Available online at www.sciencedirect.com

SCIENCE DIRECT

Brain Research 1049 (2005) 156 – 164

BRAIN RESEARCH

www.elsevier.com/locate/brainres

Research Report

Arginine vasopressin and vasoactive intestinal polypeptide fibers make appositions with gonadotropin-releasing hormone and estrogen receptor cells in the diurnal rodent *Arvicanthis niloticus*

Megan M. Mahoney[a,b,*], Laura Smale[c,d,e]

[a]*Reproductive Sciences Program, University of Michigan, Ann Arbor, MI 48109-1109, USA*
[b]*Department of Psychology, University of Michigan, Ann Arbor, MI 48109-1109, USA*
[c]*Department Zoology, Michigan State University, East Lansing, MI 48843, USA*
[d]*Department of Psychology, Michigan State University, East Lansing, MI 48843, USA*
[e]*Neuroscience Program, Michigan State University, East Lansing, MI 48843, USA*

Accepted 19 April 2005
Available online 3 June 2005

Abstract

Diurnal and nocturnal animals differ with respect to the timing of a host of behavioral and physiological events including those associated with estrus, but the neural bases of these differences have not been elucidated. We investigated this issue by examining the distribution of cells containing gonadotropin-releasing hormone (GnRH) as well as estrogen receptors (ERs) in relation to fibers containing peptides present in the suprachiasmatic nucleus (SCN) in a diurnal animal, *Arvicanthis niloticus* (the unstriped Nile grass rat). We found that fibers containing two peptides found in SCN cells, arginine vasopressin and vasoactive intestinal polypeptide appeared to be in contact with GnRH and ER positive cells. These data suggest that temporal information is carried along the same direct pathways from the SCN to GnRH and ER neurons in day- and night-active species.

Theme: Other systems of the CNS
Topic: Comparative neuroanatomy

Keywords: Arvicanthis; Diurnal; Gonadotropin-releasing hormone; Estrogen receptor; Arginine vasopressin; Vasoactive intestinal polypeptide

Abbreviations: 3V, 3rd ventricle; ac, anterior commissure; AHA, anterior hypothalamic area; AVP, arginine vasopressin; AVPV, anteroventral portion of the periventricular nucleus; BNST, bed nucleus of the stria terminalis; d3V, dorsal portion of 3rd ventricle; DAB, diaminobenzidine; DBB, diagonal band of Broca; ER, estrogen receptor-α; GnRH, gonadotropin-releasing hormone; hDBB, horizontal portion of DBB; LH, luteinizing hormone; LPOA, lateral portion of POA; LS, lateral septum; LSPV, lower subparaventricular zone; LSV, ventral portion of LS; lv, lateral ventricle; mPOA, medial portion of POA; MS, medial septum; oc, optic chiasm; OVLT, organum vasculosum of the lamina terminalis; PBS, phosphate-buffered saline; PeVN, periventricular nucleus; POA, preoptic area; PVN, paraventricular nucleus of the hypothalamus; RCH, retrochiasmatic area; SCN, suprachiasmatic nucleus; SEM, standard error of the mean; SON, supraoptic nucleus; TX, Triton-X; vDBB, vertical portion of DBB; VIP, vasoactive intestinal polypeptide; VMPO, ventromedial portion of POA

* Corresponding author. Department of Psychology, 4031 East Hall, 530 Church Street, University of Michigan, Ann Arbor, MI 48109-1109, USA. Fax: +1 734 763 7480.
E-mail address: mahoneym@umich.edu (M.M. Mahoney).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.04.071

1. Introduction

The suprachiasmatic nucleus (SCN) is the primary site for the generation and synchronization of circadian rhythms in mammals [25]. Multiple lines of evidence have converged to indicate that the circadian clock located in the SCN is critical for the timing of estrus-related events in nocturnal rodents [3,13,18,19,24,30,36,37,55,56,70]. In these animals, the SCN appears to communicate temporal information involved in the regulation of estrus via direct synaptic contacts with both gonadotropin-releasing hormone (GnRH) and estrogen receptor-α (ER)-containing neurons [11,36]. The majority of GnRH cells contacted by SCN efferents are located in the region of the organum vasculosum of the lamina terminalis (OVLT) and the preoptic area (POA) of lab rats, and in the diagonal band of Broca (DBB) and POA of hamsters [9,61]. ER-containing cells in many regions of the hypothalamus known to be involved in the regulation of both sexual behavior and the ovulatory surge in luteinizing hormone (LH) also receive direct input from the SCN [9,65]. One such region is the anteroventral portion of the periventricular nucleus (AVPV), which is directly involved in the integration of circadian, and hormonal signals that induce the LH surge in laboratory rats [65].

The neuropeptides vasoactive intestinal polypeptide (VIP) and arginine vasopressin (AVP) may mediate the effects of the SCN on the timing of GnRH cell activation, the LH surge, and mating behavior. VIP administered into the ventricles of lab rats can stimulate the release of LH in some conditions [64] and inhibit it in others [2,54,68,69]. In female lab rats and hamsters, VIP fibers terminate on GnRH cells, and in lab rats SCN lesions eliminate most of these contacts [21,26,27,59,61]; these appositions are more numerous in females than in males, which are unable to generate a surge [21]. A role for VIP is also indicated by the presence of receptors for VIP on GnRH neurons in vivo and in vitro [39,49].

AVP also mediates the timing of estrus-related events such as LH secretion and sexual receptivity [51,50]. Like VIP, AVP inhibits LH secretion in some conditions and stimulates it in others [15,41,42,45]. AVP fibers contact GnRH neurons in the medial preoptic area (mPOA) of female lab rats and hamsters, and in the supraoptic nucleus (SON) of female cynomolgus monkeys [22,57,62]. SCN lesions in lab rats reduce the number of these contacts, suggesting that at least some of the axon terminals involved come from cells within the SCN [62]. In co-cultures of tissue containing the SCN and POA, circadian rhythms in AVP and GnRH release have identical periods, and AVP administration to single POA cultures induces GnRH release [16].

Rhythms in the timing of estrus-related events such as mating behavior, the preovulatory surge in LH, and GnRH cell activity are inverted in diurnal and nocturnal species [6,28,33,35,47,52,53,71]. The diurnal murid rodent *Arvicanthis niloticus* (grass rat) exhibits a host of rhythms that are reversed relative to those of lab rats, including rhythms in the timing of the LH surge, associated GnRH cell activation, and copulatory behavior [4,33,34,35]. Here, we used the grass rat to address the issue of how the circadian control of these estrous events might differ in nocturnal and diurnal species. At a very basic level, one possibility is that neuroanatomical pathways from the SCN to GnRH and ER neurons are the same, but that signals and/or responses to these signals are different. Alternatively, the basic pathways extending directly from the SCN to GnRH and ER neurons in nocturnal species may not be present in diurnal ones and the circadian signal may reach these neurons only through indirect pathways. Here, we evaluate these hypotheses by determining whether appositions exist between fibers that contain either VIP or AVP and cells that contain ER and GnRH and in the grass rat.

2. Materials and methods

2.1. Animals

Adult female grass rats (>60 days) bred from laboratory stock were singly housed, kept in a 12:12 light:dark cycle and provided food (Teklad rodent chow 8640, Harlan Industries) and water ad libitum. A red light (<5 lux) was left on continuously in each animal room. In order to optimize the staining of ER-containing cells, we used ovariectomized grass rats primed with estradiol in some studies. Grass rats were bilaterally ovariectomized while under Nembutal anesthesia (Abbott Laboratories) supplemented with Metofane (Mallinckrodt Veterinary). Incisions were closed with subcutaneous sutures and treated with Nolvasan (Fort Dodge Animal Health). Following ovariectomy, animals were given saline (1 cc 0.9%) and Buprenex (Reckitt & Coleman). Animals recovered for at least 7 days prior to steroid hormone injection and/or perfusion. All experiments were performed in compliance with Michigan State University All-University Committee on Animal Use and Care in accordance with the standard in the National Research Council *Guide for the Care and Use of Laboratory Animals*. All efforts were made to minimize the suffering and the number of animals used in these experiments.

2.2. Tissue processing and analysis

2.2.1. General immunocytochemical procedure

Animals were deeply anesthetized with Nembutal and perfused transcardially with 0.01 M phosphate-buffered saline (PBS; pH 7.4, 150–200 ml/animal) followed by 4% paraformaldehyde (150–200 ml/animal, Sigma) in 0.1 M phosphate buffer. Brains were post-fixed in paraformaldehyde for 4 h, transferred to 20% sucrose in 0.1 M phosphate buffer overnight, and then sectioned with a freezing microtome. Brains were cut into three series of 30-μm sections from the septum through the SON.