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Estrogen Signaling in Hypothalamic Circuits Controling Reproduction

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Abstract

It is well known that many of the actions of 17β-estradiol (E2) in the central nervous system are mediated via intracellular receptor/transcription factors that interact with steroid response elements on target genes. However, there is compelling evidence for membrane steroid receptors for estrogen in hypothalamic and other brain neurons. Yet, it is not well understood how estrogen signals via membrane receptors, and how these signals impact not only membrane excitability but also gene transcription in neurons that modulate GnRH neuronal excitability. Indeed, it has been known for sometime that E2 can rapidly alter neuronal activity within seconds, indicating that some cellular effects can occur via membrane delimited events. In addition, E2 can affect second messenger systems including calcium mobilization and a plethora of kinases to alter cell signaling. Therefore, this review will consider our current knowledge of rapid membrane-initiated and intracellular signaling by E2 in hypothalamic neurons critical for reproductive function.

Keywords

17β-estradiol (E2); estrogen receptor-α (ERα); membrane E2 receptor (mER); Proopiomelanocortin (POMC) and γ-aminobutyric acid (GABA) neurons

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It has been known for decades that 17β-estradiol (E2) has acute, rapid actions in CNS neurons including GnRH neurons (Kelly, et al., 1976; Lagrange, et al., 1995; Kelly and Rønnekleiv 2002; Kelly and Lagrange, 1998; Rønnekleiv and Kelly, 2005; Bryant, et al., 2006; Micevych and Mermelstein, 2008; Kelly and Rønnekleiv, 2009). Although the molecular mechanisms underlying these actions are still under intense investigation, it is now generally accepted that these rapid actions of E2 cannot be attributed to the classical nuclear-initiated steroid signaling of ERα or ERβ. One potential mode of rapid signaling is via ERα and ERβ signaling complexes within lipid rafts that activate multiple signaling pathways (Razandi, et al., 1999; Boulware, et al., 2005; Pedram, et al., 2006; Szegõ, et al., 2006; Dewing, et al., 2007; Micevych and Mermelstein, 2008). But it is also apparent that E2 can activate G protein-coupled receptors to modulate GnRH neuronal excitability through both pre- and postsynaptic mechanisms (Gu, et al., 1999; Toran-Allerand, 2004; Toran-Allerand, 2005; Qiu, et al., 2003; Qiu, et al., 2006b; Qiu, et al., 2008; Noel, et al.,

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2009; Zhang, et al., 2010). This review will concentrate on the rapid presynaptic actions since other chapters have focused on postsynaptic mechanisms of E2 signaling in GnRH neurons.

1. Rapid E2 signaling via a Gαq-mER in POMC and GABA neurons

POMC neurons are the “command” neurons of the hypothalamus. POMC neurons synapse directly on and modulate the excitability of neurosecretory neurons including GnRH, dopamine, corticotropin releasing hormone (CRH), oxytocin and vasopressin neurons (Khachaturian, et al., 1985; Zheng, et al., 2005). The opioid peptide β-endorphin (βEND), a major posttranslational product of POMC neurons, is released into the synaptic cleft and binds to the μ-opioid receptor postsynaptically to activate G protein-coupled, inwardly-rectifying K^+ channels (GIRK) in these neurosecretory neurons to inhibit their output (North, 1986). In women, naloxone infusion (i.v.) during the late follicular phase increases serum LH release and advances ovulation (Rossmanith, et al., 1988; Genazzani, et al., 1993). In fact, one of the the most efficacious treatments of hypothalamic amenorrhea is daily treatment with the opioid receptor antagonist naltrexone, which restores normal ovulatory cyclicity in these women (Wildt, et al., 1993). Therefore, there is evidence for tonic opioidergic control of GnRH neurons, which can go awry in pathological states. In addition to controlling neurosecretory neuronal activity, POMC (βEND) neurons also project to hypothalamic and extrahypothalamic nuclei to modulate motivated (natural reward) behaviors such as sex and maternal behavior (Koob, 1992).

So what are the physiological consequences of μ-opioid and $GABA_B$ receptor input to GnRH neurons? GnRH neurons express a number of channels that underlie burst firing activity and are only activated at membrane potentials below their resting membrane potential of −63 mV (Zhang, et al., 2007; Zhang, et al., 2009a). These channels include the hyperpolarization-activated non-selective cation channel (HCN1-4) and T-type calcium channels (Cav3.1-3.3) (see Rønnekleiv *et al.*, chapter). In order to reach potentials at which these channels are recruited, activation of K^+ (e.g., Kir) channels is critical (Hille 2001). GIRK channels expressed in GnRH neurons fulfill this role. A number of other neurotransmitters are also Gαi,o coupled to GIRK channels in GnRH and in melanin-concentrating hormone (MCH) and NPY neurons (Wu, et al., 2009; Xu, et al., 2009).

Interestingly, μ-opioid and $GABA_B$ receptors are coupled to the same population of GIRK channels in POMC, GABA, dopamine and GnRH neurons that produce a pronounced hyperpolarization when activated (Loose, et al., 1990; Kelly, et al., 1992; Lagrange, et al., 1994; Wagner, et al., 2001a; Zhang, et al., 2009b). However, in POMC and GABA neurons, which are presynaptic to GnRH neurons, a relative short (<20 min) exposure to E2 or BSA-E2 *in vitro* causes a four-fold decrease in the potency of μ-opioid and $GABA_B$ receptor agonists to inhibit those neurons through a heterologous desensitization via activation of a membrane estrogen receptor (mER) -initiated signaling cascade (Lagrange, et al., 1994; Lagrange, et al., 1996; Qiu, et al., 2003). Therefore, it is apparent that E2, via a putative mER-initiated signaling pathway, can rapidly increase POMC and GABA neuronal excitability that can subsequently hyperpolarize and recruit the channels underlying burst firing (e.g., T-type calcium channels) in GnRH neurons (Zhang, et al., 2009b).

Because of the critical role of POMC and GABA neurons in modulating neurosecretory (GnRH) neuronal activity (Lagrange, et al., 1995), a substantial effort has been exerted to elucidate the mER-mediated signaling pathway (s) using both sharp electrode intracellular and whole cell recording from guinea pig and mouse hypothalamic slices (Lagrange, et al., 1994; Lagrange, et al., 1997; Qiu, et al., 2003; Qiu, et al., 2006b). Indeed, these studies have established that E2 acts stereospecifically with a physiologically-relevant concentration

(EC_{50} = 8 nM) to attenuate the potency of μ-opioid and $GABA_B$ agonists in activating GIRK channels (Lagrange, et al., 1997; Qiu, et al., 2003). [Note: The EC_{50} is the concentration of agonist required to produce fifty percent of the maximum effect in an experimental preparation. The value is obtained from a theoretical curve fitted to experimental data points. The potency (i.e., EC_{50}) is dependent on the binding affinity, the efficacy of agonist, the receptor reserve in the tissue, and the ability of the agonist to penetrate to the site of action (Furchgott, 1978).] Furthermore, estrogenic modulation of μ-opioid and $GABA_B$ agonists potency is mimicked by stimulation of adenylyl cyclase with forskolin or by direct protein kinase A (PKA) activation with Sp-cAMP, in a concentration-dependent manner (Lagrange, et al., 1997; Qiu, et al., 2003). On the other hand, selective PKA antagonists such as KT5720 and Rp-cAMP can block the effects of E2. The activation of PKA is downstream in a signaling cascade that is initiated by a Gαq-coupled membrane ER that is linked to activation of phospholipase C (PLC)-protein kinase C (PKC)-PKA (Qiu, et al., 2003; Qiu, et al., 2006b). Hence, this receptor appears to be a Gαq-coupled mER. E2 does not compete for the μ-opioid (or $GABA_B$) receptor or alter the binding affinities of selective ligands with their receptors (Cunningham, et al., 1998). Most importantly, the ER antagonists ICI 164,384 and ICI 182,780 blocked the actions of E2 with subnanomolar affinity that is similar to ICI's affinity (K_i) for ERα (Weatherill, et al., 1988; Lagrange, et al., 1997), which is the *sine qua non* for establishing an ER-mediated mechanism. However although ICI antagonized this rapid response in POMC neurons, this Gq-coupled mER is not ERα or ERβ since it can be activated by a diphenylacrylamide compound, STX, that does not bind to ERα or ERβ (Qiu, et al., 2003; Qiu, et al., 2006b). STX (and the endogenous ligand E2) selectively target this Gαq-coupled PLC-PKC-PKA pathway in female guinea pig and mouse POMC neurons and can activate this Gαq-mER signaling pathway in mice deficient in ERα, ERβ and GPR30 (Qiu, et al., 2006b; Qiu, et al., 2008). However, identification (cloning) of the gene encoding the receptor is necessary for definitive characterization of this Gαq-mER.

2. Rapid E2 signaling in GnRH neurons via Gαq-mER

Besides the presynaptic actions of E2 to modulate POMC and GABA input onto GnRH neurons there are a number of direct effects of the steroid on GnRH neuronal excitability (see Rønnekleiv *et al*., chapter). However, one of the key actions that we identified some years ago was a rapid, direct hyperpolarization of guinea pig GnRH neurons by nanomolar concentrations of E2 via activation of inwardly rectifying K^+ channels (Kelly, et al., 1984; Condon, et al., 1989; Lagrange, et al., 1995). In addition to expression of Gαi,o receptors coupled to GIRK channels, GnRH neurons also express inwardly-rectifying K-ATP (Kir 6.2 and SUR1) channels (Zhang, et al., 2007). In fact, the K-ATP channel activity is increased by two-fold in E2-treated animals in synaptically isolated GnRH neurons (Zhang, et al., 2007). Recently, we have identified that both E2 and STX rapidly activate the Gαq-mediated signaling cascade, coupled to PLC-PKC-PKA signaling pathways, to activate K-ATP channels and hyperpolarize GnRH neurons (Zhang, et al., 2010). In addition to the synaptic activation of GIRK channels, this membrane hyperpolarization would also facilitate the recruitment of conductances critical for bursting activity (see Rønnekleiv *et al*., chapter).

3. Interaction of ER with Insulin-like Growth Factor-1 receptor and reproduction

Systemic administration of E2 in ovariectomized rats activates IGF-I receptors and induces the association between Insulin-like Growth Factor-1 (IGF-I) receptors and ERα in the hypothalamus (Quesada and Etgen, 2001; Cardona-Gómez, et al., 2002; Mendez, et al., 2003). Similar to what has been described in cortical neurons, there is an interaction (complex formation) between the p85 subunit of phosphatidylinositol 3-kinase (PI3K) and

ERα within 1–3 h, which leads to activation of protein kinase B/Akt, a serine/threonine kinase that has multiple downstream targets (Cardona-Gomez et al 2002, Mendez et al 2003) (Cardona-Gómez, et al., 2002; Mendez, et al., 2003). Also, the E2-induced activation of insulin growth factor 1 (IGF-I) receptors augments α1-adrenergic receptor signaling, which is important for reproductive functions (Quesada and Etgen, 2001). On the other hand, blockade of IGF-I receptors during E2 priming prevents E2-induced increases in α1-adrenergic receptor binding density as well as IGF-I enhancement of noradrenergic receptor signaling (Quesada and Etgen, 2002). Collectively, these findings support functional interactions between E2 and IGF-I. Therefore, these actions of E2 on the IGF-I receptor signaling pathway may be a key mechanism by which estrogen affects synaptic remodeling and neuronal plasticity during the estrous cycle. Moreover, intracerebroventricular (i.c.v.) infusion of JB-1, a selective competitive antagonist of IGF-1 autophosphorylation, inhibits the estrogen-induced LH surge and sexual behavior in ovariectomized rats (Quesada and Etgen, 2002). In addition, co-administration (i.c.v.) of inhibitors of PI3K (wortmannin) and mitogen-activated protein kinase (MAPK) (PD98059) inhibit the long-term (48 h) effects of E2 to induce the LH surge and facilitate lordosis behavior (Etgen and Acosta-Martinez, 2003). Therefore, facilitation of female sexual behavior by E2 appears to involve activation of both PI3K and MAPK signal transduction pathways. The importance of growth factors for female sexual behavior is further highlighted by observations that epidermal growth factor (EGF) and also IGF-I can, in the absence of estrogen and progesterone (within 1–4 h of i.c.v. administration), induce mating behavior in rats and mice, in part, through an ERα-dependent mechanism (Apostolakis, et al., 2000). This relatively rapid, ligand-independent ER action is in striking contrast to the well established finding that estrogen priming over a period of at least 24 h is needed for progesterone induction of female reproductive behavior (Etgen, et al., 2001). The cross-talk between estrogen signaling and membrane-initiated growth factor signaling in the hypothalamus is particularly interesting, although it is currently not well understood. The ability of both IGF-I and E2 to induce female sexual behavior may involve complex interactions between ERα, the IGF-1 receptor and the PI3K p85 subunit.

4. Cross-talk between ER and metabotropic glutamate receptors

In addition to ER cross talk with IGF-1 receptors, there is evidence for the interaction between ERα and metabotropic glutamate receptor 1a (mGluR1a) signaling in hippocampal and hypothalamic neurons (Boulware, et al., 2005; Dewing, et al., 2007; Micevych and Mermelstein, 2008). The cellular components were originally elucidated in hippocampal neurons and then subsequently studied in hypothalamic neurons (Micevych and Mermelstein, 2008). In hippocampal CA1 neuronal cultures, E2 rapidly stimulates MAPK-dependent cAMP-responsive element binding protein (CREB) phosphorylation (Boulware, et al., 2005). This effect of E2 is mimicked by the membrane-impermeable E2-BSA analog, blocked by ICI 182, 780 and inhibited by the selective mGluR1a antagonist LY367385 ((S)-(+)-α-amino-4carboxy-2-methlybenzeneacetic acid). Because transfection of hippocampal neurons with the mutant ERα abrogates the downstream activation of CREB while the ER agonist propylpyrazoletriol (PPT) stimulates CREB activation, this membrane-localized receptor in the hippocampus is thought to be ERα (Harrington, et al., 2003; Boulware, et al., 2005; Boulware, et al., 2007).

A similar scenario has been proposed for the rapid E2-induced activation and internalization of μ-opioid receptors in the medial preoptic area (mPOA) associated with induction of female sex behavior (Dewing, et al., 2007; Bondar, et al., 2009). The Micevych lab has elucidated a series of events critical for sexual receptivity in the female rodent (Micevych, et al., 2003; Mills, et al., 2004). Sexual receptivity appears to be dependent on NPY Y1 receptor activation of arcuate POMC neurons that project to the medial preoptic area

(mPOA), further highlighting the critical role of POMC neurons in reproduction (Mills, et al., 2004). Excitation of POMC neurons releases β-endorphin in the mPOA that upon binding to μ-opioid receptors causes activation, homologous desensitization and internalization (Arttamangkul, et al., 2000). 17β-estradiol and E2-conjugated to biotin (to limit membrane permeability) infused into the arcuate nucleus will initiate μ-opioid receptor activation and internalization in the mPOA and induction of full sexual receptivity in 17β-estradiol (benzoate)-primed, ovariectomized females (Dewing, et al., 2007). These actions of E2 are antagonized by ICI 182,780 and by a mGluR1a antagonist infused into the arcuate nucleus preceding E2 application (Dewing, et al., 2007). Thus, there appears to be an ER/mGluR1 signaling complex coupled to PKCθ that is responsible for the membrane-initiated E2 signaling involved in reproductive behavior (Dewing, et al., 2008). Although ERα and mGluR1a have been co-immunoprecipitated from transfected HEK cells, (Dewing, et al., 2007), the signaling complex has not been fully elucidated, but it is thought to require caveolin proteins (Boulware et al., 2007).

In other studies, E2 has been shown to rapidly activate multiple intracellular kinase cascades including MAPK, PI3K, PKA and PKC pathways relevant to reproduction (Gu, et al., 1996; Watters, et al., 1997; Bi, et al., 2001; Cato, et al., 2002; Yang, et al., 2003). In fact, the time course (15–30 min) is congruent with a membrane-initiated signaling cascade as has been revealed with electrophysiological studies (Lagrange, et al., 1994; Lagrange, et al., 1997; Qiu, et al., 2003; Qiu, et al., 2006b). In hypothalamic (mouse) GnRH neurons, the rapid phosphorylation of CREB, as measured by immunostaining of pCREB following E2 treatment, is observed within 15 min, which fits with the downstream signaling of a Gαq-mER initiated pathway in GnRH neurons (Abraham, et al., 2003; Zhang, et al., 2010). Therefore, there are multiple signaling pathways rapidly activated by E2 in arcuate (POMC) and preoptic (GABA) neurons that can modulate reproductive function.

5. Cross-talk between Gαq-mER and leptin receptor

Leptin plays a key role in energy homeostasis and reproduction and has an important role in the reproductive adaptation to starvation (Chan and Mantzoros, 2005). Serum concentrations of leptin convey nutrient information to the hypothalamic-pituitary gonadal axis (Chan and Mantzoros, 2005), and mutations in leptin or its receptor are associated with profound metabolic and physiological abnormalities such as obesity and infertility (Montez, et al., 2005). Leptin signals via its cognate receptors, leptin receptors (LRs), and there are several isoforms as a result of alternate splicing (Myers, Jr., 2004). The long isoform (LRb) is expressed abundantly in the hypothalamic arcuate, ventromedial and dorsomedial nuclei, and it is the predominant signaling form of the receptor (Bjørbæk, et al., 1997; Elmquist, et al., 1998). In fact, LRb has been localized in POMC, NPY and kisspeptin neurons but not in GnRH neurons (Håkansson, et al., 1998; Meister and Håkansson, 2001; Smith, et al., 2006; Quennell, et al., 2009). Leptin binding to its receptor causes activation of (phosphorylation) Janus Kinase-2 (Jak2) that signals via several pathways. Auto-phosphorylation of LRb allows the docking and subsequent activation of signal transducer and activator of transcription 3 (STAT3), which turns on transcription (Fig. 1) (Bates, et al., 2008; Gao, et al., 2004). The major metabolic effects of leptin are believed to be mediated by STAT3 because neuronal depletion of STAT3 results in an obese phenotype similar to leptin receptor deficient mice (Bates, et al., 2008; Gao, et al., 2004). In addition, the anorexigenic effects of E2 are thought to depend, in part, on STAT3 signaling in POMC neurons since the anorectic effects of E2 are abrogated by STAT3 knockout in female mice (Gao, et al., 2006). However, the PI3K pathway, also activated by LRb, is important for the excitatory actions of leptin since in POMC neurons the leptin-mediated depolarization/excitation is abolished by PI3K inhibition (Hill, et al., 2008; Qiu, et al., 2010). Activation of PI3K generates phosphatidylinositol-3,4,5-triphosphate (PIP_3), which appears to contribute to the

translocation and activation of PLCγ at the plasma membrane (Fig. 1) (Bae, et al., 1998). Recently, we have identified a potential point of convergence between the Gαq-mER and leptin receptor (LRb) signaling in POMC neurons (Fig. 1) (Qiu, et al., 2010). PI3K and associated proteins are targets for gene regulation by both E2 and STX (Malyala, et al., 2008). 17β-estradiol up-regulates PI3K p85α expression in the dorsomedial portion of the ventromedial hypothalamic nuclei and PI3K p55γ expression in the arcuate nucleus (Malyala, et al., 2008). STX increases the expression of phosphatidylinositol transfer protein β (PITPβ) in the arcuate nucleus (Roepke, et al., 2008). PITPβ transports lipids (phosphatidylinositols) from their site of synthesis (endoplasmic reticulum) to the cellular membrane where they are the preferred substrates for the lipid kinases such as PI3K (Cockcroft and Carvou, 2007). PITPβ activity is required for PI3K signaling and is also necessary for PLC-mediated signaling (Thomas, et al., 1993). In addition, activation of GIRK channels requires permissive levels of membrane phosphatidylinositol (4,5) biphosphate (PIP_2), which are controlled by all of these enzymes. GIRK channel activity is enhanced by Gβγ-mediated stabilization of PIP_2-GIRK binding (Huang, et al., 1998; Zhang, et al., 1999). Since PI3K- and PLC-mediated signaling are implicated in the membrane-mediated effects of E2, leptin and insulin, changes in the expression of PI3K subunits and transfer proteins may be another indirect mechanism for E2 to augment the excitability of POMC neurons. Therefore, since LRb is expressed in POMC and kisspeptin neurons but not in GnRH neurons (Balthasar, et al., 2004; Smith, et al., 2006; Qiu, et al., 2010), the cross-talk between leptin and E2 to regulate fertility are probably mediated by synaptic inputs onto GnRH neurons via POMC and kisspeptin neurons (Smith, et al., 2006; Gottsch, et al., 2009; Qiu, et al., 2010). Indeed, we have identified the direct excitatory effects of leptin on guinea pig kisspeptin neurons (Qiu et al, *unpublished findings*).

6. E2 signaling via Gαq-mER leads to new gene transcription

More recently, multiple studies have documented the cross-talk between rapid membrane-initiated and long-term nuclear-initiated steroid actions (Wagner, et al., 2001b; Vasudevan, et al., 2001; Kow and Pfaff, 2004; Malyala, et al., 2005; Qiu, et al., 2006b; Qiu, et al., 2006a; Roepke, et al., 2007). For example, it has been found that both acute effects of E2 and the transcriptional changes alter excitability of hypothalamic neurons (Kelly, et al., 2003; Malyala, et al., 2005; Qiu, et al., 2006a). In addition, the E2-induced membrane actions in the ventromedial nucleus (VMH) of the hypothalamus can potentiate its genomic effects on lordosis behavior (Kow and Pfaff, 2004). Moreover, this membrane effect in the VMH appears to be mediated by signaling pathways involving PKC and PKA (Kow and Pfaff, 2004). These findings are particularly intriguing in view of other findings that Gq-mER is coupled to PLC, PKCδ and PKA in POMC/GABA neurons (Qiu, et al., 2003; Qiu, et al., 2006b). Ultimately, E2 via both nuclear-initiated (ERα/β) and membrane-initiated signaling (Gq-mER) regulates the expression of a plethora of channels, channel binding proteins, signaling molecules and neuropeptides, as determined by custom gene microarray analysis of arcuate tissue from E2- and STX-treated female guinea pigs coupled with quantitative real-time PCR (Malyala, et al., 2004; Malyala, et al., 2005; Roepke, et al., 2008). The analysis of gene expression with STX has been used to evaluate the membrane-initiated versus membrane- and nuclear-initiated signaling of E2.

Numerous channels and signaling molecules that are involved in the modulation of channel activity are transcriptional target for ERα, ERβ and Gαq-mER receptors (Malyala, et al., 2005; Roepke, et al., 2007; Roepke, et al., 2008). 17β-estradiol treatment induces the expression of the Ca^{2+} channel subunit Cav3.1 in the arcuate nucleus of ovariectomized female guinea pigs (Roepke, et al., 2008), which increases the peak T-type Ca^{2+} current by two-fold in arcuate (POMC/GABA) neurons (Qiu, et al., 2006a). Furthermore, the E2-induced Cav3.1 mRNA expression in the arcuate is abrogated in αERKO mice (Bosch, et al.,

2009), indicating that the expression is under the control of an ERα-dependent mechanism. However, STX also up-regulates the Cav3.1 subunit in arcuate neurons in ovariectomized female guinea pigs (Roepke, et al., 2008), which would suggest that the Gq-mER and ERα-mediated transcriptional effects converge on the Cav3.1 gene (pCREB and ERE sites, Fig. 1). The increase in the T-type Ca^{2+} current augments burst firing and increases neurotransmitter (e.g., β-endorphin) release since burst firing of hypothalamic neurons is facilitated by the actions of T-type Ca^{2+} channels (Erickson, et al., 1993). Ultimately, E2 up-regulates the expression of β-endorphin in POMC neurons in ovariectomized female guinea pigs (Thornton, et al., 1994; Bethea, et al., 1995) and this increase is correlated with the increased expression of POMC mRNA (Roepke, et al., 2008). The increased POMC (and GABA) neuronal input would provide a hyperpolarizing stimulus via activation of GIRK channels in GnRH neurons (see Section 1).

Also, genes involved in calcium signaling pathways are regulated by both E2 and STX treatment. Long term (24 h and longer) treatment with E2 increases the expression of calmodulin-1, but STX treatment increases calmodulin-dependent kinase, CaM kinase II (CaMKII) in the arcuate nucleus. CaMK II is a modulator of multiple ion channels (Ca^{2+}, K^{+}, Na^{+}) and is required for the Ca^{2+}-sensitive production of long-term potentiation (LTP) in hippocampal and hypothalamic neurons (Fukunaga, et al., 2002; Pitt, 2007). The regulation of these calcium signaling molecules may have multiple effects on gene expression, neuronal excitability and synaptic neurotransmitter release. Finally, E2 down-regulates A-kinase anchoring protein (AKAP) 11 (aka AKAP220) expression, but STX increases the expression of AKAP11 in the arcuate nucleus (Roepke, et al., 2007; Roepke, et al., 2008). AKAPs are critical for scaffolding kinases (e.g., PKA, PKC) and phosphatases (e.g., phosphatase 1) close to ion channels for rapidly modulating their activity (Scott and McCartney, 1994; Wong and Scott, 2004; Hoshi, et al., 2010). Therefore, the membrane initiated signaling by E2 via Gαq-mER can have multiple consequences both in the short term to affect neuronal excitability that can ultimately lead to new gene transcription that can impact multiple synaptic inputs onto GnRH neurons.

7. Conclusions

Since the first cloning of the estrogen receptor/transcription factor ERα in 1986 (Greene, et al., 1986), it has become abundantly clear that there are not only multiple receptors (ERα, ERβ, GPR30, etc.) but also multiple modes of estrogen signaling via membrane and intracellular shuttling of these receptors. Also, this diversity of signaling allows the pleiotropic actions of E2 in the CNS, where the steroid can collaborate with neurotransmitters, growth factors and transcription factors. The actions of E2 span from rapid (seconds) to long term (hours) time frame to not only affect reproduction but other homeostatic functions that are critical for reproductive function. Certainly the recognition of the importance of membrane initiated signaling is long overdue, and its importance will only continue to escalate as we identify new tools to probe these actions.

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Abbreviations

CREB cAMP-responsive element binding protein

Gαq-mER	mER that is Gαq-coupled to activation of phospholipase Cβ
GIRK	G protein-coupled inwardly rectifying K^+
IGF-1	Insulin-like Growth Factor-1
JAK2	Janus Kinase-2
LRb	Leptin receptor b
MAPK	mitogen-activated protein kinase
mGluR1a	metabotropic glutamate receptor 1a
PKA	protein kinase A
PKB	protein kinase B also known as Akt
PKC	protein kinase C
PI3K	phosphatidylinositol 3-kinase
PLC	phospholipase C
PIP_2	phosphatidylinositol (4,5) biphosphate
PIP_3	phosphatidylinositol-3,4,5-triphosphate
PITPβ	phosphatidylinositol transfer protein β (PITPβ)
TRPC	Transient receptor potential canonical type

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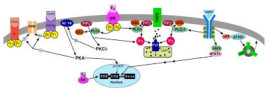

Figure 1. Cross-talk between estrogen and leptin signaling in POMC neurons

Through a putative GPCR (mER), E2 (and STX) activates a PLCβ-PKCδ-AC-PKA pathway in POMC neurons that attenuates the coupling of μ-opioid and $GABA_B$ receptors to GIRK channels (heterologous desensitization) and triggers the release of Ca^{2+} from intracellular stores. This novel pathway also activates PKA-pCREB mediated transcription and in tandem with ERE-mediated transcription will alter the mRNA expression of relevant genes (channels and signaling molecules, etc.). Leptin binds to LRb to activate PI3 kinase and PLCγ1 to augment TRPC-channel currents and POMC neuronal excitability. Leptin will also activate JAK2-STAT3 transcriptional pathways. Note: The guinea pig, similar to the primate, has all of the key cellular elements in the mediobasal hypothalamus for controlling reproduction and energy homeostasis.