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Neuronal distribution of melanin-concentrating hormone, cocaine- and amphetamine-regulated transcript and orexin B in the brain of the Djungarian hamster (*Phodopus sungorus*)

Reza M.H. Khorooshi, Martin Klingenspor*

Department of Animal Physiology, Biology Faculty, Philipps-University Marburg, Karl-von-Frisch-Street 8, 35032 Marburg, Germany

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Abstract

The distribution of melanin-concentrating hormone-, cocaine- and amphetamine-regulated transcript- and orexin B-immunoreactive elements as well as their morphological relationships in selected brain structures harbouring the neuroendocrine pathways controlling energy balance and circadian rhythmicity in the Djungarian hamster (*Phodopus sungorus*) were studied. Cocaine- and amphetamine-regulated transcript-(55–102)-immunoreactive perikarya co-expressed melanin-concentrating hormone-immunoreactivity in the lateral hypothalamic area, dorsomedial hypothalamic nucleus, zona incerta and posterior hypothalamic area. In addition, arcuate nucleus, hypothalamic periventricular nucleus, Edinger–Westphal nucleus, and the rostral aspect of the dorsal raphe nucleus contained cocaine- and amphetamine-regulated transcript-immunoreactive cell profiles. Orexin B-immunoreactive perikarya were distributed in the lateral hypothalamic area, dorsomedial hypothalamic nucleus and retrochiasmatic area. Cells immunoreactive for orexin B did not co-express melanin-concentrating hormone-immunoreactivity, but orexin B-immunoreactive fibers had close apposition to many melanin-concentrating hormone-immunoreactive cells. Whereas immunoreactivity for all examined peptides was absent in the suprachiasmatic nucleus, dense and large orexin B-immunoreactive fibers and to a lesser extent melanin-concentrating hormone- and cocaine- and amphetamine-regulated transcript-immunoreactive fibers of smaller size were present in the intergeniculate leaflet and raphe nucleus. These observations in Djungarian hamsters indicate that the neuronal distribution of the examined peptides is strongly conserved between species. In addition, the presence of fibers within the neuronal components of the circadian timing system suggests that they may indirectly influence circadian rhythms.

Keywords: Melanin-concentrating hormone; Cocaine- and amphetamine-regulated transcript; Orexin B; Djungarian hamster; Energy balance; Circadian timing system

Abbreviations: 3V, third ventricle; ARC, arcuate nucleus; CART, cocaine- and amphetamine-regulated transcript; DR, dorsal raphe nucleus; DMH, dorsomedial hypothalamic nucleus; EW, Edinger–Westphal nucleus; GHT, geniculohypothalamic tract; IGL, intergeniculate leaflet; ICV, intracerebroventricular; LHA, lateral hypothalamic area; LA, lateroanterior hypothalamic nucleus; VLPO, lateroventral preoptic area; MPOA, medial preoptic area; ME, median eminence; MnPO, median preoptic nucleus; MR, median raphe nucleus; MCH, melanin-concentrating hormone; OXA, orexin A; OXB, orexin B; PVN, paraventricular hypothalamic nucleus; PVT, paraventricular thalamic nucleus; PVA, paraventricular thalamic nucleus anterior; PVP, paraventricular thalamic nucleus posterior; Pe, periventricular hypothalamic nucleus; PHA, posterior hypothalamic area; RHT, retinohypothalamic tract; RCH, retrochiasmatic area; rDR, rostral dorsal raphe nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; PMV, ventral premammillary nucleus; VTM, ventral tuberomammillary nucleus; VMH, ventromedial hypothalamic nucleus; ZI, zona incerta

* Corresponding author. Tel.: +49 6421 28 23908; fax: +49 6421 28 28937.
E-mail address: klingens@staff.uni-marburg.de (M. Klingenspor).

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1. Introduction

Djungarian hamsters (*Phodopus sungorus*) undergo a pronounced seasonal cycle in food intake, energy expenditure and body mass triggered by acclimation to short photoperiod (Morgan et al., 2003). During the entire cycle precise adjustment of seasonally appropriate food intake, energy expenditure and body mass according to a hypothetical sliding-set point (Steinlechner et al., 1983) may require communication between neuronal components controlling energy balance, photoperiodic time measurement and circadian rhythms (Klingenspor et al., 1996; Adam et al., 2000; Mercer et al., 2000; Klingenspor et al., 2000).

The primary neuronal networks integrating and coordinating the control of energy balance are mostly located in hypothalamic structures including the arcuate nucleus (ARC), the dorsomedial hypothalamic nucleus (DMH), the ventromedial hypothalamic nucleus (VMH), the lateral hypothalamic area (LHA) and the paraventricular hypothalamic nucleus (PVN). Neuronal components of the circadian timing system are distributed throughout the brain, forming a network coordinating the temporal organization of physiological processes and behavior. The primary nodes of this network include the suprachiasmatic nucleus (SCN), the intergeniculate leaflet (IGL), the median raphe nucleus (MR) and the dorsal raphe nucleus (DR). The SCN as the pacemaker of the circadian timing system receives photic input from the retina through the retinohypothalamic tract (RHT) and from the IGL through the geniculohypothalamic tract (GHT). In addition, MR and DR influence circadian rhythmicity through innervations of the SCN and IGL, respectively (Morin and Blanchard, 1995; Meyer-Bernstein and Morin, 1996).

Chemical lesions of the ARC do not have a major impact on short day mediated downregulation of food intake and body mass (Ebling et al., 1998), whereas lesions of the SCN abolish this response (Bittman et al., 1991). Hence, the circadian timing system plays an important role in the regulation of seasonal body mass cycles through communication with the hypothalamic neuronal networks controlling energy balance. Notably, direct projections from the SCN to perikarya of neurons in the LHA producing the orexigenic peptides melanin-concentrating hormone (MCH) and orexin A (OXA) and orexin B (OXB) were found in rat and human (Abrahamson et al., 2001). Recently, the neuroanatomical basis for the possible interaction of orexins and the circadian timing system was reported in the Syrian hamster (*Mesocricetus auratus*) and Djungarian hamster (McGranaghan and Piggins, 2001; Mintz et al., 2001). Several studies of OXA and OXB which are derived from a common pro-orexin precursor, suggest a stronger orexigenic potential of OXA (Edwards et al., 1999). The neuroanatomical tracing of differential innervation patterns for these neuropeptides may increase our understanding of functional divergence. OXA and OXB display a similar distribution pattern in rats (Sakurai et al., 1998; Edwards et al., 1999; Sahu, 2002), but no information is available on OXB in a seasonal mammal like the Djungarian hamster.

Furthermore, neurons in the LHA receive projections from the neuropeptide network of the ARC, of which anorexigenic cocaine- and amphetamine-regulated transcript (CART) represents a key factor in the regulation of seasonal body mass cycles (Adam et al., 2000; Mercer et al., 2000, 2003; Mercer and Speakman, 2001). Whether the neuronal network of MCH, CART, OXA and OXB known to regulate energy balance also exerts influence on circadian behavior requires further investigation, specifically, in seasonal mammals.

The present study aimed to investigate the immunohistochemical distribution of MCH, CART and OXB in Djungarian hamsters within selected brain areas implicated in the control of food intake and circadian timekeeping processes. In addition, dual-labeling immunostaining was performed to examine the relationship between these neuropeptides.

2. Materials and methods

2.1. Tissue preparation

Adult male Djungarian hamsters (*Phodopus sungorus*, $n = 10$) were kept at room temperature in a natural photoperiod and were housed individually in Macrolon cages with free access to water and standard breeding chow diet (Altromin 7014; Altromin, Lage, Germany). Brains were collected in the month of May (14:10 light:dark) when hamsters weighed 47–55 g and had fully developed their dark-gray summer pelage. Hamsters were killed by CO_2 exposure between 13:00 and 14:00 h. Brains were dissected, fixed in 4% paraformaldehyde (48 h, 4 °C) and cryoprotected in 20% sucrose in 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 24 h at 4 °C. The brains were then cut on a cryostat into 30 μm coronal sections. Free-floating sections were stored in PBS at 4 °C prior to immunohistochemical procedures. All procedures were in accordance with German animal welfare regulation.

2.2. Immunostaining

To investigate the anatomical localization of MCH, CART and OXB in the brain of Djungarian hamsters, two different detection methods were used, namely immunofluorescence and HRP-peroxidase reaction.

Free-floating sections were rinsed in PBS and then in PBS containing 0.5% Triton-X 100 (PBS-TX). Following preincubation in blocking solution containing PBS-TX and 3% BSA, sections were incubated with primary rabbit anti-MCH (Phoenix Europe GmbH; H-070-47), CART (55-102; Phoenix Europe GmbH; H-003-62) and OXB (Phoenix Europe GmbH; H-003-32) antibodies each diluted 1:200 in blocking solution overnight at 4 °C. Following washing in