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The Anxiolytic and Antidepressant-like Effects of Testosterone and Estrogen in Gonadectomized Male Rats

Nicole Carrier, Samantha K. Saland, Florian Duclot, Huan He, Roger Mercer, and Mohamed Kabbaj

ABSTRACT

BACKGROUND: While the influence of testosterone levels on vulnerability to affective disorders is not straightforward, research suggests this hormone may confer some degree of resiliency in men. We recently demonstrated a role for the dentate gyrus in mediating testosterone's protective effects on depressive-like behavior in gonadectomized male rats. Here, testosterone may exert its effects through androgen receptor-mediated mechanisms or via local aromatization to estradiol.

METHODS: Gonadectomized male rats were implanted with a placebo, testosterone, or estradiol pellet, and subsequent protective anxiolytic- and antidepressant-like effects of testosterone and its aromatized metabolite, estradiol, were then investigated in the open field and sucrose preference tests, respectively. Moreover, their influence on gene expression in the hippocampus was analyzed by genome-wide complementary DNA microarray analysis. Finally, the contribution of testosterone's aromatization within the dentate gyrus was assessed by local infusion of the aromatase inhibitor fadrozole, whose efficacy was confirmed by liquid chromatography-tandem mass spectrometry.

RESULTS: Both hormones had antidepressant-like effects associated with a substantial overlap in transcriptional regulation, particularly in synaptic plasticity- and mitogen-activated protein kinase pathway-related genes. Further, chronic aromatase inhibition within the dentate gyrus blocked the protective effects of testosterone.

CONCLUSIONS: Both testosterone and estradiol exhibit anxiolytic- and antidepressant-like effects in gonadectomized male rats, while similarly regulating critical mediators of these behaviors, suggesting common underlying mechanisms. Accordingly, we demonstrated that testosterone's protective effects are mediated, in part, by its aromatization in the dentate gyrus. These findings thus provide further insight into a role for estradiol in mediating the protective anxiolytic- and antidepressant-like effects of testosterone.

Keywords: Anxiety, Aromatase, Depression, Estradiol, Hippocampus, Testosterone

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Androgens are suspected to serve a protective role in the development of affective disorders and can improve depressive symptoms in both men and women. However, the connection between testosterone levels and depression vulnerability is not readily apparent, as both low and high testosterone levels have been associated with depressive symptoms, along with equivocal efficacy reported in studies investigating testosterone as a standalone treatment or adjunct therapy in depressed individuals (1). Despite this, the incidence of depression in men increases with age, coinciding with a decline in testosterone levels (2–4), and testosterone replacement has some efficacy in improving depressive symptoms in this population, as well as in men with refractory depression as an adjunct treatment to antidepressant medication (5–8). Moreover, hypogonadism in young men can precipitate depressive symptomology, supporting a protective role of testosterone against the development of affective disorders. Accordingly, preclinical research has shown that testosterone has antidepressant-like effects in aged male mice (9) and protects against the development of depressive-like behavior in male rats following gonadectomy (10,11).

While it is clear that testosterone can exert modulatory effects on affective state, the underlying mechanisms remain poorly characterized. Within the brain, physiological actions of testosterone are primarily mediated by its 5α-reduced and aromatase-derived metabolites, dihydrotestosterone (DHT) and estradiol, respectively. Mounting evidence points to an active role of aromatized estradiol in the regulatory effects of testosterone on affective status (12,13). This is conceivably due, in part, to its effects on neuronal plasticity within limbic regions implicated in the pathophysiology of depression, including the hippocampus. Indeed, several reports have confirmed significant aromatase expression throughout the hippocampus of rodents and humans in a steroid- and

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sex-independent manner (14–16). Given that the hippocampal formation is rich in both androgen and estrogen receptors (14,17), it is possible that testosterone may exert antidepressant and anxiolytic effects via both androgen-dependent and estrogen-dependent mechanisms within this brain region.

We recently demonstrated that testosterone protects against depressive-like behavior in gonadectomized male rats in the forced swim and sucrose preference tests and that these effects directly involve activation of the mitogen-activated protein kinase (MAPK) signaling pathway within the dentate gyrus of the hippocampus (10,13). Importantly, these effects were likely due to testosterone's estrogenic metabolite, as supplementation of estradiol to gonadectomized rats, but not DHT, mimicked the efficacy of testosterone in the forced swim test (10). Although testosterone can activate MAPK signaling via both estrogen-dependent (18) and androgen-dependent mechanisms, it is unclear whether similar mechanisms underlie the antidepressant-like effects of estradiol supplementation in gonadectomized male rats. Further, while both hormones profoundly regulate transcription and activation of depression-relevant signaling cascades within the hippocampus, these effects are complex and can be different (even opposite) or similar in male and female subjects, owing to vast sexual dimorphisms in brain morphology, neurochemistry, and function (19–21). As such, there is a critical need to delineate the contributions of testosterone and estradiol to mood-related symptoms in a sex-specific manner. Accordingly, our previous work demonstrated that chronic testosterone supplementation protected against depressive- and anxiogenic-like consequences of social isolation stress in gonadectomized male, but not female, rats (11).

Given that, in our hands, testosterone lacked protective efficacy in ovariectomized female rats, the present work focused on male rats to determine the nature of estradiol's contribution to the protective actions of testosterone at behavioral and molecular levels. In this work, we investigated the protective anxiolytic- and antidepressant-like effects of testosterone and its aromatized metabolite in gonadectomized male rats and their influence on hippocampal gene expression. In addition, because systemically administered estradiol has widespread effects on various brain regions involved in the display of affective behaviors, we directly examined the role of testosterone's conversion to estradiol in the dentate gyrus on anxiety- and depressive-like behaviors via local infusion of the aromatase inhibitor fadrozole.

METHODS AND MATERIALS

Animals

Adult male (250–270 g) Sprague-Dawley rats (Charles River, Wilmington, Massachusetts) were pair-housed in 43 × 21.5 × 25.5 cm plastic cages and kept on a 12-hour/12-hour light/dark cycle (lights on at 0700 hours). Food and water were available ad libitum except during testing. Behavioral experiments, except the sucrose preference test, were conducted during the first 4 hours of the light phase of the light/dark cycle and all animal protocols were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Florida State University.

Surgery

Rats were anesthetized with a ketamine (70 mg/kg)/xylazine (10 mg/kg) mixture (intraperitoneal). Bupivacaine (.25% solution; .4 mL/kg) was applied topically as analgesic and the nonsteroidal anti-inflammatory drug meloxicam (1.0 mg/mL) was injected subcutaneously.

Gonadectomy and Hormone Supplementation

Gonadectomy and sham surgeries were performed as previously described (10,11). Following gonadectomy/sham surgery, 60-day slow-release testosterone (25 mg/pellet), β-estradiol 3-benzoate (.1 mg/pellet), or placebo pellets (Innovative Research of America, Sarasota, Florida) were inserted subcutaneously into male rats 10 cm from a small 2-cm incision below the shoulder blades.

Osmotic Minipumps

Rats were implanted bilaterally with cannulae (Plastics One, Roanoke, Virginia) aimed at the dentate gyrus area of the dorsal hippocampus (anterior-posterior = −4.3; medial-lateral = ±3.0; dorsal-ventral = −4.7 mm) (10). Cannula placement was verified a posteriori by sectioning on a cryostat. All rats included in analyses had correct placements. Behavioral data from seven rats were excluded from analyses due to incorrect bilateral cannula placement. Two cannulae delivered 6 μL/day of sterile saline or the sterile saline containing 1.0 μg fadrozole (Sigma-Aldrich, St. Louis, Missouri) via subscapular Alzet Osmotic Minipumps (Model 2004; Alza, Mountain View, California). This dose was chosen based on reports that intracranial infusion of fadrozole within the .8 μg to 1.378 μg dosing range effectively abolishes the local conversion of testosterone to estradiol (22,23). Before implantation, osmotic minipumps were incubated at 37°C for 48 hours in sterile saline to equilibrate and ensure accurate flow rate.

Experimental Design

Experiment 1: Depressive-like Behavior Following Gonadectomy and Hormone Replacements. Ten days following surgery, depressive-like behavior of sham-operated and gonadectomized male rats (n = 6–8/group) receiving testosterone (GDX + T), estrogen (GDX + E), or placebo (GDX) pellet replacements were investigated using the sucrose preference test.

Experiment 2: Effects of Testosterone and Estrogen Supplementation on Gene Expression in the Hippocampus of Gonadectomized Male Rats. Upon completion of behavioral testing, rats from experiment 1 were sacrificed under nonstressful conditions, and their brains were snap-frozen in 2-methylbutane and stored at −80°C until further processing for genome-wide complementary DNA (cDNA) microarray analysis. RNA was extracted from tissue punches of the dorsal hippocampus and used to generate cDNA for microarray analysis and real-time quantitative polymerase chain reaction for validation of selected targets.