Kisspeptin Excitation of GnRH Neurons

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Abstract

Kisspeptin binding to its cognate G protein-coupled receptor (GPR54, aka Kiss1R) in gonadotropin-releasing hormone (GnRH) neurons stimulates peptide release and activation of the reproductive axis in mammals. Kisspeptin has pronounced pre- and postsynaptic effects, with the latter dominating the excitability of GnRH neurons. Presynaptically, kisspeptin increases the excitatory drive (both GABA-A and glutamate) to GnRH neurons and postsynaptically, kisspeptin inhibits an A-type and inwardly rectifying K^+ (Kir 6.2 and GIRK) currents and activates nonselective cation (TRPC) currents to cause long-lasting depolarization and increased action potential firing. The signaling cascades and the multiple intracellular targets of kisspeptin actions in native GnRH neurons are continuing to be elucidated. This review summarizes our current state of knowledge about kisspeptin signaling in GnRH neurons.

Relationship Between Kisspeptin and GnRH Secretion

Kisspeptin, encoded by the *Kiss1* gene, is a key factor in the regulation of reproductive development and functions [1–6]. The *Kiss1* gene encodes a 145 amino acid protein, which is proteolytically processed to produce a 54 amino acid peptide, called kisspeptin-54, and several other smaller peptide fragments [7]. Centrally administered kisspeptins stimulate GnRH and gonadotropin secretion in prepubertal and adult animals [1, 8–11]. The central application of kisspeptin induces cFos immunoreactivity within 1–2 h in more than 85% of GnRH neurons, further suggesting that direct activation of the neurons is responsible for the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [8]. As expected, kisspeptin is not able to stimulate LH or FSH release in GPR54 knockout animals [11]. Also, the kisspeptin-mediated release of LH is completely inhibited by the GnRH antagonist, acyline [1, 8]. Importantly, CNS administration of kisspeptin in the ewe has conclusively demonstrated a correlation between kisspeptin-induced GnRH and LH release [11]. Therefore, the stimulatory actions of kisspeptin appear to be primarily on GnRH neurons and not the pituitary. Kisspeptin, when applied to GnRH neurons in vitro, potently activates these neurons and causes increased neuronal firing [12–14].

Over the past several years, there have been many publications about the regulation of *Kiss1* gene expression and the role of kisspeptins in regulating GnRH and LH secretion [1, 8, 11, 15–17]. Also, the distribution and regulation of kisspeptin mRNA (*Kiss1*) expression by

17β-estradiol (E_2) has been extensively described in the mouse and rat brain [15, 17, 18]. In these rodent species, it is known that *Kiss1* mRNA is expressed primarily in the anteroventral periventricular nucleus (AVPV) and adjacent periventricular (PeN) areas, as well as in the arcuate nucleus of the hypothalamus [19, 20]. Importantly, E_2 increases the mRNA expression of *Kiss1* in the female AVPV, but decreases the expression in the arcuate nucleus [15, 17]. These findings are consistent with data showing that the AVPV is necessary for E_2 positive feedback on GnRH and LH secretion in these species [21–23]. The number of AVPV kisspeptin neurons is significantly fewer in male rodents than in females, but the numbers are similar in the arcuate nucleus in adults of both sexes [24]. As in females, steroid treatment (testosterone or E_2) increases the number of *Kiss1* neurons in the male AVPV and decreases the number of *Kiss1* expressing cells in the arcuate nucleus [25]. The function of the AVPV kisspeptin neurons in the male rodent is not clear, but these neurons may be involved in generating the basal pulsa-tile release of LH via a stimulatory action on GnRH neurons.

In other species, such as the guinea pig, sheep, and rhesus monkey, the preoptic area (POA) appears not to be the main region responsible for E_2 positive feedback [26–30]. Thus, it appears that the basal hypothalamus may be sufficient for maintaining steroid-mediated positive feedback regulation of GnRH and LH secretion in these species. Consistent with these findings, kisspeptin neurons within the arcuate nucleus in guinea pig, sheep, and monkey appear to be involved in E_2-mediated positive as well as negative feedback regulation of GnRH neurons [31–34]. The specific kisspeptin neurons within the arcuate nucleus that mediate positive feedback regulation of LH remains to be determined, although evidence suggests that a caudal arcuate population of neurons is involved [31–34]. However, irrespective of the role of the arcuate nucleus in mediating E_2 positive feedback on LH secretion in certain species, evidence suggests that the POA is also involved [31, 34, 35]. Importantly, regardless of differential regulation of *Kiss1* neurons by E_2, in all instances, kisspeptin potently excites GnRH neurons via a phospholipase C (PLC) signaling pathway (see below).

To further study the role of kisspeptin in the regulation of GnRH neurons and LH release, kisspeptin analogs with mixed agonist/antagonist activities have been synthesized [36]. Of these, peptide 234 has primarily antagonist activities in Chinese Hamster Ovary K1 (CHO-K1) cells expressing Kiss1R and inhibits the kisspeptin response by 93%, with an IC50 of 7 nM [36]. This compound also has a binding affinity of 2.7 nM for Kiss1R stably expressed in CHO-K1 cells. Peptide 234 subsequently has been found to inhibit kisspeptin-induced GnRH neuronal firing in vitro, whereas in vivo treatment with the peptide attenuates kisspeptin-induced LH release in intact males [36]. Moreover, peptide 234 attenuates the castration rise in plasma LH levels in mouse and rat, and reduces pulsatile LH release in the ovariectomized ewe and rat [36, 37]. Also, in ovariectomized monkeys, peptide 234 attenuates pulsatile release of GnRH [36]. Collectively, these data support the concept that kisspeptin neurons (in the arcuate nucleus?) are involved in stimulating GnRH and LH release following gonadectomy.

Thus, the estrogen-mediated "negative" feedback inhibition of post-castration GnRH and LH release may be via the differential release of kisspeptin/opioid peptides since this group

of arcuate neurons also co-localizes dynorphin [38, 39]. Although there is a robust μ-opioid receptor-mediated inhibition of GnRH neurons in guinea pig [40], a κ-opioid-mediated effect has not been demonstrated.

Kisspeptin Activation of Kiss1R

Kisspeptin-54 has been identified as the endogenous ligand of the orphan G protein-coupled receptor, GPR54 [7, 41], also known as Kiss1R. In addition to kisspeptin-54, the smaller peptide fragments derived from the precursor protein (e.g., kisspeptin 14, 13, and 10) all have biological activity at Kiss1R [7, 42]. These peptides bind with low nanomolar affinities to rat and human Kiss1R expressed in Chinese hamster ovary K1 cells and stimulate PIP2 hydrolysis, Ca^{2+} mobilization, arachidonic acid release, extracellular signal-regulated protein kinase 1 (ERK1), ERK2, and p38 MAP kinase phosphorylation [7]. In mammals, Kiss1R is expressed both in the pituitary and in GnRH neurons [7, 8, 11, 12]. However, as stated above, evidence suggests that the stimulation of gonadotropin secretion by kisspeptin is via direct activation of GnRH neurons and not pituitary gonadotropes [1, 8–10, 43]. Although multiple actions of kisspeptin have been identified (see below), all of the signaling pathways have not been elucidated.

Kisspeptin Activation of Kiss1R in GnRH Neurons: Downstream Signaling Pathways

To date, kisspeptin is the most potent and efficacious neuropeptide/neurotransmitter to excite native GnRH neurons [44–49]. In most studies, kisspeptin is reported to depolarize and excite the vast majority (75–90%) of GnRH neurons (Fig. 6.1), which correlates with the expression of Kiss1R in the majority of GnRH neurons [8, 12, 14, 50]. However, Dumalska et al. found a lower percentage of GnRH neurons responding to kisspeptin and proposed that there are two physiologically distinct populations of GFP-GnRH neurons, one that responds to kisspeptin and the other that responds to the metabotropic glutamate receptor agonist, dihydroxyphenylglycine (DHPG) [51]. One explanation for these differences is that some of the recordings performed by Dumalska and coworkers were made from animals as young as 15 days of age [51], so the reduced response to kisspeptin could be age-related. Although the expression of Kiss1R is similar in juvenile as in adult male mice, and at both age levels Kiss1R can be detected in over 90% of GnRH neurons [12], the percent of GnRH neurons responding to kisspeptin is only about 27% in juvenile vs. 90% in adult males [12]. The reason for the reduced efficacy of kisspeptin in GnRH neurons from juvenile and prepubertal males is not fully understood but could be due to an immature Kiss1R signaling in the younger animals.

In the adult, kisspeptin depolarizes GnRH neurons via the coupling of Kiss1R to a phospholipase Cβ (PLCβ) signaling pathway that activates canonical transient receptor potential (TRPC) channels that allow influx of sodium and to a lesser extent calcium ions (Fig. 6.2) [14]. Besides activating TRPC channels in GnRH neurons, kisspeptin also attenuates resting and ligand-activated inwardly rectifying K^+ (Kir) channels and A-type potassium channels [13, 14, 50, 52]. The inhibition of Kir may be critical because Kir channels (e.g., K_{ATP} and GIRK channels) are highly expressed in GnRH neurons and clamp

the cells in a negative resting state of −63 mV [40, 53, 54]. This effect of kisspeptin is also vital for inhibiting GPCR- activated (μ-opioid, GABA $_B$ and perhaps melanin-concentrating hormone, MCH) GIRK (Kir) currents which are prominent in GnRH neurons [40, 54, 55]. Also, A-type K $^+$ currents are very prominent in GnRH neurons, and E $_2$ regulation of the A-current may play a role in negative feedback regulation of GnRH neurons [52, 56]. Therefore, kisspeptin inhibition of these K $^+$ currents would be of high functional significance. Moreover, kisspeptin increases calcium oscillations of mature as well as developing GnRH neurons, and these changes for the most part reflect the coupling of Kiss1R to a PLCβ signaling pathway [14, 50, 57, 58]. Therefore, by inhibiting potassium channels along with the pronounced activation of TRPC channels, kisspeptin depolarizes GnRH neurons to threshold (~45 mV) and induces sustained firing, which may be accompanied by a sustained calcium ion influx via calcium channels/TRPC channels and augmented GnRH release during positive feedback.

Kisspeptin Activation of TRPC Channels

The mammalian TRPC channel family consists of seven members, TRPC1–7, that appear to function as receptor-operated channels, analogous to the TRP channels involved in *Drosophilia* phototransduction [59]. With the exception of TRPC2, these channels are widely distributed in the mammalian brain [60]. The TRP channels are made of subunits with six membrane-spanning domains that co-assemble as tetrameric complexes similar to what has been described for K $^+$ channels [61, 62]. TRPC channels appear to co-assemble as heteromeric channels consisting of the TRPC1, 4, and 5 subfamily [63, 64], as well as TRPC3, 6, and 7 subfamily [65, 66]. It is well known that the current–voltage relationship and mechanisms of regulation of TRPC channels depend on the channel subunit composition [59]. However, the functional distinction between these channel subtypes in CNS neurons has been problematic because of a lack of selective pharmacological reagents. The main exception is the discriminatory effects of lathanides to augment TRPC4, 5 channel activity [62]. Whole-cell recording experiments, with K $^+$ channel blockers on board, have revealed that the current–voltage relationship for the kisspeptin-induced current in GnRH neurons resembles the current–voltage relationship of heteromeric complexes of TRPC 1 + 4 or TRPC 1 + 5 subunits expressed in HEK cells with the characteristic negative slope conductance and pronounced outward rectification (Fig. 6.2) [14, 59, 63]. Similar current–voltage relationships have been obtained for the leptin-induced currents in arcuate POMC and kisspeptin neurons and the mGluR1- and CCK2-induced currents in basolateral amygdala neurons [67–70]. All of these neurons have been found to express the same compliment of TRPC channels as GnRH neurons.

Interestingly, GnRH neurons express all of the "brain-type" TRPC channel subunits with the TRPC1, 4, and 5 family being the most prevalent in GnRH neurons [14]. Therefore, based on the current–voltage relationship, pharmacological profile and mRNA expression, TRPC1, 4, and 5 are key players in mediating the excitatory effects of kisspeptin in GnRH neurons (Figs. 6.2 and 6.3) [14]. Traditionally, these channels are known as "store operated calcium channels," but this description is probably the result of poorly understood signaling mechanisms [59, 71]. Therefore, current research has focused on elucidating the signaling pathway(s) by which kisspeptin activates TRPC channels, and the sources of calcium

mobilization following kisspeptin activation of GnRH neurons. Although the majority of findings seem to indicate that the initial calcium signal comes via plasma membrane channels [14, 57, 58], there is also evidence that kisspeptin induces the release of calcium from intracellular stores in GnRH neurons via inositol- 1,4,5-trisphosphate (IP3) receptors [50, 72]. However, intracellular dialysis with 2-APB, which abrogates the store release of calcium, does not inhibit the effects of kisspeptin [14]. Certainly, sustained calcium release is not required for kisspeptin's actions since calcium mobilization is transient [50].

The mammalian TRPC channels can be activated by G protein-coupled receptors and receptor tyrosine kinases (see refs. [59, 73]). In a heterologous cell expression system (i.e., Chinese hamster ovary K1 cells expressing Kiss1R), kisspeptin is capable of activating multiple signaling pathway resulting in increased IP_3 formation, calcium mobilization, arachidonic acid release, and MAP kinase phosphorylation [7]. Although the kisspeptin induction of GnRH release in hypothalamic explants from immature animals incubated in vitro is reported to involve recruitment of ERK1/2 and p38 kinases, these actions of kisspeptin have not been confirmed in adult GnRH neurons [50, 72]. In native GnRH neurons, the PLC inhibitor U73122 inhibits the effects of kisspeptin [14, 50], and indeed, it is known that all mammalian TRPC channels require PLC for activation [60]. Therefore, it appears that Gq-coupled GPR54 activates PLCβ to signal downstream to open TRPC channels in GnRH neurons, thereby allowing the influx of sodium and calcium. Interestingly, in POMC neurons, PLCγ1 appears to be the isozyme coupled to TRPC channel activation by leptin [69].

Classically, the TRPC3, 6, and 7 subfamily is DAG sensitive [59, 73]. Although TRPC3 and 7, and to a lesser extent TRPC6, transcripts are expressed in GnRH neurons, the surrogate DAG signaling molecule 2-acetyl sn-glycerol (OAG) has only a small effect to activate an inward current (~25% of the kiss-peptin-induced current) in GnRH neurons [14]. A potential explanation is that both hydrolysis of PIP_2 by PLCβ and the calcium trigger that facilitates the TRPC channel opening (i.e., the influx of Ca^{2+} through calcium channels [74]) might be missing when applying OAG alone to GnRH neurons. In addition, La^{3+} at a 100 μM concentration, which potentiates TRPC4 and 5 and blocks TRPC3, 6, and 7 channels [75], did not attenuate or augment the kisspeptin-induced current, which indicates that an ensemble of these channel subunits must exist in GnRH neurons as revealed by single-cell RT-PCR [14]. Indeed, extracellular 2-APB (100 μM), which is a potent blocker of TRPC3, 4, 5, and 6 channels, and FFA, which is a potent blocker of TRPC4 and 5 channels, inhibit the effects of kisspeptin in GnRH neurons (Fig. 6.3). These blockers have a similar effects on the leptin activation of TRPC channels in arcuate POMC and kisspeptin neurons, although lanthanum clearly potentiates the leptin- induced activation of TRPC currents in POMC and kisspeptin neurons [69, 70], suggesting subtle differences between the GnRH neurons and the other two cell types. Collectively, these data suggest that, although all of the "brain" TRPC channels are expressed in GnRH neurons, the TRPC1, 4, and 5 family appear to be major (key) players in mediating the effects of kisspeptin in GnRH neurons [14].

Kisspeptin Inhibition of Kir Channels and Their Role in GnRH Neuronal Excitability

Kisspeptin augments the activity of GnRH neurons in part via inhibition of Kir potassium channels [13, 14, 50]. In this respect, the Kir blockers barium (0.3 mM) and tetraethylammonium (20 mM) robustly inhibit the kisspeptin-induced potassium currents in GnRH neurons [13, 14, 50]. The importance of kisspeptin inhibition of Kir is further substantiated by the ability of kisspeptin to attenuate the GABA$_B$-induced hyperpolarization in GnRH neurons [54].

GABA is one of the most important neurotransmitters that regulate the excitability of GnRH neurons. Multiple studies have shown that GABA activates Cl$^-$ currents in GnRH neurons, and these effects are blocked by GABA$_A$ receptor antagonists [44, 46, 47, 76–78]. It is generally accepted that activation of GABA$_A$ receptors depolarizes and excites GnRH neurons [46, 78–80]. Several GABA$_A$ receptor subunits have been identified in GnRH neurons, including α1, α2, α3, α5, β1, β2, β3, γ1, γ2, and the rho 1 subunits [78, 81, 82]. The GABA$_B$ receptor subunits, R1 and R2, are also found in GnRH neurons [54, 83], and GABA activates GABA$_B$-receptors in GnRH neurons [54, 84]. Moreover, as has been demonstrated in numerous other hypothalamic neurons [85–90], GABA$_B$ receptors are coupled (G$\alpha_{i/o}$) to activation of G protein-coupled inwardly rectifying K$^+$ (GIRK) channels, resulting in a robust hyperpolarization of GnRH neurons.

The importance of Kir channels in modulating GnRH neuronal excitability has been well documented [13, 14, 40, 53–55, 84]. Female GnRH neurons sit at a relatively negative resting membrane potential (–63 mV) that is due, in part, to the activity of Kir channels including GIRKs and K$_{ATP}$ channels [53, 54]. For example, blocking the K$_{ATP}$ channels with the sulfonylurea tolbutamide significantly depolarizes the cells by 4–6 mV, which puts the membrane potential in the range of most parvocellular hypothalamic neurons [53, 91]. In addition, GABA release is regulated by E$_2$ through presynaptic mechanisms [85, 87, 92–94] that affect GnRH neuronal activity [40, 95]. Augmented E$_2$-induced GABA$_B$ receptor activity would further hyperpolarize the membrane through increased GIRK channel activity. However, this inhibitory tone must be attenuated during the excitatory (preovulatory) phase of GnRH neurons. One possible mechanism is that kisspeptin signaling via KissR provides the stimulus to overcome this strong inhibitory tone (Fig. 6.4). Previous investigators have shown that there is a robust kisspeptin drive during E$_2$ "positive feedback" [13, 15, 96], and kisspeptin counters the hyperpolarizing effects of activation of GIRKs by the GABA$_B$ agonist baclofen, μ-opioid receptor agonists (Zhang et al., unpublished findings), and other Ba^{2+}-sensitive inwardly rectifying K$^+$ channels in general [13, 14]. Moreover, G$\alpha_{q/11}$-coupled receptors are known to desensitize (i.e., heterologous desensitization) G$\alpha_{i/o}$-coupled receptors through PIP$_2$ hydrolysis and attenuating the GIRK-mediated hyperpolarization (Fig. 6.4) [97–99]. In addition to attenuating G$\alpha_{i/o}$-coupled receptor-mediated hyperpolarization, kisspeptin activates TRPC channels in GnRH neurons to cause further depolarization [14]. The Kiss1R-G$\alpha_{q/11}$-PLCβ signaling pathway would have a twofold effect to inhibit K$^+$ channels and activate TRPC channels, which underlies the pronounced excitatory effects of kisspeptin on GnRH neurons [12–14, 50, 52].

Interestingly MCH, although at higher concentrations, can block kisspeptin excitation of septal vesicular glutamate transporter 2 (vGluT2)-GnRH neurons by inhibiting Kir [55], which could be a mechanism by which GnRH neuronal excitability is reduced during certain physiological states.

Presynaptic Effects of Kisspeptin on GnRH Neuronal Excitability

Based on extracellular recording in GnRH neurons in slices obtained from oil- and E_2-treated mice, the kisspeptin-induced neuronal firing rate is potentiated in the E_2-treated females [13, 96]. The E_2-induced potentiation is reduced when GABA and glutamate inputs to GnRH neurons are blocked, suggesting the involvement of these fast synaptic transmitters in the E_2 effect. In addition, it is well known that kiss-peptin neurons in the AVPV are positively regulated by E_2 and are believed to contribute to positive feedback input to rodent GnRH neurons [15]. Therefore, E_2 may further augment the effects of kisspeptin in vivo via direct action on AVPV kiss-peptin neurons.

The precise localization of the kisspeptin inputs to GnRH neurons has not been identified. The AVPV is a complex nucleus that expresses other neurotransmitters in addition to kisspeptin, such as dopamine, GABA, and glutamate [100, 101]. Although projections from the AVPV to GnRH neurons have been described by a number of investigators, the functional interactions between the AVPV and GnRH neurons are just beginning to be elucidated [102]. Thus, stimulation of the AVPV and recording of responses in GnRH neurons reveals that low stimulation rates (<1 Hz) induce glutamate and GABA synaptic currents in GnRH neurons, whereas higher frequency stimulation (5–10 Hz) induces delayed excitation believed to be kisspeptin mediated since the response is absent in *Kiss1r* knockout animals and antagonized by the kisspeptin antagonist peptide 318 [102]. Therefore, the AVPV kisspeptin neurons may provide a critical excitatory input to GnRH neurons.

Kisspeptin neurons in the arcuate nucleus are negatively regulated by E_2 and are believed to be involved in negative feedback regulation of GnRH secretion [15, 37]. The mechanism by which arcuate neurons negatively regulate GnRH neurons is not completely understood, but has been proposed to also involve arcuate POMC neurons [40]. Interestingly, kisspeptin-immunoreactive fibers in the arcuate nucleus form close contacts onto POMC neurons, and kisspeptin excites POMC neurons via activation of a nonselective cation (TRPC?) channel and activation of a sodium/ calcium exchanger [103]. β-endorphin positive fibers, presumably from arcuate POMC neurons, are highly expressed in the POA, and β-endorphin synapses, as well as μ-opioid receptor expression, are found specifically on GnRH neurons [104–107]. Therefore, kisspeptin may influence GnRH neurons indirectly via actions on arcuate POMC neurons. While μ-opioid receptor activation would be inhibitory to GnRH neurons [40], recently it has been shown that an agonist of the melanocortin receptors 3 and 4 excite GnRH neurons [108]. This would suggest that POMC neurons may also excite GnRH neurons via release of αMSH, a POMC product. Clearly, further studies are needed to elucidate the role of arcuate kiss-peptin neurons in negative feedback regulation of GnRH neurons, as well as the role of these neurons in GnRH neuronal pulsatility.

Kisspeptin and Burst Firing in GnRH Neurons

It is well known that GnRH is released in a pulsatile manner, and the hypothalamic surge of GnRH and subsequent pituitary release of LH are required for triggering ovulation in the female. Although single action potential-induced calcium influx is enough to spark the release of classical transmitters, burst firing or tetanic stimulation is required for the release of neuropeptides such as vasopressin, oxytocin, substance P, and atrial natriuretic factor [109–111]. Experiments in vitro using perifused hypothalamic tissue, primary hypothalamic cultures, or a GT1 GnRH neuronal cell line have revealed that pulsatile GnRH release is evident in vitro [112–114]. Recordings in slices from genetically modified mice that express the calcium ratio-metric indicator Pericam in GnRH neurons have shown that intracellular calcium transients, generated through L-type calcium channels and amplified by calcium release from intracellular stores, are synchronized with burst firing in a subpopulation of GnRH neurons [115]. However, a recent publication suggests that kisspeptin inhibits high voltage-activated (HVA) Ca^{2+} (e.g., L-type) channels, which would attenuate the calcium-activated afterhyperpolarization and thereby promote sustained firing [116]. Regardless of the role of the HVA Ca^{2+} channels in kisspeptin's downstream signaling, T-type calcium channels and hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels and their respective currents are highly expressed in GnRH neurons [117–120]. Both the h-current and T-type calcium current contribute to burst firing [118, 121], and activation of these vital conductances is dependent on membrane (hyper) polarization [121, 122]. In fact, a hyperpolarizing stimulus removes the inactivation of T-type calcium channels and also activates the h-current. Both the GABA (via GABA$_B$) and opioids (via μ-opioid receptors) provide this hyperpolarizing stimulus to GnRH neurons [40, 54, 84]. The membrane hyperpolarization generated by Gα$_{i/o}$-coupled receptors during E$_2$ negative feedback sets the stage for recruiting both the HCN and T-type calcium channels that are critical for phasic burst firing of GnRH neurons [40, 45, 121]. Ultimately, kisspeptin attenuates the hyperpolarized state of "negative feedback" by inhibiting K$^+$ channel activity and opening up TRPC channels to cause sustained depolarization and firing [13, 14, 50, 52]. Furthermore, the calcium-activated afterhyperpolarizing currents (e.g., small conductance, calcium-activated K$^+$, SK) would serve to repolarize the cell membrane to allow the continued oscillation and burst firing [115, 123–125].

Summary

It is clear that kisspeptin has pronounced pre- and postsynaptic effects on GnRH neuronal excitability. Presynaptically, kisspeptin increases the excitatory drive (both GABA$_A$ and glutamate) to GnRH neurons, and postsynaptically kisspeptin binds to Kiss1R to activate a PLCβ signaling pathway that has multiple downstream effects to cause a robust and sustained depolarization of GnRH neurons. These downstream effects include inhibition of inwardly rectifying K$^+$ (K$_{ATP}$ and GIRK) channels and activation of TRPC1, 4, 5 channels (Fig. 6.4). Although all of the intermediary players (signaling molecules) have not been identified, it is clear that the sustained action potential firing in GnRH neurons is due to these membrane circumscribed actions of the Gαq-signaling pathway. In addition, T-type calcium channels are probably involved in the initial facilitation of TRPC channel opening (Fig. 6.4). However, future experiments need to address these nuances of Kiss1R signaling.

In addition, the effects of kisspeptin on the presynaptic glutamatergic and GABAergic neurons also need to be elucidated. Regardless, it is clear that the highly potent neuromodulator, kisspeptin, robustly depolarizes GnRH neurons and promotes burst firing via multiple cellular actions.

Acknowledgments

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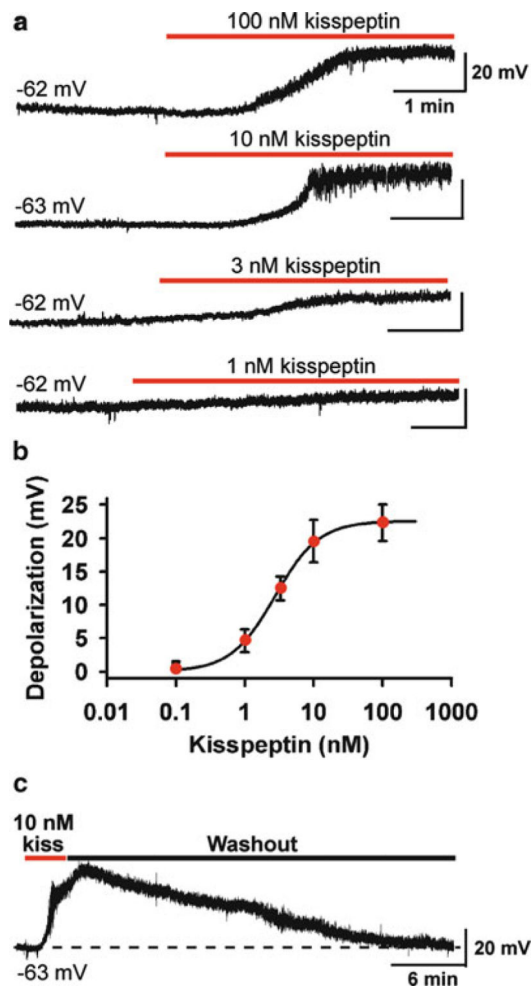
Fig. 6.1.

Kisspeptin depolarizes GnRH neurons in a concentration-dependent manner. (**a**) Representative traces showing that kisspeptin (1–100 nM) depolarized GnRH neurons in a concentration-dependent manner. The initial membrane potential for each trace is indicated. Only one cell was recorded from one slice. (**b**) Concentration–response curve of the kisspeptin-induced depolarization. Data are presented as mean $\pm$ SEM. The EC $_{50}$ for the kisspeptin-induced depolarization was 2.8 ± 0.2 nM ($n = 8$–14) based on a logistic equation fit to the data points. (**c**) The kiss-peptin (10 nM)-induced depolarization was long lasting and typically took 30 min to recover. From Zhang C, Roepke TA, Kelly MJ, Rønnekleiv OK. Kisspeptin depolarizes gonadotropin- releasing hormone neurons through activation of TRPC-like cationic channels. J Neurosci 2008; 28: 4423– 4434. Reprinted with permission from The Society for Neuroscience
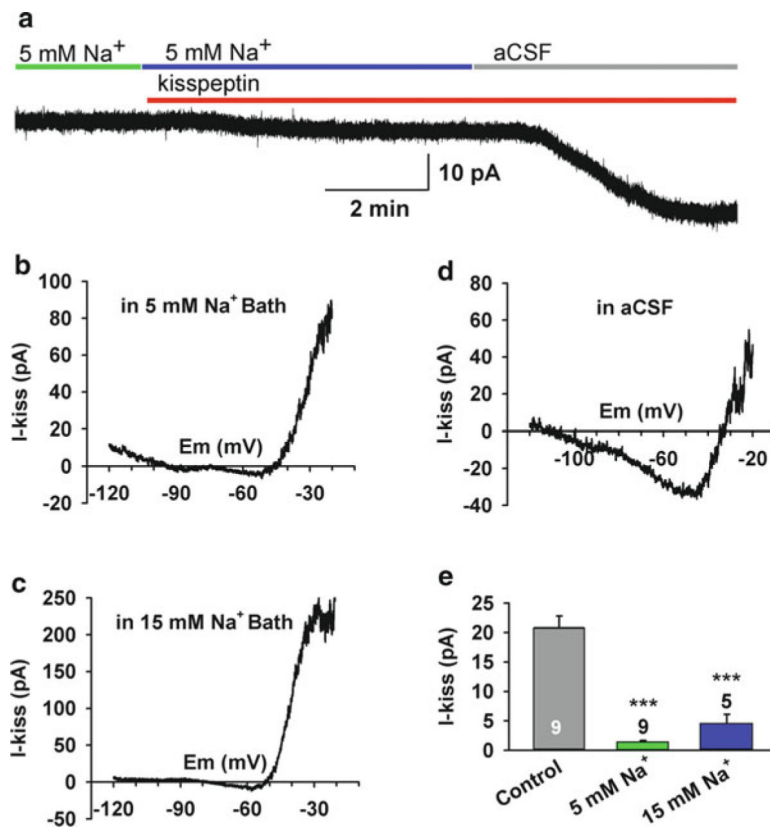
Fig. 6.2.

Kisspeptin predominantly activates a sodium-dependent, nonselective cationic (TRPC) channel. (**a**) The kisspeptin-induced inward current (at −60 mV) was greatly reduced in low Na $^+$ bath solution (5 mM Na $^+$/140 mM N -methyl-d-glucamine [NMDG $^+$]), and switching back to normal aCSF (control) solution revealed a kisspeptin sensitive inward current of 28 pA in this GnRH neuron. (**b**, **c**) The I–V relationships of the kisspeptin-evoked current in a low Na $^+$ bath solution (5 and 15 mM Na $^+$) between −20 and −120 mV showed a greatly reduced inward current. (**d**) A typical I–V relationship of the kisspeptin-induced inward current in normal aCSF (control) solution showed a larger inward current (−30 pA at −60 mV). (**e**) Summary of the effects of the extra-cellular sodium concentration on the kisspeptin-induced inward current at −60 mV. *** $p < 0.001$, significantly different from the effects of kisspeptin under control aCSF conditions. Cell numbers tested are indicated for each group. Error bars indicate SEM. From Zhang C, Roepke TA, Kelly MJ, Rønnekleiv OK. Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-like cationic channels. J Neurosci 2008; 28: 4423–4434. Reprinted with permission from The Society for Neuroscience
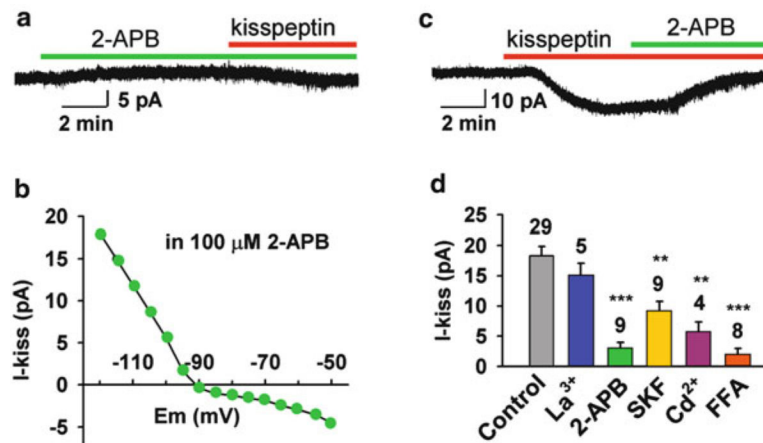
Fig. 6.3.

Effects of TRPC channel blockers on the kisspeptin-induced inward currents at −60 mV.
(**a**) A representative recording showing that 2-APB (100 μM), which had very little effect
on basal holding current, potently blocked the kisspeptin (100 nM)-evoked inward current.
(**b**) Mean *I–V* relationship of the kisspeptin-sensitive current in the presence of 2-APB
reversed at −90 mV (*n* = 4), clearly indicating that a Kir channel was inhibited by
kisspeptin. (**c**) A representative recording showing that 2-APB (100 μM) applied after
kisspeptin also strongly blocked the kisspeptin- evoked inward current. (**d**) Summary of the
effects of different TRPC channel blockers (100 μM La $^{3+}$, 100 μM 2-APB, 30 μM
SKF96365, 250 μM Cd $^{2+}$, 100 μM flufenamic acid) on the kisspeptin-induced inward
currents at −60 mV. Blockers were applied 5–7 min before or after the application of
kisspeptin (100 nM). The percent inhibition for the different blockers was as follows: 17.4%
for 100 μM La $^{3+}$, 83.6% for 100 μM 2-APB, 50% for 30 μM SKF, 68.7% for 250 μM
Cd $^{2+}$, and 89.6% for 100 μM FFA. ** $p < 0.01$ and *** $p < 0.001$, significantly different
from the kisspeptin response under control aCSF conditions. Cell numbers tested are
indicated. Error bars indicate SEM. From Zhang C, Roepke TA, Kelly MJ, Rønnekleiv OK.
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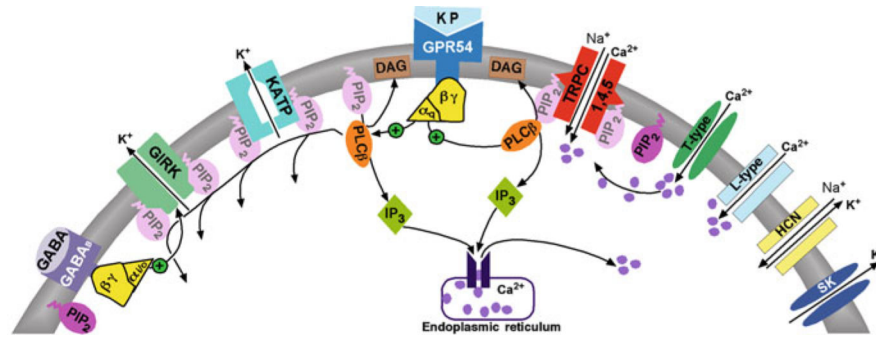from The Society for Neuroscience

Fig. 6.4.

Model of kisspeptin's actions to depolarize GnRH neurons and facilitate burst firing. Kisspeptin binds to its cognate GPR54 receptor, which is Gq-coupled to activate phospholipase Cβ. PLCβ has multiple downstream actions resultant from cleaving phosphatidylinositol 4,5 bisphosphate (PIP$_2$) to inositol 1,4,5-triphosphate (IP$_3$) and diacylglycerol (DAG). Since PIP$_2$ facilitates Kir channel opening, cleavage of this fatty acid attenuates K-ATP and GIRK channel opening. Inhibition of GIRK channels renders a number of Gα$_{i/o}$-coupled receptors ineffective to inhibit GnRH neurons (GABA$_B$, μ-opioid, NPY, MCH, etc.). On the other hand, cleavage of PIP$_2$ facilitates TRPC 4 channel opening [126]. In addition the membrane-associated fatty acid DAG probably activates the TRPC1, 4, 5 channel complex [Note: The OAG (analogue of DAG) was only weakly effective to open the TRPC channels since PIP$_2$ still exerted a strong inhibition of the TRPC channel complex]. Ca^{2+} potentiates the agonist-activated TRPC1, 4, 5 complex, and plasma membrane calcium channels appear to play a critical role. Intracellular 2-APB dialysis, which effectively blocks IP3 receptor-mediated release of Ca^{2+}, was ineffective, but extracellular Cd^{2+} potently inhibited TRPC1, 4, 5 channel activity. Therefore, we propose that low voltage-activated T-type calcium channels are initially involved in facilitating TRPC channel opening. Once depolarized, Ca^{2+} entry through high voltage-activated Ca^{2+} channels can also contribute to facilitating TRPC channel opening. Also illustrated are other channels contributing to burst firing activity such as the hyperpolarization-activated, cyclic nucleotide-gated (HCN, pacemaker) channel, and the small conductance, Ca^{2+}-activated K$^+$ channel (SK), which is involved in the repolarization of the membrane following a burst of action potentials