available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Distribution of urocortin 3 neurons innervating the ventral premammillary nucleus in the rat brain

Judney Cley Cavalcante[a], *Luciane Valéria Sita*[a], *Marcelo Betti Mascaro*[a,b], *Jackson Cioni Bittencourt*[a,c], *Carol Fuzeti Elias*[a,c,*]

[a]*Laboratory of Chemical Neuroanatomy, Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil*
[b]*Nove de Julho University Center-Uninove, São Paulo, Brazil*
[c]*Center for Neuroscience and Behavior, Institute of Psychology, University of São Paulo, São Paulo, Brazil*

ARTICLE INFO

Article history:
Accepted 15 March 2006
Available online 25 April 2006

Keywords:
Medial nucleus of amygdala
Perifornical area
Odor
Pheromone

ABSTRACT

Urocortin 3 (Ucn 3) is a recently described peptide of the corticotropin-releasing factor family. Neurons expressing Ucn 3 mRNA and peptide are distributed in specific brain areas, including the median preoptic nucleus, the perifornical area (PFx), and the medial nucleus of the amygdala (MEA). Fibers immunoreactive to Ucn 3 are confined to certain brain nuclei, being particularly dense in the ventral premammillary nucleus (PMV). In studies involving electrolytic lesions and analysis of Fos distribution according to behavioral paradigms, the PMV has been potentially implicated in conspecific aggression and sexual behavior. However, the role that Ucn 3 plays in this pathway has not been explored. Therefore, we investigated the origins of the urocortinergic innervation of the PMV of Wistar rat in an attempt to map the brain circuitry and identify likely related functions. We injected the retrograde tracer cholera toxin b subunit into the PMV and found that 88% of the Ucn 3-immunoreactive fibers in the PMV originate in the dorsal MEA, and that few originate in the PFx. As a control, we injected the anterograde tracer biotin dextran amine into both regions. We observed that the PMV is densely innervated by the MEA, and scarcely innervated by the PFx. The MEA is a secondary relay of the vomeronasal system and projects amply to hypothalamic nuclei related to hormonal and behavioral adjustments, including the PMV. Although physiological studies should also be performed, we hypothesize that Ucn 3 participates in such pathways, conveying sensory information to the PMV, which in turn modulates behavioral and neuroendocrine responses.

1. Introduction

Urocortin 3 (Ucn 3), also known as stresscopin (Hsu and Hsueh, 2001), is a recently described peptide of the corticotropin-releasing factor (CRF) family and is a selective ligand for CRF type 2 (CRF_2) receptors (Lewis et al., 2001). It has been reported that Ucn 3 modulates stress response, as well as feeding and autonomic activity (Bale et al., 2002; Hashimoto et al., 2004; Lewis et al., 2001; Valdez et al., 2003). However, exactly which brain pathways are involved in these functions is a matter of speculation.

Distribution of Ucn 3 mRNA and immunoreactive cell bodies in the rat brain is limited, restricted mainly to the

* *Corresponding author*. Laboratory of Chemical Neuroanatomy, Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 2415, Room 105, São Paulo 05508-900 SP, Brazil.
E-mail address: cfelias@usp.br (C.F. Elias).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.03.043

median preoptic nucleus, the anterior perifornical area, the principal division of the bed nucleus of the stria terminalis and the medial nucleus of the amygdala (MEA) (Lewis et al., 2001; Li et al., 2002). Distribution of Ucn 3-immunoreactive (Ucn 3-ir) fibers is particularly dense in the lateral septal nucleus, the ventromedial nucleus of the hypothalamus, and the ventral premammillary nucleus (PMV) (Li et al., 2002). The PMV is located in the posterior levels of the medial hypothalamus, a region that has been designated the "behavior control column" (Swanson, 2000). The results of studies involving electrolytic lesions of the premammillary area have suggested that the PMV participates in the regulation of aggressive behavior (van den Berg et al., 1983). In addition, studies using Fos protein as a marker for neuronal activity have shown that the PMV is activated following copulation or conspecific aggression paradigm (Kollack-Walker and Newman, 1995; Veening et al., 2005). However, it has been shown that olfactory stimulation is sufficient to induce Fos in the PMV of males exposed to soiled bedding cages where cycling females have been housed (Yokosuka et al., 1999), suggesting that the PMV plays an important role in pheromone discrimination.

Canteras et al. (1992) showed that the PMV possesses bidirectional connections with sexually dimorphic nuclei, as well as with areas such as the anteroventral periventricular nucleus, the medial preoptic nucleus, the bed nucleus of stria terminalis, and the MEA, all of which are related to reproductive control and luteinizing hormone (LH) secretion. In fact, we have recently shown that fibers originating in the PMV form close appositions with gonadotropin-releasing hormone (GnRH) in male and female rats (Rondini et al., 2004). Therefore, the PMV is apt to modulate LH secretion.

The PMV neurons also express the long form of leptin receptor (ObRb) mRNA and respond to circulating leptin (Elias et al., 2000, 2001; Elmquist et al., 1998), a hormone secreted by adipocytes. Leptin has profound effects on feeding, as well as on metabolic and neuroendocrine homeostasis (Zhang et al.,

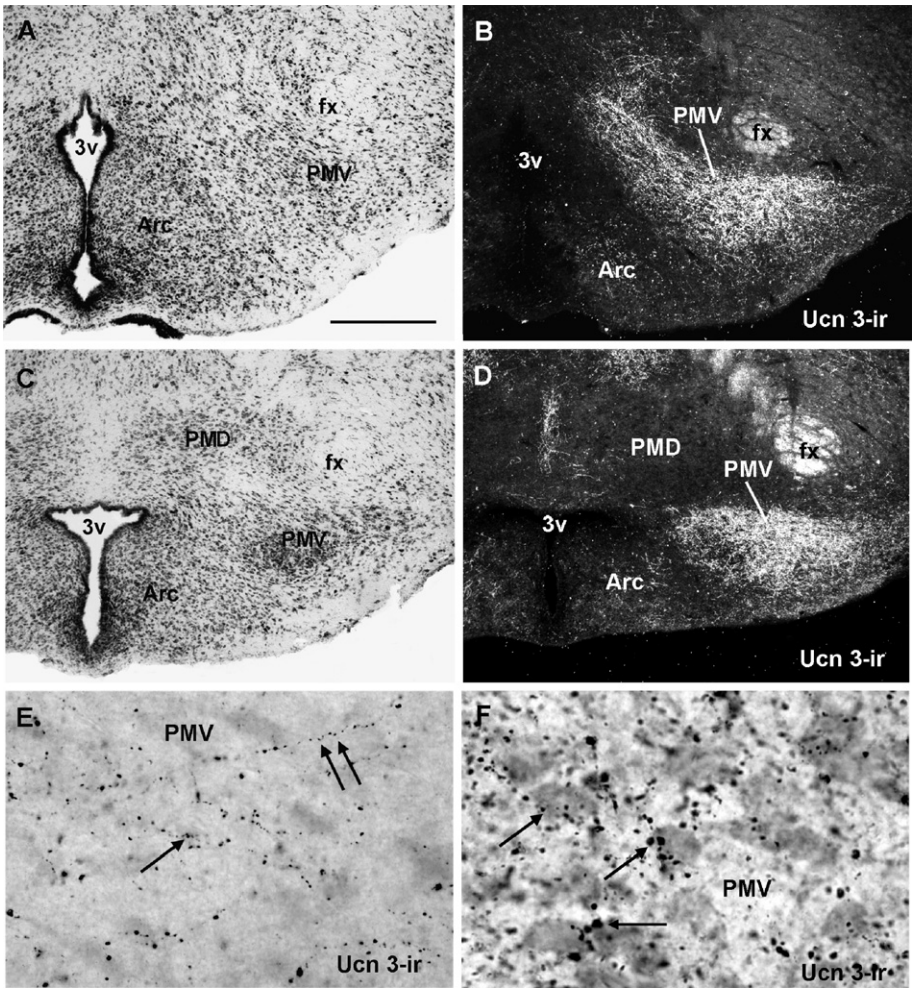

Fig. 1 – Distribution of urocortin 3-immunoreactive (Ucn 3-ir) fibers in the ventral premammillary nucleus (PMV). (A, C) Bright-field photomicrographs showing two rostrocaudal levels of the PMV in reference (thionin staining) sections. (B, D) Dark-field photomicrographs showing the distribution of Ucn 3-ir in two rostrocaudal levels of the PMV. (E, F) Bright-field photomicrographs showing Ucn 3-ir varicosities (double arrows) and terminal-like structures (single arrows). Note that, in panel F, terminal-like structures are in close apposition with PMV neurons. Abbreviations: 3v, third ventricle; Arc, arcuate nucleus; fx, fornix; PMD, dorsal premammillary nucleus. Scale bar: A–D = 400 μm; E–F = 50 μm.