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Estrogen modulation of hypothalamic neurons: Activation of multiple signaling pathways and gene expression changes

Anna Malyala *, Martin J. Kelly, Oline K. Rønnekleiv

Department of Physiology and Pharmacology, L334, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA

Abstract

Hypothalamic target neurons of estrogen include neurosecretory neurons such as gonadotropin-releasing hormone (GnRH) and dopamine neurons, and local circuitry neurons such as proopiomelanocortin (POMC) and γ-aminobutyric acid (GABA) neurons. These and other hypothalamic neurons are involved in regulating numerous homeostatic functions including reproduction, thermoregulation, stress responses, feeding and motivated behaviors. Using a combination of techniques to examine the molecular mechanisms leading to physiological changes induced by estrogen, we find that both rapid effects and transcriptional changes alter excitability of hypothalamic neurons. We have identified membrane-initiated, rapid signaling pathways through which 17β-estradiol (E_2) alters synaptic responses in these neurons using whole-cell patch recording in hypothalamic slices from ovariectomized female guinea pigs. E_2 rapidly uncouples μ-opioid and $GABA_B$ receptors from G protein-gated inwardly rectifying K^+ (GIRK) channels in POMC and dopamine neurons as manifested by a reduction in the potency of μ-opioid and $GABA_B$ receptor agonists to activate these channels. Inhibitors of phospholipase C, protein kinase C and protein kinase A block the actions of E_2, indicative that the E_2 receptor is G protein-coupled to activation of this cascade. Taking advantage of an animal model we developed to investigate estrogen's feedback actions on secretion of gonadotropin-releasing hormone (GnRH), we studied the transcriptional changes induced by estrogen using suppression subtractive hybridization (SSH) and microarray analysis. Many of the observed mRNA expression changes include transcripts encoding proteins critical for neurotransmitter release and receptor dynamics. Some of these include gec-1, PI3-kinase p55γ, rab11a GTPase, synaptobrevin2, synaptogyrin, taxilin, Ca^{2+}-dependent activator protein for secretion (CAPS) and a number of proteins containing pleckstrin homology domains—domains that are involved in plasma membrane targeting of their host protein. In situ hybridization and quantitative film autoradiography analysis on selected transcripts show differential distribution and expression in hypothalamic nuclei. Furthermore, single-cell PCR analysis reveals these genes to be expressed in neurons such as POMC (and GnRH). Whether these expression changes are mediated by the classical or membrane estrogen receptors has yet to be delineated. More detailed investigations of transcript spatial localization within neurons and their temporal expression, i.e., within minutes or hours, will provide more insight regarding how estrogen alters neuronal excitability and synaptic efficacy that ultimately lead to changes in complex behavior.

Keywords: Estrogen; Hypothalamic neurons; GABA; POMC; PI3K

1. Introduction

It is evident that the gonadal steroid hormone estrogen (17β-estradiol, E_2) imparts a multifaceted influence over synaptic transmission in the mammalian central nervous system. Not only can E_2 alter synaptic responses via genomic mechanisms, but there exists a wealth of information that indicates the steroid can also modulate cell-to-cell communication much more rapidly (for review see [1]). These synaptic alterations are brought about via changes in the cellular responsiveness to the activation of various receptor systems (both G protein-coupled and ionotropic) to their respective first messengers. For example, E_2 can modulate the cellular responsiveness to ionotropic glutamate (both *N*-methyl-D-aspartate (NMDA) and non-NMDA) receptor activation [2–4]. In addition, it can alter the linkage of G protein-coupled receptors such as opioid (both μ and κ), γ-aminobutyric acid $(GABA)_B$ and dopamine D_2 receptors to their respective effector systems [5–9]. More recently, it appears that the steroid

* Corresponding author. Tel.: +1 503 494 5833; fax: +1 503 494 4352.
E-mail address: malyalaa@ohsu.edu (A. Malyala).

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can function as a first messenger by activating an estrogen receptor that couples directly to K^+ and Ca^{2+} channels by way of a pertussis toxin-sensitive G protein [10,11]. While a clearer picture of the rapid signaling actions by estrogen is emerging, it is well established that E_2 modulates neurotransmission via genomic mechanisms. This well characterized mechanism of transactivation involves ligand binding, nuclear receptor dimerization and binding to consensus estrogen response elements (EREs) [12]. The picture however is more complicated since there are at least two estrogen receptors (ERα and ERβ) that can homodimerize or heterodimerize, sequester other DNA-binding proteins, and enable transcription by response elements other than EREs, e.g., AP-1 and CREB sites (reviewed in [13–15]). Although a number of transcripts that are regulated by E_2 have been identified [16], the full scope of estrogen's action is not known. Therefore, new approaches such as differential display and gene microarray [17,18] are being used in order to ascertain a more global picture of E_2-regulated genes and how these changes subsequently affect complex physiological processes such as reproduction, stress responses, feeding and cognition. We will focus on the integration of estrogen's rapid signaling to modulate channel activity with a particular focus on K^+ channel activity as well as gene regulation in hypothalamic neurons that mediate many of these physiological processes.

2. Estrogen modulation of G protein-coupled inwardly rectifying K^+ (GIRK) channels

One of the principal actions of estrogen is to regulate the output of gonadotropin-releasing hormone (GnRH) from the mediobasal hypothalamus and hence the reproductive cycle. Although we demonstrated direct actions of estrogen to inhibit GnRH neuronal activity over 15 years ago [10,19], it has been only recently that estrogen receptors is identified in GnRH neurons [20–22]. Our studies using the in vitro slice preparation have revealed that μ-opioid receptor-mediated inhibition of GnRH neurons arises from the activation of a member of the G protein-gated, inwardly rectifying K^+ channel subfamily known as GIRK1-4 (Kir3.1-3.4) [10,40,41]. Activation of this GIRK subfamily elicits a robust hyperpolarization in current clamp, or outward current in voltage clamp.

Estrogen responsiveness of GnRH release has been mainly attributed to neurons synapsing onto GnRH cells [23–28]. Indeed, hypothalamic POMC and GABAergic neurons, both of which provide a prominent synaptic input onto GnRH neurons, express estrogen receptors and concentrate radiolabeled estradiol [29–31]. Opioid peptides and GABAergic ligands both serve to inhibit GnRH output [32–34] and thus luteinizing hormone (LH) release [32,35–37] from the anterior pituitary. While presynaptic interactions between opioid and GABAergic nerve terminals may help regulate this process [38,39], it is clear that both μ-opioid and $GABA_B$ receptor agonists affect a direct, postsynaptic inhibition of GnRH neurons [10].

POMC and dopamine neurons are exquisitely responsive to μ-opioid receptor activation [42,43]. Acute E_2 exposure for no longer than 20 min results in a decreased potency of μ-opioid and $GABA_B$ receptor agonists to activate GIRKs in POMC and dopamine neurons [44,75]. The negative modulatory effect of estrogen (i.e., reduced potency of μ-opioid receptor and $GABA_B$ agonists in POMC and dopamine neurons) persists for at least 24 h following systemic steroid administration [6]. μ-Opioid and $GABA_B$ receptors serve as autoreceptors in their respective POMC and hypothalamic GABAergic neurons [42,46,47]. The fact that estrogen uncouples these autoreceptors from their GIRK channel implies that the steroid decreases the autoinhibition of these cells, thereby increasing the release of these inhibitory neurotransmitters. Indeed, estrogen rapidly increases extracellular GABA concentrations in the preoptic area as measured by push/pull perfusion and microdialysis [48,49]. Coupled with the attenuated $GABA_B$ receptor-mediated autoinhibition of these GABAergic neurons, it stands to reason that estrogen would dramatically increase the firing rate of these neurons during negative feedback. Given that both POMC and hypothalamic GABAergic neurons synapse onto a number of neurosecretory neurons [50–53], the collective modulation of K^+ channel activity by estrogen in these cells would greatly enhance the inhibitory tonus impinging on these neurons.

3. Cellular mechanisms of estrogen's rapid actions: activation of protein kinases

What is the underlying cause of estrogen-induced decrease in the responsiveness of hypothalamic neurons to the μ-opioid and $GABA_B$ receptor-mediated activation of GIRK channels? One insight comes from studies in which E_2 has been shown to rapidly stimulate PKA activity in peripheral (e.g., uterine) tissue, as well as to stimulate cyclic adenosine monophosphate responsive element binding protein (CREB) and c-fos expression [63–67]. Furthermore, PKA activators such as Sp-cAMP and forskolin mimic the effect of E_2 on the μ-opioid and $GABA_B$ receptor agonist potency [45,68]. In the presence of non-selective protein kinase inhibitors (e.g., staurosporine) or selective PKA inhibitors such as Rp-cAMP and KT5720, the effects of E_2 on the potency of the μ-opioid receptor agonist DAMGO or the $GABA_B$ receptor agonist baclofen are blocked. This demonstrates that the modulation by E_2 of the μ-opioid and $GABA_B$ receptor coupling to GIRK channels is due to increased PKA activity. In hippocampal CA1 pyramidal neurons, which are involved in learning and memory, estrogen activates a similar pathway to potentiate kainate currents [3]. Also, we know that multiple monoamine pathways activate PKA to inhibit the slow Ca^{2+}-activated K^+ current in CA1 hippocampal pyramidal neurons, and it has recently been shown that E_2 rapidly inhibits this current in CA1 neurons probably via a similar mechanism [69,70].