MINIREVIEW

Novel Mechanisms for Estrogen-Induced Neuroprotection

MEHARVAN SINGH,*[,1] JAMES A. DYKENS,† AND JAMES W. SIMPKINS*

*Department of Pharmacology & Neuroscience, University of North Texas Health Science Center, Fort Worth, Texas 76107; and †EyeCyte Therapeutics, San Diego, California 92024

Estrogens are gonadal steroid hormones that are present in the circulation of both males and females and that can no longer be considered within the strict confines of reproductive function. In fact, the bone, the cardiovascular system, and extrahypothalamic regions of the brain are now well-established targets of estrogens. Among the numerous aspects of brain function regulated by estrogens are their effects on mood, cognitive function, and neuronal viability. Here, we review the supporting evidence for estrogens as neuroprotective agents and summarize the various mechanisms that may be involved in this effect, focusing particularly on the mitochondria as an important target. On the basis of this evidence, we discuss the clinical applicability of estrogens in treating various age-related disorders, including Alzheimer disease and stroke, and identify the caveats that must be considered. Exp Biol Med 231:514–521, 2006

Key words: estrogen; estradiol; neuroprotection; cytoprotection; mitochondria; signaling pathways

Estrogens are gonadal steroid hormones that have classically been associated with reproductive function and, with respect to the brain, have primarily been considered within the confines of the hypothalamus. However, it is now well recognized that estrogens affect numerous extrahypothalamic regions of the brain, the consequence of which is to regulate such important functions as mood and cognitive function. In addition, a substantive and growing body of literature supports the neuroprotective actions of estrogens, which have been shown to be effective at protecting against cellular dysfunction and/or damage. For example, *in vitro* and *in vivo* studies have described estrogen's protective effects against such insults as serum deprivation (1–3), amyloid β peptide (Aβ)–induced toxicity (4–7), glutamate-induced excitotoxicity (6, 8, 9), hydrogen peroxide (H_2O_2) (4, 6, 7), oxygen-glucose deprivation (OGD) (10, 11), iron toxicity (6, 12), hemoglobin (10), and mitochondria toxins such as 3-NPA (13), *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (14), and sodium azide (10). The neuroprotective effects of estrogens have also been demonstrated in a variety of models of acute cerebral ischemia. These include transient and permanent middle cerebral artery occlusion models (15–17), global forebrain ischemia models (18, 19), photothrombotic focal ischemia models (20), and glutamate-induced focal cerebral ischemia models (21). The protective effects of estrogens have been described in rats, mice, and gerbils (22, 23). Estrogen-induced neuroprotection has been demonstrated in adult female rats, middle-aged female rats, and reproductively senescent female rats (24). Similarly, these effects of estrogens have been shown despite the presence of diabetes and hypertension (25, 26). The neuroprotective effects of estrogens have been demonstrated against subarachnoid hemorrhage, a highly prevalent form of stroke in females (27). Finally, the neuroprotective action of estrogen is not limited to the female, inasmuch as estrogen protection is also seen in males (28, 29). Collectively, these results indicate that estrogens could be valuable candidates for brain protection in both males and females. We describe the major mechanisms and cellular/subcellular targets of estrogen that mediate these protective effects.

Some of the studies described in this review were supported by grants from the National Institutes of Health (AG 010485 to J.W.S., AG 022550 to J.W.S. and M.S., and AG 023330 to M.S.).

[1] To whom correspondence should be addressed at Department of Pharmacology & Neuroscience, University of North Texas Health Science Center, Fort Worth, TX 76107. E-mail: msingh@hsc.unt.edu

1535-3702/06/2315-0514$15.00

Mechanisms of Action

Estrogen Receptors. Three major forms of estrogen exist in humans and rodents: the biologically most prevalent and potent estrogen 17β-estradiol (E2) and, in order of decreasing potency, estrone (E1) and estriol (E3). These estrogens are known to exert their actions through members of the nuclear hormone receptor superfamily, estrogen receptor-α (ER-α) (30), and the more recently identified estrogen receptor-β (ER-β) (31–33). Estrogen binding to these receptors promotes receptor dimerization and translocation to the nucleus, where subsequent association of "activated" receptor with specific DNA sequences in the promoter region of target genes (34) leads to the regulation of transcription (35). These receptors differ in their affinities for ligand, specificities for ligands (36), and tissue distribution (36, 37). Given the overlap in expression, such as the coexpression of both ER-α and ER-β mRNA in the cerebral cortex and hippocampus (37–40), studies have been performed to ascertain which receptor underlies the neuroprotective effects of estrogen.

In cell-culture systems that express, either naturally or experimentally, one of the two known estrogen receptors (ER-α or ER-β), pharmacological strategies that use estrogen-receptor antagonists, such as tamoxifen and ICI 182,780, have supported the requirement of these receptors in mediating the effects of estrogen on cell survival (41, 42). Some studies support the role of ER-α (43), whereas others implicate ER-β in mediating estrogen-induced protection (44). *In vivo* studies have also been performed to address the role of these estrogen receptors in mediating neuroprotection. For example, Dubal *et al.* (16) reported that the protective effects of low-dose estrogen (resulting in plasma levels that are approximately 25 pg/ml) against experimentally-induced stroke were abolished in ER-α knockout (ERαKO) mice. However, ERαKO mice are exposed to much higher levels of estrogen than their wild-type counterparts (45), likely because of the absence of negative feedback at the level of the hypothalamus. As such, the "threshold" for the protective effects of estrogen may have been much higher. Consistent with this idea, administration of higher concentrations of estradiol (approximately 200 pg/ml) to ERαKO mice was effective at reducing infarct volume (46, 47). Thus, depending on the region of the brain, either ER-α or ER-β may be involved in mediating estrogen's protective effects. An added complexity results from the observation that ER-α and ER-β can exist and act not only as homodimers, but also as heterodimers (32, 48, 49), which suggests a functional interaction between ER-α and ER-β. Thus, an "appropriate" balance between the levels of ER-α and ER-β may be required to mediate estrogen-induced neuroprotection, and alterations in the ratio between these receptors may determine whether estrogen is protective or damage promoting (as has been seen in reproductively senescent animals [50, 51]).

Identification of the receptor(s) that mediate the protective effects of estrogen have been complicated by the suggestion that there are membrane-associated estrogen receptors (52–55). On the one hand, it has been suggested that these membrane receptors are simply subpopulations of ER-α and/or ER-β, but other investigators have argued that the membrane estrogen receptor represents a completely distinct and novel class of estrogen receptor (56). However, the extent to which these membrane receptors are involved in mediating estrogen-induced neuroprotection is still unclear and will undoubtedly be clarified with further research and eventual cloning of potentially new estrogen receptors.

Regulation of Signal Transduction Pathways. The classical mechanism of hormone action states that, because of their lipophilicity, estrogens cross the plasma membrane to bind to intracellular estrogen receptors, resulting in translocation of the activated estrogen receptor into the nucleus and eventual regulation of gene transcription. However, this "genomic" mechanism of action is insufficient to explain the broad scope of estrogen's actions, including the rapidity of some of estrogen's actions in the brain. Alternate mechanisms have indeed been recognized, including the regulation of signal transduction pathways typically associated with growth-factor action. These include, but are not limited to, the reported ability of estrogen to elicit the Ras/Raf/ERK(MAPK) pathway (57–60), the PI-3K/Akt pathway (59, 61–63), and the cAMP/PKA/CREB pathway (63, 64) and to modulate the NFκB pathways (65, 66). These pathways have all been linked to the regulation of cell survival, although the importance of one or more of these pathways in mediating the protective effects of estrogen may be cell or context dependent. The activation of these signaling pathways in response to estrogen has been reviewed in greater detail by us and others previously (67–69). In the following sections, we highlight the mitochondria as important mechanistic targets for estrogen-induced neuroprotection.

Mitochondria and Neurodegenerative Diseases. Mitochondria are subcellular organelles that serve not only as the primary source for cellular energy, but are also the major source of intracellular free radicals. Thus, mitochondria supply high-energy ATP molecules and monitor cellular health, and they sit at a strategic position in the hierarchy of cellular organelles to make cell decisions regarding the survival or death of cells (70, 71). These mitochondrial roles are critical in the brain, given its high energy demand that is driven by the need to maintain ion gradients across the plasma membrane, which, in turn, is critical for the generation of action potentials. Although the brain represents only 2% of the body weight, it receives 15% of cardiac output and uses 20% of total body oxygen. This intense energy requirement is continuous and implies that even brief periods of oxygen or glucose deprivation can result in neuronal death.

Among the factors underlying the degeneration of neurons in such neurodegenerative diseases as Alzheimer's

disease (AD) is the generation of excessive amounts of reactive oxygen species (ROS) (72, 73). In fact, mitochondria from patients with AD are hypofunctional (74, 75) because of a catalytic defect in respiratory complex IV (C-IV) of AD-associated mitochondria (76, 77). Furthermore, when mitochondrial DNA (mtDNA) from patients with AD were inserted into transformed cells depleted of their endogenous mtDNA, the resulting phenotype included the formation of cytoplasmic hybrids (cybrids), increased oxidative stress, propensity towards apoptosis, and C-IV impairment (78, 79), suggesting that many of the cellular defects found in association with AD reflect mitochondrial defects. Although such mitochondrial impairment could be interpreted as a consequence of the disease, rather than as a primary causal factor (80), mitochondrial dysfunction is clearly involved in the progression of neuronal death and, as such, represents a viable therapeutic target.

Mitochondrial failure also contributes to cell death in more-acute circumstances, such as sudden ischemia of neurons during a stroke or of the myocardium during a heart attack. Neurons are dependent almost entirely on mitochondrial ATP production for their high energy demand, and are at risk when ATP levels drop, even transiently. Damage to mitochondria causes disruptions in ATP production and a concomitant increase in ROS that can overwhelm the antioxidant defense systems of the cell (71, 81). Such mitochondrial deficits are implicated as key events in the pathogenic cascades leading to both necrosis and apoptosis (82, 83). Oxidative stress, coupled with excessive Ca^{2+} loading, causes mitochondria to undergo a catastrophic loss of the inner mitochondrial membrane integrity, leading to an eventual collapse of the mitochondrial membrane potential ($\Delta\psi m$), a process called permeability transition (PT) (70). This collapse of $\Delta\psi m$ can be accompanied by mitochondrial swelling and release of cytochrome c and Apaf-1 (84) into the cytoplasm, leading, in turn, to the activation of caspases and apoptotic cell death (81, 83, 85, 86). This process undermines cellular and mitochondrial integrity by causing membrane peroxidation and interfering with oxidative phosphorylation. The resulting loss of ATP production causes ATPase failure, loss of ion homeostasis, and necrosis due to osmotic failure (71, 85).

Mitochondria as a Target of Estrogen-Induced Neuroprotection. The possibility that estrogens exert their potent neuroprotective effects through a mitochondrial mechanism is based on several observations. These effects may be exerted either directly or indirectly. Indirect effects of estrogen on the mitochondria may be mediated by signal transduction pathways that are not only elicited by estrogens, but are also important regulators of mitochondrial function. For example, estrogen can elicit the activation of the PI-3K/Akt pathway (59, 61–63), which, in turn, can result in the phosphorylation of the proapoptotic protein BAD. When phosphorylated, BAD is rendered inactive and prevents BAX-mediated release of cytochrome c from the mitochondria (87). Further, estrogens have been shown to affect concentrations and localization of antiapoptotic proteins (88–90), which appear to exert their antiapoptotic effects through maintenance of mitochondrial membrane potential in the face of cellular stresses (91).

Although mitochondria can be protected *via* indirect mechanisms (i.e., through regulation of signal transduction pathways or mobilization of antiapoptotic proteins), estrogen may also exert its protective actions directly. Supporting evidence comes from the observation of estrogen binding sites in the mitochondria, including the F0/F1 ATPase (92, 93). In fact, we showed that ER-β localizes to the mitochondria (90). Importantly, we have demonstrated that estrogens, after insults that are known to compromise the function of the mitochondria, are protective and help maintain the normal function of this vital organelle (13, 94). These findings are summarized as follows.

By use of oxidative stress–inducing mitochondrial toxins (13) or H_2O_2 (94), 17β-estradiol (E2) pretreatment ameliorated the insult-induced decrease in cellular ATP. One possible mechanism for these effects is that estrogens are potent lipid peroxidation inhibitors (94). Estrogens are highly lipid soluble and largely reside in the membrane component of cells (95, 96), where they are ideally suited to affect oxidation of unsaturated bonds in phospholipids. This membrane localization allows estrogens to interact synergistically with such abundant antioxidants as glutathione (97, 98), where they can apply their cytosolic reducing potential to the membrane (99). In fact, we provided evidence that estrogen prevents lipid peroxidation by sacrificing itself to oxidation, resulting in a quinol product. Interestingly, the oxidized estrogen was redox-cycled back to the parent estrogen by taking advantage of the plentiful and replenishable source of cellular reducing potential, such as glutathione or NAD(P)H (100, 101). This estrogen redox cycle is operative in the brain and serves, together with the "classic" antioxidant mechanism (102), as a defense mechanism against ROS.

Estrogens can also affect mitochondrial function by directly or indirectly influencing mitochondrial Ca^{2+} loading. Brinton *et al.* (88, 103) demonstrated that, with mild glutamate stimulation, estrogens enhance Ca^{2+} flux into cells, and this effect may be involved in estrogen's ability to increase memory function through an N-methyl-D-aspartate (NMDA)-mediated mechanism (104–106). With excitotoxic stimulation (as with high glutamate concentrations) (88, 103) or pro-oxidant stimulation (13, 94), however, estrogens prevented both cytosolic and mitochondrial influx of Ca^{2+}, thus providing a protection against excessive Ca^{2+} influx.

As stated above, $\Delta\psi m$ collapse is a critical event in promoting the death of neurons (83, 84, 107, 108). In fact, two methods of analysis revealed the protective effects of E2 against mitochondrial toxin–induced collapse of $\Delta\psi m$ in neuronal cultures. First, using rhodamine 123 (a mitochondria-specific dye), we demonstrated that pretreatment of neurons with E2 prevented mitochondrial toxin–induced mitochondrial depolarization (13). Similarly, using a

fluorescence resonance energy transfer (FRET) assay to measure $\Delta\psi m$ (109), we observed that treatment with either E2 or its diastereomer 17 α-estradiol (17 α-E2) resulted in a condition in which increased Ca^{2+} concentrations were required to cause $\Delta\psi m$ collapse (99). Collectively, these data indicated that estrogens protect mitochondria by preventing $\Delta\psi m$ collapse and could explain the ability of estrogens to prevent the release of apoptotic factors from the mitochondria (70), which is dependent on $\Delta\psi m$ collapse.

Clinical Implications of Estrogen Neuroprotection. Epidemiological studies have shown that estrogen therapy (ET) administered soon after the menopause is associated with numerous health benefits, including a reduction in the risk for cardiovascular diseases (110), decreased incidence of osteoporosis and associated bone fractures (111), decreased risk for neurodegenerative diseases (112), increased cognitive performance (113), and reduced risk for cataract (114). With respect to AD, there have been several clinical and epidemiological studies that describe estrogen's beneficial effects. For example, the seminal study by Fillit *et al.* (115) described a cognitive improvement in patients with AD who received estrogen treatment for 6 weeks. Since that study, a growing body of literature has shown that estrogen therapy may contribute to the prevention, attenuation, or even the delay of the onset of AD (116–120). Furthermore, estrogen replacement may also facilitate other treatments used for the treatment of AD. For example, in clinical trials that used Tacrine, an anticholinesterase drug used for the treatment of AD, a greater efficacy was seen in women receiving estrogen therapy than in women who were not (121).

Given the aforementioned evidence for the potent neuroprotective effects of a variety of estrogens in cell and animal models, as well as the epidemiological evidence of the efficacy of early postmenopausal treatment, it would seem to be reasonable that one or more of these compounds would be assessed in clinical trials for estrogens in stroke, AD, or other neurodegenerative conditions. However, the prospect of the clinical use of estrogens for neuroprotective therapy was dealt a severe blow with findings from the Women's Health Initiative (WHI) studies, which were published beginning in 2002. These studies assessed a variety of outcome measures that followed years of continuous daily administration of two hormone preparations, Premarin (Wyeth Pharmaceuticals, Philadelphia, PA; Refs. 122, 123) and PremPro (Wyeth; Refs. 124–126). Premarin is derived from the urine of pregnant mares and contains an abundance of equine estrogens, whereas PremPro consists of Premarin and medroxyprogesterone acetate (MPA). These studies were terminated early because the risks of therapy appeared to outweigh the benefits of treatment.

Although informative, the interpretation of the WHI studies is limited by the hormone preparations used, their route of administration, the regimen of hormone administration (i.e., continuous daily therapy versus cyclic therapy), and the advanced age of the subjects under study (127, 128). It is well known that oral administration of estrogens induces prothrombotic factors (129, 130), and this has accounted for the slight increase in the risk of deep venous thrombosis, heart attack, and stroke observed among women treated with Premarin and PremPro. It has also been suggested that the subjects in the population under study, who averaged 63 years of age at the time of study entry, had "silent" cardiovascular disease (131), and that sudden exposure to estrogens exacerbated the already-existing undiagnosed vascular disease in these women.

Given that we now have extensive knowledge of the signaling pathways that mediate estrogen-induced neuroprotection (see above), the structure-activity relationships for estrogen-induced neuroprotection (1, 132), and the route of administration that minimizes the negative effects consequent to the first-pass effect (133), novel drugs and delivery methods for estrogen neuroprotection can be investigated in future clinical studies. In brief, there are safe and effective means to administer estrogens (including nonfeminizing estrogens) for the treatment of nerve cell loss associated with chronic neurodegenerative disease and more-acute nerve cell compromising conditions, such as stroke and head injury.

1. Green PS, Gordon K, Simpkins JW. Phenolic A ring requirement for the neuroprotective effects of steroids. J Steroid Biochem Mol Biol 63:229–235, 1997.
2. Green PS, Bishop J, Simpkins JW. 17α-Estradiol exerts neuroprotective effects on SK-N-SH cells. J Neurosci 17:511–515, 1997.
3. Bishop J, Simpkins JW. Estradiol treatment increases viability of glioma and neuroblastoma cells in vitro. Mol Cell Neurosci 5:303–308, 1994.
4. Green PS, Simpkins JW. Neuroprotective effects of estrogens: potential mechanisms of action. Int J Dev Neurosci 18:347–358, 2000.
5. Green PS, Gridley KE, Simpkins JW. Estradiol protects against beta-amyloid (25–35)–induced toxicity in SK-N-SH human neuroblastoma cells. Neurosci Lett 218:165–168, 1996.
6. Goodman Y, Bruce AJ, Cheng B, Mattson MP. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. J Neurochem 66:1836–1844, 1996.
7. Behl C, Widmann M, Trapp T, Holsboer F. 17-Beta estradiol protects neurons from oxidative stress–induced cell death in vitro. Biochem Biophys Res Commun 216:473–482, 1995.
8. Zaulyanov LL, Green PS, Simpkins JW. Glutamate receptor requirement for neuronal death from anoxia-reoxygenation: an *in vitro* model for assessment of the neuroprotective effects of estrogens. Cell Mol Neurobiol 19:705–718, 1999.
9. Singer CA, Rogers KL, Strickland TM, Dorsa DM. Estrogen protects primary cortical neurons from glutamate toxicity. Neurosci Lett 212: 13–16, 1996.
10. Regan RF, Guo Y. Estrogens attenuate neuronal injury due to hemoglobin, chemical hypoxia, and excitatory amino acids in murine cortical cultures. Brain Res 764:133–140, 1997.
11. Wilson ME, Dubal DB, Wise PM. Estradiol protects against injury-induced cell death in cortical explant cultures: a role for estrogen receptors. Brain Res 873:235–242, 2000.
12. Blum-Degen D, Haas M, Pohli S, Harth R, Romer W, Oettel M,

Riederer P, Gotz ME. Scavestrogens protect IMR 32 cells from oxidative stress–induced cell death. Toxicol Appl Pharmacol 152:49–55, 1998.

13. Wang J, Green PS, Simpkins JW. Estradiol protects against ATP depletion, mitochondrial membrane potential decline and the generation of reactive oxygen species induced by 3-nitroproprionic acid in SK-N-SH human neuroblastoma cells. J Neurochem 77:804–811, 2001.
14. De Girolamo LA, Hargreaves AJ, Billett EE. Protection from MPTP-induced neurotoxicity in differentiating mouse N2a neuroblastoma cells. J Neurochem 76:650–660, 2001.
15. Alkayed NJ, Harukuni I, Kimes AS, London ED, Traystman RJ, Hurn PD. Gender-linked brain injury in experimental stroke. Stroke 29:159–165, 1998.
16. Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, Wise PM. Estradiol protects against ischemic injury. J Cereb Blood Flow Metab 18:1253–1258, 1998.
17. Simpkins JW, Rajakumar G, Zhang YQ, Simpkins CE, Greenwald D, Yu CJ, Bodor N, Day AL. Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. J Neurosurg 87:724–730, 1997.
18. He Z, He YJ, Day AL, Simpkins JW. Proestrus levels of estradiol during transient global cerebral ischemia improves the histological outcome of the hippocampal CA1 region: perfusion-dependent and -independent mechanisms. J Neurol Sci 193:79–87, 2002.
19. Sudo S, Wen TC, Desaki J, Matsuda S, Tanaka J, Arai T, Maeda N, Sakanaka M. Beta-estradiol protects hippocampal CA1 neurons against transient forebrain ischemia in gerbil. Neurosci Res 29:345–354, 1997.
20. Fukuda K, Yao H, Ibayashi S, Nakahara T, Uchimura H, Fujishima M, Hall ED. Ovariectomy exacerbates and estrogen replacement attenuates photothrombotic focal ischemic brain injury in rats. Stroke 31:155–160, 2000.
21. Mendelowitsch A, Ritz MF, Ros J, Langemann H, Gratzl O. 17β-Estradiol reduces cortical lesion size in the glutamate excitotoxicity model by enhancing extracellular lactate: a new neuroprotective pathway. Brain Res 901:230–236, 2001.
22. Culmsee C, Vedder H, Ravati A, Junker V, Otto D, Ahlemeyer B, Krieg JC, Krieglstein J. Neuroprotection by estrogens in a mouse model of focal cerebral ischemia and in cultured neurons: evidence for a receptor-independent antioxidative mechanism. J Cereb Blood Flow Metab 19:1263–1269, 1999.
23. Chen J, Xu W, Jiang H. 17β-Estradiol protects neurons from ischemic damage and attenuates accumulation of extracellular excitatory amino acids. Anesth Analg 92:1520–1523, 2001.
24. Wise PM, Dubal DB, Wilson ME, Rau SW, Bottner M, Rosewell KL. Estradiol is a protective factor in the adult and aging brain: understanding of mechanisms derived from in vivo and in vitro studies. Brain Res Brain Res Rev 37:313–319, 2001.
25. Toung TK, Hurn PD, Traystman RJ, Sieber FE. Estrogen decreases infarct size after temporary focal ischemia in a genetic model of type 1 diabetes mellitus. Stroke 31:2701–2706, 2000.
26. Carswell HV, Anderson NH, Morton JJ, McCulloch J, Dominiczak AF, Macrae IM. Investigation of estrogen status and increased stroke sensitivity on cerebral blood flow after a focal ischemic insult. J Cereb Blood Flow Metab 20:931–936, 2000.
27. Yang SH, He Z, Wu SS, He YJ, Cutright J, Millard WJ, Day AL, Simpkins JW. 17-β Estradiol can reduce secondary ischemic damage and mortality of subarachnoid hemorrhage. J Cereb Blood Flow Metab 21:174–181, 2001.
28. Toung TJ, Traystman RJ, Hurn PD. Estrogen-mediated neuroprotection after experimental stroke in male rats. Stroke 29:1666–1670, 1998.
29. Hawk T, Zhang YQ, Rajakumar G, Day AL, Simpkins JW. Testosterone increases and estradiol decreases middle cerebral artery occlusion lesion size in male rats. Brain Res 796:296–298, 1998.
30. Greene G, Gilna P, Waterfield M, Baker A, Hort Y, Shine J. Sequence and expression of human estrogen receptor complementary DNA. Science 231:1150–1154, 1986.
31. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A 93:5925–5930, 1996.
32. Ogawa S, Inoue S, Watanabe T, Hiroi H, Orimo A, Hosoi T, Ouchi Y, Muramatsu M. The complete primary structure of human estrogen receptor beta (hER beta) and its heterodimerization with ER alpha in vivo and in vitro. Biochem Biophys Res Commun 243:122–126, 1998.
33. Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett 392: 49–53, 1996.
34. Evans RM. The steroid and thyroid hormone receptor superfamily. Science 240:889–895, 1988.
35. Landers JP, Spelsberg TC. New concepts in steroid hormone action: transcription factors, proto-oncogenes, and the cascade model for steroid regulation of gene expression. Crit Rev Eukaryot Gene Expr 2: 19–63, 1992.
36. Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138:863–870, 1997.
37. Shughrue P, Scrimo P, Lane M, Askew R, Merchenthaler I. The distribution of estrogen receptor-beta mRNA in forebrain regions of the estrogen receptor–alpha knockout mouse. Endocrinology 138: 5649–5652, 1997.
38. Osterlund M, Kuiper GG, Gustafsson JA, Hurd YL. Differential distribution and regulation of estrogen receptor-alpha and -beta mRNA within the female rat brain. Brain Res Mol Brain Res 54:175–180, 1998.
39. Li X, Schwartz PE, Rissman EF. Distribution of estrogen receptor-beta–like immunoreactivity in rat forebrain. Neuroendocrinology 66: 63–67, 1997.
40. DonCarlos L. Estrogen receptor β (ER-β) mRNA in developing rat forebrain. Soc Neurosci Abstracts 23:343, 1997.
41. Chowen JA, Torres-Aleman I, Garcia-Segura LM. Trophic effects of estradiol on fetal rat hypothalamic neurons. Neuroendocrinology 56: 895–901, 1992.
42. Patrone C, Andersson S, Korhonen L, Lindholm D. Estrogen receptor–dependent regulation of sensory neuron survival in developing dorsal root ganglion. Proc Natl Acad Sci U S A 96:10905–10910, 1999.
43. Gollapudi L, Oblinger MM. Stable transfection of PC12 cells with estrogen receptor (ERalpha): protective effects of estrogen on cell survival after serum deprivation. J Neurosci Res 56:99–108, 1999.
44. Sawada H, Ibi M, Kihara T, Urushitani M, Honda K, Nakanishi M, Akaike A, Shimohama S. Mechanisms of antiapoptotic effects of estrogens in nigral dopaminergic neurons. Faseb J 14:1202–1214, 2000.
45. Couse JF, Curtis SW, Washburn TF, Lindzey J, Golding TS, Lubahn DB, Smithies O, Korach KS. Analysis of transcription and estrogen insensitivity in the female mouse after targeted disruption of the estrogen receptor gene. Mol Endocrinol 9:1441–1454, 1995.
46. McCullough LD, Alkayed NJ, Traystman RJ, Williams MJ, Hurn PD. Postischemic estrogen reduces hypoperfusion and secondary ischemia after experimental stroke. Stroke 32:796–802, 2001.
47. Sugo N, Alkayed NJ, Blizzard KK, McCullough LD, Traystman RJ, Crain BJ, Korach KS, Hurn PD. Estrogen protects ischemic brain of estrogen receptor-alpha or -beta knockout mice. Soc Neurosci Abstracts No 437.13, 2001.
48. Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA, Horne

EL, Su JL, Kliewer SA, Lehmann JM, Willson TM. Cloning and characterization of human estrogen receptor beta isoforms. Biochem Biophys Res Commun 247:75–78, 1998.

49. Pettersson K, Grandien K, Kuiper GG, Gustafsson JA. Mouse estrogen receptor beta forms estrogen response element–binding heterodimers with estrogen receptor alpha. Mol Endocrinol 11:1486–1496, 1997.
50. Nordell VL, Scarborough MM, Buchanan AK, Sohrabji F. Differential effects of estrogen in the injured forebrain of young adult and reproductive senescent animals. Neurobiol Aging 24:733–743, 2003.
51. Jezierski MK, Sohrabji F. Neurotrophin expression in the reproductively senescent forebrain is refractory to estrogen stimulation. Neurobiol Aging 22:309–319, 2001.
52. Anuradha P, Khan SM, Karthikeyan N, Thampan RV. The non-activated estrogen receptor (naER) of the goat uterus is a tyrosine kinase. Arch Biochem Biophys 309:195–204, 1994.
53. Pappas TC, Gametchu B, Watson CS. Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. Faseb J 9:404–410, 1995.
54. Pietras RJ, Szego CM. Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. Nature 265:69–72, 1977.
55. Watson CS, Pappas TC, Gametchu B. The other estrogen receptor in the plasma membrane: implications for the actions of environmental estrogens. Environ Health Perspect 103(Suppl 7):41–50, 1995.
56. Toran-Allerand CD, Guan X, MacLusky NJ, Horvath TL, Diano S, Singh M, Connolly ES Jr, Nethrapalli IS, Tinnikov AA. ER-X: a novel, plasma membrane–associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. J Neurosci 22:8391–8401, 2002.
57. Singh M, Setalo G Jr, Guan X, Warren M, Toran-Allerand C. Estrogen-induced activation of mitogen-activated protein kinase in cerebral cortical explants: convergence of estrogen and neurotrophin signaling pathways. J Neurosci 19:1179–1188, 1999.
58. Singh M, Setalo G Jr, Guan X, Frail DE, Toran-Allerand CD. Estrogen-induced activation of the mitogen-activated protein kinase cascade in the cerebral cortex of estrogen receptor–alpha knock-out mice. J Neurosci 20:1694–1700, 2000.
59. Singh M. Ovarian hormones elicit phosphorylation of Akt and extracellular-signal regulated kinase in explants of the cerebral cortex. Endocrine 14:407–415, 2001.
60. Singer CA, Figueroa-Masot XA, Batchelor RH, Dorsa DM. The mitogen-activated protein kinase pathway mediates estrogen neuroprotection after glutamate toxicity in primary cortical neurons. J Neurosci 19:2455–2463, 1999.
61. Zhang L, Rubinow DR, Xaing G, Li BS, Chang YH, Maric D, Barker JL, Ma W. Estrogen protects against beta-amyloid–induced neurotoxicity in rat hippocampal neurons by activation of Akt. Neuroreport 12:1919–1923, 2001.
62. Honda K, Sawada H, Kihara T, Urushitani M, Nakamizo T, Akaike A, Shimohama S. Phosphatidylinositol 3–kinase mediates neuroprotection by estrogen in cultured cortical neurons. J Neurosci Res 60:321–327, 2000.
63. Honda K, Shimohama S, Sawada H, Kihara T, Nakamizo T, Shibasaki H, Akaike A. Nongenomic antiapoptotic signal transduction by estrogen in cultured cortical neurons. J Neurosci Res 64:466–475, 2001.
64. Carlstrom L, Ke ZJ, Unnerstall JR, Cohen RS, Pandey SC. Estrogen modulation of the cyclic AMP response element–binding protein pathway: effects of long-term and acute treatments. Neuroendocrinology 74:227–243, 2001.
65. Wen Y, Yang S, Liu R, Perez E, Yi KD, Koulen P, Simpkins JW. Estrogen attenuates nuclear factor–kappa B activation induced by transient cerebral ischemia. Brain Res 1008:147–154, 2004.
66. Dodel RC, Du Y, Bales KR, Gao F, Paul SM. Sodium salicylate and 17beta-estradiol attenuate nuclear transcription factor NF-kappaB translocation in cultured rat astroglial cultures following exposure to amyloid A beta(1–40) and lipopolysaccharides. J Neurochem 73: 1453–1460, 1999.
67. Simpkins JW, Yang SH, Wen Y, Singh M. Estrogens, progestins, menopause and neurodegeneration: basic and clinical studies. Cell Mol Life Sci 62:271–280, 2005
68. Segars JH, Driggers PH. Estrogen action and cytoplasmic signaling cascades. Part I: Membrane-associated signaling complexes. Trends Endocrinol Metab 13:349–354, 2002.
69. Garcia-Segura LM, Azcoitia I, DonCarlos LL. Neuroprotection by estradiol. Prog Neurobiol 63:29–60, 2001.
70. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. Science 305:626–629, 2004.
71. Dykens JA. Mitochondrial free radical production and the etiology of neurodegenerative disease. In: Beal MF, Howell N, Bodis-Wollner I, eds. Neurodegenerative Diseases: Mitochondria and Free Radicals in Pathogenesis. New York: John Wiley & Sons, pp29–55, 1997.
72. Smith MA, Nunomura A, Zhu X, Takeda A, Perry G. Metabolic, metallic, and mitotic sources of oxidative stress in Alzheimer disease. Antioxid Redox Signal 2:413–420, 2000.
73. Aliev G, Smith MA, Seyidov D, Neal ML, Lamb BT, Nunomura A, Gasimov EK, Vinters HV, Perry G, LaManna JC, Friedland RP. The role of oxidative stress in the pathophysiology of cerebrovascular lesions in Alzheimer's disease. Brain Pathol 12:21–35, 2002.
74. Cottrell DA, Ince PG, Wardell TM, Turnbull DM, Johnson MA. Accelerated ageing changes in the choroid plexus of a case with multiple mitochondrial DNA deletions. Neuropathol Appl Neurobiol 27:206–214, 2001.
75. Bosetti F, Brizzi F, Barogi S, Mancuso M, Siciliano G, Tendi EA, Murri L, Rapoport SI, Solaini G. Cytochrome c oxidase and mitochondrial F1F0-ATPase (ATP synthase) activities in platelets and brain from patients with Alzheimer's disease. Neurobiol Aging 23:371–376, 2002.
76. Gibson GE, Sheu KF, Blass JP. Abnormalities of mitochondrial enzymes in Alzheimer disease. J Neural Transm 105:855–870, 1998.
77. Kish SJ. Brain energy metabolizing enzymes in Alzheimer's disease: alpha-ketoglutarate dehydrogenase complex and cytochrome oxidase. Ann N Y Acad Sci 826:218–228, 1997.
78. Trimmer PA, Swerdlow RH, Parks JK, Keeney P, Bennett JP Jr, Miller SW, Davis RE, Parker WD Jr. Abnormal mitochondrial morphology in sporadic Parkinson's and Alzheimer's disease cybrid cell lines. Exp Neurol 162:37–50, 2000.
79. Ghosh SS, Swerdlow RH, Miller SW, Sheeman B, Parker WD Jr, Davis RE. Use of cytoplasmic hybrid cell lines for elucidating the role of mitochondrial dysfunction in Alzheimer's disease and Parkinson's disease. Ann N Y Acad Sci 893:176–191, 1999.
80. Shoffner JM. Oxidative phosphorylation defects and Alzheimer's disease. Neurogenetics 1:13–19, 1997.
81. Dykens JA, Wiles ME, Wright CD, Towbin KE, Dykens EM, Pugliese RG, Hodapp RM, Evans DW. Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated Ca^{2+} and Na^{+}: implications for neurodegeneration. J Neurochem 63:584–591, 1994.
82. Lemasters JJ, Nieminen AL, Qian T, Trost LC, Herman B. The mitochondrial permeability transition in toxic, hypoxic and reperfusion injury. Mol Cell Biochem 174:159–165, 1997.
83. Kroemer G, Reed JC. Mitochondrial control of cell death. Nat Med 6: 513–519, 2000.
84. Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c–dependent activation of caspase-3. Cell 90:405–413, 1997.
85. Dykens JA. Free radicals and mitochondrial dysfunction in excitotoxicity and neurodegenerative diseases. In: Koliatsos VE, Ratan RR, eds. Cell Death and Diseases of the Nervous System. Totowa, NJ: Humana Press, pp45–68, 1999.

86. Murphy AN, Fiskum G, Beal MF. Mitochondria in neurodegeneration: bioenergetic function in cell life and death. J Cereb Blood Flow Metab 19:231–245, 1999.
87. Chan PH. Mitochondria and neuronal death/survival signaling pathways in cerebral ischemia. Neurochem Res 29:1943–1949, 2004.
88. Nilsen J, Diaz Brinton R. Mechanism of estrogen-mediated neuroprotection: regulation of mitochondrial calcium and Bcl-2 expression. Proc Natl Acad Sci U S A 100:2842–2847, 2003.
89. Wise PM, Dubal DB, Wilson ME, Rau SW. Estradiol is a neuroprotective factor in in vivo and in vitro models of brain injury. J Neurocytol 29:401–410, 2000.
90. Yang SH, Liu R, Perez EJ, Wen Y, Stevens SM Jr, Valencia T, Brun-Zinkernagel AM, Prokai L, Will Y, Dykens J, Koulen P, Simpkins JW. Mitochondrial localization of estrogen receptor beta. Proc Natl Acad Sci U S A 101:4130–4135, 2004.
91. Kroemer G. The proto-oncogene Bcl-2 and its role in regulating apoptosis. Nat Med 3:614–620, 1997.
92. Zheng J, Ramirez VD. Purification and identification of an estrogen binding protein from rat brain: oligomycin sensitivity–conferring protein (OSCP), a subunit of mitochondrial F0F1-ATP synthase/ATPase. J Steroid Biochem Mol Biol 68:65–75, 1999.
93. Zheng J, Ramirez VD. Rapid inhibition of rat brain mitochondrial proton F0F1-ATPase activity by estrogens: comparison with Na+, K+-ATPase of porcine cortex. Eur J Pharmacol 368:95–102, 1999.
94. Wang X, Simpkins JW, Dykens JA, Cammarata PR. Oxidative damage to human lens epithelial cells in culture: estrogen protection of mitochondrial potential, ATP, and cell viability. Invest Ophthalmol Vis Sci 44:2067–2075, 2003.
95. Cegelski L, Rice CV, O'Connor RD, Caruano AL, Tochtrop GP, Cai ZY, Covey DF, Schaefer J. Mapping the locations of estradiol and potent neuroprotective analogues in phospholipid bilayers by REDOR NMR. Drug Development Research 6:93–102, 2006.
96. Liang Y, Belford S, Tang F, Prokai L, Simpkins JW, Hughes JA. Membrane fluidity effects of estratrienes. Brain Res Bull 54:661–668, 2001.
97. Green PS, Gridley KE, Simpkins JW. Nuclear estrogen receptor–independent neuroprotection by estratrienes: a novel interaction with glutathione. Neuroscience 84:7–10, 1998.
98. Gridley KE, Green PS, Simpkins JW. Low concentrations of estradiol reduce beta-amyloid (25–35)–induced toxicity, lipid peroxidation and glucose utilization in human SK-N-SH neuroblastoma cells. Brain Res 778:158–165, 1997.
99. Dykens JA, Simpkins JW, Wang J, Gordon K. Polycyclic phenols, estrogens and neuroprotection: a proposed mitochondrial mechanism. Exp Gerontol 38:101–107, 2003.
100. Prokai L, Prokai-Tatrai K, Perjesi P, Zharikova AD, Simpkins JW. Quinol-based metabolic cycle for estrogens in rat liver microsomes. Drug Metab Dispos 31:701–704, 2003.
101. Prokai L, Prokai-Tatrai K, Perjesi P, Zharikova AD, Perez EJ, Liu R, Simpkins JW. Quinol-based cyclic antioxidant mechanism in estrogen neuroprotection. Proc Natl Acad Sci U S A 100:11741–11746, 2003.
102. Denisov ET, Denisova TG. Handbook of antioxidants: bond dissociation energies, rate constants, activation energies, and enthalpies of reactions. Boca Raton: CRC Press, p289, 2000.
103. Nilsen J, Chen S, Brinton RD. Dual action of estrogen on glutamate-induced calcium signaling: mechanisms requiring interaction between estrogen receptors and src/mitogen activated protein kinase pathway. Brain Res 930:216–234, 2002.
104. Morin C, Zini R, Simon N, Tillement JP. Dehydroepiandrosterone and alpha-estradiol limit the functional alterations of rat brain mitochondria submitted to different experimental stresses. Neuroscience 115: 415–424, 2002.
105. Foy MR, Xu J, Xie X, Brinton RD, Thompson RF, Berger TW. 17β-Estradiol enhances NMDA receptor–mediated EPSPs and long-term potentiation. J Neurophysiol 81:925–929, 1999.
106. Brinton RD. Cellular and molecular mechanisms of estrogen regulation of memory function and neuroprotection against Alzheimer's disease: recent insights and remaining challenges. Learn Mem 8:121–133, 2001.
107. Dykens JA. Mitochondrial radical production and mechanisms of oxidative excitotoxicity. In: Davies KJA, Ursini F, eds. The Oxygen Paradox. Padova: Cleup Press, University of Padova, pp453–467, 1995.
108. Murphy AN. Ca(2+)-mediated mitochondrial dysfunction and the protective effects of Bcl-2. Ann N Y Acad Sci 893:19–32, 1999.
109. Dykens JA, Stout AK. Assessment of mitochondrial membrane potential in situ using single potentiometric dyes and a novel fluorescence resonance energy transfer technique. Methods Cell Biol 65:285–309, 2001.
110. Paganini-Hill A. Estrogen replacement therapy and stroke. Prog Cardiovasc Dis 38:223–242, 1995.
111. Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, Ernster VL, Cummings SR. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 117:1016–1037, 1992.
112. Zandi PP, Carlson MC, Plassman BL, Welsh-Bohmer KA, Mayer LS, Steffens DC, Breitner JC. Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. JAMA 288:2123–2129, 2002.
113. Henderson V, Paganini-Hill A, Emanuel C, Dunn M, Buckwalter J. Estrogen replacement therapy in older women: comparisons between Alzheimer's disease cases and nondemented control subjects. Arch Neurol 51:896–900, 1994.
114. Harding JJ. Estrogens and cataract. Arch Ophthalmol 112:1511, 1994.
115. Fillit H, Weinreb H, Cholst I, Luine V, McEwen B, Amador R, Zabriskie J. Observations in a preliminary open trial of estradiol therapy for senile dementia-Alzheimer's type. Psychoneuroendocrinology 11:337–345, 1986.
116. Ohkura T, Isse K, Akazawa K, Hamamoto M, Yaoi Y, Hagino N. Evaluation of estrogen treatment in female patients with dementia of the Alzheimer type. Endocr J 41:361–371, 1994.
117. Kawas C, Resnick S, Morrison A, Brookmeyer R, Corrada M, Zonderman A, Bacal C, Lingle DD, Metter E. A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging [published erratum appears in Neurology 51:654, 1998]. Neurology 48:1517–1521, 1997.
118. Paganini-Hill A, Henderson V. Estrogen deficiency and risk of Alzheimer's disease in women. Am J Epidemiol 140:256–261, 1994.
119. Paganini-Hill A, Henderson VW. Estrogen replacement therapy and risk of Alzheimer disease. Arch Intern Med 156:2213–2217, 1996.
120. Tang MX, Jacobs D, Stern Y, Marder K, Schofield P, Gurland B, Andrews H, Mayeux R. Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. Lancet 348:429–432, 1996.
121. Schneider LS, Farlow MR, Henderson VW, Pogoda JM. Effects of estrogen replacement therapy on response to tacrine in patients with Alzheimer's disease. Neurology 46:1580–1584, 1996.
122. Shumaker SA, Legault C, Kuller L, Rapp SR, Thal L, Lane DS, Fillit H, Stefanick ML, Hendrix SL, Lewis CE, Masaki K, Coker LH. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. JAMA 291:2947–2958, 2004.
123. Espeland MA, Rapp SR, Shumaker SA, Brunner R, Manson JE, Sherwin BB, Hsia J, Margolis KL, Hogan PE, Wallace R, Dailey M, Freeman R, Hays J. Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. JAMA 291:2959–2968, 2004.
124. Wassertheil-Smoller S, Hendrix SL, Limacher M, Heiss G, Kooperberg C, Baird A, Kotchen T, Curb JD, Black H, Rossouw JE, Aragaki A, Safford M, Stein E, Laowattana S, Mysiw WJ. Effect of estrogen

plus progestin on stroke in postmenopausal women. The Women's Health Initiative: a randomized trial. JAMA 289:2673–2684, 2003.

125. Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, Hendrix SL, Jones BN 3rd, Assaf AR, Jackson RD, Kotchen JM, Wassertheil-Smoller S, Wactawski-Wende J. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA 289:2651–2662, 2003.
126. Rapp SR, Espeland MA, Shumaker SA, Henderson VW, Brunner RL, Manson JE, Gass ML, Stefanick ML, Lane DS, Hays J, Johnson KC, Coker LH, Dailey M, Bowen D. Effect of estrogen plus progestin on global cognitive function in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA 289:2663–2672, 2003.
127. Singh M, Simpkins JW. The Future of Hormone Therapy: What basic science and clinical studies teach us. New York: New York Academy of Science, p261, 2005.
128. Simpkins JW, Singh M. Consortium for the Assessment of Research on Progestins and Estrogens (CARPE) Fort Worth, Texas August 1–3, 2003. J Womens Health (Larchmt) 13:1165–1168, 2004.
129. Alkjaersig N, Fletcher AP, de Ziegler D, Steingold KA, Meldrum DR, Judd HL. Blood coagulation in postmenopausal women given estrogen treatment: comparison of transdermal and oral administration. J Lab Clin Med 111:224–228, 1988.
130. De Lignieres B, Basdevant A, Thomas G, Thalabard JC, Mercier-Bodard C, Conard J, Guyene TT, Mairon N, Corvol P, Guy-Grand B. Biological effects of estradiol-17 beta in postmenopausal women: oral versus percutaneous administration. J Clin Endocrinol Metab 62:536–541, 1986.
131. Harman SM, Naftolin F, Brinton EA, Judelson DR. Is the estrogen controversy over? Deconstructing the Women's Health Initiative Study: a critical evaluation of the evidence. Ann N Y Acad Sci 1052: 43–56, 2005.
132. Perez E, Liu R, Yang SH, Cai ZY, Covey DF, Simpkins JW. Neuroprotective effects of an estratriene analog are estrogen receptor independent in vitro and in vivo. Brain Res 1038:216–222, 2005.
133. Gleason CE, Carlsson CM, Johnson S, Atwood C, Asthana S. Clinical pharmacology and differential cognitive efficacy of estrogen preparations. Ann N Y Acad Sci 1052:93–115, 2005.