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Conjugated equine estrogen enhances rats' cognitive, anxiety, and social behavior

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Abstract

The ovarian hormone, 17 β -estradiol (E₂), has numerous targets in the body and brain, and can influence cognitive, affective, and social behavior. However, functional effects of commonly prescribed E₂-based hormone therapies are less known. The effects of conjugated equine estrogen (CEE) on middle-aged female rats for cognitive (object recognition), anxiety (open field, plus maze), and social (social interaction, lordosis) behavior were compared-with vehicle. Our hypothesis that CEE would enhance cognitive, anxiety, and social behavior was supported. CEE improved object recognition, increased time spent on the open arms of the plus maze, and time spent interacting with a conspecific, but did not alter open field behavior or lordosis. Thus, CEE can enhance cognitive, antianxiety, and social behavior of middle-aged rats.

Keywords

anxiety; cognition; estradiol; learning and memory; Premarin

Introduction

The ovarian steroid, 17 β -estradiol (E₂) has profound effects on several biological and behavioral processes. Indeed, cessation in ovarian function, which occurs with aging and the transition to menopause, can be associated with many unfavorable physical symptoms and changes in quality-of-life/psychological measures, such as hot flushes, night sweats, genital dryness, and changes in cognition, anxiety, and mood. Treatments for these symptoms typically include an E₂-based therapy. Although several studies have shown that E₂-based therapies can reduce anxiety and improve mood scores when administered to perimenopausal and/or postmenopausal women, there are inconsistencies in the clinical literature regarding the benefit of E₂-based therapies in older women [1].

Given the inherent limitations of clinical studies, it is important to consider E₂'s functional effects in animal models of menopause. One approach taken to model menopause is to surgically remove the primary endogenous source of E₂, the ovaries (ovariectomy; OVX). OVX of young adult rats produces cognitive impairments, increases anxiety and depressive behavior, and decreases sexual responding compared with that of intact, proestrous controls

with high physiological E₂ levels [1-4]. These effects of OVX are reversed by E₂ administration, but depend upon dosing, the E₂ regimen used, and length of E₂ deprivation [1,5]. Given the latter, that OVX produces much more dramatic decline in ovarian hormones than does reproductive senescence, and that young adult rats may have a different capacity to respond than older rats, animal models of nonsurgical meno-pause are very important. Gonadally intact 18–24-month-old mice show high levels of anxiety behavior and have low levels of E₂ [6], which can be reversed by systemic administration of E₂ and/or raloxifene (Evista; Eli Lilly and Company, Indianapolis, Indiana, USA), compared with vehicle [1]. Thus, E₂ and/or raloxifene can improve cognitive, affective, and social behavior in animal models of reproductive senescence.

Another factor that may explain why some women do not respond as favorably to E₂-based therapies, which has been predicted by reviewing the data from animal studies, is the regimen of E₂ that is used. The main circulating estrogen within the body that is produced by the ovaries, and is increased over reproductive cycles and decreased with aging, is E₂. However, the most commonly prescribed E₂-based therapy is conjugated equine estrogen (CEE) (Premarin; Wyeth Pharmaceuticals, Philadelphia, Pennsylvania, USA), which is derived from pregnant mare urine and contains trace amounts of E₂ and nearly 50 other estrogenic compounds [7]. As well, CEE is metabolized to estrone sulfate, which may not cross the blood–brain barrier as readily as 17 β -E₂ [8]. In the much-publicized Women's Health Initiative Studies, no differences were observed for incidence of mild cognitive impairments or probable dementia among women treated with CEE [9,10]. However, the age, health status, timing, and/or nature of their hormone replacement therapy may have mitigated these outcomes [11]. To begin to address these factors in an animal model, we examined the effects of CEE administration on middle-aged female rats. We hypothesized that CEE would improve cognitive performance, increase antianxiety and prosocial behavior, over that of vehicle-administered rats.

Methods

All methods used were approved by the Institutional Animal Care and Use Committee at The University at Albany-SUNY and adhered strictly to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications, no 80-23, revised 1978).

Animals and housing

Subjects ($N=20$) were adult female rats, 13 months old, which were obtained from our breeding colony (original stock from Taconic Farms, Germantown, New York, USA). Experimental rats were housed 2–3 per cage (45×24×21 cm), which contained woodchip shavings for bedding, in a temperature-controlled room (21±1°C) in the Laboratory Animal Care Facility of The Life Sciences Research Building at The University at Albany-SUNY. The rats lived in a 12/12-hour reversed light cycle (lights off at 8 : 00 h) with unlimited access to Purina Rodent Chow and tap water in their home cages.

Drug administration

CEE (Wyeth Pharmaceuticals Inc.) was dissolved in vegetable oil vehicle to a concentration of 0.625 mg/kg. OVX rats were administered vegetable oil vehicle or CEE subcutaneously. This injection regimen was based upon findings that this dosage produces physiological E₂ levels in serum [12] and studies investigating the dose-dependent effects of CEE on brain and serum levels of the progestogen, 3 α ,5 α -THP, which has robust mnemonic, anxiolytic, and prosocial effects [13-15]. The regimen used was also based upon our findings of the dose-specific and timing-specific effects of E₂ on the anxiety behavior of female rats [1].

Procedure

Rats were randomly assigned to be administered vehicle ($n=8$) or CEE ($n=12$). They were tested in the behavioral tasks described below. For object recognition, the rats were administered vehicle or CEE immediately after training. Anxiety and social behavior testing occurred 44–48 h post injection.

Behavioral testing

Rats were tested in the following tasks. Behavioral data was collected by a trained observer uninformed of the hypothesis of the study and a video tracking system (AnyMaze; Stoelting Co., Wood Dale, Illinois, USA).

Object recognition

A typical protocol for object recognition training and testing was used in our laboratory as has been reported [4]. Briefly, rats were trained in the task and then tested 4 h later. Training consisted of the rats investigating two identical objects placed in an open field. During testing, the rats were again placed in the open field and then the time spent investigating the familiar object as well as a recently placed, novel object were recorded. Both trials were 3-min long. An increased percentage of time spent investigating the novel versus the familiar object, as a function of total exploration time of both objects during testing, was considered to reflect improved performance in this task.

Open field

Rats were tested, as previously reported, in a white melamine open field that was visually divided into 16 squares [3]. The number of central and total squares into which the rat entered during the 5-min task was recorded. An increase in central entries is considered to reflect antianxiety behavior.

Elevated plus maze

Testing in the elevated plus maze was done as described [3]. Briefly, rats were placed at the junction of two open and two closed arms. The time spent in the open and closed arms was recorded during the 5-min task. An increase in open arm time is considered indicative of antianxiety behavior.

Social interaction task

Testing in the social interaction task was done as previously described [3]. In brief, the rats were placed in an open field with an OVX, E_2 -primed stimulus female. The time spent interacting with the stimulus female, as defined by touching, sniffing, following with contact, and grooming, was recorded during a 5-min task. An increased duration spent interacting is considered to reflect prosocial behavior.

Lordosis quotients

Rats were tested for sexual behavior in a Plexiglas chamber using previously described methods [2]. The frequency of lordosis (lordosis quotient) for 10 mounts or for 10 min, whichever occurred first was recorded.

Statistical analyses

One-way analyses of variance tests were used to determine the effects of CEE condition on behavioral measures. If significant main effects were found, group differences were determined by Fisher's post-hoc tests. A P value of ≤ 0.05 was considered significant.

Results

Rats administered CEE posttraining in the object recognition task had increased percentages of time spent exploring the novel object than did rats administered vehicle post training [$F(1,18)=9.56$, $P<0.01$; Fig. 1].

For the open field, there were no statistically significant effects of CEE (62.4 ± 9.2) on the mean number of central squares entered, compared with vehicle (76.5 ± 10.0). Administration of CEE, compared with vehicle, to rats increased the time spent on the open arms of the elevated plus maze [$F(1,18)=5.06$, $P<0.03$; Fig. 1].

Rats administered CEE spent more time in social interaction with a conspecific than did the rats administered vehicle [$F(1,18)=13.41$, $P<0.01$; Fig. 1]. No statistically significant effects of CEE ($83.3\pm10.1\%$) on lordosis quotients, compared with vehicle ($68.8\pm12.4\%$), were found.

Discussion

The results of this study supported our hypothesis that CEE would enhance cognitive, anxiety, and social behavior. Administration of CEE improved cognitive performance in the object recognition task compared with vehicle. Similarly, CEE, compared with vehicle, increased antianxiety behavior in the elevated plus maze and prosocial behavior in the social interaction task. As CEE did not have effects on open field behavior or lordosis, this suggests that there is some specificity in CEE's beneficial functional effects.

The present data that CEE administered to middle-aged rats improves cognitive performance and increases anti-anxiety and antidepressant behavior confirm and extend earlier studies investigating the effects of CEE on brain function. Among menopausal and postmenopausal women, CEE therapy improves memory in some [16-18], but not all, studies [9,10]. Although other behavioral studies in rodents are lacking, in-vitro studies provide additional proof-of-concept evidence that CEE may have beneficial neural effects. CEE promotes neuronal growth in brain regions important for the functional effects that we observed, such as the cortex, hippocampus, and basal forebrain [19]. CEE, or specific components of it, have neuroprotective effects on β -amyloid or glutamate toxicity models and in experimental ischemia [20-22]. Thus, CEE can have beneficial effects; however, the underlying mechanisms need to be elucidated.

What are the active components of CEE that may underlie these beneficial effects? There is very little E_2 in CEE, but there are about 10 other E_2 -like sulfates, including two (estriol and estrone), which endogenously occur among women [7]. In our laboratory, we have been particularly interested in the extent to which there may be beneficial effects of estrogens through enhancing formation of progestogens and their subsequent actions at E_2 receptors, in particular the β isoform [1]. Of note, E_2 and CEE can enhance the formation of progesterone metabolites (which have mnemonic, antianxiety, and prosocial effects) in part by the increased activity of conversion enzymes [13,14,23,24]. However, there are a number of other potential constituents of CEE, including androgens [25], which may underlie the functional effects observed herein.

Conclusion

The most typically prescribed E_2 -based hormone therapy, CEE, has cognition-enhancing, antianxiety, and prosocial effects when administered to middle-aged female rats. The mechanisms and brain targets of these effects are of continued interest.

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Fig. 1. Effects (mean±SEM) of vehicle (veh) and conjugated equine estrogen (CEE) for behavior in the object recognition (left), elevated plus maze (center), and social interaction task (right). * $P < 0.05$.