The role of the estrogen in neuroprotection: Implications for neurodegenerative diseases

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Abstract

In trying to rectify the differences in the risk, onset, and progression of neurodegenerative diseases between men and women, the gonadal hormone estrogen has been the primary focus of investigation for many years. Although this gender difference may encompass disparate and overlapping reasons, estrogen and signaling events mediated by its receptor have been shown to be neuroprotective in a number of neurodegenerative disease models such as Alzheimer’s, Parkinson’s, and Schizophrenia. Although data from human studies remains highly controversial, a large body of research findings suggests that this hormone plays a pivotal role in retarding and preventing the formation of neurodegenerative diseases through its receptor. By activating common intracellular signaling pathways and initiating “cross talk” with neurotrophins, estrogen plays an influential role in neuronal survival from injuries induced by ischemia or other environmental insults. Gaining a better understanding of these estrogen receptor mediated neuroprotective mechanisms may lead to new therapeutic strategies for the treatment or prevention of neurodegenerative diseases.

Introduction

Neurodegenerative disorders are a heterogeneous group of diseases of the nervous system that have many different etiologies. Due to the prevalence, morbidity and mortality of the neurodegenerative diseases, they represent significant medical, social, and financial burdens. These diseases are often age associated, chronic and progressive with limited treatment modalities. Neuropathologically, these diseases are characterized by abnormalities confined to specific regions of the brain. It is commonly observed in many epidemiological studies that premenopausal women experience greater protection from neurological diseases but articulating the precise bio-
logical mechanisms for this gender difference in disease risk and onset has remained elusive. Although multiple complex factors may be involved, the role of the hormone estrogen and its receptor has remained one of the central focuses of investigation since postmenopausal women enjoy none of the same protection against neurological diseases as their premenopausal counterparts [1].

The term estrogen includes thirty hormones of which the most well known are 17β-estradiol (E2) and estrone (E3). E2 is widely acknowledged as the most potent form of estrogen and serves as the focus of this speculative review. In females, E2 is produced primarily in the ovaries and can diffuse readily through the blood brain barrier like other small lipophilic molecules, but they can also be synthesized locally in the brain through the conversion of androgens by aromtase [2]. Although E2 is also produced in males, the role they play in men is somewhat unclear unless they are viewed as signaling molecules that transcend their classical roles in sexual differentiation and reproductive neuroendocrine function [3]. In both sexes, E2 exerts a wide range of physiological effects on regions of the nervous system that influence cognition, pain, fine motor skills, mood, and susceptibility to seizures [1,3]. It also shapes the developing brain by affecting the plasticity of the hypothalamus, hippocampus, midbrain and cortex [4].

As a trophic factor that strongly influences the survival of different neuronal cell types in the central nervous system (e.g. hypothalamic, amygdala, neocortical or hippocampal), E2 has been shown in a number of in vitro and animal studies to be a protective factor against different environmental stressors such as glutamate excitotoxicity [5], oxidative injury [6–7], β-amyloid induced toxicity [5,6], and the neurotoxin 6-hydroxydopamine that depletes dopamine [8]. Since neurons are under constant bombardment of such stressors at any given time, perturbations in this vital process can contribute to a microenvironment that is conducive to the neurodegenerative disease process. Moreover, different neuronal cell types respond to varying concentrations of E2 from 100 pM to 100 µM [7]. This wide variance in the effective concentrations of estrogen indicates that sensitivity to estrogen is largely receptor mediated but glutathione and nerve growth factor has been shown to enhance estrogen’s protective effect as does longer exposures to the hormone prior to the addition of the stressor [9]. While estrogen has intrinsic antioxidant properties that prevents free radical concentrations from reaching pathophysiological levels [10], we review some of more salient mechanisms by which estrogen can protect the aging brain through “classical” gene transcription and more rapid nongenomic activations of estrogen receptors in the central nervous system (CNS).

**Estrogen receptors and the brain**

It is increasingly evident that the estrogen receptor (ER) is not only a ligand-induced transcriptional enhancer but also a mediator of common intracellular signaling pathways in multiple cell types including those of the nervous system. Although ERs were originally thought to be localized intracellularly in the nuclei of cells, ERs have been isolated as membrane bound receptors coupled to second messenger systems such as G proteins and cAMP pathways [11–12]. Two functionally distinct isomers of ER (ERα and ERβ) encoded by two separate genes have been identified that differ in the C-terminal ligand binding domain and in the N-terminal transactivation domain [13].

The first studies on ERs were performed on the hypothalamus and the pituitary gland because of their relation to estrogen’s effects on reproduction [14]. Further research demonstrated that both ER subtypes were localized extensively throughout the nervous system in dendrites, presynaptic terminals, cerebral cortex, midbrain, and the brain stem [14–15]. ERα is predominantly found in the hypothalamus, amygdala and the hypothalamic preoptic areas of the brain involved in neuroendocrine, autonomic, and emotive functions [15], while ERβ is localized in learning and memory regions of the brain such as the hippocampus and cerebral cortex [16]. ERβ also appears to have an important role in brain development and is necessary for both neuronal migration and apoptosis during late embryonic development of the brain [15,17]. Both receptors are expressed to a certain extent in every cell type in the nervous system and the ability of the different ER subtypes to form heterodimers [18] suggests a scenario where the two receptors can act synergistically in response to neuronal injury. Furthermore, the robustness and complexity of ERs in mediating neuroprotective mechanisms is greatly enhanced by the expression of several alternatively spliced ER variants that are generated from different promoter sites. These ER variants alter basal activity, ligand affinity, and are differentially expressed in specific regions of the brain [19–20]. In the hippocampus, for instance, the ERβ variant lacking exon 4 exhibits diminished receptor expression and associated E2 binding [21]. Subsequently, the extent that a particular ER subtype can become impaired must be taken into consideration in the neurodegenerative process.

**Genomic ER-mediated neuroprotective mechanisms**

The neuroprotective effect of E2 is partially derived from the modulation of genes that affect neuronal survival over the course of several hours or days [22]. In the “classical” steroid transcription mediated pathways, estrogen binds to ERs, liberating them from the heat shock proteins prior to their dimerization and subsequent activation [23]. Following binding to estrogen, the ER dimers migrate to the estrogen response element (ERE) in the promoter region of a target gene [24]. Promoter analysis of the ERE has revealed several genes involved in neural function such as choline acetyltransferase [25], α1a-adrenergic receptor [26], preproenkephalin [27], glial fibrillary acidic protein (GRAP) [28], and the apoptotic genes Bcl-2, Bax, Bcl-
XL and Bad [29]. In several age related dementias such as Alzheimer’s Disease, neurons die at a vastly increased rate and thus the estrogen responsive Bcl-2 genes are particularly noteworthy in this context in that they have been shown to stimulate neurotrophic activity as well as prevent neuronal cell death [30–31]. This dual protective function greatly enhances the survival of neurons following injury by oxidative stress from reactive oxygen species [32], β-amyloid exposure [33], and excitotoxic cell death from the amino acid glutamate [34].

As ligand-induced transcription factors, ERs can also activate or suppress the expression of genes in response to environmental insults to the neural tissue by interacting with other transcription factors and coactivators such as activator protein-1 (AP-1) [35] and are independent of ERE binding. The indirect evidence for ERE independent neuroprotection is shown through ERE inhibiting peptides did not diminish protection from neurotoxins in dopaminergic neurons, whereas the estrogen antagonist ICI 182,780 was able to block protection [36]. Therefore, the data would appear to support the notion that ERs are necessary and sufficient in genomic estrogen mediated neuroprotection mechanisms.

**Estrogen and Neurotrophic “Cross Talk”**

Several studies have shown that the trophic actions of estrogens in the brain are derived from sharing pathways with neurotrophins [37–39]. In this hypothesized scenario advanced by Toran-Allerand et al., estrogen diffuses into the cell and directly activates cytoplasmic extracellular-signal regulated kinases (ERKs) that in turn relay neurotrophin signals to the nucleus [38–39]. Further supporting this intracellular “cross talk” is direct evidence that estrogen can modulate levels of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) [38–40] and the expression of neurotrophin receptors p75, trkA and trkB [38–39,41]. Moreover, ERs and neurotrophin receptors are often found to be co-localized in a number of neuronal populations and glial cells [38–39].

Although BDNF and NGF are the most common neurotrophins reported in the literature, estrogen has been shown to elevate insulin-like growth factors (IGF) that protect nerve tissue against apoptotic stimuli and can initiate recovery from many forms of neural injury [42–43]. Interestingly, the neurotrophic actions of ERs and IGF have been shown to be interdependent in the hippocampus where the administration of either J1, an IGF receptor antagonist, or ICI 182,780 can ER antagonist, can neutralize estrogen induced neuroprotection in ovariectomized rats exposed to kainic acid [45]. This experimental model provides direct evidence that ERs and IGF receptors are acting synergistically in this particular estrogen mediated neuroprotection process.

**Nongenomic actions of estrogens in the brain**

In contrast to the classical genomic pathways of steroid action that occur over the course of several hours or days, work in our lab and others have shown conclusive evidence of a rapid E2 signaling pathway mediated by cell surface ERs [45–47]. In establishing the origins of these plasma membrane ERs, Razabdi et al. have shown that ER negative CHO cells transfected with ERα and ERβ express both subtypes in the plasma membrane and nuclear membrane fractions, suggesting that both cell surface and nuclear ERs arise from the same ERα or ERβ transcript [48]. Although ERs lack potential sites for myristoylation or palmitoylation modification for plasma membrane insertion or anchoring, a subpopulation of ERs have been shown to associate with plasma membrane caveolae in endothelial cells [49].

Within a few seconds to minutes of binding to plasma membrane ER, E2 and its cell-impermeable conjugate E2-BSA both induce rapid calcium influx, stimulate cAMP generation, and modulate the production of nitric oxide (NO) [45–47, 50–51]. By maintaining basal/tonal levels of NO, nitric oxide synthase (NOS) is responsible, in part, for keeping particular types of cells in a state of inhibition and activation of these cells occurs through disinhibition [52–53]. Within the context of neurodegenerative disease, glial cells are constantly in an activated state as a result of environmental changes and basal level NO serves to dampen microenvironmental ‘noise’ which would otherwise nonspecifically and inappropriately activate them. The central nervous system (CNS) consists of neurons and glial cells in roughly equal proportions and the latter provide both protection and support for neurons by (i) supplying nutrients and oxygen to neurons; (ii) insulating neurons from one another; (iii) holding neurons in place; and (iv) removing dead neurons [54]. Estrogen has been observed to inhibit reactive gliosis that can lead to the formation of scar tissue impeding axonal regeneration [55] and to down regulate the proliferation of astrocytes in the brain lesions of rats [56].

**NO and Neurodegenerative Diseases**

In Alzheimer’s disease (AD), there are two defining pathological defects: chronic inflammation and impaired clearance of amyloid B-peptide (AB) [57–62]. In the periphery, estrogen both increases macrophage phagocytosis and has anti inflammatory effects [57–60]. Similarly in the CNS, estrogen can reverse some of the inflammatory symptoms in AD via microglia activation, particularly assisting in clearing AB deposits in the AD brain [58]. Given that cell surface ERs are coupled to constitutive nitric oxide release [52–53,63] and NO has been shown to control immune and vascular endothelial cell shape as well as scavenge free radicals under specific circumstances [63–64], lowered production of NO can lead to abnormal microglial morphological changes indicative of a proinflammatory response. We surmise that estrogen signaling would restore.
homeostasis by scavenging free radicals and inhibiting microglial shape changes associated with a pro-inflammatory process [64]. Thus, as E2 signaling through ERs is compromised, cognitive function and memory loss are subsequently reduced and may contribute to the progression of Alzheimer’s disease.

Likewise in schizophrenia, our group has proposed that macrophages/microglia may become inappropriately stimulated in schizophrenic patients. We document the significance of over reactive microglia once they become activated under abnormal circumstances leading to neural lesions that are characteristic of schizophrenia [65–68]. Since estrogen has been demonstrated to stimulate NO release from monocytes/microglia [65,68], we surmise that this process may become altered, resulting in a hyperactive cell capable of precipitating the onset of schizophrenia if the right brain region becomes affected [65–67]. Furthermore, we also propose that E2 stimulated NO release induces a state of down regulation in these cells thereby inhibiting their hyperactivity [52,53]. In a recent report, we demonstrate that E2 can down regulate microglia in invertebrate ganglia via NO, supporting the hypothesis just mentioned [69]. From this perspective, the presence of E2 related signaling through NO would also help to explain the sex related differences in the onset of schizophrenia between men and women. It would also suggest that schizophrenia is an abnormal proinflammatory state localized to the brain.

Since ERs receptors stimulate NO related pathways, the gradual age related loss of E2 can prime a genetically predisposed woman to certain cerebrovascular diseases that would become nonresponsive to estrogen replacement once lesions have formed [62]. Subsequently, the loss of tonal NO can severely impact the blood flow to the brain resulting in microstrokes [70]. Compared to pre-menopausal women, men and postmenopausal women have a higher incidence of strokes, suggesting that estrogen plays a protective role against the development of vascular related diseases. This is further supported in animal studies where Wise et al. ovariectomized rodents to mimic a menopausal state and administered small doses of estrogen weekly [71]. They then inserted a suture into the rodents’ middle cerebral arteries to replicate the effects of stroke. Upon examining the brain tissue from the rodents, they discovered that estrogen did not protect against initial cell death but did dramatically protect against cell death in the cerebral cortex, demonstrating a phenomenon similar to ischemic preconditioning [72]. Subsequent experiments were performed in ERa knockout rodents that showed that their brain is protected from further injury by ERa signaling [73].

In a recent review we discuss the phenomena of ischemic preconditioning occurring through a NO mediated process [74]. Briefly, basal nitric oxide levels serve to dampen the excitatory state of monocytes by stabilizing NF-xB, a transcription factor involved in the regulation of numerous proinflammatory genes [75]. This process can be influenced by metabolism (e.g., ATP levels, PKC activity, etc.) and free radical concentrations that arise from stimuli (e.g., signal molecules, trauma, antigenic challenge, etc.). Once the basal NO balance is perturbed in an ischemic episode, a cascading set of events ensues to protect the cell. In an ischemic reperfusion injury-protection situation, we surmise that the loss of oxygen would result in an immediate drop of ATP levels, releasing calcium from its energy requiring intracellular sequestration sites (e.g., mitochondria). Simultaneously, free radical levels would increase, triggering a momentary increase in eNOS activity that would help to scavenge the radicals generated. This rise in constitutive eNOS activity would coincide with higher levels of intracellular calcium and the tonal increase in nitric oxide during a short time frame of a few minutes would be sufficient to maintain NF-xB in its inhibitory state, since a proinflammatory situation is averted by eNOS activity.

In this scenario, the cell enters a state of suspension to survive briefly in a hypoxic environment and to avoid entering a proinflammatory state prematurely since cells are often subjected to oxygen poor environments during situations requiring sudden bursts of energy. During this suspended state, the temporary increase in free radicals serves to soak up cNOS derived nitric oxide and results in higher free radical concentrations as constitutive nitric oxide levels diminish below basal levels. Based on this model, we propose that the suspended cell is simply waiting for a return to normal oxygen and ATP levels and the sequestering of calcium required for cNOS activity. However, given this normal scenario, the cell is indeed in a counter-intuitive momentary quiescent state until oxygen levels are normalized. Furthermore, it must also be emphasized that this is not a proinflammatory state that would normally be initiated by lipopolysaccaride (LPS). In response to abnormal insults, we surmise that ischemic preconditioning and the protection afforded by it occurs by mimicking the cell’s response to cyclical dips in ATP levels and the resulting aforementioned cascade of events. By emulating this normal cellular process through eNOS derived NO, the cell is it temporarily down regulated from its excitatory state and protected from insult. Under normal circumstances, a repeat of the injury usually does not reoccur. Thus, ischemic reperfusion injury protection merely capitalizes on an preexisting protective mechanism designed to avoid premature inflammatory situations.

Within this context, E2 plays a role in this protective scenario, as well as other signaling molecules. Instead of ischemia inducing cNOS derived nitric oxide release via a drop of ATP levels, various signaling molecules like E2 can increase tonal levels of NO through their cell surface receptors, thereby initiating a NO dampening effect. Since other signaling molecules such as morphine, anandamide and interleukin-10 have been demonstrated to release NO through their respective receptors, we can expect to find a similar protection with these signaling molecules through the same downstream events [76–77].

It appears that signaling molecules that stimulate constitutive, i.e., endothelial, NOS activity confer a
protective effect by evoking a preexisting process that limits cellular activation following normal physical exertion. Thus, the initial insult, as long as it is not too traumatic, causes the system to down regulate itself. By initiating a quiescent state, latter insults can be diminished in their regard to initiate a larger proinflammatory response. Based on this hypothetical model, estrogen can stimulate NO in select areas of the brain regions where the cell surface ERs are present and protect regions of the brain associated with certain functions, such as cognition, that are susceptible to injury. Therefore, this estrogen protection scenario strongly suggests that different neural vascular disorders may have a common proinflammatory origin [78]. During menopause, estrogenic protection through NO release is largely absent since enhanced cellular down regulation is no longer required.

Conclusion

Despite the number of basic research and animal studies that have demonstrated estrogen’s neuroprotective effect, the Women’s Health Initiative Memory (WHIM) study has presented findings that show women who begin hormone treatment after 65 experience heightened risk of Alzheimer’s disease and other forms of dementia. The majority of dementia found among women participating in the study was classified as Alzheimer’s disease with vascular dementia ranking second. Based on 40 cases of dementia in the hormone group compared to 21 cases in the placebo group, the authors cite a two-fold increased risk of dementia as a result of estrogen replacement even though the total number of such cases is small compared to the 4381 participants enrolled in the study [79]. Although the study might serve to question the therapeutic value of estrogen for postmenopausal women, it must be strongly noted that the timing of estrogen replacement therapy is often administered well before the onset of menopause. We surmise that once lesions have formed that inhibit and/or diminish the release of NO, disease reversal is often difficult to accomplish since the physical apparatus is in all probability damaged. In this particular scenario, we further surmise that estrogen replacement therapy actually is supplying a free radical, accelerating the disease process rather than dampening it.

Figure 1.
The rapid and localized production of NO acts as a neuromodulator to influence functions such as behavior and memory formation. In the central and peripheral nervous systems, neuronal NOS (nNOS) coupled to ERs briefly generate NO in the picomolar range following appropriate activation signals. The timely termination of NO biosynthesis is essential for brain function, and the overproduction of the gas interferes with the functioning of neurons, eventually killing them. Although nNOS is usually reported to be a constitutive enzyme, its expression is influenced by certain physiologic and pathophysiologic stimuli such as nerve injury from ischemia or neurotoxins like glutamate. Once the onset of a neurodegenerative disorder such as Parkinson’s and Alzheimer’s has commenced, we surmise that estrogen replacement therapy can elevate NO to toxic levels where it acts as a free radical accelerating cell death rather than dampening it.
success that extends longevity, women are at a greater risk of suffering from neural degenerative diseases for a great number of years. Further basic research in elucidating how estrogen protects women from the onset of the debilitating neurodegenerative effects of these diseases is needed to provide women a greater quality of life in their twilight years.

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