BRAIN RESEARCH 1364 (2010) 53–61

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BRAIN RESEARCH

Review

Membrane rafts and GnRH receptor signaling

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ARTICLE INFO

Article history:
Accepted 2 September 2010
Available online 15 September 2010

Keywords:
GnRH receptor
GnRH
Membrane rafts
ERK
Signaling platform
Gonadotrope

ABSTRACT

The binding of hypothalamic gonadotropin-releasing hormone (GnRH) to the pituitary GnRH receptor (GnRHR) is essential for reproductive function by stimulating the synthesis and secretion of gonadotropic hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Engagement of the GnRHR by GnRH initiates a complex series of signaling events that include the activation of various mitogen-activated protein kinase (MAPK) pathways, including extracellular signal-regulated kinase (ERK). GnRHR signaling is thought to initiate within specialized microdomains in the plasma membrane termed membrane rafts. These microdomains are enriched in sphingolipid and cholesterol and are believed to be highly dynamic organizing centers for receptors and their cognate signaling molecules associated with the plasma membrane. Within this review we discuss the composition and role of membrane rafts in cell signaling and examine evidence that the mammalian type I GnRHR is constitutively and exclusively localized to these membrane microdomains in various experimental models. We conclude that membrane raft composition and organization potentially underlie the functional ability of GnRH to elicit the assembly of multi-protein signaling complexes necessary for downstream signaling to the ERK pathway that ultimately is critical for controlling fertility.

Contents

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doi:10.1016/j.brainres.2010.09.008

1. Introduction

As the central regulator of reproductive function, gonadotropin-releasing hormone (GnRH) is situated at the peak of the hypothalamic–pituitary–gonadal (HPG) axis. The pulsatile discharge of GnRH from hypothalamic neurons not only stimulates but is obligatory for synthesis and secretion of gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) from gonadotrope cells of the anterior pituitary gland. In the absence of pulsatile GnRH secretion to the anterior pituitary, proper reproductive function in mammals ceases (Clayton and Catt, 1981; Gharib et al., 1990; Belchetz et al., 1978). Numerous genetic mutations exist that result in impaired hypothalamic production or function of GnRH and lead to hypogonadism and infertility in mammals (Mason et al., 1986; Jameson, 1996; Chan et al., 2009; Bouligand et al., 2009). In humans, one well-defined clinical example of hypogonadotropic-hypogonadism is Kallmann's syndrome where patients present with delayed or absent pubertal development. This syndrome arises as a failure of GnRH neurons to migrate from the olfactory placode to the hypothalamus correctly during embryogenesis resulting in decreased levels of GnRH and subsequent gonadotropins (Hardelin, 2001; Seminara et al., 1998; Sarfati et al., 2010). In addition to various naturally occurring mutations in humans, several experimental models also underscore the critical role of GnRH/GnRH receptor (GnRHR) signaling including mutation of the GnRHR gene (Pask et al., 2005; Bedecarrats and Kaiser, 2007), genetic ablation of gonadotropes (Kendall et al., 1991), immunoneutralization of GnRH (McCue et al., 1997; Schanbacher et al., 1983), and animal models of hypothalamic–pituitary disconnection (Turzillo et al., 1995; Turzillo et al., 1997). Thus, fertility is not only dependent on proper GnRH production and release from the hypothalamus but also its subsequent binding to high affinity GnRHR on pituitary gonadotropes and the coordination of these events is of universal importance in controlling reproduction in mammals.

Studies of GnRH action have been greatly facilitated by the cloning of the GnRHR (Tsutsumi et al., 1992). To date, three forms of GnRHR have been indentified in vertebrates, GnRHR I, II, and II (Millar, 2005). For the purposes of this review, we will focus on the mammalian type I GnRHR, which is the predominate form that is expressed in anterior pituitary gonadotropes. The type I GnRHR has been cloned from several species including murine, rat, human, sheep, pig, and horse (Rispoli and Nett, 2005). The predicted amino acid structure is a 327-amino acid receptor containing 7 transmembrane domains and an extracellular ligand binding domain placing the GnRHR in the rhodopsin class of heptahelical G-protein coupled receptors (GPCR) but interestingly, the type I GnRHR is the only GPCR classified to date that lacks a carboxyl-terminal intracellular cytoplasmic domain. Unlike other GPCRs, the type I GnRHR C-terminal tail is only 1–2 amino acids (Stojilkovic et al., 1994). In more prototypical GPCRs, like β-adrenergic receptors, this cytoplasmic domain is extensive and is targeted for phosphorylation by GPCR kinases (GRK) and second-messenger regulated kinases (O'Dowd et al., 1989). In many GPCRs, phosphorylation of the C-terminus appears to be required for subsequent interaction with β-arrestin, which hinders further G-protein activation and targets the deactivated receptor for internalization (Ferguson, 2001). Experimental evidence suggests that due to the lack of an intracellular C-terminus, the mammalian GnRHR is not phosphorylated and internalization is not mediated via a β-arrestin-dependent mechanism. Indeed, the GnRHR has been described as a naturally occurring desensitization and internalization mutant (McArdle et al., 2002). Interestingly, the mammalian type I receptor does differ from GnRH receptors found in non-mammalian vertebrates such as catfish (Blomenrohr et al., 1999) and Xenopus (Hislop et al., 2001). In contrast to the mammalian type I GnRHR, the non-mammalian GnRHRs do possess prototypical C-terminal tails, are phosphorylated by GRKs and are characterized by more rapid internalization and desensitization kinetics (McArdle et al., 2002; Joseph et al., 2009).

More recent studies with the human GnRH receptor have found that, unlike its rodent counterpart, it is poorly trafficked to the plasma membrane. The majority of the human receptor appears to be intracellular and retained within the ER due to receptor misfolding (Janovick et al., 2006). Elegant work by Conn and colleagues has found that using the GnRH-mimetic indole IN3 rescues misfolded GnRH receptor from the ER. Spatial analysis of the human GnRH receptor in heterologous human cell lines further suggests that the receptor is not only associated with the ER but is also expressed and functional on the nuclear membrane (Re et al., 2010).

Considerable efforts have also been devoted toward understanding the molecular basis of GnRH action on its cognate receptor in the anterior pituitary. It is well established that agonist-occupied GnRHR undergoes a conformational change that promotes the activation of heterotrimeric G proteins, specifically, $G\alpha_{q/11}$. Upon dissociation, $G\alpha_{q/11}$ activates phospholipase C β which hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) to produce two signaling intermediates, inositol 1,4,5, bisphosphate (IP_3) and diacylglycerol (DAG). IP_3 interaction with the IP_3 receptor induces an elevation of intracellular free Ca^{2+} via endoplasmic reticulum stores. DAG, along with Ca^{2+} in many instances, leads to an activation of one or more isoforms of protein kinase C (PKC), including α, ε, and ζ in gonadotrope cells (Stanislaus et al., 1997; Kratzmeier et al., 1996). These early events underlie GnRH activation of multiple mitogen activated protein kinase (MAPK) signaling cascades including p38 MAPK (Roberson et al., 1999; Bonfil et al., 2004), c-Jun N-terminal kinase (JNK) (Levi et al., 1998; Mulvaney and Roberson, 2000) and extracellular signal-regulated kinase (ERK) (Roberson et al., 1995; Sundaresan et al., 1996; Mulvaney et al., 1999; Sim et al., 1995). Due to space constraints, a comprehensive overview of the GnRH signaling network can be found in a recent review by Naor and colleagues (Naor, 2009). For the purposes of this review, we will focus on GnRHR activation of the ERK cascade.

Regulation of the ERK signaling pathway can lead to the activation of numerous cellular targets involved in transcription, translation, and cytoskeletal remodeling (Pearson et al., 2001). Classically, activation of the ERK module proceeds through a three-tiered kinase cascade of MAPK kinase kinase (MAPKKK)-Raf-1, MAPK kinase (MAPKK)-MEK 1 and 2 and (MAPK)-ERK1 and ERK2 (Pearson et al., 2001; Seger and Krebs, 1995). Work from our laboratory and others suggests that the phosphorylation of ERKs within the gonadotrope is dependent on PKC and influx of Ca^{2+} via L-type voltage gated calcium