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MEMBRANE ESTROGEN RECEPTOR REGULATION OF HYPOTHALAMIC FUNCTION

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Abstract

Over the decades, our understanding of estrogen receptor (ER) function has evolved. Today we are confronted by at least two nuclear ERs: ERα and ERβ; and a number of putative membrane ERs, including ERα, ERβ, ER-X, GPR30 and Gq-mER. These receptors all bind estrogens or at least estrogenic compounds and activate intracellular signaling pathways. In some cases, a well-defined pharmacology, and physiology has been discovered. In other cases, the identity or the function remains to be elucidated. This mini-review attempts to synthesize our understanding of 17β-estradiol membrane signaling within hypothalamic circuits involved in homeostatic functions focusing on reproduction and energy balance.

Keywords

17β-estradiol; estrogen receptor alpha; estrogen receptor beta; GPR30 (GPER1); Gαq-coupled membrane estrogen receptor; mGluR1 receptor; GnRH neuron proopiomelanocortin (POMC) neuron

Introduction

17β-estradiol (E2) modulates circuits regulating reproduction, energy balance, temperature, circadian rhythms, stress and provides neuroprotection in cases of neurodegenerative diseases and trauma. In addition, ovarian and neurosteroidal E2 are involved in structural plasticity in the hippocampus that influences cognitive behaviors [1]. E2 signaling is one of the most fundamental aspects of reproduction. In females, it is the basis of positive and negative feedback within the hypothalamic-pituitary-ovarian axis. E2 produced by the ovary signals to the brain the endocrine status of gonads and activates circuits that regulate ovulation. Through the activation of a neural-glial network, E2 induces the release of gonadotropin-releasing hormone (GnRH) and luteininzing hormone (LH), and stimulates sexual behavior. Thus, E2 increases the probability that the ovulated egg will be fertilized. To achieve these effects, E2 binds to and activates estrogen receptors (ERs).

Our ideas about what constitutes an ER are constantly evolving as we understand more about the actions of E2, especially in the nervous system. Before ER proteins were

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characterized and cloned, ERs were defined by their ability to bind estrogens, and elicit a specific response [2]. Early, ER was considered a cytosolic receptor that upon E2 binding underwent a conformational change and translocation to the nucleus where it interacted with DNA to regulate the expression of particular genes through DNA motifs known as the estrogen (receptor) response element (ERE). It became clear that even in the absence of E2, ERs were located in the nucleus, suggesting that ERs were synthesized in the cytoplasm, but were preferentially transported back into the nucleus. Upon cloning, the nuclear ER was determined to be two proteins ERα and ERβ, coded by different genes: *ESR1* and *ESR2*, respectively [3;4]. These proteins share the same modular structure such that both bind E2 and have significant sequence homology, especially in their DNA and ligand binding domains. This bolstered the concept that ERs share a common motif and are members of a nuclear receptor family. To paraphrase Toran-Allerand [5], when there were only the two nuclear receptors, ERα and ERβ, life seemed simple. However, even in those "simple" times, it was known that ERs could interact with other transcription factors, namely Fos and Jun, which bind DNA at the activator protein-1 (AP-1) site, to regulate transcription independent of EREs [6].

Another story was developing in parallel. Early observations implicated E2 in rapid, actions in a number of neuronal and non-neuronal cells. For example, E2 membrane signaling rapidly increased levels of cAMP in the uterus [7], altered neuronal firing of hypothalamic neurons [8] and augmented the release of neuropeptides [9]. In the past two decades, there has been an explosion in the discovery of putative membrane ERs that mediate E2 membrane signaling. Many have been variants of *ESR1* and *ESR2*, but others appear to be completely novel proteins Several of these have been proposed as membrane ERs (mERs), including classical ERα and ERβ or splice variants [10–12], G protein coupled receptor 30 [13], a Gq-mER [14;15] and ER-X [16]. In addition, it has become clear that mERs can interact with other membrane receptors including insulin-like growth factor-1 [17] receptor and metabotropic glutamate receptors [18]. These mER candidates activate a variety of signaling pathways including phospholipase C, protein kinase C (PKC), protein kinase A (PKA) and MAP kinase signaling cascades [19–21]. As a starting point, we will stipulate that all mERs bind 17β-estradiol. While it would be very helpful to have a universal mER antagonist, at present none of the putative antagonists appears to be the "silver bullet" naloxone has been for the opioid receptor field [22]. The most reliable ER antagonist has been ICI 182, 780 (Fulvestrant), but some have noted agonist properties, especially at the ER-X putative mER [16]. This review is an attempt to synthesize our understanding of mERs in hypothalamic functions, to see whether a more complete picture of mERs will emerge.

E2 membrane signaling and reproduction

E2 membrane signaling and GnRH secretion

An acute, direct effect of E2 on neuronal activity in GnRH neurons from the guinea pig arcuate nucleus of the hypothalamus (ARH) was first described over twenty-five years ago [23] (reviewed in [19]). E2 rapidly hyperpolarizes GnRH neurons in guinea pigs via activation of inwardly-rectifying K^+ channels [23;24]. In mice, physiological concentrations (picomolar) of E2 rapidly augment K_{ATP} channel activity to hyperpolarize GnRH neurons via PKC and PKA signaling pathways, which are also activated by the selective Gq-mER ligand STX [25]. The K^+ channel-mediated hyperpolarization is potentially involved in recruitment of excitatory channels that are critical for burst firing of GnRH neurons including T-type calcium channels [26;27]. Nanomolar concentrations of E2 enhance action potential firing by modulating intrinsic afterhyperpolarizing and afterdepolarizing potentials via a PKA-dependent mechanism involving ERβ [28]. When synaptic input is not blocked, picomolar concentrations of E2 inhibit action potentials via an ERα-dependent mechanisms

[28]. In addition, E2 via ERβ and/or GRP30 rapidly potentiates high-voltage-activated Ca^{2+} currents (L- and R-type Ca^{2+} channels) suggesting that Ca^{2+} signaling is also a target for E2 membrane signaling GnRH neurons [29].

In primate and mouse olfactory placode GnRH (immature) neurons, E2 modulates Ca^{2+} oscillations, which synchronize with a periodicity of approximately 60 minutes [30–32], a rhythm similar to the pulsatile GnRH release [33;34]. Furthermore, nanomolar concentrations of a membrane-delimited E2 (E2-dendrimer) alter the patterns of Ca^{2+} oscillations in primate GnRH neurons [35]. The E2 membrane signaling modulation of Ca^{2+} oscillations in primate GnRH neurons is suppressed by pertussis toxin (PTX) treatment, attenuated by knockdown of GPR30 mRNA and partially mimicked by the GPR30 agonist G1 [36]. Ca^{2+} oscillations are blocked by ICI 182,780, mimicked by E2-BSA and blocked by PTX in immature mouse GnRH neurons [31;32]. However, in adult mouse GnRH neurons, nanomolar concentrations of E2 increase Ca^{2+} transients via presumably presynaptic GABA input [37]. The E2-mediated effects on Ca^{2+} oscillations are maintained in ERβKO mice [37]. However, E2 activation of cAMP response element-binding protein (CREB) in mouse GnRH neurons is absent in ERβKO mice [38]. In addition, Terasawa and colleagues have found that GPR30 and the Gq-coupled mER are also involved based on the findings that G1 and STX increase calcium oscillations and GnRH release from monkey placode neurons [36;39].

Although E2 membrane signaling may be involved in negative feedback, it has been difficult to put ERβ-, GPR30- or Gq-mER-dependent signaling into a physiological context of estrogen positive feedback, which is absent in mice with either global or neuronal-specific ERα knockdown [40]. These observations *prima facie* suggest that activation of female reproduction requires ERα-mediated E2 actions in neurons. However, these gene deletion experiments may not necessarily mean that only ERα is involved. Clearly ERα is a transcription factor affecting hundreds of genes important for cell signaling [41;42]. Some of these genes may be essential for a mER-initiated response that normally contributes to female reproduction but is defective in ERαKO mice.

In addition to the idea that E2 directly influences neurons to elicit both positive and negative feedback on GnRH neuronal activity, recent evidence supports the idea that E2 acts through a glial-neuronal network that provides an appropriate local hormonal environment for an LH surge [43;44]. This hypothesis rests on the observation that E2 facilitates progesterone synthesis in mature hypothalamic astrocytes, which if prevented abrogates estrogen positive feedback of the LH surge in rodents [43–46]. In astrocytes, E2 membrane signaling is dependent on the interaction of ERα and mGluR1a, which induces a rapid release of IP_3 receptor-sensitive Ca^{2+} stores required for progesterone synthesis [47–49]. At physiologically relevant (picomolar) concentrations, E2 elevation of $[Ca^{2+}]_i$ is blocked with LY367,385 ((S)-(+)-a-Amino-4-carboxy-2-methylbenzeneacetic acid), a mGluR1a antagonist. While glutamate is not required for E2 action, DHPG ((S)-3,5-dihydroxyphenylglycine), an mGluR1a agonist, augments the Ca^{2+} and progesterone responses to E2 stimulation *in vitro* [49;50]. These results suggest that elevation of local glutamate, presumably through neuronal activity, increases the amount of progesterone released. This would potentially generate a larger estrogen positive feedback response. Interestingly, STX also increases Ca^{2+} release and stimulates progesterone synthesis in primary adult hypothalamic cultures, mimicking the actions of E2 [51]. The activation of a novel Gq-mER signaling pathway by STX is blocked by the mGluR1a antagonist, LY367,385, suggesting convergent E2 membrane signaling in astrocytes (reviewed in [44]).

Estrogen membrane signaling and sex behavior

Traditionally nuclear-initiated E2 signaling was considered sufficient for female sexual receptivity - lordosis in rodents. This behavior characterized by the ventral arching of the spine in response to mounting by a male is displayed during behavioral estrus and is controlled by E2 through a complex hypothalamic circuitry involving the ARH, medial preoptic nucleus (MPN) and the ventromedial nucleus of the hypothalamus (VMH) [50;52]. Within the past decade, it has become clear that in addition to the direct nuclear actions of E2 in these various nuclei, membrane-initiated signaling is important for sexual behavior. One thought is that the initial E2 membrane signaling activates a signaling cascade (PKA and PKC) in VMH neurons that augments the nuclear-mediated E2 signaling and lordosis behavior [53]. One mechanism highlights the potential importance of growth factors mediating actions of E2. E2 activates IGF-I, IGF-I receptors and induces the association between IGF-1 receptors and ERα [17;54–56], leading to phosphatidylinositol 3-kinase (PI3K) and protein kinase B/Akt activation and facilitation of lordosis behavior [55–57].

A model of E2 membrane signaling that explains both facilitatory and inhibitory actions proposes that classical ERα or ERβ at the cell membrane transactivate mGluRs to initiate cell signaling (Figure 1) [18;21]. This ERα-mGluR interaction regulates the hypothalamic circuitry controlling female sexual receptivity [58]. ERα and mGluR1a are co-expressed in ARH cells and can be co-immunoprecipitated from membrane fractions [49;58;59]. Blocking either ERs or mGluR1a in the ARH prevents the rapid PKC phosphorylation and activation of MPN projecting proopiomelanocortin (POMC, the precursor of β-endorphin) neurons that are responsible for activating/internalizing μ-opioid receptors (MOR) [60–62]. Similarly, activation of ARH neurons with a membrane-impermeant E2 or DHPG stimulates MOR internalization [58]. These MPN MOR neurons, in turn, project to the VMH (Sinchak et al., unpublished findings), and the transient activation of MOR is necessary for full sexual receptivity that is apparent 30–48 hrs after E2 treatment [63]. While full sexual receptivity also requires E2-induced gene transcription, one question about membrane-initiated E2 action is how the transient, E2-induced activation and internalization of MOR in the MPN facilitate full sexual receptivity at the later time points? One possible explanation is that MOR activation produces a transient inhibition of the MPN neurons that project to the VMH. These neurons rebound from a hyperpolarized state with facilitated firing that excites VMH neurons, ultimately facilitating sexual receptivity.

In the rat ARH, E2 activation of POMC neurons involves the activation of the NPY-Y1 receptor [62]. While a direct E2 action on POMC neurons cannot be ruled out, one hypothesis is that E2 activates the ERα-mGluR1a complex in the ARH initiating multiple signaling pathways, including a Ca^{2+}-independent PKCθ [64]. Activating PKCθ in the ARH appears to be necessary for lordosis behavior since blocking PKCθ in the ARH abrogates MOR internalization, and prevents lordosis behavior [64]. Activation of protein kinases is critical for not only lordosis behavior [53;64–66] but also for feeding behavior (described below). These data collectively suggest that the membrane-initiated interactions of the ERα-mGluR1a complex via a PKC-mediated pathway are a component of the E2 control of MOR internalization in the MPN and the regulation of lordosis behavior.

Estrogen membrane signaling and energy homeostasis

Besides reproduction, E2 controls a number of hypothalamic-regulated autonomic functions including energy homeostasis, the balance between energy intake and energy expenditure. E2 signaling via ERα is a necessary component of the regulation of energy homeostasis [67]. A loss-of-function mutation in ERα has a clear metabolic phenotype in man with expression of type 2 diabetes, hyperinsulinemia and obesity [68]. However, global reinstatement of an ERα that is lacking the ERE targeting domain is sufficient for

"rescuing" the metabolic deficits in mice [69]. These findings suggest an important role for non-ERE mediated E2 signaling. Moreover, brain-specific knockout of ERα causes hyperphagia and hypometabolism, and selective knockdown of ERα in POMC neurons recapitulates the hyperphagic phenotype in female mice [70]. A caveat is that POMC-Cre is expressed also in progenitor neurons that are destined to become NPY and perhaps other hypothalamic neurons [71]. Thus, the neuronal site of action of ERα in the control of energy homeostasis remains ambiguous.

Besides the nuclear-initiated ERα signaling, compelling results point to a role for estrogen membrane signaling in the control of energy homeostasis. For example, E2 attenuates food intake within 4–6 hrs of administration into the third ventricle in fasted, ovariectomized rats and mice [72;73]. A Gq-mER signaling pathway has been elucidated in hypothalamic POMC neurons that is activated within minutes [14;15;74]. This pathway was first characterized in guinea pigs, and later shown in wild type (C57BL/6), ERα/β double-knockout and GPR30 knockout mice [14;74;75]. This Gq-mER signaling pathway is blocked by the anti-estrogen ICI 182,780 [14;15], which at present is a *sine qua non* for an ER-mediated response. Gq-mER through PLC-PKC-PKA pathways attenuates the $GABA_B$ receptors and MOR activation of G protein-coupled inwardly rectifying K^+ (GIRK) channels (Figure 1). The resulting attenuation of inhibitory presynaptic inputs increases the excitability of the anorectic POMC neurons.

Moreover, the high affinity (subnanomolar affinity for E2 [14]) Gq-mER is selectively activated by STX, an analogue of 4-OH tamoxifen, which has an even greater (20-fold) affinity for Gq-mER and no measureable binding affinity for classical ERs [76]. STX, similar to E2, reduces post-ovariectomy weight gain, inhibits food intake by reducing meal frequency, reduces abdominal fat accumulation, maintains bone density and reduces core body temperature in ovariectomized female guinea pigs [74;77;78]. Furthermore, the STX-initiated signaling pathway alters the transcription of a plethora of ARH genes involved in the control of homeostatic functions [77] (Figure 1). Therefore, Gq-mER appears to be a key player in mediating the effects of E2 on hypothalamic neurons (POMC, NPY, etc.) controlling homeostatic functions.

Summary and Conclusions

The hypothalamic regulation of homeostatic functions has traditionally been regarded as mediated by direct nuclear actions of E2, which has been the dogma for decades. However, recent results indicate that E2 actions in the hypothalamus are a composite of membrane-initiated signaling and direct nuclear effects. Although somewhat unusual for the nuclear receptor field, the idea of multiple receptors, using multiple signaling strategies is commonplace among other receptor families. For example, glutamate has several families of ionotropic and metabotropic receptors. E2 activates a cornucopia of different membrane-associated molecules that affect cell function. It is unusual however, that the same molecules are used as both nuclear and membrane ERs. In many ways, the membrane ER field is still in its infancy: which of the putative receptors are present on the membrane and how do they signal? For example, although ERα and ERβ have been localized in signaling complexes associated with the cell membrane [12;47;49;59], some controversy exists whether GPR30 (GPER1) is localized to the cell membrane. This is in spite of the fact that GRP30 resembles the canonical G protein-coupled receptor structure. One possibility is that GRP30 is localized on smooth endoplasmic reticulum, and E2 activation directly releases intracellular Ca^{2+} stores. In this way GRP30 mediates rapid E2 action but not at the cell membrane [79].

It is expected that these various ERs will have some measure of interaction. However, how and at what level these receptors interact is at present unclear. Certainly, activation of ERα-

mGluR1a or Gq-mER stimulates signaling cascades that can phosphorylate CREB and thus regulate gene expression independent of ERE (Figure 1). But how is this transcriptional activity integrated with activation of ERE and stabilization of the AP-1 site – hallmarks of direct nuclear action? Is there an adaptive advantage of estrogen membrane signaling-mediated gene expression? Over the years, the steroid receptor field has been hindered by technical limitations in addition to the dogma about nuclear-initiated signaling. However, with the advent of new technologies we realize that E2 alters the activity of a large number of proteins – most of whose functions, in a physiological sense, are unknown. Integrating these global changes is the next big challenge for the field. The localization of ERs, broadly defined, throughout the nervous system suggests that their functions are critical. Indeed, the ability of nervous tissue to synthesize steroids, and in particular E2, also argues that these messengers are vital.

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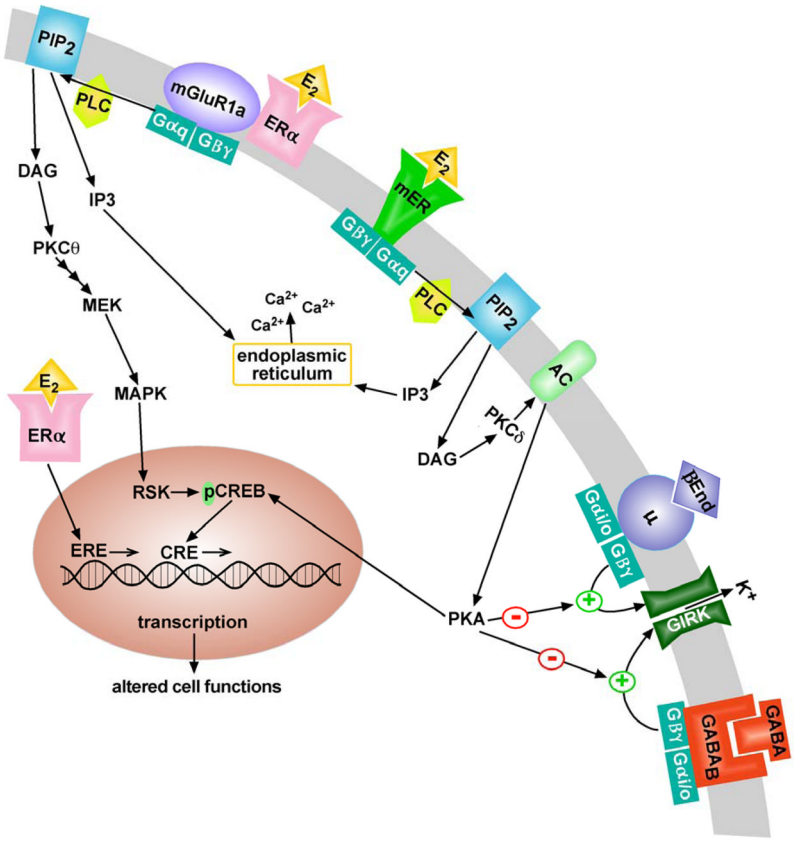

Figure 1.

Proposed schema of E2 signaling in POMC neurons: As in other non-neural cells, E2 binds to intracellular ERα to activate genes via estrogen-response elements (EREs). In addition, E2 binds to ERα localized at the neuronal (plasma) membrane and activates group 1 metabotropic glutamate receptor (mGluR1a) signaling. Activation of mGluR1a causes Gαq stimulation of phospholipase C (PLC), which leads to the hydrolysis of membrane-bound phosphatidylinositol 4,5-biphosphate (PIP_2) to inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG). IP3 releases internal calcium (Ca^{2+}) stores, while DAG activates protein kinase C-theta (PKCθ) leading to mitogen-activated kinase kinase (MEK) to mitogen-activated kinase (MAPK) to ribosomal S6 kinase (RSK)-induced phosphorylation of cAMP response element-binding protein (CREB). pCREB will activate genes expressing CREB-response elements (CREs). Also, E2 activates a membrane-associated ER (mER) that is $G\alpha_q$ -coupled (Gq-mER) to activate PLC generating IP3 and DAG. Ca^{2+} is released from the endoplasmic reticulum by IP3, and DAG activates protein kinase C-delta (PKCδ). Through phosphorylation, adenylyl cyclase (AC) activity is upregulated by PKCδ. The generation of cAMP activates PKA, which through phosphorylation can rapidly uncouple $GABA_B$ and μ-opioid (μ) receptors from G protein-coupled, inwardly rectifying K^+ (GIRK) channels. Gq-mER-mediated activation of PKA can also phosphorylate CREB. Therefore in addition to the direct effects on membrane excitability, both mGluR1 and mER signaling pathways can alter gene transcription through CREB response elements (CREs) on genes.