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Testosterone has antidepressant-like efficacy and facilitates imipramine-induced neuroplasticity in male rats exposed to chronic unpredictable stress

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ABSTRACT

Hypogonadal men are more likely to develop depression, while testosterone supplementation shows antidepressant-like effects in hypogonadal men and facilitates antidepressant efficacy. Depression is associated with hypothalamic–pituitary–adrenal (HPA) axis hyperactivity and testosterone exerts suppressive effects on the HPA axis. The hippocampus also plays a role in the feedback regulation of the HPA axis, and depressed patients show reduced hippocampal neuroplasticity. We assessed the antidepressant-like effects of testosterone with, or without, imipramine on behavioral and neural endophenotypes of depression in a chronic unpredictable stress (CUS) model of depression. A 21-day CUS protocol was used on gonadectomized male Sprague–Dawley rats treated with vehicle, 1 mg of testosterone propionate, 10 mg/kg of imipramine, or testosterone and imipramine in tandem. Testosterone treatment reduced novelty-induced hypophagia following CUS exposure, but not under non-stress conditions, representing state-dependent effects. Further, testosterone increased the latency to immobility in the forced swim test (FST), reduced basal corticosterone, and reduced adrenal mass in CUS-exposed rats. Testosterone also facilitated the effects of imipramine by reducing the latency to immobility in the FST and increasing sucrose preference. Testosterone treatment had no significant effect on neurogenesis, though the combination of testosterone and imipramine increased PSA-NCAM expression in the ventral dentate gyrus. These findings demonstrate the antidepressant- and anxiolytic-like effects of testosterone within a CUS model of depression, and provide insight into the mechanism of action, which appears to be independent of enhanced hippocampal neurogenesis.

Introduction

Hypogonadal males are more likely to develop depression, and conversely, testosterone supplementation has antidepressant action, as meta-analyses show improved mood scores in hypogonadal men following testosterone treatment (Amanatkar et al., 2014; Hintikka et al., 2009; Westley et al., 2015; Zarrouf et al., 2009). Testosterone supplementation can improve the response of patients to monoaminergic antidepressant drugs (Seidman and Rabkin, 1998), although evidence for this effect is limited, and it should be noted that side effects such as increased paranoid ideation may exist (Orengo et al., 2005; Pope et al., 2010; Seidman et al., 2005; Wilson et al., 1974). Interestingly, a sex difference is present in patient responsiveness to antidepressant treatment: women have a better outcome on mood scores with selective serotonin reuptake inhibitor (SSRI) treatment, whereas men have a better outcome on mood scores when treated by tricyclic antidepressants (TCAs), such as imipramine (Baca et al., 2004; Kornstein et al., 2000). These findings collectively indicate that the gonadal hormonal milieu and/or biological sex could impact the efficacy of antidepressant drugs, depending on the class of drug.

Stress is a major preceding factor in the development of depression (Kendler et al., 1999), where dysregulation and hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis are associated with the pathogenesis of depression (Stetler and Miller, 2011; Stokes, 1995). Antidepressant treatment normalizes HPA function in depressed patients (Schule, 2007) in a manner coincident with an improvement in mood scores (Ising et al., 2007). The hippocampus plays an essential role in the feedback regulation of the HPA axis (Jacobson and Sapolsky, 1991), and altered hippocampal morphology often coincides with

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increased HPA activity (Swaab et al., 2005). Depressed patients show reduced hippocampal volume, as evidenced by a meta-analysis (McKinnon et al., 2009) and reduced neuron density in the hippocampus (Boldrini et al., 2013). Antidepressant treatment in depressed patients, on the other hand, is associated with increased hippocampal volume, which may in part be mediated by enhanced hippocampal neurogenesis; although this effect is dependent on the age and sex of the patient (Boldrini et al., 2009; Epp et al., 2013; Lucassen et al., 2010). Neurogenesis in the hippocampus may play a key role in re-establishing basal HPA tone following stress (Snyder et al., 2011), and in facilitating the normalization of HPA feedback by antidepressant treatment (Khemissi et al., 2014; Surget et al., 2011). Thus, hippocampal neurogenesis is intimately tied to depression, HPA dysregulation, and perhaps specific aspects of antidepressant efficacy (David et al., 2009; Santarelli et al., 2003).

Depressed patients show reduced hippocampal neurogenesis (Boldrini et al., 2009, 2012), and the upregulation of neurogenesis is thought to play an integral role in antidepressant efficacy (Wainwright and Galea, 2013). Similarly, the expression of polysialylated form of the neural cell adhesion molecule (PSA-NCAM), which is involved in multiple forms of hippocampal neuroplasticity (Burgess et al., 2008; Rutishauser, 2008), is reduced by gonadectomy, by stress, and in depressed patients, while PSA-NCAM expression is enhanced by antidepressant treatment (Guirado et al., 2012; Varea et al., 2007a, 2007b; Wainwright et al., 2011, 2015). Furthermore, gonadectomy in males reduces hippocampal neuroplasticity, a phenotype that resembles the effects of CUS exposure (Carrier and Kabbaj, 2012a; Spritzer and Galea, 2007; Wainwright et al., 2011). Antidepressant drugs increase hippocampal neurogenesis via enhanced cell proliferation, with no independent effect on cell survival (Malberg et al., 2000). In contrast, chronic testosterone treatment increases hippocampal neurogenesis via an androgen receptor-dependent enhancement of cell survival, with no independent effect on cell proliferation (Hamson et al., 2013; Spritzer and Galea, 2007). Indeed, the antidepressant-like effects of testosterone do not coincide with any changes in cell proliferation in non-stressed or socially-isolated rats (Carrier and Kabbaj, 2012a,b), or cell survival in non-stressed rats (Carrier and Kabbaj, 2012a). To date, no study has thoroughly explored the effects of testosterone treatment in gonadectomized males in a model of depression with a specific focus on hippocampal neurogenesis and PSA-NCAM mediated neuroplasticity.

There are numerous animal models of depression, but the chronic unpredictable stress (CUS) model of depression produces the best combination of face, construct, and predictive validity for observing the pathogenesis of depression and assessing the efficacy of antidepressant agents (Hill et al., 2012; Willner, 2005). To our knowledge no study has fully examined antidepressant-like effects of testosterone on anhedonia, behavioral despair, and novelty-induced hypophagia within a CUS model of depression in a single study. Nor has a study fully assessed testosterone-mediated alterations to hippocampal neuroplasticity within a model of depression. This study aims to fill these gaps in the literature.

We have previously shown that gonadectomized males develop more pronounced behavioral and neurobiological depressive-like endophenotypes than intact males when exposed to CUS (Wainwright et al., 2011). Treatment with testosterone produces antidepressant-like effects in non-stressed, intact male and female rodents (Frye and Walf, 2009), rodent models of depression using isolation stress (Carrier and Kabbaj, 2012b), and rodent models of male hypogonadism (Carrier and Kabbaj, 2012a, 2012b); however, testosterone shows no antidepressant-like behavioral effects in ovariectomized females highlighting a key sex-difference in the absence of normal gonadal tone (Carrier and Kabbaj, 2012b). Though a previous study has assessed the antidepressant-like effects of testosterone in a social-isolation model of depression, measures of behavioral despair were not used, only isolated rats were behaviourally tested, and some measures of hippocampal neurogenesis including cell survival or cell differentiation were not included (Carrier and Kabbaj, 2012b).

The current study assessed the antidepressant-like efficacy of testosterone, alone and in combination with the tricyclic antidepressant imipramine, in gonadectomized males using a CUS model of depression. We examined whether testosterone alone, and in combination with imipramine, would reverse depressive-like behavioral, endocrine, and neural phenotypes in the CUS model of depression. Several components of neurogenesis (proliferation, survival, and differentiation) as well as the expression of PSA-NCAM were measured to investigate the influence of testosterone treatment on hippocampal neuroplasticity and any coincident antidepressant-like effects. We hypothesized that testosterone would show antidepressant-like effects, alone and in conjunction with imipramine treatment, in a manner that coincides with increased hippocampal neuroplasticity.

Methods

Subjects

Eighty male Sprague–Dawley rats were obtained from the Animal Care Center at the University of British Columbia. All rats weighed between 275 and 300 g (approximately 60–65 days old) upon arrival. Rats were pair-housed in Hi Temp rat cages made of polycarbonate (Ancare, Bellmore, NY) with aspen chip bedding, paper towels, and a polyvinylchloride tube in a temperature-controlled room (21 ± 1 °C) with a 12:12 h light/dark cycle (lights on at 0700 h). Food (Purina lab rat diet 5012) and water were provided ad libitum, except during periods of deprivation associated with the CUS protocol.

All rats received bilateral castration, allowed one week for recovery then randomly assigned into treatment groups according to whether they received daily injections of testosterone propionate (T) or oil, and/or treatment with the antidepressant imipramine (IMI) or vehicle (VEH), and/or received chronic unpredictable stress (CUS) or no application of stressors (noCUS). Thus there were 8 groups: Oil + Sal + noCUS; T + Sal + noCUS; Oil + IMI + noCUS; T + IMI + noCUS; Oil + Sal + CUS; T + Sal + CUS; Oil + IMI + CUS; and T + IMI + CUS. All testing and procedures were carried out in accordance with the Canadian Council for Animal Care guidelines and were approved by the Animal Care Committee at the University of British Columbia. All efforts were made to reduce the number of animals used and to minimize their suffering.

Surgery

Surgery was conducted one week after the rats arrived in the colony using aseptic procedures. The rats weighed 300–350 g on the day of surgeries. Under isoflurane anesthesia (5% in oxygen during induction, 3% in oxygen during maintenance), each rat was bilaterally castrated with both testes extracted through a small incision made at the posterior tip of the scrotum and were ligated with a monofilament suture. Immediately after surgery, Flamazine cream (1% silver sulfadiazine) was applied to the incision site and each rat was given an s.c. injection of Ketoprofen (5 mg/kg body mass) as an analgesic every 24 h for the three days following surgery. The rats were singly-housed for 3 days following surgery then pair-housed with their previous cage mate and given an additional 4 days for recovery (a total of one week) prior to further experimentation.

BrdU administration

On the day prior to commencement of CUS or noCUS treatment all rats were given one i.p. injection of the thymidine analog BrdU (5-Bromo-2-deoxyuridine; 200 mg/kg) to label dividing cells and their progeny (see Fig. 1 for timeline). The timing of BrdU injection