Hypothesis

**Estrogen-Astrocyte interactions: Implications for neuroprotection**

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**Abstract**

**Background:** Recent work has suggested that the ovarian steroid 17β-estradiol, at physiological concentrations, may exert protective effects in neurodegenerative disorders such as Alzheimer’s disease, Parkinson’s disease and acute ischemic stroke. While physiological concentrations of estrogen have consistently been shown to be protective *in vivo*, direct protection upon purified neurons is controversial, with many investigators unable to show a direct protection in highly purified primary neuronal cultures. These findings suggest that while direct protection may occur in some instances, an alternative or parallel pathway for protection may exist which could involve another cell type in the brain.

**Presentation of the Hypothesis:** A hypothetical indirect protective mechanism is proposed whereby physiological levels of estrogen stimulate the release of astrocyte-derived neuroprotective factors, which aid in the protection of neurons from cell death. This hypothesis is attractive as it provides a potential mechanism for protection of estrogen receptor (ER)-negative neurons through an astrocyte intermediate. It is envisioned that the indirect pathway could act in concert with the direct pathway to achieve a more widespread global protection of both ER+ and ER- neurons.

**Testing the hypothesis:** We hypothesize that targeted deletion of estrogen receptors in astrocytes will significantly attenuate the neuroprotective effects of estrogen.

**Implications of the hypothesis:** If true, the hypothesis would significantly advance our understanding of endocrine-glia-neuron interactions. It may also help explain, at least in part, the reported beneficial effects of estrogen in neurodegenerative disorders. Finally, it also sets the stage for potential extension of the hypothetical mechanism to other important estrogen actions in the brain such as neurotropism, neurosecretion, and synaptic plasticity.

**Background**

Over the past decade, evidence has emerged in support of a neuroprotective role for the most potent estrogen in human and rodents, 17β-estradiol. The issue of estrogen protection is important, as there is a dramatic age-related decline in estrogen levels in women, such that postmenopausal women have estrogen levels that are approximately 1% of that observed in pre-menopausal women. Coinciding with the estrogen depleted state that occurs at menopause, the risk for stroke and other neurodegenerative diseases increases dramatically. While this correlative relationship may be more coincidental than causative, a large
number of animal studies have suggested that a neuroprotective role does exist for estrogen; a finding that has propelled interest in determining the effectiveness of estrogen in the prevention of neurodegenerative and cerebrovascular disease in humans [1–9]. Additional research has suggested a beneficial role for estrogen in Alzheimer’s disease and Parkinson’s disease based on the results of both human and animal studies [10–13].

In support of a possible neuroprotective role for estrogen, it has been shown that intact adult female rats sustain lower mortality and less neuronal damage as compared to age-matched male rats following middle cerebral artery occlusion [14]. That an ovarian factor is involved in the protection was suggested by the finding that ovariectomy (OVX) eliminates the endogenous protective effect observed in females following cerebral ischemia [14]. Additionally, serum estradiol levels have been shown to be inversely correlated with ischemic stroke damage in female rats [15]. Finally, a large number of studies have shown that estrogen replacement to ovariectomized animals reinstates protection of the brain to a level similar to that observed in intact animals [3,4,6–8],[16–20]. With regards to brain regions protected by estrogen, most studies show that the cerebral cortex is most strongly protected, followed by the striatum [1]. Estrogen has also been shown to strongly protect the hippocampus region in a model of transient global ischemia, which specifically targets the hippocampal CA1 region [20].

With respect to the mechanism of action of estrogen protection, several studies have reported that a 24-hour pretreatment with physiological doses of estrogen is necessary to reduce infarct volume following cerebral ischemia in OVX female animals [4,7,16]. Pretreatment with physiological doses of estrogen for less than 24 h or at the time of middle cerebral artery occlusion fails to reduce brain injury [4]. Additional work has shown that estrogen protection is independent of effects on cerebral blood flow [4,9,17,19]. These findings have been interpreted to mean that protection by physiological doses of estrogen most likely occurs directly at the level of the brain rather than on the vasculature, and that the mechanism involves genomic activation of nuclear estrogen receptors and subsequent induction of neuroprotective factors [16,18,21,22]. In support of a critical role for estrogen receptors in estrogen protection, treatment with ICI182,780, a potent estrogen receptor antagonist, has been shown to significantly exacerbate infarct volume following cerebral ischemia in intact female rats [23].

To further elucidate the mechanism of estrogen-mediated neuroprotection, many researchers have attempted to use primary neuronal cultures and immortalized neuronal cell lines. The results of these studies have produced conflicting results. Although there are reports that physiologically relevant concentrations of estrogen protect purified neurons directly in vitro [24–27], a number of investigators have been unable to confirm a direct neuroprotection with physiological doses of estrogen [28–32]. These findings suggest that while direct protection may occur in some instances, an alternative or parallel pathway for protection may exist which could involve another non-neuronal cell type in the brain. This hypothesis is supported by the observation that physiological doses of estrogen are neuroprotective in rat organotypic cortical explant cultures, which have an intact cellular and tissue architecture and which contain multiple cell types [33].

Of the non-neuronal cell types in the brain, the astrocyte has perhaps the greatest potential for possible involvement in the mediation of estrogen neuroprotective effects. Astrocytes are the most abundant type of glial cell in the brain and are located in juxtaposition to neurons, outnumbering them by a 10:1 ratio in some regions of the brain. Astrocytes are well-known to maintain homeostasis in the brain, and have been implicated in the process of synaptic remodeling. Astrocytes also appear to have a critical role in protection/survival of neurons in the brain, as ablation of astrocytes in vivo results in a significant decrease in neuronal survival [34]. The mechanism of astrocyte-mediated neuroprotection is an area of intense investigation, with several possible mediators of this effect implicated [1,34–39]. Recent work by our laboratory has demonstrated the presence of an estrogen-astrocyte-TGF-β1 pathway, which may have implications in mediating the neuroprotective effects of estrogen in the brain [38]. That astrocyte-derived TGF-β can protect neurons has been demonstrated by Bruno et al. [39], who demonstrated that metabotropic glutamate agonists protect against neuronal injury by enhancing astrocyte-derived release of TGF-β1. Interestingly, García-Segura and coworkers have also demonstrated that estrogen can enhance levels of another neuroprotective growth factor in brain astrocytes, insulin-like growth factor-1 (IGF-1) [40].

In support of astrocytes being a target for and mediator of estrogen action in the brain, estrogen has been demonstrated to increase glial cell proliferation and enhance expression of the astrocyte specific marker, glial fibrillary acidic protein (GFAP) [41]. Furthermore, colocalization of estrogen receptors in astrocytes in a variety of brain regions has been confirmed immunocytochemically in brain sections derived from the guinea pig, rat, and human [42–47]. Estrogen receptor-α and β have also been demonstrated in rat cortical, hippocampal and hypothalamic astrocytes in vitro by a number of investigators [38,48–50]. Of significant interest is the finding that following fornix transection in the primate, an astrocyte-specific increase in ER-α expression occurs, suggesting that
astrocytes may be especially sensitive to estrogen effects after an injury [51]. In a parallel fashion, ER-α transcript has been reported to increase in the cerebral cortex following acute ischemic stroke injury in rats [16]. Taken as a whole, these studies indicate astrocytes may be targets for estrogen action in vivo, and support the concept that astrocytes could mediate, at least in part, the neuroprotective and neurotrophic effects of physiological estrogen in the brain.

Presentation of the hypothesis

It is hypothesized that estrogen-induced neuroprotection achieved with physiological doses of estrogen involves, at least in part, mediation by astrocytes, which is in addition to a possible direct neuroprotection pathway. This postulated parallel pathway of indirect and direct protection is attractive as it may explain how estrogen can achieve widespread protection of the cerebral cortex, striatum and hippocampus despite the fact that the estrogen receptor is not globally expressed in all neurons in these regions. Thus, in our hypothetical model, the ability of physiological levels of estrogen to achieve widespread protection in the brain is due to a postulated direct protection on a subpopulation of ER-positive neurons, coupled with a potential indirect protection of ER-negative neurons via an astrocyte intermediary involving release of astrocyte- derived neuroprotective growth factors.

Testing of the hypothesis

Targeted deletion of astrocytic estrogen receptors (ER-α and/or ER-β) is expected to significantly attenuate the neuroprotection observed following estrogen treatment in OVX female rodents undergoing ischemia.

Implications of the hypothesis

We propose that astrocytes play a role in mediating the neuroprotective effects of estrogen on the brain. Such a hypothetical pathway, if proven true, would go a long way to explain how estrogen may exert its beneficial effects in such important neurodegenerative disorders as Alzheimer’s disease, Parkinson’s disease and acute ischemic stroke, and may help explain how estrogen can exert widespread neuronal protection despite a limited neuronal expression pattern of its receptors.

Author Contributions

Dr. Darrell W. Brann and Krishnan M. Dhandapani shared equally in the conceptualization, preparation, writing and revision of the manuscript.

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