A Main Role for Metabotropic Glutamate Receptor 1 in the Neuroprotective Effect of Estrogen

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PharmSight on Spampinato SF et al., Estrogen receptors and type 1 metabotropic glutamate receptors are interdependent in protecting cortical neurons against β-amyloid toxicity. Mol Pharmacol 2012;81:12-20.

Abstract

Estrogen exerts neuroprotective activity under different experimental conditions through classical nuclear receptors, but mainly receptors expressed at the cell surface. Transducing mechanisms activated by these membrane estrogen receptors in the brain have been intensely investigated and, among others, interaction with G-protein coupled, metabotropic glutamate (mGlu) receptors has been considered. Besides mediating physiological estrogen functions, such as regulation of hormone production or sexual behavior in the hypothalamus, mGlu receptors, specifically mGlu1 receptor subtype, take part to the protective effect of estrogen in a model of neuronal toxicity induced by β-amyloid peptide. Coupling of estrogen receptor to mGlu1 receptor is supported by co-immunoprecipitation, similar neuroprotective effect induced by either receptor activation, lack of additivity when the two receptors are activated at the same time and prevention of the protective effect when antagonists of the other receptor are used, i.e. reduction of the protective effect of estrogen by the mGlu1 receptor antagonist and vice versa. In addition, the phosphatidylinositol-3 kinase/Akt pathway may represent the common signaling pathway to produce neuroprotection. These data introduce a novel view of the mechanisms underlying the neuroprotective activity of estrogen and open new perspectives also for future pharmacological interventions.

Keywords: Neuroprotection; Alzheimer’s disease; Estrogen; Receptor transactivation; Neurodegenerative disease; Phosphatidylinositol-3 kinase

Introduction

Widely reported and convincing data support a main role for estrogen in neuroprotection, as observed in in vitro cellular systems and in vivo animal models. Thus, addition of estradiol to cell cultures reduces neuronal death induced by exposure to excitotoxins (1-3) or β-amyloid peptide (Aβ) (4, 5) and estrogen treatment results protective in models of brain ischemia, Parkinson’s disease and Alzheimer’s disease (6-9). Neuroprotection is now mainly ascribed to membrane estrogen receptors (ERs) which generate rapid intracellular signaling upon estrogen binding (10). However, this neuroprotective activity of estrogen is not unequivocal as, specifically in in vitro models of Alzheimer’s disease, obtained by exposure of neuronal cultures to the toxic Aβ peptide, estrogen treatment can result detrimental to neuronal health, exacerbating the death of neurons, depending on the time and condition of exposure (11). This duality of estrogen effect appears intriguing and raises the question related to signaling pathways that are activated to produce such inconsistent effects. Membrane ERs are known to transactivate other receptors located in the cell membrane and, in the last few years, growing evidence has demonstrated a strict interaction of membrane ERs and metabotropic glutamate (mGlu) receptors (12). Of note, this interaction has been reported to be involved in the control of physiological aspects of estrogen function (12, 13), but also in the beneficial...
effect of estrogen in modulation of pain (14) and in neuroprotection (15)

Membrane estrogen receptors

In contrast to the classical belief that estrogen effects are mediated exclusively by genomic mechanisms, a large body of evidence has clearly established a major role for membrane ER in the actions of this hormone. Membrane ERs are known to be expressed also at the central nervous system (CNS), either in neuronal and glial cells, where estrogen exerts several different effects, regulating neuronal plasticity and excitability as well as taking part to neuroprotective and neurotrophic mechanisms (16). Acting at membrane receptors, rather than exerting a direct control of gene transcription, estrogen influences several rapid signaling pathways including activation of kinases, phosphatases and ion fluxes across membranes (17). Membrane ERs were initially described following the observation that in uterus estrogen was able to increase cAMP formation, an effect mimicked by the non-cell permeable analogs, BSA- and HRP-conjugated estrogen (18). Of note, besides their classical localization, it is the nuclear described ERα and ERβ that can also be associated with the plasma membrane and responsible for rapid, non-genomic effects of estrogen (19). What still remains to be established is how nuclear receptors are trafficked to the membrane, if they undergo post-transcriptional modifications that allow their insertion into the membrane (20, 21), and how activated receptors couple to their signaling pathways to generate a rapid cellular response.

Acting at membrane ERα and ERβ, estrogen is able to modulate ionic movements through the membrane, and electrophysiological features of neurons can be modulated very rapidly upon estrogen activation (22). The involvement of G proteins in estrogen rapid signaling has not been disregarded. In fact, ionic currents are known to be modulated by estradiol following increased formation of cAMP and activation of protein kinase A (PKA), suggesting the occurrence of mechanisms mediated by a Gs coupled protein (23). Estrogen can also activate phospholipase C (PLC)/protein kinase C (PKC), thus modulating a Gq protein (24-27), and signaling related to Gi/o protein has also been demonstrated (28, 29). In addition, more important for estrogen effects at the CNS seems to be activation of the mitogen-activated protein (MAP) kinase signaling, with ERK1/2 phosphorylation occurring minutes after estradiol addition (2), and stimulation of the phosphatidylinositol-3 (PI-3) kinase pathway, with ensuing rapid phosphorylation of Akt (30). How ERs activate G protein-mediated signaling is still controversial. One possibility is that a direct interaction occurs or, alternatively, that ERs do not come into contact with a G protein, but rather involve G protein-coupled receptors, expressed on plasma membrane, and activate their signaling. Such a mechanism would reiterate what already observed in other systems, either within or outside the CNS, where ERα and ERβ are able to transactivate other receptors, specifically those signaling through tyrosine kinase, such as epidermal growth factor (EGF) (31) and type-I insulin-like growth factor (IGF-I) receptors (32, 33).

However, removal of classical receptors, ERα or ERβ, using double knockout mice, does not prevent all estradiol binding (34) and estrogen maintains its activity (35, 36). Other estrogen recognition sites located on membranes have in fact been described. These include ER-X described in uterus, lung and neocortex, associated with caveolin proteins and functionally coupled to MAP kinase signaling (37); the more recent GPR30, a G protein coupled integral membrane protein, whose function as an estrogen receptor appears however still controversial (38); a STX binding protein, stereospecifically activated by estrogen and blocked by the estrogen antagonist ICI 182,780, whose molecular structure remains to be completely identified (27, 39).

Metabotropic glutamate receptors

mGlu receptors are a large family of G-protein coupled receptors. Eight different mGlu receptor subtypes have been identified so far and they are divided into three groups, on the basis of sequence homology and pharmacological sensitivity. Group I, including mGlu1 and mGlu5 receptors are coupled to Gq/G11 and are generally located postsynaptically. Group II, which includes mGlu2 and mGlu3 receptors, and group III, including mGlu4, mGlu6, mGlu7, and mGlu8 receptors, are all coupled to Gi/Go, as reported in heterologous expression systems, and show a preferential pre-synaptic localization (40, 41). Other signaling pathways are reported to be involved upon mGlu receptor activation. Specifically, class I receptors activate phospholipase D, Jun kinase, MAP kinase, L-type voltage sensitive Ca++ channels, K+ channels, whereas groups II and III receptors also activate MAP kinase and PI-3 kinase pathways (40, 41). The

pharmacology of all mGlu receptors has significantly grown in the last recent years and on the basis of the demonstrated role of these receptors in different CNS disorders including anxiety, schizophrenia, epilepsy, depression, pain, Alzheimer’s and Parkinson’s diseases, drugs targeting mGlu receptors have been or are now ready to be validated in clinical trials in different pathological conditions (40).

Among others, attention has been focused on mGlu1 receptors, as one of the unresolved and intriguing aspects of the function of this receptor subtype is its dual, and somehow ambiguous, role in neuroprotection/neurodegeneration processes. mGlu1 receptors produce in fact excitatory effects and consequently its activation has been related to induction and potentiation of excitotoxic neuronal death, both in vitro and in vivo (42). Divergent from these observations are data demonstrating a role for this receptor in neuroprotection, as reported, for instance, in models of cerebral hypoxia/ischemia and excitotoxic motoneuronal death (43-45). Consensus was obtained recognizing that mGlu1 can exert a dual role, depending on cellular context and environmental conditions (46, 47). An interesting explanation for the dual role of mGlu1 receptor in neuroprotection has been provided, assuming that the receptor, under conditions of excitotoxicity, can undergo conformational changes with cleavage of its intracellular tail that is necessary to signal through the protective PI-3 kinase pathway (48).

**Membrane estrogen receptors and metabotropic glutamate receptors**

The work by the groups of Mermelstein and Micevych has been pivotal in establishing the interaction occurring between membrane ERs and mGlu receptors in the CNS (reviewed in 10, 13). Interestingly, this interaction reveals region specificity and mGlu receptor as well as ER subtype selectivity, i.e. ERα and/or ERβ couple to different mGlu receptors in a region specific manner, likely to mediate different functions throughout the CNS (49). The reported ability of estrogen to inversely modulate the phosphorylation of cAMP Response Element (CREB) (23, 50, 51) in hippocampal neuronal cultures has been related to the interaction of ERs with either mGlu1 or mGlu2 receptors and activation of their signaling (24). Estrogen rapidly (within 30 sec) increases CREB phosphorylation, an effect mimicked by the mGlu1 receptor agonist (DHPG) and prevented by the mGlu1 receptor antagonist LY367385, as well as by inhibitors of the signaling cascade leading mGlu1 receptor activation to CREB phosphorylation (24). Similarly, in the same cultured hippocampal neurons, estrogen reduces L-type calcium channel currents, leading to reduction of CREB phosphorylation, an effect mimicked and blocked by the mGlu2/3 receptor agonist, LY379268, and antagonist, LY341495, respectively (24). Of note, in neuronal cultures from the striatum, estrogen triggers similar effects on CREB phosphorylation, but in this case, interaction of ERα with mGlu5 receptor elicits enhanced CREB phosphorylation, whereas interaction of ERβ with mGlu3 receptor negatively modulates CREB activation (52). Importantly, region-specific coupling of the receptors has been confirmed by co-immunoprecipitation studies showing that, in the hippocampus, ERα couples with mGlu1 receptor and both ERα and ERβ co-immunoprecipitate with mGluR2/3 (25) whereas, in the striatum, ERα couples with mGluR5 receptor and both ERα and ERβ are linked to mGluR3 (52), as summarized in Table 1.

Female lordosis, a stereotypic behaviour indicative of sexual receptivity is controlled by estrogen that induces activation and rapid internalization of MOR (μ-opioid receptor) in the medial preoptic nucleus. This effect of estrogen involves mGlu1 receptor as estradiol-induced MOR activation and lordosis is attenuated in the presence of an antagonist of mGlu1 receptor and prevention of lordosis by mGlu1 receptor blockade is only effective at the time of estradiol treatment. Furthermore, stimulation of mGlu1 receptor in the presence of low estrogen levels results in MOR internalization and consequent lordosis behavior (25).

Coupling of ERs to mGlu receptors is not restricted to the neuronal compartment but likely occurs also in astrocytes. Glial cells participate to neuronal function and ERs and mGlu receptors are known to mediate important functions also in astrocytes. Specifically, estrogen controls the production of progesterone by astrocytes in female hypothalamus (Table 1) and this effect is critical for induction of the LH surge (53). Estrogen, through mGlu1 receptor activation, increases intracellular calcium release, responsible for enhanced progesterone production. The estrogen-induced calcium flux is sensitive to blockade of mGlu1 receptor by the antagonist LY367385, whereas DHPG stimulates progesterone release similarly to estrogen (54, 55). Amplification of the calcium flux by estrogen and DHPG added together suggest that
cooperation between the two receptors is necessary for maximal progesterone production (54). Finally, the interaction between ERs and mGlu receptors has been shown to occur also in dorsal root ganglia (DRG) and to mediate pain perception (Table 1). ATP, that is responsible for activation of nociceptive C fibers (56) generates in fact a rapid Ca++ response mediated by P2X purinergic receptors and a late calcium influx through voltage-gated calcium channels (VGCC) (57). Estrogen reduces Ca++ influx by modulating L-type VGCC (14), an effect prevented by treatment with the mGluR2/3 antagonist LY341495, suggesting an interaction of ER and class II mGlu receptors in this effect (14).

Interaction of ERs and mGlu receptors in neuroprotection

It was important to establish whether the reported interaction between ERs and mGlu receptors could also be invoked in the neuroprotective effect of estradiol. As mentioned, in many instances, neuroprotection by estrogen is controversial or, more specifically, besides the largely demonstrated beneficial effects on neuronal survival, a detrimental action ascribed to the ERα subtype in neuroprotective mechanisms (58). To prove the involvement of mGlu1 receptor in the observed effect, neuroprotection is elicited also by DHPG and concomitant stimulation of the two receptors produces an effect that is less than additive. This strict interaction is further supported by the fact that treatment with receptor antagonists for ER (ICI 182,780) and mGlu1 receptor (JNJ 16259685) prevents neuroprotection elicited by their respective agonist, but also by ligand for the other receptor. Interestingly, estrogen and DHPG converge on the classical mGlu1 receptor signaling pathway (59), as stimulation of both receptors produces increased accumulation of inositol monophosphate, reflecting activation of PLC. Of note, ERα and mGlu1 receptors likely share a common signaling pathway to elicit neuroprotection against Aβ toxicity, as activation of both receptors results in increased phosphorylation of Akt and treatment with the Akt inhibitor, 10-DEBC hydrochloride, prevents the reduced neuronal death observed in neurons challenged with either estradiol or DHPG. Again, stimulation of both receptors at the same time produces an effect that is not additive and activation of ERα and mGlu1 receptors is interdependent. Thus, blockade of either receptor with the reciprocal antagonist prevents PI-3 kinase/Akt pathway activation due to the selective receptor agonist. It cannot be completely excluded that convergence of ER and mGlu1 receptors on common transducing mechanisms probably involves additional pathways activated following interaction of the two receptors, including MAP kinase, as suggested by reduction of the neuroprotective effect of estrogen, DHPG or both in the presence of the MAP kinase pathway inhibitor U0126 (our unpublished results). Interestingly,

Table 1. Coupling of ERα and ERβ and mGlu receptor subtypes in different brain areas.

<table>
<thead>
<tr>
<th>Hippocampus</th>
<th>Striatum</th>
<th>Hypothalamic astrocytes</th>
<th>Cortical neurons</th>
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<tbody>
<tr>
<td>ERα → mGluR1 → cAMP</td>
<td>ERα → mGluR5 → cAMP</td>
<td>ERα → mGluR1 → cAMP</td>
<td>ERα → mGluR1 → neurope</td>
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The co-operation between ERs and mGlu receptors, known to take part mostly in the physiological actions of estrogen, has now been demonstrated to occur also in pathological conditions, when neurons are exposed to a toxic insult. Intervention of a different receptor in the actions mediated by ERs seems to be particularly significant, as it (i) provides an answer to the unresolved issue of how membrane ERs activate rapid signaling, and (ii) assign to mGlu receptor conformation and/or function the main role of driving the final response to estrogen. Specifically, coupling of ERα and mGlu1 receptor in neurons can provide an explanation for the dual actions ascribed to ER in neuroprotection. Activation of the two receptors and their ensuing interaction before the toxic challenge can in fact be responsible for stimulation of the PI-3 kinase/Akt pathway that results in enhanced neuronal survival (Fig. 1). This may not be the case when estrogen is added after the toxic stimulus, as changes in mGlu1 receptor conformation may modify the signaling pathway activated and the final response observed.

Acknowledgements
Supported by a grant from the Italian Ministry of University and Research (Prin 2009).

Conflicts of Interest
No potential conflicts of interest to disclose.

References


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