available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Steroidogenic enzyme gene expression in the brain of the parthenogenetic whiptail lizard, *Cnemidophorus uniparens*

Brian George Dias[a], *Sonia Grace Chin*[b], *David Crews*[b,*]

[a]*Institute for Neuroscience, University of Texas at Austin, Austin, TX 78712, USA*
[b]*Section of Integrative Biology, University of Texas at Austin, PAT 30, 1 University Station Stop C 0930, Austin, TX 78712, USA*

ARTICLE INFO

Article history:
Accepted 11 November 2008
Available online 3 December 2008

Keywords:
Sexual behavior
Androgen
Estrogen
Sexual differentiation

ABSTRACT

The steroidogenic enzyme CYP17 is responsible for catalyzing the production of androgenic precursors, while CYP19 converts testosterone to estradiol. *De novo* neurosteroidogenesis in specific brain regions influences steroid hormone dependent behaviors. In the all-female lizard species *Cnemidophorus uniparens*, individuals alternately display both male-like mounting and female-like receptivity. Mounting is associated with high circulating concentrations of progesterone following ovulation (PostOv), while receptivity is correlated with estrogen preceding it (PreOv). At a neuroanatomical level, the preoptic area (POA) and ventromedial nucleus of the hypothalamus (VMN) are the foci of the male-typical mounting and female-typical receptivity, respectively. In this study, we indirectly test the hypothesis that the whiptail lizard brain is capable of *de novo* neurosteroidogenesis by cloning fragments of the genes encoding two steroidogenic enzymes, CYP17 and CYP19, and examining their expression patterns in the *C. uniparens* brain. Our data indicate that these genes are expressed in the *C. uniparens* brain, and more importantly in the POA and VMN. Using radioactive *in situ* hybridization, we measured higher CYP17 mRNA levels in the POA of PostOv lizards compared to receptive PreOv animals; CYP19 mRNA levels in the VMN did not change across the ovarian cycle. To our knowledge, these are the first data suggesting that the reptilian brain is capable of *de novo* steroidogenesis. This study also supports the idea that non-gonadal sources of steroid hormones locally produced in behaviorally relevant brain loci are central to the mediation of behavioral output.

1. Introduction

The organization and activation of sexually dimorphic behavioral repertoires by steroid hormones is a central tenet of behavioral neuroendocrinology (Phoenix et al., 1959). For example, male-typical mounting and female-typical receptivity are mediated in part by neural circuits thought to be organized in a sex-typical manner by steroid hormones during development (Schwarz and McCarthy, 2008; Gorski, 2002). Complementing this organization is the activation of these neural circuits by steroid hormones resulting in sex-typical behaviors in adulthood (Ball and Balthazart, 2004; Baum, 2003). Traditionally, the gonads have been thought to be the primary source of steroid hormones activating sexual behavior. More recently however, the idea of *de novo* steroid hormone biosynthesis within specific brain nuclei (termed neurosteroids) by the action of steroidogenic enzymes has gained prominence (Baulieu, 1998). A related possibility is that steroid

* *Corresponding author*. Fax: +1 512 471 6078.
E-mail address: crews@mail.utexas.edu (D. Crews).

0006-8993/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2008.11.071

hormones secreted by the gonads reach the brain via the circulatory system and are then converted to more relevant products in the brain as a result of steroidogenic enzyme activity.

Cholesterol is converted into steroid hormones via several enzyme-catalyzed reactions (Miller, 1988), beginning with its conversion to pregnenolone by the action of cytochrome P450 side-chain cleavage enzyme. Pregnenolone is then converted into either the active progestin, progesterone, by the enzyme 3β-hydroxysteroid dehydrogenase/isomerase or into the androgen, dehydrepiandrosterone (DHEA), by the cytochrome P450 17α-hydroxylase/C17-20lyase enzyme (CYP17) via the 17α-OH pregenenolone intermediate. DHEA can then be converted by 3β-HSD into androstenedione (AE), or AE can be derived from progesterone by the actions of CYP17 via the intermediate 17α-OH progesterone. Androstenedione can be converted into the more active androgen, testosterone (T), by the enzyme 17β-hydroxysteroid dehydrogenase. Finally, T can be converted into the active estrogen, estradiol (E_2), by the actions of the enzyme cytochrome P450 aromatase (CYP19). These enzymes are expressed in the nervous system and the neurosteroids resulting from their activity influence adult behavior (Compagnone and Mellon, 2000; Schlinger et al., 2001; King, 2008).

Most studies examining how steroids activate sex-typical behaviors in adulthood do so in gonochoristic animals wherein sex-specific genotypic complements and endocrine histories have already organized behaviorally relevant neuronal circuits in a sex-typical manner (Arnold, 2004; Crews, 2005). An alternative approach to understanding the relationship between steroid hormones and subsequent activation of behavior is to examine organisms which possess a homogenous genetic background and hormonal milieu (Crews, 2005). The parthenogenetic unisexual whiptail lizard species (*Cnemidophorus uniparens*) alternates in the expression of both male- and female-typical pseudosexual behavior across the ovarian cycle (Crews and Fitzgerald, 1980). High titers of circulating estrogen in preovulatory (PreOv) animals are correlated with female-like receptivity, while high circulating progesterone levels in postovulatory (PostOv) animals are correlated with male-like mounting. While no androgens are detected in the circulatory system of PostOv individuals (Moore et al., 1985), the nervous system of *C. uniparens* responds to exogenously administered androgen, with testosterone being a potent activator of male-like mounting in these lizards (Crews et al., 1986; reviewed in Crews, 2005). While the relationship between gonadal steroid hormones and pseudosexual behavior in *C. uniparens* is well established, several related queries remain. For example, an important question that arises from such hormonal mediation of behavior pertains to whether these hormones are synthesized in the gonad or in the brain. Also, is it possible that local synthesis of androgen in behaviorally relevant brain nuclei resolves the discrepancy between no androgens detected in the circulatory system of mounting postovulatory animals and mounting being elicited by administration of exogenous androgen? A theme common to both these questions implies that *de novo* neurosteroid synthesis in behaviorally relevant brain nuclei might result in a neurohormonal milieu that is permissive to pseudosexual behavior in *C. uniparens*. Such a possibility warrants that the nervous system possesses within local circuits the steroidogenic enzymes capable of synthesizing steroid hormones in specific brain nuclei.

Keeping in mind that the preoptic area (POA) and ventromedial nucleus of the hypothalamus (VMN) are involved in male- and female-typical pseudosexual behavior, respectively (Crews, 2005), we hypothesized that higher CYP17 levels and/

```
CYP17

Rattus norvegicus                ATAGGCACATCCTTGCCACGGTGGGAGACATCTTTGGGGCGGGCATAGAG 944
Mus musculus                     ATAAGCATATCCTTGTCACGGTGGGAGACATCTTTGGGGCAGGCATAGAG 1043
Homo sapiens                     ATAACCACATTCTCACCACCATAGGGGACATCTTTGGGGCTGGCGTGGAG 1087
Chelydra serpentina              ACGACTATCTCCTCATGACAGTGGCCGACATCTTCGGGGCGGGCGTCGAG 955
Cnemidophorus uniparens          ---------------TGACCGTGGGCGACATATTCGGGGCTGGTGTGGAG 35
Taenopygia guttata               ATGACCACCTCCTCATGACGGTGGGGGACATCTTTGGGGCCGGTGTGGAG 846
Danio rerio                      AAGATCATGTGCTCATGACGGTGGGGGACATTTTTGGGGCTGGGGTGGAA 1055
                                                  **  * *  ***** ** ***** **  * **

gi|6978730|ref|NM_012753.1|      ACAACTACCACTGTGCTCAAGTGGATCCTGGCTTTCCTGGTGCACAATCC 994
gi|42476041|ref|NM_007809.2|     ACAACTAGCTCTGTGCTGAGCTGGATCCTGGCTTTCCTGGTGCACAATCC 1093
gi|91107038|ref|NM_000102.3|     ACCACCACCTCTGTGGTTAAATGGACCCTGGCCTTCCTGCTGCACAATCC 1137
gi|46561799|gb|AY533546.1|       ACCACCAACACCGTGCTCAAGTGGGCTGTGCTCTACTTGCTCCACTACCC 1005
gi|163866769|gb|EU310876.1|      ACCACCACAACTGTTATAAATTGGTCTATACTGTATCTTCTGCTTTACCC 85
gi|32307870|gb|AY313844.1|       ACCACCACGACTGTGCTCAAATGGGCTGTGCTCTACCTGCTCCACTACCC 896
gi|47086424|ref|NM_212806.1|     ACCACTACTACTGTACTCAAATGGTCTATAGCTTACCTCGTCCACAATCC 1105
                                 ** ** *   * **  * *  ***    *    *   *  * *   * **

gi|6978730|ref|NM_012753.1|      TGAGGTGAAGAAGAAGATCCAAAAGGAGATTGACCAGTACGTAGGCTTCA 1044
gi|42476041|ref|NM_007809.2|     TGAGGTGAAGAGGAAGATCCAAAAGGAGATTGACCAGTATGTAGGCTTCA 1143
gi|91107038|ref|NM_000102.3|     TCAGGTGAAGAAGAAGCTCTACGAGGAGATTGACCAGAATGTGGGTTTCA 1187
gi|46561799|gb|AY533546.1|       GGAGGTGCAGAGGAAGGTCCAGGAGGAACTAGATCAGAAGATCGGCTTCG 1055
gi|163866769|gb|EU310876.1|      CCAGGTGCAGAGGAAGATCCAGGAAGGTT---------------------- 114
gi|32307870|gb|AY313844.1|       TGAGATCCAGAGGAAGATCCAGGAGGAAATGGACCACAAGATAGGCCAGG 946
gi|47086424|ref|NM_212806.1|     ACAGGTTCAGAGAAAGATTCAAGAGGAGCTCGACAGTAAGATTGGGAAAG 1155
                                   ** *  ***  *** *  *  * *
```

Fig. 1 – Alignment of CYP17 cDNA sequences across several vertebrates. Sequence alignment was achieved using ClustalW software. Accession numbers used to compare sequences are shown on the left hand side. Significant identity is observed across species.