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Premarin improves memory, prevents scopolamine-induced amnesia and increases number of basal forebrain choline acetyltransferase positive cells in middle-aged surgically menopausal rats

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ABSTRACT

Conjugated equine estrogen (CEE) is the most commonly prescribed estrogen therapy, and is the estrogen used in the Women's Health Initiative study. While in-vitro studies suggest that CEE is neuroprotective, no study has evaluated CEE's effects on a cognitive battery and brain immunohistochemistry in an animal model. The current experiment tested whether CEE impacted: I) spatial learning, reference memory, working memory and long-term retention, as well as ability to handle mnemonic delay and interference challenges; and, II) the cholinergic system, via pharmacological challenge during memory testing and ChAT-immunoreactive cell counts in the basal forebrain. Middle-aged ovariectomized (Ovx) rats received chronic cyclic injections of either Oil (vehicle), CEE-Low (10 μg), CEE-Medium (20 μg) or CEE-High (30 μg) treatment. Relative to the Oil group, all three CEE groups showed less overnight forgetting on the spatial reference memory task, and the CEE-High group had enhanced platform localization during the probe trial. All CEE groups exhibited enhanced learning on the spatial working memory task, and CEE dose-dependently protected against scopolamine-induced amnesia with every rat receiving the highest CEE dose maintaining zero errors after scopolamine challenge. CEE also increased number of ChAT-immunoreactive neurons in the vertical diagonal band of the basal forebrain. Neither the ability to remember after a delay nor interference, nor long-term retention, was influenced by the CEE regimen used in this study. These findings are similar to those reported previously for 17 β-estradiol, and suggest that CEE can provide cognitive benefits on spatial learning, reference and working memory, possibly through cholinergic mechanisms.

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Introduction

Conjugated equine estrogen (CEE; tradename Premarin) is the most widely used hormone therapy (HT) in the United States, given since 1942 (Stefanick, 2005), and was the estrogen used in the Women's Health Initiative (WHI) (Shumaker et al., 1998; 2004). In vitro, CEE increases neuronal growth in the basal forebrain, hippocampus and cortex (Diaz Brinton et al., 2000) and attenuates beta amyloid-induced cell death in hippocampus (Brinton et al., 2000). Clinical findings assessing CEE have been inconclusive. CEE-containing therapy improved memory via self-report (Campbell and Whitehead, 1977), case studies (Ohkura et al., 1995) and randomized psychometric evaluations (Kantor et al., 1973). Yet, findings evaluating global cognitive function in the large placebo-controlled WHI Memory Study (WHIMS), conducted by the National Institutes of Health, showed an increase in probable dementia risk and no effect on mild cognitive impairment in women 65+ years taking the combination therapy CEE+medroxyprogesterone (Shumaker et al., 2003). Unopposed treatment with CEE alone showed a trend for an increased incidence of probable dementia and mild cognitive impairment, although this did not reach statistical significance (Espeland et al., 2004; Shumaker et al., 2004). An ancillary study to the WHI testing more specific cognitive functions, the WHI Study of Cognitive Aging (WHISCA), reported that combination CEE+medroxyprogesterone therapy had a negative effect on verbal memory and a trend for positive effects on figural memory in women 65 and over free of probable dementia (Resnick et al., 2006). Together, the clinical studies indicate that CEE-containing therapy can result in both beneficial and detrimental actions on cognition in the aging brain.

Rodent models can provide insight into cognitive effects of HTs, and allow evaluation of correlative brain changes. Such studies enable

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excellent experimental control by obviating issues impacting HT use in women, such as socioeconomic status and education, as well as factors potentially affecting cognitive efficacy of HT such as time after ovarian hormone loss and age (Sherwin, 2006). The estrogen 17 β-estradiol has been shown to benefit spatial working and reference memory in young rodents (Bimonte and Denenberg, 1999; Daniel et al., 1997; Daniel et al., 2005; El-Bakri et al., 2004; Fader et al., 1999; Feng et al., 2004; Hruska and Dohanich, 2007; Korol and Kolo, 2002; Luine and Rodriguez, 1994), as well as in middle-aged rodents (Bimonte-Nelson et al., 2006; Markham et al., 2002; Talboom et al., 2008).

Abundant evidence suggests that learning and memory is mediated, in part, by the cholinergic system. Supporting data include research showing that basal forebrain (BF) cholinergic lesions impaired acquisition of a delayed match-to-position (DMP) T-maze task (Gibbs, 2002, 2007), and that DMP performance correlated with increased choline acetyltransferase (ChAT) activity in the hippocampus and frontal cortex (Gibbs, 2002). This link between cholinergic neurons, neurochemistry and cognitive performance might be relevant to estradiol's effects during cognitive testing, as estradiol treatment potentiated increases in hippocampal acetylcholine levels during maze learning (Marriott and Korol, 2003), and estradiol induced memory improvements in animals with intact BF cholinergic neurons, but not with BF cholinergic lesions (Gibbs, 2002, 2007). Further, estradiol increases the number of ChAT-immunoreactive (IR) neurons in the BF medial septum (MS) and vertical diagonal band (VDB) in young Ovx rats (Gibbs, 1997; Gibbs and Pfaff, 1992), and increases the number and size of MS ChAT-IR neurons in middle-aged mice (Granholm et al., 2002). Estradiol administration also protects against the cholinergic challenge of scopolamine-induced amnesia in young and middle-aged rats (Dohanich et al., 1994; Fader et al., 1998; Fader et al., 1999; Packard and Teather, 1997; Savonenko and Markowska, 2003).

Estradiol is the primary estrogen used to test cognitive effects of HT in animal models; CEE has not been thoroughly evaluated. Estradiol is the most potent naturally-circulating estrogen, followed by estrone and estriol, in order of receptor affinity (Kuhl, 2005; Sitruk-Ware, 2002). CEE contains sulfates of at least ten estrogens. Only two of these, estrone and estriol, are found naturally in women; all others are unique to horses (Kuhl, 2005). CEE is over 50% estrone sulfate, and contains only trace amounts of estradiol; after metabolism, the resulting biologically active circulating hormones are primarily estrone and estradiol (Bhavnani, 2003; Sitruk-Ware, 2002). Because, after CEE treatment, other weaker estrogens are present that could alter efficacy of the resulting circulating levels of estradiol (Kuhl, 2005), the animal studies done thus far testing the cognitive and cholinergic effects of estradiol cannot be generalized to potential effects of CEE.

The goal of the current study was to determine whether CEE could positively impact cognition and the cholinergic system in middle-aged rodents, while controlling for factors possibly influencing HT outcome such as Ovx duration before treatment and age, and obviating clinical variables such as socioeconomic status and education. We evaluated whether CEE influenced learning, spatial reference and working memory, including after delay and interference challenges in order to better define parameters impacted by CEE-treatment. We also investigated whether CEE affects the cholinergic system by evaluating response to pharmacological challenge during memory testing, as well as assessing number of ChAT-IR neurons in the MS and the VDB of the BF. These endpoints were chosen because in the rat model estradiol treatment after Ovx impacts spatial working and reference memory maze performance (Bimonte-Nelson et al., 2006; Bimonte and Denenberg, 1999; Daniel et al., 1997; Daniel et al., 2005; El-Bakri et al., 2004; Fader et al., 1999; Feng et al., 2004; Hruska and Dohanich, 2007; Markham et al., 2002; Talboom et al., 2008), protects against pharmacological cholinergic challenge during maze testing (Dohanich et al., 1994; Fader et al., 1998; Fader et al., 1999; Gibbs et al., 1998; Packard and Teather, 1997; Savonenko and Markowska, 2003), and increases the number of ChAT-IR neurons in the basal forebrain (Gibbs, 1997; Gibbs and Pfaff, 1992; Granholm et al., 2002).

Materials and methods

Subjects

At the start of the experiment there were 33 thirteen month old Fischer-344 female rats born and raised at the aging colony of the National Institute on Aging at Harlan Laboratories (Indianapolis, IN). Prior to surgery, rats were acclimated for several weeks, and were pair housed with an identical treatment assigned cage-mate. Animals had exposure to food and water ad-lib, and were maintained on a 12-h light/dark cycle. All procedures were approved by the local IACUC committee and adhered to NIH standards.

Surgery and hormone administration

Forty-two±2 days before behavioral testing ensued, all rats received Ovx. Rats were anesthetized with an intraperitoneal injection of a cocktail of 70 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, IA, USA)/ and 6 mg/kg xylazine (Lloyd Laboratories, Shenandoah, IA, USA) and acepromazine (10 mg/ml, Vedco Inc., St Joseph MO 64507). After bilateral dorsolateral incisions the ovaries and tips of uterine horns were ligatured and removed. The muscle was then sutured and the skin stapled. Twenty-five±3 days after surgery rats began hormone/vehicle administration after random assignment. One subcutaneous injection was given for two consecutive days followed by 2 days off, a pattern repeated throughout the study. Rats received either chronic cyclic treatment with vehicle injection (sesame oil, Ovx-Oil) or one of the following amounts of injectable CEE, in its unconstituted powder form, as prescribed to women (manufactured by Wyeth Pharmaceuticals Inc., Philadelphia, PA, obtained from a commercial pharmacy via veterinary prescription) dissolved in sesame oil: 10 (Ovx-CEE Low), 20 (Ovx-CEE Medium) and 30 μg (Ovx-CEE High). The doses used in the current study were based on the daily 0.625 mg CEE dose commonly taken by women, and used in the WHIMS. Since no similar study had been done in the rodent, we extrapolated the dose to be used in our animal model as follows. We used the average female weight of 70 kg (www.halls.md) for calculations to determine mg drug/kg body weight for the 0.625 dose, which resulted in 0.00893 mg drug/kg body weight woman. Estimating dose based on a 220 g rat, this resulted in approximately 20 μg daily administration of the powder, which was 10% CEE. This was used as the medium dose, with 10 μg lower and higher for the low and high groups, respectively. Behavioral testing began 18 days after hormone administration was initiated. The testing schedule is outlined in Table 1. The last treatment injection was administered in the morning, 2 days prior to sacrifice.

Vaginal smears and uterine weights

To confirm Ovx and CEE treatment, vaginal smears were taken daily for 17 days, beginning 1 day prior to the first CEE injection. Smears were classified as either proestrus, estrous, metestrus or diestrus (Goldman et al., 2007). Before CEE injection, all animals were in diestrus. Six days after the first CEE injection, all CEE-treated animals showed estrous smears. To examine CEE effects on uterine tissues, an additional 10 Ovx animals given injection were sacrificed at this same timepoint. The injection regimen was identical to that used in the behavior study. Six days after the initial injection of either oil (n=3), CEE-Medium (n=3) or CEE-High (n=4), uterine tissues were collected. After anesthesia, a ventral incision was made in the abdominal region, and the uterus was cut above the junction with the cervix and on the uterine horn below the ligature remaining from Ovx (Ashby et al., 1997). Uteri were trimmed of all visible fat and were immediately weighed to obtain wet weight.