NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION
RESEARCH ARTICLE
Q. Xiao et al. / Neuroscience xxx (2018) xxx–xxx

Protective Effects of 17β-Estradiol on Hippocampal Myelinated Fibers in Ovariectomized Middle-aged Rats

Qian Xiao,[a,b,c†] Yanmin Luo,[a,b,c†] Fulin Lv,[a,b] Qi He,[a,b] Hong Wu,[a,b] Fenglei Chao,[a,b] Xuan Qiu,[a,b] Lei Zhang,[a,b] Yuan Gao,[a,b,d] Chunxia Huang,[a,b,e] Sanrong Wang,[a,b] Chunni Zhou,[a,b,c] Yi Zhang,[a,b,c] Lin Jiang[a,b,c] and Yong Tang[a,b*]

[a] Department of Histology and Embryology, Chongqing Medical University, Chongqing 400016, People's Republic of China

[b] Laboratory of Stem Cells and Tissue Engineering, Department of Histology and Embryology, Chongqing Medical University, Chongqing 400016, People's Republic of China

[c] Department of Laboratory Medicine, Key Laboratory of Diagnostic Medicine (Ministry of Education), Chongqing Medical University, Chongqing 400016, People's Republic of China

[d] Department of Geriatrics, First Affiliated Hospital, Chongqing Medical University, Chongqing 400016, People's Republic of China

[e] Department of Physiology, Chongqing Medical University, Chongqing 400016, People's Republic of China

Abstract—Estrogen replacement therapy (ERT) improves hippocampus-dependent cognition. This study investigated the impact of estrogen on hippocampal volume, CA1 subfield volume and myelinated fibers in the CA1 subfield of middle-aged ovariectomized rats. Ten-month-old bilaterally ovariectomized (OVX) female rats were randomly divided into OVX + E2 and OVX + Veh groups. After four weeks of subcutaneous injection with 17β-estradiol or a placebo, the OVX + E2 rats exhibited significantly short mean escape latency in a spatial learning task than that in the OVX + Veh rats. Using stereological methods, we did not observe significant differences in the volumes of the hippocampus and CA1 subfields between the two groups. However, using stereological methods and electron microscopy techniques, the total length of myelinated fibers and the total volumes of myelinated fibers, myelin sheaths and myelinated axons in the CA1 subfields of OVX + E2 rats were significantly 38.1%, 34.2%, 36.1% and 32.5%, respectively, higher than those in the OVX + Veh rats. After the parameters were calculated according to different diameter ranges, the estrogen replacement-induced remodeling of myelinated fibers in CA1 was mainly manifested in the myelinated fibers with a diameter of <1.0 μm. Therefore, four weeks of continuous E2 replacement improved the spatial learning capabilities of middle-aged ovariectomized rats. The E2 replacement-induced protection of spatial learning abilities might be associated with the beneficial effects of estrogen on myelinated fibers, particularly those with the diameters less than 1.0 μm, in the hippocampal CA1 region of middle-aged ovariectomized rats.

Key words: ovariectomy (OVX), estrogen replacement therapy (ERT), hippocampus, myelinated fiber, stereology.

INTRODUCTION

Numerous clinical studies have shown that females have high risks of cognitive impairment and memory deterioration after surgically induced or natural menopause due to dramatic estrogen decline (Halbreich et al., 1995; Bove et al., 2014; Rocca et al., 2014; Ryan et al., 2014; Koebele et al., 2017). Accordingly, estrogen supplementation provides long-term protection against cognitive impairment in the early phase of menopause (Sherwin, 2006; Daniel et al., 2015; Engler-Chiurazzi et al., 2016; Hamson et al., 2016). Moreover, in ovariectomized animal models, a 17β-estradiol treatment improved multiple hippocampus-dependent cognitive functions, including spatial learning ability, spatial reference memory, and spatial working memory (Daniel et al., 2006; Xu and Zhang, 2006; Liu et al., 2008; Talboom et al., 2008; Kiss et al., 2012; Han et al., 2013; Uzum et al., 2016). Estrogen has been shown to improve cognitive decline caused by estrogen deficiency. However, the exact hippocampal morphological mechanisms by which estrogen improves hippocampus-related cognition are a subject of active investigation.

The integrity of the hippocampal formation is involved in the regulation of spatial learning and memory processes (Brasted et al., 2003; Broadbent et al., 2004; Suzuki, 2007; Addante, 2015). Furthermore, the hippocampal formation is highly sensitive to estrogen deficiency and estrogen replacement (Sarvari et al., 2015;

*Correspondence to: Y. Tang, Department of Histology and Embryology, Faculty of Basic Medical Sciences, Chongqing Medical University, Chongqing, People's Republic of China. Fax: +86-23-68485868.
E-mail address: ytang062@163.com (Y. Tang).
† These authors contributed equally to this article.

https://doi.org/10.1016/j.neuroscience.2018.06.006
0306-4522/

Please cite this article in press as: Xiao Q et al. Protective Effects of 17β-Estradiol on Hippocampal Myelinated Fibers in Ovariectomized Middle-aged Rats. Neuroscience (2018), https://doi.org/10.1016/j.neuroscience.2018.06.006

Yan et al., 2017) because a large number of estrogen receptors are distributed within the hippocampus, and higher ERβ levels than ERα levels have been detected in human and rat hippocampi (Shughrue et al., 1997; Foster, 2012). In a study of the relationship between the menstrual cycle and hippocampal volume, increased estrogen levels were associated with increased hippocampal volume in humans during the post-menstrual period (Protopopescu et al., 2008; Lisofsky et al., 2015), suggesting a correlation between estrogen levels and hippocampal volume. According to some cross-sectional studies, larger hippocampal volumes were observed in menopausal subjects subjected to estrogen replacement therapy (ERT) than in peers without hormone therapy (Eberling et al., 2004; Erickson et al., 2005, 2010; Lord et al., 2008). On the other hand, Albert et al. (2017) found that 3 months of estradiol administration did not change the total hippocampal volume in younger or older postmenopausal women. Using magnetic resonance imaging scans, Greenberg et al. (2006) found that the total hippocampal volume was not significantly different between elderly women (average age of nearly 71) who were or were not using hormone therapy. Low et al. (2006) also reported a negligible difference between women (aged 60–64 years) treated with ERT and untreated women. Furthermore, postmenopausal women receiving hormone therapy exhibited smaller hippocampal volumes than nonusers in other studies (Coker et al., 2009; Lord et al., 2010). Thus, the results of previous studies regarding the effects of estrogen on hippocampal volume are contradictory. Moreover, all previous human studies had a cross-sectional design and lacked well-controlled, randomized trials and accurate measurements. Therefore, studies of the effects of ERT on hippocampal volume in experimental animals are still needed.

Estrogen improves hippocampus-dependent cognition in ovariectomized animals by regulating hippocampal synaptic plasticity (Gould et al., 1990; Woolley, 1998; Xu and Zhang, 2006; Liu et al., 2008; Hara et al., 2015), altering synaptic circuitry, increasing the levels of hippocampal synaptic proteins (Frick et al., 2002; Akama and Mcewen, 2003; Choi et al., 2003; Oberlander and Woolley, 2016), and enhancing cell proliferation in the dentate gyrus (Mazzucco et al., 2006). The hippocampus contains many myelinated fibers, including those within the hippocampus and those projecting into or out of the hippocampus (Arnold and Trojanowski, 1996; Haber et al., 2009). Since myelin sheaths are well known to enhance the speed and efficacy of electrical conduction in axon, the myelination of axons in hippocampus may profoundly influence learning and memory by regulating information processing in neural circuits. Using electron microscopy, Unal et al. (2012) discovered myelin degeneration in the hippocampi of ovariectomized rats. According to the study by Luo et al. (2016), 17β-estradiol supplementation increases the volumes of myelinated fibers and myelin sheaths in the cerebral white matter of middle-aged ovariectomized rats. Estrogen receptor agonists or ligand therapy have been shown to protect against demyelination, sustain myelination, and promote remyelination in the corpus callosum of a mouse model of multiple sclerosis (Crawford et al., 2010; Kumar et al., 2013; Patel et al., 2013; Moore et al., 2014). Pintzka and Haberg (2015) reported a localized volume-sparing effect of hormone therapy on the hippocampal cornu ammonis (CA1) subfield in postmenopausal women, an area affected during the development of mild cognitive impairment (MCI) and Alzheimer's disease (AD). As shown in the study by Choi et al. (2003), estradiol increases pre- and post-synaptic protein expression in the hippocampal CA1 subregion of female rhesus macaques. Because these studies showed the sensitivity of the CA1 region to estrogen, we hypothesized that estradiol replacement might exert positive effects on myelinated fibers in the hippocampal CA1 subfield of ovariectomized females. In the current study, 10- to 12-month-old female Sprague–Dawley rats were selected to receive bilateral ovariectomy and ERT for 4 weeks. The effects of the 4-week 17β-estradiol replacement on hippocampal volume and myelinated fibers in the CA1 subfield were investigated using transmission electron microscopy and stereological methods.

EXPERIMENTAL PROCEDURES

Animals

Nineteen female Sprague–Dawley rats (10-month-old), mainly retired breeders, were provided by the Third Military Medical University (Chongqing, People's Republic of China). Rats were housed in groups of 4–5 animals per cage in a temperature-controlled room (22 ± 2 °C with a 12-h/12-h light–dark cycle) and food and water available ad libitum. Animal care and treatments were administrated according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23)

Surgical procedures and estrogen replacement

All rats were intraperitoneally anesthetized with pentobarbital sodium (1%, 4 mL/kg body weight) and underwent a bilateral ovariectomy (OVX). After a one-week recovery period, vaginal smears collected daily for 6 days indicated that all rats were acyclic (persistent estrus). Four weeks after surgery, serum estrogen concentrations were measured to ensure the complete removal of all ovarian tissues. Then, the animals were randomly divided into OVX + estrogen (E2) (n = 10) and OVX + vehicle (Veh) groups (n = 9) and received subcutaneous injections of 17β-estradiol (Abcam, ab120657, 10 mg/0.1 mL, 0.25 mL/kg) (Gould et al., 1990; Luo et al., 2016) or Veh (sesame oil, 0.25 mL/kg) subcutaneous injection, respectively, at the nape of their neck for 4 weeks.

Morris water maze task

In the last week of treatment, rats were assessed using the DMS-type 2 automatic Morris water maze (Institute of Materia Medica, Chinese Academy of Medical Sciences). The maze consisted of a pool (0.5 m tall, 1.5 m in diameter) and a transparent cylindrical platform

Please cite this article in press as: Xiao Q et al. Protective Effects of 17β-Estradiol on Hippocampal Myelinated Fibers in Ovariectomized Middle-aged Rats. Neuroscience (2018), https://doi.org/10.1016/j.neuroscience.2018.06.006