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Mitochondrial Mechanisms of Estrogen Neuroprotection

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Abstract

Mitochondria have become a primary focus in our search not only for the mechanism(s) of neuronal death but also for neuroprotective drugs and therapies that can delay or prevent Alzheimer's disease and other chronic neurodegenerative conditions. This is because mitochrondria play a central role in regulating viability and death of neurons, and mitochondrial dysfunction has been shown to contribute to neuronal death seen in neurodegenerative diseases. In this article, we review the evidence for the role of mitochondria in cell death and neurodegeneration and provide evidence that estrogens have multiple effects on mitochondria that enhance or preserve mitochondrial function during pathologic circumstances such as excitotoxicity, oxidative stress, and others. As such, estrogens and novel non-hormonal analogs have come to figure prominently in our efforts to protect neurons against both acute brain injury and chronic neurodegeneration.

Keywords

estrogens; estradiol; non-feminizing estrogens mitochondria; neuroprotection; estrogen receptors; Alzheimer's disease

1. Mitochondrial and cell death mechanisms

Mitochondrial oxidative phosphorylation is essential for neurons to meet their high ATP demand, and neuronal viability is imperiled when this ATP production is even transiently diminished. In addition to a bioenergetic crisis, mitochondrial impairment also produces a concomitant increase in production of reactive oxygen species [1]. Mitochondrial failure is the key event in the pathogenic cascade leading to ischemia-induced cell death from both necrosis and apoptosis [1,2]. Under conditions of oxidative stress and excessive cytoplasmic Ca^{2+} loading, mitochondria undergo a loss of the impermeability of the inner mitochondrial membrane that completely collapses the mitochondrial membrane potential ($\Delta\Psi m$), a process called permeability transition. Such irreversible collapse of $\Delta\Psi m$ is accompanied by mitochondrial swelling and release of cytochrome c into the cytoplasm, where it activates certain caspases and induces apoptotic cell death [2,3].

Normally, antioxidant defense systems reduce radical-induced damage by scavenging free radicals. However, accelerated mitochondrial radical production can overwhelm these

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defenses, inducing peroxidation of membrane lipids and impeding oxidative phosphorylation via inactivation of Fe–S clusters within the electron transport system. The resulting acute loss of ATP causes the transmembrane ion-dependent ATPases to fail, thereby inducing necrosis from osmotic collapse [4,5]. In addition, less severe mitochondrial dysfunction initiates apoptosis in response to a variety of stressors. The excitotoxic process, for example, entails excessive stimulation of excitatory receptors, including NMDA and other voltage and metabotropic receptors, by exposure to elevated levels of dicarboxylate neurotransmitters, like glutamate, as well as several xentobiotics, like kainic acid. Excessive elevation of Ca^{2+} during glutamate excitotoxicity undermines mitochondrial integrity and accelerates free radical production, ultimately leading to cell death [6,7]. As a result, mitochondria are seen as key modulators of neuronal viability during excitotoxicity [4,5].

2. Mitochondria and Chronic Neurodegenerative Diseases

Chronic diseases with bioenergetic and oxidative etiologies that implicate mitochondrial dysfunction include Alzheimer's disease (AD) and Parkinson's disease (PD), as well as amyotrophic lateral sclerosis (ALS), Huntington's disease and Freidreich's ataxia, among many others [8]. Although the etiology of Alzheimer's disease is multifactoral [9], a consistent finding is hypometabolism of glucose in those brain regions affected by the disease [10] that can be detected very early in the disease [11,12]. Although hypometabolism could simply reflect neuronal loss in the effected regions, mitochondrial dysfunction can be more directly implicated in AD. Amyloid-β (Aβ) causes an oxidative and bioenergetic crises [13–15], and in turn enhances the production of Aβ [16]. In addition, other aspects of normal mitochondrial physiology, such as intracellular distribution, could participate in AD progression secondary to the mitochondrial failure. For example, peri-nuclear mitochondria are more actively replicating than those elsewhere in the neuron, and are then distributed to the energetically demanding synapses. Impairment of normal axonal transport of mitochondria is likely due to the breakdown of microtubules from the hyperphosphorylation of the microtubule-associated protein, tau [17,18].

Mitochondria from AD subjects are hypofunctional [19], produce excessive reactive oxygen species [20,21], and show a defect in respiratory complex IV (C-IV) [22,23]. When inserted into transformed cells depleted of their endogenous mtDNA, mtDNA from AD patients produces a phenotype in the resulting cytoplasmic hybrids (cybrids) of increased oxidative stress, propensity towards apoptosis and C-IV impairment [24,25], suggesting that some of the cellular pathology in AD reflects mitochondrial defects. While mitochondrial impairment can be a consequence as well as a primary cause of the disease [26], mitochondrial dysfunction is clearly involved in progressive neuronal death in AD and as such represents a viable therapeutic target.

3. Estrogens and Neuroproptection

The neuroprotective effects of estrogens have been widely described against a variety of toxicities including serum deprivation [27,28], Aβ toxicity [29,30] and oxidative stress [31–34], among others, in hippocampal, amygdala, cortical and mesencephalic neurons [35–37]. Similarly, in rodent animal models, estrogens have been shown to attenuate neuronal loss following induction of cerebral ischemia [38–41], following kainic acid administration [42], and in a contusion physical injury model [43].

Retrospective epidemiological studies in post-menopausal women indicate that hormone/estrogen replacement therapy is associated with a reduction in the risk of Alzheimer's disease as well as a delay its the progression[44–47]. In addition, several clinical studies have reported improvement of cognitive functions in female AD patients receiving estrogen replacement

therapy [48–54]. The positive and protective role of estrogens in female AD patients may be in part due to the effects on mitochondrial function and/or stability.

Neuroprotection can be achieved by non-genomic mechanisms independent of interactions with the classical estrogen receptors (ERs). In many of the studies cited above using diverse cell types and cytotoxic insults, naturally occurring 17β-estradiol (17β-E2) and its diastereomer, 17α-estradiol (17α-E2), which is almost inactive as a hormone, have been found to be equally neuroprotective. Similarly, studies with a variety of novel estrogen analogues have confirmed that the structure-activity relationship for neuroprotection with this class of compounds differs significantly from the structural requirements for ER-dependent gene transcription [55–59]. For example, the complete enantiomer of 17β-E2 (ent-17β-E2) has identical physiochemical properties as 17β-E2 except for interactions with other stereospecific molecules such as ERs. Ent-17β-E2 is reported to interact only weakly with uterine-derived ERs [60,61] and lacks estrogenic effects on reproductive tissues in rodents [62,63]. In fact, some reports indicate that ent-17β-E2 exerts slight anti-uterotrophic activity and can antagonize the uterotrophic effects of 17β-E2 [63,64]. We have reported that ent-17β-E2 exerts neuroprotective effects both *in vitro* and *in vivo* in the absence of stimulation of other estrogen-responsive tissues [57]. Novel estrogen analogues such as ZYC3, 2-(1-adamantyl)-3-hydroxyestra-1,3,5(10)-trien-17-one, do not bind to estrogen receptors [59], yet possess both neuroprotective and vasoactive effects in an *in vivo* model of ischemic stroke, which offers the possibility of clinical application for stroke without the estrogen receptor mediated side effects of estrogens [65].

We defined the essential requirement for neuroprotection to be the A ring of estrogens [56] and have been able to enhance neuroprotective activity via chemical modification of the estrogen scaffold [59]. For example, increasing the stability of the A-ring phenolic radical yields an increased neuroprotective potency [59]. Other approaches to augment potency includes placement of constituents on the 2 and/or 4 position of the phenolic A ring of estradiol or estrone, unstaturation of the B or C rings of the planar steroid, synthesizing diastiomers or enantiomers of estrogens, and combinations of these approaches [59].

4. Estrogens and Mitochondrial Function

Therapeutic agents that are able to stabilize mitochondrial function during ischemia are expected to be effective neuroprotectants, preventing apoptosis by maintaining functionally intact mitochondria. A major challenge then in understanding the neuroprotective effects of estrogens and their potential for application in acute brain damage and more chronic neurodegenerative disease is determining their mitochondrial mechanism(s) of action, and the four possibilities discussed below are under active investigation.

4.1. Historic Observations

Several studies have shown that estrogens and related compounds may exert direct or indirect effects on mitochondrial function. It has been reported that 17β-E2 inhibits mitochondrial F0F1-ATPase by binding to one of its subunits, the oligomycin sensitivity conferring protein [66,67]. A testable model for cytoprotective activity is that such inhibition impedes the reverse reactions of the ATPase during ischemia, using ATP to generate a membrane potential, thereby preserving cytosolic ATP arising from glycolysis. Other investigators have found that estradiol augments sequestration of cytosolic Ca^{2+} by mitochondria in the presence of glutamate which, given the diffusion limited kinetics of the Ca^{2+} uniporter, is *prima facie* evidence of mitochondrial stabilization [68]. Moderating Ca^{2+}-induced permeability transition during excitoxicity, and in so doing maintaining OXPHOS, would clearly be beneficial. In another study, 17β-E2 has been shown to stabilize mitochondrial function and thereby protect neural cells against the pro-apoptotic action of mutant presenilin-1 [69]. Anti-apoptotic action may

also involve receptor mediated transactivation such as the upregulation of Bcl-2, known to occur upon estradiol exposure.

To further illuminate the protective effects of estrogens and other polycyclic phenols on mitochondrial integrity, we examined a number of markers of mitochondrial activity in SK-N-SH neuroblastoma cells exposed to 3-nitroproprionic acid (3-NPA). The latter inhibits oxidative phosphorylation by blocking electron entry into electron transport at the level of succinate dehydrogenase (respiratory Complex II), and so serves to model not only acute impaired energy production, seen during cerebral ischemia [38,70,71], but also more modest energy impairment associated with chronic neurodegenerative disease [72]. We reported that 17β-E2 prevents depletion of ATP, preserves $\Delta\Psi m$, and inhibits production of reactive oxygen species [1] caused by 3-NPA [73]. Further, we made similar observations of the mitochondrial effects of estrogens when H_2O_2 was used to impair mitochondrial function [74].

Estrogens have been shown to protect neurons against various other mitochondrial toxins such as MPTP, an inhibitor of respiratory complex I [75–79]. Estrogens increase the expression of longevity-associated genes, including those encoding the antioxidant enzymes superoxide dismutase and glutathione peroxidase. As a result, mitochondria from females produce fewer reactive oxygen species [1] than those from males, an effect that may contribute to increased female longevity [80–82].

4.2. Nuclear Transcriptional Effects

Estrogens regulate both nuclear and mitochondrial protein expression, although this mechanism does not pertain to estrogen analogs that do not interact with ERs. For example, estrogens increase expression of glucose transporter subunits thereby promoting glucose transport into neurons [83,84], and also increase expression of glycolytic enzymes including hexokinase, phosphofructokinase, phosphoglycerate kinase [85], and components of pyruvate dehydrogenase complex [86]. Moreover, estrogens also regulate enzymes involved in TCA cycle, such as aconitase 2 and malate dehydrogenase [86]. Estrogens enhance glycolytic activity that is coupled with an increase in glutamate turnover, evidenced by the increased expression of glutamate dehydrogenase and glutamate oxaloacetate transaminase-2 [86]. The latter impacts the generation of neurotoxic free ammonium and reduces excitotoxic free glutamate [87]. Consistent with their actions on glycolytic metabolism, estrogens also enhance the expression of F1 subunits of ATP synthase [86]. *In toto* these findings indicate that estrogens promote glucose utilization via regulation of nuclear encoded genes.

In addition, estrogens also directly regulate the expression of mitochondrial stress responsive factors encoded by nuclear DNA. The Bcl-2 family of proteins is important regulators of the mitochondrial pathway of apoptosis, determining whether the mitochondria initiate the cell death program by releasing proapoptotic factors such as cytochrome c. The family of Bcl-2 proteins consists of anti- and pro-apoptotic members and their interactions play a key role in regulating apoptosis in cells [88]. Estrogens increase anti-apoptotic proteins, Bcl-2 and Bcl-xL, which prevent permeability transition [68]. In primary neuronal cultures, estrogens upregulate expression of antiapoptotic Bcl-w and downregulate expression of proapoptotic bim in an ER-dependent manner [89].

Estrogens also enhance free radical defenses by increasing the expression and activity of glutaredoxin, gamma-glutamylcysteine synthetase, and MnSOD, all encoded by nuclear genes [88,90–93]. In addition, an increase in peroxiredoxin-V was also found upon estrogen treatment [86]. The action of estrogens on glutaredoxin, gamma-glutamylcysteine synthetase, and MnSOD expression and activation are inhibited by the ER antagonist, ICI182780, suggesting an ER-dependent mechanism.

Recently, it has been demonstrated that estradiol stimulates transcription of nuclear respiratory factor-1 (NRF-1) via binding of E2-ectivated ER to the estrogen response element [94]. In turn, NRF-1 binds to its response elements and increases the transcription of mitochondrial transcription factor A (Tfam) and mitochondrial transcription factor B types 1 and 2 (TFBs), which are imported into mitochondria where mitochondrial DNA (mtDNA) replication and transcription are increased.

4.3. Mitochondrial Transcriptional Effects

Akin to bacterial DNA, mtDNA is an intronless, circular molecule of about 16.6 kb that lacks histones and it encodes thirty-seven genes. Of the thousands of proteins in the mitochondrial proteome, mtDNA encodes thirteen proteins are the essential component of the enzyme complexes of the oxidative phosphorylation system. In mammals, the mitochondrial genome is maternally inherited and unlike the nuclear genome in non-dividing, terminally differentiated cells, mtDNA is continuously replicated during mitochondrial reproduction [95]. The strands of the DNA duplex can be distinguished as heavy (H) and light (L) strand based upon the G +T base composition which results in different buoyant densities of each stand. Most information is encoded on the H strand, with genes for two tRNAs, 14tRNAs, and 12 proteins. The L strand codes the other eight tRNAs and a single protein.

Many of the electron transport chain components encoded by mitochondrial genome are regulated by estrogens. Van Itallie and Dannies [96] found a 16-fold increase of cytochrome c oxidase subunit II mRNA upon 17β-E2 treatment in rat pituitary tumor cells. An estrogen-induced increase of cytochrome c oxidase subunit III transcript was also observed [97]. The estrogen-regulated mitochondrial encoded transcripts have been extended to all three subunits of the complex IV and subunits 6 and 8 of ATP synthase [98,99]. More recently, Nilsen et al. [86] identified 4 of the 7 subunits of complex I encoded by mitochondrial genome were regulated by 17β-E2. Given the single promoter for each strand of mtDNA and the broad range of estrogen-regulated mitochondrial transcripts, the action of estrogens on mitochondrial transcription seems universal, not specific to any single gene.

Although it is not clear how estrogens regulate mitochondrial gene expression, studies have shown that enhancement of mitochondrial transcripts by estrogens can be blocked by ER antagonist, ICI182780, suggesting an ER-dependent mechanism [100,101]. This notion is further supported by the newly-identified mitochondrial localization of ERs, especially ERβ [100,102], and up-regulation of mitochondrial complex IV by the ERβ selective ligand, diarylpropionitrile (DPN), has been demonstrated [103]. The crystal structure of ERβ has been well described and it shares a highly conserved architecture with other nuclear receptors such as ERα. Although ERα and ERβ have nearly identical DNA-binding domain, increasing evidence indicates that they regulate the expression of a distinct set of genes [104,105]. This distinction could be partly due to different compartmentalization of ERα and ERβ or recruitment of different coactivators and adaptor proteins. Most studies have been focused on the nuclear transcription regulation. However, extranuclear localization of both ER α and ERβ has been indicated [106–108]. In fact, increasing evidence has demonstrated that ERβ is mainly localized extranuclearly [100,102,106,108–110]. Consistently, most of the genes modified in ERβ knock-out mice are mitochondrial structural proteins related to oxidative phosphorylation [105]. More direct evidence has been shown with ERβ knockdown in a murine hippocampal cell line through RNA interference. This study demonstrated a phenotype change in the mitochondrial functions, which included a substantial reduction in cellular vulnerability to oxidative insults due to a more stable $\Delta\Psi m$ [109].

4.4. Signaling

Estrogens can also regulate mitochondrial function indirectly through various signaling pathways. Accumulating evidence has shown that neuroprotective effects of 17β-estradiol involve transient activation of intracellular signaling pathways *via* G proteins [111,112], extracellularly regulated kinases (phosphoinositol 3-kinase and protein kinase B/AKT, ERK and p38 MAP kinases) [113–115], phosphorylation of the cAMP response element-binding protein [116–119], and alterations in intracellular calcium levels [120–123]. Changes in the activity of these enzymes can regulate the phosphorylation of numerous intermediary signaling proteins such as Rsk, p38 and JNK, and nuclear transcriptional factors, cyclic AMP response element binding protein (CREB) and cfos/cjun, which may ultimately mediate cell survival changes (for review see [37]).

However, persistent activation of multiple kinases sends a death signal. It has been observed that both glutamate and okadaic acid (OA) [124,125] persistently activate JNK, p38 MAPK and ERK1/2 pathways. Activation of the different MAPK pathways occurs simultaneously, and there is crosstalk between ERK1/2 and p38 that is mediated by various protein phosphatases [126,127]. Moreover, it has been demonstrated that PKC phosphorylates both ERK1/2 and p38 MAPK [128–130] providing a link between PKC and MAPK signaling. In the presence of functioning protein phosphatases, estrogens, via maintenance of protein phosphatase activity [131,132], or specific inhibitors of ERK1/2 or PKC [133] prevent glutamate death signaling. However, in the face of broad protein phosphatase inhibition by OA, profound activation of multiple death-inducing kinases cannot be overcome with either estrogens or specific kinase inhibitors. 17β-Estradiol has been shown to block the persistent activation of both ERK and PKC in a variety of insults including ischemia and ethanol withdraw induced cytotoxicity [113,115,134–137].

The influence of these signaling effects of estrogens on mitochondrial function is only now being clarified. Phosphorylation events on the outer membrane of the mitochondria are well defined. Bcl-2 can be modulated by dimerization with proapoptotic family members (i.e. Bax, BAD, Bid) and by phosphorylation. The dynamic phosphorylation and dephosphorylation of Bcl-2 causes conformational changes within the protein and has been suggested to serve as a survival sensor during stressful or cytotoxic conditions [138]. Bcl-2 phosphorylation by a variety of kinases, such as PKC, ERK, Akt, PI-3 K, and others, is a cell survival signal; while dephosphorylation by PP2A and/or PP1 is associated with cell death [139]. BAD, a BH3-proapoptotic Bcl-2 family member, acts at a key nodal point in the mitochondrial apoptotic pathway in that unphosphorylated BAD binds and inactivates antiapoptotic Bcl-2 homologues. This elicits release of cytochrome c from mitochondria and consequent caspase activation cascades. PP1, PP2A, and calcineurin have been shown to dephosphorylate and activate BAD [140] and BAX [141–143]. In addition, upon dephosphorylation by PP1 and/or PP2A, BAX is thought to insert into the outer mitochondrial membrane which disrupts membrane stability and release cytochrome c.

It is also becoming clear that there is mitochondrial trafficking of an increasing number of nuclear receptors and transcription factors including but not limited to ER α, ERβ, glucocorticoid receptor, PPAR γ2, AP1, CREB, NF-κB, p53, TFAM, TFB1M, and TFB2M (for review see [144]) that have direct transcriptional effects on mitochondrial encoded proteins. For example, CREB has been shown to be present in the matrix or inner membrane of the mitochondria [145]. In addition, upon phosphorylation of external CREB by PKA, pCREB is transported into the mitochondria by TOM proteins and induces the synthesis of mitochondrial encoded subunits of oxidative phosphorylation [146]. Since estrogens have shown to mediate the phosphorylation of various signaling molecules including CREB, it is possible that estrogens may be modulating mitochondrial function and/or transcription via the singling molecules discussed in this section. Our studies indicate ERβ, along with Tom20 and/

or Hsp90 are localized to the mitochondria [147]. Receptor preference may depend on ligand induced conformational change in the receptor, and studies show that estrogen increases mitochondria localization of ERβ [100]. The dynamic state of ERβ with and without ligand may facilitate its trafficking based on hydrophobic interactions, which could be mitigated by oxidative stress.

4.5. Direct Antioxidant Effects

Estrogens have long been recognized as antioxidants in a variety of *in vivo* and *in vitro* models. This is important as many neurodegenerative disorders and brain injuries involve oxidative stress, in part because of the abundance of polyunsaturated fatty acids in neuronal membranes which increases susceptibility to peroxidative damage. Estrogens have only weak radical scavenging activity [148–150], but are able to inhibit oxidative stress markers such as lipid peroxidation [59,151–154], protein oxidation [155], and DNA damage [31,156–158]. In cell-free systems, estrogens inhibit iron-induced lipid peroxidation [159], LDL oxidation, cholesterol oxidation, and conjugated diene formation [160–169]. Given the modest activity of estrogens as mass action radical scavengers, these potent antioxidant activities are likely due to a novel redox cycling mechanism (see below). We [28] and others [55,58] have shown that estrogens, with higher capacity to donate a hydrogen radical from the phenolic hydroxyl group on the steroid A ring, are more potent neuroprotectants, underscoring the oxidative etiology of neuronal death.

Recently, we have identified additional structural modifications that improve neuroprotective potency while eliminating or reducing estrogen receptor binding [59]. For example, neuroprotection is enhanced by as much as 200-fold through addition of ba ulky hydrophobic moiety at the C2 or C4 position of the phenolic A ring. In the same screening, we were also able to establish a correlation between neuroprotective potency and inhibition of iron-induced lipid peroxidation [59]. The lipophilicity of estrogens leads to their accumulation in the hydrophobic plasma membranes and affects membrane fluid [170–173]. With a log*P* of 4.008, E2 is localized to the lipid environment of membranes, placing them at the site of key peroxidation events thereby forestalling oxidative damage. By preserving membrane integrity, which is key for OXPHOS, these molecules preserve mitochondrial function, and so moderate the injurious bioenergetic and oxidative sequales of mitochondrial failure. The lipid membrane is also the site of various signal transduction processes including PI3K/Akt signaling and phosphotidylserine flipping in apoptosis.

Oxidative stress is also a key player in normal physiological conditions. The redox state of the cell is a primary determinant for cell survival, and influences parameters such as the ratio of reduced and oxidized glutathione, oxidative state of proteins, and differentiation status. Previous work on neuroprotection has shown a synergistic interaction between E2 and glutathione (GSH) [28,174], as well as between the E2 phenoxy radical (E2O•) with other antioxidants such as α-tocopherol in vitro [175]. Estradiol has also been reported to elicit significant increases in GSH levels in HT-22, primary hippocampal, and primary neocortial cells [176]. Quinol derivitives of estradiol capable of redox cycling have also been shown to be neuroprotective [177].

Estrogen's role in maintaining the redox (homeostasis) state of cell has implications on many cellular processes. However, estrogens may also interact with proteins with sulfhydryl-containing (redox sensitive) cysteine groups. Oxidative modifications to key proteins, such as NMDA receptors [178,179], ATPase[[180,181], ryanodine receptors [181], , Keap1 [182], GAPDH [183] and tau [184] is a response to cell stress and in some cases alters signaling pathways. Many signaling pathways are redox sensitive and include AP-1, CREB, Erk, HIF-1, NFκB, JNK1/SAPK, PKB, PKC, ARE (Keap) [185], to name a few (for review, see [186]).

Interestingly, estrogens are known to affect many of these same pathways, including MAPK [137], NFκB [187], CREB [188], PKC [189], ARE [190], and PKB/Akt [191].

Thiol redox state has wide implications throughout the cell, including the mitochondria [192, 193]. Gender difference were noted above, and isolated mitochondria from female rats produced less peroxides and mitochondrial DNA damage compared to males and ovariectomized animals no doubt because the former contained higher levels of reduced GSH, plus greater activities of Mn-superoxide dismutase (MnSOD), and GSH peroxidase [194]. Diethylstilbestrol [195] and E2 [196] have been shown to increase manganese SOD activity, and E2 rreplacement in the ovariectomized rats restored mitochondrial function to levels comparable to intact female animals [186]. Aside from these classic antioxidant enzymes, E2 has also been shown to induce protein thiol/disulfide oxidoreductases, which include protein disulfide isomerase (PDI), thioredoxin, and glutaredoxin [78,197], and thereby indirectly affect thiol-dependent regeneration systems. In rat bone marrow, ovariectomy causes a decrease in total and reduced glutathione, and declines in activities of GSH reductase and thioredoxin reductase, which were normalized by E2 administration [198].

5. Conclusions

Estrogens have both direct and indirect beneficial effects on mitochondria that serve to preserve function under pathogenic circumstances. Development of non-hormonal analogs allows separation of cytoprotection via transactivation from pharmacological activities. As such, estrogens and the novel analogs are playing a central role in our effort to protect neurons against acute brain injury and chronic neurodegeneration. While the mechanism(s) of the beneficial effects of estrogens are likely multifaceted, the cytoprotective data reviewed here encourage the notion that targeting mitochondrial instability should be considered as a strategy to delay or prevent Alzheimer's disease and other chronic neurodegenerative conditions.

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References

1. Lemasters JJ, Qian T, Bradham CA, Brenner DA, Cascio WE, Trost LC, Nishimura Y, Nieminen AL, Herman B. Mitochondrial dysfunction in the pathogenesis of necrotic and apoptotic cell death. J Bioenerg Biomembr 1999;31:305–319. [PubMed: 10665521]
2. Kroemer G, Reed JC. Mitochondrial control of cell death. Nat Med 2000;6:513–519. [PubMed: 10802706]
3. Murphy AN, Fiskum G, Beal MF. Mitochondria in neurodegeneration: bioenergetic function in cell life and death. J Cereb Blood Flow Metab 1999;19:231–245. [PubMed: 10078875]
4. Dykens, JA. Mitochondrial and free radical production and the etiology of neurogenerative disease. In: Beal, MF.; Bodis-Wollner, I.; Howell, N., editors. Neurodegenerative Diseases: Mitochondria and Free Radicals in Pathogenesis. Wiley; New York: 1997. p. 29-55.
5. Dykens, JA. Free radicals and mitochondrial dysfunction in excitotoxicity and neurodegenerative diseases. In: Koliatos, VE.; Ratan, VV., editors. Cell Death and Diseases of the Nervous System. Humana Press; New Jersey: 1999. p. 45-68.
6. Dykens JA. Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated CA2+ and Na+: implications for neurodegeneration. J Neurochem 1994;63:584–591. [PubMed: 8035183]
7. Dykens JA, Stern A, Trenkner E. Mechanism of kainate toxicity to cerebellar neurons in vitro is analogous to reperfusion tissue injury. J Neurochem 1987;49:1222–1228. [PubMed: 3040909]

8. Gurney ME, Cutting FB, Zhai P, Andrus PK, Hall ED. Pathogenic mechanisms in familial amyotrophic lateral sclerosis due to mutation of Cu, Zn superoxide dismutase. Pathol Biol (Paris) 1996;44:51–56. [PubMed: 8734301]
9. Prasad KN, Cole WC, Prasad KC. Risk factors for Alzheimer's disease: role of multiple antioxidants, non-steroidal anti-inflammatory and cholinergic agents alone or in combination in prevention and treatment. J Am Coll Nutr 2002;21:506–522. [PubMed: 12480796]
10. 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 2002;23:371–376. [PubMed: 11959398]
11. Blass JP. Cerebrometabolic abnormalities in Alzheimer's disease. Neurol Res 2003;25:556–566. [PubMed: 14503009]
12. Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PL, Jones PK, Petersen RB, Perry G, Smith MA. Mitochondrial abnormalities in Alzheimer's disease. J Neurosci 2001;21:3017–3023. [PubMed: 11312286]
13. Canevari L, Abramov AY, Duchen MR. Toxicity of amyloid beta peptide: tales of calcium, mitochondria, and oxidative stress. Neurochem Res 2004;29:637–650. [PubMed: 15038611]
14. Casley CS, Canevari L, Land JM, Clark JB, Sharpe MA. Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. J Neurochem 2002;80:91–100. [PubMed: 11796747]
15. Muller WE, Kirsch C, Eckert GP. Membrane-disordering effects of beta-amyloid peptides. Biochem Soc Trans 2001;29:617–623. [PubMed: 11498039]
16. Busciglio J, Pelsman A, Wong C, Pigino G, Yuan M, Mori H, Yankner BA. Altered metabolism of the amyloid beta precursor protein is associated with mitochondrial dysfunction in Down's syndrome. Neuron 2002;33:677–688. [PubMed: 11879646]
17. Cash AD, Perry G, Ogawa O, Raina AK, Zhu X, Smith MA. Is Alzheimer's disease a mitochondrial disorder? Neuroscientist 2002;8:489–496. [PubMed: 12374431]
18. Swerdlow RH. Mitochondrial DNA--related mitochondrial dysfunction in neurodegenerative diseases. Arch Pathol Lab Med 2002;126:271–280. [PubMed: 11860299]
19. Cottrell DA, Blakely EL, Johnson MA, Ince PG, Turnbull DM. Mitochondrial enzyme-deficient hippocampal neurons and choroidal cells in AD. Neurology 2001;57:260–264. [PubMed: 11468310]
20. 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 2002;12:21–35. [PubMed: 11770899]
21. Smith MA, Nunomura A, Zhu X, Takeda A, Perry G. Metabolic, metallic, and mitotic sources of oxidative stress in Alzheimer disease. Antioxid Redox Signal 2000;2:413–420. [PubMed: 11229355]
22. Gibson GE, Sheu KF, Blass JP. Abnormalities of mitochondrial enzymes in Alzheimer disease. J Neural Transm 1998;105:855–870. [PubMed: 9869323]
23. Kish SJ. Brain energy metabolizing enzymes in Alzheimer's disease: alpha-ketoglutarate dehydrogenase complex and cytochrome oxidase. Ann N Y Acad Sci 1997;826:218–228. [PubMed: 9329693]
24. 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 1999;893:176–191. [PubMed: 10672237]
25. 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 2000;162:37–50. [PubMed: 10716887]
26. Shoffner JM. Oxidative phosphorylation defects and Alzheimer's disease. Neurogenetics 1997;1:13–19. [PubMed: 10735269]
27. Bishop J, Simpkins JW. Estradiol treatment increases viability of glioma and neuroblastoma cells in vitro. Mol Cell Neurosci 1994;5:303–308. [PubMed: 7804599]
28. Green PS, Bishop J, Simpkins JW. 17 alpha-estradiol exerts neuroprotective effects on SK-N-SH cells. J Neurosci 1997;17:511–515. [PubMed: 8987774]

29. Pike CJ. Estrogen modulates neuronal Bcl-xL expression and beta-amyloid-induced apoptosis: relevance to Alzheimer's disease. J Neurochem 1999;72:1552–1563. [PubMed: 10098861]
30. Green PS, Gridley KE, Simpkins JW. Nuclear estrogen receptor-independent neuroprotection by estratrienes: a novel interaction with glutathione. Neuroscience 1998;84:7–10. [PubMed: 9522357]
31. 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 1995;216:473–482. [PubMed: 7488136]
32. 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 1996;66:1836–1844. [PubMed: 8780008]
33. Sawada H, Ibi M, Kihara T, Urushitani M, Akaike A, Shimohama S. Estradiol protects mesencephalic dopaminergic neurons from oxidative stress-induced neuronal death. J Neurosci Res 1998;54:707–719. [PubMed: 9843162]
34. Sawada M, Alkayed NJ, Goto S, Crain BJ, Traystman RJ, Shaivitz A, Nelson RJ, Hurn PD. Estrogen receptor antagonist ICI182,780 exacerbates ischemic injury in female mouse. J Cereb Blood Flow Metab 2000;20:112–118. [PubMed: 10616799]
35. Garcia-Segura LM, Azcoitia I, DonCarlos LL. Neuroprotection by estradiol. Prog Neurobiol 2001;63:29–60. [PubMed: 11040417]
36. Green PS, Simpkins JW. Neuroprotective effects of estrogens: potential mechanisms of action. Int J Dev Neurosci 2000;18:347–358. [PubMed: 10817919]
37. Lee SJ, McEwen BS. Neurotrophic and neuroprotective actions of estrogens and their therapeutic implications. Annu Rev Pharmacol Toxicol 2001;41:569–591. [PubMed: 11264469]
38. Du C, Hu R, Csernansky CA, Hsu CY, Choi DW. Very delayed infarction after mild focal cerebral ischemia: a role for apoptosis? J Cereb Blood Flow Metab 1996;16:195–201. [PubMed: 8594050]
39. Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, Wise PM. Estradiol protects against ischemic injury. J Cereb Blood Flow Metab 1998;18:1253–1258. [PubMed: 9809515]
40. 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 1997;87:724–730. [PubMed: 9347981]
41. Yang SH, Shi J, Day AL, Simpkins JW. Estradiol exerts neuroprotective effects when administered after ischemic insult. Stroke 2000;31:745–749. discussion 749–750. [PubMed: 10700514]
42. Azcoitia I, Sierra A, Garcia-Segura LM. Estradiol prevents kainic acid-induced neuronal loss in the rat dentate gyrus. Neuroreport 1998;9:3075–3079. [PubMed: 9804319]
43. Nakamizo T, Urushitani M, Inoue R, Shinohara A, Sawada H, Honda K, Kihara T, Akaike A, Shimohama S. Protection of cultured spinal motor neurons by estradiol. Neuroreport 2000;11:3493–3497. [PubMed: 11095506]
44. 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 1996;348:429–432. [PubMed: 8709781]
45. Paganini-Hill A, Henderson VW. Estrogen deficiency and risk of Alzheimer's disease in women. Am J Epidemiol 1994;140:256–261. [PubMed: 8030628]
46. Paganini-Hill A, Ross RK, Henderson BE. Postmenopausal oestrogen treatment and stroke: a prospective study. Bmj 1988;297:519–522. [PubMed: 3139181]
47. Simpkins JW, Singh M, Bishop J. The potential role for estrogen replacement therapy in the treatment of the cognitive decline and neurodegeneration associated with Alzheimer's disease. Neurobiol Aging 1994;15(Suppl 2):S195–197. [PubMed: 7700453]
48. 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 1994;41:361–371. [PubMed: 8528351]
49. Henderson VW, Paganini-Hill A, Emanuel CK, Dunn ME, Buckwalter JG. Estrogen replacement therapy in older women. Comparisons between Alzheimer's disease cases and nondemented control subjects. Arch Neurol 1994;51:896–900. [PubMed: 8080389]
50. Dumas J, Hancur-Bucci C, Naylor M, Sites C, Newhouse P. Estradiol interacts with the cholinergic system to affect verbal memory in postmenopausal women: evidence for the critical period hypothesis. Horm Behav 2008;53:159–169. [PubMed: 17964576]

51. Grodstein F, Manson JE, Stampfer MJ. Hormone therapy and coronary heart disease: the role of time since menopause and age at hormone initiation. J Womens Health (Larchmt) 2006;15:35–44. [PubMed: 16417416]
52. Harman SM. What do hormones have to do with aging? What does aging have to do with hormones? Ann N Y Acad Sci 2004;1019:299–308. [PubMed: 15247033]
53. Hodis HN, Mack WJ, Lobo RA. Randomized controlled trial evidence that estrogen replacement therapy reduces the progression of subclinical atherosclerosis in healthy postmenopausal women without preexisting cardiovascular disease. Circulation 2003;108:e5. author reply e5. [PubMed: 12847059]
54. Maki PM. Potential importance of early initiation of hormone therapy for cognitive benefit. Menopause 2006;13:6–7. [PubMed: 16607091]
55. Behl C, Skutella T, Lezoualc'h F, Post A, Widmann M, Newton CJ, Holsboer F. Neuroprotection against oxidative stress by estrogens: structure-activity relationship. Mol Pharmacol 1997;51:535–541. [PubMed: 9106616]
56. Green PS, Gordon K, Simpkins JW. Phenolic A ring requirement for the neuroprotective effects of steroids. J Steroid Biochem Mol Biol 1997;63:229–235. [PubMed: 9459189]
57. Green PS, Yang SH, Nilsson KR, Kumar AS, Covey DF, Simpkins JW. The nonfeminizing enantiomer of 17beta-estradiol exerts protective effects in neuronal cultures and a rat model of cerebral ischemia. Endocrinology 2001;142:400–406. [PubMed: 11145603]
58. Moosmann B, Behl C. The antioxidant neuroprotective effects of estrogens and phenolic compounds are independent from their estrogenic properties. Proc Natl Acad Sci U S A 1999;96:8867–8872. [PubMed: 10430862]
59. Perez E, Cai Zu Yun, Covey Douglas F, Simpkins James W. Neuroprotective Effects of Estratriene Analogs: Structure-Activity Relationships and Molecular Optimization. Drug Development Research 2005;66:78–92.
60. Chernayaev GA, Barkova TI, Egorova VV, Sorokina IB, Ananchenko SN, Mataradze GD, Sokolova NA, Rozen VB. A series of optical structural and isomeric analogs of estradiol: a comparative study of the biological activity and affinity to cytosol receptor of rabbit uterus. J Steroid Biochem 1975;6:1483–1488. [PubMed: 1052851]
61. Payne DW, Katzenellenbogen JA. Binding specificity of rat alpha-fetoprotein for a series of estrogen derivatives: studies using equilibrium and nonequilibrium binding techniques. Endocrinology 1979;105:745–753. [PubMed: 89027]
62. Terenius L. Differential inhibition in vitro of 17 beta-estradiol binding in the mouse uterus and vagina by optical antipodes of estrogens. Mol Pharmacol 1968;4:301–310. [PubMed: 5663952]
63. Terenius L. The Allen-Doisy test for estrogens reinvestigated. Steroids 1971;17:653–661. [PubMed: 5104534]
64. Edgren RA, Jones RC. An anti-estradiol effect of ent-estradiol-17beta (1-estradiol). Steroids 1969;14:335–341. [PubMed: 5821989]
65. Liu R, Yang SH, Perez E, Yi KD, Wu SS, Eberst K, Prokai L, Prokai-Tatrai K, Cai ZY, Covey DF, Day AL, Simpkins JW. Neuroprotective effects of a novel non-receptor-binding estrogen analogue: in vitro and in vivo analysis. Stroke 2002;33:2485–2491. [PubMed: 12364742]
66. 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 1999;68:65–75. [PubMed: 10215039]
67. 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 1999;368:95–102. [PubMed: 10096774]
68. Nilsen J, Brinton RD. Mitochondria as therapeutic targets of estrogen action in the central nervous system. Curr Drug Targets CNS Neurol Disord 2004;3:297–313. [PubMed: 15379606]
69. Mattson MP, Robinson N, Guo Q. Estrogens stabilize mitochondrial function and protect neural cells against the pro-apoptotic action of mutant presenilin-1. Neuroreport 1997;8:3817–3821. [PubMed: 9427376]
70. Fink SL, Ho DY, Sapolsky RM. Energy and glutamate dependency of 3-Nitropropionic acid neurotoxicity in culture. Exp Neurol 1996;138:298–304. [PubMed: 8620928]

71. Hansford RG. Relation between mitochondrial calcium transport and control of energy metabolism. Rev Physiol Biochem Pharmacol 1985;102:1–72. [PubMed: 2863864]
72. Beal MF. Mitochondrial dysfunction in neurodegenerative diseases. Biochim Biophys Acta 1998;1366:211–223. [PubMed: 9714810]
73. 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 2001;77:804–811. [PubMed: 11331409]
74. Wang X, Dykens JA, Perez E, Liu R, Yang S, Covey DF, Simpkins JW. Neuroprotective effects of 17beta-estradiol and nonfeminizing estrogens against H2O2 toxicity in human neuroblastoma SK-N-SH cells. Mol Pharmacol 2006;70:395–404. [PubMed: 16614138]
75. Disshon KA, Dluzen DE. Estrogen as a neuromodulator of MPTP-induced neurotoxicity: effects upon striatal dopamine release. Brain Res 1997;764:9–16. [PubMed: 9295188]
76. Dluzen DE, McDermott JL, Liu B. Estrogen as a neuroprotectant against MPTP-induced neurotoxicity in C57/B1 mice. Neurotoxicol Teratol 1996;18:603–606. [PubMed: 8888025]
77. Dluzen DE, McDermott JL, Liu B. Estrogen alters MPTP-induced neurotoxicity in female mice: effects on striatal dopamine concentrations and release. J Neurochem 1996;66:658–666. [PubMed: 8592137]
78. Kenchappa RS, Diwakar L, Annepu J, Ravindranath V. Estrogen and neuroprotection: higher constitutive expression of glutaredoxin in female mice offers protection against MPTP-mediated neurodegeneration. FASEB J 2004;18:1102–1104. [PubMed: 15132975]
79. Shughrue PJ. Estrogen attenuates the MPTP-induced loss of dopamine neurons from the mouse SNc despite a lack of estrogen receptors (ERalpha and ERbeta). Exp Neurol 2004;190:468–477. [PubMed: 15530885]
80. Borras C, Gambini J, Gomez-Cabrera MC, Sastre J, Pallardo FV, Mann GE, Vina J. 17beta-oestradiol up-regulates longevity-related, antioxidant enzyme expression via the ERK1 and ERK2[MAPK]/NFkappaB cascade. Aging Cell 2005;4:113–118. [PubMed: 15924567]
81. Vina J, Borras C, Gambini J, Sastre J, Pallardo FV. Why females live longer than males: control of longevity by sex hormones. Sci Aging Knowledge Environ 2005;2005:pe17. [PubMed: 15944465]
82. Vina J, Sastre J, Pallardo F, Borras C. Mitochondrial theory of aging: importance to explain why females live longer than males. Antioxid Redox Signal 2003;5:549–556. [PubMed: 14580309]
83. Shi J, Simpkins JW. 17 beta-Estradiol modulation of glucose transporter 1 expression in blood-brain barrier. Am J Physiol 1997;272:E1016–1022. [PubMed: 9227446]
84. Shi J, Zhang YQ, Simpkins JW. Effects of 17beta-estradiol on glucose transporter 1 expression and endothelial cell survival following focal ischemia in the rats. Exp Brain Res 1997;117:200–206. [PubMed: 9419067]
85. Kostanyan A, Nazaryan K. Rat brain glycolysis regulation by estradiol-17 beta. Biochim Biophys Acta 1992;1133:301–306. [PubMed: 1531302]
86. Nilsen J, Irwin RW, Gallaher TK, Brinton RD. Estradiol in vivo regulation of brain mitochondrial proteome. J Neurosci 2007;27:14069–14077. [PubMed: 18094246]
87. Parihar MS, Brewer GJ. Mitoenergetic failure in Alzheimer disease. Am J Physiol Cell Physiol 2007;292:C8–23. [PubMed: 16807300]
88. Gottipati S, Cammarata PR. Mitochondrial superoxide dismutase activation with 17 beta-estradiol-treated human lens epithelial cells. Mol Vis 2008;14:898–905. [PubMed: 18490963]
89. Yao M, Nguyen TV, Pike CJ. Estrogen regulates Bcl-w and Bim expression: role in protection against beta-amyloid peptide-induced neuronal death. J Neurosci 2007;27:1422–1433. [PubMed: 17287517]
90. Diwakar L, Kenchappa RS, Annepu J, Saeed U, Sujanitha R, Ravindranath V. Down-regulation of glutaredoxin by estrogen receptor antagonist renders female mice susceptible to excitatory amino acid mediated complex I inhibition in CNS. Brain Res 2006;1125:176–184. [PubMed: 17109834]
91. Pedram A, Razandi M, Wallace DC, Levin ER. Functional estrogen receptors in the mitochondria of breast cancer cells. Mol Biol Cell 2006;17:2125–2137. [PubMed: 16495339]
92. Strehlow K, Rotter S, Wassmann S, Adam O, Grohe C, Laufs K, Bohm M, Nickenig G. Modulation of antioxidant enzyme expression and function by estrogen. Circ Res 2003;93:170–177. [PubMed: 12816884]

93. Urata Y, Ihara Y, Murata H, Goto S, Koji T, Yodoi J, Inoue S, Kondo T. 17Beta-estradiol protects against oxidative stress-induced cell death through the glutathione/glutaredoxin-dependent redox regulation of Akt in myocardiac H9c2 cells. J Biol Chem 2006;281:13092–13102. [PubMed: 16549430]

94. Mattingly KA, Ivanova MM, Riggs KA, Wickramasinghe NS, Barch MJ, Klinge CM. Estradiol stimulates transcription of nuclear respiratory factor-1 and increases mitochondrial biogenesis. Mol Endocrinol 2008;22:609–622. [PubMed: 18048642]

95. Falkenberg M, Larsson NG, Gustafsson CM. DNA replication and transcription in mammalian mitochondria. Annu Rev Biochem 2007;76:679–699. [PubMed: 17408359]

96. Van Itallie CM, Dannies PS. Estrogen induces accumulation of the mitochondrial ribonucleic acid for subunit II of cytochrome oxidase in pituitary tumor cells. Mol Endocrinol 1988;2:332–337. [PubMed: 2837664]

97. Bettini E, Maggi A. Estrogen induction of cytochrome c oxidase subunit III in rat hippocampus. J Neurochem 1992;58:1923–1929. [PubMed: 1373180]

98. Chen J, Delannoy M, Odwin S, He P, Trush MA, Yager JD. Enhanced mitochondrial gene transcript, ATP, bcl-2 protein levels, and altered glutathione distribution in ethinyl estradiol-treated cultured female rat hepatocytes. Toxicol Sci 2003;75:271–278. [PubMed: 12857939]

99. Chen J, Gokhale M, Li Y, Trush MA, Yager JD. Enhanced levels of several mitochondrial mRNA transcripts and mitochondrial superoxide production during ethinyl estradiol-induced hepatocarcinogenesis and after estrogen treatment of HepG2 cells. Carcinogenesis 1998;19:2187–2193. [PubMed: 9886577]

100. Chen JQ, Delannoy M, Cooke C, Yager JD. Mitochondrial localization of ERalpha and ERbeta in human MCF7 cells. Am J Physiol Endocrinol Metab 2004;286:E1011–1022. [PubMed: 14736707]

101. Chen JQ, Yager JD. Estrogen's effects on mitochondrial gene expression: mechanisms and potential contributions to estrogen carcinogenesis. Ann N Y Acad Sci 2004;1028:258–272. [PubMed: 15650251]

102. 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 2004;101:4130–4135. [PubMed: 15024130]

103. Hsieh YC, Yu HP, Suzuki T, Choudhry MA, Schwacha MG, Bland KI, Chaudry IH. Upregulation of mitochondrial respiratory complex IV by estrogen receptor-beta is critical for inhibiting mitochondrial apoptotic signaling and restoring cardiac functions following trauma-hemorrhage. J Mol Cell Cardiol 2006;41:511–521. [PubMed: 16859701]

104. Katzenellenbogen BS, Katzenellenbogen JA. Estrogen receptor transcription and transactivation: Estrogen receptor alpha and estrogen receptor beta: regulation by selective estrogen receptor modulators and importance in breast cancer. Breast Cancer Res 2000;2:335–344. [PubMed: 11250726]

105. O'Lone R, Knorr K, Jaffe IZ, Schaffer ME, Martini PG, Karas RH, Bienkowska J, Mendelsohn ME, Hansen U. Estrogen receptors alpha and beta mediate distinct pathways of vascular gene expression, including genes involved in mitochondrial electron transport and generation of reactive oxygen species. Mol Endocrinol 2007;21:1281–1296. [PubMed: 17374850]

106. Herrick SP, Waters EM, Drake CT, McEwen BS, Milner TA. Extranuclear estrogen receptor beta immunoreactivity is on doublecortin-containing cells in the adult and neonatal rat dentate gyrus. Brain Res 2006;1121:46–58. [PubMed: 17026970]

107. McEwen B, Akama K, Alves S, Brake WG, Bulloch K, Lee S, Li C, Yuen G, Milner TA. Tracking the estrogen receptor in neurons: implications for estrogen-induced synapse formation. Proc Natl Acad Sci U S A 2001;98:7093–7100. [PubMed: 11416193]

108. Milner TA, Ayoola K, Drake CT, Herrick SP, Tabori NE, McEwen BS, Warrier S, Alves SE. Ultrastructural localization of estrogen receptor beta immunoreactivity in the rat hippocampal formation. J Comp Neurol 2005;491:81–95. [PubMed: 16127691]

109. Yang SH, Sarkar SN, Liu R, Perez EJ, Wang X, Wen Y, Yan LJ, Simpkins JW. Estrogen receptor beta as a mitochondrial vulnerability factor. J Biol Chem 2009;284:9540–9548. [PubMed: 19189968]

110. Milner TA, Lubbers LS, Alves SE, McEwen BS. Nuclear and extranuclear estrogen binding sites in the rat forebrain and autonomic medullary areas. Endocrinology 2008;149:3306–3312. [PubMed: 18356271]
111. Moss RL, Gu Q. Estrogen: mechanisms for a rapid action in CA1 hippocampal neurons. Steroids 1999;64:14–21. [PubMed: 10323668]
112. Raap DK, DonCarlos L, Garcia F, Muma NA, Wolf WA, Battaglia G, Van de Kar LD. Estrogen desensitizes 5-HT(1A) receptors and reduces levels of G(z), G(i1) and G(i3) proteins in the hypothalamus. Neuropharmacology 2000;39:1823–1832. [PubMed: 10884563]
113. 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 2000;20:1694–1700. [PubMed: 10684871]
114. Toran-Allerand CD, Singh M, Setalo G Jr. Novel mechanisms of estrogen action in the brain: new players in an old story. Front Neuroendocrinol 1999;20:97–121. [PubMed: 10328986]
115. Watters JJ, Campbell JS, Cunningham MJ, Krebs EG, Dorsa DM. Rapid membrane effects of steroids in neuroblastoma cells: effects of estrogen on mitogen activated protein kinase signalling cascade and c-fos immediate early gene transcription. Endocrinology 1997;138:4030–4033. [PubMed: 9275096]
116. Murphy DD, Segal M. Morphological plasticity of dendritic spines in central neurons is mediated by activation of cAMP response element binding protein. Proc Natl Acad Sci U S A 1997;94:1482–1487. [PubMed: 9037079]
117. Watters JJ, Dorsa DM. Transcriptional effects of estrogen on neuronal neurotensin gene expression involve cAMP/protein kinase A-dependent signaling mechanisms. J Neurosci 1998;18:6672–6680. [PubMed: 9712639]
118. Zhou Q, Gedrich RW, Engel DA. Transcriptional repression of the c-fos gene by YY1 is mediated by a direct interaction with ATF/CREB. J Virol 1995;69:4323–4330. [PubMed: 7769693]
119. Zhou Y, Watters JJ, Dorsa DM. Estrogen rapidly induces the phosphorylation of the cAMP response element binding protein in rat brain. Endocrinology 1996;137:2163–2166. [PubMed: 8612562]
120. Beyer C, Raab H. Nongenomic effects of oestrogen: embryonic mouse midbrain neurones respond with a rapid release of calcium from intracellular stores. Eur J Neurosci 1998;10:255–262. [PubMed: 9753134]
121. Improta-Brears T, Whorton AR, Codazzi F, York JD, Meyer T, McDonnell DP. Estrogen-induced activation of mitogen-activated protein kinase requires mobilization of intracellular calcium. Proc Natl Acad Sci U S A 1999;96:4686–4691. [PubMed: 10200323]
122. Pozzo-Miller LD, Inoue T, Murphy DD. Estradiol increases spine density and NMDA-dependent Ca2+ transients in spines of CA1 pyramidal neurons from hippocampal slices. J Neurophysiol 1999;81:1404–1411. [PubMed: 10085365]
123. Sarkar SN, Huang RQ, Logan SM, Yi KD, Dillon GH, Simpkins JW. Estrogens directly potentiate neuronal L-type Ca2+ channels. Proc Natl Acad Sci U S A 2008;105:15148–15153. [PubMed: 18815371]
124. Kraft CA, Efimova T, Eckert RL. Activation of PKCdelta and p38delta MAPK during okadaic acid dependent keratinocyte apoptosis. Arch Dermatol Res 2007;299:71–83. [PubMed: 17256148]
125. Yoon SY, Choi JE, Yoon JH, Huh JW, Kim DH. BACE inhibitor reduces APP-beta-C-terminal fragment accumulation in axonal swellings of okadaic acid-induced neurodegeneration. Neurobiol Dis 2006;22:435–444. [PubMed: 16480887]
126. Rice AB, Ingram JL, Bonner JC. p38 mitogen-activated protein kinase regulates growth factor-induced mitogenesis of rat pulmonary myofibroblasts. Am J Respir Cell Mol Biol 2002;27:759–765. [PubMed: 12444037]
127. Wang Z, Yang H, Tachado SD, Capo-Aponte JE, Bildin VN, Koziel H, Reinach PS. Phosphatase-mediated crosstalk control of ERK and p38 MAPK signaling in corneal epithelial cells. Invest Ophthalmol Vis Sci 2006;47:5267–5275. [PubMed: 17122112]
128. Hu Y, Kang C, Philp RJ, Li B. PKC delta phosphorylates p52ShcA at Ser29 to regulate ERK activation in response to H2O2. Cell Signal 2007;19:410–418. [PubMed: 16963224]

129. Kim JY, Yang MS, Oh CD, Kim KT, Ha MJ, Kang SS, Chun JS. Signalling pathway leading to an activation of mitogen-activated protein kinase by stimulating M3 muscarinic receptor. Biochem J 1999;337(Pt 2):275–280. [PubMed: 9882625]
130. Schonwasser DC, Marais RM, Marshall CJ, Parker PJ. Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. Mol Cell Biol 1998;18:790–798. [PubMed: 9447975]
131. Yi KD, Cai ZY, Covey DF, Simpkins JW. Estrogen receptor-independent neuroprotection via protein phosphatase preservation and attenuation of persistent extracellular signal-regulated kinase 1/2 activation. J Pharmacol Exp Ther 2008;324:1188–1195. [PubMed: 18089844]
132. Yi KD, Chung J, Pang P, Simpkins JW. Role of protein phosphatases in estrogen-mediated neuroprotection. J Neurosci 2005;25:7191–7198. [PubMed: 16079401]
133. Yi KD, Covey DF, Simpkins JW. Mechanism of okadaic acid-induced neuronal death and the effect of estrogens. J Neurochem 2009;108:732–740. [PubMed: 19054278]
134. Bi R, Broutman G, Foy MR, Thompson RF, Baudry M. The tyrosine kinase and mitogen-activated protein kinase pathways mediate multiple effects of estrogen in hippocampus. Proc Natl Acad Sci U S A 2000;97:3602–3607. [PubMed: 10725383]
135. Jung ME, Gatch MB, Simpkins JW. Estrogen neuroprotection against the neurotoxic effects of ethanol withdrawal: potential mechanisms. Exp Biol Med (Maywood) 2005;230:8–22. [PubMed: 15618121]
136. Kuroki Y, Fukushima K, Kanda Y, Mizuno K, Watanabe Y. Putative membrane-bound estrogen receptors possibly stimulate mitogen-activated protein kinase in the rat hippocampus. Eur J Pharmacol 2000;400:205–209. [PubMed: 10988335]
137. Singh M, Setalo G Jr, Guan X, Warren M, Toran-Allerand CD. Estrogen-induced activation of mitogen-activated protein kinase in cerebral cortical explants: convergence of estrogen and neurotrophin signaling pathways. J Neurosci 1999;19:1179–1188. [PubMed: 9952396]
138. Ruvolo PP, Deng X, May WS. Phosphorylation of Bcl2 and regulation of apoptosis. Leukemia 2001;15:515–522. [PubMed: 11368354]
139. Ruvolo PP, Deng X, Ito T, Carr BK, May WS. Ceramide induces Bcl2 dephosphorylation via a mechanism involving mitochondrial PP2A. J Biol Chem 1999;274:20296–20300. [PubMed: 10400650]
140. Wang HG, Pathan N, Ethell IM, Krajewski S, Yamaguchi Y, Shibasaki F, McKeon F, Bobo T, Franke TF, Reed JC. Ca2+-induced apoptosis through calcineurin dephosphorylation of BAD. Science 1999;284:339–343. [PubMed: 10195903]
141. Chatfield K, Eastman A. Inhibitors of protein phosphatases 1 and 2A differentially prevent intrinsic and extrinsic apoptosis pathways. Biochem Biophys Res Commun 2004;323:1313–1320. [PubMed: 15451440]
142. Hsiung SC, Tin A, Tamir H, Franke TF, Liu KP. Inhibition of 5-HT1A receptor-dependent cell survival by cAMP/protein kinase A: role of protein phosphatase 2A and Bax. J Neurosci Res 2008;86:2326–2338. [PubMed: 18459133]
143. Zecchin KG, Seidinger AL, Chiaratti MR, Degasperi GR, Meirelles FV, Castilho RF, Vercesi AE. High Bcl-2/Bax ratio in Walker tumor cells protects mitochondria but does not prevent H2O2-induced apoptosis via calcineurin pathways. J Bioenerg Biomembr 2007;39:186–194. [PubMed: 17431754]
144. Lee J, Sharma S, Kim J, Ferrante RJ, Ryu H. Mitochondrial nuclear receptors and transcription factors: who's minding the cell? J Neurosci Res 2008;86:961–971. [PubMed: 18041090]
145. Schuh RA, Kristian T, Fiskum G. Calcium-dependent dephosphorylation of brain mitochondrial calcium/cAMP response element binding protein (CREB). J Neurochem 2005;92:388–394. [PubMed: 15663486]
146. De Rasmo D, Signorile A, Roca E, Papa S. cAMP response element-binding protein (CREB) is imported into mitochondria and promotes protein synthesis. FEBS J 2009;276:4325–4333. [PubMed: 19614745]
147. Simpkins JW, Yang SH, Sarkar SN, Pearce V. Estrogen actions on mitochondria--physiological and pathological implications. Mol Cell Endocrinol 2008;290:51–59. [PubMed: 18571833]

148. Romer W, Oettel M, Menzenbach B, Droescher P, Schwarz S. Novel estrogens and their radical scavenging effects, iron-chelating, and total antioxidative activities: 17 alpha-substituted analogs of delta 9(11)-dehydro-17 beta-estradiol. Steroids 1997;62:688–694. [PubMed: 9366006]

149. 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 1997;29:345–354. [PubMed: 9527626]

150. Vegeto E, Bonincontro C, Pollio G, Sala A, Viappiani S, Nardi F, Brusadelli A, Viviani B, Ciana P, Maggi A. Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia. J Neurosci 2001;21:1809–1818. [PubMed: 11245665]

151. Bayir H, Marion DW, Puccio AM, Wisniewski SR, Janesko KL, Clark RS, Kochanek PM. Marked gender effect on lipid peroxidation after severe traumatic brain injury in adult patients. J Neurotrauma 2004;21:1–8. [PubMed: 14987460]

152. Jung ME, Rewal M, Perez E, Wen Y, Simpkins JW. Estrogen protects against brain lipid peroxidation in ethanol-withdrawn rats. Pharmacol Biochem Behav 2004;79:573–586. [PubMed: 15582030]

153. Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. Steroids 1994;59:383–388. [PubMed: 7940617]

154. Vedder H, Anthes N, Stumm G, Wurz C, Behl C, Krieg JC. Estrogen hormones reduce lipid peroxidation in cells and tissues of the central nervous system. J Neurochem 1999;72:2531–2538. [PubMed: 10349864]

155. Telci A, Cakatay U, Akhan SE, Bilgin ME, Turfanda A, Sivas A. Postmenopausal hormone replacement therapy use decreases oxidative protein damage. Gynecol Obstet Invest 2002;54:88–93. [PubMed: 12566750]

156. Park OJ. Comparison of estrogen and genistein in their antigenotoxic effects, apoptosis and signal transduction protein expression patterns. Biofactors 2004;21:379–382. [PubMed: 15630230]

157. Sierens J, Hartley JA, Campbell MJ, Leathem AJ, Woodside JV. Effect of phytoestrogen and antioxidant supplementation on oxidative DNA damage assessed using the comet assay. Mutat Res 2001;485:169–176. [PubMed: 11182547]

158. Thibodeau PA, Kachadourian R, Lemay R, Bisson M, Day BJ, Paquette B. In vitro pro- and antioxidant properties of estrogens. J Steroid Biochem Mol Biol 2002;81:227–236. [PubMed: 12163134]

159. Ruiz-Larrea B, Leal A, Martin C, Martinez R, Lacort M. Effects of estrogens on the redox chemistry of iron: a possible mechanism of the antioxidant action of estrogens. Steroids 1995;60:780–783. [PubMed: 8585103]

160. Ayres S, Tang M, Subbiah MT. Estradiol-17beta as an antioxidant: some distinct features when compared with common fat-soluble antioxidants. J Lab Clin Med 1996;128:367–375. [PubMed: 8833885]

161. Berco M, Bhavnani BR. Differential neuroprotective effects of equine estrogens against oxidized low density lipoprotein-induced neuronal cell death. J Soc Gynecol Investig 2001;8:245–254.

162. Bhavnani BR, Cecutti A, Gerulath A, Woolever AC, Berco M. Comparison of the antioxidant effects of equine estrogens, red wine components, vitamin E, and probucol on low-density lipoprotein oxidation in postmenopausal women. Menopause 2001;8:408–419. [PubMed: 11723413]

163. Clemente C, Caruso MG, Berloco P, Notarnicola M, D'Attoma B, Osella AR, Guerra V, Buonsante A, Giannandrea B, Di Leo A. Antioxidant effect of short-term hormonal treatment in postmenopausal women. Maturitas 1999;31:137–142. [PubMed: 10227007]

164. Martin C, Barturen K, Martinez R, Lacort M, Ruiz-Larrea MB. In vitro inhibition by estrogens of the oxidative modifications of human lipoproteins. J Physiol Biochem 1998;54:195–202. [PubMed: 10225411]

165. McManus J, McEneny J, Young IS, Thompson W. The effect of various oestrogens and progestogens on the susceptibility of low density lipoproteins to oxidation in vitro. Maturitas 1996;25:125–131. [PubMed: 8905603]

166. Miller CP, Jirkovsky I, Hayhurst DA, Adelman SJ. In vitro antioxidant effects of estrogens with a hindered 3-OH function on the copper-induced oxidation of low density lipoprotein. Steroids 1996;61:305–308. [PubMed: 8738836]

167. Schwenke DC, Wagner JD, Adams MR. In vitro lipid peroxidation of LDL from postmenopausal cynomolgus macaques treated with female hormones. J Lipid Res 1999;40:235–244. [PubMed: 9925652]
168. Tang M, Abplanalp W, Ayres S, Subbiah MT. Superior and distinct antioxidant effects of selected estrogen metabolites on lipid peroxidation. Metabolism 1996;45:411–414. [PubMed: 8609824]
169. Sack MN, Rader DJ, Cannon RO. 3rd, Oestrogen and inhibition of oxidation of low-density lipoproteins in postmenopausal women. Lancet 1994;343:269–270. [PubMed: 7905101]
170. Dicko A, Morissette M, Ben Ameur S, Pezolet M, Di Paolo T. Effect of estradiol and tamoxifen on brain membranes: investigation by infrared and fluorescence spectroscopy. Brain Res Bull 1999;49:401–405. [PubMed: 10483916]
171. Liang Y, Belford S, Tang F, Prokai L, Simpkins JW, Hughes JA. Membrane fluidity effects of estratrienes. Brain Res Bull 2001;54:661–668. [PubMed: 11403993]
172. Whiting KP, Restall CJ, Brain PF. Steroid hormone-induced effects on membrane fluidity and their potential roles in non-genomic mechanisms. Life Sci 2000;67:743–757. [PubMed: 10968404]
173. Wiseman H. The antioxidant action of a pure antioestrogen: ability to inhibit lipid peroxidation compared to tamoxifen and 17 beta-oestradiol and relevance to its anticancer potential. Biochem Pharmacol 1994;47:493–498. [PubMed: 8117317]
174. Gridley KE, Green PS, Simpkins JW. A novel, synergistic interaction between 17 beta-estradiol and glutathione in the protection of neurons against beta-amyloid 25–35-induced toxicity in vitro. Mol Pharmacol 1998;54:874–880. [PubMed: 9804622]
175. Winterle JS, Mill T, Harris T, Goldbeck RA. Absolute kinetic characterization of 17-beta-estradiol as a radical-scavenging, antioxidant synergist. Arch Biochem Biophys 2001;392:233–244. [PubMed: 11488597]
176. Schmidt AJ, Krieg JC, Vedder H. Differential effects of glucocorticoids and gonadal steroids on glutathione levels in neuronal and glial cell systems. J Neurosci Res 2002;67:544–550. [PubMed: 11835321]
177. 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 2003;100:11741–11746. [PubMed: 14504383]
178. Sucher NJ, Lipton SA. Redox modulatory site of the NMDA receptor-channel complex: regulation by oxidized glutathione. J Neurosci Res 1991;30:582–591. [PubMed: 1666131]
179. Tang LH, Aizenman E. Allosteric modulation of the NMDA receptor by dihydrolipoic and lipoic acid in rat cortical neurons in vitro. Neuron 1993;11:857–863. [PubMed: 8240809]
180. Shivakumar BR, Kolluri SV, Ravindranath V. Glutathione and protein thiol homeostasis in brain during reperfusion after cerebral ischemia. J Pharmacol Exp Ther 1995;274:1167–1173. [PubMed: 7562484]
181. Pessah IN, Kim KH, Feng W. Redox sensing properties of the ryanodine receptor complex. Front Biosci 2002;7:a72–79. [PubMed: 11991848]
182. Zhang DD, Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol Cell Biol 2003;23:8137–8151. [PubMed: 14585973]
183. Brune B, Mohr S. Protein thiol modification of glyceraldehyde-3-phosphate dehydrogenase and caspase-3 by nitric oxide. Curr Protein Pept Sci 2001;2:61–72. [PubMed: 12369901]
184. Sayre LM, Autilio-Gambetti L, Gambetti P. Pathogenesis of experimental giant neurofilamentous axonopathies: a unified hypothesis based on chemical modification of neurofilaments. Brain Res 1985;357:69–83. [PubMed: 2412663]
185. Bloom D, Dhakshinamoorthy S, Jaiswal AK. Site-directed mutagenesis of cysteine to serine in the DNA binding region of Nrf2 decreases its capacity to upregulate antioxidant response element-mediated expression and antioxidant induction of NAD(P)H:quinone oxidoreductase1 gene. Oncogene 2002;21:2191–2200. [PubMed: 11948402]
186. Allen RG, Tresini M. Oxidative stress and gene regulation. Free Radic Biol Med 2000;28:463–499. [PubMed: 10699758]

187. 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 2004;1008:147–154. [PubMed: 15145751]

188. Aronica SM, Kraus WL, Katzenellenbogen BS. Estrogen action via the cAMP signaling pathway: stimulation of adenylate cyclase and cAMP-regulated gene transcription. Proc Natl Acad Sci U S A 1994;91:8517–8521. [PubMed: 8078914]

189. Jung ME, Watson DG, Wen Y, Simpkins JW. Role of protein kinase C in estrogen protection against apoptotic cerebellar cell death in ethanol-withdrawn rats. Alcohol 2003;31:39–48. [PubMed: 14615010]

190. Ansell PJ, Espinosa-Nicholas C, Curran EM, Judy BM, Philips BJ, Hannink M, Lubahn DB. In vitro and in vivo regulation of antioxidant response element-dependent gene expression by estrogens. Endocrinology 2004;145:311–317. [PubMed: 14551226]

191. Singh M. Ovarian hormones elicit phosphorylation of Akt and extracellular-signal regulated kinase in explants of the cerebral cortex. Endocrine 2001;14:407–415. [PubMed: 11444439]

192. Lin TK, Hughes G, Muratovska A, Blaikie FH, Brookes PS, Darley-Usmar V, Smith RA, Murphy MP. Specific modification of mitochondrial protein thiols in response to oxidative stress: a proteomics approach. J Biol Chem 2002;277:17048–17056. [PubMed: 11861642]

193. McStay GP, Clarke SJ, Halestrap AP. Role of critical thiol groups on the matrix surface of the adenine nucleotide translocase in the mechanism of the mitochondrial permeability transition pore. Biochem J 2002;367:541–548. [PubMed: 12149099]

194. Borras C, Sastre J, Garcia-Sala D, Lloret A, Pallardo FV, Vina J. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. Free Radic Biol Med 2003;34:546–552. [PubMed: 12614843]

195. Oberley TD, Schultz JL, Oberley LW. In vitro modulation of antioxidant enzyme levels in normal hamster kidney and estrogen-induced hamster kidney tumor. Free Radic Biol Med 1994;16:741–751. [PubMed: 8070677]

196. Moorthy K, Sharma D, Basir SF, Baquer NZ. Administration of estradiol and progesterone modulate the activities of antioxidant enzyme and aminotransferases in naturally menopausal rats. Exp Gerontol 2005;40:295–302. [PubMed: 15820610]

197. Ejima K, Nanri H, Araki M, Uchida K, Kashimura M, Ikeda M. 17beta-estradiol induces protein thiol/disulfide oxidoreductases and protects cultured bovine aortic endothelial cells from oxidative stress. Eur J Endocrinol 1999;140:608–613. [PubMed: 10366417]

198. Lean JM, Davies JT, Fuller K, Jagger CJ, Kirstein B, Partington GA, Urry ZL, Chambers TJ. A crucial role for thiol antioxidants in estrogen-deficiency bone loss. J Clin Invest 2003;112:915–923. [PubMed: 12975476]

Table 1

Estrogen Interaction with Mitochondria

Direct	Indirect
Stabilization of the ΔΨm	Increased nuclear mediated transcription
Inhibition of F0F1 – ATPase	Increased mitochondrial mediated transcription
Augment mitochondrial Sequestration of Ca^{+2}	Activation of intracellular signaling proteins