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Review

Estrogen positive feedback to gonadotropin-releasing hormone (GnRH) neurons in the rodent: The case for the rostral periventricular area of the third ventricle (RP3V)

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ABSTRACT

Increasing levels of circulating estradiol during the follicular phase of the ovarian cycle act on the brain to trigger a sudden and massive release of gonadotropin-releasing hormone (GnRH) that evokes the pituitary luteinizing hormone surge responsible for ovulation in mammals. The mechanisms through which estrogen is able to exert this potent “positive feedback” influence upon the GnRH neurons are beginning to be unravelled. Recent studies utilizing mouse models with global and cell-specific deletions of the different estrogen receptors (ERs) have shown that estrogen positive feedback is likely to use an indirect pathway involving the modulation of ER α -expressing neurons that project to GnRH neurons. Conventional tract tracing studies in rats, and experiments involving conditional pseudorabies virus tract tracing from GnRH neurons in the transgenic mouse, indicate that the dominant populations of ER α -expressing neuronal afferents to GnRH neurons reside in the anteroventral periventricular, median preoptic and periventricular preoptic nuclei. Together these estrogen-sensitive afferents to GnRH neurons form a periventricular continuum that can be referred to as rostral periventricular area of the third ventricle (RP3V) neurons. The neurochemical identity of some RP3V neurons has been determined and there is mounting evidence for important roles of glutamate, GABA, kisspeptin and neurotensin-expressing RP3V neurons in estrogen positive feedback. The definition of the key cluster of estrogen-sensitive neurons responsible for activating the GnRH neurons to evoke the GnRH surge (and ovulation) should be of substantial value to on-going efforts to understand the molecular and cellular basis of the estrogen positive feedback mechanism.

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Abbreviations: AV3V, anteroventral third ventricle; AVPV, anteroventral periventricular nucleus; GnRH, gonadotropin-releasing hormone; MEPO, median preoptic nucleus; PR, progesterone receptor; RP3V, rostral periventricular area of the third ventricle; rPOA, rostral preoptic area; PRV, pseudorabies virus; PV, periventricular nucleus

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1. Introduction

The gonadotropin-releasing hormone (GnRH) neurons represent the final output pathway of the neuronal network controlling fertility in all mammalian species. As a consequence of the pattern of GnRH neuron migration into the brain along the terminal and vomeronasal axons during embryogenesis (Wray, 2002), the GnRH neuron cell bodies exist as a scattered continuum throughout the basal forebrain of adult mammals. However, the majority of GnRH neurons extend axons to a highly circumscribed region within median eminence of the hypothalamus from where they release GnRH into the pituitary portal system to control gonadotrophin secretion (Herbison, 2006). This population of “neuroendocrine” GnRH neurons exhibits marked functional plasticity in the adult female brain; at mid-cycle, the GnRH neuron switches from delivering an episodic pattern of GnRH secretion to one of sustained high level output for several hours. This massive secretion of GnRH, termed the GnRH surge, is the primary trigger of the pituitary luteinizing hormone (LH) surge and, in turn, ovulation in all mammals (Caraty et al., 1995, 1989; Ching, 1982; Clarke and Cummins, 1985; Levine et al., 1982, 1985; Moenter et al., 1992a,b; Moenter et al., 1990; Pau et al., 1993; Sarkar et al., 1976; Xia et al., 1992).

It is now recognized that changes in circulating estrogen levels are primarily responsible for driving the plasticity in GnRH neuron function that generates the GnRH surge in mammals. This paper intends to review what is known about (i) the changes in GnRH neuron activity at mid-cycle and (ii) our present understanding of how estrogen initiates these changes. In doing so, the case will be made for defining the rostral periventricular area of the third ventricle (RP3V) as a functional anatomical construct containing the key estrogen-regulated inputs to GnRH neurons necessary for estrogen positive feedback.

2. GnRH neuron excitability and secretion at mid-cycle

Studies in sheep have enabled the pattern of GnRH secretion into the portal vasculature to be studied in great detail. Analyses in the ewe have shown that the pattern of GnRH release leading up to the surge changes from a strictly pulsatile pattern to one of significant non-episodic GnRH release intermingled with high frequency pulsatile GnRH secretion through to a sudden “explosion” of extremely high GnRH release (Fig. 1) (Caraty et

al., 1989; Clarke, 1993; Evans et al., 1995; Moenter et al., 1992a). This massive increase in GnRH secretion continues for a period of >24 h, considerably beyond the duration of the LH surge it induces, before returning to a strictly episodic pattern of release (Caraty et al., 1995; Moenter et al., 1990).

The results of GnRH measurement studies in other species are similar to that of ewes but, because of technical constraints, less detailed information has been available. In the monkey, both median eminence and third ventricular monitoring of GnRH concentrations have shown that a marked increase in GnRH release occurs at the time of the LH surge (Pau et al., 1993; Xia et al., 1992). The precise profile of GnRH release over this time has not been studied, but seems likely to be comprised of pulsatile events superimposed upon a high basal outpouring of GnRH (Xia et al., 1992). Like the

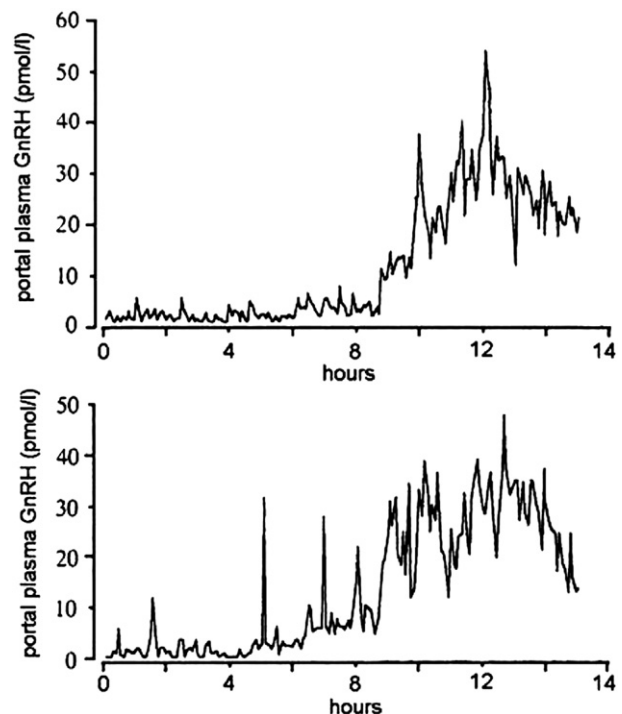


Fig. 1 – Dynamics of GnRH surge in the portal vasculature of two ovariectomized, estrogen-treated ewes. Adapted from Caraty, A., Locatelli, A. and Martin, G.B., 1989, *Journal of Endocrinology*. 123, 375–382 © Society for Endocrinology (1989). Reproduced by permission.

sheep, the GnRH surge in the monkey long outlasts the LH surge (Xia et al., 1992).

Secretion studies in the rat have used push-pull or microdialysis approaches to sample GnRH levels in the median eminence/arcuate nucleus region. Although hampered by a lack of good temporal resolution, these studies have, nevertheless, reported a clear increase in GnRH secretion correlated with the LH surge (Ching, 1982; Levine and Ramirez, 1982; Sarkar et al., 1976). The dynamics of GnRH surges in the rat appear to take the form of a marked increase in GnRH pulse amplitude with more minor variations in GnRH pulse frequency and inter-pulse secretion (Levine, 1997; Levine and Ramirez, 1982; Park and Ramirez, 1989; Sisk et al., 2001). Whether the proestrous increase in GnRH secretion persists for several hours after the end of the LH surge, as it does in the sheep and monkey, is not clear.

Together, these studies suggest that the GnRH neurons exhibit a fundamentally different pattern of secretory activity for several hours in each cycle to evoke the GnRH/LH surge. Experiments using the expression of the immediate-early gene *c-Fos*, as a marker of electrical activation, have indicated that GnRH neurons are indeed activated co-incident with the occurrence of the GnRH surge (Hoffman et al., 1993). Furthermore, a recent extracellular electrophysiological study using a daily LH surge mouse model, has reported that the mean firing rate of GnRH neurons is elevated in the afternoon of the expected surge (Christian et al., 2005). Thus, as would be expected, the increase in GnRH secretion into the portal vasculature at the time of the surge results from a change in the electrical excitability of GnRH neurons, and this occurs, at least in part, at the level of their cell bodies.

An unresolved issue is that of precisely which populations of neuroendocrine GnRH neurons may be involved in the GnRH surge. There is ample evidence for quite marked heterogeneity within the GnRH neuronal population (Herbison, 2006) and the *c-Fos* studies mentioned above indicated that it is a sub-population of GnRH neurons located in the rostral preoptic area (rPOA) around the organum vasculosum of the lamina terminalis (OVLT) that is involved in the surge in rodents (Lee et al., 1990; Wintermantel et al., 2006). In addition, changes in GnRH biosynthesis also appear to exist preferentially in GnRH neurons located around the OVLT (Rubin and King, 1994). Thus, there is some evidence for a “surge population” of GnRH neurons in rodents. Similar *c-Fos* studies in sheep and monkeys have not highlighted an anatomically delineated sub-population of GnRH neurons involved in the surge (Moenter et al., 1993; Witkin et al., 1994). Whether the GnRH neurons involved in the surge might only be “surge neurons” or also participate in generating pulsatile GnRH release or be involved in negative feedback remains an important unresolved issue. One hypothesis holds that the GnRH neurons responsible for the surge are a subset of the population that is independent of those involved in pulsatile release (Kimura and Funabashi, 1998).

3. Mechanism of estrogen positive feedback to GnRH neurons

The rising follicular-phase concentration of circulating estradiol is the key signal driving the GnRH surge in spontaneous

ovulators. In rodents, a circadian mechanism is very often coupled to the surge mechanism to ensure that the time of onset of the surge (and ovulation) is co-ordinated with sexual behavior. Thus, in rats and mice, the GnRH/LH surge only occurs when the rising estrogen and circadian clock signals coincide (Chappell, 2005; Everett and Sawyer, 1950; Legan and Karsch, 1975). However, in sheep and primates, there is no clear requirement for a circadian input in generating the GnRH/LH surge (Karsch et al., 1997; Xia et al., 1992).

How does estrogen exert such a potent stimulatory effect on GnRH neuron secretion once every cycle to generate the surge? One clue lies in the dynamic of the estrogen signal that is interpreted by the GnRH neuron network. In primates, sheep and rodents, the LH/GnRH surge is only induced by an estrogen signal of sufficiently high or increasing levels that last for several hours (Bronson, 1981; Caraty et al., 1989; Legan et al., 1975; Moenter et al., 1990; Sarkar and Fink, 1980; Xia et al., 1992; Yamaji et al., 1971). Experiments examining the exact period of elevated estrogen exposure required in the ewe have suggested that a 7–14 h period is the absolute minimum (Evans et al., 1997). Importantly, studies in both the rat (Legan et al., 1975) and sheep (Evans et al., 1997) have demonstrated that estrogen does not need to be present at the actual time of surge initiation for a normal GnRH surge to occur. This indicates that the GnRH neuronal network “reads” a prolonged, high concentration estrogen state as the appropriate signal to initiate positive feedback. The requirement for prolonged estrogen exposure would be compatible with the idea that estrogen acts, at least in part, in a classical genomic manner through estrogen receptors (ERs) to alter gene expression within the GnRH neuronal network. This concept has very recently been supported by a study showing that mice expressing a mutated ER α that is unable to bind to DNA estrogen response elements, are unable to generate positive feedback (Glidewell-Kenney et al., 2007).

Where is estrogen acting within the GnRH neuronal network? As it would appear that classical ERs are involved, one strategy has been to evaluate the roles and locations of the different ER isoforms (ER α and ER β) involved in estrogen positive feedback. The generation of mutant mouse models with deletions of ERs has been especially useful in this regard. The global ER α KO mouse lines are infertile whereas ER β KO mutants show a range of reproductive phenotypes (Couse et al., 2003; Dupont et al., 2000; Kregel et al., 1998; Lubahn et al., 1993). To evaluate estrogen positive feedback in these mouse lines, we used an ovariectomized, estrogen-only replacement paradigm to evaluate the LH surge and *c-Fos* expression in GnRH neurons. These studies revealed that estrogen positive feedback was normal in ER β KO mice, but absent in ER α KO mice (Wintermantel et al., 2006). Studies were then undertaken using ER α - and ER β -selective agonists (Hegele-Hartung et al., 2004) in wild-type mice to examine which receptor isoform was sufficient to generate positive feedback. These studies found that the ER α agonist generated normal positive feedback (Wintermantel et al., 2006) whereas the ER β agonist was unable to initiate *c-Fos* expression in GnRH neurons or generate an LH surge (unpublished observations, Porteous, Wintermantel and Herbison).

Together, the above studies clearly indicated that ER α was both necessary and sufficient for estrogen positive feedback to

occur in mice. As GnRH neurons do not express ER α (Herbison and Pape, 2001), this indicated that estrogen acted indirectly upon GnRH neurons to bring about their activation. Previous studies had suggested roles for ER α -expressing neurons, glia and endothelial cells in the positive feedback mechanism (Herbison, 1998; Petersen et al., 2003; Prevot, 2002; Rage et al., 1997). To refine further the cell types involved in this pathway, we used a Cre-LoxP approach to delete ER α specifically from neurons in the forebrain. Neuron-specific ER α KO mutants failed to exhibit estrogen positive feedback (Wintermantel et al., 2006) and thereby revealed that neurons expressing ER α are the critical cell type interacting with GnRH neurons to bring about estrogen positive feedback (Fig. 2). This does not, of course, discount roles for ER α -expressing glia or endothelial cells in this mechanism, but does indicate that they are unable to bring about positive feedback by themselves.

The hypothesis that estrogen acted via ER-expressing interneurons to regulate GnRH neurons was generated initially in the early 1980s by Shivers et al. (1983) who reported that GnRH neurons were unable to bind radiolabeled estradiol. That experiment has now been repeated with more sensitive reagents that have shown that some GnRH neurons do indeed bind estradiol (Hrabovszky et al., 2000). Nevertheless, the new evidence from ER mutant mice clearly demonstrates that direct actions of estradiol on GnRH neurons through ER β or other non-classical estrogen-binding molecules are not sufficient for positive feedback.

What is the location and identity of ER α -expressing neurons involved in the estrogen positive feedback mechanism? Lesion studies undertaken in rats have clearly demonstrated the key importance of cells within the anteroventral periventricular nucleus (AVPV), more than any other brain

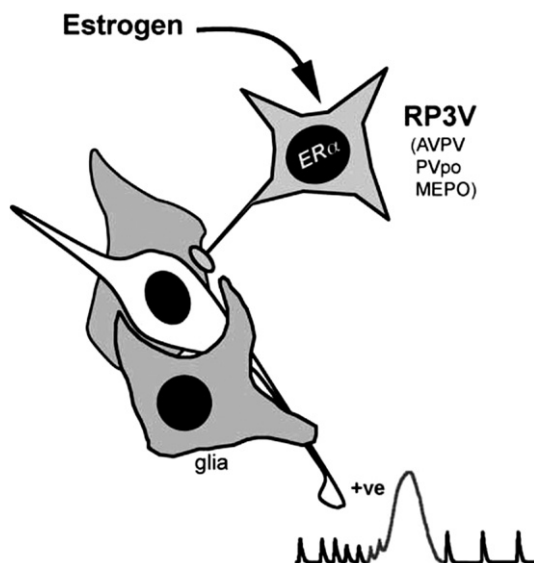


Fig. 2 – Schematic representation of the key pathway underlying estrogen positive feedback in the rodent. A population of neurons scattered through the anteroventral periventricular (AVPV), periventricular preoptic (PVpo) and median preoptic (MEPO) nuclei project directly to GnRH neurons and express ER α . Together, they are referred to as the rostral periventricular area of the third ventricle (RP3V) population of neurons. The pale cell is a GnRH neuron.

region, in the positive feedback mechanism. Lesions of the AVPV were shown to result in persistent estrus and the abolition of the estrogen- or estrogen plus progesterone-induced LH surges (Ronnekleiv and Kelly, 1988; Wiegand and Terasawa, 1982; Wiegand et al., 1980). Although not administered specifically into the AVPV, estradiol implants placed in the medial preoptic area (but not elsewhere) were found to be able to generate an LH surge in the rat (Goodman, 1978; Kalra and McCann, 1975) while similarly positioned implants of anti-estrogens inhibited the estrogen-induced LH surge (Petersen and Barraclough, 1989; Petersen et al., 1989). There is also evidence for abundant ER α and ER β expression within neurons of the AVPV (Herbison and Theodosis, 1992b; Orikasa et al., 2002; Shughrue et al., 1997) and conventional tracing studies have indicated that ER-expressing AVPV neurons are likely to project directly to the GnRH neurons (Gu and Simerly, 1997; Simonian et al., 1999). Furthermore, cells in the AVPV express c-Fos at the time of the surge suggesting that they are also activated by estrogen positive feedback (Le et al., 1999). As ER α -containing neurons of the AVPV receive direct inputs from the suprachiasmatic nucleus in the rat (Watson et al., 1995), they may also represent a site of integration of circadian and estrogen inputs in the regulation of GnRH neurons in rodents (Herbison, 1998; Petersen et al., 2003; Simerly, 2002). Together, these experimental findings strongly suggest that estrogen regulates AVPV neurons to activate the GnRH neurons.

Using a conditional pseudorabies virus (PRV) tracing approach (DeFalco et al., 2001), we have been able to determine the locations of neurons that innervate the sub-population of GnRH neurons residing in the rPOA of the mouse (Campbell, 2007; Wintermantel et al., 2006). By combining this approach with ER α immunocytochemistry, it has been possible to establish the locations of ER α -expressing neurons that project to the sub-population of GnRH neurons likely involved in generating the GnRH/LH surge (Fig. 3). We have found that virtually all ER α -afferents reside in the median preoptic nucleus (MEPO), AVPV, and preoptic and anterior hypothalamic divisions of the periventricular nucleus (PVpo/a), with a very few ER α -expressing primary afferents identified in the arcuate nucleus (Fig. 3) (Wintermantel et al., 2006). While confirming the long-suspected arrangement between the AVPV and GnRH neurons, this study also highlighted that neurons adjacent to the AVPV in the PV and MEPO, were also direct ER α -expressing primary afferents.

4. The case for the RP3V

Whereas the role of AVPV cells in estrogen positive feedback is well documented (see above), neurons within the MEPO and PV have received relatively little attention in terms of the positive feedback mechanism. A series of Nissl-stained sections from an adult female C57BL/6J mouse through these areas is shown in Fig. 4. Interestingly, in the landmark lesioning studies by Wiegand and Terasawa, lesions of the PVpo (termed ASR in that paper) blocked the estrogen-induced LH surge in rats (Wiegand and Terasawa, 1982). Furthermore, in c-Fos mapping studies undertaken by Hoffman and colleagues, it was clear that increased numbers of c-Fos-

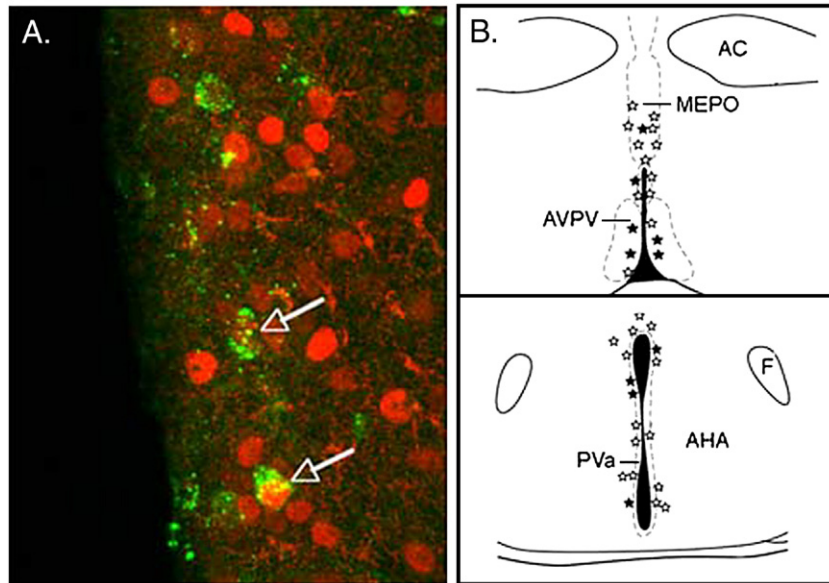


Fig. 3 – Definition of ER α -expressing neurons projecting to rostral preoptic area GnRH neurons using Cre-conditional pseudorabies virus tract tracing. (A) Neurons in the anteroventral periventricular nucleus (AVPV) projecting to GnRH neurons are defined by the expression of green fluorescent protein. Immunolabeling for ER α (red) reveals two of these neurons (arrows) to be ER α -expressing afferents to GnRH neurons. (B) Schematic coronal representation of the location of neurons projecting to GnRH neurons (stars) at the level of the preoptic area (top) and anterior hypothalamic nuclei (AHA) (bottom). Filled stars represent primary afferents that express ER α . AC, anterior commissure; F, fornix; MEPO, median preoptic nucleus; PVA, periventricular nucleus, anterior hypothalamic area division. Adapted and reproduced from Neuron, Vol 52, Wintermantel TM, Campbell RE, Porteous R, Bock D, Grone HJ, Todman MG, Korach KS, Greiner E, Perez CA, Schutz G, Herbison AE, “Definition of estrogen receptor pathway critical for estrogen positive feedback to gonadotropin-releasing hormone neurons and fertility” 271–280, ©2006 with permission from Elsevier.

expressing neurons at the time of the surge were located with in the AVPV as well as more caudally within the PVpo (Le et al., 1999). Similarly, following the identification of ER α -expressing afferents in the PVpo/a with the PRV approach, we re-evaluated c-Fos expression in the PV of mice and found that the number of cells positive for c-Fos was increased in the PVpo at the time of positive feedback. A re-evaluation of the MEPO in the same study failed, however, to find any evidence for increased c-Fos in this region in response to estrogen (Wintermantel et al., 2006). Evidence in support of the PVpo, in particular, as a brain region containing primary afferents to the GnRH neurons, including ER α -expressing afferents, also comes from conventional tracing approaches in the rat (Hahn and Coen, 2006; Le et al., 1999; Simonian et al., 1999).

Thus, on the basis of neuroanatomical tracing, c-Fos activity and lesioning studies, it would appear that the key estrogen-sensitive neurons projecting to GnRH neurons are located in a periventricular continuum that includes the AVPV, but also extends caudally into the PVpo and dorsally into the MEPO. Thus, it is important to recognize that neurons outside the AVPV are also likely to be involved in the estrogen positive feedback mechanism. As such, the definition of a functional anatomical construct (RP3V=MEPO+AVPV+PVpo) comprised of the neuronal afferents involved in estrogen feedback to GnRH neurons should be useful (Fig. 2). This is a similar concept to the naming of the anteroventral third ventricle (AV3V) by Johnson to represent a functionally related group of neurons residing in different rostral hypothalamic

nuclei involved in controlling body fluid balance (Johnson, 1985). The RP3V concept also neatly expands the boundaries of the AVPV as the periventricular visceromotor pattern generator for neuroendocrine GnRH neurons (Thompson and Swanson, 2003).

5. Neurochemical phenotypes of RP3V neurons

The neurochemical identity of the RP3V ER-expressing neurons is not well established at present. A variety of classical neurotransmitters and neuropeptides are synthesized by rostral periventricular neurons and several of these populations are known to express ER α ; these include glutamate (Eyigor et al., 2004; Ottem et al., 2004), GABA (Herbison, 1997; Ottem et al., 2004), dynorphin (Simerly, 1991), enkephalin (Simerly, 1991; Yuri and Kawata, 1994), galanin (Bloch et al., 1992), kisspeptin (Smith et al., 2005), substance P (Okamura et al., 1994), calcitonin gene-related peptide (Herbison and Theodosios, 1992a) and neurotensin (Herbison and Theodosios, 1992b) neurons. At present, a strong case can be made for the involvement of ER α -expressing glutamate, GABA, kisspeptin and neurotensin RP3V neurons in the estrogen positive feedback mechanism (for reviews see Herbison, 2006; Petersen et al., 2003; Smith and Jennes, 2001). A brief outline of the data available for each candidate is provided below.

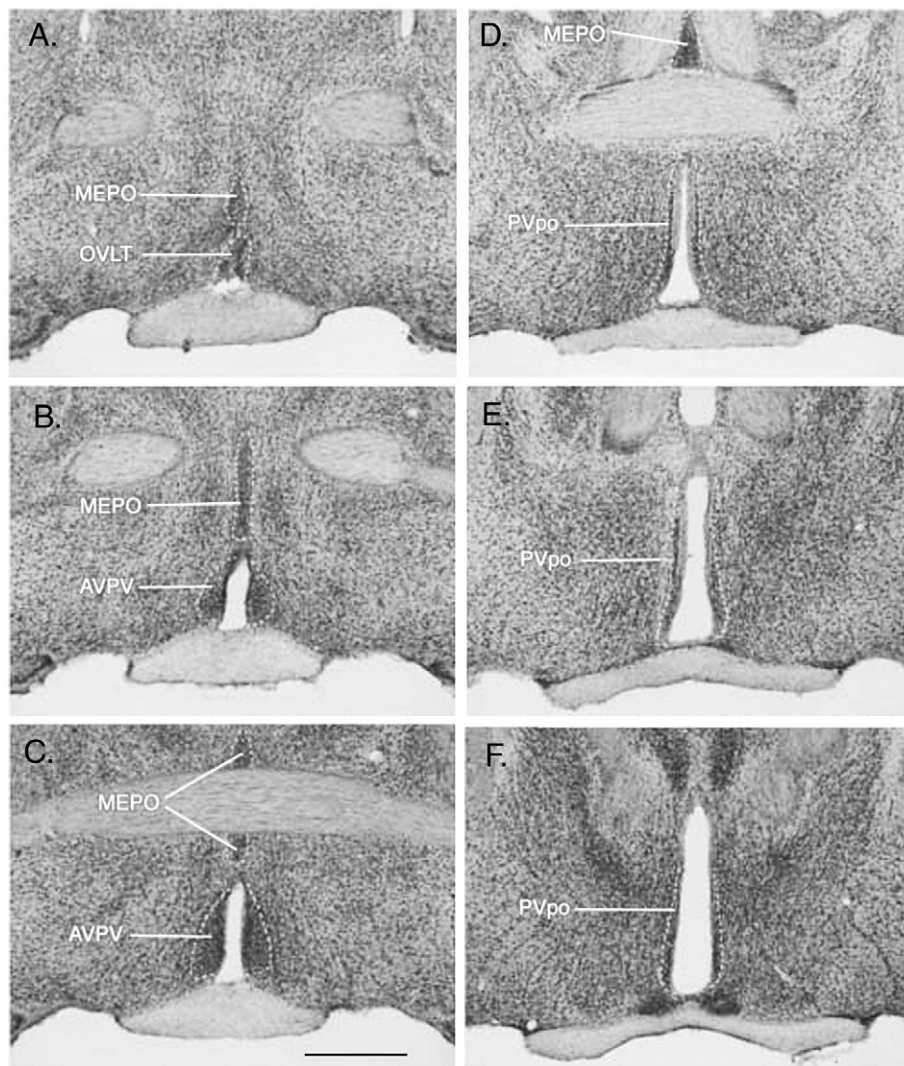


Fig. 4 – Nissl-stained coronal sections through the preoptic area of an adult female C57BL/6J mouse highlighting the three brain regions (anteroventral periventricular (AVPV), median preoptic (MEPO) and periventricular preoptic (PVpo) nuclei) in which ER-expressing neuronal afferents to GnRH neurons are found (RP3V neurons). The 40 μm -thick sections are arranged in rostral to caudal order (A–F) with 40 μm between sections. Scale bar=500 μm . OVLT, organum vasculosum of the lamina terminalis.

5.1. Glutamate

Glutamate receptor antagonists administered into the ventricular system of rats blocked the occurrence of the LH surge (Brann and Mahesh, 1991; Brann et al., 1993; Lopez et al., 1990) and glutamate levels within the vicinity of the GnRH neuron cell bodies, but not their terminals, increased at the time of the surge (Jarry et al., 1995; Ping et al., 1994). Although such studies have not addressed the RP3V glutamatergic cells themselves, the data are consistent with the hypothesis that estrogen activates these cells to stimulate the GnRH neurons. Dual-label immunocytochemical studies have shown that the numbers of glutamatergic inputs to GnRH neurons are likely to increase at the time of the surge (Ottem et al., 2004). Intriguingly, these glutamatergic inputs may be dual-phenotype GABA-glutamate terminals originating from the AVPV in which the synthesis of glutamate goes up and that of GABA goes down prior to the surge (Ottem et al., 2004).

5.2. GABA

There is a consistent set of data that show GABA levels within the general vicinity of the GnRH cell bodies rise in the morning and then fall precipitously in the afternoon just before the onset of the LH surge (Jarry et al., 1995, 1988; Mitsushima et al., 2002; Robinson et al., 1991; Tin Tin Win et al., 2004). Pharmacological studies with GABA_A receptor antagonists have further indicated that this fall in GABA release is necessary for the GnRH surge to proceed (Herbison and Dyer, 1991; Kimura and Jinnai, 1994; Seltzer and Donoso, 1992). These data are in agreement with the idea that a reduction in net inhibitory GABAergic input to the GnRH neurons (dis-inhibition) must occur to enable GnRH neuron activation to generate the surge. However, it is unclear how this afternoon decline in GABA release occurs, particularly as estrogen has been shown to exert a predominant stimulatory effect upon preoptic GABAergic neurons (Herbison, 1998; Mitsushima et al., 2002).

5.3. Kisspeptin

The recent discovery of the key importance of kisspeptin-GPR54 signaling in puberty (de Roux et al., 2003; Seminara et al., 2003) has led to the evaluation of its role in the GnRH surge mechanism. To date, the results of these studies look very promising and suggest that kisspeptin is a “high-order” component of the surge generating mechanism. Kisspeptin neurons are (i) located within the AVPV and PVpo (Clarkson and Herbison, 2006; Smith et al., 2006), (ii) exhibit a female-dominant, sexually dimorphic distribution (Clarkson and Herbison, 2006; Kauffman et al., 2007), (iii) express ER α (Smith et al., 2005) and (iv) are activated to express c-Fos at the time of estrogen positive feedback in both the rat (Smith et al., 2006) and mouse (Clarkson and Herbison, unpublished). Together with unpublished evidence that RP3V kisspeptin neurons expressing ER α are primary afferents to rPOA GnRH neurons (Campbell, Clarkson and Herbison), these observations provide a strong neuroanatomical case for kisspeptin in estrogen feedback. In addition, however, there is functional evidence that kisspeptin is required for the GnRH surge to occur (Kinoshita et al., 2005), that GnRH neurons express GPR54 (Irwig et al., 2004), and that kisspeptin is the most potent activator of GnRH neuron firing yet discovered (Han et al., 2005). Hence, it seems very likely that the rising follicular phase concentrations of estrogen stimulate directly RP3V kisspeptin neurons that, in turn, activate GnRH neurons to evoke the GnRH surge.

5.4. Neurotensin

The infusion of neurotensin into the preoptic area of the rat increases the magnitude of the LH surge (Akema et al., 1987; Ferris et al., 1984) whereas the administration of neurotensin antisera reduces the size of the LH surge (Alexander et al., 1989). Neither treatment influences the timing of the LH surge, indicating that neurotensin neurons are not involved in the circadian component of surge generation in the rat. Neurotensin neurons in the AVPV express ER α (Herbison and Theodosis, 1992b) and estrogen increases their biosynthesis of neurotensin (Alexander and Leeman, 1994; Alexander et al., 1989). As GnRH neurons were shown recently to express neurotensin receptors (Smith and Wise, 2001), it is possible that RP3V neurotensin neurons are stimulated by estrogen to help activate the GnRH neurons prior to the surge.

5.5. Molecular Mechanisms

Although progress has been made towards identifying the RP3V population of neurons and determining the phenotypes of neurons within it, little headway has been made in terms of understanding how estrogen modulates the excitability of RP3V neurons. What are the downstream gene targets for estrogen in RP3V neurons? The progesterone receptor (PR) is one possibility as estrogen clearly increases its expression in the AVPV and the receptor may then be modulated in a ligand-independent manner (Levine, 1997) or by astroglial-derived progesterone (Micevych et al., 2003, 2007). Although the role of ovarian-derived progesterone in positive feedback

is unclear, the antagonism or deletion of PRs interrupts the LH surge (Levine, 1997). For populations such as the neurotensin and kisspeptin neurons, there is evidence suggesting that the slowly increasing estradiol levels of the follicular phase act to gradually increase neuropeptide mRNA expression by these cells (Smith et al., 2006; Smith and Wise, 2001). However, the relationship of slowly increasing mRNA expression to an abrupt increase in neuropeptide secretion from terminals is not known. Whereas a circadian input might appear to be an attractive candidate underlying the sudden onset of the surge in rodents, it does not explain the equally abrupt onset of the GnRH surge in sheep and primates where circadian factors are of little or no consequence. These pieces of information illustrate the complexity of the molecular machinery that is likely to underlie the activation of RP3V neurons by estrogen. Indeed, it seems almost certain that estrogen will regulate multiple genes involved in diverse cellular functions within individual RP3V neurons. The elucidation of these pathways and their interactions will represent a major step forward in our understanding of estrogen-regulated brain function and the generation of the GnRH/LH surge.

6. Summary

There is little doubt that the estrogen positive feedback signal responsible for driving rodent GnRH neurons into a surge mode of secretion results from the activation of ER α -expressing neuronal afferents to GnRH neurons (Fig. 2). Evidence for a role of AVPV ER α -expressing neurons in this pathway is now overwhelming. However, new findings, considered alongside a re-evaluation of older data, indicate that adjacent ER α -positive neurons in the PVpo and MEPO are also likely to be involved in this mechanism. While the AVPV may indeed be the central nucleus in the pathway, it remains that there is a continuum of ER α -expressing GnRH neuron afferents running the length of the rostral periventricular nuclei. As such, it is proposed that the topographic description of “AVPV, PVpo and MEPO estrogen-receptive afferents to GnRH neurons” be replaced by the functional neuroanatomical construct of “RP3V neurons” (Fig. 2).

The studies identifying the RP3V have been undertaken in rodents. Whether a similar neuroanatomical grouping exists for estrogen positive feedback to GnRH neurons in other species is not known. Certainly, studies in the ewe indicate that the key site of estrogen action in evoking the GnRH/LH surge is not within the preoptic area but located in the mediobasal hypothalamus (Blache et al., 1991; Caraty et al., 1997). Equally, the limited studies that have been undertaken in primates suggest that a mediobasal hypothalamic network is sufficient for positive feedback to occur (Ferin et al., 1974). Thus, it is very likely that the locations of the relevant estrogen-sensitive neurons in sheep and primates are different from those of the rodent. Nevertheless, available evidence in other species suggests that the positive feedback pathway is organized in a similar indirect manner requiring ER-expressing afferent inputs to GnRH neurons (see Herbison, 2006). Thus, it seems likely that RP3V-equivalent populations exist in other mammals and these await elucidation.

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