Hormones and Behavior 63 (2013) 144–157

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

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

17β-Estradiol, but not estrone, increases the survival and activation of new neurons in the hippocampus in response to spatial memory in adult female rats

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ARTICLE INFO

Article history:
Received 1 June 2012
Revised 18 September 2012
Accepted 22 September 2012
Available online 12 October 2012

Keywords:
Hippocampus
Morris water maze
17β-estradiol
Estrone
Dentate gyrus
Adult neurogenesis
Zif268
Immediate early genes
Females
Spatial learning

ABSTRACT

Estrogens fluctuate across the lifespan in women, with circulating 17β-estradiol levels higher pre-menopause than estrone and circulating estrone levels higher postmenopause than 17β-estradiol. Estrone is a common component of hormone replacement therapies, but research shows that 17β-estradiol may have a greater positive impact on cognition. Previous studies show that acute estrone and 17β-estradiol impact hippocampus-dependent learning and cell proliferation in the dentate gyrus in a dose-dependent manner in adult female rats. The current study explores how chronic treatment with estrone and 17β-estradiol differentially influences spatial learning, hippocampal neurogenesis and activation of new neurons in response to spatial memory. Adult female rats received daily injections of vehicle (sesame oil), or a 10 μg dose of either 17β-estradiol or estrone for 20 days. One day following the first hormone injection all rats were injected with the DNA synthesis marker, bromodeoxyuridine. On days 11–15 after BrdU injection rats were trained on a spatial reference version of the Morris water maze, and five days later (day 20 of estrogens treatment) were given a probe trial to assess memory retention. Cell proliferation was assessed by the endogenous cell cycle marker, Ki67, cell survival was assessed by counting the number and density of BrdU-ir cells in the dentate gyrus and cell activation was assessed by the percentage of BrdU-ir cells that were co-labelled with the immediate early gene product zif268. There were no significant differences between groups in acquisition or retention of Morris water maze. However, the 17β-estradiol group had significantly higher, while the estrone group had significantly lower, levels of cell survival (BrdU-ir cells) in the dentate gyrus compared to controls. Furthermore, rats injected with 17β-estradiol showed significantly higher levels of activation of new neurons in response to spatial memory compared to controls. These results provide insight into how estrogens differentially influence the brain and behavior, and may provide insight into the development of hormone replacement therapies for women.

Introduction

Estrogens fluctuate across the lifespan for women. There are three main forms of estrogens: estrone, estradiol and estriol. Estrone and estradiol shift dramatically during the transition from pre-menopause to post-menopause. Although both estrogens decline during perimenopause, there is a shift in the ratio between these two estrogens after menopause, such that while estradiol is found in greater amounts pre-menopause, estrone is more abundant post-menopause (Rannevik et al., 1995).

Hormone replacement therapy (HRT) has often been prescribed to peri- and post-menopausal women to combat the somatic and cognitive symptoms of menopause (Coope et al., 1975). Intriguingly the most prescribed HRT, Premarin, is comprised of a mixture of natural conjugated equine estrogens (CEE) but over 50% sulphated estrone. Considering that post-menopausal women have higher levels of estrone compared to estradiol, it seems counterintuitive that the most popular therapy uses estrone as the primary component. Studies have found conflicting results when investigating the effects of HRTs on cognition, perhaps due to factors such as the timing of replacement, age of subject, and formulation of HRT with different therapies having different concentrations of estrogens (Hogervorst et al., 2000; Ryan et al., 2008). Indeed a meta-analysis by Ryan (2008) revealed that the HRTs showing more beneficial impacts on cognition were composed primarily of 17β-estradiol, as opposed to Premarin, which is primarily estrone. These studies suggest that more investigation into how different estrogens impact cognition is needed.

Estrogens influence spatial performance on cognitive tasks. Early work provides support that high endogenous levels of estrogens are negatively correlated with spatial performance in the young adult female rodent (Frye, 1995; Galea et al., 1995) and women (Hampson, 1990). Furthermore, lower doses of 17β-estradiol led to better spatial working memory while higher doses of 17β-estradiol led to poorer spatial working and reference memory in young adult female rats (Daniel et al., 1997; Galea et al., 2001; Holmes et al., 2002). These results suggest

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http://dx.doi.org/10.1016/j.yhbeh.2012.09.011

a dose-dependent relationship between the level of 17β-estradiol and spatial performance, with lower levels facilitating but higher levels of 17β-estradiol impairing spatial performance. Studies suggest that 17β-estradiol works directly in the hippocampus to exert its effects on hippocampus-dependent learning and memory in female rodents (Sinopoli et al., 2006; Zhao et al., 2010). Furthermore, there are a number of studies showing a link between menstrual cycle status, estrogens, cognition and activity in the hippocampus and cortex in primates (Dietrich et al., 2001; Hao et al., 2003; Schöning et al., 2007), suggesting that the effects of estrogens are not limited to rodents.

Fewer studies have examined the influence of other estrogens, such as estrone, on cognition. Recently we found that while both 17β- and 17α-estradiol (a naturally occurring optical isomer of 17β-estradiol) showed a dose-dependent facilitation of hippocampus-dependent contextual fear conditioning, estrone either had no significant effect or impaired contextual fear conditioning (Barha et al., 2010). Intriguingly, post-training hippocampal infusions of either 17β-estradiol or estrone led to improved retention on the T-maze footshock avoidance task, in which performance is not dependent on the integrity of the hippocampus (Farr et al., 2000). Furthermore, treatment with Premarin, which is composed mainly of estrone and significantly increases circulating levels of estrone, impairs hippocampus-dependent spatial working and reference learning (Barha and Galea, in press). These findings collectively suggest that the effects of estrogens on cognition depend on dose, type of estrogens and cognitive task.

Estrogens also influence neuroplasticity in the hippocampus. In the adult female, 17β-estradiol influences apical spine density in the CA1 region of the hippocampus (MacLusky et al., 2005; Woolley et al., 1990) and alters neurogenesis in the hippocampus (Barker and Galea, 2008; Galea and McEwen, 1999; Gould et al., 1999; Ormerod and Galea, 2001; Ormerod et al., 2003). Fewer studies have examined the influence of estrone on neuroplasticity in the hippocampus. Acute estrone treatment increases both the synaptic protein synaptophysin and cell proliferation (Barha et al., 2009b) in the hippocampus, but to date no studies have examined the influence of chronic estrone treatment on neuroplasticity.

Adult neurogenesis occurs in the dentate gyrus of the hippocampus of most mammalian species studied, including humans (Eriksson et al., 1998; Gould et al., 1997). Adult neurogenesis in the dentate gyrus of the hippocampus consists of at least four processes: cell proliferation (production of new cells), migration (migration of new cells to the appropriate place), differentiation (the phenotype of new cells) and cell survival (cells surviving to maturity). The number of new neurons can be increased by enhancing cell proliferation, increasing the percentage of cells that become neurons and/or by enhancing the survival of new neurons (for review see Barha et al., 2009a). It is possible to increase the number of cells surviving without influencing cell proliferation, as well as increase the number of cells proliferating without influencing cell survival. For example, chronic exposure to antidepressants upregulates cell proliferation but has no independent effect on cell survival (Malberg et al., 2000), while exposure to an enriched environment or chronic testosterone upregulates cell survival, but has no independent effect on cell proliferation (Olson et al., 2006; Spritzer and Galea, 2007). Adult hippocampal neurogenesis is modulated by many factors including hippocampus-dependent learning and gonadal hormones, such as estradiol (Barha et al., 2010; Barker and Galea, 2008; Epp et al., 2011a, 2011b; Galea and McEwen, 1999; Holmes et al., 2002). For example, repeated estradiol treatment decreases the survival of new neurons in the dentate gyrus of the hippocampus in adult female, but not male, rodents (Barker and Galea, 2008). Hippocampus-dependent learning can either increase or decrease hippocampal neurogenesis in male rats, an effect dependent on task difficulty (Epp and Galea, 2009), quality of learning (Epp et al., 2007; Sisti et al., 2007) and age of new neurons at the time of exposure and perfusion (Epp et al., 2007, 2011a, 2011b). To our knowledge, only two studies have investigated how neurogenesis might be impacted in females after training on a hippocampus-dependent task with equivocal results, likely depending on the type of task (Chow et al., submitted for publication; Dalla et al., 2009), but no studies have investigated the role of estrogens to modulate this effect.

Increases in hippocampal neurogenesis do not necessarily equate to enhancements in certain forms of learning and memory (Barha and Galea, in press; Butz et al., 2006; Jessberger et al., 2007). The role of new neurons in the function of the dentate gyrus is still under investigation and it is possible that these new neurons may not be necessary for the successful completion of all tasks dependent on the hippocampus (for review see Koehl and Abrous, 2011). New evidence suggests that it is important to determine whether new cells in the dentate gyrus are also properly activated in response to learning and memory performance. One such method involves assessing the expression of immediate early genes (IEGs) in new cells. There are several IEGs that respond to learning and memory including the IEG product zif268 that shows increased expression in response to the Morris water maze (Clark et al., 2012). Recent research shows that new neurons in the hippocampus may preferentially respond to spatial, compared to cued, learning in male rodents as assessed by expression of IEG (Epp et al., 2011a; Kee et al., 2007). Importantly, treatment with the HRT Premarin decreased activation of new neurons in the dentate gyrus of female rats, which was correlated with impaired spatial working and reference learning and memory (Barha and Galea, in press). However, no studies have investigated activation (as assessed by IEG) of new neurons in response to hippocampus-dependent learning after administration of 17β-estradiol or estrone.

The aim of this study was to explore how chronic high doses of estrone and 17β-estradiol impact hippocampus-dependent spatial reference memory, hippocampal neurogenesis and activation of new neurons in response to spatial memory using a rodent model of surgical menopause. To examine this, ovariectomized rats were given daily injections of either a vehicle (sesame oil), or a 10 μg dose of either 17β-estradiol or estrone for 20 days and trained on a spatial reference memory version of the Morris water maze. We hypothesized that chronic 17β-estradiol and estrone would differentially impact hippocampal neurogenesis and performance on the Morris water maze. We also hypothesized that chronic 17β-estradiol would significantly increase activation of new neurons, while estrone would significantly decrease activation of new neurons in response to spatial memory compared to controls.

Experimental procedures

Subjects

Subjects were 27 adult female Sprague–Dawley rats bred and raised at the University of British Columbia. The rats weighed between 208 and 260 g at the start of testing and were approximately 3 months of age. All subjects were bilaterally ovariectomized through bilateral flank incisions while under anaesthesia using 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether (isofluorene, Boxter, Mississauga, ON, Canada). Rats were induced at a flow rate began at 5% and maintained at 2.5–3% to sustain a stable respiratory rate. Animals were also given 5 mL of lactate ringer solution and 5 mg/kg injection of a nonsteroidal anti-inflammatory analgesic (Anafen, MERIAL Canada Inc., Baie d'Urfe´, Quebec, Canada). The animals were left for a week undisturbed but monitored to recover from surgery. Subjects were housed individually in standard cages supplemented with a polyvinylchloride tube, paper towel, in addition to food and water *ad libitum* and on a 12:12 h light/dark schedule with temperature maintained between 21° and 22 °C. All the testing was conducted in accordance with the Canadian Council for Animal Care guidelines and was approved by the Animal Care Committee at the University of British Columbia.