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Androgen regulation of corticotropin-releasing hormone receptor 2 (CRHR2) mRNA expression and receptor binding in the rat brain

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ABSTRACT

Stress-induced affective disorders, such as depression and anxiety, are more prevalent in females than in males. The reduced vulnerability to these disorders in males may be due to the presence of androgens, which are known to dampen the stress response and reduce anxiety-like behaviors. However, a neurobiological mechanism for this sex difference has yet to be elucidated. Corticotropin-releasing hormone receptor 2 (CRHR2) has been implicated in regulating anxiety-type behaviors and is expressed in stress-responsive brain regions that also contain androgen receptors (AR). We hypothesized that androgen may exert its effects through actions on CRHR2 and we therefore examined the regulation of CRHR2 mRNA and receptor binding in the male rat forebrain following androgen administration. Young adult male Sprague/Dawley rats were gonadectomized (GDX) and treated with the non-aromatizable androgen, dihydrotestosterone propionate (DHTP) using hormone filled Silastic capsules. Control animals received empty capsules. Using quantitative real-time RT-PCR, CRHR2 mRNA levels were determined in block-dissected brain regions. DHTP treatment significantly increased CRHR2 mRNA expression in the hippocampus, hypothalamus, and lateral septum ($p<0.01$) when compared to vehicle-treated controls. A similar trend was observed in amygdala ($p= 0.05$). Furthermore, *in vitro* autoradiography revealed significantly higher CRHR2 binding in the lateral septum in androgen-treated males, with the highest difference observed in the ventral lateral region. Regulation of CRHR2 mRNA by AR was also examined using an *in vitro* approach. Hippocampal neurons, which contain high levels of AR, were harvested from E17–18 rat fetuses, and maintained in primary culture for 14 days. Neurons were then treated with dihydrotestosterone (DHT; 1 nM), DHT plus flutamide (an androgen receptor antagonist), or vehicle for 48 h. CRHR2 mRNA levels were measured using quantitative real-time RT-PCR. Consistent with *in vivo* studies, DHT significantly increased CRHR2 mRNA expression in hippocampal neurons ($p<.02$) compared to vehicle-treated controls. Flutamide treatment prevented the effect of DHT on CRHR2 mRNA indicating that DHT's effect on CRHR2 expression is AR-mediated. Thus, the CRHR2 gene appears to be a target for regulation by AR and these data suggest a potential mechanism by which androgen may alter mood and anxiety-related behaviors.

Introduction

Stress-related psychiatric disorders like major depressive disorder and generalized anxiety disorder affect well over 10% of the population in the United States (Kessler et al., 2005). Underlying these pathologies is an apparent dysregulation of stress-responsive neuroendocrine function (Varghese and Brown, 2001; Barden, 2004), suggesting a critical role for stress sensitivity in affective disorders. Furthermore, several studies have consistently reported that major depressive episodes are over twice as common in women as compared to men (Weissman et al., 1993; Kornstein, 1997; Llewellyn et al., 1997), a difference that emerges at puberty (Angold and Worthman, 1993), suggesting a hormonal component. Importantly, numerous studies have confirmed the involvement of hypothalamic–pituitary–adrenal (HPA) axis dysregulation with depression and anxiety disorders (Holsboer and Barden, 1996; Ehlert et al., 2001; Varghese and Brown, 2001; Barden, 2004). Taken together, these data indicate a potential interplay between gonadal steroids, stress reactivity, and the development of mood disorders. The mechanism of estrogen's effect on mood and stress sensitivity has been extensively studied. However, the method by which androgens alter mood and HPA axis function has not been as well explored.

The HPA axis is the major neuroendocrine axis that responds to stress. Activation of the HPA axis following a physical or emotional stressor is characterized by a series of neuronal and hormonal responses in an attempt to maintain homeostasis (see de Kloet et al., 2005 for review). Activity of the HPA axis is controlled by a subset of neurons in the parvocellular part of the paraventricular nucleus (PVN) of the hypothalamus that receive afferent stress-related input, and

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secrete corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) into the hypophyseal portal system. Afferents from the brainstem, basal forebrain, and limbic brain regions provide stressor-related input directly and indirectly to these neuroendocrine neurons. At the anterior pituitary, CRH, and to a lesser extent AVP, stimulates the synthesis and secretion of adrenocorticotropic hormone (ACTH), which in turn drives glucocorticoid production by the adrenal cortex (cortisol in humans, corticosterone (CORT) in rodents). Thus, the rapid activation of the HPA axis in response to a stressor is directed by hypothalamic CRH.

In male rodents, removal of the endogenous source of androgen by gonadectomy (GDX), causes increased anxiety- and depressive-type behaviors which are reversed by systemic testosterone treatment (Slob et al., 1981; Adler et al., 1999; Frye and Seliga, 2001). Moreover, GDX males have higher stress-induced CORT and ACTH, which is also reversible via testosterone or dihydrotestosterone (DHT) treatment (Handa et al., 1994; Viau and Meaney, 2004). Gonadectomy is not accompanied by changes in pituitary sensitivity to CRH (Handa et al., 1994) or changes in levels of circulating corticosteroid binding globulin (CBG; Lund et al., 2004b) suggesting that the actions of androgens on HPA axis reactivity to stress are mediated centrally. Similarly, in men, aging is associated with a concomitant decline in androgen levels that may lead to a host of behavioral symptoms that overlap greatly with those of major depression (Amore, 2005).

Dysregulation of CRH signaling, in particular, plays a major role in the development of depression and anxiety (Heuser et al., 1998; Arborelius et al., 1999; Reul and Holsboer, 2002). Both receptors for CRH, CRHR1 and CRHR2, have integral roles in regulating stress sensitivity and alterations in receptor expression can be linked to behavioral disorders (for review see Bale and Vale, 2004). For example, CRHR1 knockout (KO) animals show hyporesponsivity, whereas CRHR2KO animals show hyperesponsivity to a restraint stress. Furthermore, CRHR1KO animals exhibit reduced, whereas CRHR2KO animals display increased anxiety (Smith et al., 1998; Timpl et al., 1998; Bale et al., 2000; Kishimoto et al., 2000). Thus, it appears that CRHR1 and CRHR2 act in an opposing fashion, where CRHR1 is responsible for activation of the HPA axis and anxiety-related behaviors, while CRHR2 may be responsible for attenuating these responses.

Sex hormones play a key role in regulating central CRH expression (for review see Ni and Nicholson, 2006). The CRH promoter contains estrogen receptor response element (ERE) half sites and at least one androgen response element (ARE) (Vamvakopoulos and Chrousos, 1993; Bao et al., 2006). In females, CRH mRNA expression in the PVN is reduced by ovariectomy, and can be restored via estrogen replacement treatment (Roy et al., 1999). Additionally, in gonadectomized males, estradiol benzoate increases restraint-induced CRH hnRNA within the PVN (Lund et al., 2004a). On the other hand, androgens appear to have an inhibitory effect on CRH expression. Basal CRH expression is lower in males than in females, and gonadectomy in males causes an increase in basal CRH expression that can be restored by dihydrotestosterone propionate (DHTP) treatment (Haas and George, 1988; Bingaman et al., 1994). Furthermore, treatment of gonadectomized males with DHTP reduces restraint-induced CRH hnRNA (Haas and George, 1988; Lund et al., 2004a).

It is currently unknown whether sex steroids regulate the expression, function, or activity of CRH receptors. Interestingly, the CRHR2 promoter contains EREs and AREs, which suggests a potential role for sex hormones in the modulation of CRHR2 expression (Catalano et al., 2003). Furthermore, in the male vole, CRHR2 binding is higher in the bed nucleus of the stria terminalis (BST) than in the female vole (Lim et al., 2005). Additionally, the expression pattern for CRHR2 in brain overlaps considerably with that of androgen receptor (AR) (Van Pett et al., 2000). Thus, the connection between androgens, HPA axis sensitivity, and stress-related disorders may be partially explained by androgen regulation of CRHR2 signaling. To explore this possibility, we examined androgen regulation of CRHR2 expression in the male rodent forebrain. The results of these studies demonstrate that androgen upregulates CRHR2 mRNA in most areas of overlap between CRHR2 and AR, and this regulatory function is mediated specifically via AR. Thus, androgen regulation of the CRHR2 gene is a potential mechanism for androgen modulation of stress and stress-related behavioral disorders.

Materials and methods

Animals

Young adult male (300–400 g), and timed pregnant female Sprague–Dawley rats were obtained from Charles River Laboratories (Wilmington, MA), caged in pairs (adult males), or individually housed (pregnant dams) in shoebox type cages in the Colorado State University laboratory animal research facility and maintained on a 12:12-h light schedule (lights on at 0700 h) with *ad libitum* access to rat chow and water. All animal protocols were approved by the Animal Care and Use Committee at Colorado State University. One week following arrival, the adult male rats were gonadectomized under isofluorane anesthesia. Two weeks following gonadectomy, animals were implanted with two 2.5 mm Silastic capsules (Dow Corning, Midland, MI; 0.062" ID, 0.125" OD) containing either crystalline 5alpha-androstan-17beta-ol-3-one propionate (DHTP, Steraloids, Newport, RI), or nothing (blank). One week following capsule implantation, the animals were sacrificed and brains immediately removed and either flash-frozen in isopentane at −30 °C (for receptor autoradiography) or chilled on ice and immediately microdissected for subsequent RNA isolation. Trunk blood was collected into pre-chilled tubes containing 0.5 M EDTA. For *in vitro* studies, pregnant dams were halothane anesthetized on gestational day 17 or 18 and the rat fetuses were delivered by Cesarean section. Brains were removed and placed in ice-cold CMF Ringer's-glucose solution until the hippocampi were dissected and cells were dispersed. Primary hippocampal neurons were harvested as described below.

Dihydrotestosterone radioimmunoassay

Plasma concentrations of DHT were measured via radioimmunoassay by a commercially available kit (Diagnostic Systems Laboratories, Inc., Webster, TX). Plasma obtained from each animal was run in duplicate alongside a standard curve of known DHT concentrations ranging from 0 pg to 2500 pg. The sensitivity of the assay was 4 pg/ml and intra-assay variance was 4.2%. All samples were run in a single assay to prevent interassay variation from influencing the results.

Tissue block dissection and RNA isolation

Freshly harvested tissue was dissected on ice from coronal sections, according to the atlas of Paxinos and Watson (1998). For hypothalamus, a coronal slice was made from optic chiasm rostral to the anterior edge of the mammillary bodies caudal and the hypothalamic sulcus lateral to the top of the third ventricle superior. This dissection included the entire rostral–caudal extent of the hypothalamus. The lateral septum was dissected in its entire dorsal to ventral aspect from a coronal slice made of approximately 1.0 mm anterior to bregma through −0.5 mm posterior to bregma. Medial septum was excluded from this dissection. Hippocampus and amygdala were dissected from the same coronal slice used for hypothalamus. Dissected tissue was placed into microcentrifuge tubes on ice containing GIT extraction buffer (4 M guanidinium thiocyanate, 25 mM sodium citrate, 0.5% N-laurel sarcosine, 0.1 M β-mercaptoethanol) and homogenized. Total RNA was isolated using previously described protocols (Chomczynski and Sacchi, 1987). The