Hormones and Behavior 55 (2009) 398–403

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Sexually dimorphic estrogen receptor α mRNA expression in the preoptic area and ventromedial hypothalamus of green anole lizards

Laurel A. Beck [a,*], Juli Wade [a,b,c]

[a] Neuroscience Program, Michigan State University, East Lansing, MI 48824, USA
[b] Department of Psychology, Michigan State University, East Lansing, MI 48824, USA
[c] Department of Zoology, Michigan State University, East Lansing, MI 48824, USA

ARTICLE INFO

Article history:
Received 10 November 2008
Revised 6 January 2009
Accepted 8 January 2009
Available online 20 January 2009

Keywords:
Amygdala
Estrogen receptor-alpha
Lizard
Preoptic area
Sex difference
Ventromedial hypothalamus

ABSTRACT

Estradiol (E2) is important in activation of male reproductive behaviors, and masculinizes morphology of associated brain regions in a number of mammalian and avian species. In contrast, it is testosterone, rather than its metabolites, that is the most potent activator of male sexual behavior in green anole lizards. As in other vertebrate groups, however, E2 is critical for receptivity in females of this species. Aromatase, the enzyme which converts testosterone to E2, is more active in the male than female green anole brain, and appears to be actively regulated on a seasonal basis, suggesting some role for E2 in males. This study was designed to enhance our understanding of potential E2 actions by localizing and quantifying relative levels of estrogen receptor-alpha (ERα) mRNA in forebrain regions involved in masculine and feminine behaviors in anoles. These areas include the preoptic area (POA), ventromedial amygdala (AMY) and ventromedial hypothalamus (VMH). *In situ* hybridization was conducted in adult males and females collected during both breeding and non-breeding seasons. ERα mRNA was expressed in each brain region across sexes and seasons. However, expression was up to 3 times greater in the VMH compared to the POA and AMY. In the POA and VMH, expression was higher in females compared to males, independent of season. The increased receptor expression in females is consistent with E2 playing a larger role in female than male reproductive behaviors.

Introduction

Estradiol (E2) is a potent activator of both male and female sex behaviors in a variety of mammalian and avian species (reviewed in Ball and Balthazart, 2004; Meisel and Sachs, 1994). In females, E2 is released into circulation from the ovaries (reviewed in Blaustein and Erskine, 2002). In males testosterone (T) secreted primarily from the testes is converted to E2 locally in the brain via aromatization (Morali et al., 1977; Roselli et al., 1985). E2 then acts within the brain to promote sexual behaviors in both sexes.

In rodents, gonadectomy greatly diminishes male sex behaviors, and E2 administered either systemically (Dalterio et al., 1979; Wallis and Luttge, 1975) or locally within the forebrain (Davis and Barfield, 1979; Nyby et al., 1992) restores them. The same is true in male Japanese quail, where E2 treatment following castration reinstates courtship and copulatory behaviors (Adkins et al., 1980). E2 is also critical to the activation of receptive behaviors in a variety of female vertebrates. These include rodent species (Rubin and Barfield, 1983; reviewed in Blaustein and Erskine, 2002), whiptail lizards (Wade and Crews, 1991), and Japanese quail (Delville and Balthazart, 1987).

The preoptic area (POA) and medial amygdala are critical for the production of male-typical sexual behaviors. These behaviors include courtship and copulation in rodents (reviewed in Meisel and Sachs, 1994), birds (medial preoptic nucleus and nucleus taeniae, respectively; Balthazart and Surlemont, 1990; Watson and Adkins-Regan, 1989), and lizards (Kingston and Crews, 1994; Greenberg et al., 1984; Wheeler and Crews, 1978). In females, the ventromedial hypothalamus (VMH) is involved in the control of receptive behaviors, including the extensively studied rodent lordosis (Emery and Moss, 1984; La Vaque and Rodgers, 1975; Pfaff and Sakuma, 1979). E2 is thought to bind to estrogen receptors (ER) located within these brain regions to facilitate reproductive behaviors.

ERs are expressed in male and female brains in mammals (Lauber et al., 1991; Warembourg and Leroy, 2004; Wood and Newman, 1995), birds (Halldin et al., 2006; reviewed in Gahr, 2001), and reptiles (Crews et al., 2004). Additionally, the extent of ER expression may differ between the sexes. For example, in mammals ER expression in the VMH is usually greater in females compared to males (Brown et al., 1996; Lauber et al., 1991; Scott et al., 2000). Additionally, forebrain steroid hormone receptor expression can vary seasonally. For example, estrogen receptor-alpha (ERα) expression increases following exposure to short day-lengths in the medial amygdala of Syrian hamsters (Mangels et al., 1998) and bed nucleus of the stria terminalis of California mice (Trainor et al., 2007). Similarly, ERα decreases

* Corresponding author. 108 Giltner Hall, Michigan State University, East Lansing, MI 48824, USA. Fax: +1 517 432 2744.
E-mail address: becklau2@msu.edu (L.A. Beck).

0018-506X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2009.01.003

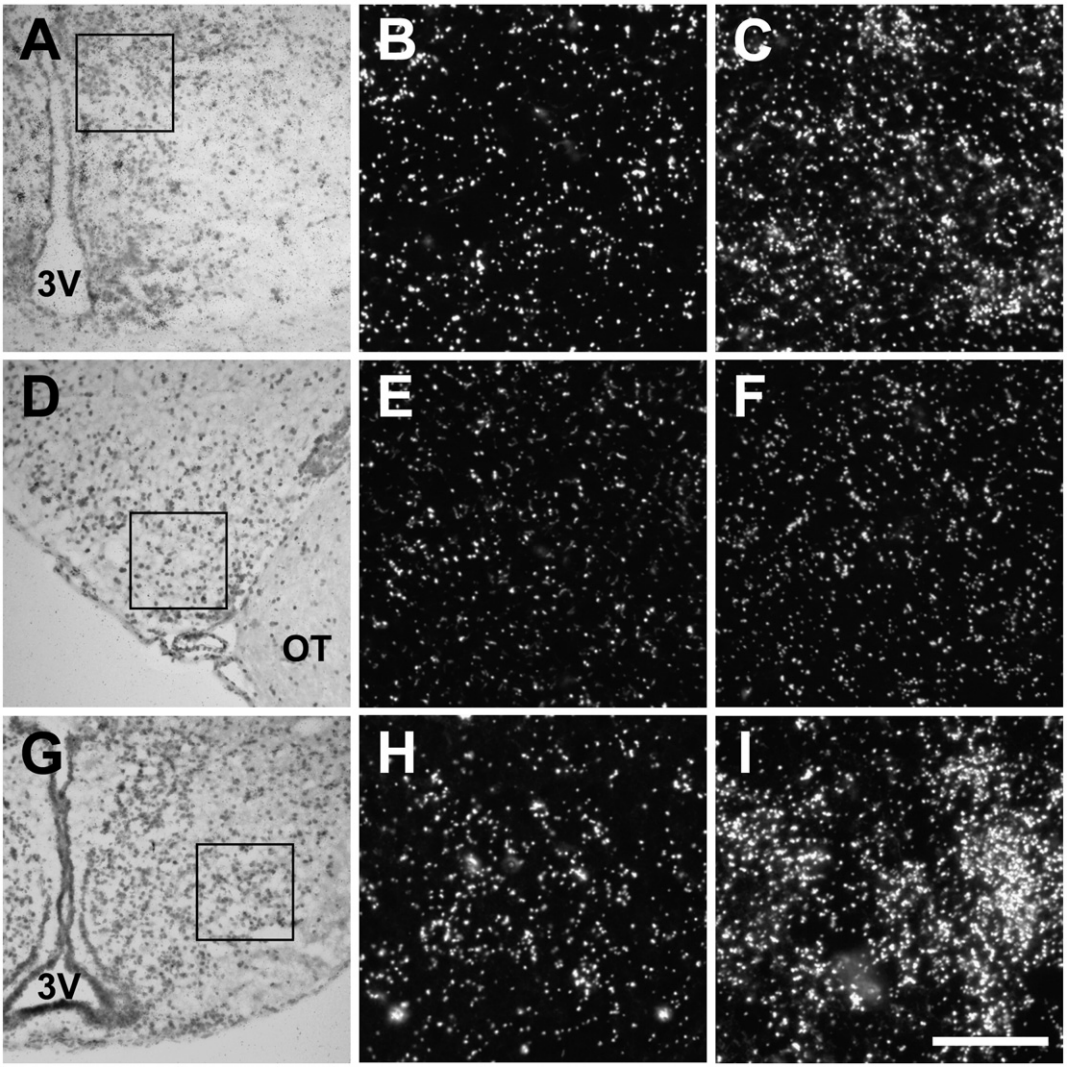

Fig. 1. Photomicrographs in the preoptic area (A–C), ventromedial amygdala (D–F), and ventromedial hypothalamus (G–I). Boxes in the brightfield images (left column; A, D, and G) are 150 μm×150 μm, and indicate locations of the darkfield images in males (middle column; B, E, and H) and females (right column; C, F, and I). Sampling regions covered approximately one quarter of the area within these boxes/images. 3V=third ventricle, OT=optic tract. Scale bar in panel I represents 50 μm for all darkfield photographs.

during the non-breeding season (NBS) in the song nucleus HVC (used as a proper name; Reiner et al., 2004) of canaries (Gahr and Metzdorf, 1997) and POA of spotted antbirds (Canoine et al., 2007). Seasonal fluctuations in circulating E2 may lead to these differences in receptor expression, as occurs with E2 treatment in some mammals (Hamada et al., 2005; Lauber et al., 1991; Meredith et al., 1994; Simerly and Young, 1991).

While much is known about E2 modulation of reproductive behavior in mammals and birds, less is known about the reproductive behavioral effects of E2 in the seasonally-breeding green anole lizard. One role of E2 in female anoles is clear; it activates receptivity (McNicol and Crews, 1979; Tokarz and Crews, 1980; Winkler and Wade, 1998), similar to other species (reviewed in Blaustein and Erskine, 2002). The strength of this behavioral response to E2 is reduced in the NBS compared to the breeding season (BS; Wu et al., 1985), which might be associated with differences in receptor expression. The role(s) that circulating and/or locally produced E2 may play in male green anoles is unclear. E2 does not activate male sex behaviors, and the aromatase inhibitor fadrozole does not inhibit T's facilitation of them (Winkler and Wade, 1998). Yet, brain aromatase activity is higher in males than females (Rosen and Wade, 2001) and within males, aromatase activity is higher during the summer BS than the winter NBS (Rosen and Wade, 2001). Therefore, local synthesis of E2 from T via aromatase can occur in the male anole brain and is actively regulated between the seasons. This effect is similar to other species (reviewed in Ball and Balthazart, 2004; Roselli et al., 2004). However, the function of this regulated E2 synthesis is unknown.

As in mammals, the POA and amygdala (ventromedial portion, AMY; also known as ventral posterior amygdala, Bruce and Neary, 1995) are involved in the control of male courtship and copulatory behaviors in the green anole lizard (Greenberg et al., 1984; Wheeler and Crews, 1978). Also similar to other species, the VMH likely regulates sexual behavior in female anoles. In other reptiles, such as the whiptail lizard, the VMH controls receptivity (Kendrick et al., 1995; Wade and Crews, 1991) and lesions to the medial basal hypothalamus, which encompasses the VMH, in green anoles inhibits female receptivity (Farragher and Crews, 1979). Together, this information suggests that the VMH is likely involved in female receptive behaviors in anoles.

Expression of ERα specifically has not been quantified in the adult green anole. However, autoradiography has detected binding of ^{3}H-estradiol in the forebrain (Martinez-Vargas et al., 1978; Morrell et al., 1979; see Discussion section). Therefore, the following experiment was designed to assess ERα expression in the POA, AMY, and VMH in males and females during both BS and NBS. The goal was to quantify ERα mRNA with the hope of elucidating where