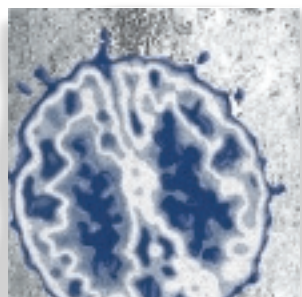


Estrogen and neuroprotection: from clinical observations to molecular mechanisms

Dena B. Dubal, PhD; Phyllis M. Wise, PhD



We now appreciate that estrogen is a pleiotropic gonadal steroid that exerts profound effects on the plasticity and cell survival of the adult brain. Over the past century, the life span of women has increased, but the age of the menopause remains constant. This means that women may now live over one third of their lives in a hypoestrogenic, postmenopausal state. The impact of prolonged hypoestrogenicity on the brain is now a critical health concern as we realize that these women may suffer an increased risk of cognitive dysfunction and neurodegeneration due to a variety of diseases. Accumulating evidence from both clinical and basic science studies indicates that estrogen exerts critical protective actions against neurodegenerative conditions such as Alzheimer's disease and stroke. Here, we review the discoveries that comprise our current understanding of estrogen action against neurodegeneration. These findings carry far-reaching possibilities for improving the quality of life in our aging population.

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Estrogen is a pleiotropic hormone that acts beyond the scope of its reproductive functions and exerts protective actions on multiple tissues including the brain. The protective actions of estrogen carry tremendous implications for the promotion of health and the prevention of disease in postmenopausal women. Since the life span of women has increased from approximately 50 to 80 years, but the age of the menopause remains at about 51 years, women may now live *over three decades* of their lives in a hypoestrogenic, postmenopausal state. The impact of prolonged hypoestrogenicity is now a critical health concern, since we realize that these women may suffer from an increased vulnerability to a variety of diseases. Conversely, replacement with estrogen appears to act in the primary prevention of many disease processes, including neurodegeneration. Estrogen, however, is not always beneficial, as high and unopposed levels may increase the risk for certain cancers in some women. Our challenge, therefore, is to design hormone replacement therapies that exert only beneficial effects in the body. To this end, we must gain a more complete understanding of the spectrum of estrogen's actions and, more specifically, we must dissect the mechanisms that underlie its actions.

The broad spectrum of estrogen's actions includes significant protection of the brain and primary prevention against neurodegeneration. Clinical observations indicate that estrogen replacement in postmenopausal women can (i) ameliorate cognitive dysfunction, and (ii) decrease the risk and delay the onset of degenerative conditions such as Alzheimer's disease (AD) and stroke.

Keywords: estrogen; estradiol; estrogen replacement therapy; menopause; stroke; cerebral ischemia; Alzheimer's disease; cognition; brain injury; neuroprotection

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Basic research

Basic science studies have revealed a strong cellular and molecular basis for these clinical observations. Recent insights into the molecular events that underlie estrogen-mediated neuroprotection encompass actions that range from its pharmacological, antioxidant mechanisms to its physiological, estrogen receptor (ER)-dependent mechanisms. The results of the studies that reveal estrogen's neuroprotective actions and mechanisms carry exciting and far-reaching possibilities for improving the quality of life of our aging population. As we continue to discover how estrogens act in the brain to promote enhanced neural function and exert protective effects against degeneration, we will be able to design hormones that exert only beneficial effects in the body.

Estrogen, the menopause, and hormone replacement

Estrogen

Estrogens are synthesized predominantly in the ovary as 18-carbon steroids with an aromatic A-ring. They act on multiple endocrine targets and are synthesized in many forms. Most clinical and basic science studies have focused attention on the actions of estradiol, the most potent and biologically active form of estrogen that circulates in the body prior to the menopause.

Menopause

Because the menopause impacts the health of so many women, investigators have focused on understanding the driving factors that govern this change. For many years, it was accepted that the menopause resulted simply from the depletion of the postmitotic pool of ovarian follicles that is set down during embryonic development.¹ Clearly the exhaustion of this reservoir necessarily means that a woman is permanently postmenopausal and can no longer produce offspring with her genetic makeup. As importantly, since the ovarian follicles are not only the source of germ cells, but are also the primary source of estradiol, plasma concentrations of this hormone drop precipitously during the postmenopausal years and remain low for the remainder of a woman's life, unless she chooses to take hormone replacement therapy. Whether or not the brain plays a role in the transition to the menopause has been a topic of active debate. Results from studies using animal models have suggested that

aging of the brain and a declining ability to provide coordinated neurochemical signals that are required for ovulation contribute to reproductive senescence. However, whether these findings are relevant to the human menopause has been less clear. Recently, an increasing number of researchers have begun to appreciate that the brain may play an important role in the sequence of events leading to menopause in humans. Several findings lead to this conclusion. First, the pattern of luteinizing hormone (LH) secretion and the levels of follicle-stimulating hormone (FSH) secretion change before women enter the perimenopausal period. These changes are likely to reflect changes in the pattern of hypothalamic hormone secretion. Second, the responsiveness of the brain to estradiol treatment and the ability of this hormone to induce gonadotropin surges are greatly compromised in middle-aged women as they enter the transition to the menopause. Finally, estradiol concentrations remain normal or are elevated during the perimenopausal period, suggesting that the ovary remains capable of secreting this critical hormone. Interestingly, these findings are strikingly similar to what has been observed in middle-aged laboratory rodents, as they become less fertile and cease to have reproductive cycles.² Since many studies to examine the role of the brain cannot be performed in women because they are invasive and involve experimental manipulations that cannot be performed in humans, laboratory animals provide the only means through which we can gain a better understanding of the role of the brain in the menopause. The striking similarities between many of the events that occur during middle age give us reason to believe that rodents serve as excellent models in which to examine the factors that initiate the process of reproductive aging during middle age. We hope that information gained from these species can be extrapolated to humans and will allow us to uncover and explore concepts that can be generalized to human reproductive aging.

Estrogen replacement therapy in postmenopausal women

The process of reproductive aging in women ultimately results in a hypoestrogenic, postmenopausal state. As our understanding of estrogen action in the body grows, the consequences of prolonged hypoestrogenicity and the profound impact of estrogen replacement therapy (ERT) on postmenopausal women become increasingly

clear. We now know that, although estrogen can promote disease in some women,³ it acts in a broad spectrum of tissues to promote health and overall well-being.⁴⁻⁸ Insight into many of the protective actions of estrogen is gained from observations that oophorectomized young women suffer increased pathophysiology, such as bone resorption,⁹ compared with their counterparts with normal reproductive function. Further, hypoestrogenic, postmenopausal women often suffer increased disease compared with premenopausal women and age-matched men. Studies demonstrate that estrogen acts in the brain to enhance cognitive function and decrease the risk and/or delay the onset of neurodegenerative conditions.^{6,10-13} Further, estrogen decreases the risk and/or mortality for cardiovascular disease, potentially through its beneficial effects on the lipid profile and on the endothelium,^{7,14,15} though recent evidence suggests that estrogen-mediated protection of the heart may not persist in women with preexisting cardiovascular disease.¹⁶ Finally, estrogen is crucial in the positive remodeling of bone; the loss of estrogen in postmenopausal women is accompanied by a dramatic increase in osteoporosis.⁵

Estrogen: effects on cognition and Alzheimer's disease

The focus of this review—and the focus of our recent discoveries—is the action of estrogen in the injured and aging brain. Our interest in this area stemmed from clinical observations that estrogen acts beyond its traditional targets in the hypothalamus and pituitary, and influences other facets of brain function involved in memory and neurodegeneration. Since neurodegenerative events occur frequently in elderly women who are chronically hypoestrogenic, the results of these studies carry profound implications in the clinical setting. Thus, it is important to determine whether and how ERT can ameliorate neural dysfunction.

Estrogen and cognition

Clinical studies demonstrate that estrogen influences memory and cognition,^{6,17-22} and can protect against neurodegenerative diseases such as AD.^{10,11,23-30} These findings, however, are not without controversy. As the results of more clinical studies become available, we are beginning to appreciate that the protective actions of estrogen do not apply in all situations.³¹⁻³³

Many studies have examined whether ERT improves cognitive function. The majority of data show that estrogen can enhance cognitive function in both young²² and older women.^{19,21,34} It appears that by maintaining normal cognitive function, estrogen may further act to decrease the risk and delay the onset of AD.^{10,26-28,35} Importantly, estrogen does not exert actions on all aspects of memory. It is critical to consider that memory is a broad term describing several distinct neural functions, many of which originate in different and overlapping regions of the brain. Thus, it is not surprising that estrogen may influence specific subtypes of memory. For example, some,^{17,19,34} but not all,³⁶ studies show that ERT appears to specifically enhance immediate and delayed recall of verbal information. Other reports indicate that the beneficial actions of ERT on cognition include improvement of visuospatial memory.^{37,38}

Estrogen and Alzheimer's disease

Several studies have examined whether ERT decreases the risk, delays the onset, or stops the progression of neurodegeneration caused by AD. Estrogen has been shown to decrease the risk for AD¹⁰ or induce a modest improvement in cognitive function in individuals with AD.^{11,35,39,40} However, other studies have reported no difference in cognitive function between estrogen- and placebo-treated individuals.⁴¹⁻⁴³ A recent cohort study⁴³ failed to detect slowing of AD progression or improvement of cognitive and functional outcomes in women with mild-to-moderate AD treated with Premarin®. The results of this study and those of other studies³¹⁻³³ strongly suggest that estrogen may fail to reverse or even halt a disease process that has already been initiated. Therefore, while it is clear that estrogen can influence certain aspects of cognitive function and decrease the risk for AD, it remains controversial whether it can act against an existing neurodegenerative condition. Taken together, the findings of these studies indicate that estrogen may protect the brain through primary prevention.

Estrogen and stroke

Our studies on estrogen action against neurodegeneration have focused attention on whether estradiol plays a role in stroke injury. We, and others, have aggressively investigated this question since stroke: (i) is a major form of neurodegeneration that grossly impacts our

Basic research

aging population, and (ii) has been successfully reproduced in several animal models. To date, most clinical studies have assessed whether ERT alters the risk and mortality of stroke,⁴⁴ but have not addressed whether estradiol decreases the degree of brain injury resulting from stroke.

Stroke is a neurodegenerative condition that greatly impacts the health and livelihood of our aging population. It is the third leading cause of death for middle-aged and older women, and a major health problem that affects 500 000 Americans each year.⁴⁵ Interestingly, premenopausal women are at a lower risk for stroke than men of the same age.⁴⁶ However, after the menopause the incidence of cerebrovascular disease rises rapidly.⁴⁷ These clinical observations parallel statistics on the prevalence of stroke with regard to age and sex: at a younger age, women appear to be protected against stroke, compared with men, but lose this advantage in their postmenopausal years (*Figure 1*).⁴⁸ Together, these data suggest that endogenous estrogen plays a protective role against stroke. Since stroke imposes major morbidity and mortality in postmenopausal women, it is critical to determine whether ERT may decrease the risk and/or severity of cerebrovascular disease.

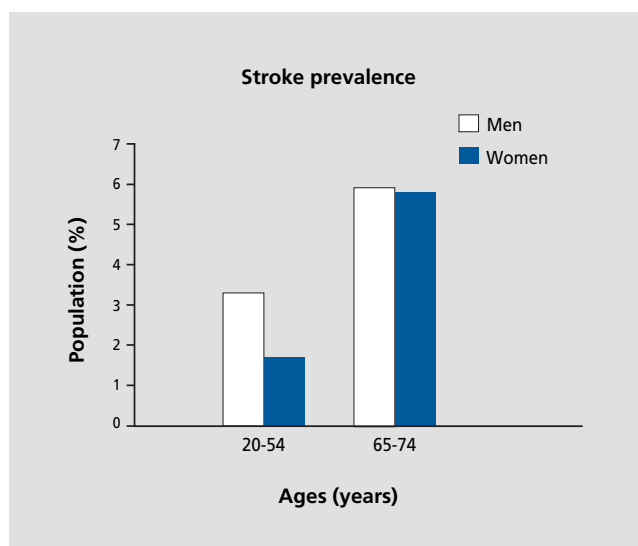


Figure 1. Endogenous estrogen may decrease the risk for stroke. Prior to the menopause, women appear to be protected against the occurrence of stroke, compared with age-matched men (left). However, this protection is lost at some time after the menopause (right), suggesting that endogenous estrogen may play a protective role against stroke. Data modified from the American Heart Association (2001).⁴⁸

Classification of stroke

Stroke results in infarction of the brain. Major causes and risk factors for stroke include coronary artery disease, cardiac failure, diabetes, hypertension, atherosclerosis, and thrombotic conditions. The overlapping and often mixed etiologies of stroke can result in two major types of pathology: ischemic stroke or hemorrhagic stroke. Briefly, clot(s) in the cerebrovasculature produce ischemic infarct, and the bursting of cerebral vessel(s) causes subarachnoid hemorrhage.

Estrogen and stroke risk

Several lines of evidence suggest that ERT may reduce the likelihood for stroke by modifying risk factors that underlie both stroke and coronary heart disease (CHD).⁴⁴ For example, estrogen may protect by exerting beneficial effects on diabetes and on the serum lipid profile.^{49,50} Interestingly, CHD doubles the risk for stroke and ERT greatly reduces the risk for CHD (by 30% to 40%). It thus follows that estrogen may decrease the risk for stroke in parallel with its protective actions on CHD.

In contrast to protection, estrogen may, under some circumstances, impose an increased risk for stroke by influencing coagulation and fibrinolysis. Concerns of the thrombotic potential of estrogen arose from early observations that oral contraceptives appeared to increase the risk of venous thrombosis, pulmonary embolism, and stroke.⁵¹ Similarly, ERT in postmenopausal women appears to be associated with a higher risk of venous thrombosis during the first year of use.⁵² However, whether ERT imposes a risk for ischemic stroke in postmenopausal women is unclear. We now understand that the dose of estrogen administered and the route of estrogen delivery are key components in determining clotting potential. At higher doses, oral estrogen, which enters the body via the enterohepatic system, can stimulate the production of thrombogenic factors^{53,54} predominantly through its actions on the liver. Alternatively, lower doses of estrogen, delivered orally or transdermally, may not significantly affect hemostasis.^{53,55-57} Importantly, transdermal delivery of estrogen bypasses enterohepatic circulation and may thus prevent estrogen-mediated stimulation of thrombogenic factors in the liver. Collectively, these findings highlight the importance of low, physiological doses in estrogen replacement of postmenopausal women.

ERT and stroke: overview of clinical studies

Studies have only begun to explore the actions of hormone replacement on stroke in the clinical setting. Data from several human studies clearly indicate that estrogen exerts protection against stroke⁵⁸⁻⁶¹; however, many studies report either protective trends or no significant effect of estrogen^{58,59,62-72} and few report deleterious effects of estrogen.^{62,66,73,74} Preliminary results from the latest clinical study, the Women's Estrogen for Stroke Trial (WEST), indicate that estrogen does not protect against the rate of either nonfatal stroke or death in postmenopausal women with a history of stroke.⁷⁵ In parallel with studies that fail to detect estrogen-mediated protection of the heart in women with cardiovascular disease,¹⁶ or of the brain in women with AD,⁴³ the results of WEST suggest that estrogen does not effectively protect against or reverse a disease process that has already been initiated.

To date, clinical studies have mainly probed whether ERT significantly affects the incidence and mortality of stroke. The outcomes of many of these studies are varied and often appear to be contradictory. Thus, we cannot yet draw clear conclusions from the existing data due to several confounding issues. The lack of uniformity among the data in clinical reports may result from several inconsistencies.⁴⁴ First, stroke is a mixed group of diseases with varying etiologies. If ERT decreases or increases the risk of specific stroke subtypes, effects of estrogen may be distorted and/or masked when strokes are grouped together and classified differently among the studies. Second, it is important to distinguish between ever and current users of ERT, as these categories represent two distinct populations of women; ever users of ERT may demonstrate long-lasting actions of estrogen, while current users of ERT may exhibit primarily short-term actions of estrogen. Clearly, mixing the two populations can obscure important effects of estrogen. Finally, it is critical to identify and probe the effects of different modes and regimens of ERT since estrogen action can vary dramatically if it is administered (i) in physiological versus pharmacological doses; (ii) alone or combined with progesterone (cyclic or continuous); and (iii) orally or transdermally. Since the distinctions among ERT regimens are not clear in most clinical studies, it is often difficult to interpret results or, further, to compare results with the outcomes of other studies.

Cerebral ischemia: an animal model of stroke

Realistically, even the best, well-designed clinical studies may not benefit from the experimental advantages of many basic science studies including clear and unconfounded controls, well-controlled environments, and lack of selection or recall bias. Thus, investigators have developed animal models of stroke to investigate the pathophysiology and potential treatments for stroke.

Cerebral artery occlusion

Experimental methods developed to emulate stroke in animal models produce brain ischemia by blocking blood flow to the cerebral vasculature. The varieties of techniques used to induce ischemia differ with regard to the means of producing ischemia, the site of occlusion, and the duration of occlusion. We utilized an animal model of stroke, permanent middle cerebral artery occlusion (MCAO), to examine the effects of estrogen in neurodegeneration. Since ischemic infarcts represent the majority (>70%) of cerebrovascular disease in the aging population, we adopted an animal model that reproduces ischemic infarcts. MCAO has been thoroughly developed and characterized to study the pathophysiology and therapeutic possibilities of ischemic injury. Occlusion, or the blocking of blood flow, of the middle cerebral artery predominantly affects two major areas: the cortex and underlying striatum. Permanent blockage of this artery at its base causes severe metabolic impairment in the striatum because this region receives no alternative blood perfusion; this characterizes the "ischemic core." The cortex, on the other hand, undergoes moderate metabolic impairment and is potentially salvageable by effective therapeutic agents because it receives some perfusion from the anterior cerebral artery and the vertebral artery⁷⁶; this characterizes "ischemic penumbra."

Ischemia results in cell death

The ischemic brain is exposed to excessive amounts of glutamate, which leads to massive influxes of calcium into cells. Although the exact mechanisms of ischemic injury are not clear, glutamate neurotoxicity is a key player in the pathogenesis of an ischemic lesion.⁷⁷⁻⁷⁹ Inappropriate rises in intracellular calcium due to glutamate and ion dyshomeostasis can cause moderate or

Basic research

irreversible injury, depending on the severity of the insult.⁷⁹ An evolving view of trauma in focal cerebral ischemia is that the severely impaired “ischemic core” dies by necrotic cell death, a mode of death characterized by inflammation and cellular bursting. The moderately injured “ischemic penumbra” dies, in part, by apoptotic cell death, an orchestrated event of cellular signaling that results in distinct morphological changes resembling autodigestion.⁸⁰ While the exact modes of ischemic cell death are controversial, several apoptotic factors have been identified as pathogenic or survival components in ischemic injury.⁸¹⁻⁸⁷ As discussed below, many studies, including our own, have investigated whether estradiol can attenuate cell death resulting from ischemic injury and whether the mechanisms of protection against cell death involve suppression of apoptotic signaling.

Estrogen and neuroprotection: insights from basic science studies

Estrogen protects against in vivo brain injury

In 1991, a single in vivo report suggested that estradiol may play a role in protection of the brain. This study, carried out by Hall and colleagues, demonstrated that female gerbils sustained less neuronal pathology following global ischemia than males.⁸⁸ Since then, the field of estrogen and neuroprotection has rapidly expanded and numerous laboratories have demonstrated that estrogen exerts profound neuroprotective actions in a variety of paradigms of brain injury.⁸⁹ The results of these studies have clearly shown that estradiol decreases the severity of injury in several in vivo models including cerebral ischemia,⁹⁰⁻⁹⁵ cerebral contusion,⁹⁶⁻⁹⁸ hypoxia,⁹⁹ and drug-induced toxicity.¹⁰⁰

Studies performed using animal models of stroke provide strong evidence that estradiol is a neuroprotective factor that profoundly attenuates the degree of ischemic brain injury. These studies clearly establish that females uniformly endure less stroke injury than males. Female gerbils demonstrate less neuronal pathology than males after ischemia induced by unilateral carotid artery occlusion.⁸⁸ Likewise, gonadally intact female rats sustain over 50% less infarction than gonadally intact males and ovariectomized female rats following ischemia induced by transient occlusion of the middle cerebral artery.^{94,101} Further, gonadectomized females^{90-93,102} and males⁹² that

are treated with estradiol suffer less MCAO-induced injury than estradiol-depleted controls.

Our work has significantly contributed to the understanding of the neuroprotective actions of physiological levels of estradiol. We have found that low, physiological doses of estradiol replacement are sufficient to exert dramatic protection in the brains of young female rats (*Figure 2*).⁹⁰ Further, we found that middle-aged female rats remain responsive to the neuroprotective effects of low estradiol levels.¹⁰³ Collectively, the results of these studies suggest that postmenopausal women that are estrogen-replaced may suffer a decreased degree of brain injury following a stroke, compared with their hypoestrogenic counterparts. However, we must be careful in extrapolating from rodents to humans until the appropriate clinical studies are performed.

Estrogen protects against in vitro neural injury

In addition to in vivo studies, several in vitro studies have greatly contributed to our understanding of estrogen action against degeneration. Many paradigms have been utilized to investigate whether estrogen can protect neural cells, in vitro. Studies have been performed in primary neuronal cultures, mixed astrocyte/neuron cultures, cell lines, and organotypic cultures. Using these paradigms, investigators have aimed to reproduce the deleterious environments found in various neurodegenerative conditions such as AD and stroke and have then tested whether estrogen protects against cell death.

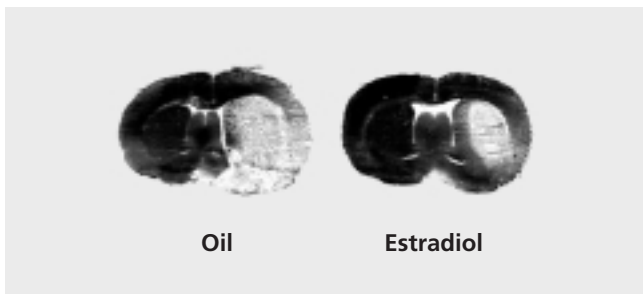


Figure 2. Physiological levels of estradiol decrease ischemic brain damage following stroke injury. Representative coronal sections obtained from oil-treated (left) and estradiol-treated (right) rat brains collected 24 h after the onset of ischemia and stained with hematoxylin and eosin. Ischemic injury, produced by permanent middle cerebral artery occlusion, appears unstained and is distributed across the cerebral cortex and striatum. Pretreatment with low levels of estradiol dramatically decreases the extent of stroke injury, compared with oil-treated controls.

In vitro studies clearly establish that estrogen exerts profound protective effects against a variety of neurotoxic insults. Studies have induced injury through conditions that mimic AD toxicity,¹⁰⁴⁻¹⁰⁷ hypoxia and oxidative stress,¹⁰⁷⁻¹¹³ excitotoxicity,^{107,111,114-116} and physical injury.¹¹⁷ Thus, studies have examined whether estradiol can salvage cells from death induced by inhibition of mitochondrial function, suppression of glucose metabolism, alteration of nitric oxide production, or administration of substances such as β -amyloid peptide, excitatory amino acids, free radicals, and glycoprotein 120. Though the differing modes of injury are distinct, they may share similar mechanisms of toxicity and face final common pathways in the induction of cell death. It remains to be determined whether estradiol protects against cell death through parallel or divergent pathways in the different modes of injury.

Estrogen does not always protect

It is important to appreciate that estrogen does not always exert beneficial effects. The actions of estrogen appear to be dictated by the type of estrogen administered, dose of estrogen given, and the animal model utilized. The type of estrogen administered impacts the efficacy of its neuroprotective actions. Most studies have focused attention on the effects of 17β -estradiol since it is the most biologically active and potent endogenous estrogen. However, we have gained major insight into estrogen action through studies that have probed the effects of 17α -estradiol, an “inactive” stereoisomer that does not effectively bind and activate ERs. The studies show that at physiological levels, 17β -estradiol protects and 17α -estradiol fails to protect against brain injury,¹¹⁰ indicating that ERs are critical to the mechanisms of hormone-mediated protection.^{12,118} However, the picture becomes more complex when we consider the dose of estrogen administered. Although physiological levels of 17α -estradiol fail to protect, pharmacological levels of both 17β - and 17α -estradiol act to protect^{12,119} through mechanisms that are likely to bypass ERs. The results of these findings highlight the importance of dose and type/potency of estrogen administered in achieving neuroprotection. Since a variety of estrogenic compounds are components of ERT preparations and several “designer” estrogens are administered or being developed, it will be critical to assess the efficacies of the wide variety of estrogens in promoting beneficial actions in the brain.

Under certain circumstances, 17β -estradiol can either fail to protect or even harm the brain. While estradiol can decrease brain injury in the vast majority of studies, estradiol fails to attenuate cell death in some animal models.^{96,120,121} It is possible that when the degree of injury is too severe, as may be the case in the hippocampus following prolonged global ischemia,¹²⁰ the actions of estradiol are not sufficient to prevent cell death. Under other circumstances, estradiol can be deleterious to neural function. In animal models of epilepsy, estradiol lowers the threshold for seizures and facilitates the induction and duration of excitatory neural firing.^{115,122} These data suggest that ERT may not always exert only beneficial actions in the brains of postmenopausal women, particularly in those with a medical history of epilepsy. As we continue to learn about the complexity of estrogen action with regards to dose, type of estrogen, and neurological condition, we will be better able to modify and transform estrogen replacement into therapy that exerts only beneficial actions in the brains of postmenopausal women.

Molecular mechanisms of estrogen-mediated neuroprotection

Estrogen may exert neuroprotective effects through several mechanisms: estrogen can act through ER-dependent and ER-independent, genomic as well as nongenomic means to attenuate neural injury. Collectively, studies demonstrate that the pathway of estrogen action is influenced by the dose of estrogen administered. In general, pharmacological levels of estradiol protect the brain through mechanisms that do not require ERs, while physiological levels of estradiol protect the brain through mechanisms that depend upon ERs, as discussed below.

Estrogen receptor-mediated mechanisms

ERs are critical to our understanding of the mechanisms of estrogen action. Two ER subtypes, or ERs, exist: the classic ER α and the recently discovered ER β . Although, portions of ER α and ER β are quite similar in structure, their distributions throughout the body and the brain are unique. Their unique regional distributions suggest that the receptors play very different roles in the body. Both ER α and ER β are transcription factors. They bind estradiol through their ligand-binding domains and,

Basic research

upon activation, homodimerize or heterodimerize through zinc finger structures located in the DNA and ligand-binding domains. The ER dimer then translocates to the nucleus and binds to the estrogen-responsive elements located in the promoters of estrogen-responsive genes to regulate transcription (Figure 3). ER-regulated transcription is enhanced by cofactors (coactivators and corepressors) that bind the ER–DNA complex to either amplify or diminish transcriptional activation or repression (Figure 3).

Our long-standing and traditional view of ER action¹²³ is rapidly transforming as we discover novel and unique roles for ERs, beyond direct transcriptional modulation. We now know that ERs interact with signal transduction pathways,^{124,125} such as adenylyl cyclase, phosphoinositol-3-kinase, and/or mitogen-activated kinase (MAPK), or involve cross-talk with growth factor receptors, such as *trkA* and insulin-like growth factor–I (IGF-I) receptor.^{114,124,126–128} These novel ER-mediated mechanisms may lead to downstream altered gene expression and/or

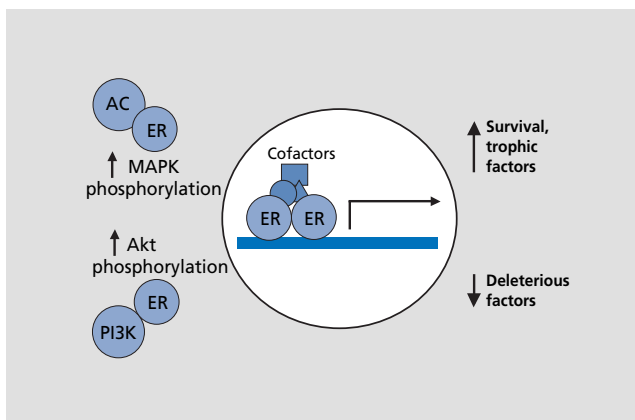


Figure 3. Estrogen receptors (ERs) act through traditional and novel mechanisms. This diagram illustrates ERs in their classical roles as transcription factors and in their newfound roles as components of signal transduction pathways. As transcription factors, ERs bind estradiol, dimerize, and then translocate to the nucleus. In the nucleus, ERs bind to estrogen-responsive elements in the promoters of target genes to induce or to suppress transcription. Cofactors can modulate the ER–DNA interactions to either amplify or diminish transcription. In addition to gene transactivation, ERs may also activate signal transduction pathways. For example, estradiol stimulates adenylyl cyclase (AC), one of several ways that it increases mitogen-activated protein kinase (MAPK) phosphorylation. Further, estradiol stimulates phosphoinositol-3-kinase (PI3K), which increases Akt (protein kinase B) phosphorylation. These effects on signal transduction may turn key proteins on or off, or ultimately induce genomic actions.

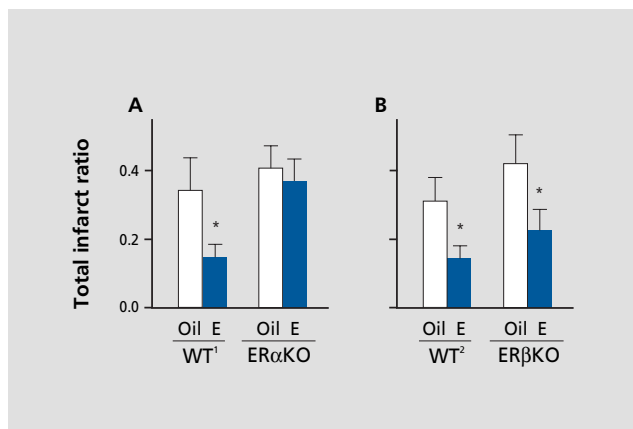


Figure 4. Estrogen receptor– α (ER α) is critical in estradiol-mediated protection of the brain following stroke injury. Estradiol (E) reduces ischemic infarct in both wildtype mice, WT¹ (A) and WT² (B), compared with corresponding oil-treated controls (n=6–10/group) (* P <0.02). Estradiol fails to protect in ER α knock-out (ER α KO) mice, compared with oil-treated controls (n=13/group) (A), but continues to protect in (B) ER β KO mice, compared with oil-treated controls (n=8–9/group) (* P <0.02). Values represent means \pm SE.

Reproduced from reference 140: Dubal DB, Zhu B, Yu B, et al. Estrogen receptor- α , not - β , is a critical link in estradiol-mediated protection against brain injury. *Proc Natl Acad Sci U S A*. 2001;98:1952–1957. Copyright © 2001, National Academy of Sciences, USA.

altered phosphorylation of proteins to promote estradiol action (Figure 3).

These traditional and novel ER-mediated interactions may induce a variety of cellular responses that promote trophic and protective effects in the brain. Physiological levels of estradiol can enhance synaptic plasticity,^{129–133} regulate the expression of neurotrophins and cognate receptors,^{134–137} and elevate the expression of cell survival factors.^{106,138,139} Any or all ER-mediated actions of estradiol that enhance the integrity and plasticity of the brain may ultimately promote neuroprotection.

We investigated the functional roles for ERs in estradiol-mediated protection against stroke injury and discovered a novel and unique role for ER α in the brain. Our data revealed that physiological levels of estradiol require ERs to exert protection against cerebral ischemia.^{110,140} Specifically, we utilized transgenic mice that were knocked out for either ER α or ER β and found that the classic ER, ER α , is the critical mechanistic link in the ability of low levels of estradiol to exert neuroprotection (Figure 4). We have begun to identify the repertoire of downstream genomic targets of estradiol action through ERs and, to date, have reported that

estradiol modulates the expression of a several players in ischemic brain injury including survival factors,¹³⁹ immediate early genes,¹⁴¹ neuropeptides,¹⁴² and trophic factors.¹⁴³

Estrogen receptor-independent mechanisms and actions

Though our studies focus attention on the mechanisms of ER-dependent, physiological actions of estrogen, it is very important to understand the mechanisms underlying pharmacological actions of estrogen since these effects are rapid, immediate, and may be critical to development of acute treatment of brain injury.

While physiological levels of estradiol generally require receptor-mediated genomic or nongenomic function for neuroprotection, pharmacological levels of estradiol appear to protect through non-ER-mediated effects. Pharmacological levels of estradiol can rapidly and reversibly decrease *N*-methyl-D-aspartate (NMDA)-induced currents,¹¹⁶ suggesting that it may reduce excitatory cell death caused by neurodegenerative injury. Furthermore, estrogens can influence members of the nitric oxide synthase family to induce vasodilatory actions on cerebral blood vessels¹⁴⁴ and thus improve blood flow to compromised brain regions. Estrogens can also act as potent antioxidants and inhibit lipid peroxidation^{88,105,107,112,145-148} through actions that have been shown

to occur via the C3 hydroxyl group located on the phenolic A-ring of the steroids.^{112,145} These studies^{88,105,112,145,146} confirm that this antioxidant mechanism requires supra-physiological levels of estrogen, and these findings may be key in the development of therapeutic approaches aimed at achieving neuroprotection against injury induced by oxidative stress.

Conclusion

In summary, a large breadth of clinical and basic science studies have led to a new appreciation that estradiol acts far beyond the reproductive axis and exerts profound protective actions in the adult and aging brain. Though we have only just begun to identify potential cellular and molecular mechanisms of this protection, our growing knowledge of estrogen action in the injured brain will ultimately lead to a more complete understanding of the precise mechanisms underlying estradiol-mediated protection. This knowledge is crucial to developing both preventative and acute therapies for neurodegenerative conditions and carries great promise for improving the quality of lives in our aging population. □

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Basic research

Estrógenos y neuroprotección: desde las observaciones clínicas a los mecanismos moleculares

Actualmente nosotros sabemos que los estrógenos son esteroides gonadales pleiotrópicos que ejercen efectos significativos en la plasticidad y en la supervivencia celular del cerebro adulto. Durante el siglo pasado la duración de la vida de las mujeres ha aumentado, pero la edad de la menopausia se ha mantenido constante. Esto significa que ahora las mujeres pueden vivir más de un tercio de sus vidas en un estado hipoestrogénico postmenopáusicas. El impacto en el cerebro del hipoestrogenismo prolongado constituye ahora una preocupación crítica de salud ya que nos damos cuenta que estas mujeres pueden tener un aumento del riesgo de disfunción cognitiva y de neurodegeneración debido a una variedad de enfermedades. La evidencia que se ha acumulado tanto de los estudios clínicos como de ciencias básicas indica que los estrógenos ejercen acciones protectoras críticas ante condiciones neurodegenerativas como la enfermedad de Alzheimer y los accidentes vasculares cerebrales. En este artículo nosotros revisamos los descubrimientos que engloban nuestra comprensión actual de la acción de los estrógenos contra la neurodegeneración. Estos hallazgos conducen a posibilidades de gran alcance para mejorar la calidad de vida en nuestra población que envejece.

Estrogènes et neuroprotection : de l'observation clinique aux mécanismes moléculaires

Nous savons maintenant que les estrogènes sont des stéroïdes gonadiques pléiotropes qui exercent d'importants effets sur la plasticité et la survie cellulaire du cerveau de l'adulte. Si l'espérance de vie des femmes a augmenté au siècle dernier, l'âge de la ménopause, quant à lui, reste constant. Cela signifie que les femmes peuvent maintenant passer plus d'un tiers de leur vie dans un climat postménopausique, hypoestrogénique. L'impact sur le cerveau d'une hypoestrogénicité prolongée est devenu un souci de santé préoccupant car nous réalisons que ces femmes peuvent être confrontées à un risque accru de dysfonctionnement cognitif et de neurodégénérescence secondaires à de nombreuses maladies. L'accumulation des constatations issues des études scientifiques cliniques et fondamentales indique que les estrogènes exercent des actions protectrices cruciales contre les pathologies neurodégénératives telles que la maladie d'Alzheimer et l'accident vasculaire cérébral. Nous passons ici en revue les découvertes qui constituent nos connaissances actuelles sur l'action des estrogènes contre la neurodégénérescence. Ces découvertes ouvrent la voie à des possibilités d'une portée considérable pour améliorer la qualité de vie de notre population vieillissante.

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