Neuroprotective Role of Estrogen Receptor-β in Alzheimer's Disease Pathology

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Abstract

Sex is one of the main risk factors for Alzheimer's disease (AD). Estrogen signaling has thus been implicated as an important factor in the incidence and development of AD. Decreased expression of estrogen receptor-β (ERβ) in female patients with AD has been linked to impaired mitochondrial function and increased markers of oxidative stress. Furthermore, evidence from ERβ-knockout mice suggests that ERβ deficiency increases mitochondrial vulnerability to amyloid-β (Aβ) toxicity, which may contribute to aggravation of AD pathogenesis. Similarly, it has been shown that overexpression of ERβ protects against Aβ-induced cytotoxicity via multiple mechanisms in the absence of 17β-estradiol. The latter finding presents a new strategy for the development of treatments that will selectively activate ERβ in a ligand-independent manner, potentially allowing for efficacious neuroprotection, while avoiding the side effects associated with estrogen replacement therapy. The purpose of the current manuscript is to review the available research on the role of ERβ in pathological cellular events associated with AD.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive loss of neurons and marked histological abnormalities. The disease is typically diagnosed after the age of 65, and its prevalence is expected to rise tremendously as baby boomers age.[1] The growing prevalence, together with the severity of the symptoms, has led to an increased interest in investigating the causal factors and nature of the pathophysiology underlying the disease. Some of the important molecular processes associated with the disease include tau protein phosphorylation, aggregation of neurofibrillary tangles, and β-amyloid plaque formation.[1] Other cellular phenomena, such as increased oxidative stress and activation of apoptotic pathways, are also significant contributors to disease progression.[2] While numerous studies have been able to identify causal factors and describe the pathogenesis of the disease, a successful cure has yet to be developed.

Sex appears to be one of the main risk factors for AD. Concordantly, the incidence of AD in females is substantially higher than in males. Although this observation may partially be explained by the fact that female AD patients tend to live longer,[1] additional evidence suggests that decreased levels of estrogens following menopause may constitute a risk factor for the development of AD.[3] As such, there is rational interest in studying the effects of sex hormones on the onset and progression of AD. Recently, a longitudinal study on elderly women using hormone replacement therapy (HT) to alleviate the effects of menopause suggested a neuroprotective role for estrogens.[4] According to the study, women undertaking HT showed delayed onset[5] and diminished risk of AD,[6] as compared to age-matched controls. In addition, transdermal estradiol treatment in women with mild to moderate AD profile resulted in enhanced cognition, especially on measures of visual, semantic, and verbal memory.[7] However, while it is evident that estradiol has beneficial effects on maintenance of the central nervous system (CNS), there are a number of side effects associated with HT, such as an increased risk for breast cancer.[8] As such, the development of novel treatments for AD that bypass the negative outcomes associated with HT has been the focus of research in the past decade.[9]

Several lines of evidence have implicated estrogen receptors (ER) as important mediators of estrogen-induced neuroprotection. The two ER subtypes, ERα and ERβ, differ in terms of their activity and distribution. While ERα is primarily expressed in the reproductive system, ERβ shows substantial distribution in the CNS, notably in the frontal lobes and hippocampus.[9] These regions may be the mechanistic location of estrogen-induced neuroprotection; indeed, neuroprotective properties of ERβ have been shown using both *in vitro* and in vivo models.[2,10] For instance, it was shown that ERβ activation prevents damage from oxidative stress by activating the mitogen activated protein kinase pathway.[2] Similarly, studies of ERβ agonists were shown to reduce neuronal loss in mice following transient global ischemia.[10]

Due to its prominent expression in the frontal lobe and hippocampus, as well as its neuroprotective properties, ERβ may be a potentially efficacious therapeutic target in AD patients. Therefore, the aim of this article is to review the role of ERβ in the pathogenesis of AD, as well as to examine the possibility of developing new therapeutic targets based on selective ERβ activation.

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ER-β Deficiency

Multiple lines of evidence indicate that mitochondrial dysfunction and elevated oxidative damage are reliable precursors to the development of AD pathology. Although mitochondria play an integral role in cellular bioenergetics, it is also the site of reactive oxygen species (ROS) production, which damage macromolecules through oxidative stress.[11] Moreover, accumulation of oxidative damage to cellular components appears to be an early stage in the pathogenesis of AD, preceding the accumulation of plaques and tangles.[12] The essential role of estrogen in maintaining mitochondrial bioenergetics is well established.[11] For instance, physiological concentrations of 17β-estradiol substantially reduced the rate of superoxide production in the mitochondria, an effect blocked with ICI-182,780, an ER-antagonist.[13] Thus, it is suggested that the role of 17β-estradiol in maintaining mitochondrial efficiency is ER-dependent. A possible modulatory role of ERβ on mitochondrial function is further supported by observed co-localization between ERβ and mitochondria in the cortical and hippocampal neurons of rodents.[14]

To establish the association between ERβ and AD, Long *et al.*[15] examined ERβ expression and localization patterns in brain samples from women with AD and healthy age-matched controls.[15] In accordance with the study in rodents, immunohistochemical analysis revealed co-localization of ERβ and mitochondria in the human brain samples from both groups. However, relative to the age-matched controls, the AD group showed reduced expression of ERβ in whole cell lysates as well as mitochondrial fractions, indicating decreased ERβ expression and association with the mitochondria.

Confirming the necessity of ER in the maintenance of optimal mitochondrial performance, Long *et al.* demonstrated that the decrease in mitochondrial ERβ expression was accompanied by reductions in mitochondrial function.[15] As an example, the activities of cytochrome C oxidase (COX) and succinate dehydrogenase, two enzymes involved in the electron transport chain, were substantially reduced in AD brains.

Consistently, Western blot analysis revealed substantial reductions in COX 1 and COX 2 subunits encoded by the mitochondrial DNA. On the other hand, nuclear DNA-encoded COX 4 levels remained unchanged. These findings suggest that deficits in mitochondrial function could result from oxidative damage to mitochondrial DNA. Likewise, it was previously documented that COX activity was reduced in aged subjects, suggesting that this could occur as a result of accumulated oxidative damage to mitochondrial DNA.[16] Cumulative damage to cellular structures is further supported by an observation that brain samples from AD patients were characterized by elevated levels of carbonylated proteins, which are products of ROS damage.

Altogether, the results by Long *et al.*[15] suggested that female AD patients could have greater accumulation of oxidative damage as a consequence of mitochondrial dysfunction, relative to controls. Furthermore, this perturbation was correlated with decreased neuronal ERβ expression. However, while the association between decreased neuronal ERβ and AD pathology is evident, it is not clear whether ERβ deficiency causes AD, or whether this deficiency is a secondary consequence of AD pathogenesis.

Considering the association between ERβ expression and mitochondrial function in human brains, the second part of Long *et al.* study aimed to investigate the immediate effect of ERβ deficiency on mitochondrial function by comparing female ERβ knockout mice to the wildtype (WT) animals.[15] Oxygen consumption, mitochondrial membrane potential (MMP), and ROS generation were assessed following treatment with amyloid-β (Aβ) protein of varying concentrations (5-200μM) in WT, ERβ heterozygotes (+/-), and ERβ null (-/-) mice. These studies revealed that ERβ knockout mice did not differ in terms of basal oxygen consumption, however treatment with Aβ resulted in significant reductions in oxygen consumption at all concentrations, although this effect was not significantly different between the genotypes. In terms of ROS generation, the three genotypes did not differ in their baseline measurements; however, $ER\beta^{-/-}$ mice showed the greatest increase in ROS production following treatment with Aβ peptides, as compared to the WT animals. These results suggest that ERβ deficiency does not interfere with oxygen metabolism under normal conditions, but rather makes the mitochondria more vulnerable to Aβ-induced toxicity.

Finally, the role of ERβ in maintaining the MMP appears to be a critical one, as both $ER\beta^{+/-}$ and $ER\beta^{-/-}$ mice showed significant reductions in basal MMP relative to WT mice, which was further exacerbated by treatment with Aβ. The necessity of ERβ in maintaining the MMP was also confirmed in platelets isolated from $ER\beta^{-/-}$ mice,[17] as well as in human lens epithelial cells, whereby reducing ERβ expression with siRNA resulted in depolarization of the MMP, thereby driving the cell towards a proapoptotic state.[18]

Several lines of evidence suggest that mitochondrial energy metabolism can be impaired by Aβ. The Aβ protein has been shown to directly interact with mitochondrial proteins, disrupting the electron transport chain, and increasing ROS generation.[19] The resulting increase in oxidative damage in turn promotes Aβ generation, which further enhances oxidative stress.[20]

The conclusions from the Long *et al.* study are two-fold: first, treatment with Aβ protein impaired mitochondrial respiration in female mouse brains, an observation consistent with previous research.[21] Secondly, the observed impairment was greater in $ER\beta^{-/-}$ animals, suggesting that deficiency in ERβ is a potential contributor to the pathogenesis of AD.

Although the possibility remains that the mice were more vulnerable to Aβ toxicity due to other factors,[22] such as developmental abnormalities, there is additional evidence to support the role of ERβ deficiency in AD pathology. For instance, an earlier study demonstrated that $ER\beta^{-/-}$ mice exhibited pathological changes characteristic of AD, such as accumulation of Aβ and apolipoprotein E (ApoE) in cortical and limbic regions.[23]

In summary, while it is well documented that accumulation of oxidative damage decreases mitochondrial function and increases ROS production, the Long *et al.* study provides strong evidence that ERβ deficiency disrupts normal mitochondrial function and thus promotes deposition of Aβ, and the aggravation of AD progression.

ERβ Overexpression

Further evidence in support of the role that ERβ plays in the development of AD comes from a recent study by Wang *et al.* complementing the results of Long *et al.*,[15] the study shows that overexpression of ERβ protects against Aβ toxicity in rat adrenal pheochromocytoma cells (PC12) transfected with the ERβ gene.[24] This study showed an increase in ERβ expression and subsequently utilized two experimental conditions: one group was treated with Aβ, while the other received Aβ together with the protein kinase B-inhibitor, Abi-2.[24]

Protein Kinase B, a serine/threonine specific protein kinase involved in cell survival, was shown to be abnormally expressed in the frontal and temporal lobes of AD patients.[25] Furthermore, estrogen exerted a neuroprotective effect through a protein kinase B-dependent pathway in rat cortical neurons.[26] Consistent with these observations, Wang *et al.* showed that overexpression of ERβ protected the PC12 cells against Aβ-induced apoptosis, although this effect was abolished in cells treated with protein kinase B-inhibitor. These observations are consistent with previous work showing estradiol-induced protein kinase B activation alleviated the cytotoxic effects of Aβ in hippocampal neurons.[27] Thus, ER-mediated protein kinase B recruitment constitutes one form of protection against Aβ-induced cell death.

These findings confirm the interaction of ERβ and protein kinase B as mediators of neuroprotection, and suggest that these effects can be induced in a ligand-independent manner, since these treatments did not involve estradiol administration. This latter observation is in agreement with previous research indicating that ERβ is capable of inhibiting apoptosis in the absence of its main ligand, estradiol, by binding to the proapoptotic protein Bad and limiting its interactions with downstream targets.[28]

The results of these studies provide support for ERβ-mediated protection from Aβ-induced cytotoxicity. As these neuroprotective effects are estradiol-independent, the findings have substantial implications for AD treatment development, as discussed below.

Implications for Treatment Development

Available evidence suggests that estrogen loss associated with menopause may contribute to the development of AD. Research on the efficacy of estrogen replacement therapy has produced mixed results. While some studies showed cognitive improvements from estradiol treatment in female AD patients,[7] estrogen therapy does not seem like a feasible long-term treatment. Sex hormone therapy has a number of adverse side effects, including increased probability of developing breast cancer[30] and venous thromboembolism,[31] among others. As such, recent findings implicating ERβ as a neuroprotective factor in AD provide important insights for the development of new therapeutics that will not rely on estradiol administration and hence avoid the associated side effects.

Increasing evidence suggests that selective ERβ activation may be capable of improving cognition. A recent study by Zhao *et al.* demonstrated that an ERβ-specific activator, 2,3-bis(4-hydroxyphenyl) propionitrile (DPN), was able to promote neuronal survival in rat hippocampus by increasing the expression of antiapoptotic protein Bcl-2.[32] Enhanced hippocampal function following ERβ activation was also observed by Liu *et al.*, who demonstrated elevated levels of synaptic proteins, enhanced long-term potentiation, and increased numbers of dendritic spines, following treatment with selective ERβ-agonists in rat hippocampi.[33] These findings were also accompanied by improved performance on spatial memory tests, suggesting that ERβ treatment can enhance hippocampal synaptic plasticity, and ultimately learning and memory, in the absence of its primary ligand, 17β-estradiol. It remains to be established whether similar mechanisms exist in humans.

The findings by Zhao *et al.* were recently corroborated by an *in vitro* study with rat hippocampal neurons, which showed that DPN, a selective ERβ activator, was able to increase cell viability following treatment with Aβ,[32,34] Relative to the control condition, treatment with DPN resulted in lower cellular ROS levels, decreased expression of pro-apoptotic caspase-3 and Bax, and upregulation of anti-apoptotic Bcl, thereby attenuating the pro-apoptotic effects of Aβ. In addition, the authors have noted that cells treated with DPN exhibited enhanced phosphorylation of protein kinase B and extracellular regulated kinase 1/2, both of which are inhibited by β-amyloid protein. Furthermore, DPN treatment alone at test concentrations did not result in toxicity. Altogether, the authors concluded that DPN protected the neurons against Aβ-induced damage through a number of mechanisms involving anti-apoptosis, anti-oxidation, and anti-inflammation, while not causing toxicity in untreated cells.

Together these studies suggest that targeting ERβ has therapeutic potential in countering the cytotoxic effects of Aβ protein seen in the pathogenesis of AD. Considering that ERβ is widely distributed in the brain and to a much lower extent in peripheral and reproductive tissues,[35] favourable outcomes may be achieved by designing subtype-selective ERβ-based therapeutics. Such treatments will be able to harness the neuroprotective effects of ERβ in the brain, without eliciting the adverse effects of HT. Furthermore, therapeutic benefits will not be limited to women, as ERβ is also found in male brains.

Conclusion

The growing prevalence of AD and lack of available treatments has led researchers to examine new contributing factors in AD etiology. The association between sex and AD incidence suggests a role for estrogen in neuroprotective mechanisms against the pathological processes associated with the disease. Particularly, ERβ has been implicated as a modulator of neuroprotection, which can be stimulated without use of its endogenous ligand. ERβ deficiency is associated with reduced mitochondrial function and increased oxidative stress in female AD patients. Furthermore, decreased expression of ERβ renders mitochondria more vulnerable to Aβ toxicity. Likewise, ERβ overexpression appears to protect neuronal cells from Aβ cytotoxicity and promote neuronal survival via multiple pathways. Because the neuroprotective actions of ERβ are ligand-independent, the design of new treatments for AD is afforded an opportunity. The selective targeting of ERβ, because of its preferential distribution in the CNS, might allow elicitation of the beneficial effects of estrogen against neurodegeneration, while reducing the negative side effect of HRT.

Acknowledgments

The authors would like to thank Dr. Janelle LeBoutillier, professor at the University of Toronto Scarborough Campus, for her guidance and assistance. Her encouragement and advice was invaluable in completion of this manuscript.

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