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REVIEW

Guarding the Blood–Brain Barrier: A Role for Estrogen in the Etiology of Neurodegenerative Disease

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Although the effect of estrogen replacement therapy on the incidence of the neurodegenerative disease such as Alzheimer's disease is controversial, experimental studies indicate that estrogen replacement to young adult animals is neuroprotective and that perimenopausal estrogen replacement is associated with a decreased incidence of Alzheimer's disease. Estrogen affects a wide variety of cellular processes that can protect neuronal health. This article considers the disruption of the blood–brain barrier in Alzheimer's disease and forwards the hypothesis that estrogen may preserve neural health by maintaining the integrity of the blood–brain barrier.

Key words: Alzheimer's disease; Hormone therapy; Junction proteins; Endothelial cells; Cytokines

ESTROGEN AND ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is characterized by profound deterioration in cognitive ability and affect. Neuronal damage in the end-stage patient is diffuse and widespread, and postmortem pathological findings include significant neuronal death and the presence of amyloid plaques. Although the incidence of AD is not gender biased, the cumulative risk for dementia in women is significantly greater than in men (2,35). Because the risk for AD is greater following menopause, the loss of endogenous ovarian hormones has been proposed as a risk factor for AD among women. Vasomotor symptoms that present at menopause are usually treated with a combination of estrogen and progestins (hormone therapy) or estrogen alone (estrogen therapy), and considerable basic and clinical research has focused on the effect of menopausal hormone replacement on the incidence of neurodegenerative disease and dementia.

Retrospective studies correlating past use of hormone replacement with present disease have reported a decreased risk for AD among women who had taken hormone therapy [reviewed in (45)]. This was further supported by later prospective studies where use of hormone replacement was also correlated with a reduced incidence of AD (56,99). However, the Women's Health Initiative (WHI), a large-scale, double-blind, primary intervention study, demonstrated that hormone replacement to postmenopausal women increased the incidence of all cause dementia (90), while estrogen replacement increased the probability of mild cognitive impairment (31), frequently considered a precursor to dementia. In view of the large number of basic science studies that have shown a neuroprotective effect for estrogen and progesterone,

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several hypotheses have been forwarded to explain the discrepancy between these studies and the WHI. Some authors have speculated that the timing of estrogen replacement may be a crucial variable in determining its effectiveness (44,93). Thus, estrogen replacement to perimenopausal females is more likely to be effective in protecting brain function, while hormone therapy to postmenopausal women, as in the WHI studies, may not be beneficial. In fact, this idea is underscored by findings of the Cache County study where perimenopausal, but not postmenopausal, use of estrogen replacement was most effective in reducing the incidence of AD (112). Similarly, in the experimental literature, studies comparing young adult females with acyclic older females have shown that estrogen replacement has trophic effects in the former but deleterious effects in the latter (50,52,71).

NEUROPROTECTIVE ACTIONS OF ESTROGEN

Although estrogen exerts neuroprotective actions in animal models of ischemic stroke (29,91) and excitotoxic injury (67,94), the precise mechanisms of estrogen's actions are not clearly understood. Most studies have taken the approach of examining estrogen's effects on pathophysiological events related to AD. AD is associated with the loss of trophic factors (46,77), loss of cholinergic cells (78,107), extracellular β-amyloid deposition (5,6,51), and neural inflammation (1,66,109). Thus, estrogen has been shown to regulate growth factors such as NGF and BDNF (38,49,92,95), which support forebrain neurons that are lost in AD and to increase the availability of cholinergic substrates and enzymes (14,32,62,85). Estrogen has also been shown to reduce β-amyloid-induced cell death (41,42), although estrogen replacement to the transgenic amyloid-expressing mouse has equivocal results (40,113). In the case of neural inflammation, estrogen treatment to brain resident microglia in vitro (28,104,105) and in young adult females in vivo (71,72,103) is immunosuppressive; however, estrogen replacement to older females in vivo paradoxically increases the inflammatory response following neural injury (63,71).

ALZHEIMER'S DISEASE AND INFLAMMATION

Because neuroinflammatory diseases disproportionately affect women, estrogen's ability to modulate the neural inflammatory response is a significant concern. Some studies have shown that estrogen suppresses local inflammation when an inflammagen is administered within the nervous system (72,103), while others have shown that estrogen is, in fact, necessary for mounting an adequate inflammatory response (96). However, peripheral transfer of inflammatory cytokines and immune cells into the brain across the blood–brain barrier (BBB) can potently affect neuronal health and cognitive function (11), and estrogen's action on this process is less well understood.

Among the aging population it is common to find a chronic low-grade inflammation, called "inflammaging" (34), marked by increased circulating levels of cytokines such as IL-1β, TNF-α, and IL-10. Tissues/organs are therefore exposed to constant high levels of proinflammatory molecules (IL-1β and TNF-α), and recent studies have reported a correlation between these high circulating levels of cytokines with chronic diseases that target the elderly such as atherosclerosis (17). Similarly, AD patients have increased levels of TNF-α (16), a cytokine that modulates the activity of γ-secretase and consequently amyloid production and deposition (61). High circulating levels of inflammatory cytokines in elderly patients with metabolic disease are also associated with cognitive impairment (110). In fact, a recent study shows that indicators of metabolic risk such as larger waist circumference, lower plasma concentrations of low-density lipoproteins, and higher plasma concentrations of triglycerides and glucose are positively associated with AD patients compared to cognitively normal controls (81), although a prospective study of the Honolulu-Asia cohort indicated that metabolic risk increased vascular dementia but not AD (54). Interestingly, a study with a larger Finnish cohort also underscored the strong correlation between metabolic syndrome and AD; however, this study also showed the prevalence of AD was higher among women with metabolic syndrome than those without the syndrome (102). Such an association was not seen in males, suggesting that female genetic or hormonal factors may modify the risk for AD.

BLOOD–BRAIN BARRIER DISRUPTION IN ALZHEIMER'S DISEASE

Ordinarily, the brain is believed to be an immune-privileged site protected by the BBB from circulating immune cells. The BBB consists of a physical barrier of endothelial cells, astrocytes, and pericytes, all of which regulate transport of substances from general circulation to the brain. Transcellular passage of proteins requires specific receptors and transporters, while paracellular passage of cells is strictly limited

by junctional proteins located in the clefts between endothelial cells (79,108). Unlike other organs with immune privilege such as the gonads (ovaries and testes) and the fetal–placental unit, where the purpose of such a barrier is transparent, the brain's immune privilege is more puzzling. One hypothesis that has been forwarded is that the barrier serves to protect neurons that may synthesize novel macromolecules postnatally, which will appear "foreign" to the matured immune system. An interesting corollary to this idea is that neurons that are involved with learning and memory may produce new molecules associated with this cognitive process and that disruption of the barrier may cause selective death of these cells, as in AD (7).

Although brain microvasculature changes have been noted in AD patients, evidence for a true impairment of the BBB is only now gaining ground. In AD, capillary density (33) and capacity (12) is altered, and nerve plexuses that innervate adjacent blood vessels are lost (87), while nitric oxide synthase activity (27) and lipid peroxidation (4) in these vessels is elevated. However, routine assays for BBB permeability such as Evan's dye and albumin extravasation or radioimaging tools have failed to confirm changes in BBB permeability (53). Ironically, the principal risk factor for AD, namely the cluster of cardiovascular events such as stroke, hypertension, and atherosclerosis, all cause a disruption of the BBB, which has prompted a reexamination of the BBB integrity in AD. One set of evidence comes from the disease-related presence of large proteins that are typically excluded from the brain. In a recent study, increased severity of AD, staged by Braak levels, was associated with a stepwise increase in aberrant CNS localization of prothrombin, an anticoagulant peptide synthesized by the liver (114). Similarly, an eightfold increase in IgG-positive neurons was reported in the hippocampus and entorhinal regions of AD brains compared to age-matched controls (25). D'Andrea has further suggested that AD may well be an autoimmune disease where neurons are targeted following disruption of the barrier (26). Other supporting evidence for barrier dysfunction is related to the deposition of amyloid, the hallmark lesion of the disease. Mutation of the APP gene leading to Aβ overproduction results in amyloid accumulation not only in the brain but also in cerebral blood vessels, and concomitant barrier disruption (47,48,116). In fact, a recent study indicates that only cerebral vessel amyloid deposition correlates with recognized criteria for diagnosing AD (8). These neuropathological findings are supported by experimental evidence from the triple transgenic AD mouse where both the memory deficits and the amyloid deposition in this animal are preceded by a disruption of the BBB (101).

ESTROGEN AND THE BLOOD–BRAIN BARRIER

Speculation of estrogen's action at the BBB was first prompted by studies on sex differences in vascular integrity and barrier permeability. For example, gender differences in the integrity of the lateral striatal artery have been implicated in 3-nitroproprionic acid-induced selective striatal lesions, where males are disproportionately affected compared to females (70). Sex differences have also been reported for blood–brain permeability, although these may not always be related to a disruption of the physical barrier. For example, the greater brain level of peripherally injected fluorescein in females is mainly concentrated in midline circumventricular organs that are external to the barrier (64), and may therefore be related to gender differences in vascular beds rather than endothelial disjunctions. Similarly, increased brain concentration of metabolites of the anticonvulsant dimethylbenzamide (65) and the opioid agonist fluoropropyl-*N*-nordiprenorphine (20) in females is more simply related to sex differences in the production of metabolites capable of penetrating the BBB.

When coupled to peripheral or central injury models, however, female gender appears to be a risk factor for greater barrier permeability. In an acute sepsis model, brain penetration of selenium was greater in females than males (68), as was Evan's blue dye extravasation in a hyposmolarity model (74). Furthermore, while both males and females experienced an increase in barrier permeability after acute hyperosmolarity, a subsequent exposure to seizure-inducing pentylenetetrazol further increased barrier permeability in females but not males (75). In a subsequent study, this group also reported that barrier permeability resulting from bicuculline-induced seizures was greater in intact females than ovariectomized age-matched animals (73), suggesting that ovarian hormones might be detrimental for the BBB in some conditions. In contrast, however, blood to brain transfer of aminoisobutyric acid was reportedly greater in ovariectomized females compared to intact females (86). In humans, studying gender differences in blood to brain transfer of biomolecules is challenging, although one study reported that that IgG expression in CSF increases significantly with age, and was higher in males than females at all ages tested, although this latter difference was not statistically significant (76).

Gender differences in barrier function and differ-

ences in intact versus ovariectomized females implicate the ovarian hormones, principally estrogen and progesterone, in the maintenance of this structure. Many more studies have focused on estrogen compared to progesterone, although a clear picture of estrogen's effects on the BBB is yet to emerge. Ethinyl estradiol, a synthetic estrogen commonly used in birth control pills, increases permeability of the brain to albumin (36), water (82), inulin, and sucrose (115). The endogenous estrogen, 17β-estradiol, on the other hand, increases glucose uptake and transport (13,88) and acts synergistically with myelin basic protein to cause mast cell infiltration into the brain parenchyma (100). However, when coupled to a neural injury, such as ischemia (22,23) and VEGF- (21) or 3NP-induced (70) vascular toxicity, 17β estradiol reduces the leakiness of the BBB, as do oral conjugate estrogens in Aβ40- and Aβ42-induced vascular lesions and leukocyte permeability (83). In view of the data that BBB permeability is greater in females than males in other injury models (described earlier), while estrogen replacement decreases this permeability, it raises the possibility that other ovarian hormones or hypothalamic–pituitary hormones may counteract the effects of estrogen in intact animals. Another related, but poorly investigated, issue is that of barrier changes that result from "ovarian" aging, namely menopause in women or reproductive senescence in animals, where endogenous gonadal hormone synthesis is reduced. Recent studies from our laboratory, using Evan's blue dye, indicate a two- to threefold increase in the amount of dye that is transferred into the brain in reproductive senescent females compared to young adult females (10). Furthermore, estrogen replacement to young females decreased permeability across the BBB but paradoxically increased dye permeability in the hippocampus of reproductive senescent females.

Much less is known of the specific aspect of barrier function that is affected by estrogen treatment, although estrogen has profound effects on brain vasculature [for review, see (57)]. Estrogen promotes the survival of endothelial cells in vitro and in vivo (98), and protects these cells against hypoxia- (80) and TNF-α-induced (97) apoptosis. Both the known estrogen receptor subtypes ER-α (19,58,106) and ER-β (3,60) are expressed in endothelial cells, including a novel 46 kDa N-terminal truncated ER-α as well as a nontraditional membrane-bound ER-α located in caveolae (18,24,59). Experiments with knockout mice suggest that the ER-α receptor is crucial for estrogens actions on endothelial cells (15,37,84).

Endothelial cells, aided by astrocytes and pericytes, retrict both transcellular and paracellular transport of biomolecules and cells. Immune cells, for example, use paracellular means to enter the brain and this passage is restricted by the presence of junction proteins that occupy the cleft between adjacent endothelial cells. Although the estrogen receptor is a ligand-dependent transcription factor and regulates a wide array of gene families, very little is known about estrogen's effects on the expression of junction proteins in the brain. In the uterus, estrogen has been shown to decrease cell–cell adhesion in vitro (43), and regulates aquaporin (AQ), the water channel protein to increase water content. In the brain, estrogen has been shown to reduce connexin-43 in the SCN (89) and reduce edema (69) and bleeding after hemorrhagic stroke (9). Interestingly, estrogen treatment prevents AQP-4 mRNA in perivascular glial cells, suggesting a mechanism by which estrogen may reduce edema formation in insults such as stroke. In the case of the junction proteins, estrogen increases the expression of occludin in brain microvessels (55) as well as in human cervico-vaginal endothelial cells (39). Furthermore, there is a dose-dependent regulation of this protein by estrogen, such that low doses of estrogen increase occludin levels but high doses decrease this protein (111). Very little is known of estrogen's effects on other junction proteins such as Claudin-5 and the family of junctional adhesion molecule (JAMs). A related, and little studied, issue is the effect of estrogen on the pattern and localization of these proteins (i.e., does estrogen promote junctional localization of these proteins, where they would be most effective in maintaining barrier function).

CONCLUSION

Because dysfunction of the BBB can expose the brain to proteins and macromolecules that harm neurons and potentiate inflammatory cascades, age- and hormone-related changes in this structure can have far-reaching consequences for mental health. Furthermore, the preponderance of autoimmune inflammatory diseases in females, both young and old, also underscores the necessity for understanding the effects of endogenous hormones on the physical and physiological aspects of the BBB, as well as the effects of estrogens contained in birth control preparations and menopause-related hormone replacement preparations. Although estrogen's effects have been examined more thoroughly than other ovarian hormones, there are significant gaps in our understanding of (a) the specific aspects of barrier function controlled by estrogen, whether these are intercellular junction proteins or intracellular transport elements, (b) estrogen's effects on the cellular components of

the barrier such as astrocytes, pericytes, and endothelial cells, cell types that are vulnerable to aging effects, and (c) the consequences of ovarian aging or reproductive senescence in the context of BBB function and whether aging alters the effects of hormone and estrogen therapy. Finally, such studies will need to examine the effects of the major ovarian hormones, estrogen and progesterone, administered separately and concurrently, as well gonadotrophins, such as FSH, whose levels are significantly increased after menopause.

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