ELSEVIER

Neurobiology of Aging 34 (2013) 986–1004

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

The hormone therapy, Premarin, impairs hippocampus-dependent spatial learning and memory and reduces activation of new granule neurons in response to memory in female rats

Cindy K. Barha[a], Liisa A.M. Galea[a,b,c,*]

[a] Department of Psychology, University of British Columbia, Vancouver, British Columbia, Canada
[b] Graduate Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, Canada
[c] Brain Research Centre, University of British Columbia, Vancouver, British Columbia, Canada

Received 5 April 2012; received in revised form 29 June 2012; accepted 11 July 2012

Abstract

Estrogens have been implicated as possible therapeutic agents for improving cognition in postmenopausal women and have been linked to neurodegenerative disorders such as Alzheimer's disease. However, the utility of Premarin (Wyeth Pharmaceuticals, Markham, ON, Canada), a conjugated equine estrogen and the most commonly prescribed hormone therapy, has recently been questioned. The purpose of this study was to investigate the effects of Premarin at 2 different doses (10 or 20 μg) on hippocampus-dependent spatial learning and memory, hippocampal neurogenesis, and new neuronal activation using a rodent model of surgical menopause. Rats were treated daily with subcutaneous injections of Premarin and trained on the spatial working/reference memory version of the radial arm maze. Premarin impaired spatial reference and working learning and memory, increased hippocampal neurogenesis, but either decreased or increased activation of new neurons in response to memory retrieval as indexed by the expression of the immediate early gene product zif268, depending on the maturity of cells examined. This activation of new neurons was related to impaired performance in Premarin-treated but not control-treated female rats. These results indicate that Premarin may be impairing hippocampus-dependent learning and memory by negatively altering the neurogenic environment in the dentate gyrus thus disrupting normal activity of new neurons.

Keywords: Premarin; Hormone therapy; Adult hippocampal neurogenesis; Zif268; Spatial reference memory; Spatial working memory; Conjugated equine estrogens; immediate early genes; females

1. Introduction

Neurodegenerative diseases and cognitive decline associated with aging affect millions of people every year and may be related to gonadal hormone levels. For example, some studies have shown that older men and women with lower levels of gonadal hormones have poorer cognition and increased incidence of neurodegenerative diseases (Baum, 2005; Veiga et al., 2004). Furthermore, natural menopause, the cessation of ovarian function and subsequent reduction in gonadal hormones (i.e., 17β-estradiol) that occurs around 50 years of age in women, and surgical menopause are associated with a decline in certain domains of cognition (Henderson and Sherwin, 2007; Hogervorst and Bandelow, 2010; Sherwin and Henry, 2008). It is important to note that normal aging impairs certain cognitive domains, including long-term memory and working memory, while other cognitive domains remain intact, such as vocabulary memory (Buckner, 2004).

Estrogens, such as 17β-estradiol and estrone, have been suggested as possible therapeutic agents for improving cognition in postmenopausal women. Indeed certain types of hormone therapy (HT) improve cognition and reduce the incidence of Alzheimer's disease in postmenopausal women

* Corresponding author at: University of British Columbia, 2136 West Mall, Vancouver, BC V6T 1Z4, Canada. Tel.: +1 604 822 6536; fax: +1 604 822 3697.
E-mail address: lgalea@psych.ubc.ca (L.A.M. Galea).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.07.009

(Hogervorst et al., 2000; Ryan et al., 2008; Seshadri et al., 2001). The effects of HTs on cognition vary dramatically across studies in humans, with some studies finding improvements and other studies findings impairments. There may be many reasons for these discrepancies including the healthy cell bias hypothesis (Brinton, 2008), the critical period hypothesis (Gibbs, 2010), and the formulation of HT.

The healthy cell bias of estrogen action hypothesis purports that discrepant results of HT to improve cognition may be related to the health status of females and cells, with positive effects of estrogens seen in healthy women and cells and negative effects seen in unhealthy women and cells (Brinton, 2005). A key component of this hypothesis is that timing of estrogen treatment is critical, with beneficial outcomes seen when treatment is begun before neurodegenerative decline commences or before aging negatively influences cells. Thus study outcomes are dependent on estradiol's ability to enhance mitochondrial function in the healthy cell, maintain mitochondrial bioenergetics, and prevent dysregulation of intracellular calcium homeostasis (Brinton, 2008).

Another important determinant of the effectiveness of HT on cognition relates to the critical period hypothesis that states that the ability of estrogens to influence cognition greatly decreases as the time without endogenous estrogens increases (Gibbs and Gabor, 2003). Gibbs (2000) showed that treatment with 17β-estradiol prevented impairments in spatial learning when treatment was initiated immediately or 3 months after ovariectomy but not if initiated 10 months after ovariectomy. Furthermore, 17β-estradiol enhanced spatial working memory performance when treatment began immediately, but not 5 months after ovariectomy (Daniel et al., 2006). The importance of timing for initiating HT may be related to the loss of basal forebrain cholinergic function, the decline of which is accelerated after ovariectomy (Gibbs, 2010). Importantly, clinical studies in women also support the critical period hypothesis (for review see Maki, 2006; Rocca et al., 2011; Sherwin, 2009). Together, these studies support the existence of a critical period of time within which HT must be commenced in order to confer beneficial effects on plasticity and behavior.

In addition to the healthy cell bias and the critical period hypotheses, the formulation of HT may be important. A meta-analysis suggested that HTs containing 17β-estradiol had more positive effects on certain aspects of cognition than HTs containing conjugated equine estrogens (CEE) (Hogervorst et al., 2000; Ryan et al., 2008). Furthermore, the Women's Health Initiative Memory Study (WHIMS), the largest randomized controlled trial conducted thus far, did not find evidence of a beneficial effect of the HT Premarin on cognition or risk for dementia in women (Espeland et al., 2004; Rapp et al., 2003; Shumaker et al., 2003). Importantly Premarin, the most common type of HT prescribed in the United States in the 1990s (Wysowski et al., 1995), is comprised of CEEs and has at least 10 different estrogenic compounds but more than 50% are sulfated forms of estrone. Despite the large number of participants, many important methodological issues have been raised with the WHIMS in recent years. Specifically, the women in the WHIMS suffered from many serious health issues, were between 65 and 79 years of age, and would have been without significant ovarian functioning for more than 15 years, which may have impacted the ability of HT to influence the brain (for review see Henderson, 2006; Sherwin and Henry, 2008). Furthermore, the type of HT given to participants is of central concern, as CEE-based HTs, such as Premarin, do not confer the same benefits on cognition and dementia risk early in menopause as do 17β-estradiol-based HTs (Hogervorst et al., 2000; Ryan et al., 2008). Indeed, Premarin treatment has been associated with greater hippocampal atrophy in postmenopausal women compared with placebo treatment (Resnick et al., 2009).

Estrone and 17β-estradiol, 2 main estrogens used in HTs, influence both brain and behavior. Experimental studies have shown that 17β-estradiol has potent neuroprotective properties (Chen et al., 2006), and can greatly increase neuroplasticity in the hippocampus (Barha and Galea, 2010, 2011; Barha et al., 2009, 2010; Frick et al., 2010). Importantly, 17β-estradiol, at certain doses, can have positive effects on learning and memory (Barha and Galea, 2010). The relationship between 17β-estradiol and spatial performance is complex with many moderating variables, including dose of 17β-estradiol, length of treatment, type of memory system under investigation, age of animals, route of administration, memory demand, and strategy use (Daniel, 2006; Frick, 2009; Korol, 2004). Estrone also influences neuroplasticity (Barha and Galea, 2011; Barha et al., 2009; Bhavnani et al., 2003; Budziszewska et al., 2001) but impairs hippocampus-dependent memory at certain doses (Barha et al., 2010). Furthermore, in middle-aged female rats Premarin can have positive or negative effects on spatial memory depending on dose and type of exposure (cyclic or tonic; Acosta et al., 2009; Engler-Chiurazzi et al., 2011).

The hippocampus mediates many forms of learning and memory (Sutherland et al., 1983) and shows a remarkable degree of plasticity throughout the lifespan (Galea et al., 2008). In adulthood progenitor cells in the dentate gyrus of the hippocampus retain the ability to proliferate into daughter cells that can become neurons in most mammalian species including humans (Eriksson et al., 1998; Gould et al., 1997). Evidence suggests that adult hippocampal neurogenesis is involved in hippocampus-dependent learning and memory, as inhibition of neurogenesis severely impairs some forms of hippocampus-dependent learning and memory (Clelland et al., 2009; Koehl and Abrous, 2011). Furthermore, findings suggest adult-generated neurons are functional and are activated by spatial learning and memory (Epp et al., 2011a; Kee et al., 2007). Interestingly, short-term treatment with 17β-estradiol and estrone increases cell proliferation, whereas longer-term treatment with 17β-es-