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Central 5-alpha reduction of testosterone is required for testosterone's inhibition of the hypothalamo-pituitary–adrenal axis response to restraint stress in adult male rats

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ABSTRACT

In rodents, the hypothalamo-pituitary–adrenal (HPA) axis is controlled by a precise regulatory mechanism that is influenced by circulating gonadal and adrenal hormones. In males, gonadectomy increases the adrenocorticotropic hormone (ACTH) and corticosterone (CORT) response to stressors, and androgen replacement returns the response to that of the intact male. Testosterone (T) actions in regulating HPA activity may be through aromatization to estradiol, or by 5α-reduction to the more potent androgen, dihydrotestosterone (DHT). To determine if the latter pathway is involved, we assessed the function of the HPA axis response to restraint stress following hormone treatments, or after peripheral or central treatment with the 5α-reductase inhibitor, finasteride. Initially, we examined the timecourse whereby gonadectomy alters the CORT response to restraint stress. Enhanced CORT responses were evident within 48 h following gonadectomy. Correspondingly, treatment of intact male rats with the 5α-reductase inhibitor, finasteride, for 48 h, enhanced the CORT and ACTH response to restraint stress. Peripheral injections of gonadectomized male rats with DHT or T for 48 h reduced the ACTH and CORT response to restraint stress. The effects of T, but not DHT, could be blocked by the third ventricle

Abbreviations: 5αR, 5-alpha reductase; 3β-diol, 5-alpha androstane 3β,17βdiol; 3V, third ventricle; 5αDHP, 5α-dihydroprogesterone; ACTH, adrenocorticotropic hormone; BnST, bed nucleus of the stria terminalis; CORT, corticosterone; CRH, corticotropin releasing hormone; CSF, cerebrospinal fluid; DHT, dihydrotestosterone; DHTP, dihydrotestosterone propionate; E, estradiol; ER, estrogen receptor; HPA, hypothalamo-pituitary-adrenal; icv, intracerebroventricular; ir, immunoreactivity; MPOA, medial preoptic area; PBS, phosphate-buffered saline; PVN, paraventricular nucleus; s.c, subcutaneous; T, testosterone; TP, testosterone propionate; Veh, vehicle

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administration of finasteride prior to stress application. These data indicate that the actions of T in modulating HPA axis activity involve 5α-reductase within the central nervous system. These results further our understanding of how T acts to modulate the neuroendocrine stress responses and indicate that 5α reduction to DHT is a necessary step for T action.

1. Introduction

In rodents, adrenal corticosterone (CORT) secretion is controlled by the activity of a neuroendocrine axis that involves the hypothalamus, the anterior pituitary and the adrenal gland. This hypothalamo-pituitary–adrenal (HPA) axis represents a cascade of neural and humoral signals driven by both the circadian pacemaker as well as the environment. Changing environmental conditions or perceived threats to homeostasis activate the HPA axis by funneling information through neurons located in the paraventricular nucleus of the hypothalamus (PVN), a critical brain region that integrates positive and negative inputs to achieve a proper endocrine output. Central to HPA axis regulation are selective neurons in the parvocellular part of the PVN that contain corticotropin-releasing hormone (CRH).

Evidence that sex steroid hormones can interact with the regulatory elements of the HPA axis comes from studies showing that gonadectomy of both males and females alters the reactivity of the HPA axis (Handa et al., 1994a). Depending on dose, timing, or age of treatment, estradiol (E) treatment of gonadectomized animals can either enhance or inhibit (Serova et al., 2010; Evuarherhe et al., 2009; Weiser and Handa, 2009; Young et al., 2001) HPA activity, whereas androgen treatment has consistently been shown to inhibit HPA reactivity in rodents, monkeys and humans (Kalil et al., 2013; Williamson and Viau, 2008; Handa et al. (1994); Toufexis and Wilson, 2012; Rubinow et al., 2005). To date, the mechanism(s) by which T and E act to influence HPA function have not been completely resolved. Evidence for T and E acting in part at the adrenal gland (Kitay, 1965), anterior pituitary (Coyne and Kitay, 1969, 1971; Viau and Meaney, 2004) and hypothalamus (Viau and Meaney, 1996; Viau, 2002; Handa et al. (1994)) exists. Furthermore, recent studies have also suggested that the actions of E can be differentially mediated by estrogen receptor (ER) alpha and beta. Whereas ERalpha agonists increase the CORT response to stress, ERbeta agonists have been shown to inhibit the response (Lund et al., 2006, Weiser and Handa, 2009).

Although androgens suppress HPA axis reactivity (Handa et al. (1994)) and reduce CRH-immunoreactivity (ir) in the PVN (Bingaman et al., 1994a), androgen receptors (ARs) are not expressed in neuroendocrine neurons within the PVN (Bingham et al., 2006, Bingaman et al., 1994b). PVN neurons that express AR are found in the dorsal and the ventral medial parvocellular parts of the PVN, which are non-neuroendocrine neurons that project to spinal cord and brainstem pre-autonomic nuclei (Bingham et al., 2006). Consequently, it has been hypothesized that androgens regulate PVN neuropeptide expression and secretion transsynaptically. In support of this, implantation of testosterone (T) into the medial preoptic area (MPOA) and bed nucleus of the stria terminalis (BnST), brain regions that provide afferent input to the PVN, can reduce the CORT response to acute stress (Williamson and Viau, 2008; Viau, 2002; Viau and Meaney, 1996). Further, retrograde tracing studies show that AR-ir can be specifically found in neurons of the BnST, MPOA and anteroventral periventricular nucleus that project to the PVN (Williamson and Viau, 2007). By contrast, these areas may not be the only brain sites mediating the inhibitory effect of androgens on HPA reactivity since stereotaxic application of dihydrotestosterone (DHT) to a region just above the PVN (to prevent mechanical disruption of the PVN) was as effective as peripherally administered DHT in inhibiting HPA function (Lund et al., 2006). Such results raise the possibility that androgens may work at multiple brain sites to regulate the gain of the HPA axis.

It is now well established that T, the principle circulating androgen in males, can be intracellularly converted to E in brain tissue by the aromatase enzyme (Roselli et al., 1997), or to DHT by the 5-alpha reductase enzyme (5αR; Lephart, 1993). Although both T and DHT bind the AR with high affinity, DHT has classically been used in studies of androgen action as it is considered to be a more potent and selective agonist for ARs and is not a substrate for aromatization to estradiol. However, whether central 5-alpha reduction of T to DHT is a necessary step for the inhibitory effects of androgens on HPA function has not been determined. Consequently, in these studies, we have examined the role of the central 5αR enzyme in the androgenic modulation of the HPA reactivity to restraint stress by using centrally or peripherally administered finasteride, an inhibitor of 5αR enzyme activity. In addition, as a prelude to this exploration, we also characterized the time course of changes in HPA stress responsiveness following gonadectomy of male rats.

2. Results

2.1. *Time course of changes in stress-reactive levels of CORT following gonadectomy of male rats*

Restraint stress-responsive levels of plasma CORT were measured at 1, 2, 3, 5 and 7 days following gonadectomy of adult male rats. The effect of gonadectomy over days was first analyzed by comparing the CORT responses to stress using a 2 way ANOVA with gonadal state (GDX, Sham) and days post-gonadectomy as main factors. The analysis yielded a main effect of gonadectomy ($F_{1,88}=10.64$, $p<0.002$). As shown in Fig. 1, a one-way ANOVA including intact animals (0 days GDX) and all times following GDX revealed increased levels of CORT at day 2, 3, 5 and 7 days following gonadectomy compared to those of intact animals. A one-way follow-up