Steroids 99 (2015) 16–25

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Pharmacological blockade of the aromatase enzyme, but not the androgen receptor, reverses androstenedione-induced cognitive impairments in young surgically menopausal rats

Sarah E. Mennenga [a,b], Stephanie V. Koebele [a,b], Abeer A. Mousa [a], Tanya J. Alderete [a], Candy W.S. Tsang [a], Jazmin I. Acosta [a,b], Bryan W. Camp [a,b], Laurence M. Demers [c], Heather A. Bimonte-Nelson [a,b,*]

[a] Department of Psychology, Arizona State University, Tempe, AZ 85287, United States
[b] Arizona Alzheimer's Consortium, Phoenix, AZ 85006, United States
[c] The Pennsylvania State University College of Medicine, The M. S. Hershey Medical Center, Hershey, PA 17033, United States

ARTICLE INFO

Article history:
Received 24 June 2014
Accepted 2 July 2014
Available online 23 August 2014

Keywords:
Androstenedione
Aromatase
Estrone
Cognition
Memory

ABSTRACT

Androstenedione, the main circulating ovarian hormone present after menopause, has been shown to positively correlate with poor spatial memory in an ovary-intact rodent model of follicular depletion, and to impair spatial memory when administered exogenously to surgically menopausal ovariectomized rats. Androstenedione can be converted directly to estrone via the aromatase enzyme, or to testosterone. The current study investigated the hormonal mechanism underlying androstenedione-induced cognitive impairments. Young adult ovariectomized rats were given either androstenedione, androstenedione plus the aromatase inhibitor anastrozole to block conversion to estrone, androstenedione plus the androgen receptor blocker flutamide to block androgen receptor activity, or vehicle treatment, and were then administered a battery of learning and memory maze tasks. Since we have previously shown that estrone administration to ovariectomized rats impaired cognition, we hypothesized that androstenedione's conversion to estrone underlies, in part, its negative cognitive impact. Here, androstenedione administration impaired spatial reference and working memory. Further, androstenedione did not induce memory deficits when co-administered with the aromatase inhibitor, anastrozole, whereas pharmacological blockade of the androgen receptor failed to block the cognitive impairing effects of androstenedione. Anastrozole alone did not impact performance on any cognitive measure. The current data support the tenet that androstenedione impairs memory through its conversion to estrone, rather than via actions on the androgen receptor. Studying the effects of aromatase and estrogen metabolism is critical to elucidating how hormones impact women's health across the lifespan, and results hold important implications for understanding and optimizing the hormone milieu from the many endogenous and exogenous hormone exposures across the lifetime.

1. Introduction

By the year 2050, the population over the age of 65 in the U.S. is projected to reach 88.5 million people, more than double what it was in the year 2010, and more than half of the population will be female [47]. Around the fifth decade of life, most females experience menopause, whereby eggs stop maturing, and eventually ovulation and menstruation cease. With this reproductive senescence, there is a drastic loss of ovarian-derived estrogen and progesterone, and the androgen androstenedione becomes the principal hormone released by the ovaries [46]. This androgen-rich hormone milieu is also seen in a rodent model of transitional menopause via treatment with 4-vinylcyclohexene diepoxide (VCD), an industrial chemical that induces gradual depletion of primary and primordial follicles in the female rat [35,34,3,2].

Accumulating evidence in the female rat suggests that androstenedione has a negative impact on cognition. Our laboratory previously demonstrated that VCD-induced, transitional menopause in middle-aged, female rats impairs cognitive performance across multiple domains, compared to rats that had undergone surgical

* Corresponding author at: Arizona State University, Dept. of Psychology, Behavioral Neuroscience Division, Arizona Alzheimer's Consortium, P.O. Box 871104, Tempe, AZ 85287, United States. Tel.: +1 480 727 0766; fax: +1 480 965 8544.
E-mail address: bimonte.nelson@asu.edu (H.A. Bimonte-Nelson).

http://dx.doi.org/10.1016/j.steroids.2014.08.010
0039-128X/

menopause via ovariectomy (Ovx). Of note, this finding was not apparent in animals that had undergone Ovx following VCD treatment, such that the follicle-deplete ovaries were removed after follicular depletion had ensued [3]. An unexpected finding from this study was that higher serum levels of androstenedione, which is released from the follicle-deplete menopausal ovary [46], correlated with poorer memory scores in follicle-deplete, VCD-treated rats [3]. In a follow-up study, we again found that higher androstenedione levels correlated with impaired spatial performance in transitionally menopausal rats demonstrating an androgen-rich serum profile [2]. This correlation was evident for multiple types of errors representing several domains of memory, including reference memory, a form of long-term memory that remains constant across all days and trials, as well as two orthogonal measures of working memory, a form of short-term memory that requires updating of information [2]. We surmised that if androstenedione was truly related to poorer memory, impairments should be revealed after administration of androstenedione to a "blank" ovarian hormone template. To test this hypothesis, we performed a study in which middle-aged (14 month old) Ovx rats were administered either vehicle or one of two doses of androstenedione, and then tested with a battery of mazes that assess learning and memory. Relative to vehicle treatment, androstenedione administration impaired spatial reference memory on the Morris water maze, was detrimental to performance on the water radial-arm maze (WRAM) when the working memory load was most demanding, and impaired memory retention on a win-stay delay match to sample (DMS) task [12]. Thus, in several different studies we have shown that androstenedione, released from the follicle-deplete ovary in both women and rats, markedly impairs memory.

Elucidating the effects of androstenedione on the brain and its function is critically important to understanding the cognitive impact of natural menopause; ovarian-derived androstenedione is present in menopausal women who maintain their ovaries, an effect observed for at least ten years after menopause ensues [18]. Drugs that block the activity of the aromatase enzyme [42], which catalyzes the conversion of androstenedione to the estrogen estrone, are some of the tools used to treat metastatic breast cancer prevalent in menopausal women [25], as well as manage estrogen-dependent endometrial carcinoma [20]. Here, we seek to decipher the hormone mechanism(s) underlying the negative cognitive impact of androstenedione using a rat model. Androstenedione could be exerting cognitive effects through a multitude of mechanistic pathways; it is a direct precursor to testosterone via the 17β-hydroxysteroid dehydrogenase (17β-HSD) enzyme, and to estrone via the aromatase enzyme, and, further, it binds to androgen receptors [28,30]. In the rodent model, testosterone administration has been shown to enhance spatial working memory [10], spatial reference memory [5], and performance on avoidance tasks [17,14]. There is also evidence that higher relative levels of testosterone are associated with better spatial ability performance in women, while lower relative levels of salivary testosterone were related to better spatial ability performance in men [26]. We have previously shown that estrone administration in Ovx rats produces cognitive impairments [15]. Given these results, we now hypothesize that androstenedione's conversion to estrone underlies its negative cognitive impact, rather than its actions on the androgen receptor.

The primary purpose of the current study was to systematically evaluate whether androstenedione's conversion to estrone, or its effects on the androgen receptor, are responsible for the negative cognitive effects of androstenedione administration in the surgically menopausal young adult rat. Herein, we tested the hormonal mechanism underlying our previously observed androstenedione-induced cognitive impairments using pharmacological manipulations that either block androstenedione's conversion to estrone, or block androstenedione's androgenic effects by blocking activation of the androgen receptor. Anastrozole, a non-steroidal aromatase inhibitor, or flutamide, a non-steroidal anti-androgen, were co-administered with androstenedione to determine whether androstenedione impairs memory via its conversion to estrone, or via its action on the androgen receptor, respectively. A secondary purpose of this study was to test the effects of anastrozole given alone. Indeed, aromatase inhibitors such as anastrozole are currently used to treat breast cancer and prevent breast cancer recurrence [42]. Elucidating the impact of aromatase and estrogen metabolism on the brain and its function is critical to our understanding of the systems-level alterations that occur with changes in both endogenous and exogenous steroid hormones.

2. Materials and methods

2.1. Subjects

Forty-nine four-month-old Fischer-344 virgin female rats born and raised at the National Institute on Aging colony at Harlan Laboratories (Indianapolis, IN) were used. Upon arrival, rats were pair housed, had access to food and water ad-lib, and were maintained on a 12-h light/dark cycle at the Arizona State University animal facility. All procedures were approved by the local IACUC committee and adhered to NIH standards. Rats arrived two weeks before experiment initiation (See Fig. 1).

2.2. Ovariectomy and hormone treatment

All rats received Ovx 13–14 days before the start of behavioral testing. Animals received bilateral dorsolateral incisions in the skin and peritoneum, the ovaries and tips of the uterine horns were ligatured and removed, and the muscle and skin were then sutured closed. During surgery, rats received subcutaneous injections of Rimadyl (5 mg/ml/kg) for pain and saline (2 ml) to prevent dehydration. Hormone or vehicle treatment began 2–3 days after surgery (11 days before behavioral testing ensued) and continued until sacrifice. All assigned treatments were administered daily via subcutaneous injection into the scruff of the neck at an injection volume of 0.5 ml. Rats were randomly assigned to one of five treatment groups: Vehicle ($n = 10$), Androstenedione ($n = 10$), Androstenedione + Anastrozole ($n = 10$), Androstenedione + Flutamide ($n = 10$), and Anastrozole ($n = 9$). Vehicle-treated animals received 0.5 ml of polyethylene glycol (PEG) (Sigma–Aldrich, St. Louis, MO, USA) only. All rats receiving androstenedione (Steraloids, Newport, RI, USA) were given 2 mg daily dissolved in PEG; this dose of androstenedione was based on previous literature [32,44,12] and has been shown to produce working memory impairments in middle-aged Ovx rats [12]. Animals in the Androstenedione + Anastrozole group received 0.025 mg/day anastrozole (Tocris, Minneapolis, MN, USA) co-administered with 2 mg androstenedione treatment, in order to block activity of the aromatase enzyme, preventing the conversion of androstenedione to estrone. Animals in the Androstenedione + Flutamide treatment group received 27.5 mg of flutamide (Sigma–Aldrich, St. Louis, MO, USA) co-administered with 2 mg androstenedione treatment, to block the action of testosterone on androgen receptors. The Anastrozole treatment group received 0.025 mg/day anastrozole dissolved in PEG.

Eleven days after the initiation of hormone treatment administration, behavioral testing began. Behavioral testing commenced approximately one hour after injections each day, and all treatment groups were counterbalanced across testing squads. All rats were subjected to the complete battery of behavioral evaluations. The order of behavior tests is concordant with our prior studies showing correlations between serum androstenedione levels and memory [3,2,12].