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Research report

Estrogen receptor α and vasopressin in the paraventricular nucleus of the hypothalamus in *Peromyscus*

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Abstract

The purpose of this study was to determine the presence of estrogen receptor alpha (ERα) and the relationship between neurons that express ERα and produce vasopressin (AVP) in the paraventricular nucleus of the hypothalamus (PVN) in new world mice of the genus *Peromyscus*. Brains were collected from male and female *Peromyscus californicus*, *Peromyscus leucopus*, *Peromyscus maniculatus*, and *Peromyscus polionotus*, and double labeled for the expression of ERα and AVP immunoreactivity (IR). The number of cells expressing ERα-IR and AVP-IR was determined in the medial and posterior region of the PVN. The results indicate that *Peromyscus* is the first taxonomic group reported to have ERα widely distributed in the PVN, occurring in both medial and posterior regions of the PVN. While estrogen can regulate the production of AVP, AVP and ERα were rarely colocalized. There was, however, a significant inverse relationship between the number of cells that expressed ERα-IR and the number expressing AVP-IR. There were no sex differences in the expression of ERα-IR or AVP-IR.

Theme: Other systems of the CNS
Topic: Comparative neuroanatomy

Keywords: AVP; Colocalization; Deer mice; ER alpha; Monogamy; PVN

1. Introduction

Estrogen and testosterone, after aromatization to estradiol, affect many aspects of reproduction and sociosexual behavior. These effects are mediated primarily by one of two estrogen receptor subtypes, α (ERα) and β. Despite the relatively wide distribution of ERα, there are regions of the hypothalamus in which ERα is all but absent. Based on the patterns observed in standard laboratory species, the paraventricular nucleus (PVN) of the hypothalamus is one such region; in rats (*Rattus norvegicus*) and mice (*Mus musculus domesticus*), only the β isoform is found in the PVN. However, the patterns observed in rats and lab mice might not generalize to other species. The distribution of ERα has been examined thoroughly in several other species, but has been reported in the PVN only in sheep [31,51] and guinea pigs [44,58,59], suggesting that the presence or absence of ERα in the PVN may be species-specific. Therefore, one of the goals of this study was to determine if ERα is present in the PVN of new world mice, specifically *Peromyscus*.

In addition to containing ER, the PVN is a major site of production of the neuropeptide arginine vasopressin (AVP) for both central and peripheral release. The AVP neurons in the PVN project to multiple central sites including the central, anterior, basal, and lateral amygdala and the locus coeruleus [55,56], and centrally released AVP has a role in the regulation of social behavior and aggression (e.g., Refs. [1,5,7,16]). Central production and release of AVP is steroid-

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dependent in some nuclei. For example, in rodents, AVP is reduced in the bed nucleus of the stria terminalis (BST) and the medial amygdala (MeA) following gonadectomy and restored with steroid replacement [12,35]. Neonatal castration results in a permanent reduction in the number of neurons that express AVP [32,57], but when neonatally castrated rats are given estradiol as neonates, central AVP is increased [22]. The role of estrogen in AVP production is also supported by data demonstrating that aromatase knock out mice have reduced AVP in the MeA and BST [45]. Both BST and MeA contain substantial amounts of ERα; presumably, AVP is regulated in these nuclei through ERα. The PVN is a notable exception in that AVP production in central projections of the PVN does not appear to be steroid dependent [12], perhaps because the species examined do not have the α subtype in the PVN.

Estrogen can affect the production of AVP in at least two ways. The AVP gene has an estrogen response element [52] and estrogen can act non-genomically through AP-1 sites [40]. Which ER subtype is present appears to be functionally significant, as transcription of the vasopressin gene is differentially regulated by estrogen depending on whether it acts through ERα or ERβ [52]. In in vitro luciferase reporter assays, cells transfected with ERα increased luciferase activity in response to estrogen while estrogen was inhibitory in cells transfected with ERβ [52]. This suggests different functions for the two isotypes and, thus, the distribution of AVP neurons might differ between species with ERα in the PVN and those in which ERβ predominates.

To test this prediction we compared the expression and colocalization of ERα with AVP in the PVN of four species of *Peromyscus*: *Peromyscus californicus*, *Peromyscus leucopus*, *Peromyscus maniculatus*, and *Peromyscus polionotus*. *Peromyscus* were chosen for several reasons. AVP plays a major role in the behavior and reproduction of *Peromyscus* [4,5] leading to the prediction that AVP production may differ from other species, such as rats and mice. The distribution of the AVP receptor ($V_{1a}R$) has been linked to social system; the distribution of $V_{1a}R$ differs between polygynous and monogamous species [26,27,61]. The species chosen include both polygynous (*P. leucopus* and *P. maniculatus*) and monogamous (*P. californicus* and *P. polionotus*) species. By using *Peromyscus* it will be possible to not only compare the expression of estrogen receptors and AVP across species and sex, but also to make comparisons based upon social organization all within the same genus.

2. Materials and methods

2.1. Animals

Peromyscus were obtained from the Peromyscus Stock Center (University of South Carolina, Columbia, South Carolina). All animals were individually housed, sexually naive adults. Animals were maintained on a 14:10 h light/dark cycle and provided food (Purina Rat Chow) and water ad libitum. Brains were collected from males and females of each species (*P. maniculatus*, BW stock, n=7 males, 6 females; *P. leucopus*, LL stock, n=5 males, 5 females; *P. californicus*, IS stock, n=5 males, 4 females; *P. polionotus*, PO stock, n=6 males, 6 females). To avoid complications associated with estrus, vaginal lavages were done to determine stage of estrus and tissue was collected only during diestrus. All procedures reported in this study were approved by the appropriate IACUC and were within the guidelines established by the National Institutes of Health Guide for the Care and Use of Animals. The experiments were designed to minimize pain and discomfort and the number of animals required.

2.2. Localization of ERα and AVP

Mice were deeply anesthetized with a mixture of Ketamine (67.7 mg/kg) and Xylazine (13.33 mg/kg) prior to transcardial perfusion with 4% paraformaldehyde and 2.5% acrolein in 0.1 M potassium phosphate buffered saline (KPBS; pH 7.6). Brains were removed and stored in 25% sucrose at 4 °C until sectioned at 30 μm using a freezing sliding microtome. Sections were stored in cryoprotectant [60] at −20 °C until processed using ABC immunocytochemistry (ICC) staining for ERα and AVP. Tissue sections were rinsed 6 times over 60 min in 0.05 M KPBS. Sections were then incubated at room temperature in 1% sodium borohydride for 20 min to neutralize acrolein used during fixation and then rinsed repeatedly in KPBS. Next, sections were incubated with rabbit ERα polyclonal antibody (anti-ERα C1355, Upstate Biotechnology, Waltham, MA) at a concentration of 1:100,000 in 0.05 M KPBS +0.4% Triton X for 1 h at room temperature and then for 48 h at 4 °C. This antibody binds to both free and bound receptors [36], reducing variation in staining due to potential differences in circulating hormone levels. The antibody was generated against the last 15 C-terminal amino acids of the rat ERα protein, a region that shares no homology with ERβ. The specificity of this antibody was tested by omitting the primary antibody from the ICC procedure and by performing ICC after pre-adsorption with the synthetic peptide (10× the concentration of antibody) against which the antibody was raised. Sections were rinsed 10 times over 60 min in KPBS before being incubated for 1 h at room temperature in biotinylated goat, anti-rabbit IgG (Vector Laboratories, Burlingame, CA) at 1:600 dilution in KPBS + 0.4% Triton X. Sections were rinsed 5 times over 50 min in KPBS and incubated for 1 h at room temperature in an avidin–biotin peroxidase complex (Vectastain ABC kit-elite pk-6100 standard, 4.5 μl A and 4.5 μl B per 1 ml solution, Vector Laboratories) in KPBS + 0.4% Triton X. Sections were rinsed 3 times in KPBS followed by three rinses in 0.175 M sodium acetate. Finally, ERα was visualized by incubation