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Original article

Surgical menopause enhances hippocampal amyloidogenesis following global cerebral ischemia

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Abstract

Background: Prematurely menopausal women have a doubled lifetime risk of dementia and a 5-fold increased risk of mortality from neurological disorders, but the molecular mechanisms underlying these risks remain unclear. We hypothesized that ischemia-induced amyloidogenesis may be enhanced in the hippocampus following prolonged loss of ovarian 17β-estradiol (E2), which could contribute to these phenomena.
Methods: The current study used a rat model of premature surgical menopause (10-week bilateral ovariectomy) with E2 therapy either initiated immediately (short-term E2 deprivation (STED)) or delayed to the end of the ovariectomy period (long-term E2 deprivation (LTED)). One week after continuous, subcutaneous E2 therapy, we subjected animals to 10-min global cerebral ischemia (GCI) to assess the effect of LTED on ischemia-induced amyloidogenesis in the hippocampal CA1.
Results: The present study revealed that while hippocampal β-amyloid (Aβ) is not typically enhanced following GCI, there is a rapid, robust elevation of endogenous Aβ in LTED females after GCI. In STED females, we observed that GCI attenuates and E2 maintains A Disintegrin and Metalloprotease 10 (ADAM 10) expression in the hippocampal CA1, and concurrently, GCI increases and E2 decreases BACE1 levels in the same region. Intriguingly, however, we observed a loss of E2 regulation of ADAM 10, ADAM 17, and BACE1 levels in the hippocampal CA1 of LTED females, which provides mechanistic evidence for the enhanced post-ischemic Aβ load following LTED. We also observed loss of E2 regulation of tau hyperphosphorylation in LTED females subjected to GCI.
Conclusion: Collectively, these studies partially explain the enhanced risk of dementia and mortality from neurological disorders seen in prematurely menopausal women and support timely initiation of E2 therapy to yield maximum neurological benefit.

Keywords: Amyloid; Dementia; Estrogen; Ischemia; Surgical menopause

1. Introduction

Women that enter menopause prematurely, or before the age of 40, due to bilateral oophorectomy incur a doubled lifetime risk of dementia and a 5-fold increased risk of mortality from neurological disorders.[1,2] The molecular mechanisms underlying the enhanced risks remain poorly understood, but prolonged loss of the neuroprotective ovarian steroid hormone 17β-estradiol (E2 or estrogen) is thought to play a key role, as estrogen therapy administered at the time of surgery and continued until the median age of natural

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menopausal onset normalizes these risks.[3] Studies in our lab have provided a potential clue as to why surgical menopause may lead to an increased risk of dementia and mortality from neurological disorders. Along these lines, recent work has shown that the hippocampus sustains more damage from global cerebral ischemia (GCI) following 10-week ovariectomy (long-term E2 deprivation (LTED)); this includes previously unseen neuronal cell death in the hippocampal CA3 region, which is usually highly resistant to GCI, and a worse cognitive outcome following GCI.[4] Intriguingly, additional work demonstrated that the hippocampus may become more susceptible to non-ischemic stressors following LTED as well, since the hippocampus of LTED female rats was also significantly more damaged following exposure to β-amyloid (Aβ)(1−42), the most neurotoxic form of amyloid.[4]

Of the different types of dementia, Alzheimer's disease (AD) is the most common, and it is characterized by two neuropathological hallmarks: senile plaques of Aβ and neurofibrillary tangles (NFTs) of hyperphosphorylated tau.[5] Excess neural deposits of Aβ and NFTs are neurotoxic, causing extensive synapse loss and neurodegeneration, as well as an irreversible cascade of progressive memory loss, psychological disturbances, motor dysfunction, and eventually, death.[6] The amount of Aβ present in the brain is largely dependent on the processing of amyloid precursor protein (APP), a Type I transmembrane protein that is sequentially cleaved by enzymes to create intracellular and extracellular fragments.[7−9] APP has two main processing pathways: non-amyloidogenic and amyloidogenic. During non-amyloidogenic processing, APP is sequentially cleaved within the Aβ sequence domain by an α-secretase, such as A Disintegrin and Metalloprotease 10 or 17 (ADAM 10 or ADAM 17), followed by a gamma secretase enzyme complex.[7,9] As such, non-amyloidogenic APP processing precludes formation of Aβ and produces three non-toxic fragments.[7,8] Conversely, during amyloidogenic processing, APP is cleaved by the β-secretase Aβ cleaving enzyme 1 (BACE1) prior to cleavage by the γ-secretase machinery. This results in the formation of the insoluble, neurotoxic 40−42 amino acid Aβ protein. If not successfully cleared from the brain, Aβ monomers form oligomers that then aggregate into extracellular deposits termed senile plaques.[7]

Intriguingly, E2 has been credited with a protective role in AD.[10] Observational studies revealed that postmenopausal women exposed to exogenous estrogens mid-life had a 29%−44% decreased risk of dementia,[11−13] and a recent study suggested that longer cumulative lifetime durations of estrogen exposure, including both endogenous and exogenous sources of E2, were associated with a lowered risk of AD, with each additional month of E2 exposure translating to a 0.5% decrease in AD risk.[14] With respect to basic science studies, E2 has also been repeatedly shown to protect against the neuropathological hallmarks of AD both *in vitro* and *in vivo*.[10,15] For instance, E2 was found to prevent phosphorylation of the microtubule-associated protein tau following cerebral ischemia in rodents, which mitigates subsequent formation of NFTs.[15,16] Furthermore, exogenous E2 is well known to protect against Aβ neurotoxicity,[15,17,18] and brain-specific E2 depletion was found to accelerate Aβ deposition and hinder Aβ clearance in a transgenic mouse model of AD.[19] Collectively, these studies suggest that E2 tends to reduce the neural load of Aβ, and they corroborate post-mortem studies, which found significantly reduced levels of E2 in the brains of female AD patients, compared with age- and gender-matched controls.[19] In regard to the mechanism(s) through which E2 modulates neural Aβ, scientific evidence supports E2 influence of both Aβ deposition and Aβ clearance. Along these lines, E2 is purported to regulate expression of at least two major proteins responsible for removal of neurotoxic Aβ: insulin degrading enzyme and neprilysin.[20−24] With respect to Aβ deposition, several studies suggest that E2 may regulate APP processing at several steps, thereby promoting the non-amyloidogenic pathway. As evidence, BACE1, the rate-limiting enzyme for Aβ formation, has several estrogen response elements (EREs) within its promoter region,[25] and E2 has been shown to decrease BACE1 expression both in mixed neuronal cultures and in neurons *in vivo*.[15,20,26,27] Conversely, E2 has also been hypothesized to regulate two putative α-secretases ADAM 10[4,27−30] and ADAM 17,[26,31] which is also known as TNFα-converting enzyme (TACE).

While E2's neuroprotective role in AD has been well studied *in vitro*, E2's neuroprotection from AD has not been completely characterized *in vivo,* particularly considering the development of AD-like neuropathology following GCI. Furthermore, aside from a single observed decrease of neprilysin expression in the brain 45 days post-ovariectomy,[24] and our lab's recent finding of a switch to amyloidogenic APP processing in the hippocampal CA3 region following GCI in long-term ovariectomized females,[4] the effect of LTED (surgical menopause) on critical pathways affecting Aβ load in non-transgenic rodents is largely unknown. Along these lines, the current study attempted to determine whether surgical menopause enhanced amyloidogenesis in the hippocampal CA1 following a stressor (GCI). Furthermore, the current study also aimed to definitively characterize acute E2 regulation of APP processing (ADAM 10, ADAM 17, and BACE1 expression) in the hippocampal CA1 following GCI and to determine whether E2 regulation of APP processing is lost following long-term ovariectomy, as these events could mechanistically explain the enhanced risk of dementia and mortality from neurological disorders observed in prematurely menopausal women.

2. Methods

2.1. Animals

All procedures were approved by the Georgia Regents University Institutional Animal Care and Use Committee (Animal Use Protocols: 09-03-174 and 2012-0474) and were conducted in accordance with the National Institutes of Health guidelines for animal research. Young adult (3-month-old) female Sprague−Dawley rats were utilized for these studies. All animals were group housed on a 10 h/14 h light−dark