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# Minireview: Neuroprotective Effects of Estrogen—New Insights into Mechanisms of Action

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**ABSTRACT**

An accumulating body of evidence clearly establishes that estradiol is a potent neuroprotective and neurotrophic factor in the adult: it influences memory and cognition, decreases the risk and delays the onset of neurological diseases such as Alzheimer's disease, and attenuates the extent of cell death that results from brain injuries such as cerebrovascular stroke and neurotrauma. Thus, estradiol appears to act at two levels: 1) it decreases the risk of disease or injury; and/or 2) it decreases the extent of injury incurred by suppressing the neurotoxic stimulus itself or increasing the resilience of the brain to a given injury. During the past century, the average life span of women has increased dramatically, whereas the time of the menopause has remained essentially constant. Thus, more women will live a larger fraction of their lives in a postmenopausal, hypoestrogenic state than ever before. Clearly, it is critical for us understand the circumstances under which estradiol exerts protective actions and the cellular and molecular mechanisms that underlie these novel, nonreproductive actions. (*Endocrinology* **142:** 969–973, 2001)



DURING the past decade, our appreciation that estradiol is a potent protective factor in many physiological systems has increased remarkably. The intense interest in understanding the circumstances under which estradiol protects and the mechanisms of it protective actions results from three converging areas of understanding. First, we have begun to appreciate that, in addition to its well-recognized effects on the reproductive axis and traditional reproductive target organs, estradiol is a pleiotropic hormone that influences numerous nonreproductive functions such as bone and mineral metabolism, cardiac and vascular function, memory, cognition and mood, and the incidence and progression of age-related diseases (reviewed in Refs. 1–3). Second, the discovery of three types of estrogen receptors (ER$\alpha$, ER$\beta$, and membrane ER) (4, 5) has led us to reevaluate potentially new targets and diverse mechanisms of estradiol action that have not been considered previously. Researchers have launched aggressive investigations into the specific biological actions of each receptor in anticipation that we will be able to selectively induce the protective actions of estrogens. Third, during the past century, the average life span of women has increased dramatically from 50 yr to over 80 yr; whereas the age of menopause has remained essentially fixed at 51 yr. Hence, a greater proportion and a greater total number of women will spend over 30 yr of their lives in the hypoestrogenic postmenopausal state. Because estradiol influences multiple physiological systems, the cessation of menstrual cyclicity and resulting hypoestrogenicity broadly impacts women's health. Clearly, understanding the circumstances under which estradiol exerts protective actions and the cellular and molecular mechanisms that underlie these novel, nonreproductive actions will prove crucial to preventing the deleterious consequences of prolonged hypoestrogenicity and to improving women's health.

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## Neuroprotective Actions of Estradiol in the Adult

We have long appreciated that estradiol is a potent neurotrophic and neuroprotective factor during embryonic and neonatal development (reviewed in Refs. 6–8). Our appreciation that estradiol exerts important protective actions on the adult brain is more recent and has grown through the results of studies in both humans and animal models. Clinical studies establish that estradiol influences aspects of memory, cognition, and mood in healthy young and postmenopausal women. In addition, it appears to delay the onset of and slow the decline in cognitive function associated with neurodegenerative diseases, such as Alzheimer's or Parkinson's disease, and may attenuate the extent of acute injury associated with stroke and brain trauma (reviewed in Refs. 9 and 10). Finally, recent data suggest that estradiol may protect against neurotoxic HIV proteins, gp120 and Tat (11, 12). It is more controversial as to whether estrogen replacement therapy can ameliorate memory and cognitive function after the disease or injury process has been initiated (13–15).

The vast majority of studies that investigate the potential neuroprotective actions of estrogen have been performed using experimental animal models and *in vitro* methodologies. The *in vivo* studies allow basic scientists to use experimental paradigms that mimic clinical forms of brain trauma and to create different controlled hormonal environments to follow the evolution of injury and the mechanisms that explain the differential extent and rate of cell death. The power of *in vitro* methods (explant cultures, primary dispersed cells, or neuronal cell lines) lies in the fact that investigators can take advantage of more simple systems where direct and indirect actions of estradiol can be deciphered. These basic science studies complement clinical results and support the conclusion that estradiol exerts protective actions during adulthood. They reveal the breadth of mechanisms that es-

trogens use and uncover the interactive and complex nature of the cellular and molecular mechanisms that are involved.

### Evidence that Estradiol Protects the Brain against Injury

Interestingly, although it has been known that estradiol may influence memory and cognition and protect against neurodegenerative diseases, its role in acute injury has only recently been a focus of study. Until a decade ago, most studies that investigated the etiology, evolution, and pathophysiological mechanisms of acute injury used males and assumed that conclusions drawn from studies would apply to females. The results of more recent studies clearly reveal that sex, and estradiol, in particular, are critical considerations in the outcome of brain injury. Several lines of evidence suggest that females are less vulnerable to acute insults associated with cerebral ischemia (for reviews see Refs. 9, 16, 17), neurotrauma (for review see Ref. 10), hypoxia (18) and drug-induced toxicity (19). First, there are striking sex differences in the incidence, the pathophysiology and the outcome of neurological injury. Premenopausal women suffer from cerebrovascular stroke less frequently than men (20), but this sex-related difference disappears in older postmenopausal women compared with age-matched men (21). Likewise, young female rodents consistently sustain less neural cell death and smaller infarcts, experience fewer behavioral signs of injury, and survive for a longer period of time after ischemia, hypoxia, or traumatic brain injury (22–27) (reviewed in Ref. 10).

Second, administration of estradiol or estrogenic compounds protects against stroke-like (reviewed in Refs. (9, 16, 17) or traumatic brain injury (28); treatment diminishes the extent of injury, and, in some studies, decreases mortality and behavioral dysfunction. Researchers have used several different treatment protocols. Hormone has been administered at physiological and pharmacological levels before, simultaneously with, or after brain injury. In general, it appears that pharmacological doses of estradiol or estrogen-like compounds protect even when given up to 3 h following the onset of injury (29); whereas lower physiological concentrations of estradiol must be administered before the injury to exert protective actions. We have found that administration of low physiological levels of 17$\beta$-estradiol for 1 week before permanent occlusion of the middle cerebral artery leads to a dramatic decrease in the extent of the infarct (30) (Fig. 1). Although the varying estrogen treatment protocols collectively decrease neural injury, the mechanisms by which they achieve neuroprotection appear to be diverse and complex (see below).

Third, estradiol protects against cell death in numerous *in vitro* models of brain injury. In parallel with *in vivo* studies, investigators have used a wide range of concentrations of estradiol ($10^{-5}$–$10^{-12}$ M), diverse neurotoxic stimuli (glucose deprivation, hypoxia, oxidative stress, excitotoxicity, and physical injury), and various culturing methods (primary neuronal cells, tumor-derived neuronal cell lines, mixed neuron/astrocyte cultures, and organotypic explant cultures) (reviewed in Ref. 9). The *in vitro* approach has helped us immensely to decipher the underlying molecular mechanisms by which estradiol attenuates the extent of injury. Explant cultures allow us to eliminate the indirect effects of estradiol on nonneural systems, while maintaining local synaptic connections and interactions with the glial environment. Use of dispersed neuronal cell cultures allows us to address the role of direct actions of estradiol on neurons while eliminating indirect actions through the immune system, the vasculature or glia.

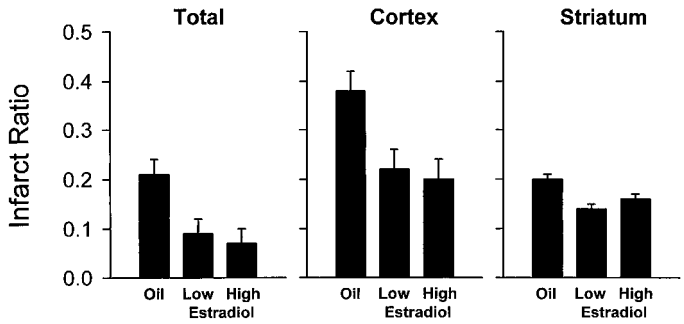


FIG. 1. Infarct volume in ovariectomized and ovariectomized estradiol-treated rats. Estradiol pretreatment significantly reduced the total and cortical infarcts produced by permanent middle cerebral artery occlusion. Low and high physiological levels of estradiol pretreatment significantly reduced overall and cortical infarct size, as compared with oil-pretreated controls. Estradiol did not significantly reduce striatal injury. Values represent mean ± SE. Modified from Ref. 30.

### Mechanisms of Estradiol Action

It has become increasingly clear that estradiol protects the brain by directly affecting neuronal viability and by acting on other cell types, such as vascular endothelial cells, astrocytes, and microglia via traditional and novel, estrogen receptor-dependent and receptor-independent mechanisms of action. The predominant mechanisms may depend upon the brain region under investigation, the type of neural injury or stimulus-induced, and/or the dose of hormone administered. In general, it appears that physiological levels of estradiol protect via mechanisms that require pretreatment and involve estrogen receptors and changes in gene expression. The interactions of estradiol with its receptors may lead to the expected classic downstream events: receptor dimerization, receptor binding to estrogen response elements on DNA, and induction of transcription of target genes. Alternatively, interactions with receptors may also elicit novel cross-talk with second messenger molecules that lead to phosphorylation and activation of key proteins. In contrast, pharmacological levels of estradiol appear to by-pass estrogen receptors and invoke mechanisms that involve blood flow, antioxidant actions, and/or nitric oxide (NO) production. A note of caution should be considered: recent work by Green *et al.* (31) shows that the presence of glutathione in cell cultures dramatically reduces the effective concentration at which estrogen exerts protective effects *in vitro* under circumstances where the receptor is not required. Thus, under some circumstances, low levels of estradiol may protect via receptor-independent mechanisms.

#### *Estrogen receptor-dependent neuroprotection*

Studies performed both *in vivo* and *in vitro* suggest that physiological concentrations of estradiol protect through estrogen receptor-dependent mechanisms that lead to transcription of critical genes that ultimately promote cell survival. Our labo-

ratory recently discovered that within 24 h of middle cerebral artery occlusion, ERα messenger RNA (mRNA) is dramatically up-regulated and that estradiol pretreatment prevents injury-induced down-regulation of ERβ in the cerebral cortex (Fig. 2). These data suggest that brain injury may influence responsiveness of the injured cerebral cortex to estradiol and induce differential actions that are mediated by each receptor subtype (32, 33). It is important to note that ERα is only transiently expressed in the cerebral cortex during neonatal development when this region of the brain undergoes dramatic neurogenesis, neuritogenesis, and differentiation. Its expression virtually disappears thereafter. We speculate that the dramatic up-regulation of ERα in the cerebral cortex may allow a recapitulation of the developmental actions of estradiol in promoting neurogenesis and redifferentiation of the cortex. Several studies support the concept that following stroke injury, specific features of brain function (*e.g.* bilateral motor control and the capacity to reorganize cortical representational maps) revert to those seen during early stages of development, with the process of recovery recapitulating ontogeny (reviewed in Ref. 34). Further, we have recently reported that physiological levels of estradiol do not protect against ischemic injury in ERα knock-out mice (35). These data clearly establish that ERα is a critical mechanistic link that mediates the neuroprotective effects of physiological levels of estradiol. Using explant cultures of the neonatal cerebral cortex, we (36) have shown that low concentrations of estradiol protect against cell death. Our studies strongly suggest that estrogen receptors are critical because the protection cannot be achieved using 17α-estradiol and is blocked by coincubation with ICI 182,780, an estrogen receptor antagonist (Fig. 3). These findings complement those of Gollapudi and Oblinger (37, 38) who showed that PC12 cells transfected with the full-length rat ERα respond to the protective effects of estradiol, but cells transfected with vector DNA alone are not protected by estradiol. Further, investigators have found that neuronal glutamate-induced cell death is blocked by estrogen receptor antagonists, tamoxifen (39, 40) and ICI 182,780 (36, 41, 42). On the other hand, many *in vitro* studies demonstrate that high concentrations of estradiol protect cultured neurons that do not express estrogen receptors (31, 43).

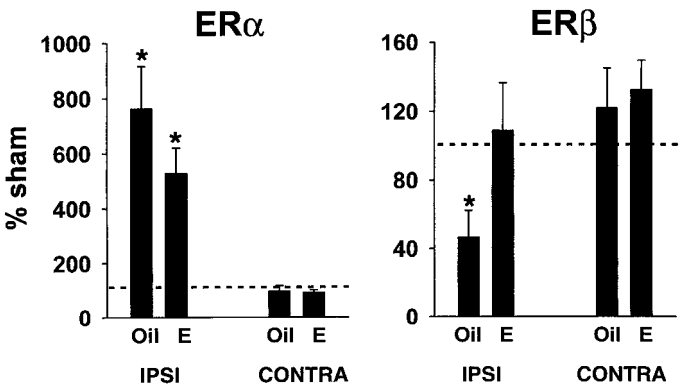


FIG. 2. Estrogen receptors were differentially modulated in ischemic injury. A, *ERα* mRNA increased in the ipsilateral cortex of oil- and estradiol-treated rats, compared with the contralateral cortex. Estradiol prevented the injury-induced down regulation of *ERβ* mRNA in the ipsilateral cortex. In the absence of estradiol, *ERβ* expression in injury declined significantly below constitutive levels. Data are graphed as a percentage of sham expression. Data are represented as mean ± SE. Modified from Ref. 70.

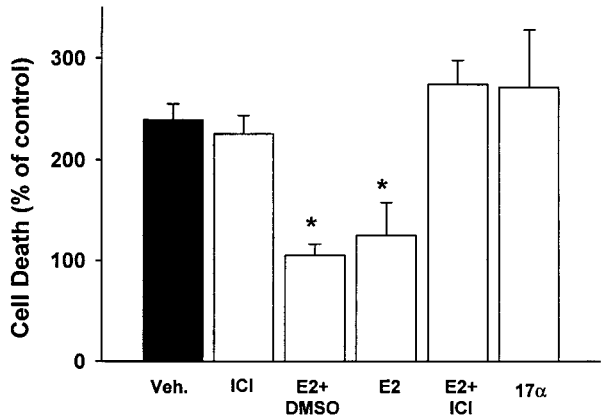


FIG. 3. LDH release in controls and following ischemic injury following treatment with 17α-estradiol or 17β-estradiol in the presence of ICI 182,780. *Bars* represent the mean of cell death expressed as a percent of control ± SE. Modified from Ref. 36.

What genes are influenced by estradiol and how do these downstream events drive neuroprotection? It is well accepted that estradiol influences the expression of numerous genes in multiple regions of the brain, including the hippocampus and cortex, that are theoretically relevant to estradiol's ability to protect. For example, estradiol affects, in complex ways, the expression of genes that are involved in the balance of apoptosis and cell survival (44), mitochondrial function (45), the function of astrocytes (46, 47), synthesis and secretion of neurotransmitters that modulate neuronal excitability or neuron/astrocyte interactions (48, 49), expression of neurotrophins, growth factors, and their receptors leading to enhanced neuronal viability (42, 49–55), and expression of factors that influence dendritic or axonal elongation and synaptogenesis (56, 57). In addition, we know that injury induces alterations in the expression of many of the same or functionally related genes (58–67). Thus, it is tempting to speculate that estradiol protects through modulation of these genes. However, few studies (68–72) have directly tested whether estradiol influences these factors in the context of injury. In general, these studies have shown an interaction between injury and the presence of estradiol that favors the survival of neurons after injury. However, to date, no studies have established that such alterations are functional links to estradiol's ability to protect against injury-induced cell death. We have demonstrated that estradiol's ability to protect correlates with differential expression of *galanin* (72), *bcl-2* (70), *c-fos* (71), and *ERα* and *ERβ* (70) mRNA in the cerebral cortex after ischemic injury and are beginning to probe the functional roles of these estradiol-mediated changes in gene expression.

### *Nonreceptor-mediated protective actions of estradiol*

High levels of estradiol increase vasodilation and increase cerebral blood flow by affecting the microcirculation and vasoactive substances in the vasculature through estrogen receptor-independent mechanisms. Estradiol increases cerebral perfusion in some species and under some conditions (73, 74). However, investigators have also reported estradiol-induced protection in the absence of changes in cerebral blood flow (22, 30). Therefore, it is unclear whether such changes can explain

the protective effects of estradiol or whether they only correlate with protection. Estrogens inhibit the vasoconstrictor endothelin (75, 76) and stimulate the vasodilator endothelium-derived relaxing factor (NO) (77). It appears that estradiol enhances the expression and activity of two isoforms of nitric oxide synthase (NOS), endothelial NOS and neuronal NOS (78). Pelligrino and colleagues (79, 80) reported that transient forebrain ischemia leads to a greater reduction in cerebral blood flow in ovariectomized female rats than intact females. Further, they found that this difference correlated with differences in NOS levels in the brain.

Estrogens may also protect through receptor-independent mechanisms by attenuating the formation of free radicals. At high concentrations (in the $\mu$M range *in vitro*), the phenolic A ring of estrogenic compounds acts as a highly effective electron donor and free radical scavenger, preventing the lipid peroxidation-induced membrane damage (for review see Refs. 16, 43, 81–83). Several investigators (84–86) reported that estradiol reduces lipid peroxidation in several different neuronal cell systems and that this correlates with reduced cell death. Further, estradiol attenuates lipid peroxidation induced by various toxic stimuli, including exposure to amyloid-$\beta$ protein or iron sulfate. The doses of estradiol required for antioxidant activity parallel those required for neuroprotection in these systems.

Finally, exciting new evidence suggests that estradiol may protect against injury via receptor-dependent or receptor-independent mechanisms that involve cross-talk with other second messenger signaling molecules such as cAMP (87, 88), MAP kinases (89, 90) or molecules of PI-3K/Akt pathway (91). These mechanisms may allow estradiol to act rapidly through phosphorylation and activation of preexisting critical proteins and/or to act after some delay through phosphorylation-dependent genomic actions.

In summary, our understanding that estradiol is a complex pleiotropic hormone that plays important nonreproductive functions in the adult brain has emerged rapidly. We now appreciate that estradiol appears to act at two levels: it appears to decrease the risk of disease, and also to attenuate the extent of injury incurred by suppressing the neurotoxic stimulus itself or increasing the resilience of the brain to a given injury. Nonetheless, studies have only begun to decipher and probe the cellular and molecular bases of the novel actions of estrogen. As we continue to gain greater insights into the mechanisms of estradiol-mediated protection, we will be better able to develop estrogen-like compounds that selectively elicit protective effects for use as therapeutic agents to ameliorate cognitive dysfunction and diminish the risk and severity of neurodegenerative diseases and neurotrauma.

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