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Multiple Pathways Transmit Neuroprotective Effects of Gonadal Steroids

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Numerous preclinical studies suggest that gonadal steroids, particularly estrogen, may be neuroprotective against insult or disease progression. This paper reviews the mechanisms contributing to estrogen-mediated neuroprotection. Rapid signaling pathways, such as MAPK, PI3K, Akt, and PKC, are required for estrogen's ability to provide neuroprotection. These rapid signaling pathways converge on genomic pathways to modulate transcription of E2-responsive genes via ERE-dependent and ERE-independent mechanisms. It is clear that both rapid signaling and transcription are important for estrogen's neuroprotective effects. A mechanistic understanding of estrogen-mediated neuroprotection is crucial for the development of therapeutic interventions that enhance quality of life without deleterious side effects.

Key Words: Neuroprotection; in vitro model; transcription.

Introduction

There Is Evidence of Gender Differences in Susceptibility to Neurological Disease or Insult

Data from epidemiological studies suggest that women are less susceptible to Alzheimer's disease (AD) *(1)*, Parkinson's disease (PD) *(2,3)*, and stroke *(4)*. However, there seems to be a reversal of this phenomenon subsequent to menopause *(1,2,4)* that is correlated with declining levels of circulating estrogen. In recent years, many have begun to appreciate the fact that gonadal steroids, particularly estrogen, may be neuroprotective against insult or disease progression. In order to address this public health issue in an aging population, there has been increased interest in determining whether estrogen replacement decreases the risk for stroke and AD in postmenopausal women. Similarly, preclinical research aimed at elucidating the molecular mechanisms underlying estrogen's neuroprotective effects have also intensified.

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The Benefits of Hormone Replacement Therapy for Menopausal Women Are in Question

The Women's Health Initiative (WHI) clinical trials were designed to assess the benefits and risks of estrogen replacement therapy (ERT) or combined estrogen + progesterone hormone replacement therapy (HRT) in postmenopausal women *(5)*. The WHI monitored the effects of HRT on several risk factors, including, among others, the incidence of dementia, heart disease, cancer (breast, colorectal, endometrial), and stroke. The WHI terminated prematurely due to increased risk for coronary heart disease, stroke, as well as lack of overall benefit *(5,6)*. Interpretation of the WHI findings is complicated by several methodological issues. For instance, approx 67% of women enrolled in the WHI clinical trials were 10 yr postmenopause and 75% had not taken any sort of ERT in that interval *(7)*. This contrasts with previous ERT trials, which were initiated during perimenopause and suggested potential cardioprotection *(8)*. Finally, participants in the WHI clinical trials received the commonly prescribed conjugated equine estrogen (CEE) and medroxyprogesterone acetate (MPA) rather than estrogen and progesterone. Data indicate that CEE and MPA have divergent effects from those of estrogen and progesterone *(7,9)*. It is clear that findings from clinical studies published to date have not resolved the issue of when and how to administer ERT. Although clinical studies have provided conflicting results *(5–8,10)*, preclinical data provide a more consistent picture of the putative mechanisms responsible for estrogen-mediated neuroprotection.

Estrogen Regulates Transcription via "Classical" and "Nonclassical" Mechanisms

The prevailing "classical" genomic model of estrogen receptor (ER) action states that, in the absence of hormone, members of the steroid hormone receptor superfamily reside in a complex with inhibitory chaperone proteins. Upon ligand binding, the receptor undergoes a conformational change that allows it to dimerize with another ligand-bound receptor, enter the nucleus, and bind DNA. The steroid–receptor complex has direct contact with estrogen response elements (EREs) on the DNA. The ER dimer may recruit basal accessory proteins *(11)* to provide the necessary complement to relax the chromatin and initiate transcription at specific

estrogen response elements (EREs). The consensus ERE sequence is an inverse palindromic repeat consisting of GG TCAnnnTGACC *(12)*.

In addition to "classical" ERE-mediated transcription, alternative mechanisms for regulation of target genes have been described where ERs modulate transcription indirectly at other response elements. These "nonclassical" genomic responses do not require a direct ER–DNA interaction. ERs can be part of the basal transcriptional machinery that initiate transcription of genes at a variety of response elements, including serum response elements (SRE), cyclic-AMP response elements (CRE), activator protein-1 (AP1), signal transducer and activator of transcription (STAT), and GC-rich regions *(13–17)*. The transcription factors that interact directly with the DNA in this model are regulated by rapid signaling pathways initiated at the plasma membrane or in the cytoplasm.

Estrogen Has Multiple Rapid Signaling Effects That Indirectly Modulate Transcription

Within the last few years, accumulating evidence supports the idea that estrogen also activates rapid non-genomic signaling pathways at the plasma membrane or in the cytoplasm. These include ER coupling to G-proteins, adenylyl cyclase, protein kinases A (PKA), PKB (also known as AKT), PKC, c-src, cyclic AMP response element binding protein (CREB), fos, c-jun *(18)*, mitogen activated protein kinases (MAPK's), nuclear factor kappa B (NFκB), cyclic GMP, and nitric oxide synthase, among others *(17, 19–22)*. Rapid signaling effects are believed to be responsible for membrane electrophysiological responses *(23)* to estrogen, which cannot be explained by slower transcriptional mechanisms. Very recently there has been increased emphasis on integrating what is known about the transcriptional and rapid non-transcriptional effects of steroids in the CNS *(17,24)*.

Some Comments About Terminology

There are several ways to categorize the molecular effects of estrogen. The simplest model distinguishes between rapid signaling events that occur at the plasma membrane and/or in the cytoplasm (nongenomic) from events that alter RNA transcription (genomic) in the nucleus. This model is complicated by the fact that, estrogen regulates transcription classically (via ERE) *and* nonclassically (via other transcription factors and response elements). Furthermore, nonclassical and classical (see ref. *25*) transcriptional responses can be modulated by rapid signaling events (Fig. 1). Therefore, within the context of neuroprotection, the term non-genomic may be inappropriate because rapid signaling events may in some cases be a prerequisite for transcription. In order to provide clarity on this issue, the terminology ERE-dependent or ERE-independent will be used. Rapid signaling events initiated at the plasma membrane or in the cytoplasm that modulate transcription at ERE are considered ERE-dependent mechanisms of action. Conversely, rapid signaling events that modulate transcription in an ERE-independent manner (as the preponderance of data suggests) are subsumed under the ERE-independent heading. These events are often termed "rapid signaling" events because they are initiated at the plasma membrane or in the cytoplasm and occur on a much faster time scale than transcriptional events.

Gonadal Steroids Are Neuroprotective via Rapid Signaling Pathways and Transcriptional Mechanisms

This review will focus on evidence supporting the concept that estrogen-mediated neuroprotection is dependent on rapid signaling *and* transcriptional mechanisms. There are other potentially important mechanisms involving antioxidant effects of estrogenic compounds that will not be covered in this review. Finally, although much of the current discussion is focused on estrogen, evidence suggests that the gonadal steroids progesterone (P) and testosterone (T) are neuroprotective and that they utilize similar mechanisms of action *(9,26–28)*. However, space limitations preclude an extensive discussion of the potential neuroprotective effects of P and T.

Rapid Signaling Pathways Are Necessary for E_2-Mediated Neuroprotection

Early Evidence Correlated Rapid Signaling with Neuroprotection

In order to elucidate the mechanisms responsible for estrogen's neuroprotective effects, our laboratory has used cell culture models, which are more amenable to mechanistic experiments than in vivo neuroprotection models. Initial neuroprotection studies noted that 17β-estradiol (E_2) protected primary cortical neurons from glutamate excitotoxicity *(29)*. Furthermore, there was evidence that this was an ER-dependent effect. We subsequently noted that that Bcl-2 expression was correlated with the protective effects of E_2 in the NT-2N neuronal cell line *(30)*. It was reasonable to assume that ERE-dependent transcriptional mechanisms mediated E_2's effects. However, evidence from this laboratory *(19)* and others *(31)* raised the possibility that E_2 might also be neuroprotective via rapid signaling pathways. This work led to the demonstration that 5-min pre-exposure to E_2 could be neuroprotective against glutamate toxicity. Furthermore, E_2's neuroprotective effects were correlated with transient activation of rapid signaling pathways including the tyrosine kinase src, tyrosine phosphorylation of $p21^{ras}$-guanine nucleotide activating protein, and phosphorylation of the MAPK pathway *(32)*. Blockade of ERK 1/ 2 phosphorylation with the MAPK kinase (MEK) inhibitor PD98059 abrogated E_2's neuroprotective effects, implicating the MAPK pathway in E_2-mediated neuroprotection (for

review see ref. *33*). Studies have subsequently demonstrated that E_2 rapidly activates MAPK in vivo *(34–36)* and ERK phosphorylation is required for neuroprotection in the hippocampus *(37,38)*. Furthermore, ER expression is sufficient to render otherwise unresponsive cell lines responsive to E_2 signals *(39,40)*. In order to determine the relative contribution of ERα and ERβ to E_2's neuroprotective effects, murine hippocampal-derived HT22 cells were stably transfected with cDNAs encoding ERα or ERβ *(25,41)*. Either ERα or ERβ expression was found to be required for E_2-mediated neuroprotection in HT22 cells.

While rapid signaling via the src/MAPK pathway is required for neuroprotection in HT22 cells, it was still unclear whether these events act independently of ERE-mediated gene transcription in eliciting neuroprotection. Mize et al. *(25)* examined the effect of a mutated ERα that does not bind ERE. Data from this study indicated that although both the "wild-type" and mutant ER could activate MAPK, the mutated receptor demonstrated reduced (50%) neuroprotection when compared to wild type. This clearly demonstrates that rapid signaling as well as ERE-dependent mechanisms are important for neuroprotection *(25)*.

Rapid Signaling Can Be Initiated by Putative Membrane ER

Because activation of signaling pathways, such as ERK, phosphatidylinositol-3-kinase (PI3K), nitric oxide (NO), and calcium flux generally occur at the plasma membrane, the question arises: Do ERs act at the plasma membrane? Numerous reports have suggested the existence of functional ERs at, or near, the plasma membrane using binding assays *(42–48)*, antibody staining *(49–51)*, and membrane-delimited estrogens *(40,45,52)*. Together, these results support the existence of functional ERα and ERβ at the plasma membrane in both neurons *(53–55)* and glia *(56)*. It has been hypothesized that these membrane ERs transduce E_2's rapid signaling effects important for neuroprotection. For example, double ERα/ERβ knockout mice are unable to initiate E_2-induced rapid signaling necessary for neuroprotection *(35,57)*.

A number of membrane associated proteins have been identified that associate with ERs. Several reports show interactions between ERα and caveolar proteins in endothelial cells *(58–61)*. Epidermal growth factor receptor (EGFR) has also been shown to promote ERα localization to the membrane *(62)*. In neurons, the scaffolding protein striatin interacts with the DNA-binding domain of ERα and is necessary for rapid activation of NOS *(63)*. Another scaffolding protein termed modulator of non-genomic activity of estrogen receptor (MNAR) has been shown to interact with the ligand-binding domain of ERα and is required for estrogen-mediated activation of c-src *(63)*. Additionally MNAR mRNA and ERα mRNA are co-localized in the rat brain *(64)*. This is supportive of an interaction between these proteins. Finally, MNAR also interacts with the p85 subunit of PI3K *(65)*, providing a possible molecular link between ER and the PI3K-AKT pathways (see below).

Several Novel ER/ER Candidates Have Been Described

ERα and ERβ may not be the only receptors capable of binding estrogen and initiating signaling events. GPR30 *(66)* is a seven-membrane-spanning protein, which is widely expressed and can modulate signaling events in cells in response to E_2. ER-X is an as yet, uncloned receptor which is ICI-insensitive and mediates MAPK activation in ERα knockout mice *(67)*. ER splice variants have been described such as the truncated ER46 *(68)*. Although E_2 is presumed to be the preferred ligand for ER splice variants and possibly GPR30, there is evidence that the preferred ligand for ER-X is 17α-estradiol *(67,69,70)*. Previously, 17α-estradiol had been regarded as an ER-inactive compound that elicited neuroprotective effects only at high concentrations via antioxidant mechanisms (review see ref. *71*), but other recent reports have suggested that this compound can signal via receptors to produce neuroprotective effects *(72, 73)*. Finally, there are data indicating that another novel G protein–coupled membrane ER exists *(20)*. Although this uncloned ER has not been linked to neuroprotection, it does modulate protein kinase C (PKC) activity, which is one mechanism by which estrogen is neuroprotective (see below).

Membrane-Delimited E_2 is Neuroprotective via MAPK, PI3K, and TGF-β

Membrane-impermeant estrogens have been used to differentiate between cell-surface and intracellular actions of estrogen in a variety of cell lines *(40,74)*. Activation of ERK by membrane-impermeant E_2 occurs via Raf-1 in SN56 cells and has been correlated with neuroprotection against Aβ toxicity *(75,76)*. Membrane-impermeant E_2 has also been shown to protect neuronal-astrocyte co-cultures via activation of PI3K/Akt signaling pathway and release of TGF-β *(77,78)*. Neuroprotective E_2-mediated MAPK activation has also been shown to attenuate microglial superoxide release and phagocytic activity in N9 microglial cells *(79)*.

Gonadal Steroid Neuroprotection Is Also Mediated by the PI3K-AKT Pathway

E_2 and P have been found to activate PI3K and AKT in neuronal cell lines *(80)*. T has likewise been observed to elicit rapid activation of the PI3K pathway to produce neuroprotective effects (for review, see ref. *81*). E_2-mediated activation of AKT can be neuroprotective in a variety of neurons *(80,82,83)*. E_2-mediated neuroprotection has also been shown to be dependent on PI3K activity in rat primary cortical neurons challenged with glutamate and NO *(84)* or staurosporine *(85,86)*. PI3K activity is required for E_2-mediated neuroprotection in retinal neurons exposed to H_2O_2 toxicity *(87)*. Activation of the PI3K/AKT pathway is required for E_2 action on cortical astrocytes, which release neuroprotective cytokines, transforming growth factor (TGF)-

β1, and TGF-β2 to promote neuroprotective effects in the brain *(77)*.

Activation of PI3K and AKT can inhibit the activity of GSK3β, a constitutively active kinase involved in the phosphorylation of tau. E_2-mediated inhibition of GSK3β has been demonstrated to be neuroprotective in rat hippocampal slice cultures *(88)* and the rat hippocampus *(89)*. ERα is, in turn, a substrate for GSK3, and inhibition of GSK3β activity results in both a decrease in the phosphorylation of ERα and reduction in its transcriptional activity *(90)*.

Protein Kinase C (PKC) Has Been Implicated in E_2-Mediated Neuroprotection

E_2-mediated neuroprotection has been shown to involve PKC isoforms in a variety of neuronal models. PKC includes a large family of protein kinases separated into the calcium and diacylglycerol (DAG)–dependent common isoforms (α, β, δ), the DAG-dependent novel isoforms (δ, ε, η, and θ), and the atypical isoforms (ι/λ and ζ). Hayashi et al. have demonstrated that a G protein–coupled ER activates PKCγ, which leads to neuroprotection in a middle cerebral artery occlusion (MCAO) model *(91)*. Similarly, blockade of PKC activity abrogates neuroprotection elicited by E_2 and the ERα-specific agonist PPT, but not the ERβ selective agonist DPN, in primary cultured neurons challenged by Aβ toxicity *(73,92)*. Activation of PKC occurs downstream of E_2 signaling via phospholipase C (PLC) (for review, see ref. *93*). Rapid activation of PKC by a membrane-impermeant E_2 has been shown to potentiate transcriptional activity of subsequent "chase" doses of E_2 in SK-N-BE neuroblastoma cells, demonstrating a direct link between rapid and classic signaling *(94)*. Recently, however, it has been demonstrated that it is the inhibition of PKCε that mediates neuroprotection in HT22 cells *(95)*. Future studies will need to clarify the role of the various PKC isoforms in neuroprotection.

There Is Cross-Talk Between Insulin Growth Factor 1 (IGF1) Receptors and ER

Estrogen has been shown to directly interact with growth factor signaling pathways such as the IGF-1 pathway. Recent findings indicate that IGF-1 participates in physiologically relevant neuroprotective mechanisms and can protect against neuronal and glial cell degeneration in animal models of stroke and other insults (for review, see ref. *96*). Azcoitia et al. have shown estrogen-mediated neuroprotection against kainic acid (KA) toxicity involves IGF1R *(97)*. Estrogen interacts with the IGF1 signaling pathway at many levels (for review, see ref. *98*). These interactions begin at the level of ligand:receptor interaction. ERα co-immunoprecipitates (IPs) with IGF1R within 1–3 h of E_2 treatment, while after the 3 h, ERα co-IPs with the p85 subunit of PI3K *(98)*. These signaling molecules also share several second messenger pathways. Although IGF and E_2 signals synergize to activate AKT, activation of ERK occurs in response to either IGF or E_2, but not in a synergistic fashion *(82)*. GSK 3β is another key molecular target shared by E_2 and IGF-1 receptor signaling in the promotion of neuroprotection.

Transcriptional (Genomic) Regulation of Estrogen Neuroprotection

In the preceding section, we reviewed the contribution of rapid signaling to E_2's neuroprotective effects. Although the rapid signaling and transcriptional modes of E_2 action were once viewed as separate events elicited by the hormone, it has recently been experimentally demonstrated that E_2's rapid effects have direct and functional consequences on transcription *(94,99,100)*. Thus, estrogen neuroprotection likely involves the integration of rapid signaling with the transcription of genes required for repair and the prevention of further injury. This section of the review will address how estrogen-initiated rapid signaling pathways converge to modulate the transcription of target genes involved in neuroprotection.

The Transcription of Estrogen Responsive Genes Is Modulated by Insult

Estrogen provides neuroprotection using multiple pathways and its effects on transcription depend on the model of injury. Estrogen has been shown to provide neuroprotection from apoptotic and necrotic insults *(71)*, which use different pathways to achieve cell death. Apoptosis, for example, involves caspases to induce cell death without releasing the intracellular contents, whereas necrotic cell death typically involves an inflammatory response and cell lysis *(101,102)*. Thus, the genes and gene products that are regulated by estrogen should depend on the type of injury that induced them.

Injury models relying on apoptotic signaling mechanisms have demonstrated neuroprotection via estrogen inhibition of pro-apoptotic gene transcription (for review, see ref. *103*). Pro-apoptotic gene products regulated by estrogen include caspases *(104–108)*, cytochrome c *(109)*, calpain *(108)*, Nip-2 *(110,111)*, and Par-4 *(112)*, whereas E_2 increases the anti-apoptotic gene thioredoxin *(113,114)*. Estrogen can also protect against necrosis through inhibition of inflammatory gene products activated by the necrotic pathway, such as pro-inflammatory cytokines *(115–118)*, COX-2/prostaglandins *(118–120)*, complement *(121)*, NO/NOS *(21,115,118,122, 123)*, and matrix metalloproteinases *(119,121)*. In addition, some injury models use both necrotic and apoptotic pathways, as in the case of cerebral ischemia where as much as 50% of damage involves apoptotic pathways *(124)*. Thus, E_2-mediated neuroprotection against ischemic damage involves gene products from both pathways (for reviews, see refs. *71* and *125–127*). Other gene products are regulated by estrogen across injury models such as Bcl-2 *(30,86,108,127–134)*, IGF-1 *(135–138)*, NGF *(133,139–144)*, BDNF *(145–150)*, and TGF-β1 *(77,151)*. Thus, estrogen's ability to regulate genes and gene products may depend on the signaling pathways elicited by the insult. These signaling pathways converge at the genomic level to differentially regulate transcription.

Evidence Favors Neuroprotection via ERE-Independent Transcription

Although E_2-mediated transcription at estrogen response elements (EREs) has long been documented in the field of reproductive biology (for review, see ref. *152*), evidence directly linking classical ERE-mediated transcription to neuroprotection is surprisingly lacking. Most estrogen-responsive genes lack ERE palindromes and instead have non-palindromic EREs or ERE half-sites *(153)* that mediate transcription *(12,154–156)*. One of the most cited examples involved in estrogen neuroprotection is the Bcl family, including Bcl-x_L *(132)* and Bcl-2 *(30)*, which are maintained at steady-state levels by estrogen in spite of injury *(128, 129)*. Recently it has been experimentally demonstrated that estrogen rescues Bcl-2 levels through ERE- rather than CRE-mediated transcription in breast cancer cells sufficient to prevent apoptosis *(157)*. Although these pathways are similar to those occurring in the brain, the extent to which ER–ERE interactions are involved in neuroprotection remain unclear. For example, the anti-apoptotic effect of E_2 in dopaminergic neurons (cultured from the substantia nigra, a region damaged in PD) was blocked by the ERE transcriptional antagonist, ICI 182,780, but not a synthetic peptide that blocks ER dimer formation *(107)*. Moreover, data from our laboratory have demonstrated that HT22 cells expressing an ERα mutant incapable of interacting with EREs have a reduced neuroprotective response to E_2 compared to cells expressing ERα or ERβ *(25)*, indicating that ERE-dependent transcription contributes, at least in part, to E_2-mediated neuroprotection.

Estrogen Is Neuroprotective via ERE-Independent Transcription

Estrogen can modulate transcription in an ERE-independent manner *(19)*. It has been estimated that one third of ER-regulated genes do not contain ERE sequences in their promoters *(156)*. Other response elements that have been shown to mediate transcription of estrogen-regulated genes include AP-1 (via fos/jun), SRE (via Elk-1/SRF), STAT (via Stat/Stat), CRE (via CREB and ATF/jun), GC rich sites (via SP1), and NFκB (via NFkB) *(17,156,158–162)*. ERE-independent transcription can be activated by E_2 through either ER binding to transcription factors (tethering) or via direct phosphorylation of transcription factors by estrogen-induced signaling cascades, such as ERK 1/2 phosphorylation of CREB for CRE-mediated transcription (for review, see ref. *17*). While examples of ERE-independent transcription of genes by E_2 has been described in the brain and periphery *(15,17,156,162–169)*, an understanding of their contribution to neuroprotection is just beginning.

E_2 Regulates Transcription of Neuroprotective Genes via AP-1 Sites

Both ERα and ERβ can form a complex with Fos/Jun and promote transcription at AP-1 elements in a ligand-specific manner *(14,170,171)*. As a result, many gene products involved in E_2 neuroprotection are regulated in this manner, including IGF-1 *(172)*, TNFα *(163)*, GAP-43 *(173,174)*, and several matrix metalloproteinases (for review, see ref. *175*). IGF-1, for example, can then activate Akt and CREB, resulting in the upregulation of Bcl-2 via CRE-mediated transcription *(176)*. Thus, E_2's induction of one genomic pathway, i.e., activation of IGF-1 through AP-1, can affect other ERE-independent pathways and potentially contribute to E_2's neuroprotective effects. In addition, the E_2-induced release of TGFβ1 from glia has been shown to be neuroprotective and capable of enhancing c-jun/AP-1 binding in neurons; inhibition of AP-1 binding was sufficient to block the E_2-mediated neuroprotection *(177)*. Thus, E_2 can confer neuroprotection intercellularly as well as intracellularly using non-ERE promoter sites.

E_2 Regulates the Transcription of Neuroprotective Genes via CRE Sites

Estrogen has been demonstrated to rapidly increase intracellular calcium levels via L-type *(178)* and N-type *(94)* calcium channels. Alterations in intracellular calcium levels are the first step in many rapid signaling events leading to gene transcription. MAPK/ribosomal s6 kinase (rsk), calmodulin-dependent protein kinase (CAMK) II and IV, and PKA pathways are all affected by changes in calcium. These pathways increase CREB phosphorylation and subsequent CRE-mediated gene transcription (for reviews, see refs. *72* and *179–183*). Therefore, genes whose promoters contain CREs, such as BDNF *(184)*, thioredoxin *(185)*, and Bcl-2 *(186)*, are all potential E_2 targets. A recent study demonstrated that E_2 can rapidly induce Ca^{2+} influx, Src/ERK activation, and CREB phosphorylation leading to subsequent upregulation of Bcl-2 protein expression in hippocampal and cortical neurons *(178)*. Although the direct effect on neuroprotection was not tested, CRE-mediated transcription may play a role as siRNA knockdown of CREB prevented the E_2-induced CREB phosphorylation and upregulation of Bcl-2 *(178)*.

Rapid Phosphorylation Events by Estrogen May Lead to ERE-Independent Transcription

CRE transcription can also be modulated through direct phosphorylation of the NMDA NR2 subunit *(187)*. Estrogen treatment phosphorylates NMDA NR2 through activation of the MAPK pathway *(188–190)*, although downstream effects on CRE transcription have not yet been examined. CRE transcription via NMDA NR2 phosphorylation can be modulated by GSK3β and protein phosphatase 1 (PP1) *(191)*, two gene products that have recently been shown to be regulated by E_2 in the brain *(88,89,192)*. Together, these studies suggest that rapid signaling pathways can have direct consequences on transcription and that E_2 may be able to modulate ERE-independent transcription through multiple mechanisms not yet been elucidated.

Integration of Transcription: Multiple Response Elements

The cooperation of multiple response elements to induce transcription may bridge ERE-dependent and ERE-independent transcriptional effects of estrogen. Many genes have ERE half-sites capable of transcription *(167,193–195)* and some of these ERE half-sites require Sp1 sites nearby to achieve maximal activation and estrogen responsiveness (for review, see refs. *156* and *161*). Genes with Sp1 sites that have been linked to estrogen neuroprotection include *c-fos (195)* and *Bcl-2 (194)*. These genes also contain EREs *(157, 196,197)*, although it is unclear whether coordinate binding of these sites mediates E_2 neuroprotection. Other examples of coordinate response element binding inducing transcription include E_2-induction of tyrosine hydroxylase through overlapping ERE and CRE/CaRE sequences *(15)*, GnRH receptor induction via Smad binding element (SBE) and *AP-1 (198)* and *TGFβ1* induction of adenine nucleotide translocator 1 (*Ant-1*) through cooperative binding of both Smad and Sp1 to their response elements *(199)*. Thus, cooperative binding of multiple response elements is emerging as an important step for transcriptional regulation and potentially neuroprotection.

Summary and Conclusions

On average, preclinical data support the idea that estrogen is neuroprotective. Mechanisms underlying estrogen's protective effects include the activation of nuclear, cytoplasmic, and putative membrane-localized ER. These ER may directly bind EREs to modulate transcription. ER can also couple with rapid signaling pathways that involve kinases, phosphatases, and transcription factors. These rapid signaling mechanisms converge on response elements in the promoters of estrogen responsive genes to alter transcription indirectly. The link between E_2-initiated rapid signaling and any form of transcription is readily apparent as many rapidly activated proteins interact directly (including or excluding ER) with response elements as part of the basal transcriptional machinery (Fig. 1). Selective agents that act via one mechanism and not others may represent a means to capitalize on the beneficial effects of estrogen without the potentially deleterious effects of HRT.

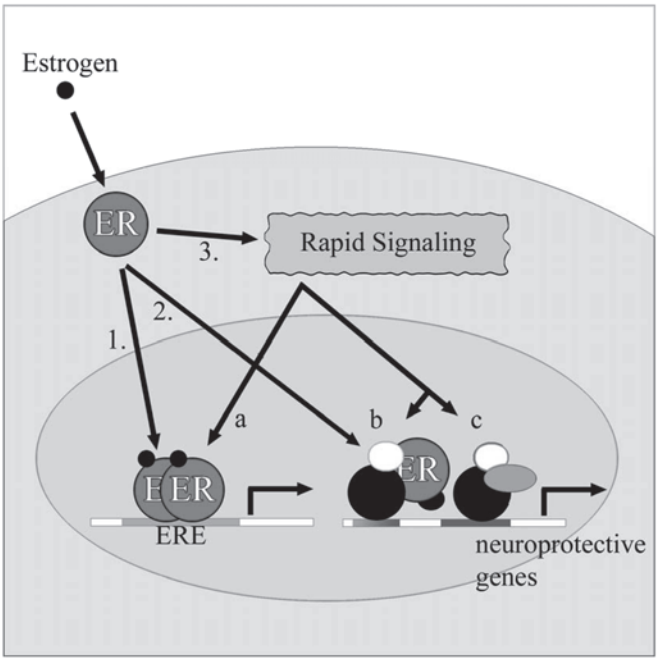

Fig. 1. Mechanisms of estrogen neuroprotection include (1) Regulation of transcription via direct interactions with estrogen response elements (EREs). (2) Regulation of transcription indirectly via other transcription factors such as CRE and AP-1. (3) Activation of cytoplasmic rapid signaling pathways (src/MAPK, PI3K-AKT, PKC) which indirectly modulate transcription: (a) in an ERE-dependent manner; (b) in an ERE-independent manner, including ER in the basal transcription machinery; and (c) in an ERE-independent manner, excluding ER from the basal transcription machinery.

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