available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Aromatase immunoreactivity in the bluehead wrasse brain, *Thalassoma bifasciatum*: Immunolocalization and co-regionalization with arginine vasotocin and tyrosine hydroxylase

*K. Erica Marsh, Lela M. Creutz, M. Beth Hawkins, John Godwin**

North Carolina State University, Department of Zoology and W.M. Keck Center for Behavioral Biology, Raleigh, NC 27606, USA

ARTICLE INFO

Article history:
Accepted 7 September 2006
Available online 11 October 2006

Keywords:
Aromatase
Arginine vasotocin
Dopamine
Estrogen
Teleost
Hypothalamus

ABSTRACT

Sex steroid hormones regulate various neural functions that control vertebrate sociosexual behavior. A number of sex steroids can be synthesized de novo in the brain, including estrogens by the enzyme aromatase. Aromatase, the neuropeptides arginine vasotocin/vasopressin, and the monoamine neurotransmitter dopamine have all been implicated in the control of male sexual and aggressive behavior in a variety of vertebrates. This study examined the expression of brain aromatase in the bluehead wrasse (*Thalassoma bifasciatum*), a teleost fish that exhibits socially controlled behavioral and gonadal sex change. We used immunocytochemistry (ICC) to characterize distributions of aromatase-immunoreactive (ir) cells, and to examine their relationship with AVT-ir neurons and tyrosine hydroxylase-ir (TH-ir) neurons in key sensory and integrative areas of the brain of this species. Aromatase-ir appeared to be in glial cell populations, and was found in the dorsal and ventral telencephalon, the preoptic area of the hypothalamus, and the lateral recess of the third ventricle, among other brain areas. Aromatase-ir fibers are closely associated with AVT-ir neurons throughout the preoptic area, indicating the potential for functional interactions. Aromatase-ir cell bodies and fibers were also co-regionalized with TH-ir neurons, suggesting possible interaction between the dopaminergic system and neural estrogen production. The presence of aromatase in brain regions important in the regulation of sexual and aggressive behavior suggests that local estrogen synthesis could regulate sex change through effects on signaling systems that subserve reproductive behavior and function.

1. Introduction

In all vertebrate taxa including mammals, mating behavior is a complex sequence of behavioral responses requiring the ability to integrate endogenous hormonal and neurochemical changes with environmental information. The most important environmental information for many species can come from conspecifics. These social signals are often sexual in nature and have profound effects on both neural function and behavioral profiles. The mechanisms underlying behavioral adaptations to changing social conditions have not been comprehensively identified as yet and understanding the

* *Corresponding author*. Department of Zoology, NC State University, Campus Box 7617, 127 D. Clark Labs, Raleigh, NC 27695-7617. Fax: +1 919 515 2698.
E-mail address: John_Godwin@ncsu.edu (J. Godwin).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.09.017

molecular basis of this transduction of social information is a key challenge for behavioral neuroscience.

Sex hormones play key roles in neural modulation of behavioral processes. Both testosterone (T) and estradiol 17β (E_2) stimulate male sexual behavior in a variety of vertebrates (Cross and Roselli 1999). While 'classical' genomic pathways are clearly important for many of these effects, increasing evidence also points to rapid steroid actions on neurons and in the mediation of sexual behavior (Revankar et al. 2005; Remage-Healey and Bass 2004). For example, changes in the conversion of androgens to estrogens by aromatase can be seen within minutes in the quail brain. This suggests that estrogen production in the brain could potentially be regulated over short time courses and such rapid alterations would be consistent with observed estrogen effects on behavior (Balthazart et al. 2001, Balthazart and Ball, 2006). Rapid alterations in neural estrogen production have also been documented in sex-changing fishes (see below).

We are focusing on modulation of neural estrogen through aromatase as a potential mechanism underlying rapid adaptation to changing social conditions in a sex-changing coral reef fish, the bluehead wrasse (*Thalassoma bifasciatum*). Several studies have highlighted the importance of estrogens and the aromatase pathway in the gonadal sex change processes (Cardwell and Liley 1991; Cochran and Grier 1991; Godwin and Thomas 1993; Chang et al. 1994, 1995; Kroon and Liley 2000; Kroon et al. 2003; Nakamura et al., 1989, 2003) and have suggested that it is a decrease in E_2 levels that permits male development (Kroon et al. 2005). Similarly, we found that when the estrogen synthesis blocker 1,4,6-androstatrien-3,17-dione (ATD) is given alone or coadministered with T, complete color and gonadal sex change is induced in female *T. bifasciatum* (Austin et al. unpublished).

Many sex-changing fishes, including the bluehead wrasse, show very rapid behavioral changes during the sex change process. Male behaviors are often expressed within minutes or hours as an individual assumes social dominance (Robertson 1972; Warner and Swearer 1991; Godwin et al. 1996; Black et al. 2005). This short time scale of behavioral change is consistent with the rapid steroid actions on behavior in other systems discussed above. The neural form of aromatase (cytochrome P450b or AROb) is abundantly expressed in the brains of teleost fishes (Callard et al. 2001), including in key regions regulating sexual behavior (Schlinger et al., 1999; Forlano et al. 2001; Forlano and Bass, 2005a,b; Chang et al. 2005; Kishida and Callard, 2001; Menuet et al. 2003, 2005). As with the important role of aromatase in gonadal sex change processes, neural estrogen synthesis via AROb appears to be critical in transducing social signals regulating male-typical sexual behavior under changing social conditions. In *Lythrypnus dalli*, another gobiid species with socially controlled sex change, neural aromatase activity in dominant females decreases significantly within hours of male removal (Black et al. 2005). This decrease in aromatase activity is correlated with rapid increases in aggressive and territorial behavior in the transitional females.

Other neural signaling systems have also been implicated in the process of behavioral and gonadal sex change. Arginine vasotocin (AVT), the neuropeptide found in fishes that is homologous to tetrapod arginine vasopressin (AVP), is of particular interest in the bluehead wrasse system. AVT affects reproductive behaviors in a broad range of vertebrates (Thompson and Moore 2003; Moore 1992; Moore and Lowry 1998; Goodson and Bass 2001), including several fishes (Salek et al., 2001; reviewed by Moore 1992; Moore and Lowry 1998; Goodson and Bass 2001). Expression of AVT is higher in dominant male bluehead wrasses than females and increases rapidly with sex change (Godwin et al., 2000). This increased expression of AVT is driven by social dominance and is independent of gonads (Semsar and Godwin 2003). Additionally, administration of AVT increases aggressive and courtship behavior typical of dominant males (Semsar et al. 2001) while an AVT receptor antagonist blocks territorial acquisition in large males and behavioral sex change in females (Semsar and Godwin 2004).

Monoamine neurotransmitters represent other neurochemical systems that can influence and be influenced by steroid hormone signaling. Behavioral studies in mice, quail, teleost fishes, and primates have demonstrated rapid steroid actions involving neurotransmitters (Hull et al. 2004). The dopamine system regulates sexual behavior and function in a variety of vertebrates and is often responsive to the steroid hormone environment (see Hull et al. 2004). Both human and non-human animal studies strongly suggest that dopamine facilitates male sexual behavior (Dominguez and Hull 2005). Levels of dopamine and other monoamines change over the course of female-to-male sex change in a congener of the bluehead wrasse (*Thalassoma duperrey*, Larson et al. 2003a) and manipulations of dopaminergic signaling can influence this process (Larson et al. 2003b). In quail, estradiol rapidly modulates male sexual behavior and this correlates with changes in levels of dopamine in the brain (Cornil et al. 2006). Dopamine has also been shown to down-regulate neural aromatase activity in quail (Balthazart et al. 2002; Balthazart and Ball, 2006), providing strong evidence for the involvement of monoamines in the neurochemical pathway controlling male sexual behavior.

The bluehead wrasse (*T. bifasciatum*) offers an experimentally tractable model in which to investigate the neurochemical pathways that underlie behavioral adaptation to changing social conditions. This common Caribbean reef fish has three sexual phenotypes: large, brightly colored terminal phase (TP) males and smaller, yellow and brown striped, initial phase (IP) males and females. TP males develop from either sex-changing females or role-changing IP males. Most TP males maintain territories on reefs and court and mate with females within these territories, although there is variation among males in these behaviors (see Semsar et al., 2001). Females display the yellow and brown coloration and live within groups on reefs that normally include a dominant TP male. IP males are non-territorial, usually mate in large aggregations ('group spawns'), and may also mimic females to 'sneak' or 'streak' spawn with TP male/female pairs. When the TP males are removed from a social group, the largest female or IP male present will change sex and/or role to become a TP male (Warner and Swearer, 1991). Gonadal change takes place over 8–10 days, but male-typical behavior is often exhibited by sex-changing females within minutes of removal of the TP male (Godwin et al. 1996).

This unpredictable social system requires rapid adaptation to changing dominance hierarchies. The first step in determining how estrogen might influence these behaviors and other