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Sexually dimorphic expression of CREB binding protein in the green anole brain

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ABSTRACT

Green anoles are seasonally breeding lizards in which male sexual behavior is primarily regulated by an annual increase in testosterone. This hormone activates stereotyped behaviors, as well as morphological and biochemical changes in the brain, with greater effect in the breeding season than in the non-breeding season. This study is the first description of CREB binding protein (CBP) in the reptilian brain, and investigates the possibility that changes in CBP, an androgen receptor coactivator, may facilitate differences in responsiveness to testosterone across seasons. A portion of this gene was cloned for the green anole, and *in situ* hybridization was performed to examine the expression of CBP in the brains of gonadally intact male and female green anoles across breeding states. Additionally, hormonal regulation of CBP was evaluated across sex and season in animals that were gonadectomized and treated with testosterone or a control. Similar to other vertebrates, CBP was expressed at relatively high levels in steroid-sensitive brain regions. In the anole ventromedial amygdala, CBP mRNA levels were nearly twice as high in gonadally intact females compared to males. In contrast, CBP expression did not differ across seasons or hormone manipulation in this brain region. No significant effects were detected in the preoptic area or ventromedial hypothalamus. This pattern suggests that CBP might influence female-biased functions controlled by the ventromedial amygdala, but is not consistent with a role in mediating seasonal differences in responsiveness to testosterone in these areas associated with reproductive function.

1. Introduction

Sexual behavior is regulated by steroid hormones and their cognate receptors across vertebrate species. The receptors and hormones that facilitate sexual behavior have largely been identified, but many of the molecular mechanisms involved in steroid action in the brain have yet to be elucidated (McCarthy et al., 2012). In particular, a number of factors leading to sex and seasonal differences in reproductive behaviors remain to be determined. The green anole lizard is an excellent model in which to address this topic.

Green anoles have a breeding season (BS) that lasts from April through July, followed by a non-breeding season (NBS) in which the gonads of both sexes regress and reproductive behaviors cease (Wade, 2011). Male sexual behavior in this species is primarily regulated by testosterone (T), rather than its metabolites, dihydrotestosterone and estradiol (Wade, 2011). T facilitates stereotyped reproductive behaviors, as well as morphological and biochemical changes within the brain. However, responsiveness to this hormone varies across seasons. Specifically, the same dosage of T given to castrated males in the NBS has reduced effects compared to the BS on courtship, copulation, copulatory muscle fiber size and the size of cells in brain regions responsible for male sexual behavior (Holmes and Wade, 2004; Lovern et al., 2004; Neal and Wade, 2007; O'Bryant and Wade, 1999, 2002). In addition, T increases brain aromatase activity only in males and only during the BS (Cohen and Wade, 2010).

To investigate how these changes in hormone responsiveness may occur at the molecular level, we initially examined whether androgen receptor expression changes across season in three regions of the anole brain: the preoptic area (POA), ventromedial amygdala (AMY) and ventromedial hypothalamus (VMH) (Fig. 1). Across vertebrates, the POA and AMY (or its homolog) are critical

Abbreviations: AMY, ventromedial amygdala; BS, breeding season; CBP, CREB binding protein; NBS, non-breeding season; POA, preoptic area; SRC-1, steroid receptor coactivator-1; T, testosterone; TP, testosterone propionate; VMH, ventromedial hypothalamus.

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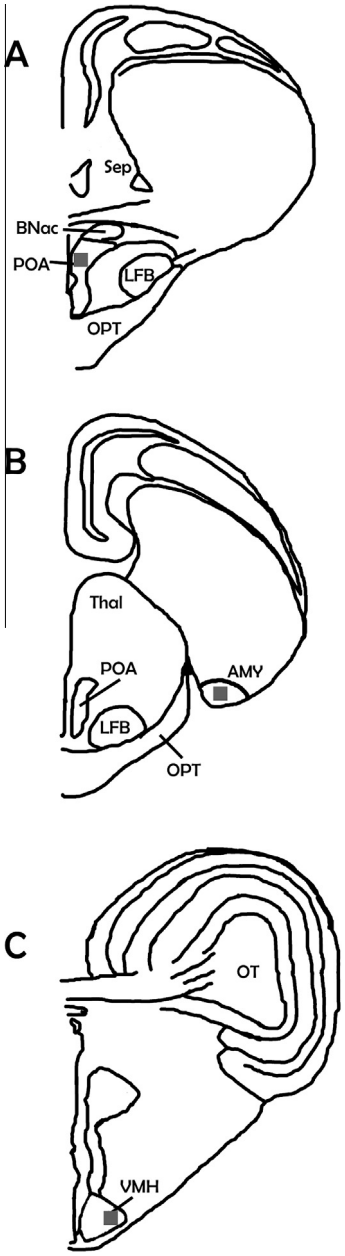

Fig. 1. Representative drawings of hemi cross-sections through portions of the anole brain. Gray boxes depict the sampling sites for the preoptic area (A; POA), ventromedial amygdala (B; AMY), and ventromedial hypothalamus (C; VMH). In the VMH, quantification was from the lateral portion of the region, as that is where androgen and estrogen receptors are primarily located. Other abbreviations: Sep = septum, BNac = bed nucleus of the anterior commissure, LFB = lateral forebrain bundle, OPT = optic tract, Thal = thalamus, OT = optic tectum.

for male typical sexual behaviors (Ball and Balthazart, 2004; Christensen and Clemens, 1974; Wheeler and Crews, 1978; Wood and Newman, 1995), with this ventromedial portion of the amygdala, specifically, being important for male reproductive behavior in the green anole (Greenberg et al., 1984). The VMH regulates female receptivity across vertebrates (Flanagan-Cato, 2011; Meisel et al., 1987). While androgen receptor mRNA is detected in all three regions in the green anole, the pattern of its expression is not consistent with a role in facilitating a seasonal responsiveness to T. No differences in expression are found between the seasons for either sex (Kerver and Wade, 2014).

We then began to consider steroid receptor coactivators. These proteins are rate-limiting factors for transactivation of the nuclear receptor complex (McKenna et al., 1999). When a ligand-activated androgen receptor complex binds to an androgen response element on DNA, it recruits coactivators to the complex, which increase transcriptional activity. Coactivators serve to bridge the receptor complex with basal transcriptional machinery and recruit RNA polymerase II. They can also have endogenous histone acetyltransferase activity, which alters how tightly DNA is wound around histones and thus allows transcription factors to bind more easily (Heinlein and Chang, 2002).

Two nuclear receptor coactivators of particular interest are steroid receptor coactivator-1 (SRC-1) and cAMP response element binding protein (CREB)-binding protein, abbreviated as CBP. They act synergistically to coactivate androgen and other nuclear receptors (Smith et al., 1996). We recently investigated whether SRC-1 might play a role in the seasonal responsiveness to T. While the patterns of relative mRNA levels were consistent with facilitation of male sexual behavior within the BS, they did not suggest a role in modulating responsiveness to T across seasons in the anole (Kerver and Wade, 2015). The present set of studies investigates CBP mRNA within the anole brain.

CBP was discovered in 1993 as a protein that binds specifically to phosphorylated CREB, and thus was named CREB-binding protein (Chrivia et al., 1993). It is expressed in steroid-sensitive brain areas that are involved with reproduction in rodents and interacts with hundreds of transcription factors (Auger et al., 2002a; Tetel, 2009; Vo and Goodman, 2001). Immunocytochemistry studies demonstrate that CBP is widely expressed in the adult rat brain, with high levels in the POA, amygdala, hypothalamus, thalamus, hippocampus, cortex and cerebellum (Molenda et al., 2002; Stromberg et al., 1999).

CBP is necessary for AR-dependent transactivation (Aarnisalo et al., 1998). The concentration of available coactivators can cause genome-wide changes due to competition for their recruitment (Rosenfeld et al., 2006). We therefore hypothesized that CBP availability could influence the responsiveness to T across seasons. We examined the expression of CBP mRNA in males and females from both the BS and NBS in three brain regions critical for sexual behavior in the anole – the POA, AMY and VMH. Additionally, we followed up on group differences from these gonadally intact animals by investigating whether CBP expression in the brain is regulated by circulating T levels.

2. Materials and methods

2.1. Animals

Male and female green anoles were wild caught and shipped to us during the BS and NBS by Charles Sullivan Co. (Nashville, TN). BS animals were received in April and NBS animals in October. Male/female pairs were housed in individual 10-gallon aquaria, which each contained peat moss substrate, a rock and stick for basking, as well as a water dish. Cages were misted daily with water. Black dividers between the cages prevented visual contact. Animals were exposed to fluorescent lighting, full spectrum bulbs and a heat lamp directly over each cage. BS conditions were maintained on a 14:10 light:dark cycle with temperatures ranging from 28 to 38 °C in the daytime, depending on the proximity to the heat lamp. Temperatures averaged 18 °C when the lights were off overnight. During NBS conditions, the light cycle was 10:14 light:dark and daytime temperatures ranged from 24 to 30 °C and averaged 15 °C at night. Humidity was maintained at 60–70% in both seasons. Animals were fed crickets or meal worms three times per week in the BS and two times per week in the NBS. All procedures