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Testosterone and imipramine have antidepressant effects in socially isolated male but not female rats

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ABSTRACT

Rationale: Affective disorders are twice as likely to occur in women as they are in men suggesting a critical role for gonadal hormones in their etiology. In particular, testosterone has been shown to have protective effects in men.
Objective: To investigate antidepressant effects and interactions between testosterone and imipramine in socially isolated male and female rats.
Methods: A chronic social isolation model was used to induce an anxiety and depressive-like state in adult gonadectomized (Gnx) male and ovariectomized (Ovx) female rats receiving chronic testosterone and imipramine treatments. Their anxiety and depression-like behaviors were examined using the light–dark box, elevated plus maze, open field, sucrose preference and novelty induced hypophagia tests.
Results: In socially isolated rats, the anxiolytic and antidepressant effects of testosterone and imipramine were limited to male rats. Additionally, testosterone enhanced the neurogenic effect of imipramine on hippocampal cell proliferation in male rats. Although female rats exhibited signs of anxiety and depressive-like behaviors following social isolation, testosterone and/or imipramine administration had no anxiolytic or antidepressant effects in Ovx females.
Conclusions: Testosterone and imipramine had anxiolytic and antidepressant effects in socially isolated male, but not female rats. Testosterone enhanced the effect of imipramine on cell proliferation in the hippocampus of male rats.

Introduction

There are well documented sex differences in the prevalence of anxiety and depressive disorders, where females are twice as likely as men to be affected (Angst et al., 2002; Bebbington et al., 2003; Earls, 1987; Kessler, 2003). Evidence from human and animal studies suggests that females react differently to stressors than males (Frye and Walf, 2009; Vermeulen and Kaufman, 2002). Although it is well established that women are more susceptible to the development of affective disorders, considerably less attention has been given to sex differences in the development, presentation, and features of depressive disorders as well as the response to antidepressant treatments.

Biological, psychological, and sociological theories attempt to explain the preponderance of women suffering from anxiety and depressive disorders, however, the prevailing hypothesis implicates a critical role for gonadal hormones in both the onset and regulation of mood disorders (Colangelo et al., 2008). In particular, testosterone had antidepressant effects in humans and rodents. Indeed, hypogonadal men with low circulating levels of testosterone have increased incidence of major depressive disorder and testosterone replacement effectively improved mood (Cunningham et al., 1989; McIntyre et al., 2006; McNicholas et al., 2003). Testosterone administration had protective effects against the development of anxiety and depressive-like symptoms and had antidepressant effects in aged male and female mice (Frye and Walf, 2009; Solomon et al., 2009). These studies suggested that testosterone is protective against the development and progression of affective disorders.

The onset of anxiety and depressive disorders is highly correlated with the occurrence of stressful life events (Agliati et al., 2006). In the laboratory, chronic social isolation constitutes an environmentally-induced stressful experience that may be effectively used to model a depressive-like state (Migues et al., 2005). In our recently published work (Carrier and Kabbaj, 2012), we have shown in stress-free conditions, that testosterone had antidepressant effects in male rats despite a lack of change in neurogenesis. In this work, Gnx male rats were subjected to a chronic social isolation protocol to examine if testosterone has protective effects and/or can affect neurogenesis under stressful conditions. We then extended our study to investigate potential anxiolytic and antidepressant effects of testosterone and imipramine administration in socially isolated Ovx female rats. We report prominent effects of testosterone and imipramine treatments in the response to social isolation stress in male, but not female rats.

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Methods

Animals

Adult male (weighing 250–270 g) and female (weighing 200–225 g) Sprague–Dawley rats, were purchased from Charles River (Wilmington, MA, USA). All rats were initially pair-housed in 43×21.5×25.5 cm Plexiglas cages and kept on a 12 h:12 h light: dark cycle (lights on at 0700 h). Food and water was available ad libitum except during testing. All behavioral experiments, except the sucrose preference test, were conducted during the first 4 h of the light phase of the light/dark cycle and all animal protocols were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Florida State University.

Surgery

Immediately following gonadectomy/ovariectomy, male and female rats received placebo/testosterone pellet and saline/imipramine osmotic minipumps. Rats were anesthetized with a mixture of ketamine/xylazine (70 mg/kg of ketamine and 10 mg/kg xylazine; i.p.) and bupivicaine (0.25% solution; 0.4 mL/kg) was applied topically as analgesic. The non-steroidal anti-inflammatory drug (NSAID) meloxicam (1.0 mg/mL) was injected subcutaneously.

Gonadectomy/ovariectomy

Following disinfection of the skin (with alcohol and betadine), a 1–2 cm ventral midline incision was made in the scrotum of adult male rats to expose the tunica. The tunica was pierced and both testes were extracted to expose the underlying blood vessels, which were ligated with silk suture. The testes were excised and all vessels and ducts were placed back into the tunica prior to suturing. A slightly larger 2–3 cm ventral midline incision was made in the lower abdominal region of adult female rats to expose the uterus. Visible blood vessels were ligated, the ovaries removed, and the muscle layers and skin were sutured.

Testosterone supplementation

Sixty-day slow release testosterone (25 mg/pellet) or placebo pellets (Innovative Research of America, Sarasota FL) were inserted subcutaneously 7–10 cm from a small 1–2 cm incision below the shoulder blades. We have shown that these pellets reproduce the physiological levels of testosterone found in male rats (Carrier and Kabbaj, 2012).

Osmotic minipumps

Alzet Osmotic Minipumps (Alza, Mountain View, CA) for 28-day administration (Model 2ML4), containing imipramine HCl (Sigma-Aldrich, St. Louis, MO; 20 mg/kg/day) or 0.9% saline were implanted subcutaneously in the dorsal rear flank region. Imipramine was prepared in 0.9% sterile saline.

Experimental Design

Experiment 1: Validation of chronic social isolation as an anxiety and depression model in male rats

Adult male rats (2–3 months of age at the start) were either pair-housed or socially isolated for 3 weeks prior to behavioral testing. During this time they were only handled twice a week for cage maintenance. Their anxiety and depression-like behaviors were examined using the light–dark box and sucrose preference tests respectively.

Experiment 2: Social isolation, testosterone, and imipramine exposure in male rats

Immediately following Gnx surgeries, all rats were socially isolated for 2 weeks to induce an anxiety and depressive-like state. Anxiety-like behaviors were assessed 13–16 days following isolation using the light dark box, open field, and elevated plus maze tests. Depressive-like behaviors were examined 3 weeks after social isolation using the sucrose preference and novelty-induced hypophagia tests. To label proliferating cells in the dentate gyrus, rats were injected with 5-bromo-2'-deoxyuridine (BrdU) under non-stress conditions, 3 days after all behavior testing was completed. Rats were transcardially perfused 24 h later, and their brains processed for BrdU immunohistochemistry.

Experiment 3: Validation of chronic social isolation as an anxiety and depression model in female rats

Adult female rats (2–3 months of age at the start) were either pair-housed or socially isolated for 3 weeks prior to behavioral testing. During this time they were only handled twice a week for cage maintenance. Their anxiety and depression-like behaviors were examined using the light–dark box and sucrose preference tests respectively.

Experiment 4: Social isolation, testosterone, and imipramine exposure in female rats

Immediately following Ovx surgeries, all rats were socially isolated for 2 weeks to induce an anxiety and depressive-like state. Anxiety-like behaviors were assessed 13–16 days following isolation using the light dark box, open field, and elevated plus maze tests. Depressive-like behaviors were examined 3 weeks after social isolation using the sucrose preference and novelty-induced hypophagia tests.

Behavioral tests

Light–dark box

Rats were placed into the dark compartment (200×310 mm) of the dual chamber apparatus (Model LE-812, EB Instruments, Pinellas Park, FL) and allowed to freely explore both compartments for 10 min. Total duration spent and frequency of entries in the light compartment (310×310 mm) were analyzed using PPCWIN software. The apparatus was cleaned with 70% ethanol between trials.

Open field test

Rats were placed in a large (1 m×1 m) open field under dim light and were allowed to freely explore the arena for 10 min. Rat behavior was recorded by a digital camcorder placed directly above the open field. Total duration spent in the center was analyzed in EthoVision XT version 6 (Noldus Information Technology, Leesburg, VA). The open field arena was cleaned with 70% ethanol between trials.

Elevated plus maze

Rats were placed into the elevated plus maze (MED Associates Inc., St. Albans, VT) facing a closed arm and were allowed to freely explore the maze for 10 min under dim light. Rats' behavior was recorded by a digital camcorder placed directly above the elevated plus maze. Open arm duration and number of entries into open arms were analyzed in EthoVision XT version 6 (Noldus Information Technology, Leesburg, VA). The elevated plus maze was cleaned with 70% ethanol between trials.