Continuous estrone treatment impairs spatial memory and does not impact number of basal forebrain cholinergic neurons in the surgically menopausal middle-aged rat

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ABSTRACT

CEE (conjugated equine estrogens) is the most widely prescribed estrogen-only menopausal hormone therapy in the United States, and is comprised of over 50% estrone (E1) sulfate. Following CEE administration, E1 is the principal circulating estrogen. However, the cognitive and neurobiological effects of E1 in a middle-aged rodent model have not yet been evaluated. We assessed cognitive effects of continuous E1 treatment in middle-aged surgically menopausal rats using a maze battery. We also quantified number of choline acetyltransferase-immunoreactive (ChAT-IR) neurons in distinct basal forebrain regions known in earlier studies in to be impacted by the most potent naturally-circulating estrogen in rodents and women, 17β-estradiol (17β-E2), as well as CEE. On the spatial working memory delayed-match-to-sample water maze, the highest E1 dose impaired memory performance during acquisition and after delay challenge. E1 did not impact ChAT-IR neuron number in the medial septum (MS) or horizontal/vertical diagonal bands. In a comparison study, 17β-E2 increased MS ChAT-IR neuron number. Findings indicate that E1 negatively impacts spatial working memory and memory retention, and does not increase ChAT-IR neuron number in basal forebrain, as does 17β-E2. Thus, data from prior studies suggest that 17β-E2 and CEE can enhance cognition and increase number of ChAT-IR basal forebrain neurons, while here we show that E1 does not induce these effects. Findings from preclinical basic science studies can inform the design of specific combinations of estrogens that could be beneficial to the brain and cognition. Accumulating data suggest that E1 is not likely to be among these key beneficial estrogens.

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Introduction

Conjugated equine estrogens (CEE) have been given to menopausal women since 1942 (Stefanick, 2005) and was the estrogenic component tested in the Women's Health Initiative Memory study (Shumaker et al., 1998, 2004). It is the most widely prescribed estrogenic component of menopausal hormone therapy in the United States, even despite a decrease in use after the 2002 publication of clinical trial results (Hersh et al., 2004). CEE has been shown to have both positive and negative effects on cognition in menopausal women (for review see Hogervorst et al., 2000; Sherwin and Henry, 2008), and can enhance memory in the middle-aged Ovx rat, which is a dose-specific effect (Acosta et al., 2009b; Engler-Chiurazzi et al., 2011). CEE is a complex estrogen formulation comprised of 50% estrone (E1) sulfate, and it contains the sulfates of at least ten other estrogens (Kuhl, 2005), many of which have yet to be individually evaluated for cognition in women or rodent models. Determining effects of the specific estrogen components of this complex formulation could help determine why it sometimes enhances, and why it sometimes impairs, cognition. Furthermore, this strategy may identify a group of cognitively enhancing estrogens to be combined into optimal hormone therapy formulations for specific populations of women, as well as identify estrogens detrimental to the brain and cognition to be excluded from future formulations. In women, 17β-estradiol (17β-E2), present only in trace amounts in CEE, is the most potent naturally-circulating estrogen, followed by E1 and estriol, in order of receptor affinity (Kuhl, 2005). 17β-E2 and E1 are biologically interconvertible; in vivo, they readily get converted into one another (Kuhl, 2005; Prokai-Tatrai and Prokai, 2005). Circulating levels of E1 increase following treatment with CEE in menopausal and post-menopausal women (Yasui et al., 1999), and following administration of CEE to middle-aged ovariectomized (Ovx) rats (Acosta et al., 2009b; Engler-Chiurazzi et al., 2011). Although we have shown that the CEE component Δ[8,9]-dehydroestrone exerted beneficial cognitive effects in middle-aged, Ovx rats (Talboom

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et al., 2010), the cognitive impact of the principle circulating estogen following CEE administration, E1, is unclear. We hypothesize that E1 will impair cognition in middle-aged Ovx rats. Indeed, one paper in young rats has shown a single subcutaneous E1 injection impairs contextual fear conditioning memory when given 30 min before training (Barha et al., 2009). Furthermore, although not all in vitro studies report negative effects with E1 administration (Zhao and Brinton, 2006), for most measures in which other estrogenic CEE components (e.g., equilin and $\Delta^{8,9}$-dehydroestrone) were neuroprotective in vitro, E1 was ineffective (Zhao and Brinton, 2006).

The basal forebrain cholinergic system is important for learning and memory, is susceptible to age-related changes, and is impacted by ovarian hormone removal and 17β-E2 replacement (for review see Gibbs, 2010). For example, in aged female rats, less choline acetyltransferase (ChAT) protein activity was found in the vertical diagonal bands (vDB), relative to younger counterparts (Luine and Hearns, 1990). Also, in adult Ovx rats, 17β-E2 treatment increased ChAT protein activity in the horizontal diagonal bands (hDB; Luine, 1985), as well as ChAT-immunoreactive (ChAT-IR) neuron counts in the medial septum (MS; Gibbs, 1997). Evidence from Gibbs' laboratory suggests that the effects of 17β-E2 on cognition require a functioning basal forebrain cholinergic system; for example, 17β-E2 was ineffective in animals with basal forebrain lesions, and enhanced memory only in non-lesion controls (Gibbs, 2002, 2007). Although it has been established that 17β-E2 impacts the basal forebrain cholinergic system, an effect which is likely related to cognitive enhancements (for review see Bimonte-Nelson et al., 2010; Gibbs, 2010), there has been no study evaluating whether E1 impacts basal forebrain cholinergic neurons.

In the present study, we evaluated the cognitive impact of subcutaneously administered continuous E1 treatment in middle-aged Ovx rats, utilizing several spatial memory mazes previously shown to be sensitive to the effects of aging (Frick et al., 1995; Talboom et al., 2008), and estrogen administration (Acosta et al., 2009b; Bimonte-Nelson et al., 2006; Engler-Chiurazzi et al., 2011; Walf et al., 2009), such that a potential pattern of E1's effects on specific memory types could be revealed. Several classic peripheral markers of estrogenic action, including vaginal smears and uterine weights, were measured to confirm effects of Ovx and E1 treatment. Lastly, we evaluated the impact of E1 on the basal forebrain cholinergic system by quantifying the number of ChAT-IR neurons in the MS and the hDB/vDB of the basal forebrain in the cognitively tested animals. Because 17β-E2 has been shown to impact ChAT protein activity (Luine, 1985) and ChAT-IR neuron counts (Gibbs, 1997) in the basal forebrain, to aid in interpretation of potential E1 ChAT-IR effects, we performed a separate study evaluating ChAT-IR neuron numbers after treatment with 17β-E2 using the same quantification procedures as those used in the E1 study. Determining the impact of E1 on spatial memory and the cholinergic system will help to characterize the unique cognitive and neurobiological impacts of this estrogen, which is a primary circulating estrogen after administration of the commonly used hormone therapy, CEE.

Materials and methods

Subjects

We used 32 middle-aged (13 months old at the beginning of the study) Fischer-344 female rats born and raised at the National Institute on Aging colony at Harlan Laboratories (Indianapolis, IN). Animals were pair-housed, acclimated for several weeks at Arizona State University, had exposure to food and water ad libitum, and were maintained on a 12-h light/dark cycle at 23 °C. Experimental procedures were approved by the Arizona State University Institutional Animal Care and Use Committee and adhered to Guidelines for the Care and Use of Laboratory Animals and NIH standards.

Hormone treatments

Fig. 1 displays a timeline of the experimental protocol. Approximately 28 days before behavioral testing ensued, under isoflurane inhalant anesthesia, all rats underwent Ovx surgery to remove endogenous ovarian hormones. Dorsolateral incisions were made in the skin and peritoneum, and ovaries and tips of uterine horns were ligated and removed. Rats were then separated into the following groups: Ovx with Vehicle only (polyethylene glycol, PEG) (Vehicle, n=9), Ovx plus 2.6 μg/day of E1 (E1-Low, n=7), Ovx plus 4.0 μg/day of E1 (E1-Med, n=8), and Ovx plus 8.0 μg/day of E1 (E1-High, n=8). All hormones were purchased from Sigma (St. Louis, MO). The E1-Low dose was based on the most efficacious dose of 17β-E2 found in a dose response study conducted in our laboratory evaluating spatial working memory (unpublished observations). The E1-Med dose was based on findings from Beyer et al. (1976) in which 4.0 μg/day of E1 induced lordosis behavior and increased uterine weights. To assess the cognitive and physiological impact of a broad range of E1 doses, the E1-High dose was double the E1-Med dose. Corresponding to published studies evaluating E1 and other estrogens (Barha et al., 2009; Talboom et al., 2010) in which subjects were given approximately one to two weeks between Ovx and hormone treatment, in the current study, hormone treatment began 19±1 days after Ovx. In parallel with other studies (Engler-Chiurazzi et al., 2011; Talboom et al., 2010), Vehicle and E1 treatments were administered continuously using Alzet osmotic pumps (Model 2004; Durect Corporation, Cupertino, CA). Briefly, E1 was dissolved in PEG (Sigma, St. Louis, MO) and inserted into the pumps as per manufacturer's instructions. For the Vehicle group, pumps were filled with PEG only. For pump insertion, under isoflurane anesthesia, a small incision was made in the dorsal scruff of the neck, and a subcutaneous pocket was created. One pump filled with Vehicle or the appropriate E1 dose was inserted into the pocket and the skin was stapled. Nine days after pump insertion surgery, cognitive testing began. All animals had Vehicle or E1 exposure until sacrifice.

Markers of peripheral estrogenic action

To confirm the effects of Ovx as well as E1 treatments, we assessed several peripheral physiological markers that routinely change with estrogen treatment. Notably, E1 has been found to impact peripheral tissues, including the uterus (Beyer et al., 1976). We therefore performed vaginal smears (Goldman et al., 2007) and measured uterine weights (Westerlind et al., 1998), the latter of which was done upon animal sacrifice. Smears were classified as proestrus, estrous, metestrus, or diestrus (Acosta et al., 2009b; Engler-Chiurazzi et al., 2011; Goldman et al., 2007). Vaginal smears were first conducted to confirm the lack of uterine stimulation and complete Ovx 18 days after Ovx, which was one day before E1 administration via pump
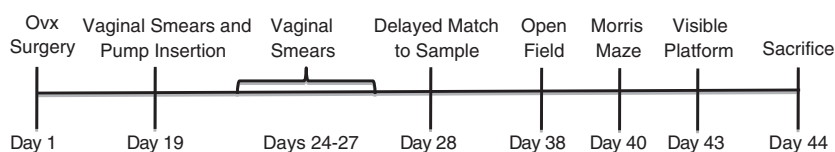

Fig. 1. Study timeline. A timeline summarizing the effects of Ovx surgery, pump insertion surgery for hormone administration, vaginal smears, and start day for each maze in the behavioral battery.