Published in final edited form as:

Mol Cell Endocrinol. 2010 January 27; 314(2): 158. doi:10.1016/j.mce.2009.05.015.

Pulsatile GnRH secretion: Roles of G protein-coupled receptors, second messengers and ion channels

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Abstract

The pulsatile secretion of GnRH from normal and immortalized hypothalamic GnRH neurons is highly calcium-dependent and is stimulated by cAMP. It is also influenced by agonist activation of the endogenous GnRH receptor (GnRH-R), which couples to multiple G proteins. This autocrine mechanism could serve as a timer to determine the frequency of pulsatile GnRH release by regulating Ca^{2+} - and cAMP-dependent signaling and GnRH neuronal firing. The firing of individual and/or bursts of action potentials (APs) in spontaneously active GnRH neurons is followed by afterhyperpolarization (AHP) that lasts from several milliseconds to several seconds. GnRH-induced activation of GnRH neurons causes a significant increase in medium AHP that is partially sensitive to apamin. GnRH-induced modulation of Ca^{2+} influx and the consequent changes in AHP current suggest that the GnRH receptors expressed in hypothalamic GnRH neurons are important modulators of their neuronal excitability. The coexistence of multiple regulatory mechanisms could provide a high degree of redundancy in the maintenance of this crucial component of the reproductive process. It is also conceivable that this multifactorial system could reflect the gradation from simple to more complex neuroendocrine control systems for regulating hypothalamo-pituitary function and gonadal activity during the evolution of the GnRH pulse generator.

1. Introduction

Mammalian reproduction is controlled by integrated sets of interactions between the hypothalamus, pituitary gland and gonads. Each component of the reproductive system is regulated by feedback mechanisms that coordinate the processes leading to gonadotropin secretion, gamete production and continuation of the species (Knobil, et al. 1980, McCann, et al. 1998). The generation of pulsatile GnRH release at the median eminence is the central and essential element governing reproductive function, and depends on the coordinated activities of the 1500 or so GnRH neurons that are located in the hypothalamus (Schwanzel-Fukuda, et al. 1992, Wray 2001). In most mammalian species the GnRH neurons are distributed in the preoptic area and adjacent sites in the rostral region of the hypothalamus, rather than concentrated in a discrete nucleus (Lehman, et al. 1997). These scattered neurons are believed to form a diffuse neural network that functions coordinately as a GnRH pulse generator (Knobil 1990). The episodic mode of GnRH secretion from hypothalamic neurons, and of GnRH receptor activation in pituitary gonadotrophs, are essential for optimal gonadotropin synthesis and secretion from hypothalamic neurons, and ultimately for normal reproductive function

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(Knobil 1988). The genesis of GnRH pulse generation in the hypothalamus is still incompletely understood, but episodic neuropeptide secretion appears to be an intrinsic property of the GnRH neuron that is dependent on intracellular signaling and mechanism(s) leading to coordinated bursts of GnRH release (Krsmanovic, et al. 1999).

2. Regulation of calcium and cAMP in GT1-7 neurons

Calcium and cyclic AMP (cAMP) are important factors in the mechanism of episodic signaling in hypothalamic GnRH neurons (Vitalis, et al. 2000). The observation that cAMP production in GT1-7 neurons is stimulated by increased extracellular Ca^{2+} and the Ca^{2+} channel agonist, BK-8644, and is diminished by low extracellular Ca^{2+} and treatment with dihydropyridine analogs, is consistent with activation and/or inhibition of the calcium-dependent adenylyl cyclase type I (ACI) expressed in these cells (Krsmanovic, et al. 2001). Agonist activation of the endogenous GnRH receptor in GT1-7 neurons elicits a bell-shaped change in cAMP production, the inhibitory phase of which is dependent on coupling of the GnRH receptor to G_i -related proteins. The stimulation of cAMP production by activation of endogenous LH receptors is enhanced by nanomolar concentrations of GnRH but is abolished by micromolar concentrations of the peptide, in a PTX-sensitive manner (Mores, et al. 1996). These findings indicate that cAMP production in GnRH neuronal cells is maintained by Ca^{2+} entry through voltage-sensitive calcium channels, leading to activation of ACI, and that Ca^{2+} influx-dependent activation of ACI acts in conjunction with AC-regulatory G proteins to determine basal and agonist-stimulated levels of cAMP production (Krsmanovic, et al. 2001). Another important factor is the ability of cAMP to activate cyclic nucleotide-gated channels (CNG), further stimulating Ca^{2+} entry and promoting GnRH secretion (El Majdoubi and Weiner 2002, Weiner and Charles 2001).

3. Mechanism of pulsatile GnRH secretion in GnRH neurons and GT1-7 cells

The pulsatile secretion of gonadotropin-releasing hormone (GnRH) from normal and immortalized hypothalamic GnRH neurons is highly calcium-dependent and is stimulated by cAMP (Krsmanovic, et al. 1992, Vitalis, et al. 2000). Upon agonist activation the endogenous GnRH-R becomes coupled to $\text{G}_{q/11}$, leading to release of membrane-bound $\alpha_{q/11}$ subunits, activation of PLC- β , and increased inositol phosphate/ Ca^{2+} signaling (Krsmanovic, et al. 2003, Naor 1997, Naor, et al. 1995). Conversely, GnRH antagonists increase membrane-associated $\alpha_{q/11}$ subunits and abolish receptor signaling and pulsatile GnRH secretion (Krsmanovic, et al. 2003). GnRH also stimulates cAMP production, but at high concentrations has a PTX-sensitive inhibitory effect, indicative of receptor coupling to G_i . The capacity of agonist-activated GnRH-R to activate both G_s and G_i proteins was also demonstrated by the ability of GnRH to reduce membrane-associated α_s and α_{i3} levels, and to increase or decrease cAMP production at appropriate agonist concentrations. Conversely, membrane-associated α_{i3} is increased by GnRH antagonist and pertussis toxin (PTX) treatment, with concomitant loss of pulsatile GnRH secretion (Krsmanovic, et al. 2003). Treatment with cholera toxin reduces α_s and increases cAMP production in parallel with the responses induced by high nanomolar GnRH concentrations. Treatment with cholera toxin and 8-bromo-cAMP amplified episodic GnRH pulses but did not affect their frequency. These findings suggest that an agonist concentration-dependent switch in coupling of the GnRH-R between specific G proteins modulates neuronal Ca^{2+} signaling via G_s -cAMP stimulatory and G_i -cAMP inhibitory mechanisms. Activation of G_i also inhibits GnRH neuronal function and episodic secretion, probably in part by regulating membrane ion currents (Krsmanovic, et al. 2003). This autocrine mechanism could serve as a timer to determine the frequency of pulsatile GnRH release by regulating Ca^{2+} - and cAMP-dependent signaling and GnRH neuronal firing.

The secretion of GnRH is mainly regulated and controlled by signaling pathways initiated at the plasma membrane. In contrast, the control of gene expression is usually a long-term process and is responsible for the changes in transcription of the gene (Naor 2009). In GT1-7 cells, agonist binding to the GnRH-R causes phosphorylation of MAPKs that is mediated by protein kinase C (PKC)-dependent transactivation of the EGFR. An analysis of the mechanisms involved in this process showed that GnRH stimulation of GT1-7 cells causes release/shedding of the soluble ligand, heparin binding epidermal growth factor (HB-EGF), as a consequence of metalloprotease activation. The signaling characteristics of HB-EGF closely resembled those of GnRH and EGF in terms of the phosphorylation of EGFR, Shc, ERK1/2, and RSK-1, as well as the nuclear translocation of RSK-1 (Naor, et al. 2000, Shah, et al. 2006). However, neither the selective Src kinase inhibitor PP2 nor the overexpression of negative regulatory Src kinase or dominant negative Pyk2 had any effect on HB-EGF-induced responses. In contrast to GT1-7 cells, HEK 293 cells expressing the GnRH-R did not exhibit metalloprotease induction and EGFR transactivation during GnRH stimulation. These data indicate that the GnRH-induced transactivation of the EGFR and subsequent ERK1/2 phosphorylation result from ectodomain shedding of HB-EGF through PKC-dependent activation of metalloprotease (s) in GT1-7 neuronal cells (Shah, et al. 2005).

4. Role of serotonin receptors in signaling and neurosecretion in GnRH neurons

Hypothalamic GnRH neurons and immortalized GT1-7 cells express several GPCRs and ion channels, exhibit spontaneous electrical activity, and release GnRH in a pulsatile manner at a frequency similar to that of GnRH secretion *in vivo* (Krsmanovic, et al. 1999, Krsmanovic, et al. 1996). Agonist activation of the neuronal GnRH receptor stimulates the phospholipase C/inositol 1,4,5-triphosphate/calcium (PLC/InsP₃/Ca²⁺) signaling pathway, increases or inhibits cAMP production in a dose-dependent manner, and modulates the frequency and amplitude of pulsatile GnRH release. These features are dependent on coupling of the GnRH receptor to diverse G proteins (Grosse, et al. 2000, Janovick, et al. 2007, Krsmanovic, et al. 2003). Five families of G protein-coupled 5-HT receptors (5-HT₁, primarily coupled to G_i; 5-HT₂, primarily coupled to G_q; 5-HT₄, 5-HT₆, and 5-HT₇, primarily coupled to G_s) have been classified by structural and transductional criteria and regulate two major intracellular second messenger pathways, adenylyl cyclase and phospholipase C (Hoyer, et al. 2002). Synaptic contacts have been observed between tritiated 5-hydroxytryptamine-labelled boutons and GnRH-immunoreactive dendrites. GnRH-immunoreactive axon terminals function as presynaptic elements in contact with unidentified dendritic spines, shafts or perikarya. These observations provide a morphological basis for the finding that 5-hydroxytryptamine-containing neurons can act directly on GnRH release (Kiss and Halasz 1985).

Treatment of perfused GT1-7 neurons with serotonin, a non-selective 5-HT receptor agonist, causes a transient increase in GnRH release followed by reduction of pulsatile GnRH secretion, dose- and time-dependent stimulation of inositol phosphate production (InsP), and both stimulation and inhibition of cAMP production. The inhibitory action of serotonin on cAMP production is abolished by prior treatment with PTX. These findings indicate that PLC/InsP₃/Ca²⁺ and AC/cAMP stimulatory and inhibitory signaling pathways are activated during treatment of GT1-7 neurons with serotonin (Wada, et al. 2006). Deconvolution of the complex actions of serotonin on GT1-7 neuronal signaling and GnRH release was performed with selective 5-HT receptor agonist and antagonist analogs. Specific activation of the G_q-coupled 5-HT₂ receptors with both α -methylserotonin and 2,5-dimethoxy-4-iodoamphetamine [($\pm$)-DOI] stimulated InsP₃ production, inhibited cAMP production, and caused a delayed increase in GnRH release. Ketanserin, the selective antagonist of 5-HT₂ receptors, reversed the changes induced by the agonist analog (Wada, et al. 2006) (Fig. 1).

Treatment of perfused GT1-7 cells with SC 53116, a selective agonist for the G_s -coupled 5-HT₄ receptors, caused a robust and sustained increase in GnRH release. The increased GnRH release resembles that caused by treatment with 8-Br-cAMP, and indicates that cAMP is essential but not alone sufficient for the maintenance of pulsatile GnRH release. The exact manner in which 5-HT₄ treatment and the consequent increase in cAMP contribute to the episodic mode of GnRH secretion has yet to be determined, but its importance in the control of GnRH release is indicated by the above findings. It is possible that cAMP is essential, but not alone sufficient, for the maintenance of pulsatile GnRH release (Wada, et al. 2006) (Fig. 1).

Treatment of perfused GT1-7 cells with methoxyphenyl piperazine (2-MPP) and R(±)-8-hydroxy-DPAT, a selective 5-HT_{1A} agonist that couples to G_i , caused profound inhibition of pulsatile GnRH release. The inhibitory action of 2-MPP was abolished by prior treatment with methiothepin mesylate (MTTP), a selective 5-HT_{1A} receptor antagonist. cAMP production was also inhibited, and InsP₃ remained unchanged. The marked inhibitory effect of 5-HT_{1A} receptors on pulsatile GnRH secretion provides further evidence for the role of G_i -dependent mechanisms in the operation of the GnRH pulse generator (Wada, et al. 2006) (Fig. 1).

The ability of G protein-coupled 5HT receptor subtypes expressed in GnRH neurons to activate single or multiple G proteins in a time- and dose-dependent manner regulates the PLC/InsP₃/Ca²⁺ and AC/cAMP signaling pathways, and ultimately modulates the frequency and amplitude of pulsatile GnRH release. This process, in conjunction with the spontaneous electrical activity of the GnRH neuron, contributes to the control of the episodic mode of pulsatile neuropeptide secretion that is characteristic of GnRH neuronal function *in vivo*.

5. Modulation of spontaneous firing of action potentials (APs) by GnRH agonist and antagonist analogs

Spontaneous firing of APs in the cell-attached current-clamp mode was evident in both native and immortalized GnRH neurons and was similar to that observed in olfactory placode GnRH neurons (Kusano, et al. 1995), as well as GnRH neurons expressing green fluorescent protein (Kuehl-Kovarik, et al. 2002, Spergel, et al. 1999, Suter, et al. 2000), and immortalized GnRH neurons (Costantin and Charles 1999, LeBeau, et al. 2000, Van Goor, et al. 1999). In our study, GnRH treatment of both native and GT1-7 neurons consistently increased the frequency of AP firing (Xu, et al. 2004). Such enhanced AP firing was GnRH receptor-dependent, and was diminished by a GnRH antagonist. Interestingly, the basal firing of APs in GnRH neurons was also GnRH receptor-dependent and was abolished during GnRH antagonist treatment. Basal electrical activity returned during washout of the antagonist, indicating that the GnRH autoregulatory system participates in the control of spontaneous APs firing in both native and GT1-7 neurons (Martinez-Fuentes, et al. 2004). Cultured native GnRH neurons and immortalized GnRH neurons have closely similar electrical properties, and GT1-7 neurons can serve as surrogates for native neurons in electrophysiological studies. Action potentials and the associated Ca²⁺ influx are followed by slow afterhyperpolarizations (sAHPs) caused by a voltage-insensitive, Ca²⁺-dependent K⁺ current (Nunemaker, et al. 2003). Slow AHPs are common in mammalian neurons and are present in both peripheral and central nervous systems. The firing of individual and/or bursts of action potentials (APs) in spontaneously active GnRH neurons is followed by hyperpolarization that lasts from several milliseconds (ms) to several seconds (s). Such hyperpolarization is mediated by the activation of two families of Ca²⁺-activated K⁺ channels (Liu and Herbison 2008) (Fig. 2). Big conductance (BK) channels contribute to action potential repolarization, whereas small conductance (SK) channels underlie the AHP and mediate firing frequency and spike-frequency adaptation. Fast afterhyperpolarizations (fAHP) and sAHPs were not observed during spontaneous firing of fast rhythmic APs (Fig. 2). Treatment of GnRH neurons with GnRH caused a significant

increase in medium afterhyperpolarization (mAHP) that was partially sensitive to apamin. Also, treatment of GnRH neurons with 10 nM GnRH increased the occurrence of high-frequency broad APs, with unchanged decay constants for fAHP and mAHP. In contrast, treatment of GnRH neurons with 1 μ M GnRH abolished mAHP current, but did not affect the occurrence of fAHP current. That was followed by subthreshold afterdepolarization potential (ADP) and significant reduction of the frequency of AP firing (Jager and Grissmer 2004). These data indicate that AP- and GnRH-driven Ca^{2+} influx in GnRH neurons determine the profile of after-hyperpolarization currents and consequently mediate firing frequency and the spike-profile (Fig. 2). The GnRH-induced modulation of Ca^{2+} influx, and the consequent changes in AHP current, suggest that the GnRH receptors expressed in hypothalamic GnRH neurons are important modulators of their neuronal excitability.

6. Gonadotropin receptor-mediated neurosecretion, second messengers, and electrical activity of native and immortalized GnRH neurons

Activation of luteinizing hormone/human chorionic gonadotropin (LH/hCG) receptors in cultured hypothalamic cells and GT1-7 neurons stimulates adenylyl cyclase-cAMP signaling and reversibly abolishes pulsatile GnRH release (Mores, et al. 1996). The agonist-induced cAMP response is time-dependent and shows early stimulatory and delayed inhibitory phases. The time-dependent decrease in cAMP production was prevented by pertussis toxin, indicating that LH/hCG receptors are coupled to both G_s and G_i/G_o proteins. The apparent switch of coupling between G_s and G_i/G_o was prevented by the protein kinase A (PKA) inhibitor, H89, suggesting that PKA-mediated phosphorylation of the LH/hCG receptor promotes its coupling to G_i/G_o (Daaka, et al. 1997). Treatment with hCG also inhibited potassium-induced increases in cytosolic calcium ($[\text{Ca}^{2+}]_i$), indicating that activated LH/hCG receptors may influence the gating of voltage-sensitive calcium channels and membrane excitability (Fig. 3). In recent studies on cultures derived from the E13 olfactory placode, the firing of spontaneous APs in both identified GnRH neurons and GT1-7 cells was reversibly abolished by LH/hCG treatment (Hu, et al. 2006). In excised inside-out patches, potassium current was effectively blocked by hCG in both native GnRH neurons and GT1-7 neurons. This inhibitory action of hCG was prevented by prior treatment with pertussis toxin (PTX), indicating that the effect is mediated by liberated subunits of G_i/G_o . These findings demonstrate that agonist-activation of LH/hCG receptors expressed in native and immortalized GnRH neurons slows the firing of spontaneous APs by affecting the gating properties of potassium and calcium channels, and consequently abolishes pulsatile GnRH release (Hu, et al. 2006) (Fig. 3).

Conclusion

Hypothalamic and immortalized GnRH neurons express several G protein-coupled receptors and ion channels, exhibit spontaneous electrical activity, and release GnRH in a pulsatile manner with frequency similar to that of GnRH secretion *in vivo*. These features are dependent on coupling of the GnRH receptor to diverse G proteins.

Serotonin, the endogenous non-selective 5-HT receptor agonist, activates the $\text{InsP}_3/\text{Ca}^{2+}$ signaling pathway and exerts both stimulatory and inhibitory action on cAMP production and GnRH release in GT1-7 cells. Selective activation of the G_q -coupled 5-HT receptor, stimulates InsP_3 production, inhibits cAMP production and causes a delayed increase in GnRH release. In contrast, pulsatile GnRH release was profoundly inhibited during activation of the G_i -coupled 5HT receptor. Activation of the G_s -coupled 5-HT receptor with the selective agonist stimulated cAMP production, inhibited InsP_3 production, and caused a sustained and robust increase in GnRH release.

The ability of G protein-coupled receptor subtypes expressed in GnRH neurons to activate single or multiple G proteins in a time- and dose-dependent manner regulates the PLC/InsP₃/Ca²⁺ and AC/cAMP signaling pathways, and ultimately modulates the frequency and amplitude of pulsatile GnRH release. This process, in conjunction with the spontaneous electrical activity of the GnRH neuron, contributes to the control of the episodic mode of pulsatile neuropeptide secretion that is characteristic of GnRH neuronal function *in vivo*.

The firing of individual and/or bursts of action potentials in spontaneously active GnRH neurons is followed by hyperpolarization that lasts from several milliseconds to several seconds. Treatment of GnRH neurons with low nanomolar GnRH concentrations caused a significant increase in mAHP that was partially sensitive to apamin. In contrast, treatment of GnRH neurons with micromolar GnRH concentrations abolished mAHP current, but did not affect the occurrence of fAHP current. That was followed by subthreshold after-depolarization potential and significant reduction of the frequency of AP firing. These data indicate that AP- and GnRH-driven Ca²⁺ influx in GnRH neurons determines the profile of after-hyperpolarization currents and consequently mediates firing frequency and the spike-profile. GnRH-induced modulation of Ca²⁺ influx and the consequent changes in AHP current suggest that the GnRH receptors expressed in hypothalamic GnRH neurons are important modulators of their neuronal excitability.

Activation of the LHR in cultured hypothalamic cells and GT1-7 neurons transiently stimulates and subsequently inhibits cAMP production and pulsatile GnRH release. LH-induced release of membrane-bound G_{as} and G_{ai3} subunits are indicative of differential G protein coupling to the LHR. Action potential firing in identified GnRH neurons was also initially increased and then progressively decreased during LH treatment. The inhibitory action of LH on AP firing was prevented by PTX. RT-PCR analysis of GT1-7 neurons revealed the expression of G protein-gated inwardly rectifying potassium (GIRK) channels in these cells. The LH-induced currents were inhibited by PTX and were identified as GIRK currents. These responses indicate that agonist-stimulation of endogenous LHR expressed in GnRH neurons activates GIRK channels, leading to suppression of membrane excitability and inhibition of AP firing. These findings demonstrate that regulation of GIRK channel function is a dominant factor in gonadotropin-induced abolition of pulsatile GnRH release. Furthermore, this mechanism could contribute to the suppression of pituitary function during pregnancy.

References

- Costantin JL, Charles AC. Spontaneous action potentials initiate rhythmic intercellular calcium waves in immortalized hypothalamic (GT1-1) neurons. *J Neurophysiol* 1999;82:429–435. [PubMed: 10400969]
- Daaka Y, Luttrell LM, Lefkowitz RJ. Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature* 1997;390:88–91. [PubMed: 9363896]
- El Majdoubi M, Weiner RI. Localization of olfactory cyclic nucleotide-gated channels in rat gonadotropin-releasing hormone neurons. *Endocrinology* 2002;143:2441–2444. [PubMed: 12021210]
- Grosse R, Schmid A, Schoneberg T, Herrlich A, Muhn p, Schultz G, Gudermann T. Gonadotropin-releasing hormone receptor initiates multiple signaling pathways by exclusively coupling to G(q/11) proteins. *J Biol Chem* 2000;275:9193–9200. [PubMed: 10734055]
- Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* 2002;71:533–554. [PubMed: 11888546]
- Hu L, Wada K, Mores N, Krsmanovic LZ, Catt KJ. Essential role of G protein-gated inwardly rectifying potassium channels in gonadotropin-induced regulation of GnRH neuronal firing and pulsatile neurosecretion. *J Biol Chem* 2006;281:25231–25240. [PubMed: 16825187]
- Jager H, Grissmer S. Characterization of the outer pore region of the apamin-sensitive Ca²⁺-activated K⁺ channel rSK2. *Toxicon* 2004;43:951–960. [PubMed: 15208028]

- Janovick JA, Brothers SP, Knollman PE, Conn PM. Specializations of a G-protein-coupled receptor that appear to aid with detection of frequency-modulated signals from its ligand. *FASEB J* 2007;21:384–392. [PubMed: 17172315]
- Kiss J, Halasz B. Demonstration of serotonergic axons terminating on luteinizing hormone-releasing hormone neurons in the preoptic area of the rat using a combination of immunocytochemistry and high resolution autoradiography. *Neuroscience* 1985;14:69–78. [PubMed: 3883231]
- Knobil E. The neuroendocrine control of ovulation. *Hum Reprod* 1988;3:469–472. [PubMed: 3292570]
- Knobil E. The GnRH pulse generator. *Am J Obstet Gynecol* 1990;163:1721–1727. [PubMed: 2122728]
- Knobil E, Plant TM, Wildt L, Belchetz PE, Marshall G. Control of the rhesus monkey menstrual cycle: permissive role of hypothalamic gonadotropin-releasing hormone. *Science* 1980;207:1371–1373. [PubMed: 6766566]
- Krsmanovic LZ, Martinez-Fuentes AJ, Arora KK, Mores N, Navarro CE, Chen HC, Stojilkovic SS, Catt KJ. Autocrine regulation of gonadotropin-releasing hormone secretion in cultured hypothalamic neurons. *Endocrinology* 1999;140:1423–1431. [PubMed: 10067871]
- Krsmanovic LZ, Mores N, Navarro CE, Arora KK, Catt KJ. An agonist-induced switch in G protein coupling of the gonadotropin-releasing hormone receptor regulates pulsatile neuropeptide secretion. *Proc Natl Acad Sci U S A* 2003;100:2969–2974. [PubMed: 12591945]
- Krsmanovic LZ, Mores N, Navarro CE, Tomic M, Catt KJ. Regulation of Ca²⁺-sensitive adenylyl cyclase in gonadotropin-releasing hormone neurons. *Mol Endocrinol* 2001;15:429–440. [PubMed: 11222744]
- Krsmanovic LZ, Stojilkovic SS, Catt KJ. Pulsatile gonadotropin-releasing hormone release and its regulation. *Trends Endocrinol Metab* 1996;7:56–59. [PubMed: 18406726]
- Krsmanovic LZ, Stojilkovic SS, Merelli F, Dufour SM, Virmani MA, Catt KJ. Calcium signaling and episodic secretion of gonadotropin-releasing hormone in hypothalamic neurons. *Proc Natl Acad Sci U S A* 1992;89:8462–8466. [PubMed: 1326758]
- Kuehl-Kovarik MC, Pouliot WA, Halterman GL, Handa RJ, Dudek FE, Partin KM. Episodic bursting activity and response to excitatory amino acids in acutely dissociated gonadotropin-releasing hormone neurons genetically targeted with green fluorescent protein. *J Neurosci* 2002;22:2313–2322. [PubMed: 11896170]
- Kusano K, Fueshko S, Gainer H, Wray S. Electrical and synaptic properties of embryonic luteinizing hormone-releasing hormone neurons in explant cultures. *Proc Natl Acad Sci U S A* 1995;92:3918–3922. [PubMed: 7537379]
- LeBeau AP, Van Goor F, Stojilkovic SS, Sherman A. Modeling of membrane excitability in gonadotropin-releasing hormone-secreting hypothalamic neurons regulated by Ca²⁺-mobilizing and adenylyl cyclase-coupled receptors. *J Neurosci* 2000;20:9290–9297. [PubMed: 11125008]
- Lehman MN, Goodman RL, Karsch FJ, Jackson GL, Berriman SJ, Jansen HT. The GnRH system of seasonal breeders: anatomy and plasticity. *Brain Res Bull* 1997;44:445–457. [PubMed: 9370210]
- Liu X, Herbison AE. Small-conductance calcium-activated potassium (SK) channels control excitability and firing dynamics in gonadotropin-releasing hormone (GnRH) neurons. *Endocrinology*. 2008
- Martinez-Fuentes AJ, Hu L, Krsmanovic LZ, Catt KJ. Gonadotropin-releasing hormone (GnRH) receptor expression and membrane signaling in early embryonic GnRH neurons: role in pulsatile neurosecretion. *Mol Endocrinol* 2004;18:1808–1817. [PubMed: 15184526]
- McCann SM, Kimura M, Walczewska A, Karanth S, Rettori V, Yu WH. Hypothalamic control of gonadotropin secretion by LHRH, FSHRF, NO, cytokines, and leptin. *Domest Anim Endocrinol* 1998;15:333–344. [PubMed: 9785037]
- Mores N, Krsmanovic LZ, Catt KJ. Activation of LH receptors expressed in GnRH neurons stimulates cyclic AMP production and inhibits pulsatile neuropeptide release. *Endocrinology* 1996;137:5731–5734. [PubMed: 8940408]
- Naor I, Benard I, Seger I. Activation of MAPK Cascades by G-protein-coupled Receptors: The Case of Gonadotropin-releasing Hormone Receptor. *Trends Endocrinol Metab* 2000;11:91–99. [PubMed: 10707049]
- Naor Z. GnRH receptor signaling: cross-talk of Ca²⁺ and protein kinase C. *Eur J Endocrinol* 1997;136:123–127. [PubMed: 9116903]

- Naor Z. Signaling by G-protein-coupled receptor (GPCR): studies on the GnRH receptor. *Front Neuroendocrinol* 2009;30:10–29. [PubMed: 18708085]
- Naor Z, Shacham S, Harris D, Seger R, Reiss N. Signal transduction of the gonadotropin releasing hormone (GnRH) receptor: cross-talk of calcium, protein kinase C (PKC), and arachidonic acid. *Cell Mol Neurobiol* 1995;15:527–544. [PubMed: 8719039]
- Nunemaker CS, DeFazio RA, Moenter SM. Calcium current subtypes in GnRH neurons. *Biol Reprod* 2003;69:1914–1922. [PubMed: 12904316]
- Schwanzel-Fukuda M, Zheng LM, Bergen H, Weesner G, Pfaff DW. LHRH neurons: functions and development. *Prog Brain Res* 1992;93:189–201. [PubMed: 1480748]
- Shah BH, Baukal AJ, Shah FB, Catt KJ. Mechanisms of extracellularly regulated kinases 1/2 activation in adrenal glomerulosa cells by lysophosphatidic acid and epidermal growth factor. *Mol Endocrinol* 2005;19:2535–2548. [PubMed: 15928312]
- Shah BH, Shah FB, Catt KJ. Role of metalloproteinase-dependent EGF receptor activation in alpha-adrenoceptor-stimulated MAP kinase phosphorylation in GT1-7 neurons. *J Neurochem* 2006;96:520–532. [PubMed: 16336626]
- Spergel DJ, Kruth U, Hanley DF, Sprengel R, Seeburg PH. GABA- and glutamate-activated channels in green fluorescent protein- tagged gonadotropin-releasing hormone neurons in transgenic mice. *J Neurosci* 1999;19:2037–2050. [PubMed: 10066257]
- Suter KJ, Wuarin JP, Smith BN, Dudek FE, Moenter SM. Whole-cell recordings from preoptic/hypothalamic slices reveal burst firing in gonadotropin-releasing hormone neurons identified with green fluorescent protein in transgenic mice. *Endocrinology* 2000;141:3731–3736. [PubMed: 11014229]
- Van Goor F, Krsmanovic LZ, Catt KJ, Stojilkovic SS. Coordinate regulation of gonadotropin-releasing hormone neuronal firing patterns by cytosolic calcium and store depletion. *Proc Natl Acad Sci U S A* 1999;96:4101–4106. [PubMed: 10097170]
- Vitalis EA, Costantin JL, Tsai PS, Sakakibara H, Paruthiyil S, Iiri T, Martini JF, Taga M, Choi AL, Charles AC, Weiner RI. Role of the cAMP signaling pathway in the regulation of gonadotropin-releasing hormone secretion in GT1 cells. *Proc Natl Acad Sci U S A* 2000;97:1861–1866. [PubMed: 10677547]
- Wada K, Hu L, Mores N, Navarro CE, Fuda H, Krsmanovic LZ, Catt KJ. Serotonin (5-HT) receptor subtypes mediate specific modes of 5-HT-induced signaling and regulation of neurosecretion in gonadotropin-releasing hormone neurons. *Mol Endocrinol* 2006;20:125–135. [PubMed: 16109737]
- Weiner RI, Charles A. Regulation of gonadotropin-releasing hormone release by cyclic AMP signalling pathways. *Growth Horm IGF Res* 2001;11:S9–S15. [PubMed: 11527095]
- Wray S. Review Article: Development of Luteinizing Hormone Releasing Hormone Neurons. *J Neuroendocrinol* 2001;13:3–11. [PubMed: 11123510]
- Xu C, Xu XZ, Nunemaker CS, Moenter SM. Dose-Dependent Switch in Response of Gonadotropin-Releasing Hormone (GnRH) Neurons to GnRH Mediated through the Type I GnRH Receptor. *Endocrinology* 2004;145:728–735. [PubMed: 14576189]

Abbreviation used

AP	Action potential
AHP	afterhyperpolarization
fAHP	fast afterhyperpolarization
mAHP	medium afterhyperpolarization
sAHP	slow afterhyperpolarization
ADP	afterdepolarization potential
ACI	calcium-dependent adenylyl cyclase type I
CNG	cyclic nucleotide-gated

EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ERK	extracellular-signal-regulated kinase
HB-EGF	heparin binding epidermal growth factor
GnRH	gonadotropin-releasing hormone
GnRH-R	gonadotropin-releasing hormone receptor
GIRK	G protein-activated inwardly rectifying potassium channels
MAPK	mitogen-activated protein kinase
PTX	pertussis toxin
PKC	protein kinase C
PLC/InsP ₃ /Ca ²⁺	phospholipase C/ inositol 1,4,5-triphosphate/calcium
VSCC	voltage-sensitive calcium channels

Figure 1. Expression and signaling pathways of serotonin receptors expressed in GnRH neurons

In addition to the GnRH receptor, RT-PCR analysis of RNA isolated from cultured GT1-7 neurons revealed the expression of mRNA for 5-HT₂ G_q-coupled receptors, G_s-coupled 5-HT₄ and 5-HT-7 receptors and 5-HT_{1A} G_i-coupled receptors. In electrophysiological studies, activation of 5-HT₂ receptors in hypothalamic GnRH neurons with the specific receptor agonist significantly increased the frequency of AP firing. This was associated with membrane depolarization, increased [Ca²⁺]_i, and transient stimulation of GnRH secretion. The spontaneous electrical activity of hypothalamic GnRH neurons also increased during treatment with a 5-HT₄ receptor agonist. The increase in spontaneous AP firing was associated with increased bursting activity and the appearance of lower-amplitude broad APs. Selective activation of 5-HT₄ receptors in GT1-7 neurons increased cAMP production in a dose-dependent manner, and caused a robust and sustained increase in GnRH secretion during perfusion studies. In contrast, treatment of identified hypothalamic GnRH neurons with a 5-HT_{1A} receptor agonist caused pronounced inhibition of spontaneous AP firing. In GT1-7 neurons, treatment with the selective 5-HT_{1A} receptor agonist activated the G_i-mediated AC/cAMP-inhibitory signaling pathway. This response was prevented by PTX, consistent with coupling to an inhibitory G_i protein. The resulting decrease in cAMP production was associated with marked inhibition of pulsatile GnRH release. VGCC, voltage-sensitive calcium channels; ACI, calcium-dependent adenylyl cyclase type I; CNG, cyclic nucleotide gated channels; GIRK, G protein-activated inwardly rectifying potassium channels. Green and red lines indicate stimulatory and inhibitory actions, respectively.

Figure 2. Spontaneous action potential firing and afterhyperpolarizing current in hypothalamic GnRH neurons

A, a majority of recorded GnRH neurons fired spontaneous APs which alternate between slow and fast tonic firing. Action potential-driven Ca^{2+} influx in GnRH neurons determines the profile of afterhyperpolarization currents and consequently mediates firing frequency and the spike-profile. B, Small conductance (SK) channels underlie the afterhyperpolarization (AHP) and mediate firing frequency and spike-frequency adaptation.

Figure 3. LH/hCG-induced modulation of pulsatile GnRH release, cAMP production, and electrical activity in native and immortalized GnRH neurons

Activation of LH/hCG receptors in perfused hypothalamic cells causes initial stimulation and subsequent inhibition of pulsatile GnRH release. Treatment with LH caused time-dependent biphasic changes in the frequency of AP firing in both identified hypothalamic GnRH neurons and GT1-7 cells. LH treatment initially increased AP firing, which was followed by a significant reduction of AP firing during prolonged treatment. In addition to its initial transient stimulation of AP firing in hypothalamic GnRH neurons, LH subsequently caused pronounced and time-dependent inhibition of spontaneous AP firing. This effect was reversible, and spontaneous firing of APs recovered during the washout of LH. In both hypothalamic cells and GT1-7 neurons, treatment with LH activates time-dependent stimulatory and inhibitory changes in cAMP production and GnRH release. VSCC, voltage-sensitive calcium channels; ACI, calcium-dependent adenylyl cyclase type I; CNG, cyclic nucleotide gated channels; GIRK, G protein-activated inwardly rectifying potassium channels. Green and red lines indicate stimulatory and inhibitory actions, respectively.