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Research paper

3β-HSD expression in the CNS of a manakin and finch

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ABSTRACT

The prohormone, dehydroepiandrosterone (DHEA) circulates in vertebrate blood with the potential for actions on target tissues including the central nervous system (CNS). Many actions of DHEA require its conversion into more active products, some of which are catalyzed by the enzyme 3β-hydroxysteroid-dehydrogenase/isomerase (3β-HSD). Studies of birds show both expression and activity of 3β-HSD in brain and its importance in regulating social behavior. In oscine songbirds, 3β-HSD is expressed at reasonably high levels in brain, possibly linked to their complex neural circuitry controlling song. Studies also indicate that circulating DHEA may serve as the substrate for neural 3β-HSD to produce active steroids that activate behavior during non-breeding seasons. In the golden-collared manakin (*Manacus vitellinus*), a sub-oscine bird, low levels of courtship behavior are displayed by males when circulating testosterone levels are basal. Therefore, we asked whether DHEA circulates in blood of manakins and whether the brain expresses 3β-HSD mRNA. Given that the spinal cord is a target of androgens and likely important in regulating acrobatic movements, we also examined expression of this enzyme in the manakin spinal cord. For comparison, we examined expression levels with those of an oscine songbird, the zebra finch (*Taeniopygia guttata*), a species in which brain, but not spinal cord, 3β-HSD has been well studied. DHEA was detected in manakin blood at levels similar to that seen in other species. As described previously, 3β-HSD was expressed in all zebra finch brain regions examined. By contrast, expression of 3β-HSD was only detected in the manakin hypothalamus where levels were greater than zebra finches. In spinal cord, 3β-HSD was detected in some but not all regions in both species. These data point to species differences and indicate that manakins have the substrate and neural machinery to convert circulating DHEA into potentially active androgens and/or estrogens.

1. Introduction

Steroid hormones are critical for the expression of adaptive phenotypes in vertebrates living in a variety of social and/ or biotic environments (Nelson, 2011). Often, the underlying mechanisms are difficult to discern by only measuring circulating hormone levels, because steroid levels in blood are not always congruent with those found in tissues (Pradhan et al., 2015; Schmidt et al., 2008), and some circulating hormones require conversion into more active metabolites by the locally expressed steroid-metabolic enzymes (London et al., 2009; Vanson et al., 1996). Among these enzymes, 3β-hydroxysteroid dehydrogenase (3β-HSD) is critically positioned in the steroidogenic pathway and is well studied in the gonads and adrenals (Freking et al., 2000; Schlinger et al., 2008). Nevertheless, 3β-HSD is also expressed in other vertebrate tissues including liver, heart, aorta, kidney, spinal cord and brain (Coirini et al., 2002; Nakamura et al., 2005; Payne and Hales, 2004; Zhao et al., 1991; Zhao et al., 1990) where its function is only recently being fully appreciated. This enzyme is essential for the conversion of pregnenolone to progesterone and

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of dehydroepiandrosterone (DHEA) to androstenedione, which, in turn, is the substrate for the production of the more potent androgens, testosterone (T) and perhaps also 5α-dihydrotestosterone, that binds strongly to the androgen receptor.

Studies of birds show both expression and activity of 3β-HSD in brain (Tsutsui, 2011; Vanson et al., 1996) as well as possible neurobehavioral functions. For example, in the oscine songbird brain, 3β-HSD functions coordinately with the estrogen synthetic enzyme aromatase to convert DHEA into estrogens (Pradhan et al., 2010a; Rohmann et al., 2007; Tam and Schlinger, 2007). These estrogens may then activate neural estrogen receptors to regulate social behaviors, such as estrogen-dependent aggressive behavior expressed during the non-breeding season when circulating testosterone is basal (Soma et al., 2000; Soma and Wingfield, 2001; Pradhan et al., 2010a,b).

Birds of the Order Passeriformes are separated into the oscine songbirds (such as the zebra finch), that learn complex songs and possess a complex neural circuitry that underlies song learning (Nottebohm et al., 1976), and the sub-oscines, species that lack complex song and most neural structures comprising the oscine song system (Sibley and Ahlquist, 1985). Elevated neural expression of 3β-HSD may be associated with the presence of the oscine neural song system or it might be a general property of the Passeriform brain, a question that can be explored by examination of RNA expression in different Passeriform species. In zebra finches, 3β-HSD has been shown in organotypic brain slices and microdissected brain regions during both development and adulthood (London et al., 2006; Tam and Schlinger, 2007), where it is regulated by stress (Soma et al., 2004) as well as by 17β-estradiol (Pradhan et al., 2010a, 2008). Thus, whereas 3β-HSD has been examined using multiple levels of analysis in zebra finches, similar studies are lacking for a sub-oscine passerine bird.

Our laboratory has studied the neuroendocrine basis of behavior in a sub-oscine species, the golden-collared manakin (*Manacus vitellinus*) of Panamanian rainforests. Males of this species perform physically elaborate courtship displays daily over the course of the 6–7 month-long reproductive season. These displays depend on androgens (Feng et al., 2010; Fusani et al., 2007; Fuxjager et al., 2012b; Schlinger et al., 2013); nevertheless, even during the breeding season, circulating T levels in males are extremely variable with some displaying males having little or no measurable T levels in the blood (Day et al., 2007; Fusani et al., 2007). Interestingly, juvenile males, as well as adult males during the nonbreeding season, exhibit low levels of courtship even with low circulating levels of T (Day et al., 2007; Fusani et al., 2007). One mechanism that could explain these observations, is that DHEA circulates in manakin blood and functions as a substrate for 3β-HSD in brain to activate courtship behavior in breeding or non-breeding birds with low levels of circulating T. To address this question, we first asked if DHEA is present in manakin blood with greater levels in courting males as compared to females or non-courting (juvenile) males. Next, we asked whether 3β-HSD is expressed in the manakin central nervous system (CNS) to potentially utilize circulating DHEA substrate for the formation of more active androgens and/or estrogens. We used quantitative PCR to measure 3β-HSD mRNA expression in micro-dissected regions of the brain and spinal cord of adult males and female manakins. To evaluate potential differences between the sub-oscine manakin and an oscine songbird, we included adult male and female zebra finches in the expression analysis. CNS regions of interest were selected based on previous studies showing significant androgen and/or estrogen binding or receptor expression in manakins suggesting their possible function in activating and controlling male courtship displays (Fusani et al., 2014; Fuxjager et al., 2012b; Schultz and Schlinger, 1999).

2. Materials and methods

2.1. Animals

All research was conducted with approval of appropriate governmental agencies and under the strict guidelines of the Animal Care and Use Committee at the University of California, Los Angeles (UCLA) and the Smithsonian Tropical Research Institute (STRI). Manakin blood (n = 25) and tissue samples (n = 12) were collected during the courtship season (February–April 2010, 2011) from forests in and around Gamboa, Panama. Reproductively active zebra finches (n = 24) were obtained from our UCLA colony.

2.2. Tissue collection

Blood samples were collected in Panama from adult (n = 14) and juvenile males (n = 5) and adult females (n = 6). Animals were captured using mist-nets and bled by venipuncture within 10 min of capture. Blood was kept at 4 °C and then centrifuged at 1000*g* within 3 h to yield on average 65 μl (30–100 μl) plasma. Manakin brain tissues were collected immediately upon decapitation, dissected into the cerebellum (Cb), hypothalamus (Hyp), and left telencephalon (Tel), placed on dry ice and then stored either on dry ice or in a −80 °C freezer at the Smithsonian Tropical Research Institute facilities in Panama City until shipped to UCLA; spinal cords were dissected into the cervical, thoracic and lumbosacral regions and placed in RNAlater solution. Appropriate aliquots were based off of weight of each sample and then refrigerated 2–8 °C. All of the same brain tissues were collected from male (n = 6) and female (n = 6) zebra finches, with the exception that the whole Tel was collected. Spinal cords were collected from a separate group of male (n = 6) and female (n = 6) zebra finches. All tissues were frozen in dry ice immediately upon dissection and then stored at −80 °C until assays. We have previously found no difference in RNA expression levels for zebra finch tissues placed in RNAlater or frozen immediately on dry ice (Fuxjager et al., 2015).

2.3. DHEA measures

Concentration of DHEA was measured using a commercial kit (DSL 8900, Diagnostic Systems Laboratories, Webster, Texas, USA) with modifications as described previously Granger et al. (1999) to increase assay sensitivity. This assay has been validated for a number of bird species (Chin et al., 2008; Goodson et al., 2005; Newman et al., 2008a,b). Briefly, plasma samples were extracted twice, each time using 3 mL dichloromethane (Newman et al., 2008b) using the freeze-decanting method, in which the water phase is snap-frozen on a mixture of ethanol and dry ice and the organic phase decanted (Canoine, 2001). Extracted samples were dried down and re-suspended in 220 μl PBS with 0.1% BSA and then assayed in duplicate. The initial sample volume was on average 53 μl. The detection limit was 128 pg/mL and the intra-assay coefficient of variation was <12%. We did not measure recovery in this study, though this method typically yields steroidal recoveries of 80–90% (Newman et al., 2008).

2.4. RNA and PCR

Total RNA was extracted from tissue samples by TRIzol© Reagent (Invitrogen, Carlsbad, CA) and following the manufacturer's instructions. Tissues were homogenized in TRIzol© for ~40 s at medium/high speed with a standard stator homogenizer. Note that for RNA extractions, half the Tel (left) was used for manakin and the whole Tel was used for zebra finch. RNA concentration was measured with a Nanodrop System 1000 (Thermo Scientific,

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