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NEUROTROPHIC AND NEUROPROTECTIVE ACTIONS OF ESTROGENS AND THEIR THERAPEUTIC IMPLICATIONS

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■ **Abstract** Originally known for its regulation of reproductive functions, estradiol, a lipophilic hormone that can easily cross plasma membranes as well as the blood-brain barrier, maintains brain systems subserving arousal, attention, mood, and cognition. In addition, both synthetic and natural estrogens exert neurotrophic and neuroprotective effects. There is increasing evidence that estrogen actions are mediated by nongenomic as well as direct and indirect genomic pathways. Although in vitro models have provided the most extensive evidence for neurotrophic and neuroprotective actions to date, there are also in vivo studies that support these actions.

INTRODUCTION

Since its discovery and recognition as a "female" sex hormone (1), estradiol has been studied for its effects on the female reproductive tissues as well as its actions in the reproductive neuroendocrine system, and most recently for its ability to affect other aspects of brain function. The pioneering work of Jensen and others, demonstrating intracellular estrogen receptors (ERs) (2), introduced the use of tritium-labeled steroid hormones, and this led quickly to the discovery of estrogen-concentrating cells in the pituitary gland, hypothalamus, and other brain regions (3–5). Only much more recently has interest in estrogen actions on the brain shifted toward brain areas associated with cognitive and other functions not directly connected to reproduction (6). These studies have revealed actions of estrogens on many major neurotransmitter systems and brain regions subserving cognitive, emotional, and vegetative functions, as well as an important role in brain development and in the emerging area of protecting nerve cells from damage.

Estradiol 17β is a steroid hormone with many diverse cellular effects in neural tissue. It is produced by the ovaries and brain, as well as fat tissue by the aromatization of testosterone. Thus, estradiol is a hormone in males as well as in females. The neural effects of estrogens include a neurotrophic role in such processes as cell proliferation and differentiation, neuronal survival, and synaptogenesis during a sensitive developmental period, which leads to the establishment of the sexually differentiated brain (7–9). Estrogens also have neurotrophic effects on the adult brain, promoting collateral axonal sprouting in the deafferented hypothalamus (10) and promoting synaptogenesis in the female rat hippocampus (11, 12).

Estradiol also has neuroprotective effects in the aged or injured brain, which are evident in relation to Alzheimer's disease (13–15) and damage from ischemia (16–19). Estradiol has been shown to (*a*) activate nuclear ERs, (*b*) interact with a form of ER that activates second messenger systems, (*c*) induce anti-apoptotic gene expression, (*d*) maintain intracellular calcium homeostasis, (*e*) promote antioxidant activity, and (*f*) modulate actions of neurotrophins. Our aim in this article is to highlight the neuromodulatory role of estrogen as a neurotrophic and neuroprotective agent.

SITES OF ESTROGEN ACTION IN BRAIN

Intracellular ERs are found in both sexes starting early in development, along with aromatizing enzymes that convert testosterone and androstenedione to estradiol and estrone, respectively (7, 20). In rodent brains ERs are expressed along with aromatase in the hypothalamus starting around day 14 of gestation. ERs are later expressed in other brain areas such as the amygdala, midbrain, hippocampus, and spinal cord. In the rodent hippocampus and cerebral cortex there is a transient peak of ER expression in the first week after birth (7, 21). In general, besides the hypothalamus and the amygdala in the adult brain, ER is expressed in cholinergic, 5-serotonergic, noradrenergic, and dopaminergic neural systems as well as in specific GABA interneurons of the hippocampus and cerebral cortex (6).

There are two currently known primary ERs, referred to as ERα and ERβ; knockout mice for both ER isoforms have been generated (for reviews see 22, 23). ERα-deficient (αERKO) mice are infertile and show major deficits in sexual behavior, whereas ERβ-deficient mice (βERKO) appear to be less profoundly affected. From both immunocytochemical and mRNA studies, ERα is localized to the hypothalamus, amygdala, and scattered neurons in other brain regions such as the midbrain, hippocampus, and cerebral cortex. ERβ mRNA is found in the hypothalamus and is even more evident in other brain areas such as the midbrain, cerebellum, hippocampus, and cerebral cortex, but the antibodies for ERβ have been less reliable, and localization of ERβ immunoreactivity remains problematic (6). Both ER isoforms are expressed in the hypothalamus and midbrain and to some extent in the hippocampus and cerebral cortex, whereas ERβ appears to be the exclusive isoform in the substantia nigra and cerebellum (24, 25).

Complementary to the in situ and immunocytochemical findings, in vivo ligand-binding studies originally performed with ^{3}H-estradiol (4) and recently performed by Shughrue & Merchenthaler, using ^{125}I-estrogen (26), show binding to all high-affinity ER isoforms. The ^{125}I-estrogen uptake method appears to be more sensitive than ^{3}H-estradiol uptake and reveals some ERs where previously none had been detected. For example, in the original ^{3}H-estradiol uptake study only hippocampal interneurons were labeled (4, 27), whereas in the recent ^{125}I-estrogen binding studies, cell nuclear estrogen binding sites were reported in pyramidal cells of the ventral CA1–3 of the hippocampus and laminae II–VI of the isocortex.

Although both ER isoforms are found primarily in cell nuclei of target cells (25, 28), there are also indications of the presence of ER in other parts of the cell (29–33). Recent ultrastructural evidence showed the presence of ERα-immunoreactivity (Ir) in extranuclear sites in the hippocampus, namely in some axon terminals, dendritic spines, and glial processes (32). Although the functional significance of these extranuclear receptors is not yet known, the discussion below points to possible intracellular signaling mechanisms for the extranuclear forms of ER. Additionally, the enzyme that converts testosterone to estradiol, the cytochrome p450 aromatase complex, is localized throughout the neuronal perikarya, including dendrites, axon processes, and synaptic vesicles in the hypothalamic and limbic cell groups in Japanese quail, rat, monkey, and human (30). Taken together, these findings suggest the possibility that some brain cells express a local mechanism to regulate rapid estrogen synthesis and action at the synaptic level. This is a dramatic change from the dogma of nuclear ER and, as such, requires that substantial additional data be considered as fact.

This new view of estrogen action may be relevant to the fact that estrogen regulates synaptic connectivity in the hypothalamus as well as in the hippocampus in vivo (12, 34, 35). Similarly, in vitro studies on primary cultures of hippocampal neurons have revealed that estrogen induction of new dendritic spines and synapses involves the interactions of GABAergic interneurons and brain-derived neurotrophic factor (BDNF) (36). Estradiol treatment induces biphasic responses in GABAergic cells, initially decreasing GABA-Ir and GAD-Ir (synthesizing enzyme for GABA) and subsequently increasing both GABA and GAD (37). Hippocampal interneurons contain cell nuclear ERα-Ir (37, 38). Reminiscent of the developmental expression in vivo, ERα-Ir increases in primary hippocampal culture for 7 days, during which time neurons undergo proliferation and differentiation, and then slowly decreases by day 28 in vitro (38). Others have shown that at 3–5 days in vitro, hippocampal neurons exhibit low levels of nuclear staining for ERα but exhibit abundant nonnuclear, peri-plasma membrane neurite labeling (33).

It is conceivable that both nuclear and membrane-associated forms of ERα and ERβ may be involved in mediating neurotrophic and neuroprotective estrogen effects. As shown below, nonnuclear estrogen effects are likely to involve activation of second messenger systems and transcription factors that are regulated via phosphorylation and dephosphorylation, some of which are linked to cell survival. Other nonnuclear estrogen actions are likely to occur independently of

either nuclear or nonnuclear ER, based upon their structure-activity profile and concentration dependence in the micromolar range.

MECHANISMS OF ESTROGEN ACTION

The actions of estrogens will therefore be categorized as (*a*) direct genomic actions, (*b*) indirect genomic actions via second messenger cascades, (*c*) nongenomic effects at low estrogen levels, and (*d*) nongenomic effects at high estrogen levels involving antioxidant actions. It is important to keep in mind that there is a lack of information in some cases that would clearly identify which of these mechanisms may be involved in particular cellular processes or effects of estrogens (see Figure 1).

Direct Genomic Mechanism

The classical direct genomic mechanism involves the nuclear form of ERα or ERβ. Once bound by estrogen, the nuclear ligand-receptor complex acts as a transcription factor by binding either to an estrogen response element (ERE) or to fos-jun heterodimers, which in turn bind to an activation protein-1 (AP-1) response element (39–41). This direct genomic mechanism requires >45 min for new protein synthesis and probably much longer to alter cellular response (42).

Indirect Genomic Mechanism

The newly discovered indirect genomic actions of estradiol are postulated to occur when activation of a form of ER, possibly associated with cell membranes, stimulates a second messenger system such as adenylyl cyclase (AC), protein kinase A (PKA), protein kinase B (PKB), also known as Akt, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK), also known as extracellular signal-related kinase (ERK) (43–48). This results in phosphorylation of various cellular substrates ranging from membrane and cytoplasmic proteins to transcriptional regulators, such as cAMP-response element–binding protein (CREB by PKA) (49–52) or serum response factor (SRF)-Elk-1 complex (by MAPK/ERK) (for review see 48). These specific gene regulatory proteins (CREB, SRF-Elk-1) act at the DNA regulatory regions cAMP responsive element (CRE) and serum response element (SRE), respectively. The resulting cascades are capable of regulating non-ERE-containing genes such as MAP2, β-tubulin (53, 54), and GAP43 (55), presumably by activating the MAPK/ERK pathway.

Although steroids have long been suspected of affecting second messenger systems, based upon studies of oocytes (56) and cancer cells (46), recent evidence reinforces a direct coupling of estrogen effects to a G-protein-linked second messenger cascade. The most recent, direct evidence comes from work in the hypothalamus (43), where estrogen has been shown to modulate receptor/effector coupling and/or expression of the genes encoding all major classes of G-protein-coupled

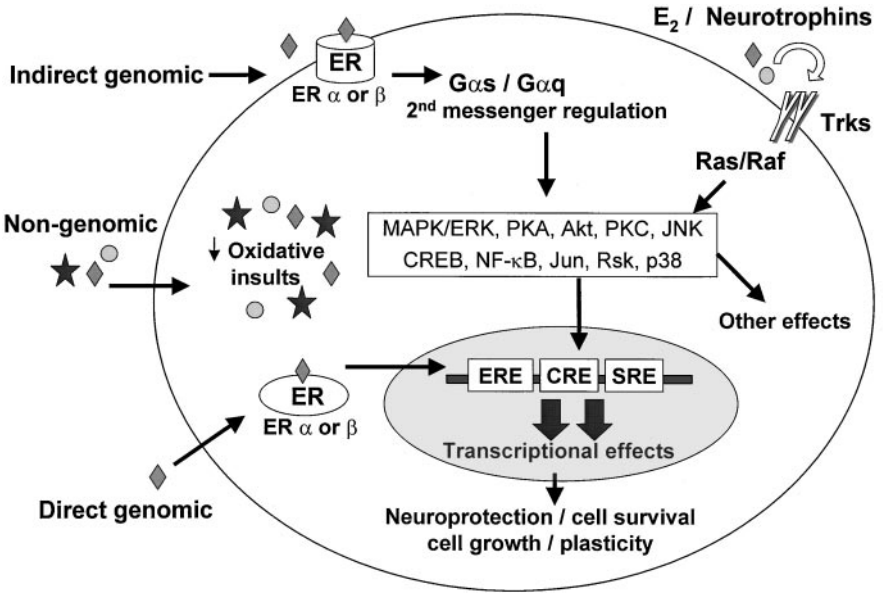

Figure 1 Putative mechanisms of estrogen action. In the direct genomic mechanism the nuclear form of ERα or ERβ associates with either the ERE or fos/jun heterodimers that bind, in turn, to AP-1 sites. Indirect genomic mechanisms include the activation of an ER linked to second messenger systems such as AC/PKC, cAMP/PKA and MAPK/ERK, converging with the genomic pathway. In one of these pathways, Ras activates Raf, which leads to sequential phosphorylation and activation of MAPK/ERK. Activated ERK then translocates into the nucleus to interact directly with nuclear transcription factors (e.g. CREB, cfos/cjun), and indirectly through the activation of intermediary signaling proteins (e.g. Rsk, p38, JNK) to bind to the DNA regulatory regions CRE and SRE. Neurotrophins and estrogens may influence each other's actions by regulating receptors and/or ligand availability through reciprocal regulation at the genomic level. Nongenomic estrogen effects at high concentrations involve antioxidant effects not mediated by known intracellular ERs. ERE, AP-1, SRE, and CRE are regulatory regions in DNA sequences that are recognized by specific gene-regulatory proteins. ERE is recognized by estrogen-ER complexes; AP-1 is recognized by fos/jun heterodimers; CRE is recognized by phospho-CREB (phosphorylated by PKA in response to a rise in cAMP levels); SRE is recognized by SRF-Elk-1 complex phosphorylated by MAPK/ERK. The MAPK/ERK migrates from the cytoplasm to the nucleus and phosphorylates Elk-1, thereby activating it to turn on the transcription of the *fos* gene. MAPK/ERK and PKC can phosphorylate jun protein, which combines with the newly formed fos to form heterodimers that ultimately bind to AP-1. ★, estriol; ◆, 17α-estradiol; ●, 17β estradiol; ERE, estrogen response element; CRE, cAMP response element; SRE, serum response element. See text for other abbreviations.

receptors. Specifically, estrogen activates a membrane-associated ER that stimulates PKC, which can rapidly uncouple the μ (opioid) and $GABA_B$ receptors via a G-protein to K+ channel. This, in turn, reduces β-endorphin and GABA binding or increases β-endorphin and GABA release, implying that estrogen modulates signaling pathways shared by both μ-opioid and $GABA_B$ receptor systems.

Furthermore, the activated PKC can also stimulate AC to produce cAMP, which in turn activates the PKA. The rapid ER-mediated activation of PKA leads to the phosphorylation of CREB (pCREB), which can then alter gene transcription through interaction with the regulatory region on a DNA sequence, CRE, altering gene expression. Thus, estrogen can rapidly increase PKA activity, which has the ability to modulate the coupling of G-protein-coupled receptors to their effector systems (43). A selective PKA antagonist (KT5720 and Rp-cAMP) can block the effects of estrogen. Whether this general mechanism exists throughout the central nervous system remains to be seen.

Second, a critical study by Levin (44) has provided further information about the source of the estrogen receptor involved in coupling to second messenger systems. This study demonstrated that a single transcript arising from transfection of either ERα or ERβ cDNA into Chinese hamster ovarian cells leads to expression of around 3% of the receptor as a membrane-associated form of ER and 97% as the nuclear ER. Both forms of ER show similar estrogen affinities and specificities. The membrane ER activates the α subunits of several GTP-binding proteins (e.g. Gαq and Gαs) and rapidly stimulates inositol phosphate and adenylyl cyclase activity. This leads to downstream signal transduction cascades, such that Gαq activates PLC and increases IP3 and Ca2+ and then activates ERK activity. On the other hand, Gαs stimulates AC, increases cAMP generation and activates PKA. This study further demonstrated that estradiol promotes and acts broadly through a G-protein-coupled receptor–dependent mechanism in a wide variety of cellular responses throughout the CNS.

These second messenger pathways (AC $\rightarrow$ PKA; PKC $\rightarrow$ AC $\rightarrow$ PKA; MAPK/ERK; Ras $\rightarrow$ ERK $\rightarrow$ Rsk) may be activated in response to an influx of extracellular Ca^{2+}, the binding of neurotrophins (nerve growth factor or BDNF) to their cognate receptors, or the binding of estrogens to a membrane-associated ER. The MAPK/ERK cascade is a major signal transduction pathway, which can be turned on by a wide range of extracellular proliferation- and differentiation-inducing signals. Activated MAPK/ERK can translocate to the nucleus and interact directly with the nuclear regulatory sequences: CRE, SRE, and AP-1 (57). These are the DNA sites to which the factors bind, which can subsequently lead to the phosphorylation and activation of transcription factors in the nucleus, such as CREB and immediate early genes such as *c-fos/c-jun* and *AP-1*. Ras (a member of a large family of GTP-binding proteins that help relay signals from membrane receptors to the nucleus) activates Raf, which sequentially phosphorylates and activates MAPK kinase (MEK) and MAPK/ERK. MAPK/ERK can phosphorylate another kinase, Rsk, which can then also translocate to the nucleus to interact indirectly with

nuclear response elements (57). Figure 1 shows a schematic overview of known ER signaling events, genomic and nongenomic, as well as those linked to the form of ER that activates second messengers.

The MAPK pathway is thought to play an important role in the action of neurotrophins and in synaptic plasticity (58). Moreover, as discussed below, estrogen-mediated neuroprotection may involve the tyrosine kinase/MAPK signal transduction cascade, because estrogen can rapidly activate tyrosine kinase and MAPK activity and because its effects are blocked by inhibitors of tyrosine kinase and MAPK (59).

In summary, although further studies must demonstrate that membrane-associated ER actually activates second messenger functions in vivo, the existence of ER in dendritic spines and some presynaptic endings in the hippocampus (32) suggest possible signaling from the synapse to the cell nucleus.

Nongenomic Effects at Low Estrogen Concentrations

In addition to the above mechanisms, estradiol has rapid nongenomic effects that enhance the amplitude of kainate-induced currents of CA1 neurons (60, 61) or inhibit calcium currents in striatal neurons (62). Although these effects are produced by nanomolar or lower levels of estradiol, the pharmacology of these estrogen effects is somewhat different from those likely to involve intracellular ER, based upon the lack of effects of nonsteroidal estrogen antagonists. Thus, a different estrogen receptor mechanism may be involved (6). It should also be noted that all of the intracellular ER–related second messenger pathways depicted in Figure 1 and discussed above can result in the phosphorylation of membrane and cytoplasmic proteins and that, in principle, estradiol is thus capable of affecting a host of nongenomic events throughout the cell. However, based upon the report of Razandi et al (45), it would be expected that these pathways should show inhibition of estrogen action by nonsteroidal estrogen antagonists.

Nongenomic Effects at High Estrogen Concentrations Involving Antioxidant Effects

Other nongenomic effects clearly involve a receptor system other than the intracellular ER. This is so because of the structure-activity profile in which 17α and 17β estradiol are equally potent and because, generally speaking, micromolar, rather than nanomolar, concentrations of estradiol are required for this class of effects, which are largely neuroprotective in various cell culture models. Neuroprotective actions of estradiol in vitro were first described using SK-N-SH human neuroblastoma cells under serum deprivation (63). Estradiol 17β (2 μM) increased total live cell number for up to 48 h without increasing thymidine incorporation, indicating an effect on cell survival and not cell division. Since then there have been numerous studies, all reporting the neuroprotective effects against various neurotoxic conditions (see Table 2).

At varying concentrations of 17α or 17β estradiol (from pM-μM) with different cell types, the responses range from enhancement of survival, to facilitation of neurite outgrowth, to prevention of cell death. These various effects were blocked by an NMDA receptor antagonist (AP5) but not by an estrogen antagonist (ICI 182,780), again confirming that nuclear ERs are not involved. Because both 17α and 17β estradiol have been shown to be effective, with 17α being more effective than 17β in many cases, this may reflect a novel estrogen receptor distinct from the intracellular ER α and β. However, there are also numerous protective effects that involve second messenger activation, and these may involve intracellular ER α or β, which are discussed below, along with other examples of the neuroprotective actions that involve micromolar levels of various estrogens.

NEUROTROPHIC EFFECTS OF ESTROGENS

Neurotrophic factors promote the growth, survival, and maintenance of neurons. Since the discovery of nerve growth factor, a large number of neurotrophic factors have been discovered. Cell culture techniques have become the standard methods for assaying putative neurotrophic factors and identifying responsive cell types. Estrogens, which have neurotrophic effects, are also able to interact with the neurotrophins. The neurotrophic effects of estrogen are summarized in Table 1.

The first neurotrophic actions of estrogens on cell survival/growth were demonstrated using organotypic slice cultures of the developing hypothalamus, preoptic area, and cerebral cortex (48, 64). At about the same time, estrogens came to be recognized for their role in sexual differentiation of the brain as metabolites of testosterone (see above). These estrogenic actions bear a general similarity to the neurotrophins, a family of growth factors, and their cognate receptors, tropomyosin-related kinase receptors (trk): (*a*) nerve growth factor (nerve growth factor binds trkA), (*b*) brain-derived neurotrophic factor (BDNF binds trkB), (*c*) neurotrophin-3 (NT-3 binds trkC), and (*d*) neurotrophin 4/5 (NT4/5 binds trkB).

Widespread colocalizations of ER and neurotrophin receptors (trks) are found mainly in neurons of the cerebral cortex, hypothalamus, hippocampus, and sensory ganglia. This receptor co-expression helps to explain the fact that estradiol and neurotrophins generally appear to exert reciprocal regulation upon each other's actions at the level of gene transcription. For example, EREs are present in the low-affinity neurotrophin receptor and the pan-neurotrophin receptor, $p75^{NTR}$, as well as in trkA. As a result, following ovariectomy, ER mRNA expression peaks while trkA mRNA expression decreases, or vice versa (48, 65). In addition, it has been reported that estrogen treatment alters the neurotrophin receptor ($p75^{NTR}$/trkA) ratio, which can subsequently alter ligand autophosphorylation as well as neurotrophin binding affinities. Thus, the intricate reciprocal regulation of trks and ERs emphasizes the advantages of having multiple "fail-safe" neurotrophic mechanisms that converge upon and cross-couple with known MAPK signaling pathways (48).

TABLE 1 Neurotrophic effects of estrogen

Brain Regions/ Neuronal/ Cell Types	Steroid	Modulation	Effective Concentration	Ref.
Hypothalamic explant	17 β-estradiol	Neurite growth	100 ng/ml	48
Hypothalamic neurons	17 β-estradiol	Up-regulates IGF	10^{-9} M	100
Hypothalamic neurons	17 β-estradiol	Neurite outgrowth	0.1 μM	101
Hypothalamic neurons	17 β-estradiol	Neuronal survival	10^{-12} M	
Amygdala neurons	17 β-estradiol	Neurite outgrowth	100 nM	102
		Neuronal differentiation	100 nM	102
		Dendritogenic growth	100 nM	102
Cortical neurons	17 β-estradiol	Neuronal differentiation	10–100 nM	103
		No proliferation	10–100 nM	103
Dorsal root ganglion	17 α- and β-estradiol	Up-regulates Bcl-x	1 nM	104
		Neuronal survival	1 nM	104
Hippocampal neuronal	17 β-estradiol	Up-regulates BDNF		105
Hippocampal neuronal	17 β-estradiol	Down-regulates BDNF		37
Dopaminergic neurons	17 β-estradiol	Neurite outgrowth	1 pM–10 nM	106
	17 β-estradiol	Increase TH mRNA	1 pM–10 nM	106
Dopaminergic neurons	17 β-estradiol	Increase DA uptake	10^{-14} M	107
Neocortical cells	17 β-estradiol	Neurite outgrowth	1 nM	108

An additional way in which estrogens seem to fine-tune neurotrophin responses is seen in the hippocampus in vivo and in vitro. Ovarian hormones regulate hippocampal BDNF mRNA levels in vivo, showing fluctuations across the estrous cycle (66). Moreover, whereas estrogen treatment has been reported to increase BDNF mRNA levels in the whole hippocampus of ovariectomized rats (67), estradiol treatment has also been reported to transiently decrease BDNF expression in inhibitory hippocampal interneurons; this decrease, along with transiently

decreased GABAergic activity in the same cells, is hypothesized to result in disinhibition of pyramidal cells, allowing the activity-dependent increase in dendritic spine density (37). BDNF treatment can block the estradiol-induced increase in spine density, whereas blocking BDNF with neutralizing antibodies mimics the effects of estradiol. It is noteworthy that the BDNF gene contains a putative estrogen response element (68).

Estrogens and neurotrophins can rapidly phosphorylate the estrogen receptor (48). In neural and nonneural tumor cell lines, estrogen effects that are presumably plasma membrane–mediated are very rapid, occurring in seconds to minutes; this may involve a shared estrogen and growth factor signaling pathway for cell proliferation and ER phosphorylation. For example, in mammary tumor cells (MCF-7) estradiol can elicit maximal phosphorylation of Src within 10 seconds (46).

It is still not clear whether the estrogen receptors that mediate the growth-promoting properties of estrogen in the developing brain are the classical receptors, ERα and ERβ, or perhaps another yet unidentified receptor subtype. However, there is growing evidence showing that estrogen receptors can mediate extracellular signaling in both an estrogen-dependent and estrogen-independent manner through growth factor signaling pathways (MAPK/ERK) (69). Toran-Allerand and colleagues (48) have suggested another potential pathway for estrogen-induced MAPK/ERK activation. This involves a multimeric caveolar-associated complex consisting of RAF, hsp 90, ER, Src, and ERK (58). This pathway has been implicated in signal transduction and vesicular trafficking (for review see 48).

Interestingly, like many of the neurotrophic factors, estrogen stimulation of neurite growth is developmentally regulated and hence is not seen in normal adults. However, after neural damage or loss of estrogens, responsiveness to estrogen can once again be demonstrated. Manifestations of these effects include reinnervation of deafferented neurons in the surgically isolated hypothalamus (10) and the induction of new dendritic spines and synapse in the undamaged hippocampus of ovariectomized female rats (11, 12, 70).

Although the maintenance of the capacity for growth and regeneration is a component of neuroprotection, the protection from damage is another aspect. As noted above and discussed in detail below, there are estrogen effects that reduce free radical production that are not stereospecific for 17β estradiol and may therefore involve receptors other than ERα or ERβ.

NEUROPROTECTIVE EFFECTS OF ESTROGENS

The decline of estrogen levels, whether natural or surgically induced, has been implicated in the etiology or the progression of age-associated neurodegenerative states. Estrogen replacement therapy has been helpful for some postmenopausal women. The benefits include improved cognitive function/mental performance, particularly verbal memory (6), or delayed onset of neurodegenerative disorders, such as Alzheimer's (13, 15) and possibly also Parkinson's diseases (71), as well as

reduced incidence of osteoporosis and cardiovascular diseases (72). There are also reports of estrogen neuroprotection from ischemic damage (16–19). However, much of the progress regarding mechanism has involved the use of cell culture (see Table 2).

Anti-Apoptotic Effects

Another mechanism by which estrogens might exert their neuroprotective effects is via modulation of molecules involved in the programmed cell-death pathway. The negative regulators of cell death are anti-apoptotic proteins, Bcl-2 and Bcl-xl, whereas the positive regulators of apoptosis are Bax, Bad, and Bid. In cultured hippocampal neurons, estrogen increases Bcl-xl expression and decreases both caspase-mediated proteolysis and cell death induced by β-amyloid (73). In fact, Pike (73) showed colocalization of ER and Bcl-x in human neuronal populations that exhibit relative resistance to Alzheimer's disease neurodegeneration. It has been suggested that estrogen treatment can enhance neuronal resilience to apoptotic insult via an ER-dependent genomic pathway that involves increased anti-apoptotic protein Bcl-xl, with subsequent inhibition of pro-apoptotic steps and capase-mediated proteolysis.

In substantia nigra dopaminergic neurons, estrogens provide neuroprotection against apoptosis via an ER mechanism that involves the AP-1 response element and the ERβ receptor, which predominates in this brain region (74). It appears likely that regulation of Bcl-2 expression is involved in the dopaminergic neurons (74).

An estrogen-mediated increase in Bcl-2 expression, associated with neuroprotection, has been reported in the cerebral cortex after ischemia (16). This study also showed that other members of the apoptotic family (bax, bcl-xl, bcl-xs, and bad) were not changed by estrogen treatment. In addition, estradiol increased the ratio of ERβ/ERα in the ischemic model, and it was suggested that ERβ-dependent signaling may be linked with neuroprotection (40), although the data for this suggestion are correlative. In relation to Bcl-2 regulation, recent studies (75–77) demonstrated that in the neuroblastoma cell line (SK-ER3), the mRNA of the pro-apoptotic gene Nip2, which interacts with Bcl-2, is significantly decreased following estradiol treatment (75, 77). The exact role of Nip2-Bcl-2 in neural cell apoptosis is not known. However, Nip2 has been shown to influence brain maturation such that Nip2 mRNA transiently increases (embryonic day 15–20) and then decreases by embryonic day 21 (77). Thus, the Nip2 gene, a target for estrogenic activity, may be involved in the complex apoptotic-anti-apoptotic mechanism, and estrogens may interact to shift this balance. Further studies are needed to show specific localization of ER and Nip2 and the exact interaction of Nip2 with Bcl-2.

Oxidative Stress

Neurotoxins, amyloid β-protein, hydrogen peroxide, glutamate, and NMDA can cause oxidative stresses, which induce cell death. These stresses have been linked to

TABLE 2 Neuroprotective effects of estrogen

Brain Regions/ Neuronal/Cell Types	Type of Damage	Steroid	Receptor Involved	Modulation[a]	Effective Concentration	Ref.
In vivo—adult arcuate nucleus	None			Inc. Bcl-2		76
In vivo hippocampal/cortical	Amyloid β-peptide			Inc. Bcl-X_L		73
Primary mouse hypothalamic	Serum deprivation	Estradiol	?			109
Primary rat amygdala	Serum deprivation					110
Primary rat hypothalamic	Serum deprivation	17β-estradiol	Yes		pM range	111
Primary hippocampal	Excitotoxicity	17α- and β-estradiol	No		100 nM–10 mM	91
	Excitotoxicity	17β-estradiol	Yes	Inc. pCREB	10 nM	38
	Oxidative stress	17α- and β-estradiol	No		100 nM–10 mM	91
	Amyloid β-peptide	17α- and β-estradiol	No		100 nM–10 mM	112
	gp120	17β-estradiol	?		1 nM +	59
Primary cortical	Glutamate toxicity		No			108
	Serum deprivation		No		nM range	59
	Glutamate toxicity	17β-estradiol	Yes		15–50 nM	113
Primary mesencephalic	Excitotoxicity					113
	Superoxide					113
	H_2O_2					113

Neuroblastoma	Serum deprivation	17β-estradiol	No		2 μM	63
C6-glioma 2B	Glutamate toxicity	17β-estradiol		Dec. cell death	0.01 mM	114
Human Sk-ER3	—	17β-estradiol		Dec. Nip2	1 nM	77
Human SK-N-SH	Serum deprivation	17α- and β-estradiol	No		0.2–2 nM	85
	Amyloid β-peptide		No		0.2–2 nM	86
Human SK-N-MC	Excitotoxicity		No?			82
	H_2O_2		No?			82
Human B103	Amyloid β-peptide					115
Rat PC12	Excitotoxicity					116
	H_2O_2					116
	Amyloid β-peptide					117
Rat NT2	Excitotoxicity					118
	H_2O_2					118
Mouse HT22	H_2O_2	17α- and β-estradiol	No		10 μM	119
	Amyloid β-peptide	17α- and β-estradiol	No		10 μM	119
	Glutamate toxicity	17α- and β-estradiol	No		10 μM	119

[a]Inc., increase; Dec., decrease.

diseases such as atherosclerosis and neurodegenerative disorders (both Alzheimer's and Parkinson's disease). They stimulate free radical accumulation induced by lipid peroxidation in neurons. As noted above, both 17β and 17α estradiol promote neuroprotection in these models via an antioxidant effect (78). In addition, glutamate causes a significant drop in glutathione levels, which also leads to an increase in intracellular peroxides and ultimately causes cell death (79). Estrogens, which possess intrinsic antioxidant activity, particularly in the μM range, may serve as scavengers for free radicals (80). Estriol, 17α-estradiol, and 17β-estradiol all have significant antioxidant properties, with estriol (a weak estrogen) being the most potent antioxidant (81). The differences in antioxidant potency are due to minor variations in the phenolic structure (82), and the structure-activity profile as well as the μM concentration range indicate that these effects are independent of the activation of the classical intracellular ERs.

Glutamate and NMDA

Glutamate (range 1–3 μM) is required for optimal survival of normal hippocampal neurons in culture, but exposure to very high concentrations of glutamate (3–10 μm) is toxic to neurons (83). Estrogen pretreatment produced an effective neuroprotection from glutamate excitotoxicity, whereas other nonestrogenic steroids did not show protection (80, 84).

Whether or not ER is present, many factors, such as neuronal cell types, nature of excitotoxic agent, and length of treatment, contribute to substantial differences in neuroprotective estrogen concentrations. The effective concentration of estrogen-mediated neuroprotection ranges from a low 0.1 nM (63, 85, 86) to 10–50 nM (38, 84) to 50 μM (82, 87). At low levels of estrogen, it is likely that the direct genomic or indirect genomic cascades (see above) are involved, because antagonists of the intracellular ER block the effects, whereas at high levels of estrogen, the antioxidant-nongenomic pathway has been implicated (80, 88).

In the primary hippocampal cultures, excitotoxic damage produced by NMDA (0.1–1 μm) can be prevented by preincubation (24–48 h) with estrogens as determined by reduced cellular lactate dehydrogenase release and by an increased phosphorylation of CREB (pCREB) (38), which suggests that this neuroprotective effect may involve changes in transcriptional regulation of CREB.

β-AMYLOID PEPTIDES

It is well known that accumulation of β-amyloid protein is thought to be a causal factor in the development of cerebral plaques and tangles associated with the neuropathology of Alzheimer's disease. Estrogen treatment is reported to inhibit the formation of toxic β amyloid and favor the production of the naturally secreted form of the amyloid precursor protein (89, 90). At the same time, estrogens exert protective effects against β-amyloid toxicity in both primary and slice cultures of

the hippocampus (80, 91), as well as in the HT22 neuronal cell line that reportedly lacks functional ER (78), and in a neuroblastoma cell line (90). Consistent with the antioxidant effects described above, the protective effects of estrogen against β-amyloid toxicity show equal potency between 17β estradiol (active at the ER) and 17α-estradiol (inactive at the intracellular ER) (88). Furthermore, the fact that estrogens protected HT22 neurons that lack functional ER suggests a non-ER-mediated effect against β-amyloid toxicity.

Role of Astroglial Cells and Aromatase

Astroglial cells may play an important role in neuroprotection, at least in part via their ability to express aromatase. This may be part of a response pattern in which glial cells are activated to produce specific growth factors, including the production of estradiol from testosterone to prevent further cell damage (92). Although estrogens increase the activity of brain aromatase by acting through the genome, it has been shown that aromatase activity decreases in the presence of ATP, Ca^{2+}, and Mg^{2+} in brain homogenates, a condition that promotes protein phosphorylation (93). This suggests that the local estrogen synthesis in the brain can be changed rapidly when needed and act in a paracrine fashion on neighboring cells (30, 94).

Role of ER in Neuroprotection and the Usefulness and Limitations of SERMs

As noted above, not all neuroprotective effects of estrogens involve such high levels of estrogen or lack of stereospecificity for estradiol 17β. Also noted above, a number of studies have reported that activation of ERK/MAPK by estradiol, possibly via an intracellular ER, mediates some forms of neuroprotection (58, 59, 74). These may be manifestations of the direct coupling of an intracellular ER to a second messenger system, as discussed earlier. Moreover, as noted above, estradiol can also directly regulate gene expression at the nuclear level through binding of ER to ERE or to fos-jun heterodimers that bind to the AP-1 site. However, the binding of ERβ to fos/jun heterodimers suppresses gene transcription from the AP-1 element, whereas nonsteroidal anti-estrogens such as tamoxifen have the opposite effect (40). Thus, tamoxifen blocks ERα and ERβ actions via the ERE but acts as an agonist when ERβ is acting via the AP-1 site. Therefore, it is conceivable that anti-apoptotic effects of estradiol may be mediated by activation of anti-apoptotic genes through ERE or by the suppression of pro-apoptotic genes through the AP-1 enhancer element.

Tamoxifen and raloxifene are examples of partial agonists for ER. These are also known as selective estrogen receptor modulators (SERM). SERMs have the advantage of providing beneficial estrogen-like effects on some tissues (bone, heart, and perhaps neurons) while acting as anti-estrogens in other tissue (breast and uterus). Furthermore, as noted above, SERMs have differential effects through

ERα and ERβ and their interactions with ERE and AP-1 sites. The problem in the brain is that there are many actions of estrogens that are mediated via different intracellular mechanisms, some in which a SERM may be an agonist and others in which a SERM may be an antagonist (for review see 6). Agonistic actions of SERMs are known for the induction of choline acetyltransferase in the basal forebrain and hippocampus (95, 96), whereas antagonistic actions of SERMs are evident in blocking the estrogen induction of dendrite spines in the hippocampus (6). Moreover, a recent study shows somewhat different regional patterns of agonist-like effects of both tamoxifen and raloxifene in regulating 5HT-2A receptors in cerebral cortex and striatum of ovariectomized, adult rats (97).

In addition, SERMs appear to block estrogen effects through membrane-associated ER to the extent to which they can stimulate second messenger systems (45). Yet they are ineffective in blocking estrogen effects on calcium currents in striatal neurons (62) and they mimic estrogen in affecting kainate currents in hippocampal neurons (98, 99). Thus, the usefulness of SERMs as substitutes for estrogen replacement in postmenopausal women must be evaluated with great care.

CONCLUSIONS

The study of neurotrophic and neuroprotective actions of estrogens has emphasized the fact, evident from the investigation of other actions of estrogens in the nervous system, that estrogen effects in neural tissue are highly diverse and that some of these effects involve mechanisms of action that are not mediated by estrogen binding to a nuclear ER acting via the classical ERE. In particular, the possibility of second messenger pathways triggered by estrogens and the identification of estrogen receptors and aromatizing enzymes in some, but not all, nerve terminals, dendritic spines, and glial cell processes raise the possibility of anatomically specific intracellular signaling processes. Furthermore, the cross talk between estrogens and neurotrophins at the receptor and second messenger levels indicates that growth-promoting effects and neuroprotective actions have some degree of overlap in terms of receptors and initial pathways. Finally, these findings, together with the development of SERMs for mimicking or blocking intracellular ER, as well as the increasing evidence of antioxidant actions of estrogens, albeit at micromolar concentrations, make the study of estrogen actions in neural tissue an exciting and challenging topic and a target for pharmaceutical development.

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