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Regulation of Endogenous Conductances in GnRH neurons by Estrogens

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Abstract

17 β -estradiol (E2) regulates the activity of the gonadotropin-releasing hormone (GnRH) neurons through both presynaptic and postsynaptic mechanisms, and this ovarian steroid hormone is essential for cyclical GnRH neuronal activity and secretion. E2 has significant actions to modulate the mRNA expression of numerous ion channels in GnRH neurons and/or to enhance (suppress) endogenous conductances (currents) including potassium (K_{ATP} , A-type) and calcium low voltage T-type and high voltage L-type currents. Also, it is well documented that E2 can alter the excitability of GnRH neurons via direct action, but the intracellular signaling cascades mediating these actions are not well understood. As an example, K_{ATP} channels are critical ion channels needed for maintaining GnRH neurons in a hyperpolarized state for recruiting T-type calcium channels that are important for burst firing in GnRH neurons. E2 modulates the activity of K_{ATP} channels via a membrane-initiated signaling pathway in GnRH neurons. Obviously there are other channels, including the small conductance activated K^+ (SK) channels, that maybe modulated by this signaling pathway, but the ensemble of mER-, ER α -, and ER β -mediated effects both pre- and post-synaptic will ultimately dictate the excitability of GnRH neurons.

Keywords

17 β -estradiol; estrogen receptors; ion channels; hyperpolarization; excitability

1. Introduction

Recent technological advances, such as the development of ER mutants and transgenic animals expressing enhanced green fluorescent protein (eGFP) in GnRH neurons, have greatly facilitated studies to understand the cellular mechanisms by which GnRH neurons are regulated by estrogens and neurotransmitters/neuromodulators (Spergel, et al., 1999; Suter, et al., 2000a; Kato, et al., 2003; Han, et al., 2005; Abraham, et al., 2003; Smith, et al.,

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2006; Wintermantel, et al., 2006; Zhang, et al., 2007). Many of these actions are presynaptic, whereas other actions are postsynaptic directly on GnRH neurons. This review will for the most part focus on the mechanism by which estrogens directly modulates endogenous conductances leading to alteration in GnRH neuronal excitability.

2. Control of GnRH neurosecretion during negative and positive feedback

Gonadotropin releasing hormone (GnRH) neurosecretion and the control of the ovulatory cycle in females is dependent on estrogen feedback, primarily 17 β -estradiol (E2) secreted from the ovaries which reaches the pituitary and brain via the circulation. In spite of recent advances in cellular physiology, we still do not fully understand all of the signaling mechanism(s) by which E2 regulates GnRH neurons. It has been obvious for a number of years that GnRH neurons are modulated by E2 in a complex manner. For example, depletion of serum estrogens by ovariectomy disrupts GnRH regulation of pituitary luteinizing hormone (LH) secretion and results in elevated levels of plasma LH within one or two days. This effect is due to the loss of negative feedback actions of E2. However, both negative and positive (induction of the LH surge) feedback regulation of LH (GnRH) secretion can be restored by replacement with E2. At least in rodents, the positive feedback is believed to be by an action of E2 in the anteroventral periventricular (AVPV) nucleus (Wiegand, et al., 1978; Petersen, et al., 1989; Petersen and Barraclough, 1989; Han, et al., 2005; Smith, et al., 2006; Wintermantel, et al., 2006; Clarkson, et al., 2008), whereas negative feedback may involve the arcuate nucleus of the hypothalamus (Lagrange, et al., 1995; Kauffman, et al., 2007; Gottsch, et al., 2009). Neurons in the AVPV express GABA, opioid peptides and kisspeptin, a neuropeptide encoded by the *Kiss 1* gene, all of which are important for regulation of GnRH neurosecretion (Simerly, et al., 1988; Wagner, et al., 2001; Jackson and Kuehl, 2002; DeFazio, et al., 2002; Smith, et al., 2006; Christian and Moenter, 2007; Clarkson, et al., 2008). The AVPV area expresses high levels of estrogen receptor α (ER α) and also ER β , and the actions of the gonadal steroids are mediated, in part, via the nuclear-initiated signaling (genomic) mechanism (Shughrue, et al., 1997; Wintermantel, et al., 2006; Clarkson, et al., 2008). Kisspeptin (Kiss-1) mRNA expression is greatly increased in the AVPV following E2 treatment. This finding combined with previous observations that the AVPV is necessary for positive feedback has led to the hypothesis that E2 acts on AVPV kisspeptin neurons to induce GnRH super secretion and the GnRH (LH) surge. Kisspeptin neurons in the arcuate nucleus, which co-express dynorphin and neurokinin B, are strongly inhibited by E2 and, interestingly, this inhibition by E2 may use, at least in part, a non-ERE signaling pathway (Smith, et al., 2005; Gottsch, et al., 2009; Navarro, et al., 2009). Therefore, it is now generally believed that the inhibition by E2 of arcuate kisspeptin may represent an important contribution to negative feedback. The exact mechanism(s) is not known, but could involve a combination of kisspeptin/dynorphin/neurokinin B actions at the GnRH cell bodies or directly at the GnRH nerve terminals in the median eminence region. Clearly, further studies are needed on this important issue.

3. Circulating levels of E2 and Cellular actions of E2 *in vitro*

Many years ago it was shown that radioactive E2 injected systemically was concentrated in preoptic area and basal hypothalamus above levels observed in plasma (Eisenfeld and Axelrod, 1965). Therefore, circulating levels of E2 may not be a true determinant of physiological versus non-physiological levels of E2 in the hypothalamus (Herbison, 2009). In particular, when applying E2 in a bath superfusing brain slices, physiological versus non-physiological actions would depend on the pharmacokinetics of E2 in a slice preparation. Usually one reports the EC₅₀ as a measure of the relative potency of a drug in a given preparation. The EC₅₀ 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). In the arcuate nucleus the EC_{50} value for the actions of E2 to acutely attenuate the μ -opioid response was 7.5 nM (Lagrange, et al., 1997). In GnRH neurons, the EC_{50} for E2 to augment the ATP-sensitive K^+ (K_{ATP}) current is 0.6 nM (Zhang et al., 2010). Most importantly, the ER antagonist ICI 164,384 or ICI 182,780 blocks the actions of E2 with subnanomolar affinity that is similar to ICI's affinity (K_i) for $ER\alpha$, further supporting the specificity and physiological relevance of these acute E2 actions in the slice (Weatherill, et al., 1988; Lagrange, et al., 1997).

4. Multiple estrogen receptors and signaling cascades are involved in 17β -estradiol modulation of GnRH neuronal activity

Because $ER\alpha$, the first cloned receptor/transcription factor for E2, has not been localized to native GnRH neurons, the predominant view has been that estrogen affects GnRH neurons exclusively through pre-synaptic mechanisms as outlined above. However, a second ER, $ER\beta$, is expressed in GnRH neurons (Herbison and Pape, 2001; Hrabovszky, et al., 2001; Kallo, et al., 2001) and a number of studies have illustrated that the acute actions of E2 on GnRH neurons are $ER\beta$ -dependent (see below). Still, the functional significance of $ER\beta$ in GnRH neurons remains uncertain. In addition, synaptically isolated GnRH neurons are rapidly hyperpolarized by E2, an effect that inhibits their firing (Kelly, et al., 1984; Condon, et al., 1989; Lagrange, et al., 1995). It is thought that these hyperpolarizing actions of E2 on GnRH neurons are via a G protein ($G_{\alpha_{i,0}}$)-coupled receptor. Indeed, in GT1-7 cells, an immortalized GnRH neuronal cell line, E2 inhibits adenylyl cyclase activity (cAMP production) via a pertussis toxin-sensitive $G_{\alpha_{i,0}}$ coupling mechanism (Navarro, et al., 2003). Because of the rapid onset of these E2 effects the involvement of transcription is highly unlikely, but an E2-responsive - $G_{\alpha_{i,0}}$ -coupled receptor has not been identified. E2 can also act acutely to increase calcium oscillations in primate GnRH neurons via G protein-receptor 30 (GPR30) (Terasawa, et al., 2010).

The observation that E2 treatment *in vivo* can within 15 min induce phosphorylation of cyclic AMP response element binding protein (pCREB) in GnRH neurons, was a clear indication of E2 rapid signaling within the hypothalamus. Moreover, this effect was lost in animals with a deletion mutation of $ER\beta$, but not $ER\alpha$, suggesting a role for $ER\beta$ in the E2-induced rapid induction of pCREB in mouse GnRH neurons (Abraham, et al., 2003). A role for $ER\beta$ has been further supported by studies using whole-cell recordings of GnRH neurons to illustrate that high physiological levels (0.1–100 nM) of E2 and the $ER\beta$ agonist diarylpropionitrile (DPN) both of which act acutely (within 15 min) to enhance action potential firing, reduce the afterhyperpolarization potential (AHP) and increase the amplitude of a slow afterdepolarization (ADP) in GnRH neurons (Chu, et al., 2009). These effects were observed in the presence of ionotropic GABA and glutamate blockade (although other inputs such as kisspeptin were not blocked), suggesting direct, rapid excitatory actions by E2 on GnRH neurons that involve $ER\beta$. Other rapid actions of E2 to alter calcium transients in GnRH neurons are thought to involve modulation of presynaptic GABA input and appears to be dependent on $ER\alpha$. Thus physiological levels (1–100nM) of E2 (within 15 min) can increase calcium transients in GnRH neurons via an $ER\alpha$ -dependent release of GABA from presynaptic terminals (Romanò, et al., 2008). However, E2 (10 pM) may also act indirectly to rapidly inhibit GnRH neurons in an $ER\alpha$ -dependant manner (Chu, et al., 2009). Therefore, the cumulative findings would suggest rapid presynaptic actions of E2 via $ER\alpha$ and rapid postsynaptic actions via $ER\beta$ to either excite or inhibit GnRH neurons.

The ability of E2 to acutely modify GnRH neurons through calcium signaling has been further explored in mouse hypothalamic neuronal explants and in primate nasal placode

cultures. In these models, E2 (0.2–1 nM) augments synchronous intracellular Ca^{2+} oscillations in GnRH neurons (Temple, et al., 2004; Temple and Wray, 2005; Abe, et al., 2008). This relatively rapid effect is observed within 10–30 min of E2 application. The calcium oscillations in the presence of TTX are most likely the result of E2 acting directly on GnRH neurons. Furthermore, E2 conjugated to BSA at the C-17 position (E2-17-BSA) or to a large non-degradable poly(amido)amine-dendrimer macromolecule (EDC) (Harrington, et al., 2006) mimics the effects of E2 on intracellular calcium oscillations (Temple, et al., 2004; Temple and Wray, 2005; Abe, et al., 2008). In the mouse explants experiments, the E2-induced calcium oscillations are blocked by ICI 182,780 suggesting that an ER is involved (Temple, et al., 2004; Temple and Wray, 2005). The actions of E2-BSA in GnRH neuronal cultures are abrogated by pertussis toxin treatment supporting a role for a G protein-coupled membrane receptor (Temple, et al., 2004; Temple and Wray, 2005; Noel, et al., 2009). In mouse, the E2-induced calcium oscillations are observed in 7 division explants which express ER β , but not in 21 division explants which lack ER β . Therefore, it is hypothesized that these rapid membrane actions in mouse GnRH neurons are ER β -dependent (Temple, et al., 2004). In contrast, E2-induced calcium oscillations in the primate placode cultures are not blocked by the antagonist ICI 182,780, and ICI 182,780 alone has no effect (Abe, et al., 2008; Noel, et al., 2009).

Interestingly, GPR30 is expressed in primate GnRH neurons and may, in part, be responsible for the rapid membrane-initiated actions of E2 in these neurons (Noel, et al., 2009). Thus E2 conjugated EDC failed to induce calcium oscillations in cultured GnRH neurons transiently transfected with human GPR30 siRNA, but not with the control siRNA, further supporting a role for GPR30 (Noel, et al., 2009). GPR30 has received considerable attention because it exhibits binding and signaling characteristics of a membrane ER (Revankar, et al., 2005; Thomas, et al., 2005; Filardo and Thomas, 2005; Funakoshi, et al., 2006; Matsuda, et al., 2008). Although there is some controversy whether this receptor is expressed in the plasma membrane or localized to the endoplasmic reticulum (Revankar, et al., 2005; Thomas, et al., 2005; Filardo and Thomas, 2005; Funakoshi, et al., 2006; Matsuda, et al., 2008), there is general agreement on the estrogen-mediated signaling. For example in breast cancer cells that are transfected with GPR30, estrogen activates the MAP kinases, ERK1 and ERK2 (Filardo, 2002; Filardo and Thomas, 2005). In addition, E2 can also stimulate adenylyl cyclase activity, which leads to PKA-mediated suppression of epidermal growth factor (EGF)-induced ERK1/2 activity. However, the E2-induced GPR30 signaling pathway in GnRH neurons is currently unknown (Terasawa, et al., 2010). Collectively, these data suggest a rapid direct action of E2 through ER β and perhaps GPR 30 to excite GnRH neurons and a rapid indirect action via ER α to excite or suppress GnRH neuronal excitability. How these acute actions contribute to the overall physiology of GnRH neurons is currently not well understood.

Genetic models have been generated from ER α knockout (α ERKO) animals that possess an ER-knock-in mutation, which allows *in vivo* distinction between estrogen response element (ERE)-dependent and ERE-independent mechanisms of E2 action (McDevitt, et al., 2008; Glidewell-Kenney, et al., 2008). This ERE-independent model was used to study GnRH neurons, using loose cell attached recordings (Christian, et al., 2008). Based on these studies it was concluded that an E2-induced decrease in GnRH neuronal firing during the morning (negative feedback model) as well as increased neuronal firing during the afternoon (positive feedback model) are both dependent on ER α binding to ERE. In contrast, based on measurements of E2-induced inhibition of plasma LH in ovariectomized wildtype (WT), α ERKO and ER-knock-in mutant animals (E207A/G208A; "AA"), negative feedback regulation of LH (GnRH) is at least in part dependent on a mechanism other than ER binding to ERE (McDevitt, et al., 2008). Similarly, the E2 inhibition of arcuate kisspeptin neurons, believed to mediate negative feedback, is also via an ERE-independent mechanism

(Gottsch, et al., 2009). These data would indicate that feedback regulation of E2 to inhibit or excite GnRH neurons is quite complex involving multiple receptors and both pre- and post-synaptic E2 actions, requiring further experiments for a more complete understanding.

5. 17 β -estradiol regulation of GnRH neuronal excitability

Recent findings have revealed that GnRH neurons express ATP-sensitive K⁺ (K_{ATP}) channels and the agonist-induced outward (K_{ATP}) current is increased by approximately two-fold in E2-treated animals (Zhang, et al., 2007). The increased channel activity, which occurs in the presence of TTX and GABA and glutamate blockade, is not the result of increased expression of the channel based on quantitative real-time PCR results from harvested GnRH neurons. Therefore, it appears that there is an E2-induced change in signaling molecules (kinases) that impinge on the channel, part of which may be due to acute phosphorylation events that non-the-less are long acting effects on cell function. Indeed, our recent findings suggest that E2 may signal via a mER to hyperpolarize GnRH neurons, as defined by the mER ligand STX and the inhibition of the response by ICI 182,780 (Qiu, et al., 2003; Qiu, et al., 2006; Zhang, et al., 2010). Previously, we found that E2 could hyperpolarize guinea pig GnRH neurons via activation of a K⁺ channel and hypothesized that this could contribute to the negative feedback actions of the hormone (Lagrange, et al., 1995). In our original studies we found that E2 activated an inwardly rectifying K⁺ (Kir) channel (Lagrange, et al., 1995). In mouse GnRH neurons, we do not see a change in the holding current (indicative of a direct K⁺ channel activation) but rather an augmentation of the K_{ATP} channel opener (diazoxide)-activated current. The apparent direct activation of Kir channels in the guinea pig versus the augmentation of a Kir channel activity in the mouse is most likely due to the different recording conditions, sharp electrode recordings in the guinea pig versus the whole-cell patch recordings (whole-cell dialysis) in the mouse GnRH neurons. Alternatively, it could reflect a subtle species difference. Regardless, in both species E2 acutely augments a Kir current that results in a membrane hyperpolarization, which critically sets the stage for recruiting “excitatory” channels (see below). In addition, there are neurotransmitters (GABA and opioids) that activate G protein-activated inwardly rectifying K⁺ (GIRK) channels (Lagrange, et al., 1995; Zhang, et al., 2009b) and these channels will also contribute to the membrane hyperpolarization (see the Kelly and Qiu chapter, this volume).

It is well known that Kir channels are critical for maintaining excitable cells in a hyperpolarized resting state closer to E_K, but once cells are depolarized the channels allow for the quick transition to long depolarizing responses because of their inwardly rectifying properties (Hille 2001). With greater membrane hyperpolarization of GnRH neurons there is an increase in the number of Na⁺ spikes during rebound excitation (Zhang, et al., 2009a). Indeed, K_{ATP} and GIRK channels appear to be the critical Kir channels in GnRH neurons for maintaining the membrane in a relatively negative resting state (Zhang, et al., 2007; Zhang, et al., 2009b). Blocking K_{ATP} channels with the sulfonylurea tolbutamide significantly depolarizes GnRH neurons (Fig. 1), which is indicative of tonic K_{ATP} channel activity that is significantly augmented with E2 treatment (Zhang, et al., 2007). We also know that GABAergic, opioid, neuropeptide Y and perhaps melanin-concentrating hormone (MCH) input via their respective GABA_B, μ -opioid, Y5 and MCH1 receptors activates GIRK channels to hyperpolarize GnRH neurons (Lagrange, et al., 1995; Zhang, et al., 2009b; Xu, et al., 2009; Wu, et al., 2009). This combination of membrane hyperpolarization would allow for the recruitment of multiple excitatory conductances that are critical for generating burst firing in CNS neurons (Huguenard and McCormick, 1992; Erickson, et al., 1993; Kelly and Wagner, 2002).

In addition, it has been known for some time that the peptide GnRH functions by a short-circuit feedback pathway to modulate the excitability of GnRH neurons and reduce GnRH secretion (Padmanabhan, et al., 1995; Krsmanovic, et al., 1999; Xu, et al., 2004; Herbison, 2006). Indeed, GnRH activates the M-current in a sub-population of GnRH neurons (Xu, et al., 2004; Xu, et al., 2008). The receptor mediating this effect is the GnRH receptor 1 (GnRH-R1) (Xu, et al., 2004; Todman, et al., 2005; Xu, et al., 2008). In TTX, which blocks synaptic input, the activation of this persistent K^+ current by GnRH causes a hyperpolarization (Xu, et al., 2008; Han, et al., 2010) that along with activation of other K^+ currents would contribute to the inhibition of cell firing. GnRH has also been found to depolarize GnRH neurons and to act transsynaptically to regulate the activity of GnRH neurons via modulation of GABAergic input to these cells (Todman, et al., 2005; Chen and Moenter, 2009; Han, et al., 2010). In immortalized GnRH neurons, GnRH in nanomolar concentrations activates GnRH-R1, which results in Gq/11 activation of PLC signaling along with coupling to Gs and increased cAMP production (Krsmanovic, et al., 2003). However, the complete signaling mechanism in native GnRH neurons is not known.

Studies in EGFP-GnRH mice have illustrated that GnRH neurons often fire in episodes of repetitive bursts (Fig. 1) (Suter, et al., 2000b; Kuehl-Kovarik, et al., 2002; Kuehl-Kovarik, et al., 2005). Currently, it is not known how the bursts are generated in GnRH neurons, although depolarizing afterpotentials (DAP; also called afterdepolarizing potentials, ADP) are proposed to be involved (Kuehl-Kovarik, et al., 2005; Chu and Moenter, 2006). Based on current clamp and voltage clamp recordings in neurosecretory hypothalamic neurons, models have been developed that include an ensemble of channels that appear to be critical for burst firing in parvocellular neurosecretory neurons (Kelly and Rønnekleiv 1994; Kelly and Wagner, 2002; Lee, et al., 2010). Based on these models, calcium T-type channels together with the hyperpolarization-activated cation current (I_h) and calcium-dependent small (SK)-type channels (underlying an AHP) are crucial for burst firing.

Three subunits of the T-type channel (Cav 3.1, 3.2, 3.3) have been cloned (Perez-Reyes, et al., 1998; Lee, et al., 1999), with the specific gating properties of the T-type channel being dependent on the subunit composition. Thus, the Cav3.1 and 3.2 current profiles are more similar compared to Cav3.3, and compelling evidence suggests that these subunits are involved in short burst firing in thalamocortical relay neurons (Chemin, et al., 2002). GnRH neurons express all three of the T-type calcium channel subunits and the expression of these subunits are regulated by E2 in a subunit-specific and diurnal manner (Kato, et al., 2003; Zhang, et al., 2009a). Of importance is that T-type calcium currents can be measured in 95% of GnRH neurons (Kato, et al., 2003; Zhang, et al., 2009a), and the $V_{1/2}$ for T-channel de-inactivation is -80 mV (potential at which half of the T-type calcium channels are recruited) (Zhang, et al., 2009a). The role of the T-type Ca^{2+} currents, as found in other neuronal systems, is to facilitate rhythmic intracellular Ca^{2+} transients by initiating membrane depolarization that recruits high voltage activated (HVA) Ca^{2+} currents (Cueni, et al., 2008; Liu and Shipley, 2008). This is of functional significance since HVA Ca^{2+} currents are prevalent in GnRH neurons but they can only be recruited from a more depolarized state, whereas, low voltage activated (LVA) T-type currents are recruited from a more hyperpolarized state, i.e. they could provide the “spark” for setting off burst firing (Kato, et al., 2003; Nunemaker, et al., 2003; Spergel, 2007; Zhang, et al., 2009a; Sun, et al., 2010; Tanaka, et al., 2010). Importantly, the T-type channel subunit expression, current density and rebound excitation of GnRH neurons are significantly enhanced with a surge-inducing E2-treatment paradigm (Zhang, et al., 2009a). Therefore, the E2-dependent increase in T-type calcium channels may serve to augment the excitability of GnRH neurons in preparation for the GnRH (LH) surge. Not surprisingly, L-type calcium currents are also regulated by E2 in GnRH neurons (Sun, et al., 2010). In ovariectomized mice treated with E2 to induce afternoon LH surges, the peak L-type current was reduced during the morning

at the time of negative feedback but increased during the afternoon at the time of positive feedback. This would indicate that L-type currents may be significant contributors to the afternoon surge of GnRH (LH). However, the diurnal signal that precisely activates this current is unknown. In addition, GnRH neurons express a time-dependent, hyperpolarization-activated, cation current (I_h or pacemaker current) that contributes to rhythmic firing (Lagrange, et al., 1995; Kelly and Wagner, 2002; Zhang, et al., 2007).

Neuronal excitability is also determined by the afterhyperpolarization (AHP) that follows an action potential, and SK channels underlie a component of the median afterhyperpolarization (mAHP) K^+ currents in CA1 pyramidal cells and other neurons (Bond, et al., 2004). SK channel subunits are expressed in GnRH neurons (Bosch, et al., 2002; Kato, et al., 2006), and SK channel activation exerts a powerful influence on firing properties of GnRH neurons. In particular, these channels and other AHP channels appear to regulate both intraburst and interburst neuronal firing in GnRH neurons and may play a key role in modulating GnRH neuronal excitability (Kato, et al., 2006; Liu and Herbison, 2008; Lee, et al., 2010).

Finally, Kir channels are negatively modulated by a number of Gq-coupled receptors including the kisspeptin receptor GPR 54 in GnRH neurons, which allows the rapid transition to heightened excitatory states (Kobrinisky, et al., 2000; Hille 2001; Liu, et al., 2008; Zhang, et al., 2008). Kisspeptin potently depolarizes hypothalamic GnRH neurons through a combination of inhibiting an inwardly rectifying potassium (Kir) conductance and activating a transient receptor potential canonical (TRPC) conductance (Zhang, et al., 2008; Liu, et al., 2008; Pielecka-Fortuna, et al., 2008). To date, kisspeptin is the most potent and efficacious neuropeptide/neurotransmitter to modulate GnRH neuronal activity (Lagrange, et al., 1995; Spergel, et al., 1999; Kuehl-Kovarik, et al., 2002; DeFazio, et al., 2002; Suter, 2004; Kuehl-Kovarik, et al., 2005; Han, et al., 2002; Herbison, 2006; Zhang, et al., 2008). Besides activating TRPC channels, kisspeptin also attenuates a resting (barium and cesium sensitive) Kir current in GnRH neurons (Fig. 2). The inhibition of Kir may be critical because Kir channels (e.g., K_{ATP} channels) are highly expressed in GnRH neurons and clamp the cells in a negative resting state of -63 mV (Lagrange, et al., 1995; Zhang, et al., 2007; Zhang, et al., 2009b). This effect of kisspeptin would also be vital for inhibiting GPCR-activated (μ -opioid, $GABA_B$ and MCH) Kir currents which are active in GnRH neurons (Lagrange, et al., 1995; Zhang, et al., 2009b; Wu, et al., 2009). Therefore by inhibiting Kir channels along with the pronounced activation of TRPC channels, kisspeptin depolarizes the cells to threshold (~ -45 mV) and induces sustained firing. Interestingly, GnRH neurons express all of the brain TRPC channel subunits (TRPC1,3,4,5,6,7) with TRPC1, 4 and 5 being the most prevalent in GnRH neurons (Zhang, et al., 2008). Based on the current - voltage relationship, pharmacological profile and mRNA expression, TRPC1, 4, and 5 are key players in mediating the effects of kisspeptin in GnRH neurons. Moreover, the kisspeptin receptor GPR54 couples to a Gq and phospholipase C (PLC) pathway in GnRH neurons (Zhang, et al., 2008; Liu, et al., 2008; Constantin, et al., 2009), and kisspeptin activation of this pathway results in the release of calcium from intracellular stores after stimulation of inositol-1,4,5-trisphosphate (IP3) receptors, which would activate the store-operated TRPC1,4 and 5 channels in GnRH neurons (Fig. 2) (Liu, et al., 2008).

6. Conclusions

Remarkable progress has been made in the understanding of GnRH neuronal regulation since the production of eGFP-GnRH expressing mice and rats, which have allowed direct targeting of the otherwise scattered GnRH neurons. Numerous publications dealing with calcium signaling, neuronal activity, diurnal rhythms and gene expression changes in GnRH neurons have advanced the field. However, challenging questions remain concerning

membrane-initiated signaling of estrogen and how this relates to the nuclear-initiated signaling of estrogen, the mechanism underlying pulsatility of GnRH neurosecretion, the integration of presynaptic and postsynaptic E2 actions, as well as the mechanism of kisspeptin actions in GnRH neurons **combined with circadian signals** for induction of negative and positive feedback. Future studies need to be directed towards elucidating the expression of ion channels **and signaling molecules** and how the integrative sum of these contribute to the burst firing of GnRH neurons.

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Abbreviations

K_{ATP} channels	ATP-sensitive potassium channels
GIRK channels	G-protein activated inwardly rectifying potassium channels
TRPC channels	canonical transient receptor potential channels
SK channels	small conductance calcium-activated K ⁺ channels
eGFP	enhanced green fluorescence protein
pCREB	phosphorylated cyclic AMP response element binding protein
DPN	diarylpropionitrile
AHP	afterhyperpolarization potential
DAP	depolarizing afterpotential

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Figure 1. Contribution of K_{ATP} channels to burst firing in GnRH neurons

a, An example showing how hyperpolarization facilitates the burst firing in GnRH neurons. Initially, the cell had a hyperpolarized membrane potential of -68 mV relative to the average resting membrane potential of -63 mV (Zhang et al, 2007) and fired in a bursting mode. After a burst firing the membrane was further hyperpolarized to -73 mV by the afterhyperpolarization (AHP). However, when the cell was depolarized to -63 mV by blocking K_{ATP} channels with tolbutamide ($100 \mu\text{M}$), it fired in a continuous mode. **b**, The expanded scale of the burst firing showing the robust AHP following the burst firing. **c**, The expanded scale of the continuous firing mode in the presence of tolbutamide.

Figure 2. Cellular model of G protein-coupled receptor (GPCR) signaling in GnRH neurons
Schematic overview showing kisspeptin/GPR54 signaling, 17 β -estradiol (E2) membrane-initiated signaling and GnRH signaling in GnRH neurons. (This obviously incomplete illustration focuses primarily on findings from the authors laboratory). Kisspeptin (KP) binds to its G protein-coupled (Gq11) receptor (GPR54) to activate phospholipase C (PLC). PLC activation leads to opening of TRPC channels and inhibition of GIRK (Kir) channels causing a pronounced excitation of GnRH neurons. On the other hand, GABA binding to GABA_B receptors activates GIRK channels and suppresses kisspeptin excitation of GnRH neurons. E2 acts via a putative GPCR (Gq) mER to activate PLC leading to activation of protein kinase C (PKC) and PKA resulting in K_{ATP} channel opening and enhanced K_{ATP} current. Activation of PKA may also alter the excitability of T-type calcium channels. Another downstream target of PKA is CRE binding protein (CREB) which is phosphorylated and through interaction with CRE leads to new gene transcription. E2-mediated PKA activation, or E2 via ER β , can increase the mRNA expression of the T-type calcium channel subunits, Cav 3.1, 3.2 and 3.3. E2 potentially may also regulate the mRNA expression of L-type channel subunits, Cav 1.2 and 1.3, as well as the HCN 1-4 and SK 2, 3 subunits. E2 and the GPR30 agonist, G1, induce calcium oscillations in primate GnRH neurons via actions on GPR30 (Terasawa, et al., 2010). Finally, autoregulatory feedback of GnRH onto GnRH neurons, can activate KCNQ channels leading to M-current-induced hyperpolarization.