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Regulation of Immunity by Estrogen Through Sympathetic Nervous System in Aging

Yaşlanmada Sempatik Sinir Sistemi Aracılığıyla Östrojen Tarafından Bağışıklığın Düzenlenmesi

Ramasamy VASANTHAREKHA[1], Lalgi HIMA[1], Prabhu THANDAPANI[1], Sanjana KUMARAGURU[1], Ramesh Amirtha PRIYA[1], Poornima ANANTHASUBRAMANIAN[1], Srinivasan THYAGARAJAN[1]

Abstract

Women are more prone to autoimmune diseases, hormone-dependent cancers, osteoporosis, and neurodegenerative diseases with advancing age. The age-associated increase in the incidence and development of diseases and cancer is the result of a decline in immunocompetence facilitated by dysfunctions of nervous system and endocrine system. Reciprocal interactions between the brain and primary (bone marrow and thymus) and secondary (spleen and lymph nodes) lymphoid organs, via neurotransmitters and immune molecules determine an individual's health or disease status. One of the major contributing factors for this imbalance in homeostatic functioning of the neuroendocrine-immune system is estradiol (E2) that exerts its effects through alterations in the production of neurochemicals and immune mediators. Estrogen's reported beneficial effects such as anti-inflammatory and neuroprotective functions and deleterious effects of cancer progression are dependent upon age of women, type of cells and receptors, and the intracellular pathways and signaling molecules involved in mediating its effects. It is imperative that the diverse effects of estrogen on organ systems should be investigated via a longitudinal study beginning with early middle-aged rats to understand the long-term of exposure of estrogen on health and development of diseases. In this review, we present evidence for the biphasic effects of E2 on neural-immune interactions in the thymus, spleen, and lymph nodes and brain areas of early middle-aged female rats. These effects were dependent on pro/antioxidant status, and expression of growth factors and intracellular signaling molecules that are crucial to the neuronal plasticity influencing neuroprotection and inflammatory processes causing neurodegeneration.

Keywords: Inflammation, Cytokine, Hormone, Immunosenescence

Öz

Kadınlar, yaşlanma sırasında, otoimmün hastalıklara, hormona bağlı kanserlerin gelişimine, osteoporoza ve nörodejeneratif hastalıkları geliştirmeye daha çok eğilimlidir. Bu hastalıkların ileri yaşlarda daha çok ortaya çıkmasının sebebi, sinir sistemi ve endokrin sistemdeki bozukluklara bağlı olarak bağışıklıkta meydana gelen zayıflıktır. Beyin ile kemik iliği ve timus gibi birincil ve dalak ve lenf nodları gibi ikincil lenfoid organlar arasında nörotransmitterler aracılığı ile süren haberleşme, kişinin sağlıklı ya da hasta olmasını belirleyici niteliktedir. Nöroendokrin ve immün sistem arasındaki homeostatik işlevleri en çok belirleyen faktörlerin başında, sinirden salınan kimyasallar ve bağışıklık düzenleyen estrojen (E2) gelir. Estrojenin yangıyı azaltan, sinir koruyucu etkilerinin yanı sıra, yaşa, hücre tipleri ve reseptörlerin varlığına ve bu reseptörlerin etkinleştirdiği hücre içi moleküllerine bağlı olarak tümörün ilerlemesini artırıcı etkisi de bulunmaktadır. Estrojenin uzun süreli olarak organ sistemlerine, yaşlılığa ve sağlığa olan etkisini araştırmak için orta yaşlı sıçanlarda başlayan ve yıllar içinde devam eden çalışmalara gerek bulunmaktadır. Bu derlemede, E2'nin orta yaşlı dişi sıçanlarda timus, dalak, lenf nodları ve beyin bölgelerine olan ikili etkileri ile ilgili kanıtlar sunulmuştur. Bu etkiler, sinirlerin, sinir koruyucu ya da yangısal süreçler sonucunda siniri haraplayan durumlarını etkileyen, oksidan ya da anti-oksidan etkilere bağlı olarak oluşan ve büyüme faktörlerinin ifadesi ve hücre içi sinyaller ile ilgili moleküllere bağlı olarak meydana gelen sinirsel gelişim ile ilişkilidir.

Anahtar Kelimeler: Yangı, sitokin, hormon, bağışık-yaşlanma

[1]SRM Institute of Science and Technology, Biotechnology, Kattankulathur, India

Correspondence:
Srinivasan THYAGARAJAN, B.V.Sc., Ph.D.
Integrative Medicine Laboratory
Department of Biotechnology
School of Bioengineering
SRM Institute of Science and Technology,
Kattankulathur 603203,
Tamil Nadu, INDIA
Phone: 91-9940201794
E-mail: thyagarajan.s@ktr.srmuniv.ac.in

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Introduction

The notion that immune system functions autonomously was reversed by the research findings in the past few decades that demonstrated that neuroendocrine system regulates immune system and vice versa through the release of neurochemicals and

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hormonal secretion to maintain homeostasis and health.[1] Neurotransmitters and neuropeptides influence the secretion of releasing hormones from the hypothalamus which in turn cause the release of pituitary hormones and target endocrine gland hormones governing various physiological and metabolic activities, and immunity in the periphery.[2,3] Central nervous system activities are also controlled by the immune molecules and cytokines that cross the blood-brain barrier to maintain homeostasis.[4] The presence of sympathetic noradrenergic (NA) nerve fibers that innervate the primary (bone marrow and thymus) and secondary (spleen and lymph nodes) lymphoid organs which are in contact with the immune cells provided direct evidence for immunomodulation.[5,6] Aging is marked by reduced functional capacity of the neuroendocrine-immune network resulting in the cessation of regular reproductive cycles in the females, altered hormonal secretion, and enhanced incidence of cancers, metabolic, autoimmune, and neurodegenerative diseases.[2,5–7] Alterations in neuroendocrine-immune interactions with advancing age are more specific in nature and are characterized by differences in hormonal secretion that is associated with changes in reproductive cycles beginning in the middle age in both rodents and women as they become acyclic and menopausal, respectively, with advancing age.[3,8,9] Gonadal hormones, estrogen (17β-estradiol; E2) and progesterone, have been found to be responsible for these effects because of their multiple pathophysiological effects on the cells depending on their receptor status and modulatory effects.[10] Estrogen's effects have been purported to have both neuroprotection and risk of development of stroke and cancer [11,12] indicating its dual effects necessitate in-depth studies involving its role in continued exposure beginning at puberty, during regular and irregular reproductive cycles, and later during acyclicity that may be responsible for altered neural-immune interactions, development of diseases and cancer.

E2 is the major circulating hormone in animals and controls epithelial proliferation, apoptosis, and differentiation that are critical in female reproductive cycle and lactation.[9] In addition, estrogen regulates other physiological functions such as metabolism, vascular functions, reproductive characteristics and behavior, and pathological outcomes such as cancer, osteoporosis, and neurodegenerative and autoimmune diseases.[10] The effects of E2 are mediated through the nuclear receptors, ERα and ERβ, directly influencing the expression of genes and GPR30, a receptor present in endoplasmic reticulum and plasma membrane modulating non-genomic responses.[9–11] Published studies from our laboratory form the basis for this short review where we discuss the role of E2 modulating neural-immune interactions in the brain areas (frontal cortex, striatum, medial basal hypothalamus, and hippocampus) and lymphoid organs (thymus, spleen, and lymph nodes) of early middle-aged rats mediated through the antioxidant enzymes, growth factors, and intracellular signaling molecules and transcription factors (ERK, CREB, Akt, mTOR, and NF-κB).[13–16]

Age-related alterations in the neuroendocrine and immune systems

Regular estrous cycles in young (2-to 8-month-old) female rats are followed by irregular cycles in early middle-aged rats (8-to 9-month-old) characterized by distinct fluctuations in hypothalamic, pituitary, and gonadal hormones and the most marked one is prolonged E2 secretion and delayed ovulation during middle age.[2] These stages are followed by pseudopregnancy and persistent anestrus/acyclicity states due to progressive loss of estrogen feedback on luteinizing hormone (LH) secretion that may promote the aging process.[2,7] The characteristic delay and attenuated LH surge observed in middle-aged female rats is because of decline in gonadotropin-releasing hormone (GnRH) neuronal activity influenced by altered afferent neuronal activities of aminergic and peptidergic neurons.[3,9] Further proof for this notion was observed in our studies where we had demonstrated altered NE release from the hypothalamic areas, medial preoptic area and medial basal hypothalamus, in middle-aged female rats.[17,18] In addition to hypothalamic catecholamines, several other neurotransmitters and neuropeptides modulate hypothalamic-pituitary-gonadal axis to not only alter reproductive cycles but also, have a key role in the development of female-specific diseases.[17,18]

Besides alterations in reproductive cycles with advancing age, alterations are observed in immunity such as suppression of acquired immune responses and enhanced inflammatory immune responses that favor chronic inflammation.[19] Impaired signaling of Toll-like receptors, increased NF-κB activation, and thymic involution resulting in imbalance in naïve and memory T cells, loss of T cell receptor numbers, and functional alterations in Th1, Th2, Th17, and Treg cells are some of the key changes in innate and acquired immunity that in turn, promote altered intracellular signaling which may

be ultimately liable for the inability of an individual to fight against antigenic challenges.[19,20] Although there is no published literature examining longitudinal changes in innate and acquired immunity during reproductive aging in females, we have provided evidence for a decline in natural killer (NK) cell activity, and Concanavalin (Con A)-induced T cell proliferation and IL-2 production in early middle-aged female rats.[21]

Sympathetic noradrenergic neuronal activity in the lymphoid organs influences immunity

Studies demonstrating the presence of noradrenergic and peptidergic nerve fibers in the primary (bone marrow and thymus) and secondary (spleen, lymph nodes, and lymphoid tissues) lymphoid organs, release of neurochemicals by these nerve fibers, and subsequent interactions of these neurochemicals with the appropriate receptors on T and B lymphocytes, macrophages, and other immune cells dispelled the long-held view that immune system functions independently of other systems.[5,6] Experimental studies involving pharmacological and surgical methods provided evidence for the release of NE by the sympathetic NA nervous system and its binding to the adrenergic receptors on the macrophages and lymphocytes to modulate immune responses.[1,5,6]

Thymus: Sympathetic NA nerve fibers are observed in the cortical regions which are in close contact with the thymocytes and other non-functional cells in the thymus of young rats.[5,6] With advancing age, the density of NA nerve fibers increased in the thymus of female rats similar to male rats accompanied by an increase in thymic NE concentration and NE content that may be the result of thymic involution due to an increase in the levels of gonadal hormones.[21] These changes in sympathetic NA innervation may influence thymopoiesis during aging.

Spleen: In the young male and female rats, sympathetic NA nerve fibers coursing along the splenic artery enter at the hilar region of the spleen, travel along the subcapsular and trabecular areas, and ultimately, enter the lymphoid parenchyma of the spleen, white pulp, where dense innervations is observed in T cells-rich area, periarteriolar lymphatic sheath and also, the outer area of the white pulp, marginal zone.[5,6] In contrast to the thymus, there was a drastic reduction in the sympathetic NA innervation in the spleen accompanied by a reduction in NE concentration in the hilar region of the spleens of old female rats.[21] Interestingly, NE content in the end region and whole spleen declined in the early middle-aged rat indicating that age-related alterations in NA innervations begins at this age.[21] Paralleling these alterations in splenic NA innervation, there was a decline in NK cell activity and IL-2 production by splenocytes in early middle-aged rats while there was a decline in T cell proliferation, IFN-γ and IL-2 production, and NK cell activity in the spleens of old female rats.[21]

Lymph nodes: In young rats, NA nerve fibers enter as plexuses along with the blood vessels into the lymph nodes which then extend to the subcapsule and into the medullary regions.[5,6] Subsequently, NA nerve fibers enter cortical parenchyma and paracortical regions that are rich in T lymphocytes.[21] Similar to the spleen, NA nerve fibers are scant or nearly absent in regions rich in B lymphocytes. However with advancing age, a marked reduction in the density of NA nerve fibers in all the compartment of mesenteric lymph nodes (MLN) was observed including the paracortical regions of early middle-aged and old female rats accompanied by a significant reduction in NE concentration.[21]

These findings involving age-related alterations in sympathetic NA innervations in primary and secondary lymphoid organs accompanied by changes in immune responses demonstrate that gonadal hormones especially, estrogen, may play a crucial role in neural-immune interactions in health and diseases.

Neuroimmunomodulation by estrogen in the central nervous system and lymphoid organs

Numerous studies have demonstrated the beneficial effects of estrogen such as neuroprotection in ischemic brain injury and Alzheimer's disease, reducing the risk of heart diseases, etc in animal models.[21] These findings necessitated a study, Women's Health Initiative (WHI) study, involving treatment of postmenopausal women with estrogen alone or estrogen and progesterone and the results from this multicentric study showed that there was an increased chance of developing cancer and stroke, and hormonal treatment interfered with cognitive performance.[12,22,23] Although these results allude to the deleterious effects of estrogen alone or in combination with progesterone, subsequent analysis of these data have

suggested that these effects are dependent upon the age of women, hysterectomy status, and type of estrogen used and emphasized the need for a longitudinal study during the reproductive aging process.[24]

In an attempt to understand the role of estrogen on neural-immune interactions, we performed a study in which ovariectomized (OVX) early middle-aged animals (8-to 9-mo-old female Sprague-Dawley rats) were treated with two doses of E2 (30-day implantation of 0.6 μg or 300 μg pellets of 17β-estradiol) and its effects investigated on various areas of brain [frontal cortex (FC), striatum (STR), medial basal hypothalamus (MBH), and hippocampus (HP)], thymus, spleen, and lymph nodes.[13–16]

Brain: Treatment with E2 augmented p-tyrosine hydroxylase expression in the frontal cortex and hippocampus, decreased NF-κB expression in the frontal cortex and striatum, and enhanced cholinergic neuronal activity in hippocampus and striatum accompanied by estrogen-induced reversal of age-and OVX-related decline in intracellular signaling molecules (p-ERK, p-CREB, and p-Akt).[15] Also, there was an increase in the activities of antioxidant enzymes [superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), glutathione-S-transferase (GST)] and nitric oxide (NO) production, and a simultaneous decline in lipid peroxidation. Altogether, these results demonstrated that estrogen in early middle-aged female rats provided neuroprotection on catecholaminergic and cholinergic neurons mediated through downregulation of inflammatory mediators and upregulation of cellular survival mediators. Our results are in agreement with the findings from published literature which had reported similar beneficial effects of E2 on cognition and behavior facilitated through a number of intracellular molecules to achieve neuroprotection, and anti-inflammatory effects.[11] However, earlier studies have reported that treatment with physiological levels of estrogen is known to cause lesions in the hypothalamic arcuate nucleus interfering with cytoarchitecture and functions of neurons, microglia and astrocytes indicating that estrogen's effects on neuronal and immune functions may be dependent on several factors including age, dose, and duration of treatment.[25]

Thymus: Estrogen treatment inhibited the parathormon expression in the thymus and increased the expression of p-ERK, p-CREB, and p-Akt indicating that E2 causes neurodegeneration in the thymus possibly through increased production of free radicals.[16] These effects may accentuate E2-induced inhibition of the early thymic progenitor cell population and arrest T cell maturation through ERα and Fas/FasL pathway to create inflammatory environment.[26,27]

Spleen: Administration of E2 altered tyrosine hydroxylase expression and immune responses in a dose-dependent manner in the spleen: low dose E2 suppressed IFN-γ production, increased Con A-induced lymphoproliferation, IL-2 production, expression of intracellular signaling molecules (p-ERK, p-CREB, and p-Akt), antioxidant enzyme activities (SOD, CAT, and GST), and NO production while high dose E2 inhibited Con A-induced lymphoproliferation but enhanced the expression of p-TH, NGF, intracellular signaling molecules (p-ERK and p-CREB), and activities of antioxidant enzymes (SOD and GST).[13] However, these beneficial effects were associated with increase in lipid peroxidation and protein carbonyl formation by both low and high doses indicating that estrogen may exert neurotoxic effects with prolonged exposure.[13] Similar effects of estrogen were demonstrated in the hypothalamic preoptic area where it enhanced proinflammatory cytokine production and decreased NE synthesis while it promoted sympathetic neuronal degeneration in the myometrium and vagina accompanied by inhibition of growth factor production and axon guidance molecules.[28,29] T cells especially CD4+ T cells and B cells have increased expression of β2-ARs suggesting that immune responses are regulated by several factors influencing their activation. Coincubation of E2 and β2-AR agonist influenced immune responses via ERα involving cell survival (ERK, PKA and PKC) and inflammation-inducing (NF-κB and NO) pathways indicating that it may differentially regulate acquired and inflammatory immune responses.[30]

Lymph nodes: Treatment of early middle-aged OVX female rats with estrogen enhanced lymphoproliferation, IFN-γ and TNF-α, ROS production, expression of p-NF-κB (p50 and p65), p-mTOR, and p-Akt/Total Akt and decreased cytochrome C oxidase activity in the draining lymph nodes (axillary and inguinal lymph nodes).[14] Both doses of estrogen increased extent of lipid peroxidation and NO production.[14] However, similar treatment with E2 had different effects in MLN where it enhanced the activities of antioxidant enzymes without an increase in free radical generation suggesting that estrogen exerts neuroprotective effects on sympathetic NA activity in MLN.[16] The differential effect of estrogen on these two

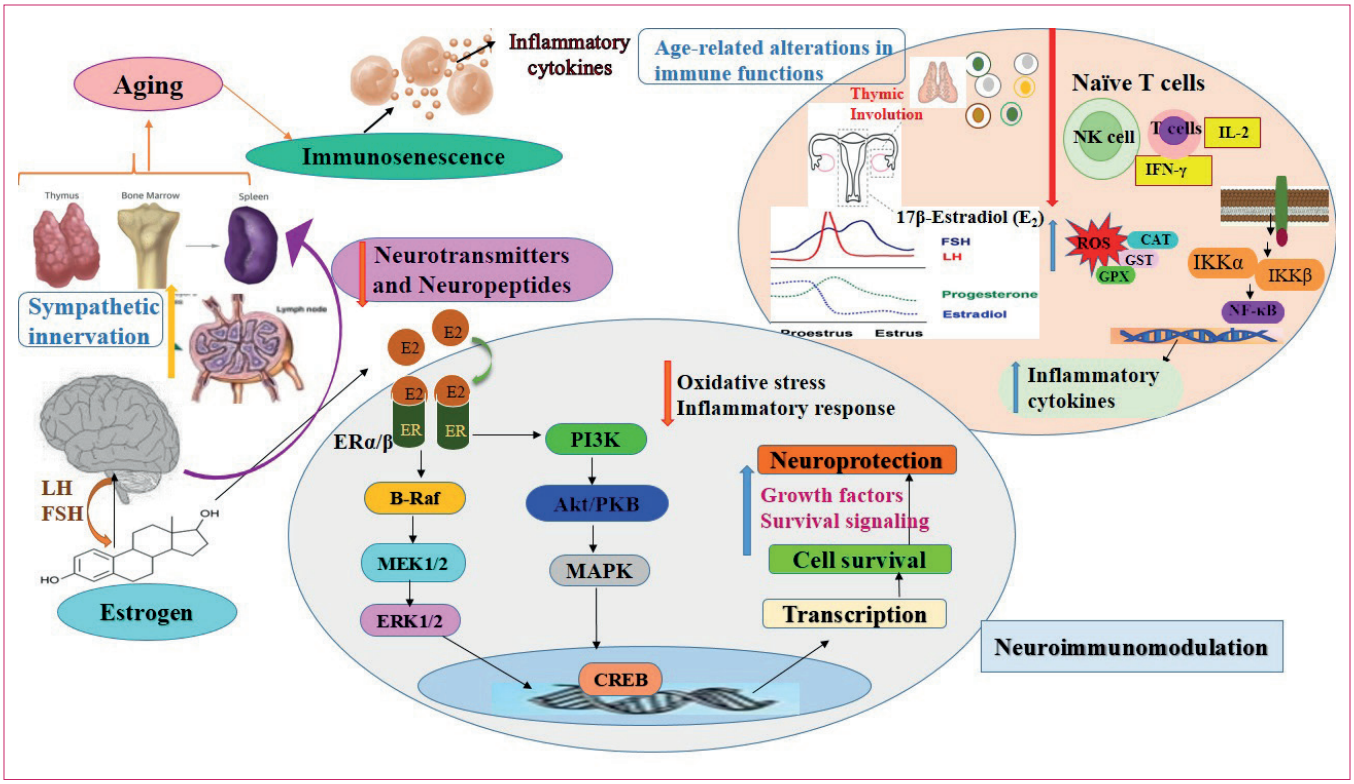

Figure 1. Summary of the results involving pathways that mediate the neuroprotective and neurodegenerative properties of estrogen in brain areas and lymphoid organs. Abbreviations; **ER**- Estrogen receptor, **LH-** luteinizing hormone, **FSH-** follicle stimulating hormone, **ERK**-Extracellular signal regulated kinase, **Akt**-Protein kinase B, **PI3K**- Phosphoinositide 3-kinase, **CREB**-cAMP response element binding protein, **NF-κB-** NF-κB-Nuclear factor kappa-chain-enhancer of activated B cells, **CAT**- catalase, **GPX**- glutathione peroxidase, **GST**- glutathione-s-transferase, **Cyt C**- Cytochrome C, **ROS**- reactive oxygen species

lymph nodes may be due to possible differences in stromal cells that may regulate trafficking of immune cells and immune molecules through the lymph nodes.[31]

Neuroendocrine-immune interactions in women: Effects of estrogen

Reproductive aging in women is characterized by similar changes in rodents such as irregular menstrual cycles preceding menopause, fluctuations in estrogen levels during perimenopause, and an increase in estradiol/progesterone ratio in the perimenopausal stage.[8,32] As discussed earlier in this review, a multicentric Women's Health Initiative study was conducted to investigate whether the beneficial effects of estrogen observed in experimental animals are applicable to women. But the results from these studies involving hormone replacement therapy showed that it would increase the risk of breast cancer and cardiovascular diseases that may be related to several factors including, age, hysterectomy status, dose and type of estrogen, and duration of treatment.[12,22,23] Lack of concrete evidence for estrogen-induced modulation of neural-immune interactions through longitudinal studies in both rodents and women hampers the understanding of its physiological and intracellular effects in health and diseases. We have demonstrated that a decline in estrogen and progesterone levels in middle-aged perimenopausal women was accompanied by a decrease in T lymphocyte proliferation, IFN-γ production, NGF and p-CREB expression, SOD and catalase activities while there was an increase in lipid peroxidation.[33] In another study, we found a similar decrease in T cell proliferation, expression of intracellular signaling molecules (p-ERK, p-CREB, and p-Akt), and activities of SOD, catalase, and GST accompanied by an increase in TNF-α production in PBMCs of middle-aged perimenopausal women suggesting that a decline in acquired immune responses and enhanced inflammatory immune responses may predispose women to the onset of diseases and cancer.[unpublished data] Recently, we have presented evidence for an association between altered immune responses, cholesterol metabolism, heme metabolism, calcium homeostasis, and enzyme profiles and cognitive decline in elderly men and women with mild cognitive impairment and Alzheimer's

disease emphasizing the importance of understanding the neuroendocrine-immune interactions in the development of age-associated development of diseases and cancer.[34]

Conclusions

The results from our studies demonstrate that estrogen causes neurodegeneration of sympathetic NA activity in the thymus, simultaneously confers neuroprotection and induces inflammatory environment in the spleen and lymph nodes, and has anti-inflammatory and neuroprotective effects in the brain areas of early middle-aged female rats. These changes were accompanied by alterations in compensatory factors involving activities of antioxidant enzymes and growth factors, and intracellular signaling molecules. Such effects were also reflected in middle-aged women suggesting that estrogen is critical to the remodeling of neural-immune network and the modalities of this effect may influence the development of diseases and cancer in elderly women.

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