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Cognition in female rats after blocking conversion of androgens to estrogens

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ABSTRACT

Women and non-human females have surprisingly high levels of circulating testosterone, yet the effects of androgens on non-reproductive behaviors, including cognition, of females are not well characterized. The current project used an aromatase inhibitor, letrozole, to block conversion of androgens to estrogens. Adult female rats were ovariectomized and administered either vehicle only, testosterone propionate only (400 μg/kg, TP only), letrozole only (1 mg/kg, Letro only), or the combination of letrozole and testosterone (TP + Letro) over 4 weeks. A gonadally intact group was used for comparisons. During the last 3 weeks, the animals were tested for working memory in both a spatial task (radial arm maze) and a non-spatial task (object recognition). At sacrifice, uterine weights and serum testosterone and estradiol were determined. Behavioral results were the intact animals showed better working memories on the object recognition task, but that there were no differences among the ovariectomized groups. In the radial arm maze task, groups with best to worst performance were TP only > Intact = TP + Letro > vehicle = Letro only. Highest to lowest serum titers, for testosterone, were TP + Letro > TP only > Intact = Letro only > vehicle and, for estradiol, Intact > TP only > Vehicle > Letro only = TP + Letro. Our interpretation is that testosterone enhanced spatial performance when bioavailability of both TP and E2 are high, and high testosterone can rescue spatial memory when E2 bioavailability is low.

1. Introduction

The influences of testosterone on female brain regions and behaviors unrelated to reproduction are not well studied. Yet, circulating testosterone has been reported in women, female monkeys, and rats to be at significant levels, usually circa 10% but also as high as 40% of male values (Charles and Alexander, 2011; Labrie et al., 2009; Maliqueo et al., 2013; Sfikakis et al., 2014; Zhao et al., 2005). Moreover, serum titers follow the ebb and flow of ovarian hormones, mainly estradiol, in cycling females and during pregnancy (Beehner et al., 2005; Burger, 2002; Enea et al., 2008). Interestingly, serum levels of testosterone may not decrease during the transition to menopause and comparisons with natural menopause or oophorectomy suggests that the testosterone was from the post-menopausal ovaries (Davison et al., 2005).

Our interest in steroid metabolic pathways (Taylor et al., 2011; Taylor et al., 2012; Taylor et al., 1994) led to an experiment examining the relative role played by the direct effects of testosterone on female cognition rather than testosterone being aromatized to estradiol (E2). A swath of research holds that spatial and non-spatial memories of females can be enhanced by E2 and its binding of estrogen receptors (ERs) (Hammond et al., 2009; Holmes et al., 2002; Luine et al., 1998; Taylor et al., 2012; Walf and Frye, 2006; Wide et al., 2004). Administration of testosterone may also improve spatial memory in females (Roof and Havens, 1992), presumably after aromatization. However, the androgens and the androgen receptor (AR) also are implicated in hippocampal dependent memory in females (Isgor and Sengelaub, 1998; Isgor and Sengelaub, 2003). An important question on mechanisms is the relative role of E2 and testosterone in female cognition. Female cognition may be influenced by testosterone directly binding the AR, either before or after metabolism to dihyrotestosterone (DHT), or by binding estrogen receptors following conversion to E2 (Edinger and Frye, 2007; Handa et al., 2011).

The present study was designed to tease apart the effects of E2 from the androgens in spatial memory by targeting aromatase (Vierk et al., 2014). The strategic location of aromatase in the steroid metabolic cascade suggests that the aromatase inhibitor letrozole could be used to block conversion of testosterone to E2. Female rats were ovariectomized (OVX) and administered testosterone propionate (TP) with or without concurrent letrozole treatment. Control groups included an OVX letrozole only group and OVX or gonadally intact groups receiving

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only vehicle. Our logic was to assess cognition in groups of females in which testosterone is not converted to E2 by blocking aromatization. Because evidence suggests testosterone is central for spatial learning in males, we also employed a non-spatial task that is less sensitive to testosterone. Circulating levels of testosterone and E2, as well as hormone sensitive uterine weights, were measured at sacrifice at the end of the study.

2. Methods

2.1. Animals

A total of 40 female Long-Evans rats 3–6 months of age from the animal colony maintained at the University of Missouri-St. Louis served as subjects in the experiment. The females were either gonadally intact (N = 8) or ovariectomized (N = 32). All rats were individually housed in flat bottom plastic cages measuring 48.3 × 25.4 × 20.3 cm. Standard lab diet and water were available ad libitum except as dictated by a food restriction protocol described below. The colony room lighting was a 12:12 h reversed light/dark cycle; room temperature (20–22 °C) and relative humidity (50%) were controlled automatically. The Institutional Animal Care and Use Committee of the University of Missouri-St. Louis approved the experimental protocol.

2.2. Materials

For behavioral sessions, overhead lighting in the experimental room was turned off, and a red light was used as dim illumination of the area surrounding the apparatus. Movement of the animal was tracked by the ANYmaze tracking system (Stoelting Co, Wood Dale, IL, U.S.A.) via an overhead, infrared camera.

A radial arm maze (RAM) was used for tests of spatial learning. Briefly, the apparatus consists of a center platform with 8 arms radiating out from the center (120 cm diameter, 40 cm center diameter, 40 cm arm length, 10 cm arm width). At the end of each arm, there is a small hole to place food to prevent visual identification of the reward. Extramaze cues were located on walls surrounding the RAM apparatus for animals to use as spatial cues. The apparatus was cleaned with a solution of diluted soap after each session.

An object recognition (ObjRec) paradigm was used as the test for non-spatial learning and memory. The task is also a test of working memory in which the natural tendency of animals to explore unfamiliar objects is exploited as motivation to learn. Objects chosen from our collection of trinkets of various shapes and textures were placed at opposite sides of a glass terrarium (50 cm × 25 cm × 25 cm). Positions of the objects were counterbalanced within and between animals. All objects were cleaned with soap solution after each session. Assessment of locomotor activity was conducted using an open field apparatus (122 cm × 91.5 cm) onto which black lines divided the surface of the table into 15 squares of equal size (Taylor et al., 2012).

Testosterone propionate and letrozole were purchased (Sigma-Aldrich Chemical Company, St. Louis, MO, USA) and suspended in an olive oil vehicle. Materials for the radioimmunoassays of testosterone and estradiol in serum also were purchased (Boeringer, Mannheim, Germany).

2.3. Experimental design

Animals were either OVX or sham operated (intact) one week prior to the start of drug treatments. OVX rats were assigned at random to 1 of 4 groups (N = 8 per grp) to be subcutaneously (SC) injected with either vehicle (Veh), testosterone propionate (400 μg/kg body wt, TP only), letrozole (1 mg/kg body wt, Letro only) or both substances (400 μg/kg TP/ and 1 mg/kg body wt, TP + Letro). The gonadally intact females injected only with vehicle comprised the fifth group.

2.4. Procedures

The OVX surgeries were completed a week before the beginning of the experiment. Anesthesia was induced using 5% isoflurane administered via inhalation and maintained at 2–3%. Following recovery from surgery, animals received daily SC injections of experimental drug or vehicle for 4 weeks with behavioral sessions in the open field, RAM and ObjRec apparatus conducted during weeks 2–4. Food restriction began during week 2. Subjects were fed 12–14 g of rat chow per day to restrict animals to no less than 90% of their ad lib weight.

RAM procedures were conducted over a 2-week period, and ObjRec procedures were conducted over a 1-week period. Order of the two procedures was counterbalanced between and within groups. Using a procedure described earlier (Taylor et al., 2012), tests of general locomotor activity were 6 min in the open field apparatus completed weekly on days in which RAM and ObjRec procedures were not conducted.

The RAM procedure required two weeks for habituation, training, and testing. Pieces of Honey Nut cereal were used as reward. Prior to exposure to the RAM procedure, pieces of cereal were placed in a Petri dish in the home cage of each rat to become familiar with the new food. Animals were habituated to the apparatus for three days; all 8 arms of the RAM were baited, and the animal was allowed to explore for 15 min or until all cereal pieces were eaten. Training continued for 4 days in which the animals were removed after all cereal pieces were eaten or a maximum of 5 min had elapsed.

The final phase was the RAM test day in which all 8 arms were baited, and the rat introduced to the apparatus. Measuring the order of choices in the RAM assessed spatial working memory. Entering an arm that had not been visited previously was considered a correct choice. Working memory errors were defined as reentries into arms that had already been visited (Burgos et al., 2005; Nott and Levin, 2006). The numbers of correct choices before making a mistake by reentering an arm already visited (Brucato et al., 1994; Dellu-Hagedorn, 2005; Levin and Torry, 1996) were tallied for a subset of animals in each group. Animals were removed from the apparatus immediately upon consuming all food or until 5 min had elapsed.

A habituation session and a test session were used to assess behavior in the ObjRec task. Habituation consisted of two 15 min sessions over 2 days in the terrarium. For a subsequent test day, a pair of novel objects was placed in the terrarium and the animal given two trials. In Trial 1, the animal was allowed to freely investigate the objects for 5 min. Time directly investigating, defined as the snout being within 2 cm of the object (OShea et al., 2004; Taylor et al., 1999), was recorded for each object. The animal was returned to a holding cage for an inter-trial interval of 80 min. Upon being returned to the terrarium for Trial 2, the animal was confronted with one of the previous, and now familiar, objects and a novel object. Time spent during the second trial of 5 min investigating a novel object relative to the familiar object defined recognition of the old, familiar object (Taylor et al., 2004).

On the final day of the experiment, a subset of animals from each group were administered their normal drug treatment and sacrificed via guillotine 1 h later to correspond to the time after drug exposure that the animals were tested in the behavioral paradigms. Both uterine horns were excised and weighed as a measure of target organ responsiveness, and trunk blood was collected for hormone assays from 4 animals from each group. Concentrations of E2 and testosterone were determined by radioimmunoassays using a method similar to one previously published (Taylor et al., 2004). Serum was obtained by centrifugation and frozen at —80C until hormone assays were performed with Panted extraction kits (Boeringer, Mannheim, Germany). Detection limits were 5 pg/ml for estradiol and 0.2 ng/ml for testosterone. Inter-assay variation and intra-assay variation were, respectively, 6% and 8.3% for testosterone and 8.1% and 5.7% for estradiol.