Brain Research Bulletin 124 (2016) 238–253

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Estrogen-induced neuroprotective and anti-inflammatory effects are dependent on the brain areas of middle-aged female rats

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ARTICLE INFO

Article history:
Received 28 December 2015
Received in revised form 24 May 2016
Accepted 26 May 2016
Available online 27 May 2016

Keywords:
Tyrosine hydroxylase
Nerve growth factor
Cholinesterase
ERK
CREB
Akt

ABSTRACT

Background: Reproductive aging in females is characterized by fluctuations and precipitous decline in estrogen levels, which may lead to reduction in cognitive function and age-associated neurodegenerative disorders. The nature of estrogen-mediated neuronal plasticity is unknown during reproductive aging. We hypothesize that estrogen treatment of early middle-aged ovariectomized rats may exert specific effects in the brain by modulating signaling pathways regulating metabolic enzymes, inflammatory markers, antioxidant status, cholinergic function and survival signals.

Purpose: To investigate the mechanisms of estrogen-induced effects on neuroprotection and neuroinflammation through the involvement of intracellular signaling pathways in brain areas of ovariectomized (OVX) middle-aged (MA) female rats.

Methods: Ovariectomized early MA female Sprague-Dawley rats (n = 8/group) were implanted with 17β-estradiol (E_2) 30-day release pellets (0.6 μg and 300 μg). At the end of the treatment period, frontal cortex (FC), striatum (STR), medial basal hypothalamus (MBH), and hippocampus (HP) were isolated and examined for the expression of tyrosine hydroxylase (p-TH), nerve growth factor (NGF), p-NF-κB (p50 and p65)and p-ERK, p-CREB, p-Akt, and activities of cholinesterases and antioxidant enzymes, key regulatory enzymes of metabolic pathways, and nitric oxide production.

Results: E_2 enhanced p-TH expression in FC and HP, reduced NGF expression in HP, and suppressed p-NF-κB expression in FC and STR. It also increased the expression of molecular markers (p-ERK, p-CREB and p-Akt), and nitric oxide production in various brain areas, while differentially regulating the activities of metabolic enzymes and cholinesterases.

Conclusion: Estrogen modulates the neural and inflammatory factors, and intracellular markers depending on the brain areas that may influence differential remodeling of neuronal circuitry which can be used to develop therapeutic strategies in cognitive impairment and neurodegenerative disorders in aging.

1. Introduction

Reproductive senescence in female rats is characterized by a gradual transition from regular reproductive cycles to irregular estrous cycles and finally, to anestrus due to alterations in pituitary and gonadal hormones (Meites, 1991; Wise, 1982a). During middle age, prior to the irregular estrous cycles, the ability of 17-β estradiol to induce gonadotropin releasing hormone/luteinizing hormone (GnRH/LH) surge is diminished that may be attributed to age-related changes in the synthesis, release and metabolism of catecholamines in discrete areas of the hypothalamus (Downs and Wise, 2009; MohanKumar et al., 1994; MohanKumar et al., 1995; MohanKumar et al., 1997; ThyagaRajan et al., 1995; Wise, 1982b; Wise, 1984). Estrogen's, 17β-estradiol, neuroprotective and anti-inflammatory actions involving astrocytes and microglia interactions have been suggested as a potential therapeutic tool in the treatment of neurodegenerative disorders (Chakrabarti et al., 2014). These effects are also mediated through neurotrophins that promote growth, survival, and plasticity of neurons in central and peripheral nervous system (Babayan and Kramár, 2013; Mónica Brauer and Smith, 2015). Studies from our laboratory

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http://dx.doi.org/10.1016/j.brainresbull.2016.05.015
0361-9230/

and others have shown that during aging, estrogen exerts a dual role by protecting central neurons and mediating peripheral denervation depending upon the dose and duration of estrogen exposure and areas examined (Priyanka et al., 2013c; Zoubinaet al., 2001; ThyagaRajan and Priyanka, 2011). Centrally, administration of estrogen was found to increase the tyrosine hydroxylase-positive(TH+) nerve fibers in the prefrontal cortex of old rats, while in the periphery, it reduced the sympathetic innervation in uterus (Chisholm et al., 2012; Mónica Brauer and Smith, 2015; Zoubina et al., 2001). Recently, we have provided evidence for an increase in p-TH expression in the spleen in association with IFN-γ production by lymphocytes that may be modulated by estrogen receptor subtype-dependent actions (Kale et al., 2014; Priyanka et al., 2013b).

These neuroprotective effects of estrogen are mediated through its regulatory actions on various intracellular signaling pathways such as activation of ERK expression in dorsal hippocampus, and induction of phosphorylation of the cyclic AMP response element binding protein (CREB) (Fernandez et al., 2008; Carlstrom et al., 2001; Wade and Dorsa, 2003). Similar to MAPK and CREB, neuroprotective role of Akt pathways has been examined in several studies (Grove-Strawser et al., 2010; Sanchez et al., 2012). A decline in estrogen levels during reproductive aging in women and rodents has been associated with cognitive impairment and development of neurodegenerative disorders (Jacome et al., 2010; Tang et al., 1996). This may be due to a decline in basal forebrain cholinergic neurons as they are known to be a key neurochemical in the maintenance of cognitive functions (Gibbs, 2010; Norbury et al., 2003). In addition, nitric oxide (NO) is another neurotransmitter synthesized in the central nervous system (CNS) which is involved in memory and learning (Paul and Ekambaram, 2011). Inhibition of NO synthesis has been shown to cause a decrease in ACh release that may lead to altered conditioned response in rats (Kopf et al., 2001). Similar to its neuroprotective role, anti-inflammatory effects of estrogen have been examined in several studies (Ghisletti et al., 2005; Zhang et al., 2014). Treatment with estrogen in ovariectomized mutant presenilin 2 mice protects against presenilin 2-driven Alzheimer disease (AD) development by inhibiting the expression of NF-κB (Hwang et al., 2016).

The adult brain requires disproportionate amount of energy and majority of it is acquired through uninterrupted supply of glucose to meet the physiological demands of the central nervous system (Shulman et al., 2003). Increased energy demand may be achieved through the estrogen's ability to upregulate the expression of glucose transporter subunits influencing increased transport of glucose across the blood brain barrier and also, through increased activities of glycolytic enzymes (Kostanyan and Nazaryan, 1992; Shi and Simpkins, 1997). Furthermore, a sizable portion of ATP in the brain is consumed by Na+/K+- ATPase, a key enzyme that is crucial to the energy metabolism and an important target for estrogens and estrogen-like molecules (Magistretti, 2008). Mitochondrial dysfunction in Alzheimer's disease and Parkinson's disease accounts for the hypometabolic state which may exist prior to the incidence of the diseases and hasten the disease progression (Brinton, 2008; Shi and Xu, 2008). Therefore, these key enzymes are linked either directly or indirectly to neuronal survival and any metabolic dysfunctions in mitochondria may have a significant role in the development of neurodegenerative diseases (Atamna and Frey, 2007; Soane et al., 2007).

The present study examines whether estrogen supplementation in early middle-aged rats is neuroprotective and anti-inflammatory through the involvement of nerve growth factor and compensatory factors such as enzymes of the energy metabolism and antioxidant enzymes mediated through intracellular signaling molecules. For this purpose, early middle-aged female OVX Sprague-Dawley rats were implanted with 30-day subcutaneous (s.c.) placebo or estrogen pellets and the brain areas (frontal cortex, striatum, medial basal hypothalamus, and hippocampus) were examined for the expression of tyrosine hydroxylase (p-TH), nerve growth factor (NGF), p-NF-κB, intracellular signaling pathway molecules (p-ERK, p-CREB, and p-Akt), and compensatory factors (nitric oxide production, cholinesterase activity, and enzymes involved in energy metabolism and maintenance of antioxidant status).

2. Materials and methods

2.1. Animals

Young and early middle-aged female Sprague-Dawley (SD) rats were purchased from the National Institute of Nutrition, Hyderabad and housed for acclimatization at the University's Animal House. The experiments began when the young rats reached the age of 3 months and the early middle-aged rats were 8–9 months old. Estrous cycles were monitored by vaginal smears for 8–10 days to establish regular cyclicity in young and middle-aged rats. Food pellets and water were provided *ad libitum* and animals were housed under hygienic conditions. All animal experiments were conducted in accordance with the principles and procedures outlined and approved by the University's Institutional Animal Ethics Committee.

2.2. Treatment

The early middle-aged SD female rats (MA) were either sham-operated or ovariectomized and randomly distributed into a sham-operated control group (MA Sham; n = 8), a placebo-treated ovariectomized group (MA OVX + Placebo; n = 8) and two estrogen treatment groups (MA OVX + E 0.6 μg; n = 8 and MA OVX + E 300 μg; n = 8). A separate group of young 3-month old female rats (Young; n = 8) served as control animals. Initially the middle-aged female rats were either sham operated or bilaterally ovariectomized and after a week of recovery, 30-day placebo or estrogen pellets [17β-estradiol (0.6 μg or 300 μg), Innovative Research America, Florida, USA] were implanted subcutaneously in the nape of the neck. The doses were selected based on preliminary study in our laboratory and a published study (Kasturi et al., 2009). At the end of the treatment period, the animals were sacrificed by decapitation between 08:00 to 10:00 h. The brain areas [frontal cortex (FC), striatum (STR), medial basal hypothalamus (MBH), and hippocampus (HP)] were collected and frozen at −80 °C for Western blotting, molecular markers and enzyme assays.

2.3. Western blot analysis

Western blot analysis has been described previously (Priyanka et al., 2013a). Briefly, brain tissues were homogenized in lysis buffer, centrifuged at 1500 rpm for 10 min and the supernatants obtained were used for Western blotting. Protein concentration was estimated using Folin and Ciocalteu's phenol reagent (Sigma, St. Louis, MO). Thirty (30) μg of total protein was electrophoresed on 10% SDS-polyacrylamide gels and blotted on 0.2 μm nitrocellulose membranes (Sigma, St. Louis, MO). The membranes were blocked for 1 h and incubated overnight in blocking buffer containing primary antibody [p-TH (Ser 40; 1:750); NGF (M-20; 1:750); NF-κB (p50; NLS;1:750); NF-κB (p65;Ser 536;1:750) and β-Actin (C4; 1:3000)] (Santa Cruz Biotechnology, Santa Cruz, CA). The blots were then washed with Tris-buffered saline, incubated with HRP-anti rabbit IgG (1:10000) and HRP-anti mouse IgG(1:10000) (Santa Cruz Biotechnology, Santa Cruz, CA) and developed using 3,3′,5,5′-tetramethylbenzidine (TMB) Liquid Substrate System (Sigma, St. Louis, MO). Western Blotting was performed for at least 4 samples