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Estrogenic regulation of the GnRH neuron

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Reproductive function is regulated by the secretion of luteinizing hormone (LH) and follicle-stimulating hormone from the pituitary and the steroid hormones from the gonads. The dynamic changes in the levels of the reproductive hormones regulate secondary sex characteristics, gametogenesis, cellular function, and behavior. Hypothalamic GnRH neurons, with cell bodies located in the basal hypothalamus, represent the final common pathway for neuronally derived signals to the pituitary. As such, they serve as integrators of a dizzying array of signals including sensory inputs mediating information about circadian, seasonal, behavioral, pheromonal, and emotional cues. Additionally, information about peripheral physiological function may also be included in the integrative signal to the GnRH neuron. These signals may communicate information about metabolic status, disease, or infection. Gonadal steroid hormones arguably exert the most important effects on GnRH neuronal function. In both males and females, the gonadal steroid hormones exert negative feedback regulation on axis activity at both the level of the pituitary and the hypothalamus. These negative feedback loops regulate homeostasis of steroid hormone levels. In females, a cyclic reversal of estrogen feedback produces a positive feedback loop at both the hypothalamic and pituitary levels. Central positive feedback results in a dramatic increase in GnRH secretion (Moenter et al., 1992; Xia et al., 1992; Clarke, 1993; Sisk et al., 2001). This is coupled with an increase in pituitary sensitivity to GnRH (Savoy-Moore et al., 1980; Turzillo et al., 1995), which produces the massive surge in secretion of LH that triggers ovulation. While feedback regulation of the axis in males is in part mediated by estrogen receptors (ER), there is not a clear consensus as to the relative role of ER versus AR signaling in males (Lindzey et al., 1998; Wersinger et al., 1999). Therefore, this review will focus on estrogenic signaling in the female.

Keywords: GnRH, kisspeptin, GPR54, estrogen receptor, feedback, progesterone receptor, arcuate, AVPV

EVIDENCE FOR REGULATION OF GnRH GENE EXPRESSION BY ESTROGEN

Estrogen has a bimodal effect on the hypothalamus with both an inhibitory and stimulatory influence on GnRH secretion. The stimulatory effect of estrogen is seen at the end of the follicular phase where estrogen triggers the preovulatory GnRH surge (Sarkar et al., 1976; Karsch et al., 1997; Levine, 1997; Herbison, 1998; Simerly, 2002). The ability of estrogen to induce a surge declines with age in female rodents and women (Wise, 1982; Shaw et al., 2011) This appears to be due in large part to a decrease in estrogen induction of GnRH neurons, particularly in rodents (Downs and Wise, 2009), although a decline in pituitary responsiveness to GnRH contributing to attenuated luteinizing hormone (LH) surges has also been proposed (Shaw et al., 2009).

The inhibitory effect of estrogen on GnRH secretion and GnRH gene expression has been shown in *in vivo* studies in several mammalian species (Zoeller et al., 1988; Petersen et al., 1995). Studies in ewes indicate that estrogen inhibits GnRH pulse amplitude in the early follicular and luteal phase of the cycle (Caraty et al., 1989; Chongthammakun and Terasawa, 1993). *In vivo* and *in vitro* studies in the rat hypothalamus (Sarkar and Fink, 1980; Spratt and Herbison, 1997) indicate that estrogen inhibits GnRH mRNA expression and that this effect is localized to the rostral preoptic area of the hypothalamus. The inhibitory effect of estrogen seems to involve different anatomical sites in the hypothalamus than those associated with the stimulatory effect of estrogen on GnRH (Shander and Barraclough, 1980; Wiegand et al., 1980; Wray et al., 1989; Gibson et al., 1997; Caraty et al., 1998), which had indicated that the inhibitory and stimulatory effects may occur independently from one another. Negative feedback was localized to the arcuate and median eminence of the medial basal hypothalamus in these studies while positive feedback was mapped to the preoptic and suprachiasmatic nucleus. It is proposed that the biological substrate for these effects is kisspeptin, the hypothalamic protein previously found to be essential in pubertal onset (de Roux et al., 2003; Seminara et al., 2003). Estrogen has been shown to mediate a decrease in kisspeptin in the arcuate nucleus in contrast to increasing expression in the AVPV (Discussed below: Smith et al., 2006; Dungan et al., 2007).

In vitro evidence of negative estrogen regulation of rat GnRH gene expression includes transfection studies in both placental JEG-3 cells (Wierman et al., 1992) and in GT1-7 GnRH-expressing neuronal cells co-transfected with the estrogen receptor alpha (ER)α cDNA (Kepa et al., 1992). These *in vitro* studies indicated that estrogen decreases expression of the rat GnRH gene promoter. Studies of the-human GnRH promoter in transient transfection

experiments in JEG-3 cells co-transfected with ERα cDNA indicate estrogen-mediated regulation of the-human GnRH promoter (Radovick et al., 1991; Dong et al., 1996). Studies performed by Roy et al. (1999) demonstrated a decrease in GnRH mRNA levels in the GnRH-expressing neuronal cell line, GT1-7, treated with 17β-estradiol over a 48-h time course, starting as early as 12 h. Our laboratory has confirmed and extended these findings and observed a decrease in GnRH expression and secretion by estradiol in both the GN11 and GT1-7 GnRH-expressing cell lines and determined that these effects were primarily mediated by ERα in GT1-7 cells and by both ERα and ERβ in GN11 cells (Ng et al., 2009).

In addition, we have shown that estrogen down-regulates GnRH gene expression in a castration–estrogen replacement paradigm using a transgenic mouse model. This model was developed by targeting GnRH neurons with construct containing the GnRH gene promoter fused to a luciferase reporter gene (Radovick et al., 1994; Wolfe et al., 1995; Kim et al., 2007; −3446/+5-luc mice). These GnRH promoter elements were shown to specifically and reproducibly target hypothalamic GnRH neurons in transgenic mice. After treatment with estradiol, gonadectomized female mice exhibited an 80% reduction in hypothalamic luciferase expression. Although these *in vivo* studies do not prove that ER directly regulates GnRH expression in the hypothalamus, they do establish that GnRH gene expression is negatively regulated by estrogen at a transcriptional level.

THE ESTROGEN RECEPTOR

Estrogen is known to exert its effect through binding and activation of ERs. The ER is a member of the superfamily of nuclear receptors (Beato et al., 1995; Mangelsdorf et al., 1995), and is involved in transcriptional regulation of target genes. Two isoforms of ER (ERα and ERβ), with variable tissue distribution, have been described (White et al., 1987; Couse et al., 1997; Tremblay et al., 1997). ER isoforms share a common structural organization that includes six distinct functional domains, A to F, characteristic of members of the superfamily of nuclear receptors (Beato et al., 1995; Mangelsdorf et al., 1995). The A/B domain located at the N-terminal portion of the receptor contains a weak constitutive ligand-independent transcription activating function-l (AF-1); the C domain contains zinc finger-like motifs that are involved in binding to an estrogen response element (ERE); the D domain contains a hinge region that modulates DNA-binding; and the E/F domains contain the ligand-binding domain and a strong ligand-dependent activating function-2 (AF-2). Mouse ERα and ERβ share considerable homology in their DNA and ligand-binding domains (97 and 60% respectively), but display no sequence homology in their amino terminal domains (Tremblay et al., 1997). Mouse ERα has a slightly higher affinity for binding to a consensus ERE when compared to ERβ (Tremblay et al., 1997). Both ER isoforms, however, activate natural estrogen-responsive promoters to a similar extent in transient transfection studies.

THE CLASSICAL ER SIGNALING PATHWAY

The classic ER signaling pathway involves estrogen binding to ERs (Katzenellenbogen et al., 1993; Beato et al., 1995; Mangelsdorf et al., 1995) inducing a conformational change leading to receptor dimerization (Wang et al., 1995; Pettersson et al., 1997; Ogawa et al., 1998) and subsequent binding to an ERE located on promoter regions of target genes (Beato et al., 1995; Mangelsdorf et al., 1995). Binding of the ER complex to DNA activates gene transcription through its activating function domains, AF-1 and AF-2. AF-l and AF-2 can act independently, or synergize with one another to stimulate positive regulation of gene transcription (Tora et al., 1989; Berry et al., 1990). The ability of AF-1 and AF-2 to contribute to ER transcriptional activity seems to be cell- and promoter-specific (Tora et al., 1989; Tzukerman et al., 1994), and involves binding of AF-l and/or AF-2 to cofactors (Tzukerman et al., 1994; Shibata et al., 1997; Tremblay et al., 1999). Co-activators of ER include a family of related proteins known as the p160s: the SRC family, pCIP, and others (Onate et al., 1995; Horwitz et al., 1996; McKenna et al., 1999; Xu and O'Malley, 2002; Smith and O'Malley, 2004); and recruitment of p160 cofactors is sufficient for activation of the ER (Shang et al., 2000). p160s, in turn, interact with other co-activator proteins such as CREB-binding protein (CBP; Chakravarti et al., 1996), p300, and CBP-associated factor (P/CAF; Korzus et al., 1998). Other ER co-activators include RIP140 (Cavailles et al., 1995). Co-repressors of ER have also been described, including SHP (Seol et al., 1998; Johansson et al., 1999), NcoR (Lavinsky et al., 1998), and SMRT (Misiti et al., 1998).

THE RELATIVE ROLES OF ERα AND ERβ AND KISSPEPTIN IN THE CONTROL OF THE REPRODUCTIVE AXIS

The recent description of a patient with idiopathic hypogonadotropic hypogonadism with a mutation in a G-protein coupled receptor, referred to as GPR54, has added to our knowledge of signaling pathways involved in this complex system (de Roux et al., 2003; Seminara et al., 2003). GPR54 binds kisspeptin (kiss1) to regulate GnRH secretion (Kotani et al., 2001; Smith et al., 2005, 2006; d'Anglemont de Tassigny et al., 2008). Several studies have confirmed that GPR54 co-localizes with GnRH neurons (Irwig et al., 2004; Parhar et al., 2004; Han et al., 2005; Messager et al., 2005; Novaira et al., 2009). Central or systemic administration of kiss1 leads to GnRH and gonadotropin secretion in both prepubertal and adult animals (Gottsch et al., 2004; Matsui et al., 2004; Messager et al., 2005; Navarro et al., 2005; Shahab et al., 2005; Zhang et al., 2008). Confirming that kiss1 lies upstream of the GnRH neuron, mice who have a knock-out of GPR54, as well as humans that have mutations in GPR54, respond normally to GnRH.

In rats, ER isoform selective ligands have provided evidence that ERα is the predominant receptor isoform mediating negative feedback (Sanchez-Criado et al., 2006). However E2 is known to regulate gene transcription by binding to high affinity receptor dimers and by facilitating dimer formation between ERs (Katzenellenbogen et al., 1993). In fact, ERα and ERβ can form heterodimers in tissues where they are co-expressed (Pace et al., 1997; Powell et al., 2010) and the DBD was sufficient to allow for heterodimerization (Pace et al., 1997). This might explain the higher LH levels observed in ERα and ERβ double gene knock-out (KO) mice compared to ERα KO mice (Couse et al., 2003). The studies demonstrating the key role for ERα in negative feedback build on previous studies demonstrating an important role for kisspeptin for LH secretion during the entire estrous cycle despite dynamic changes in estrogen levels (Roa et al., 2006). Kiss1 neurons are activated during

the preovulatory period (Smith et al., 2006), and blockade of kiss1 secretion results in a lack of LH surge generation (Kinoshita et al., 2005). Two principal populations of kiss1 neurons are described in the hypothalamus; one in the arcuate nucleus and one in the AVPV which match well with the anatomical loci of estrogen positive and negative feedback discussed above (model is summarized in **Figure 1**). The arcuate population has been proposed to mediate estrogen negative feedback while the specific subset of neurons in the AVPV have been shown to be critical intermediates in transducing estradiol positive feedback required to induce ovulation (Popolow et al., 1981; Wiegand and Terasawa, 1982; Simerly and Swanson, 1987; Gu and Simerly, 1997). Within the AVPV of female mice, *Kiss1*-expressing neurons are activated by estrogen (Smith et al., 2005, 2006). Interestingly, perinatal treatment with the environmental estrogenic compound bisphenol A (BPA) produces males with female-like development of the kisspeptin neurons in the AVPV that correlates with the ability of BPA treated male mice to generate LH surges in response to high levels of estrogen (Bai et al., 2011). Kiss1 neurons have been shown to express ERα (and some ERβ), and kiss1 neurons in the AVPV secrete kiss1 in response to estrogen treatment. Since these neurons contact GnRH neurons, it has been proposed that kiss1 neurons in the AVPV are the locus of estrogen positive feedback leading to the gonadotropin surge. This complements studies from Wintermantel et al. (2006) that have shown neuronal ERα was required for generation of the LH surge in mice. The identity of these AVPV neurons is not entirely clear. While some have proposed that both ARC and AVPC kiss1 neurons may be GABA neurons (Petersen et al., 2003; Cravo et al., 2011) or glutaminergic neurons (Cravo et al., 2011) recent compelling evidence exists that a subset of both the ARC and AVPV kiss1 neurons also express galanin (Porteous et al., 2011; Kallo et al., 2012) and that a large number of ARC kiss1, and even some AVPV kiss1 neurons, co-express neurokinin B and dynorphin (Navarro et al., 2009). Whether kisspeptin is permissive or active in regulating GnRH neurons at the time of the LH surge is unknown (Dungan et al., 2007). Nonetheless, GnRH neuronal activity is increased by kiss1 and modulated by changes in estradiol levels (Pielecka-Fortuna et al., 2008).

EVIDENCE FOR ERs IN THE GnRH NEURON

Although the presence of ERs in the gonadotroph is well accepted and hence estrogen action on the gonadotroph is direct. The mechanism by which estrogen regulates GnRH neurons is not well understood. The more prevalent hypothesis is that the influence of estrogen on GnRH is not direct but is conveyed to GnRH neurons via presynaptic afferents from adjacent cells that express ER (now thought to be kiss1 neurons). This hypothesis is based on several *in vivo* double-labeling immunohistochemical studies examining the hypothalamus of many mammalian species where co-expression of GnRH and ER was not demonstrated (Shivers et al., 1983; Herbison and Theodosis, 1992; Watson et al., 1992; Herbison et al., 1993, 1995; Kalra, 1993; Lehman and Karsch, 1993; Navas et al., 1995; Sullivan et al., 1995; Lopez et al., 1996; Warembourg et al., 1998; Kelly et al., 2003; Garcia-Segura and McCarthy, 2004). Results from these studies indicate that, although ERs are highly expressed in the hypothalamus, few if any GnRH neurons express ER. Additionally then, the effects of estrogen on GnRH neurons would occur in an indirect manner by ERα and/or ERβ expressing neurons, or glial cells (Rage et al., 1997; Smith and Jennes, 2001; Prevot, 2002; Petersen et al., 2003). However, the original studies may have been hampered by an inability to detect a lower concentration of ERs in tissues, or by the castration paradigms that were used, which could alter ER expression levels.

An accumulating body of *in vitro* evidence currently indicates the presence of functional ERs in GnRH neurons. A number of these studies have used immortalized GnRH-expressing neuronal cell lines and have shown that they express ERα and/or ERβ (Shen et al., 1998; Butler et al., 1999; Roy et al., 1999; Hrabovszky et al., 2000; Kallo et al., 2001; Martinez-Morales et al., 2001) and respond to estrogen treatment by increasing galanin (Shen et al., 1998) and androgen receptor gene expression (Poletti et al., 1994). These studies show evidence for a direct estrogen effect on GnRH neurons, and suggest the presence of a functional ER signaling pathways in these neurons. ERβ may be the predominant ER in GnRH neurons (Hrabovszky et al., 2001; Kallo et al., 2001; Legan and Tsai, 2003; Petersen et al., 2003; Skinner and Dufourny, 2005); however, a separate immunohistochemistry study of female rats found ERα transcripts in GnRH neurons in the preoptic area (Butler et al., 1999). Prenatal GnRH neurons have been shown to express ERβ transcripts, however, the number of cells expressing ERβ decreased over time (Sharifi et al., 2002). These studies led to the most recent evidence in humans that ERβ colocalized with GnRH (Hrabovszky et al., 2007). With mounting evidence that

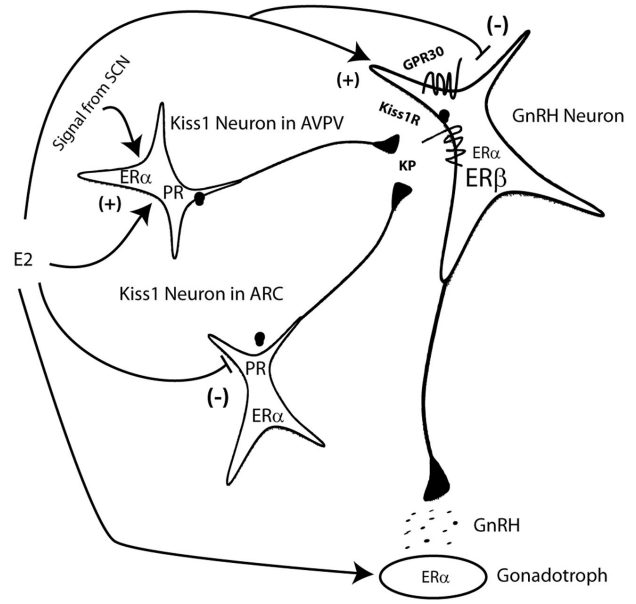

FIGURE 1 | Model of estrogenic regulation of the GnRH neuron. Estrogen (E2) from the ovary regulates the pituitary gonadotroph, the kiss1 neurons in the AVPV and the arcuate (ARC) nuclei and can also directly regulate the GnRH neuron. E2 regulation of kiss1 in both nuclei is regulated by ERα and progesterone receptor (PR). A daily signal from the SCN to the kiss1 neurons of the AVPV helps coordinate surge generation. Direct effects at the level of the GnRH neuron can be mediated by ERα, ERβ, or the membrane associated GPR30. Kiss1 (KP) regulates GnRH neurons by binding to and activating the kiss1 receptor (also known as GPR54).

GnRH neurons contain ERs, the functional significance remains to be determined as well as the intracellular signaling processes used. However, a direct, transcription-dependent mechanism on GnRH has been shown (Temple et al., 2004).

ESTROGEN RECEPTOR KNOCK-OUT PHENOTYPES

Studies from mice with generalized ERα disruption (ERαKO) indicate that ERα plays a major role in regulating the reproductive neuroendocrine–gonadal system (Lubahn et al., 1993; Couse et al., 1999; Schomberg et al., 1999; Dupont et al., 2000). ERα is widely expressed throughout the reproductive axis, including the hypothalamus, pituitary, gonads (both ovaries and testis), and uterus. Therefore, a generalized disruption of ERα would be predicted to affect the axis at various levels centrally and peripherally (Lubahn et al., 1993; Schomberg et al., 1999; Dupont et al., 2000), rendering the isolation of the effect of estrogen at any level impossible. Homozygous ERα KO mice display significant gonadal defects and impaired feedback regulation of the neuroendocrine axis. Homozygous ERα KO females were also infertile and were found to have hypoplastic uteri, large hemorrhagic cysts, and absent corpus luteum in their ovaries (Couse et al., 1995; Korach et al., 1996; Schomberg et al., 1999; Dupont et al., 2000).

In addition, studies in homozygous ERα KO females indicate that they exhibit 10-fold higher serum estrogen level than wild type controls. LH levels were also significantly increased in intact ERαKO females compared to those in WT females (Lindzey et al., 1998; Wersinger et al., 1999). Estrogen feedback regulation is thought to occur both at the level of the hypothalamus and at the level of the pituitary. Thus, elevated LH levels may indirectly reflect elevated GnRH levels due to loss of negative estrogen regulation at the level of the hypothalamus or may also reflect loss of negative estrogen regulation at the level of the pituitary. Similarly, LH and follicle-stimulating hormone (FSH) mRNA levels were higher in intact ERα KO compared to those in intact WT females, and were comparable to LH and FSH mRNA levels in ovariectomized, non-replaced WT females (Scully et al., 1997; Lindzey et al., 1998). These results suggest a role for estrogen in negative regulation of LH and FSH, but do not localize the effect to the level of the hypothalamus, and/or the pituitary.

Initial studies of ERβKO mice failed to identify a significant reproductive phenotype. However, it was noted later that ER knock-out mice produced by insertion of an ER allele containing a neomycin resistance gene could produce a chimeric protein that is partially estrogen-responsive (Couse et al., 1995; Antal et al., 2008; Chen et al., 2009). Complete ERβKO mice demonstrated subfertility (Krege et al., 1998; Dupont et al., 2000; Antal et al., 2008) though gonadotropin levels appear to be unchanged compared to control mice. These data suggest that ERβ may not be important for central E2 negative feedback of the axis. However, gonadotropin levels are less elevated in ERαKO versus the combined ERαβ KO mice, indicating that ERβ may have a negative feedback role in the axis (Couse et al., 2000). Interestingly, the role of ERβ may be to increase progesterone mediated effects at the level of the hypothalamus to produce the gonadotropin surge (Chappell and Levine, 2000).

Additional studies in ERαKO mice indicated a role for ERα in the positive regulation of LH secretion by the pituitary and inferred that negative regulation occurs at the hypothalamic level (Lindzey et al., 2006). Recently, ERα expression in neuronal cells was shown to be required for positive feedback of estradiol (Wintermantel et al., 2006). Furthermore, a pituitary-specific ERα KO mouse model, although being infertile, was shown to have normal levels of LH, suggesting that positive feedback regulation was impaired but negative regulation by estrogen was intact (Gieske et al., 2008). In contrast, we also generated a pituitary-specific KO of ERα and find elevated serum LH levels (Singh et al., 2009). It is not clear why these two models differ in regards to LH levels and may reflect differences in sampling or LH assay methodologies.

NON-CLASSICAL ER SIGNALING PATHWAYS

In the classical, or genomic pathway, ERs bind to an ERE on DNA to alter the transcription of genes (O'Malley and Tsai, 1992; Arbogast, 2008). However, ERα has been shown to signal through ERE-independent, non-classical, as well as non-genomic pathways (McEwen and Alves, 1999; Kousteni et al., 2001) through protein–protein interactions by tethering to other transcription factors such as AP-1 and NF-kB and the transcriptional complex (Stein and Yang, 1995; Ray et al., 1997; Kushner et al., 2000; Pedram et al., 2002). Furthermore, ERα has been shown to display antagonistic effects on ERα mediated transcription by interfering with recruitment of an AP-1 protein complex on DNA (Matthews et al., 2006). ER mediated, ligand-dependent signaling has been shown to involve cross-talk with growth factor mediated pathways through phosphorylation by MAPK activation (Kato et al., 1995; Couse and Korach, 1999; Feng et al., 2001; Masuhiro et al., 2005), and ERα has also been shown to induce rapid membrane-initiated signaling (Levin, 2005; Revankar et al., 2005; Pedram et al., 2006; Vasudevan and Pfaff, 2007).

Non-genomic effects of estradiol have been reported in several studies. For example, estradiol increased the phosphorylation of cAMP response element binding protein (CREB) in GnRH neurons (Abraham et al., 2003) as well as calcium oscillations (Abe et al., 2008) and potassium currents (DeFazio and Moenter, 2002; Farkas et al., 2007; Roepke et al., 2007; Zhang et al., 2007). Immunocytochemical localization was performed for ER isoforms in GT1-7 cells, localizing both receptors at the cell membrane to some degree (Navarro et al., 2003) and providing some evidence that the ERα isoform was responsible for norepinephrine responsiveness (Morales et al., 2007). Rapid signaling effects of estradiol have also been linked in hypothalamic neurons to activated protein kinase C pathways (Qiu et al., 2003). A membrane ER that is distinct from either ERα or ERβ has recently been identified. The GPR30 orphan receptor, a member of the G-protein coupled family of receptors, has been identified as a putative ER. GPR30 located in the endoplasmic reticulum, Golgi apparatus, and nuclear membranes have been shown to be activated by estradiol and other estrogen agonists (Filardo, 2002; Brailoiu et al., 2007; Prossnitz et al., 2008). GPR30 has been found in primate and mouse GnRH neurons (Noel et al., 2009; Sun et al., 2010) and is proposed to be located at the plasma membrane as membrane impermeable BSA conjugated E2 can exert rapid effects on GnRH function (Noel et al., 2009). Further complexity has recently been added with the report of a novel ER, acting as a G-protein coupled receptor in the hypothalamus (Qiu et al., 2008).

ROLE OF PROGESTERONE RECEPTORS (PR_A AND PR_B) IN THE POSITIVE FEEDBACK ACTIONS OF E_2

The preovulatory release of GnRH consists of a 2- to 4-h increase in the overall amount of GnRH secreted, occurring between 1600 and 2000 hours on the afternoon of proestrus (Levine and Ramirez, 1982). Classical neuroendocrine studies demonstrated that the CNS mechanisms that mediate the release of a GnRH surge require the integration of two obligatory signals – the preovulatory estrogen surge, and a daily neural signal that is synaptically conveyed from the circadian clock resident in the suprachiasmatic nucleus (Levine, 1997). The major action of estrogen is to "couple" the daily neural signal to the neuronal circuitries that mediate release of GnRH surges (Karsch and Foster, 1975; Legan and Karsch, 1975). In the absence of a sufficient elevation of estrogen, the daily signal is not communicated to neurons controlling the release of GnRH, and no surge takes place; with exposure of the hypothalamus to a sufficient estrogen stimulus, the pathways that convey the daily neural signal for the surge are rendered patent, and the GnRH is released into the hypophysial portal vasculature. At the same time, estrogen greatly enhances the responsiveness of the gonadotrophs to this burst of GnRH (Taga et al., 1982; Bauer-Dantoin et al., 1995; Shupnik, 1996). Both of these processes, along with the ability of GnRH to "self-prime" the estrogen-exposed pituitary to its own actions (Kamel et al., 1987) culminate in the release of a massive, but transient increase of LH on the afternoon of proestrus, which in turn triggers ovulation on the following morning of estrous.

How does estrogen couple the daily neuronal signal to the neurons responsible for the release of GnRH surges? One major locus of this integrative activity is the AVPV nucleus of the hypothalamus, where estrogen appears to activate ERα in neurons that receive afferents from the SCN and to project to GnRH neurons (possibly kiss1 neurons; Van der Beek et al., 1997; Tsukahara et al., 2008). We have examined the cellular actions of estrogen that may mediate these effects, focusing on the roles that PRs may play. Estrogen induces the expression of both isoforms of PR, PR_B, and the N-terminally truncated PR_A, in the AVPV, as well as other hypothalamic and preoptic nuclei. We have determined that induction of PRs is obligatory for the successful release of GnRH surges; thus, GnRH and LH surges are absent in ovarian intact and estrogen-treated PR gene knock-out (PRKO) mice (Chappell et al., 1997, 1999), and in rats treated with a progesterone receptor antagonist or intra-cerebroventricular PR antisense oligonucleotides (Chappell and Levine, 2000). We have proposed a model for the release of GnRH surges that includes (1) the induction of PRs by estrogen in AVPV neurons, (2) delivery of the daily neural signal from the SCN to AVPV neurons by neurotransmitter circuitries, and (3) the neurotransmitter-mediated activation of intracellular second messenger production that in turn transactivates the PRs in a ligand-independent manner; thereafter, (4) induces signals to kiss1 neurons that evoke the neurosecretion for the GnRH surge, which is (5) further amplified by ovarian progesterone release in response to the LH surge. The net result of all of these integrated physiological events is the release of a robust GnRH surge that is timed to trigger ovulation in concert with behavioral heat and maximal wakefulness, and uterine proliferation and differentiation thereby maximizing the chances for successful fertilization and implantation. The downstream targets of PR signaling that in turn initiate the GnRH surge process are not known, although we have provided evidence that one such target may be the neuropeptide Y (NPY) gene (Bauer-Dantoin et al., 1993). It is not clear how activated PRs may regulate NPY transcription, as there appear to be no classical palindromic PRE/GRE sites in the promoter of the preproNPY gene.

Estrogen's positive feedback actions also include a massive stimulation of pituitary responsiveness to GnRH stimulation. That E2-induced PR expression is obligatory for the manifestation of GnRH self-priming was demonstrated by the finding that this phenomenon is absent in PRKO animals (Chappell et al., 1999). The ability of progesterone to induce the GnRH self-priming response is rapid and depends upon mRNA and protein synthesis, but the cellular targets of PRs, whether activated by GnRH or progesterone, remain unclear.

Progesterone receptor in the ARC also likely contributes to negative feedback of the axis. Microimplants of the progesterone antagonist RU486 in the ARC, but not in the POA, blunt the negative feedback effects of progesterone in ewes (Goodman et al., 2011). The locus of action of the progesterone is likely the kisspeptin/dynorphin/neurokinin B expressing neurons in the arcuate (Goodman et al., 2004, 2011; Navarro et al., 2011).

CLASSICAL VERSUS NON-CLASSICAL PROGESTERONE RECEPTOR SIGNALING

Rapid effects of P have been documented in a variety of tissues, each potentially being mediated by one or more of several "non-classical" signaling mechanisms. Some of the rapid actions of P have been attributed to the ability of bound PR_A and PR_B to interact with the Src tyrosine kinase localized to the plasma membrane, which in turn prompts cellular responses via activation of the Src/Ras/Raf-1/MAPK signaling pathway (Faivre et al., 2008). At least three $PR_{A/B}$-independent pathways have also been identified that may mediate the effects of P in a variety of tissues and cell types. Progesterone is known to be rapidly metabolized in the brain to several neurosteroids, including allopregnanolone (3α-hydroxy-5α-pregnan-20-one; 3α5αTHP), which has been shown to modulate $GABA_A$ receptors in the brain (Majewska et al., 1986). Secondly, a progesterone membrane binding protein, progesterone receptor membrane component 1 (PGRMC1), has been suggested to mediate the ability of P to activate protein kinase G or other rapid signaling mechanisms in certain cells (Falkenstein et al., 1996).

The involvement of PRs in the positive feedback actions of E_2, nevertheless, is unequivocal, as both GnRH surges and GnRH self-priming mechanisms are inoperative in PRKO mice.

In summary, studies on estrogen effects on GnRH neuronal activity and secretion are wrought with complexity due to the various ER isoforms, direct versus indirect effects, classical versus non-classical estrogen signaling and the role of other hormones, such as testosterone (Eagleson et al., 2000; McGee et al., 2012) and progesterone, in modulating estrogen action on GnRH neurons. To overcome these difficulties, investigators have used a wide array of *in vitro* models, pharmacological tools, anatomical mapping, and transgenic and knock-out models. While these studies have helped clarify the role and mechanisms of estrogen action on GnRH neurons, many questions remain to be answered.

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