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Neuroprotection and Estrogen Receptors

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Abstract

This review is intended to assess the state of current knowledge on the role of estrogen receptors (ER) in the neuroprotective effects of estrogens in models for acute neuronal injury and death. We evaluate the overall evidence that estrogens are neuroprotective in acute injury and critically assess the role of estrogen receptor (ER)α, ERβ, GPR 30 and non-receptor mediated mechanisms in these robust neuroprotective effects of this ovarian steroid hormone. We conclude that all three receptors, as well as non-receptor mediated mechanisms can be involved in neuroprotection, depending on the model used, the level of estrogen administrated and the mode of administration of the steroid. Also, the signaling pathways used by both ER-dependent and ER-independent mechanisms to exert neuroprotection are considered. Finally, further studies that are needed to parse out the relative contribution of receptor versus non-receptor-mediated signaling are discussed.

Keywords

Estradiol; estrogens; non-feminizing estrogens; estrogen receptor alpha; estrogen receptor beta; neuroprotection; traumatic brain injury; cerebral ischemia; stroke; spinal cord injury

1. Background and Other Recent Reviews on the Subject

Prior to 2002, the efficacy of estrogens was being assessed in preclinical studies in a large number of conditions caused by or associated with neurodegeneration. With the publication of the first results from the Women's Health Initiative (WHI) Studies in 2002 [1], the field of estrogen and neuroprotection underwent a major change. In as much as there are about 450 reports of estrogen neuroprotection, we have chosen to limit our consideration to studies that assess the role of estrogen receptors (ERs) in acute neuronal loss from injury, either in vitro or in vivo. As such, in vivo studies that assess the effects of estrogens in chronic neurodegenerative disease models for Alzheimer's disease (AD) or Parkinson's disease (PD) are not herein considered. The reader is refereed to other excellent reviews on these topics [2, 3].

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A PubMed search of reviews on ERs and neuroprotection since 2000 resulted in 58 hits. More than half of these (33) deal with specific disease states (AD, PD, schizophrenia, HIV dementia); specific mechanisms of interest to the authors, such as signaling pathways, mitochondria; astrocytes and very specific brain or eye regions. The remaining 25 dealt with acute neuroprotection, the major issue considered herein. Of these, we refer you to the following reviews that are recent and address the role of ER in neuroprotection [4–10]. Finally, this review focuses on the actions of estrogens on neurons, and does not discuss the neuroprotective effects of this steroid on oligodendrocytes or astrocytes. For consideration of these effects of estrogens in neuroprotection the reader is referred to several excellent recent reviews on the topic [11–14].

2. Overview of Evidence for Neuroprotection by Estrogens

Endogenous ovarian hormones were suspected to be neuroprotectants against global cerebral ischemia-reperfusion injury based upon studies demonstrating that gonadally intact adult female rodents sustain less neuronal damage compared to age-matched males [15]. Our laboratory first demonstrated that 17β-estradiol (17βE2) is a potent neuroprotectant *in vitro* [16] and is very effective against ischemia-induced brain damage [15]. There is now abundant evidence for neuroprotection by estrogens both *in vitro* and *in vivo*.

Multiple independent and lethal mechanisms are involved in cerebral ischemia-induced neuronal death, and estrogens have been identified as multi-faceted hormones that antagonize many aspects of damage-inducing cascades resulting from cerebral ischemia. Protective effects of estrogens have been widely reported in a variety of neurons against different toxicities, which mimic cerebral ischemia *in vitro* [17]. Antioxidant effects of the steroid [18] and attenuation of N-methyl-D-aspartate (NMDA) receptor activation [19] have been implicated as mechanisms for the neuroprotective effects of estrogens. Also, two major signaling pathways, ERK and PI-3K-Akt, have been well characterized as being able to mediate inhibition of apoptosis and support neuronal survival. Both signaling pathways can be activated by estrogens [20,21].

In *in vivo* studies, the neuroprotective effects of estrogens have been demonstrated in a variety of models of acute cerebral ischemia. These include transient and permanent middle cerebral artery occlusion models [15, 22, 23], global forebrain ischemia models [24], photothrombotic focal ischemia models [25], and glutamate-induced focal cerebral ischemia models [26]. The protective effects of estrogens have been described in rats, mice and gerbils [15, 27, 28]. Estrogen-induced neuroprotection has been demonstrated in adult female, middle-aged female as well as reproductively senescent female rats, in some [29], but not all [30, 31] studies. Similarly, these effects of estrogens can persist despite the presence of diabetes and hypertension [32, 33]. The neuroprotective effects of estrogens have also been demonstrated against subarachnoid hemorrhage, a highly prevalent form of stroke in females [34]. Finally, the neuroprotective action of estrogens is not limited to females, inasmuch as estrogen protection is also seen in males [35, 36]. Collectively, these results indicate that estrogens could be valuable candidates for brain protection during acute stroke in both males and females.

Estrogens in concentrations ranging from low physiological to high pharmacological produce protective effects in stroke models. Administration of a physiological level of 17βE2 at the onset of an ischemic event is not neuroprotective [29], but neuroprotective effects of pharmacological doses of 17βE2 were clearly demonstrated with acute treatment at the time of or just before an ischemic event, as well as after its onset [15, 37, 38]. The therapeutic window of 17βE2 at the dose of 100μg/kg lasts up to 3 hours after insult [39] and this therapeutic window can be extended up to 6 hours after ischemic insult with doses of 500 to 1,000 μg/kg [40]. This long post-event efficacy of estrogens is promising, since the therapeutic window for estrogen neuroprotection could be insult severity-dependent and may differ between species. For example, the infarct penumbra, which can be protected, develops over a longer period in human subjects than in rodents [41]. So, it is reasonable to predict that estrogens could have a longer therapeutic window in humans than the 6 hour window that we have described in rodents.

3. Clinical Studies of Estrogens in Neurodegenerative Conditions

Post-menopausal women are in an estrogen-deprived state and are at risk for stroke and other neurodegenerative diseases [42]. Epidemiological evidence suggests that post-menopausal estrogen therapy (ET) reduces the risk or delays the onset of AD [43, 44]. Estrogen loss from natural or surgical menopause is associated with a decline in cognitive function that is reversed by ET [45–47]. ET affects cognitive function during brain aging as well [48–50]. Clinical trials, however, have not shown a protective effect of estrogens on dementia or cognitive function in older women [51–54] or an alleviation of disease in women with mild to moderate AD [55]. Others find estrogen-amelioration of disease in Parkinson's disease (PD) [56, 57] and recovery from neurotrauma such as stroke [4, 58] in the vast majority but not all cases [59]. Mortality from stroke is reduced in post-menopausal subjects who were taking ET at the time of stroke [60–62]. Finally, estrogen status plays role in recovery from brain injury [63, 64].

The WHI studies reported that Premarin® (and PremPro®) not only failed to positively affect conditions associated with neuroprotection, but that ET or combined hormone therapy (HT) may worsen cognition [51–55] and increase strokes [1, 65, 66] and cardiac arrests [1, 65]. It is only now that reanalysis of the WHI data indicates that early post-menopausal treatment with these compounds provides benefits [66, 67], as was reported previously in observational trials [43–49] and a great deal of animal model data as indicated above. Similarly, animal studies are completed [10] or ongoing to assess the "window of opportunity hypothesis", which states that there is a limited period after the menopause during which ET or HT is effective. In view of these new studies, there is a need to re-assess the potential role of estrogens as a therapy for neurodegeneration.

4. Role of ERα in the Neuroprotective Effects of Estrogens

4.1. Focal ischemia.

Because there is intense interest in the possibility that estrogens can reduce stroke-induced neuronal injury, a large number of investigators have assessed the impact of estrogens on neuronal survival after middle cerebral artery occlusion (MCAO), the most well studied

animal model of stroke (focal ischemia). More than a decade of research in these models points to classical ERs as the mediator of 17βE2-induced neuroprotective actions in both males and females when the hormone is administered at physiological doses prior to injury [29, 68, 69]. An early study [70] reported that the broad-spectrum ER antagonist ICI 182,780 (ICI) exacerbates the extent of MCAO-induced injury, thereby implicating classical ERs. ICI can also block the neuroprotective actions of acutely administered estradiol in this model [71].

When ER subtype-specific agonists are given to wild type animals prior to MCAO, or when estradiol is administered prior to MCAO in mice with selective ablation of ERα (ERKO) or ERβ (BERKO, the vast majority of studies show that ERα rather than ERβ is responsible for neuroprotection in this model of focal ischemia. The Wise laboratory was the first to use ERKO and BERKO mice, and they reported that 17βE2 was unable to reduce MCAO-induced injury in ERKOs but was as effective in BERKOs as in wild type mice [72]. This finding has since been replicated by other groups using knockout mice [69] or an ERα-selective agonist [73, 74]. A recent study of 17βE2's neuroprotective efficacy after permanent MCAO in male and female mice with ERα selectively ablated in neurons or in myeloid cells (microglia) demonstrated that neuronal ERα is the mediator of neuroprotection [75]. Similarly, an ERβ agonist is unable to reduce infarct size or improve sensorimotor function in female rats undergoing permanent MCAO [76]. The finding that levels of ERα mRNA and protein are often upregulated in the injured brain after MCAO has also been interpreted as evidence that this receptor subtype is important for neuroprotection in focal ischemia. In contrast, Sampei [77] questioned the role of ERα because MCAO did not produce more damage in female ERKO mice than in wild type controls. However, these latter experiments were carried out in intact females, raising the possibility that ERα may not mediate the effects of endogenous estrogens. As discussed below, an ERβ agonist has also been reported to attenuate neuronal damage after transient MCAO and reperfusion [74].

4.2. Global ischemia

Global ischemia models the type of neuronal injury induced by transient cardiac arrest or other instances when the entire forebrain is temporarily subjected to loss of blood perfusion. It is produced experimentally in rodents (usually rats or gerbils) by transient (5-15 minute) blockade of the blood vessels supplying the forebrain and results in delayed, selective death of hippocampal pyramidal neurons, primarily in the CA1 subfield [78]. 17βE2 pretreatment for days or weeks is highly neuroprotective in this model, and ICI can abrogate the protective actions of estradiol [79–82]. Interestingly, ICI also blocks the ability of 17βE2 to reduce kainic acid-induced death of hilar neurons in the hippocampus [83]. There is strong evidence from studies with selective agonists that ERα can mediate neuroprotection after global ischemia [81, 84]. However, as described in the next section, several pharmacological studies also implicate ERβ as a mediator of 17βE2 neuroprotection in global ischemia. Most recently, the Brann laboratory showed that the hippocampus of aged and long-term hormone-deprived rats exhibits increased ubiquitination and degradation of ERα. ERβ was also reduced in the aged hippocampus. This reduction in hippocampal ERs was associated with a loss of estrogen neuroprotection after global ischemia [85].

4.3. β-amyloid toxicity, oxidative stress, and glutamate excitotoxicity

Many laboratories utilize cell cultures to address mechanistic questions about the ability of estrogens to protect neurons and astrocytes from cell death due to oxidative stress, excitotoxicity (predominantly in response to glutamate) and β-amyloid. Although a few studies with ER subtype selective agonists failed to document a role for classical ERs in protecting cultured neurons from excitotoxicity or oxidative stress (e.g., [86]), the bulk of empirical evidence indicates that ERα is an important (although not the exclusive) mediator of estrogen protection in cultured neurons and astrocytes. Early reports showed that 17βE2 protected immortalized HT22 hippocampal neurons [87] and a cholinergic cell line [88] from β-amyloid-induced toxicity and that transfection with ERα could confer this neuroprotection. A later study in primary neuronal cultures exposed to ER subtype-selective agonists also pointed to ERα as the primary mediator of protection from β-amyloid toxicity [89]. Subsequent work by the Marin group indicates that a membrane-bound form of ERα, which associates with caveolin and a voltage-dependent anion channel in lipid rafts, may be especially critical for conferring protection from β-amyloid [90]. In addition, 17βE2 is reported to reduce β-amyloid toxicity via ERα in fetal neuroepithelial cells [91]; these authors also implicated ERα in 17βE2-induced neuroprotection from oxidative stress. Most recently, an interaction between ERα and the metabotropic glutamate receptor mGluR1 was shown to mediate the neuroprotective effects of 17βE2 in primary mixed cultures of cortical neurons challenged with β-amyloid [92].

Similarly, studies in primary cultures of hippocampal neurons and in HT22 cells implicate ERα as a mediator of 17βE2 protection of glutamate toxicity [93–95]. 17βE2 also reduces glutamate-induced death of cultured astrocytes [96] and immortalized mouse hippocampal neurons [97] in an ICI-sensitive manner, although the ER subtype mediating this protection was not identified. There is also an interesting report that a brief pretreatment with 17βE2 or an ERα selective agonist can protect female but not male cortical neurons from glutamate toxicity [98]. Studies in human neuroblastoma cells (SK-NM-C) challenged with a calcium ionophore also indicate that transfection with ERα but not ERβ confers neuroprotection [99].

4.4. Other models

A contribution of ERα to neuroprotection has also been documented in several other models of neuronal injury. Studies using ER subtype-selective agonists or knockout mice in in vivo and in vitro models of neurotoxin-induced dopamine neuronal loss [100–104] implicate ERα. This classical ER subtype may also contribute to 17βE2 neuroprotection after traumatic brain injury [105], spinal cord injury [79], and experimental autoimmune encephalitis [106]. An ERα-selective agonist can mimic the ability of long-term pretreatment with 17βE2 to reduce death of immortalized mouse brain endothelial cells [107] subjected to oxygen-glucose deprivation. Studies with an ERα-selective antagonist show that this receptor may also contribute to estradiol reduction of lipopolysaccharide-induced death of cultured rat mesencephalic neurons [108] and TNFα-induced apoptosis in VSC4.1 motoneurons [79]. Finally, there is also evidence that classical ER antagonists can block the neuroprotective actions of 17βE2 in the retina [109–110], although the relevant ER subtype has not been identified.

5. Role of ERβ in the Neuroprotective Effects of Estrogens

5.1. Focal ischemia.

As noted above, most experimental studies in animal models subjected to MCAO suggest that ERα is the sole or primary mediator of 17βE2–induced protection when the hormone is provided at physiological levels prior to ischemia. However, there is a report that an ERβ-selective agonist can attenuate MCAO-induced autonomic dysfunction when administered 30 min prior to insult, but only if reperfusion is instituted after 30 min of MCAO. This same agent was ineffective if the MCAO was permanent, whereas pretreatment with an ERα agonist was effective after permanent MCAO [74]. Both ERα and ERβ may also be required to mediate 17βE2 enhancement of neurogenesis after focal ischemia [111]. In mice subjected to MCAO, 17βE2 is also reported to work via ERβ to attenuate neuroprotection induced by ischemic preconditioning [112].

5.2. Global ischemia.

There is more evidence that ERβ contributes to 17βE2's ability to reduce neuronal loss induced by transient global ischemia than by focal ischemia. Two independent groups using different agonists showed that pretreatment with ERβ-selective agents can be neuroprotective. In mice, DPN reduced ischemic damage to the caudate nucleus [113], and in rats, WAY enhanced survival of hippocampal CA1 pyramidal neurons in some animals [81]. In the rat study, the ERα agonist PPT was also effective in a subset of animals, suggesting that either ER receptor subtype can mediate protection in this model. There is also a report that the phytoestrogen genistein can reduce ischemia-induced functional and histological damage in gerbils and that an ERβ-selective antagonist can reverse the beneficial effects of some doses of genistein [114].

5.3. β-amyloid toxicity, oxidative stress, and glutamate excitotoxicity

There is considerable evidence that ERβ participates in neuroprotective actions of 17βE2 in cultured cells. For example, many studies documenting a role for ERα also showed that transfection of cells with ERβ or treatment with ERβ-selective agonists was neuroprotective against glutamate [93–95] and β-amyloid toxicity [87]. In contrast, a recent study that employed both ER subtype-selective agonists and BERKO mice concluded that ERβ mediates 17βE2-induced protection against NMDA-induced toxicity in hippocampal slices [115]. Interestingly, estradiol increased protein levels of both ERα and ERβ in the latter study, whereas Cimarosti [116] reported selective up-regulation of ERβ in 17βE2-treated hippocampal explants subjected to oxygen-glucose deprivation.

5.4. Other models.

ERβ has been implicated as a mediator of neuroprotection in an acoustic trauma model of brain injury. This conclusion was based on studies of ERKO, BERKO and aromatase knockout mice treated with 17βE2 and on evaluation of ER subtype selective agonists [117]. Studies with an ERβ agonist also indicate that this receptor can attenuate TNFα-induced apoptosis in VSC4.1 motoneurons [79]. In a related study, genistein was shown to decrease apoptosis of cultured motoneurons exposed to supernatants containing microglial cytokines;

this protection was reversed by ICI and was associated with increased expression of ERβ [118]. An ERβ agonist has also been reported to increase oligodendrocyte differentiation, improve myelination, and enhance axon conduction in a mouse model of chronic experimental autoimmune encephalomyelitis [119].

6. Role of GPR 30 and Other Non-Classical Estrogen Receptors in the Neuroprotective Effects of Estrogens

While most studies implicate either ERα or ERβ in the protective effects of estrogens, studies in which estradiol exerts positive effects in models that lack functional ERα and ERβ but express GPR30 [120–122] support the role of membrane associated estrogen receptors in the protective effects of estrogens. With respect to GPR30, not only has its activation been associated with neuroprotection [123], but also with improved spatial learning [124–127]. GPR30 is an important component of estrogen-mediated neuroprotection because, as G-protein coupled receptor, its activation can lead to rapid activation of intracellular signaling cascades (see examples of such cascades below) which in turn, could act in conjunction with the classical neuroprotective mechanisms associated with activation of the intracellular/nuclear receptors, ERα and ERβ. For example, activation of GPR30 can increase cAMP and calcium mobilization in neurons [128, 129]. Additionally, activation of this estrogen receptor in mouse cortical neurons can protect cells through a non-genomic mechanism in which ERK activation leads to increased Bcl-2 and decreased pro-caspase-3 [126]. Activation of the neuroprotection-associated PI3K-Akt pathway has also been implicated as a pathway elicited following GPR30 activation [130].

Other non-classical estrogen receptors have also been implicated in the protective effects of estrogens. For example, the Toran-Allerand laboratory proposed a novel estrogen receptor, termed ER-X [131], as a key mediator of estrogens' actions on the brain. Interestingly, this receptor has also been proposed as the receptor for the endogenous diastereomer of 17βE2, 17α-estradiol [132]. And though its role in neuroprotection, per se, has not been specifically addressed, its expression increases in response to ischemic injury. This increase has been proposed as a potential compensatory mechanism to promote cell viability and function. Although other putative estrogen-binding proteins exist in the both nervous and non-nervous tissue, their roles in mediating estrogen-induced neuroprotection have yet to be determined.

7. Non-Receptor Mediated Effects of Estrogens in Neuroprotection

We made the seminal finding that the ERα/β ligand 17βE2 and its presumed inactive diastereomer, 17α-estradiol, which binds to these ER-isoforms with an approximately 40-fold lower affinity [133, 134], were equally potent in protecting neuronal cells from the effects were not blocked by an ER antagonist [135]. This observation prompted a closer evaluation of the structural requirements for neuroprotection among steroids [136, 137]; our data showed that estrogen analogues with little classical ER binding affinity were just as protective as 17βE2; therefore, we reasoned that a substantial portion of the neuroprotective activity of estrogens was ER-independent.

We [136] and others [137, 138] have determined that the phenolic nature of the 17βE2 molecule is essential for neuroprotection. Therefore, we synthesized estrogen analogues to perform structure-activity relationship (SAR) studies using rationally designed compounds in 17βE2-neuroprotection assays. Over 100 compounds were tested in HT-22 (murine hippocampal) cells for their ability to inhibit cytotoxicity against glutamate and iodoacetic acid. The EC_{50} (or IC_{50}) values for neuroprotection, ER binding, and protection against lipid peroxidation were determined to ascertain potency in comparison with 17βE2 [133; Simpkins, unpublished observations]. Neuroprotective estratrienes that have electron donating constituents increase the redox potential of the phenoxy radical, providing better neuroprotective properties [133]. The donated hydrogen radical can quench free radicals formed in oxidative stress conditions. A-ring derivatives with electron donating constituents that stabilize the phenoxy radical were more potent than 17βE2 in protecting these cells from oxidative stress-induced toxicity. Our primary synthetic strategy was to replace the hydrogen with a bulky alkyl group at the 2- or 4-positions of the A-ring. For those compounds that included the addition of a single group to the 2-carbon position of the A-ring or 4-carbon position of the A-ring, there was an increase in potency by approximately ~2- to 170-fold as compared to the parent compounds, 17βE2 and estrone. When two groups flanked the 3-OH position, neuroprotection was enhanced, with approximately 9- and 4-fold decrease in EC_{50} values for protection against neurotoxicity, respectively. Preventing the ability of the phenolic A-ring to undergo redox chemistry by converting the phenolic OH to an OCH_3 group eliminated the neuroprotection of the 2-substituted analogues. Switching the hydroxyl group to the 2-position and adding bulky groups (1-adamantyl or *tert*-buty) to the 3-position greatly improved neuroprotective potencies of the parent compound [133].

Introducing conjugated double bonds into the B- or C-rings of estratrienes is another way to increase the stability of the phenoxy radical. Compounds with these modifications were approximately 180- and 490-fold more potent than 17βE2 against glutamate and indoacetic acid toxicity, respectively. D-ring substituents alter lipophilicity; however, the addition of different alkyl side chains, esterification with benzoic acid and introduction of methyl ethers to the 17-position did not result in better neuroprotection, but instead decreased the potency of the parent compound.

In summary, we have determined critical features of the 17βE2 structure that dictate neuroprotective efficacy and thus advance our understanding of estrogen-mediated neuroprotection. A key observation is that stabilization of the phenoxy radical state increases potency against oxidative stress damage.

8. Signaling Pathways From ERs to Neuroprotection

In addition to promoting neuroprotection via classical genomic mechanisms (i.e., those which involve increased expression of genes and their associated cytoprotective proteins), activation of intracellular signaling pathways serves as another important mechanism by which estrogens promote brain cell health and viability. Given the multiplicity of estrogen receptors that can activate signaling pathways, however, the extent of overlap in the regulation of neuroprotection-associated signaling pathways triggered by these different receptors is still unclear. As stated above, GPR30 is a trans-membrane protein making it

ideal for transducing an intracellular signaling cascade. However, because both ERα and ERβ can localize to the plasma membrane, they are not excluded as mediators of estrogen-induced rapid signaling [139–147]. Indeed ICI 182,780-sensitive estrogen receptors (ERα and ERβ) are responsible for neuroprotective signaling initiated by a membrane impermeable form of estrogen [148]. In other cases, GPR30 is the receptor that initiates protective signaling pathways 7]. Additionally, protein complexes consisting of B-Raf, hsp90, and the ERα are present in cortical tissue providing an intracellular junction by which ERs can converge with growth factor-associated signaling pathways 9].

Several groups have reported that activation of the ERK/MAP kinase pathway is essential for estrogen-induced neuroprotection [150–152]. Activation of the ERK/MAP kinase pathway is generally initiated with the activation of B-Raf by Ras, and through a series of sequential phosphorylation/activation events, leads to the phosphorylation and activation of ERK1/2. Activated ERK can, in turn, translocate to the nucleus and interact with various transcription factors to influence the expression of various cytoprotection-associated genes.

Estrogens may also activate the ERK/MAP kinase pathway through the interaction of ERα with metabotropic glutamate receptors at the plasma membrane, where such interaction has been reported to leads to the activation of a variety of second messengers [153]. In fact, membrane-associated estrogen receptors are able to activate metabotropic glutamate receptors even in the absence of glutamate, and in striatal neurons, can alter the phosphorylation of CREB, which can increase the expression of BDNF and other protective proteins [154].

Estrogens also activate the PI3K/Akt pathway in cortical neurons [155–157]. With respect to cell survival, activated Akt is known to decrease pro-apoptotic proteins within cells. Further, activation of the PI3K pathway by estrogen has been shown to increase the phosphorylation of 4E-binding protein 1 (4EBP1), which allows for the promotion of protein translation without altering mRNA levels [156].

Another signaling pathway affected by estrogens that has strong implications in maintaining brain function is the Rho A pathway. A recent study revealed that estrogen, acting through ERβ, is a positive modulator of the Rho A pathway, whose downstream signaling sequelae include alterations in synaptic physiology and long term potentiation [157–159].

While this is not a comprehensive list of all the cytoprotection-associated signaling pathways known to be activated by estrogen, and other pathways that include the Wnt/beta-catenin signaling pathway [160] or p53 [161] may be equally relevant, the examples provided are intended to support the fact that activation of intracellular signaling pathways, through one or more estrogen receptors, is a viable means by which estrogen promotes brain cell health, function and viability.

9. Role of ERs in Protective Effects of Other Hormones

Androgens, which include testosterone and its 5α- reduced metabolite, dihydrotestosterone (DHT), also exert protective effects on the brain. These effects of androgens can be mediated by the androgen receptor (AR) or, alternatively, through metabolites that bind to and activate

ERs. For example, testosterone can be metabolized by the p450 enzyme aromatase to βE2. In contrast, DHT has long been considered the non-aromatizable androgen, and thus, used as a more “pure” AR-activating hormone. However, recent evidence suggests that the cytoprotective effects of DHT may also be mediated by the ERs, through prior conversion of DHT to 5α-androstane-3β, 17β-diol (3β-diol) by the enzyme, 3β-hydroxysteroid dehydrogenase [162]. 3β-diol does not bind appreciably to ARs but rather binds with nM affinity to the ERs [163]. These data suggest that androgens can have biologically meaningful effects through their metabolites, which elicit their effects through activation of ERs.

10. Future Research Needs and Conclusions

The discovery of selective and effective compounds for the treatment of estrogen deprivation symptoms, including vulnerability to neurodegeneration after the menopause, requires the determination of the receptor(s) involved in mediating there neuroprotective effects. As described above, there is strong evidence for the involvement of classical ERs, membrane ERs and non-ER mediated pathways in estrogen neuroprotection. This raises the possibility that estrogens either affect a multitude of receptors and/or pathways simultaneously, or that their effects are dose-, duration-, insult-, and/or animal model-specific. Resolving this issue is critical, not only in determining the targets for drug discovery programs for improved estrogens and for determining biomarkers of women who may benefit from estrogen therapy in the post-menopause, but also for our understanding of the mechanism(s) by which estrogen-deprivation leads to increased vulnerability of neurodegeneration following insult.

Intriguingly, estrogens are better studied for their neuroprotective effects than any other class of compounds, and despite this, and the rationale for clinical use of estrogens in injured patients [164] there is only one ongoing clinical trial of estrogen neuroprotection against traumatic brain Injury and hemorrhagic shock (Wigginton et al. unpublished observations). We submit that resolution of the ERs (or lack thereof) involved in estrogen-neuroprotection is critical to moving the field forward and to conducting appropriate clinical trails of selective estrogens for specific acute neurodegenerative insults.

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