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A component of Premarin® enhances multiple cognitive functions and influences nicotinic receptor expression

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ABSTRACT

In women, ovarian hormone loss at menopause has been related to cognitive decline, and some studies suggest that estrogen-containing hormone therapy (HT) can mitigate these effects. Recently, the Women's Health Initiative study found that conjugated equine estrogens, the most commonly prescribed HT, do not benefit cognition. Isolated components of conjugated equine estrogens (tradename Premarin®) have been evaluated in vitro, with delta^{8,9}-dehydroestrone (Δ^8E1) and equilin showing the strongest neuroprotective profiles. It has not been evaluated whether Δ^8E1 or equilin impact cognition or the cholinergic system, which is affected by other estrogens and known to modulate cognition. Here, in middle-aged, ovariectomized rats, we evaluated the effects of Δ^8E1 and equilin treatments on a cognitive battery and cholinergic nicotinic receptors (nAChR). Specifically, we used ^{125}I-labeled epibatidine binding to assay brain nicotinic receptor containing 4α and 2β subunits (α4β2-nAChR), since this nicotinic receptor subtype has been shown previously to be sensitive to other estrogens. Δ^8E1 enhanced spatial working, recent and reference memory. Δ^8E1 also decreased hippocampal and entorhinal cortex α4β2-nAChR expression, which was related to spatial reference memory performance. Equilin treatment did not affect spatial memory or rat α4β2-nAChR expression, and neither estrogen impacted $^{86}Rb^+$ efflux, indicating lack of direct action on human α4β2 nAChR function. Both estrogens influenced vaginal smear profiles, uterine weights, and serum luteinizing hormone levels, analogous to classic estrogens. The findings indicate that specific isolated Premarin® components differ in their ability to affect cognition and nAChR expression. Taken with the works of others showing Δ^8E1-induced benefits on several dimensions of health-related concerns associated with menopause, this body of research identifies Δ^8E1 as a new avenue to be investigated as a potential component of HT that may benefit brain health and function during aging.

Introduction

By the year 2050, an estimated 45 million postmenopausal women in the United States will have to make the choice of whether to utilize hormone therapy (HT; U.S. Census Bureau, 2007). Ovarian hormone loss due to surgical or natural menopause has been associated with cognitive decline in women (Nappi et al., 1999; Phillips and Sherwin, 1992; Sherwin, 1988); however, the question of how HT impacts cognition is unclear. Premarin®, a complex conjugated equine estrogen preparation synthesized from the urine of pregnant mares, has been given since 1942 and is the most widely used estrogen component of HT in the United States (Hersh et al., 2004; Stefanick, 2005). Some studies in women demonstrate that Premarin®-containing HT improved memory (Campbell and Whitehead, 1977; Kantor et al., 1973; Ohkura et al., 1995). However, the largest placebo-controlled double blind study conducted to date evaluating HT effects on all-cause dementia, the Women's Health Initiative Memory Study (WHIMS), found that conjugated equine estrogen treatment given alone did not reduce incidence of dementia or mild cognitive impairment (Shumaker et al., 2004), and that treatment with conjugated equine estrogens plus the progestin medroxyprogesterone acetate increased the risk of cognitive decline and probable dementia, and did not prevent mild cognitive impairment (Rapp et al., 2003; Shumaker et al., 2003). A recent follow-up study evaluating WHIMS participants found that Premarin® treatment, with or without medroxyprogesterone acetate, was associated with brain atrophy as assessed via MRI scans (Resnick et al., 2009).

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Thus, while noting many factors likely affected the WHIMS findings, including the global nature of the cognitive measure, older age of participants, and duration of hormone deprivation before treatment initiation (for discussion see Sherwin, 2005), the collected findings suggest that the absence of ovarian hormones is not optimal for cognition, but neither is the most commonly utilized HT.

Premarin® contains the sulfates of more than 10 estrogens, is over 50% estrone, 20–25% equilin, 3.5% delta^{8,9}-dehydroestrone (Δ^8E1), and contains only trace amounts of 17β-estradiol (the most potent naturally circulating estrogen in women and rats); after metabolism, the resulting biologically active circulating hormones are primarily estrone and equilin, and after estrone's conversion, 17β-estradiol (Bhavnani, 2003; Bhavnani et al., 1998; Mayer et al., 2008; Sitruk-Ware, 2002). In the last decade, landmark basic science research from the Brinton laboratory has led to several discoveries regarding the neuroprotective properties of estrogens. This work demonstrated that some components of Premarin® enhanced markers of neuroprotection, while others showed little benefit (Brinton et al., 1997; Zhao and Brinton, 2006). The estrogens Δ^8E1 and equilin, found naturally in horses but not in women or rats, were the two primary Premarin® components that showed the most consistent and potent neuroprotective effects in vitro (Zhao and Brinton, 2006). Δ^8E1- and equilin-mediated neuroprotection in vitro could potentially translate to improved function of neural networks and brain regions mediating cognitive function, resulting in mnemonic enhancements in vivo.

Basal forebrain cholinergic neurons project to the hippocampus and surrounding cortical areas, and play an important role in learning and memory (Hasselmo, 2006). 17β-estradiol enhances basal forebrain cholinergic function, as evidenced by expression of different cholinergic markers and pharmacological cholinergic challenges (e.g., Gibbs, 2000a; Markowska and Savonenko, 2002; Packard and Teather, 1997). In fact, choline acetyltransferase (ChAT) increases in the basal forebrain months after transient exposure to 17β-estradiol, noting effects are sensitive to several variables including timing and dose (Bohacek et al., 2008; Gibbs, 1997; Rodgers et al., 2010). Our laboratory has demonstrated Premarin®-induced benefits to the cholinergic system, whereby Premarin® treatment prevented scopolamine-induced amnesia and increased number of ChAT positive neurons in the vertical diagonal band of the basal forebrain in ovariectomized (Ovx) rats (Acosta et al., 2009b). Cholinergic signals are mediated by muscarinic acetylcholine receptors (mAChR) and nicotinic acetylcholine receptors (nAChR), which are present in brain regions mediating memory (Clarke et al., 1985; Court and Clementi, 1995; Tice et al., 1996; Vaucher et al., 2002) and are implicated in mnemonic processing (Hasselmo, 2006; Konopacki et al., 1992; Rouse et al., 1999). Nicotine administration improves memory in humans (Buccafusco et al., 2005), and we and others have noted nicotine-induced memory enhancement in rats across multiple memory domains (Arendash et al., 1995; French et al., 2006; Riekkinen and Riekkinen, 1997; Socci et al., 1995). 17β-estradiol and ethinyl β-estradiol bind to the α4β2-nAChR, the most abundant nAChR subtype in the brain, and directly potentiate the function of human α4β2-nAChRs (hα4β2-nAChR); ethinyl β-estradiol, but not 17β-estradiol, potentiates rat α4β2-nAChRs (rα4β2-nAChR) (Curtis et al., 2002; Paradiso et al., 2001). Recently, in vivo research has demonstrated that nicotine co-administered with estradiol potentiates visual spatial memory in Ovx rats, beyond that of either compound administered alone (Taylor and Maloney, 2010). Thus, there is a link between estrogens, nAChRs, and memory.

Numerous studies have demonstrated that 17β-estradiol benefits spatial working and reference memory in young Ovx rats (Bimonte and Denenberg, 1999; Daniel et al., 1997, 2005; El-Bakri et al., 2004; Fader et al., 1998; Feng et al., 2004; Gibbs, 2007; Hruska and Dohanich, 2007; Korol and Kolo, 2002; Luine and Rodriguez, 1994), as well as in middle-aged Ovx rats (Bimonte-Nelson et al., 2006; Foster et al., 2003; Markham et al., 2002; Talboom et al., 2008). Premarin® can also benefit cognition in middle-aged Ovx rats, although these effects are dose and task specific (Acosta et al., 2009b; Engler-Chiurazzi et al., in press; Walf and Frye, 2008). The current study utilized a model similar to the one used in this prior research, the middle-aged Ovx rat, to test the impact of Δ^8E1 and equilin on memory and rα4β2-nAChR expression and function. We used a battery of water-escape mazes previously shown to be influenced by age as well as ovarian hormone loss and estrogen replacement (Acosta et al., 2009a,b; Bimonte-Nelson et al., 2006; Engler-Chiurazzi et al., in press; Talboom et al., 2008). These mazes evaluate working memory, which is information that needs to be updated and is pertinent for a short time, and reference memory, which is information that remains constant over time (see Jarrard et al., 1984; Jones, 2002). ^{125}I-labeled epibatidine (I-epi) radioligand binding assays were used to evaluate rα4β2-nAChR expression levels in the hippocampus and entorhinal cortex, and cell culture was used to evaluate whether Δ^8E1 and equilin directly altered hα4β2-nAChR function via $^{86}Rb^+$ efflux experiments. Lastly, several peripheral markers routinely noted to change with 17β-estradiol treatment, including serum luteinizing hormone (LH) levels, vaginal smears, and uterine weights, were assessed.

Materials and methods

Subjects

Subjects were 50 middle-aged (12–13 month old) Fischer-344 female rats born and raised at the National Institute on Aging colony at Harlan Laboratories (Indianapolis, IN). Animals were acclimated for several weeks to the vivarium at Arizona State University, had exposure to food and water ad-lib, and were maintained on a 12-h light/dark cycle at 23 °C. Procedures were approved by Arizona State University IACUC and adhered to the Guide for the Care and Use of Laboratory Animals and NIH standards.

Hormone manipulation

Ovariectomy, group assignment and hormone dosing

Thirty days after arrival, all rats received Ovx under isoflurane anesthesia. Dorsolateral incisions were made in the skin and peritoneum, and ovaries and tips of uterine horns were ligated and removed. Rats were randomly assigned into a control group receiving vehicle only or groups receiving one of three doses of hormone delivered via osmotic minipump. Specifically, the groups were: Ovx plus vehicle (Veh, n = 8), Ovx plus 2.6 μg/day of Δ^8E1 (Δ^8E1-Low, n = 7), Ovx plus 17.5 μg/day of Δ^8E1 (Δ^8E1-Med, n = 7), Ovx plus 35 μg/day of Δ^8E1 (Δ^8E1-High, n = 7), Ovx plus 2.6 μg/day of equilin (Equilin-Low, n = 7), Ovx plus 6.25 μg/day of equilin (Equilin-Med, n = 7), and Ovx plus 12.5 μg/day of equilin (Equilin-High, n = 7). All hormones were purchased from Steraloids Inc. (Newport, RI). Low doses of Δ^8E1 and equilin were derived from our studies demonstrating cognitive enhancement using 3.6 μg of Premarin®. Doses were adjusted to account for Δ^8E1 and equilin being in its unconjugated form,[1] and to approximate the rat body weight equivalent of the

[1] The 20–36 μg of Premarin® powder that we have shown to enhance cognition was actually 2.0–3.6 μg of estrogens, respectively, since it was ≈10% hormone and ≈90% filler (Acosta et al., 2009b; Engler-Chiurazzi et al., in press). Premarin® is a mixture of estrogens conjugated to sulfate; the hormones must be deconjugated by the liver to become bioactive (Bhavnani et al., 1998). We used bioactive, unconjugated Δ8E1 and equilin in this study. To attain an approximately equivalent amount of estrogen molecules between our current Δ8E1 and equilin-low dose, and our previous Premarin dose, we had to account for the fact that estrogens in Premarin are conjugated, and conjugated estrogens weigh more. Since the molecular weights of Δ8E1 sulfate and equilin sulfate (the conjugated estrogens) are each 370.08 (as monosodium salts), while that of Δ8E1 and equilin (as unconjugated estrogens) are each 268.35, the final concentrations for the low doses were reduced by 28% (i.e., 3.6 μg/day–28%≈2.6 μg/day).