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Membrane-Localised Oestrogen Receptor α and β Influence Neuronal Activity Through Activation of Metabotropic Glutamate Receptors

P. G. Mermelstein

Department of Neuroscience, University of Minnesota, Minneapolis, MN, USA

Abstract

Until recently, the idea that oestradiol could affect cellular processes independent of nuclear oestrogen receptors (ERs) was controversial. This was despite the large number of carefully controlled studies performed both within and outside the nervous system demonstrating that oestrogens regulate various intracellular signalling pathways by acting at the membrane surface of cells and/or at biological rates incompatible with the time course of genomic-initiated events. At present, it is far less controversial that oestradiol acts at surface membrane receptors to regulate nervous system function. Recent studies have demonstrated that the classical intracellular ERs, ER α and ER β , are major players in mediating the actions of oestradiol on the membrane surface. This review focuses on one potential mechanism by which surface-localised ER α and ER β stimulate intracellular signalling events in cells of the nervous system. After oestradiol treatment, both ER α and ER β are capable of activating different classes of metabotropic glutamate receptors (mGluRs). Oestradiol activation of mGluRs is independent of glutamate, but requires expression of several different caveolin proteins to compartmentalise the different ERs with mGluRs into functional signalling microdomains. ER/mGluR signalling is a potential means by which oestrogens can both rapidly and for extended periods, influence a variety of intracellular signalling processes and behaviours.

Keywords

oestradiol; mGluR; lordosis; nociception; pain; oestrogen cycle; membrane; rapid actions

Oestrogen receptor (ER) signalling via direct actions on gene expression

Researchers have studied the effects of gonadal hormones on brain function for over 60 years (1). Across the lifetime of an animal, hormones such as oestrogens affect nervous system function through alterations in both anatomy and physiology. It is well established that oestrogens regulate sexual development, maturation and reproductive behaviours. Subsequent to the cloning of the first ER (2,3), ER α was determined to be a ligand-regulated transcription factor (4). This was consistent with previous data demonstrating the actions of oestradiol were dependent on the translation of new protein (5,6). Furthermore, the distribution of ER α (7,8) was highly correlated with steroid autoradiography studies (9,10), which found the highest levels of oestrogen binding in brain regions critical for reproductive success (11). In addition, ER α was found to be located primarily in the nucleus (12,13), where it would bind DNA at

oestrogen response elements (EREs) as a dimer once the receptors became occupied by their steroid ligand (14).

These ER α -mediated changes in gene expression and protein synthesis are often referred to as the classical mechanism of action of oestrogen. Although once thought to be a single, straightforward model of the action of oestrogen in the brain, the complexity of ER-mediated gene expression has proven extensive. Expansion of the model was required, for example, following the discovery of ER β (15) and diverse ER interactions with various co-activators and other transcription factors (16–18). This interplay between ERs and various other nuclear machinery involved with gene transcription accounts for some of the diversity of oestrogen-regulated genes, including those which lack EREs. Yet, even with the many revisions required to expand the original model of the action of oestrogen to fit these additional findings, much support was lacking within the field of neuroendocrinology for oestradiol affecting cell function outside of a transcriptionally-initiated event.

Mechanisms of the action of oestrogen working at the membrane surface

Beside the evidence that the nuclear effects of oestradiol in brain went far beyond the simple model of ER α -induced gene expression, another paradigm shift (i.e. oestrogens act at the surface membrane to regulate neuronal function) attempted to gain traction. This was in response to three principal findings that were often incongruous with an ER transcriptionally-initiated response. The first comprised multiple discoveries of the action of oestrogen within areas of the nervous system not being associated with reproduction, and the corresponding discovery that various nonreproductive behaviours are affected by oestrogens. The second finding was that many of these additional effects of oestradiol occurred on a time scale too rapid to be accounted for by the nucleus-initiated mechanism of action. Third, many of these rapid effects appeared to be initiated by oestradiol acting at the surface of the neuronal membrane.

In neurones, Kelly *et al.* (19) were the first main proponents of oestradiol having rapid effects via membrane actions, and showed that, within seconds, the hormone altered the electrical activity of preoptic and septal neurones. Fast actions of oestrogen are observed not just within the nervous system, but in various other tissues. One of the first reported rapid effects of oestrogens was on the accumulation of cAMP in uterine tissue. Szego and Davis (20) reported that concentrations of cAMP increased within 15 s of oestrogen application.

Recent studies have demonstrated oestrogen modification of cell excitability through modulation of ion channels in many other brain regions (21–23). Various intracellular signalling proteins are also affected by membrane oestrogen signalling, including activation of protein kinase A, protein kinase B, protein kinase C, phospholipase C, inositol triphosphate and mitogen-activated protein kinase (MAPK) (24–35). Of importance, these pathways often converge and/or interact with one another. Not surprisingly then, through these alternative mechanisms, oestradiol can affect gene expression and protein synthesis via the activation of transcription factors such as cAMP response element binding protein (CREB) (26,36–38). These rapid actions of oestrogen are relevant to a whole host of behaviours, such as learning and memory, motor control, mood, and pain perception (39–42). Of particular interest, the regions of the nervous system critical to these behaviours were originally thought to have little or no expression of the classical ERs: ER α and ER β . Thus, the question was raised as to the mechanism by which oestradiol was able to exert these additional effects.

Although there was increasing evidence that oestradiol could have rapid actions on cell biology, the mechanism(s) by which the steroid acts has remained controversial. A consistent finding has been that many of the reported rapid effects appear to be initiated at the membrane surface, as determined through the use of membrane impermeable oestrogen analogs (43,44). The

persistence of a rapid action of oestradiol following the intracellular dialysis (i.e. filling) of a cell with the steroid also supports this mechanism of action (23). Indeed, oestradiol had been shown to bind to the membrane of endothelial cells as early as 1977 (45).

In the 1980s, researchers began testing the hypothesis that the ER α and ER β could localise to the membrane surface (46,47). These reports were often improperly discredited, on the claim that these findings were due to a technical artefact (i.e. contamination of membrane fractions with transposed receptors from the nucleus during the isolation procedure). In addition, the known structure of ER α and ER β provided no clue as to how they would be membrane-localised, as well as be able to activate intracellular signalling even if they were trafficked to this region of the cell. However, in 1999, overexpression of ER α and ER β revealed that a portion of the ER protein was targeted to the membrane and activated intracellular signalling (48). Even with the caveats of using of overexpressed protein, this simple and elegant experiment demonstrated that the same protein is capable of mediating both intracellular and membrane actions of oestradiol.

ER interactions with metabotropic glutamate receptors (mGluRs)

One often studied indication of rapid oestradiol action has been the phosphorylation (i.e. activation) of the transcription factor CREB (26,35,36,38,49). Phosphorylation of CREB is an important convergence point in cell signalling, and is critically involved in various forms of neuronal plasticity. Several studies have found that the activation of surface ERs leads to CREB phosphorylation via stimulation of the MAPK/extracellular regulated kinase signalling pathway. In turn, activated CREB regulates gene expression through interaction with DNA at CREB response elements. These and other rapid oestradiol actions are blocked by the ER antagonist ICI 182,780, whereas ER α and ER β agonists frequently mimic the actions of the steroid (36,50). Such results provided pharmacological evidence that classical ERs play a role in the novel actions of oestradiol. Although these data still provide room to argue for a unique membrane ER, the debate as to whether classical ERs were at least partially responsible for mediating some of the reported rapid effects essentially ended when Abraham *et al.* (51), using ER knockout mice, determined that the rapid actions of oestradiol-mediating the phosphorylation of CREB and MAPK were dependent on ER α and ER β (51).

Based on this accumulating evidence of rapid oestrogen signalling, we and others hypothesised that membrane-localised ER α and ER β are responsible for many of the membrane-initiated actions of oestradiol. When testing this hypothesis, two questions immediate arise. First, how do classical ERs initiate cell signalling when localised to the membrane? Second, how are ERs trafficked to the membrane in the first place?

Clues to the mechanism by which membrane-localised ER α and ER β exert effects on cell function included numerous studies reporting the action of oestrogen as being sensitive to G-protein manipulation (23,31). Based upon these data, a relatively straightforward hypothesis (i.e. ER α and ER β directly bind and activate G-proteins) was proposed (52,53). In support of this mechanism, ER α can directly interact with at least one G-protein subunit (54). Yet, the diverse array of signalling pathways regulated by membrane ERs suggests additional mechanisms of action. Outside of the nervous system, membrane ERs have been found to directly bind and activate surface receptors linked to various second messenger systems (55–57). In parallel, we find membrane localised ER α and ER β capable of activating various metabotropic glutamate receptors (mGluRs).

Our initial experiments performed in cultured hippocampal neurones from female rat pups found that oestradiol stimulation of ER α resulted in increased CREB phosphorylation through activation of mGluR1 (36). mGluR1 and mGluR5 comprise the group I mGluRs, which are Gq linked and were previously shown capable of activating CREB (58,59). Mechanistically,

mGluR1 stimulation leads to MAPK-dependent CREB phosphorylation via activation of phospholipase C, protein kinase C and inositol trisphosphate signalling. Interestingly, the activation of ER α by oestradiol was only effective in triggering CREB phosphorylation in cultures derived from female, and not male, hippocampus. The underlying cause for this sex difference is currently being investigated.

The actions of ER α on mGluR1 require physiological (i.e. picomolar) concentrations of oestradiol. Furthermore, CREB phosphorylation was observed within 30 s of steroid application (with maximal responses at 2 min following oestradiol administration). With the additional evidence that nonpermeable oestrogen analogs and ER α agonists mimicked the response of oestradiol, and that the pure ER antagonist ICI 182,780 blocked the actions of oestradiol, we concluded ER α at the membrane was responsible for triggering CREB phosphorylation.

As noted, oestradiol has been observed to stimulate a variety of intracellular signalling cascades. In striatal neurones, we had previously reported that oestradiol, through activation of a G-protein coupled receptor, could decrease L-type calcium channel currents (23). This has subsequently been confirmed in various other neuronal systems (60,61). This is of particular importance because calcium entry through L-type calcium channels can rapidly trigger CREB phosphorylation through activation of calcium calmodulin-dependent protein kinase IV (CaMKIV). Consequently, we found oestradiol to also decrease L-type calcium channel-dependent CREB phosphorylation. Oestrogen inhibition of L-type calcium channel-dependent CREB phosphorylation was dependent upon activation of the group II mGluRs, mGluR2 and/or mGluR3 (36). These mGluRs are functionally linked to Gi/o second messenger signalling. The only major difference between oestradiol activation of mGluR1 versus mGluR2/3 was that the latter was triggered by both ER α as well as ER β .

It was of particular interest that we observed the bidirectional affects (i.e. activation of both mGluR1 and mGluR2/3 signalling) of oestradiol upon CREB phosphorylation within the same population of cells. Isolation of one pathway versus the other was first achieved through pharmacological manipulation. However, in a follow-up study, we were able to independently disrupt one signalling pathway or the other through modification of caveolin expression and/or function (62). Caveolin proteins are membrane proteins that organise signal transduction machinery, and have been previously demonstrated to interact with both mGluRs and steroid hormone receptors (63). Originally described outside of the nervous system, caveolin proteins were found essential for various membrane ER α responses (64). In hippocampal neurones, the caveolin-1 protein (CAV1) is essential for the functional coupling of ER α with mGluR1. Conversely, caveolin-3 (CAV3) is necessary for ER α and ER β activation of mGluR2/3 (62). To our knowledge, this is the first demonstration of functionally discrete microdomains within the same cell being generated by different caveolin proteins.

Recent studies have examined potential ER/mGluR interactions in other brain regions. We have focused our efforts on the striatum, where rapid effects of oestrogens have been reported for some time (65). Analogous to our results in hippocampal tissue, activation of ER α leads to the phosphorylation of CREB, whereas ER α and ER β attenuate L-type calcium channel dependent CREB phosphorylation. Both pathways were similarly dependent upon CAV1 and CAV3. To our surprise, however, the mGluRs responsible for oestrogen signalling to the nucleus were different. Although ER α is coupled to mGluR1 in the hippocampus, it is coupled to mGluR5 in the striatum. Similarly, recent findings suggest ER α /ER β activate mGluR2 signalling in hippocampus but mGluR3 in the striatum (66). These results are particularly intriguing because all four mGluRs are expressed in both the hippocampus and striatum. Regardless of the mechanism for differential pairing of ERs and mGluRs across neuronal subtypes, these data suggest that ERs may be coupled to various GPCRs, and not a select

population. This may account for the widespread action of oestrogen within the nervous system. At the very least, ERs can have diverse effects on neuronal cell excitability through pairing with different caveolin and mGluR proteins (Fig. 1).

Physiological relevance of ER/mGluR signalling

Although our work has focused on elucidating the mechanisms by which membrane ERs regulate neuronal function, and thus has used a more reductionist approach, it is also essential to demonstrate that these same signalling pathways are present and relevant in more intact preparations, and thus show physiological relevance. The laboratory of Paul Micevych has examined three separate model systems previously demonstrated to be regulated by membrane ERs. In each of these systems, ER/mGluR coupling was deemed crucial.

The first set of experiments examined oestradiol signalling in the arcuate nucleus and its role in female sexual receptivity. In the female rat, oestradiol acts on a limbic-hypothalamic circuit to allow the expression of lordosis, a stereotypic sexually receptive behaviour (67–69). It has been known for almost 30 years that oestradiol-induced lordosis behaviour is dependent on the transcription of new proteins (6,70,71). However, priming animals with a membrane-constrained oestradiol (E₂-6-BSA) followed with a subthreshold dose of oestradiol was equally efficacious as two oestradiol injections (72). Thus, membrane actions of the steroid are also important for lordosis. Previous studies had determined that membrane ERs in the arcuate nucleus act to affect a circuit influencing lordosis that also includes the medial preoptic nucleus (73,74). In our recent work, we found that in the arcuate nucleus, ER α was co-localised with mGluR1a. Furthermore, the effects of oestrogen in the arcuate nucleus upon the medial preoptic nucleus and subsequent lordosis behaviour was critically dependent on mGluR1 (75).

This first demonstration of ER/mGluR coupling in hypothalamic neurones was followed by an equally elegant demonstration in hypothalamic glial cells. Previous studies have shown that oestradiol administration results in an increase in intracellular calcium within these cells. The resulting rise in calcium is believed to be critical in the synthesis of neuroprogesterone and the luteinising hormone surge (76). Similar to the findings in neurones, the oestrogen-dependent rise in intracellular calcium within glial cells was reliant upon interactions between membrane ER and mGluR1a (77). In a third preparation, the functional coupling of ERs to mGluR2/3 was confirmed.

The cell bodies of primary visceral spinal afferent neurones are located in the dorsal root ganglia (DRG). These cells transmit nociceptive information to the spinal cord. One such activator of these DRG cells is ATP, which has emerged as a putative signal for visceral pain. Noxious stimuli such as distention of the viscera or tissue damage release ATP (78), which then transmits this signal by activating purinergic, ATP-gated P2X receptors on primary afferent fibers (79). The opening of P2X channels results in membrane depolarisations sufficient to trigger multiple action potentials and calcium influx through voltage-gated calcium channels (80). The vast majority of ATP-sensitive DRG neurones responds to oestradiol (60). Oestradiol was found to inhibit ATP-mediated calcium influx in small diameter DRG neurones, though modulation of L-type calcium channel currents. Furthermore, oestradiol inhibition of L-type calcium channels was dependent on mGluR2/3 (81).

Conclusions

It has now become widely accepted that the actions of oestrogen within the brain far exceed its effects upon sexual behaviour and maturation via nuclear-initiated processes. Indeed, oestradiol has been shown to induce a multitude of rapid, membrane-initiated events within various regions of the brain. These additional effects have been demonstrated to play a crucial role in diverse behaviours, such as learning and memory, motor control, mood, and pain

perception. Recent findings indicating that membrane localised ER α and ER β are functionally coupled to different classes of mGluRs may account for many of these novel actions of oestrogens on nervous system function.

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Fig. 1. Oestrogen receptor (ER) activation of metabotropic glutamate receptor (mGluR) signalling through interactions with caveolin proteins. (A) Model system of oestradiol-induced activation of mGluRs via caveolin-based caveolae. (B) Summary of distinct signalling pathways by which ERα and ERβ can affect cAMP response element binding protein (CREB) phosphorylation.