

Hypothesis

Estrogen-Astrocyte interactions: Implications for neuroprotection

Krishnan M Dhandapani and Darrell W Brann*

Address: Institute of Molecular Medicine and Genetics, Neurobiology Program and Department of Neurology, 1120 15th Street, Medical College of Georgia, Augusta, GA 30912

E-mail: Krishnan M Dhandapani - mp5403gs@mail.mcg.edu; Darrell W Brann* - dbrann@mail.mcg.edu

*Corresponding author

Published: 7 June 2002

Received: 16 April 2002

BMC Neuroscience 2002, 3:6

Accepted: 7 June 2002

This article is available from: <http://www.biomedcentral.com/1471-2202/3/6>

© 2002 Dhandapani and Brann; licensee BioMed Central Ltd. Verbatim copying and redistribution of this article are permitted in any medium for any purpose, provided this notice is preserved along with the article's original URL.

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 postmeno-

pausal 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 con-

flicting 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- β_1 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- β_1 . Interestingly, Garcia-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 sub-population of ER-positive neurons, coupled with a potential indirect protection of ER-negative neurons via an astrocyte intermediacy 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.

Acknowledgements

The research of the authors is supported by research grants (HD28964 and AG17186 to DWB) from the National Institutes of Health, NICHD and NIA.

References

- Dhandapani K, Brann DW: **Protective effects of estrogens and SERMs in the brain.** *Biol Reprod* 2002
- Hall ED, Pazara KE, Linseman KL: **Sex differences in postischemic neuronal necrosis in gerbils.** *J Cereb Blood Flow Metab* 1991, **11**:292-298
- Simpkins JW, Rajakumar G, Zhang YQ, Simpkins CE, Greenwald D, Yu CJ, Bodor N, Day AL: **Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat.** *J Neurosurg* 1997, **87**:724-730
- Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, Wise PM: **Estradiol protects against ischemic injury.** *J Cereb Blood Flow Metab* 1998, **18**:1253-1258
- Shi J, Panickar KS, Yang SH, Rabbani O, Day AL, Simpkins JW: **Estrogen attenuates over-expression of β -amyloid precursor protein messenger RNA in an animal model of focal ischemia.** *Brain Res* 1998, **810**:87-92
- Zhang YQ, Shi J, Rajakumar G, Day AL, Simpkins JW: **Effects of gender and estradiol treatment on focal brain ischemia.** *Brain Res* 1998, **784**:321-324
- Rusa R, Alkayed NJ, Crain BJ, Traystman RJ, Kimes AS, London ED, Klaus JA, Hurn PD: **17 β -estradiol reduces stroke injury in estrogen-deficient animals.** *Stroke* 1999, **30**:1665-1670
- Toung TJK, Traystman RJ, Hurn PD: **Estrogen-mediated neuroprotection after experimental stroke in males.** *Stroke* 1998, **29**:1666-1670
- Alkayed NJ, Murphy SJ, Traystman RJ, Hurn PD: **Neuroprotective effects of female gonadal steroids in reproductively senescent female rats.** *Stroke* 2000, **31**:161-168
- Resnick SM, Maki PM: **Effects of hormone replacement therapy on cognitive and brain aging.** *Ann N Y Acad Sci* 2001, **949**:203-214
- Schonknecht P, Pantel J, Klinga K, Jensen M, Hartmann T, Salbach B, Schroder J: **Reduced cerebrospinal fluid estradiol levels are associated with increased beta-amyloid levels in female patients with Alzheimer's disease.** *Neurosci Lett* 2001, **307**:122-124
- Leranth C, Roth RH, Elsworth JD, Naftolin F, Horvath TL, Redmond DE Jr: **Estrogen is essential for maintaining nigrostriatal dopamine neurons in primates: implications for Parkinson's disease and memory.** *J Neurosci* 2000, **20**:8604-8609
- Dluzen DE, McDermott JL: **Gender differences in neurotoxicity of the nigrostriatal dopaminergic system: implications for Parkinson's disease.** *J Genet Specif Med* 2000, **3**:36-42
- Alkayed NJ, Harukuni I, Kimes AS, London ED, Traystman RJ, Hurn PD: **Gender-Linked Brain Injury in Experimental Stroke.** *Stroke* 1998, **29**:159-66
- Liao S, Chen W, Kuo J, Chen C: **Association of serum estrogen level and ischemic neuroprotection in female rats.** *Neurosci Lett* 2001, **297**:159-162
- Dubal DB, Shughrue PJ, Wilson ME, Merchenthaler I, Wise PM: **Estradiol modulates bcl-2 in cerebral ischemia: a potential role for estrogen receptors.** *J Neurosci* 1999, **19**:6385-6393
- Fukada K, Yao H, Ibayashi S, Nakahura T, Uchimura H, Fujishima M, Hall ED: **Ovariectomy exacerbates and estrogen replacement attenuates photothrombotic focal ischemic brain injury in rats.** *Stroke* 2000, **31**:155-160
- Roof RL, Hall ED: **Gender differences in acute CNS trauma and stroke: neuroprotective effects of estrogen and progesterone.** *J Neurotrauma* 2000, **17**:367-388
- Dubal DB, Wise PM: **Neuroprotective effects of estradiol in middle-aged female rats.** *Endocrinology* 2001, **142**:43-48
- Jover T, Tanaka H, Calderone A, Oguro K, Bennett M, Etgen A, Zukin RS: **Estrogen protects against global ischemia-induced neuronal death and prevents activation of apoptotic signaling cascades in the hippocampal CA1.** *J Neurosci* 2002, **22**:2115-2124
- Cyr M, Calon F, Morissette M, Grandbois M, Di Paolo T, Callier S: **Drugs with estrogen-like potency and brain activity: potential therapeutic application for the CNS.** *Curr Pharm Des* 2000, **6**:1287-1312
- Kuppers E, Ivanova T, Karolczak M, Beyer C: **Estrogen: a multifunctional messenger to nigrostriatal dopaminergic neurons.** *J Neurocytol* 2000, **29**:375-385
- Sawada M, Alkayed NJ, Goto S, Crain BJ, Traystman RJ, Shaivitz A, Nelson RJ, Hurn PD: **Estrogen receptor antagonist ICI162,780 exacerbates ischemic injury in female mouse.** *J Cereb Blood Flow Metab* 2000, **20**:112-118

24. Singer CA, Rogers KL, Strickland TM, Dorsa DM: **Estrogen protects primary cortical neurons from glutamate toxicity.** *Neurosci Lett* 1996, **212**:13-16
25. Singer CA, Figueroa-Masot XA, Batchelor RH, Dorsa DM: **The mitogen-activated protein kinase pathway mediates estrogen neuroprotection after glutamate toxicity in primary cortical neurons.** *J Neurosci* 1999, **19**:2455-2463
26. Honda K, Sawada H, Kihara T, Urushitani M, Nakamizo T, Akaike A, Shimohama S: **Phosphatidylinositol 3-kinase mediates neuroprotection by estrogen in cultured cortical neurons.** *J Neurosci Res* 2000, **60**:321-327
27. Honda K, Shimohama S, Sawada H, Kihara T, Nakamizo T, Shibasaki H, Akaike A: **Nongenomic antiapoptotic signal transduction by estrogen in cultured cortical neurons.** *J Neurosci Res* 2001, **64**:466-475
28. Harms C, Lautenschlager M, Bergk A, Katchanov J, Freyer D, Kapinya K, Herwig U, Megow D, Dimagl U, Weber JR, Hortnagl H: **Differential mechanisms of neuroprotection by 17beta-estradiol in apoptotic versus necrotic neurodegeneration.** *J Neurosci* 2001, **21**:2600-2609
29. Bae YH, Hwang JY, Kim YH, Koh JY: **Anti-oxidative neuroprotection by estrogen in mouse cortical cultures.** *J Korean Med Sci* 2000, **15**:327-336
30. Regan RF, Guo Y: **Estrogens attenuate neuronal injury due to hemoglobin, chemical hypoxia, and excitatory amino acids in murine cortical cultures.** *Brain Res* 1997, **764**:133-140
31. Behl C, Skutella T, Lezoualc'h F, Post A, Widmann M, Newton CJ, Holsboer F: **Neuroprotection against oxidative stress by estrogens: structure-activity relationship.** *Mol Pharmacol* 1997, **51**:535-541
32. Goodman Y, Bruce AJ, Cheng B, Mattson MP: **Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons.** *J Neurochem* 1996, **66**:1836-1844
33. Wilson ME, Dubal DB, Wise PM: **Estradiol protects against injury-induced cell death in cortical explant cultures: a role for estrogen receptors.** *Brain Res* 2000, **873**:235-242
34. Cui W, Allen ND, Skynner M, Gusterson B, Clark AJ: **Inducible ablation of astrocytes shows that these cells are required for neuronal survival in the adult brain.** *Glia* 2001, **34**:272-282
35. D'Onofrio M, Cuomo L, Battaglia G, Ngomba RT, Storto M, Kingston AE, Orzi F, De Blasi A, Di Iorio P, Nicoletti F, Bruno V: **Neuroprotection mediated by glial group II metabotropic glutamate receptors requires the activation of the MAP kinase and the phosphatidylinositol-3-kinase pathways.** *J Neurochem* 2001, **78**:435-445
36. Wang XF, Cynader MS: **Pyruvate released by astrocytes protects neurons from copper-catalyzed cysteine neurotoxicity.** *J Neurosci* 2001, **21**:3322-3331
37. Lamigeon C, Bellier JP, Sacchettoni S, Rujano M, Jacquemont B: **Enhanced neuronal protection from oxidative stress by coculture with glutamic acid decarboxylase-expressing astrocytes.** *J Neurochem* 2001, **77**:598-606
38. Buchanan C, Mahesh VB, Brann DW: **Estrogen-astrocyte-luteinizing hormone-releasing hormone signaling: a role for transforming growth factor- β_1 .** *Biol Reprod* 2000, **62**:1710-1721
39. Bruno V, Battaglia G, Casabona G, Copani A, Caciagli F, Nicoletti F: **Neuroprotection by glial metabotropic glutamate receptors is mediated by transforming growth factor beta.** *J Neurosci* 1998, **18**:9594-9600
40. Duenas M, Luquin S, Chowen JA, Torres-Aleman I, Naftolin F, Garcia-Segura LM: **Gonadal hormone regulation of insulin-like growth factor-I-like immunoreactivity in hypothalamic astroglia of developing and adult rats.** *Neuroendocrinology* 1994, **59**:528-538
41. Jung Testas I, Renoir M, Bugnard H, Greene GI, Baulieu EE: **Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells.** *J Steroid Biochem Mol Biol* 1992, **41**:621-631
42. Langub MC Jr, Watson RE Jr: **Estrogen receptor immunoreactive glia, endothelia, and ependyma in guinea pig preoptic area and median eminence: electron microscopy.** *Endocrinology* 1992, **130**:364-372
43. Azcoitia I, Sierra A, Garcia-Segura LM: **Localization of estrogen receptor- β immunoreactivity in astrocytes of the adult rat brain.** *Glia* 1999, **26**:260-267
44. Milner TA, McEwen BS, Hayashi S, Li CJ, Reagan LP, Alves SE: **Ultrastructural evidence that hippocampal alpha estrogen receptors are located at extranuclear sites.** *J Comp Neurol* 2001, **429**:355-371
45. Jakab RL, Wong JK, Belcher SM: **Estrogen receptor beta immunoreactivity in differentiating cells of the developing rat cerebellum.** *J Comp Neurol* 2001, **430**:396-409
46. Donahue JE, Stopa EG, Chorsky RL, King JC, Schipper HM, Tobet SA, Blaustein JD, Reichlin S: **Cells containing immunoreactive estrogen receptor-alpha in the human basal forebrain.** *Brain Res* 2000, **856**:142-151
47. Savaskan E, Olivieri G, Meier F, Ravid R, Muller-Spahn F: **Hippocampal estrogen beta-receptor immunoreactivity is increased in Alzheimer's disease.** *Brain Res* 2001, **908**:113-119
48. Santagati S, Melcangi RC, Celotti F, Martini L, Maggi A: **Estrogen receptor is expressed in different types of glial cells in culture.** *J Neurochem* 1994, **63**:2058-2064
49. Hosli E, Ruhl W, Hosli L: **Histochemical and electrophysiological evidence for estrogen receptors on cultured astrocytes: colocalization with cholinergic receptors.** *Int J Dev Neurosci* 2000, **18**:101-111
50. Su JD, Qiu J, Zhong YP, Li XY, Wang JW, Chen YZ: **Expression of estrogen receptor (ER)-alpha and -beta immunoreactivity in hippocampal cell cultures with special attention to GABAergic neurons.** *J Neurosci Res* 2001, **65**:396-402
51. Blurton-Jones M, Tuszyński MH: **Reactive astrocytes express estrogen receptors in the injured primate brain.** *J Comp Neurol* 2001, **433**:115-123

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMedcentral will be the most significant development for disseminating the results of biomedical research in our lifetime."

Paul Nurse, Director-General, Imperial Cancer Research Fund

Publish with **BMC** and your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours - you keep the copyright



Submit your manuscript here:

<http://www.biomedcentral.com/manuscript/>

editorial@biomedcentral.com