JMS 2022; Vol5(3):104-108
UDC:615.357.651.1.035.4:616.831-084
DOI:10.55302/JMS2253104p

ESTROGENS- MECHANISM OF NEUROPROTECTION

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Abstract

Estrogen is an important steroid hormone signal that regulates multiple tissues and functions in the body. The brain is an important target organ for estrogen. In addition to direct effects, estrogen influences brain function through effects on the vasculature and the immune system.

Estrogen influences several neurotransmitter systems, including acetylcholine, serotonin, noradrenalin, and glutamate.

There are complex mechanisms that underlie estrogen neuroprotective and neurotrophic actions (genomic, non-genomic kinase signalic pathways and membrane estrogen receptors pathways). Estradiol induces gene transcription and rapid membrane signaling mediated by estrogen receptor alpha (ERα), estrogen receptor-beta (ERβ), and a recently characterized G-protein coupled estrogen receptor, each with distinct distributions and ability to influence estradiol-dependent signaling.

Vector-mediated expression of estrogen receptors in the hippocampus provides an innovative research approach and suggests that memory depends on the relative expression of ERα and ERβ interacting with estradiol levels.

The potential role of estrogen as a neuroprotective factor and a multicellular mode of estrogen action in the regulation of neuronal survival and neurotrophism is discussed, as are potential future directions for the field.

***Key words*:** estrogen; estrogen receptors; steroid hormone; neuroprotection**;**

Introduction

Estrogen is an important steroid hormone signal that regulates multiple tissues and functions in the body. Classically, it is considered a "reproductive" hormone, due to its well-known role in feedback signaling in the hypothalamic-pituitary-ovarian axis [1,2]. This review will focus on the "non-reproductive" effects of estrogen in the brain, specifically on the neuroprotective actions of estrogen.

Cerebral ischemia

Studies on cerebral ischemia-induced brain damage in gerbils revealed that female gerbils had a lower incidence and less severe brain damage following carotid artery occlusion than male gerbils [3]. A number of studies have documented that women are "protected" against stroke relative to men – at least until the years of menopause, when estrogen levels decline due to follicular depletion and stroke incidence increases in women [4].

That the protective factor is estrogen was supported by studies showing that removal of the ovaries in rats and mice eliminates the protective effect observed in females following cerebral ischemia [5]. Furthermore, many groups have shown that exogenous administration of estrogen dramatically reduces infarct volume following focal or global cerebral ischemia in ovariectomized female mice, rats and gerbils [6].

The neuroprotective effect of estrogen in global cerebral ischemia, which affects primarily the CA1 region of the hippocampus, was shown to be correlated with significant improvements in recognition, working memory and spatial memory [7].

Additional studies showed that aromatase inhibitors caused a similar increase in cortical and striatal damage following cerebral ischemia [8], and that hippocampal sensitivity to kainic acid-induced neuronal death was markedly increased in gonadectomized rats that received an aromatase inhibitor as compared to vehicle-treated controls [9]. Protective effects of estrogens have been widely reported in a variety of neurons against different toxicities, which mimic cerebral ischemia *in vitro* [10].

Antioxidant effects of the steroid and attenuation of N-methyl-D-aspartate (NMDA) receptor activation have been implicated as mechanisms for the neuroprotective effects of estrogens. Also, two

major signaling pathways, ERK and PI-3K-Akt, have been well characterized as being able to mediate inhibition of apoptosis and support neuronal survival. Both signaling pathways can be activated by estrogens [11].

Role of estrogen receptors (ERs) in the neuroprotective effects of estrogen

More than a decade of research in these models points to classical estrogen receptors (Ers) as the mediator of 17βE2-induced neuroprotective actions in both males and females when the hormone is administered at physiological doses prior to injury [12,13].

When ER subtype-specific agonists are given to wild type animals prior to middle cerebral artery occlusion (MCAO), or when estradiol is administered prior to MCAO in mice with selective ablation of ERα (ERKO) or ERβ (BERKO, the vast majority of studies show that ERα rather than ERβ is responsible for neuroprotection in this model of focal ischemia [14,15].

A recent study of 17βE2's neuroprotective efficacy after permanent MCAO in male and female mice with ERα selectively ablated in neurons or in myeloid cells (microglia) demonstrated that neuronal ERα is the mediator of neuroprotection [16].

Similarly, an ERβ agonist is unable to reduce infarct size or improve sensorimotor function in female rats undergoing permanent MCAO [17]. There is strong evidence from studies with selective agonists that ERα can mediate neuroprotection after global ischemia [18].

Most experimental studies in animal models subjected to MCAO suggest that ERα is the sole or primary mediator of 17βE2–induced protection when the hormone is provided at physiological levels prior to ischemia. However, there is a report that an ERβ-selective agonist can attenuate MCAO-induced autonomic dysfunction when administered 30 min prior to insult, but only if reperfusion is instituted after 30 min of MCAO. This same agent was ineffective if the MCAO was permanent, whereas pretreatment with an ERα agonist was effective after permanent MCAO [15].

Both ERα and ERβ may also be required to mediate 17βE2 enhancement of neurogenesis after focal ischemia. In mice subjected to MCAO, 17βE2 is also reported to work via ERβ to attenuate neuroprotection induced by ischemic preconditioning [19]. Studies with an ERβ agonist also indicate that this receptor can attenuate TNFα-induced apoptosis in VSC4.1 motoneurons [20].

In a related study, genistein was shown to decrease apoptosis of cultured motoneurons exposed to supernatants containing microglial cytokines; this protection was reversed by ICI and was associated with increased expression of ERβ [21]. An ERβ agonist has also been reported to increase oligodendrocyte differentiation, improve myelination, and enhance axon conduction in a mouse model of chronic experimental autoimmune encephalomyelitis [22].

GPR30 is also an important component of estrogen-mediated neuroprotection because, as G-protein coupled receptor, its activation can lead to rapid activation of intracellular signaling cascades which in turn, could act in conjunction with the classical neuroprotective mechanisms associated with activation of the intracellular/nuclear receptors, ERα and ERβ. For example, activation of GPR30 can increase cAMP and calcium mobilization in neurons [23].

Potential mechanisms of estrogen neuroprotection

With respect to genes regulated by estrogen that may facilitate its neuroprotection, estrogen has been shown to increase the expression of the anti-apoptotic gene, *bcl-2*, in the ischemic penumbra following MCAO and global ischemia [24]. Estrogen also increases *bcl-2 in vitro* in rat hippocampal neurons [25] and human NT2 neurons, while it inhibits expression of proapoptotic BAD (bcl-2-antagonist of cell death) [24].

Additionally, estrogen has also been demonstrated to reduce cytochrome c translocation [26], as well as caspase 3 activation and DNA fragmentation [27], further implicating an anti-apoptotic action of estrogen in cerebral ischemia. In addition to a genomic effect, nongenomic effects of estrogen may also play a role in mediating its neuroprotective effects in the brain. For instance, estrogen can rapidly activate the extracellular signal-regulated kinases (ERK) and phosphoinositol-3-kinase (PI3K)-Akt pathways in cortical and hippocampal cells *in vitro*, effects implicated in estrogen neuroprotection action [28].

Estrogen has also been shown to enhance Akt activation in the cerebral cortex and CA1 of the hippocampus following focal or global cerebral ischemia [29].

In addition to a potential direct protective action on neurons, there is evidence that estrogen may also have indirect effects on other cell types such as astrocytes and microglia that may facilitate its neuroprotective actions in cerebral ischemia and other neurodegenerative disorders. With respect to astrocytes, physiological concentrations of estrogen are clearly protective in organotypic cortical explants and neuronal-astrocyte cocultures, and there is evidence of astrocyte mediation of estrogen effects [30,31].

Astrocytes may participate in the production of brain-derived estrogen as they have been shown to express aromatase and this expression is enhanced following cerebral ischemia and following brain injury [32].

Thus, local production of estrogen by astrocytes could be another mechanism of astrocyte-mediated protection of neurons. After neuronal injury and in many neurodegenerative disorders, activated microglia are known to secrete proinflammatory factors that can contribute to the progressive neural damage. A number of studies have suggested that estrogen may act upon microglia to suppress their activation, an effect that could help mediate estrogen neuroprotection.Estradiol reduces inflammatory responses during transient cerebral ischemia[33].

Recent work has further implicated reactive oxygen species (ROS), particularly superoxide, as playing a key role in neuronal cell death following cerebral ischemia. The superoxide anion radical (O_2) is the product of a one electron reduction of oxygen and it is the precursor of most ROS and a mediator in oxidative chain reactions. In both permanent and transient cerebral ischemia, ROS have been shown to increase significantly following onset of cerebral ischemia [34].

Estradiol significantly attenuated hydrogen peroxide production in the striatum following cerebral ischemia, which was correlated with the neuroprotective effect of estrogen [35].

Estrogen (estradiol and estrone) has intrinsic free radical scavenging capability through its capture of hydroxyl radicals, which produces a nonphenolic quinol. The quinol is then converted back to estrogen by using NAD(P)H as a coenzyme, without production of reactive oxygen species. Estradiol quinol and estrone quinol were shown to be neuroprotective in hippocampal neuronal cells *in vitro* and in cerebral ischemia, respectively [36].

Other studies have confirmed estrogen neuroprotection against oxidative stress in various cell and animal models of neurodegenerative disorders [37].

Conclusions

There is a significant need for targeted research to better elucidate the mechanisms of estrogen activation of nongenomic kinase signaling pathways in neurons and non-neuronal cells, e.g. studies to identify the involved membrane estrogen receptors, mechanisms of targeting of these receptors to the plasma membrane, and further identification of scaffold proteins in the brain that may link the membrane estrogen receptor to its kinase targets and facilitate their activation. Further studies to elucidate whether the nongenomic and genomic signaling pathways induced by estrogen in the brain display crosstalk or are separate regulatory pathways are also needed. In closing, while much has been learned in the past concerning estrogen neuroprotective and neurotrophic actions in the brain, significant work lies ahead to fully unravel the complexities of estrogen action in the brain and to fully understand its mechanisms and implications.

References

1. Petersen SL, Ottem EN, Carpenter CD. Direct and indirect regulation of gonadotropin-releasing hormone neurons by estrogen. Biol Reprod. 2003;69:17771–1778.
2. Kelly MJ, Qiu J, Ronnekleiv OK. Estrogen signaling in the hypothalamus. Vitam Horm. 2005;71:123–145.
3. Alkayed NJ, Harukuni I, Kimes AS, London ED, Traystman RJ, Hurn PD. Gender-linked brain injury in experimental stroke. Stroke. 1998;29:159–166
4. Darrell W. Brann, Krishnan Dhandapani, Chandramohan Wakade, Virendra B. Mahesh, and Mohammad M. Khan. Neurotrophic and Neuroprotective Actions of Estrogen: Basic Mechanisms and Clinical Implications. Steroids.2007;72(5):381-405.l
5. Park EM, Cho S, Frys KA, Glickstein SB, Zhou P, Anrather J, Ross ME, Iadecola C. Inducible nitric oxide synthase contributes to gender differences in ischemic brain injury. J Cereb Blood Flow Metab. 2006;26:392–401.

6. Miller NR, Jover T, Cohen HW, Zukin RS, Etgen AM. Estrogen can act via estrogen receptor alpha and beta to protect hippocampal neurons against global ischemia-induced cell death. Endocrinology. 2005;146:3070–3079.
7. Plamondon H, Morin A, Charron C. Chronic 17beta-estrogen pretreatment and ischemia-induced hippocampal degeneration and memory impairments: a 6-month survival study. Horm Behav. 2006;50:361–369.
8. McCullough LD, Blizzard K, Simpson ER, Oz OK, Hurn PD. Aromatase cytochrome P450 and extragonadal estrogen play a role in ischemic neuroprotection. J Neurosci. 2003;23:8701–8705.
9. Veiga S, Azcoiitia I, Garcia-Segura LM. Extragonadal synthesis of estrogen is protective against kainic acid excitotoxic damage to the hippocampus. Neuroreport. 2005;16:1599–1603
10. Green PS and Simpkins JW: Neuroprotective effects of estrogens: potential mechanisms of action. Int J Dev Neurosci, 2000; 18: 347–358.
11. 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.
12. Hoffman GE, Merchenthaler I, and Zup SL: Neuroprotection by ovarian hormones in animal models of neurological disease. Endocrine 2006; 29: 217–231
13. Wise PM, Dubal DB, Wilson ME, Rau SW, Bottner M and Rosewell KL: Estradiol is a protective factor in the adult and aging brain: understanding of mechanisms derived from in vivo and in vitro studies. Brain Res Brain Res Rev 37: 2001; 313–319
14. Shimada K, Kitazato KT, Kinouchi T, Yagi K, Tada Y, Satomi J, Kageji T, Nagahiro S: Activation of estrogen receptor-alpha and of angiotensin-converting enzyme 2 suppresses ischemic brain damage in oophorectomized rats. Hypertension 2011; 57: 1161–1166.
15. Connell B and Saleh TM: Differential Neuroprotection of Selective Estrogen Receptor Agonists against Autonomic Dysfunction and Ischemic Cell Death in Permanent versus Reperfusion Injury. Adv Pharmacol Sci 2011; 976951.
16. Elzer JG, Muhammad S, Wintermantel TM, Regnier-Vigouroux A, Ludwig J, Schütz G, Schwaninger M: Neuronal estrogen receptor-alpha mediates neuroprotection by 17beta-estradiol. J Cereb Blood Flow Metab 2010; 30: 935–942.
17. Farr TD, Carswell HV, Gsell W, Macrae IM: Estrogen receptor beta agonist diarylpropiolnitrile (DPN) does not mediate neuroprotection in a rat model of permanent focal ischemia. Brain Res 2007; 1185: 275–282.
18. Miller NR, Jover T, Cohen HW, Zukin RS, Etgen AM: Estrogen can act via estrogen receptor alpha and beta to protect hippocampal neurons against global ischemia-induced cell death. Endocrinology 2005; 146: 3070–3079.
19. Wang L, Kitano H, Hurn PD, Murphy SJ: Estradiol attenuates neuroprotective benefits of isoflurane preconditioning in ischemic mouse brain. J Cereb Blood Flow Metab 2008; 28: 1824–1834.
20. Das A, Smith JA, Gibson C, Varma AK, Ray SK, Banik NL: Estrogen receptor agonists and estrogen attenuate TNF-alpha-induced apoptosis in VSC4.1 motoneurons. J Endocrinol 2011; 208: 171–182.
21. McDowell ML, Das A, Smith JA, Varma AK, Ray SK, Banik NL: Neuroprotective effects of genistein in VSC4.1 motoneurons exposed to activated microglial cytokines. Neurochem Int 2011; 59: 175–84
22. Crawford DK, Mangiardi M, Song B, Patel R, Du S, Sofroniew MV, Voskuhl RR, Tiwari-Woodruff SK:Oestrogen receptor beta ligand: a novel treatment to enhance endogenous functional remyelination. Brain 2010; 133: 2999–3016.
23. Revankar CM CD, Sklar LA, Arterburn JB, Prossnitz ER: A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 2005; 3071625–1
24. Alkayed NJ, Goto S, Sugo N, Joh HD, Klaus J, Crain BJ, Bernard O, Traystman RJ, Hurn PD. Estrogen and Bcl-2: gene induction and effect of transgene in experimental stroke. J Neurosci. 2001;21:7543–7550
25. Wu TW, Wang JM, Chen S, Brinton RD. 17 Beta-estrogen induced Ca2+ influx via L-type calcium channels activates the Src/ERK/cyclic-AMP response element binding protein signal

pathway and BCL-2 expression in rat hippocampal neurons: a potential initiation mechanism for estrogen-induced neuroprotection. Neuroscience . 2005;135:59–72.
26. Choi YC, Lee JH, Hong KW, Lee KS. 17Beta-estrogen prevents focal cerebral ischemic damages via activation of Akt and CREB in association with reduced PTEN phosphorylation in rats. Fundam Clin Pharmacol. 2004;18:547–557.
27. Rau SW, Dubal DB, Bottner M, Gerhold LM, Wise PM. Estrogen attenuates programmed cell death after stroke-like injury. J Neurosci. 2003;23:11420–11426.
28. Fuentes N, Silveyra P. Fuentes N, Silveyra P. Estrogen receptor signaling mechanisms.Adv Protein Chem Struct Biol.2019;116: 135-170.
29. He J, Gao Y, Wu G, Lei X, Zhang Y, Pan W, Yu H. Molecular mechanism of estrogen-mediated neuroprotection in the relief of brain ischemic injury.BMC Genet.2018;19(1):46.
30. Engler-Chiurazzi EB, Covey DF, Simpkins JW. A novel mechanism of non-feminizing estrogens in neuroprotection. Exp. Gerontol. 2017;94: 99-102.
31. Yilmaz C, Karali K, Fodelianaki G, Gravanis A, Chavakis T, Charalampopoulos I, Alexaki VI. Neurosteroids as regulators of neuroinflammation.Front Neuroendocrinol. 2019; 55:100788.
32. Wang J, Hou Y, Zhang L, Liu M, Zhao J, Zhang Z, Ma Y, Hou W. Estrogen Attenuates Traumatic Brain Injury by Inhibiting the Activation of Microglia and Astrocyte-Mediated Neuroinflammatory Responses.Mol Neurobiol. 2021;58(3):1052-1061.
33. Kong D, Yan Y, He XY, Yang H, Liang B, Wang J, He Y, Ding Y, Yu H. Effects of Resveratrol on the Mechanisms of Antioxidants and Estrogen in Alzheimer's Disease.Biomed Res Int. 2019/ 8983752.
34. Hwang WJ, Lee TY, Kim NS, Kwon JS. The Role of Estrogen Receptors and Their Signaling across Psychiatric Disorders. Int J Mol Sci. 2020;22(1):373.
35. Tsialtas I, Georgantopoulos A, Karipidou ME, Kalousi FD, Karra AG, Leonidas DD, Psarra AG. Anti-Apoptotic and Antioxidant Activities of the Mitochondrial Estrogen Receptor Beta in N2A Neuroblastoma Cells. Int J Mol Sci.2021; 22(14):7620.
36. Uddin MS, Rahman MM, Jakaria M, Rahman MS, Hossain MS, Islam A, Ahmed M, Mathew B, Omar UM, Barreto GE, Ashraf GM. Estrogen Signaling in Alzheimer's Disease: Molecular Insights and Therapeutic Targets for Alzheimer's Dementia. Mol Neurobiol.2020; 57(6):2654-2670.