B., and Brann, D. W. (1994). *Neuroendocrinology* **59,** 318–324.
72. Jarry, H., Hirsch, B., Leonhardt, S., and Wuttke, W. (1992). *Neuroendocrinology* **56,** 133–140.
73. Jarry, H., Leonhardt, S., Schwarze, T., and Wuttke, W. (1995). *Neuroendocrinology* **62,** 479–486.
74. Goroll, D., Arias, P., and Wuttke, W. (1993). *Neuroendocrinology* **58,** 11–15.
75. Carbone, S., Szwarcfarb, B., Otra Losada, M., and Moguilevsky, J. A. (1992). *Endocrinology* **130,** 1365–1370.
76. Unda, R., Brann, D. W., and Mahesh, V. B. (1995). *Neuroendocrinology* **62,** 562–570.
77. Brann, D. W., Unda, R., and Mahesh, V. B. (2002). *Neuroendocrinology* **76,** 283–289.
78. Kasuya, E., Nyberg, C. L., Mogi, K., and Terasawa, E. (1999). *Endocrinology* **140,** 705–712.
79. Leonhardt, S., Boning, B., Luft, H., Wuttke, W., and Jarry, H. (2000). *Neuroendocrinology* **71,** 8–15.
80. Urbanski, H. and Ojeda, S. (1990). *Endocrinology* **126,** 1774–1776.
81. Lopez, F., Donoso, A. O., and Negro-Vilar, A. (1990). *Endocrinology* **126,** 1771–1773.
82. Brann, D. W. and Mahesh, V. B. (1991). *Neuroendocrinology* **53,** 107–110.
83. Brann, D. W., Ping, L., and Mahesh, V. B. (1993). *Mol. Cell Neurosci.* **4,** 292–297.
84. Ping, L., Mahesh, V. B., Bhatt, G. K., and Brann, D. W. (1997). *Neuroendocrinology* **66,** 246–253.
85. Arslan, M., Pohl, C., and Plant, T. (1988). *Neuroendocrinology* **47,** 465–468.
86. Bourguignon, J., Gerard, A., and Franchimont, P. (1989). *Neuroendocrinology* **49,** 402–408.
87. Ping, L., Mahesh, V. B., and Brann, D. W. (1995). *Neuroendocrinology* **61,** 226–234.
88. Ping, L., Mahesh, V. B., and Brann, D. W. (1994). *Endocrinology* **135,** 113–118.
89. Zuo, Z., Mahesh, V. B., Zamorano, P. L., and Brann, D. W. (1996). *Endocrinology* **137,** 2334–2338.
90. Cicero, T. J., Meyer, E. R., and Bell, R. D. (1988). *Life Sci.* **42,** 1725–1732.
91. Gay, V. L. and Plant, T. M. (1987). *Endocrinology* **120,** 2289–2296.
92. Tal, J., Price, M., and Olney, J. (1983). *Brain Res.* **273,** 179–182.
93. Brann, D. W., Ping, L., Zamorano, P. L., and Mahesh, V. B. (1996). In: *Excitatory amino acids: their role in neuroendocrine function*. Brann, D. W. and Mahesh, V. B. (eds.). CRC Press: Florida.
94. Zanisi, M. and Messi, E. (1991). *J. Steroid Biochem. Mol. Biol.* **40,** 155–163.
95. Lindstrom, P. and Ohlsson, L. (1992). *Endocrinology* **131,** 1903–1907.
96. Login, I. S. (1990). *Life Sci.* **47,** 2269–2275.
97. Donoso, A. O., Lopez, F. J., and Negro-Vilar, A. (1990). *Endocrinology* **126,** 414–420.
98. Lopez, F. J., Donoso, A. O., and Negro-Vilar, A. (1992). *Endocrinology* **130,** 1986–1992.
99. MacDonald, M. C., Robertson, H. A., and Wilkinson, M. (1990). *Dev. Brain Res.* **56,** 294–297.
100. Lee, W., Abbud, R., Hoffman, G. E., and Smith, M. S. (1993). *Endocrinology* **133,** 2248–2254.
101. Saitoh, Y., Silverman, A., and Gibson, M. (1991). *Brain Res.* **561,** 11–19.
102. Hsu, C., Lee, J. N., Ho, M. L., Cheng, B. H., Li, P. H., and Yu, J. Y. (1993). *Acta Endocrinol. (Copenh.)* **128,** 385–388.
103. Bourguignon, J., Gerard, A., Mathieu, J., Simons, J., and Franchimont, P. (1989). *Endocrinology* **125,** 1090–1096.
104. Liaw, J. and Barraclough, C. A. (1993). *Mol. Brain Res.* **17,** 112–118.

105. Bourguignon, J. P., Gerard, A., Purnelle, G., et al. (1997). *J. Neuroendocrinol.* **9,** 183–191.
106. Spergel, D. J., Krsmanovic, L., Stojilkovics, S., and Catt, K. (1994). *Neuroendocrinology* **59,** 309–317.
107. Mahachoklertwattana, P., Sanchez, J., Kaplan, S., and Grumbach, M. (1994). *Endocrinology* **134,** 1023–1030.
108. Urbanski, H. F., Daschel, M., Lew, N. M., and Fahy, M. M. (1994). *J. Reprod. Fertil.* **100,** 5–9.
109. Mahesh, V. B., Zamorano, P., DeSevilla, L., Lewis, D., and Brann, D. W. (1999). *Neuroendocrinology* **69,** 397–407.
110. Abbud, R. and Smith, M. S. (1995). *Brain Res.* **690,** 117–120.
111. Urbanski, H. F. (1996). In: *Excitatory amino acids: their role in neuroendocrine function*. Brann, D. W. and Mahesh, V. B. (eds.). CRC Press: Florida.
112. Ebling, F. J., Cronin, A., and Hastings, M. H. (1996). *Soc. Neurosci.* Abstract 70410, p. 1789.
113. Spergel, D. J., Kruth, U., Hanley, D. F., Sprengel, R., and Seeburg, P. H. (1999). *J. Neurosci*. **19,** 2037–2050.
114. Eyigor, O., Lin, W., and Jennes, L. (2004). *J. Neuroendocrinol*. **16,** 26–31.
115. Suter, K. J. (2004). *Neuroscience* **128,** 443–450.
116. Kuehl-Kovarik, M. C., Pouliot, W. A., Halterman, G. L., Handa, R. J., Dudek, F. E., and Partin, K. M. (2002). *J. Neurosci*. **22,** 2313–2322.
117. Matagne, V., Lebrethon, M. C., Gerard, A., and Bourguignon, J. P. (2005). *Endocrinology* **146,** 2313–2323.
118. Ottem, E. N., Godwin, J. G., and Peterson, S. L. (2002). *Endocrinology* **143,** 4837–4845.
119. Kiss, J., Kocsis, K., Csaki, A., and Halasz, B. (2003). *Eur. J. Neurosci.* **18,** 3267–3278.
120. Hrabovszky, E., Turi, G. F., Kallo, I., and Liposits, Z. (2004). *Endocrinology* **145,** 4018–4021.
121. Ottem, E. N., Godwin, J. G., Krishnan, S., and Peterson, S. L. (2004). *J. Neurosci.* **24,** 8097–8105.
122. Urbanski, H. F. and Kohama, S. G. (1996). *Soc. Neurosci.* Abstract 143.6, p.353.
123. Blandina, P., Johnson, D., Walcott, J., and Goldfarb, J. (1992). *J. Pharmacol. Exp. Ther.* **263,** 61–68.
124. Navarro, C. E., Cabrere, R. J., and Donoso, A. O. (1994). *Brain Res. Bull.* **33,** 677–682.
125. Navarro, C. E., Cabrere, R. J., and Donoso, A. O. (1995). *Brain Res. Bull.* **37,** 119–122.
126. Johnson, M. D., Whetsell, W. O. Jr., and Crowley, W. R. (1985). *Exp. Brain Res.* **59,** 57–61.
127. Kamat, A., Yu, W., Rettori, V., and McCann, S. M. (1995). *Brain Res. Bull.* **37,** 233–235.
128. Rettori, V., Kamat, A., and McCann, S. M. (1994). *Brain Res. Bull.* **33,** 501–503.
129. Mahachoklertwattana, P., Black, S., Kaplan, S., Bristoe, J., and Grumbach, M. (1994). *Endocrinology* **135,** 1709–1712.
130. Grossman, A., Rossmanith, W., Kabigting, E., Cadd, G., Clifton, D., and Steiner, R. (1994). *J. Endocrinol.* **140,** R5–R8.
131. Herbison, A. E., Simonian, S. X., Norris, P. J., and Emson, P. C. (1996). *J. Neuroendocrinol.* **8,** 73–82.
132. Bhat, G., Mahesh, V., Aguan, K., and Brann, D. W. (1996). *Neuroendocrinology* **64,** 93–102.
133. Moretto, M., Lopez, F. J., and Negro-Vilar, A. (1993). *Endocrinology* **133,** 2399–2402.
134. Bonavera, J. J., Sahu, A., Kalra, P. S., and Kalra, S. P. (1994). *Brain Res.* **660,** 175–179.
135. Bonavera, J. J., Sahu, A., Kalra, P. S., and Kalra, S. P. (1993). *Endocrinology* **133,** 2482–2487.
136. Aguan, K., Mahesh, V. B., Ping, L., Bhat, G., and Brann, D. W. (1997). *Neuroendocrinology* **64,** 449–455.
137. Barraclough, C. A. and Sawyer, C. H. (1955). *Endocrinology* **57,** 329–337.
138. Piva, F., Maggi, R., Limonta, P., Motta, M., and Martini, L. (1985). *Endocrinology* **117,** 766–772.
139. Bonavera, J. J., Kalra, S. P., and Kalra, P. S. (1993). *Endocrinology* **133,** 178–182.
140. Bhat, G. K., Mahesh, V. B., Ping, L., Chorich, L., Wiedmeier, V. T., and Brann, D. W. (1998). *Endocrinology* **139,** 955–960.
141. Chiba, A., Kema, A., Nagami, Y., Kimura, F., and Toyoda, J. (1997). *Neuroendocrinology* **65,** 141–146.
142. Kiss, J., Csaba, Z., Csaki, A., and Halasz, B. (2005). *Eur. J. Neurosci*. **21,** 2111–2119
143. D'Aniello, A., DiCosmo, A., DiCristo, C., and Fisher, G. (1995). *Gen. Comp. Endocrinol.* **100,** 69–72.
144. DiFiore, M. M., Assisi, L., Botte, V., and D'Aniello, A. (1998). *J. Endocrinol.* **157,** 199–207.
145. Raucci, F., Assisi, L., D'Aniello, S., Spinelli, P., Botte, V., and DiFiore, M. M. (2004). *J. Endocrinol.* **182,** 365–376.
146. Assisi, L., Botte, V., D'Aniello, A., and DiFiore, M. M. (2001). *Reproduction* **121,** 803–808.
147. D'Aniello, A., DiCosmo, A., DiCristo, C., Annunziato, L., Petrucelli, L., and Fisher, G. (1996). *Life Sci.* **59,** 97–104.
148. Nagata, Y., Homma, H., Lee, J. A., and Imai, K. (1999). *FEBS Lett.* **444,** 160–164.
149. Nagata, Y., Hamma, H., Matsumoto, M., and Imai, K. (1999). *FEBS Lett.* **454,** 317–320.
150. Hu, J. H., Yang, N., Ma, Y. H., et al. (2004). *Acta Pharmacol. Sin.* **25,** 366–371.
151. Gargiulo, P. A. and Donoso, A. O. (1995). *Physiol. Behav.* **58,** 169–173.
152. Kow, L., Harlan, R., Shivers, B., and Pfaff, D. (1985). *Brain Res.* **341,** 26–34.
153. McCarthy, M., Curran, G., and Feder, H. (1991). *Neurosci. Lett.* **126,** 94–97.
154. Gargiulo, P. A., Munoz, V., and Donoso, A. O. (1992). *Physiol. Behav.* **52,** 737–739.
155. Fleming, A. S. and Kuera, C. (1991). *Behav. Neural. Biol.* **56,** 319–329.
156. Melis, M. R., Succu, S., Mascia, M. S., Cortis, L., and Argiolas, L. (2004). *Eur. J. Neurosci.* **19,** 2569–2575.