Molecular and Cellular Endocrinology 449 (2017) 42–55

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

# Mathematical modeling of gonadotropin-releasing hormone signaling

Amitesh Pratap [c, 1], Kathryn L. Garner [c, 1], Margaritis Voliotis [b, 1], Krasimira Tsaneva-Atanasova [a, b], Craig A. McArdle [c, *]

[a] Department of Mathematics, College of Engineering, Mathematics and Physical Sciences, University of Exeter, Exeter, EX4 4QF, UK
[b] EPSRC Centre for Predictive Modeling in Healthcare, University of Exeter, Exeter, EX4 4QF, UK
[c] Laboratories for Integrative Neuroscience and Endocrinology, School of Clinical Sciences, University of Bristol, Whitson Street, Bristol, BS1 3NY, UK

ARTICLE INFO

*Article history:*
Received 27 June 2016
Received in revised form
9 August 2016
Accepted 11 August 2016
Available online 17 August 2016

*Keywords:*
GnRH
GPCR
NFAT
ERK
Mathematical modeling
Mutual information

ABSTRACT

Gonadotropin-releasing hormone (GnRH) acts via G-protein coupled receptors on pituitary gonadotropes to control reproduction. These are $G_q$-coupled receptors that mediate acute effects of GnRH on the exocytotic secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as the chronic regulation of their synthesis. GnRH is secreted in short pulses and GnRH effects on its target cells are dependent upon the dynamics of these pulses. Here we overview GnRH receptors and their signaling network, placing emphasis on pulsatile signaling, and how mechanistic mathematical models and an information theoretic approach have helped further this field.



## 1. GnRH signaling: an overview

GnRH is a hypothalamic decapeptide that mediates central control of reproduction. It acts via receptors (GnRHR) on pituitary gonadotropes to control synthesis and secretion of the two gonadotropin hormones (LH and FSH) that in turn regulate gametogenesis and steroidogenesis in the gonads. LH and FSH are heterodimeric proteins with distinct β-subunits (LHβ and FSHβ) and a common α-gonadotropin subunit (αGSU) that are packaged into vesicles for release from gonadotropes. Acutely, GnRH regulates the exocytotic fusion of these vesicles with the plasma membrane whereas chronically it increases synthesis of gonadotropins and thereby controls vesicle content. There are three distinct forms of the hormone termed GnRH-I (often known simply as GnRH and also known as LHRH), GnRH-II and GnRH-III. The cloned GnRHR, which are members of the rhodopsin-like GPCR family, have been classified into three groups based on sequence homology. All of the cloned mammalian GnRHR are in groups I or II, and the type I GnRHR of humans, rats, mice, pigs, sheep, and horses share >80% amino acid sequence homology (Millar et al., 2004; Morgan and Millar, 2004). Some primates express type II GnRHR (as well as type I GnRHR), but in humans functional type II GnRHR are not expressed (Morgan and Millar, 2004; Stewart et al., 2009). The central control of reproduction is therefore mediated by GnRH-I acting via type I GnRHR, both of which are absolutely essential for mammalian reproduction (Cattanach et al., 1977; Mason et al., 1986; de Roux et al., 1997).

In gonadotropes, GnRH influences the expression of many genes(Yuen et al., 2002, 2009; Ruf et al., 2006), although most work in this area focuses on transcription of the gonadotrope signature genes for αGSU, LHβ, FSHβ and GnRHR, all of which are increased by GnRH (McArdle and Roberson, 2015). GnRHR signal primarily via $G_q$, which activates PLC to generate $IP_3$ and DAG by cleavage of phosphatidylinositol (4,5)-bisphosphate (Fig. 1A). $IP_3$ mobilizes $Ca^{2+}$ from intracellular stores and this is followed by $Ca^{2+}$ influx via L-type voltage-gated $Ca^{2+}$ channels. $Ca^{2+}$ then drives the regulated exocytotic secretion of LH and FSH, an effect that is modulated by the concomitant activation of PKC isozymes (Hansen et al., 1987; Hille et al., 1994; Stojilkovic et al., 1991; Zhu et al., 2002). Like many other GPCRs, GnRHR mediate activation of MAPKs including ERK. Mechanisms of ERK activation by GnRH differ between model systems but it is largely mediated by PKC in αT3-1 and LβT2 gonadotrope cell lines (Naor, 2009; Caunt et al., 2006). In rat

* Corresponding author.
*E-mail address:* craig.mcardle@bristol.ac.uk (C.A. McArdle).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.mce.2016.08.022

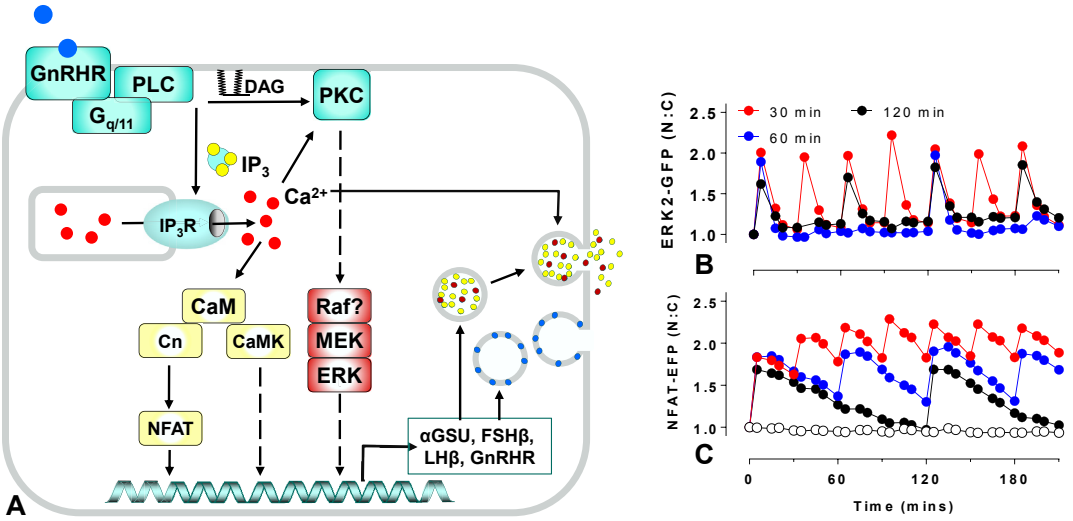


**Fig. 1. A simplified GnRHR signaling network**. Panel A: GnRH activates GnRHR causing a Gq/11-mediated activation of phospholipase C (PLC). This generates $IP_3$ which drives $IP_3$ receptor ($IP_3R$)-mediated mobilization of $Ca^{2+}$ from intracellular stores, and diacylglycerol (DAG) which (with $Ca^{2+}$) activates conventional PKC isozymes. GnRH increases cytoplasmic $Ca^{2+}$ and this drives the regulated exocytotic secretion of LH and FSH from within secretory vesicles. $Ca^{2+}$ also activates calmodulin (CaM), which activates CaM-dependent protein kinases (CaMK) and the phosphatase calcineurin (Cn), which activates the $Ca^{2+}$-dependent transcription factor NFAT (nuclear factor of activated T-cells). GnRH also activates MAPK cascades, including the (largely PKC-mediated) activation of the Raf/MEK/ERK cascade shown. NFAT and ERK-activated transcription factors (amongst others) then act in combination to control gene expression. GnRH target genes include the gonadotropin subunits; GnRH acutely regulates the rate of vesicle fusion with the plasma membrane, and chronically regulates the gonadotropin content of these vesicles. Panels B and C: data from HeLa cells transduced to express GnRHR and also ERK2-GFP (B) or NFAT-EFP (C) that translocate from the cytoplasm to the nucleus on activation, providing live cell readouts for the Raf/MEK/ERK and CaM/Cn/NFAT activation, respectively. The data shown are the nuclear:cytoplasmic ratios (N:C) and are from an experiment in which cells received 5 min pulses of $10^{-7}$ M GnRH at 30, 60 or 120 min intervals. Note that each GnRH pulse causes nuclear translocation of each reporter and the ERK2-GFP translocation responses have more rapid on-set and off-set than the NFAT-EFP responses. Note also that with the highest pulse frequency there is insufficient time for the NFAT-EFP to return to the pre-stimulation value. Similar experiments (and experimental details) are published elsewhere (Armstrong et al., 2009a,b,Armstrong et al., 2010).

pituitaries, αT3-1 and LβT2 cells, GnRH also activates JNK (Naor, 2009; Burger et al., 2004; Burger et al., 2009) and p38 (Roberson et al., 1999; Coss et al., 2007) and in LβT2 cells it has been shown to activate ERK5 (Lim et al., 2009). PKC and each of these MAPKs are implicated in control of gonadotropin signature gene expression as described elsewhere (McArdle and Roberson, 2015; Ciccone and Kaiser, 2009; Haisenleder et al., 1991). Several $Ca^{2+}$-regulated proteins are known to mediate transcriptional effects of GnRH. These include calmodulin (CaM), calmodulin-dependent protein kinases, the calmodulin dependent phosphatase calcineurin (Cn) and the $Ca^{2+}$ dependent transcription factor NFAT (McArdle and Roberson, 2015).

## 2. GnRH: a dynamic peptide

GnRH is secreted in pulses that drive pulses of gonadotropin release and are essential for normal reproduction (Dierschke et al., 1970; Clarke and Cummins, 1982). Its effects are dependent on pulse frequency, as shown in early studies where constant GnRH suppressed LH and FSH secretion, whereas restoration of GnRH pulses restored gonadotropin secretion (Belchetz et al., 1978). In humans and other primates, GnRH pulses have a duration of a few minutes and intervals of 30 min to several hours, with pulse frequency differing under different physiological conditions. For example, changes in GnRH pulse frequency drive changes in reproductive status during development, with an increase in pulse frequency driving the increased gametogenesis and gonadal steroid production at puberty (Sisk and Foster, 2004). Similarly, GnRH pulse frequency varies through the menstrual cycle, increasing before ovulation and contributing to generation of the pre-ovulatory gonadotropin surge (Ferris and Shupnik, 2006; Marshall et al., 1993). Moreover, stimulation paradigm is crucial for therapeutic intervention because agonist pulses can maintain or increase circulating gonadotropin levels whereas sustained agonist stimulation (after initial activation) reduces them, causing the chemical castration that is exploited in treatment of breast cancer, prostate cancer and other sex steroid hormone-dependent conditions (Ferris and Shupnik, 2006; Marshall et al., 1993; Bliss et al., 2010). The key observation here is that maximal GnRH effects on gonadotropin secretion are seen at sub-maximal GnRH pulse frequency and this also holds true for effects of GnRH on many of its gene targets, including the signature genes GnRHR, FSHβ and LHβ. Thus physiological and pharmacological control of the system relies on the fact that gonadotropin synthesis and secretion are low when GnRH pulse intervals are too low (i.e. before puberty) or too high (treating constant agonist stimulation as the maximal possible pulse frequency).

## 3. GnRHR: a short tail

It has long been known that sustained agonist exposure causes activation followed by desensitization of GnRH-stimulated gonadotropin secretion, that is not seen with pulsatile stimulation (Belchetz et al., 1978). GnRH causes GnRHR internalization and this could certainly contribute to desensitization of GnRH-stimulated gonadotropin secretion. Sustained stimulation of GPCRs typically causes rapid homologous receptor desensitization, where G-protein receptor kinases phosphorylate Ser and Thr residues, most often within the receptor's COOH-terminal tail, facilitating binding of non-visual arrestins (arrestins 2 and 3). The arrestins prevent G protein activation and target desensitized receptors for internalization, most often via clathrin-coated vesicles (CCVs) (Pierce and Lefkowitz, 2001). Although GnRH was known to induce GnRHR internalization via CCVs (Hazum et al., 1980; Jennes et al., 1984), the cloning of mammalian type I GnRHR revealed most remarkably that it has no COOH-terminal tail (Millar et al., 2004; Tsutsumi et al.,