Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres

Research Report

Projections from melanin-concentrating hormone (MCH) neurons to the dorsal raphe or the nuclear core of the locus coeruleus in the rat

Ye S. Yoon[a], *Hyun S. Lee*[a,b,*]

[a]*Department of Anatomy, School of Medicine, Biomedical Science Research Building #414, Konkuk University, 143-701 Seoul, Republic of Korea*
[b]*Research Institute of Medical Science, School of Medicine, Konkuk University, 143-701 Seoul, Republic of Korea*

ARTICLE INFO

Article history:
Accepted 13 August 2012
Available online 5 September 2012

Key words:
Melanin-concentrating hormone
Locus coeruleus
Dorsal raphe nucleus

ABSTRACT

Brainstem aminergic and cholinergic nuclei are essential components of reticular activating system which are under the control of hypothalamic sleep/arousal centers. In contrast to well-known role of hypocretin (Hcrt) as a potent wake-promoting substance, only recent reports stated that melanin-concentrating hormone (MCH) plays a role in the maintenance of rapid eye movement (REM) sleep. As the sequel to our report concerning the MCH/Hcrt projection to the brainstem cholinergic nuclei (Hong et al., 2011), in the present study we examined the differential projection from MCH/Hcrt neurons in medial and lateral subdivisions of the lateral hypothalamus (LH) to the dorsal raphe (DR) or the nuclear core of the locus coeruleus (LC) of the rat. Following the injection of Red Retrobeads into the LC core ($n=6$), the proportions of retrogradely labeled (retro-) MCH neurons over the total retro-cells were 4.4%±0.5% (medial subdivision) and 7.4%±0.6% (lateral one), whereas those of retro-Hcrt cells over the total retro-cells were 69.4%±3.6% (medial) and 64.4%±5.2% (lateral). Following midline-DR injections ($n=6$), the proportions of retro-MCH neurons over the total retro-cells were 14.3%±2.9% (medial) and 12.3%±1.6% (lateral), while those of retro-Hcrt cells over the total retro-cells were 46.5%±6.2% (medial) and 51.3%±9.5% (lateral). Following lateral wing-DR injections ($n=3$), the proportions of retro-MCH neurons over the total retro-cells were 15.5%±1.2% (medial) and 11.9%±3.1% (lateral), while those of retro-Hcrt cells over the total retro-cells were 48.5%±2.7% (medial) and 52.8%±2.3% (lateral). The statistical analysis showed that MCH neurons projecting to the LC core or DR were outnumbered by Hcrt cells ($P<0.01$) and that retro-MCH cells exhibited lateral predominance in LC injection cases ($P<0.05$). Based on our present as well as previous (Hong et al., 2011) observations, we suggested that MCH and Hcrt neurons in the LH provide preferential projections to the brainstem cholinergic and aminergic nuclei, respectively and that MCH projections to the nuclear core of the LC exhibit differential distribution within LH subdivisions.

*Corresponding author at: Department of Anatomy, School of Medicine, Biomedical Science Research Building #414, Konkuk Univ., 143-701 Seoul, Republic of Korea. Fax: +82 2 2049 6192.
E-mail address: hyunsook.lee@kku.ac.kr (H.S. Lee).

0006-8993/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2012.08.022

1. Introduction

The melanin-concentrating hormone (MCH) was originally known as a circulating factor mediating color changes in fish. Studies indicated that MCH neurons in the lateral hypothalamus (LH) and zona incerta (ZI) play an important role in sleep/arousal as well as feeding/energy balance (Qu et al., 1996; Shimada et al., 1998; Pissios, 2009; Bittencourt, 2011). On the other hand, brainstem aminergic and cholinergic nuclei are essential components of reticular activating system, which are under the control of hypothalamic sleep/arousal centers. Among various sleep-wake states, aminergic neurons are the most active during arousal, while cholinergic cells are active in rapid eye movement (REM) sleep as well as in wakeful state (Aston-Jones and Bloom, 1981; El Mansari et al., 1989; Burlet et al., 2002; Lu et al., 2006). In contrast to the well-known role of Hypocretin (Hcrt) as a potent wake-promoting substance, only recent reports stated that MCH play a crucial role in the maintenance of REM sleep (Verret et al., 2003; Hassani et al., 2009; Lagos et al., 2009; Peyron et al., 2009).

Various subdivisions of the perifornical region in the LH exhibit preferential, ascending or descending projections to specific brain areas; neurons located medial to the fornix provide both ascending and descending pathways toward the telencephalon and the brainstem, while those located lateral to the fornix preferentially send descending projections toward the caudal brainstem and the spinal cord (Brischoux et al., 2002; Cvetkovic et al., 2004; Hanriot et al., 2007). Likewise, there is a slight preference for Hcrt neurons projecting to the basal forebrain areas to be distributed within the medial half, whereas those projecting to the locus coeruleus (LC) and the dorsal raphe (DR) were primarily within the dorsal half (España et al., 2005; Lee et al., 2005b).

As the sequel to our report concerning the MCH/Hcrt projection to the brainstem cholinergic nuclei (Hong et al., 2011), in the present study we examined the projection from MCH/Hcrt neurons to the DR or the LC core of the rat. The retrograde tracing method was combined with double immunofluorescence for the peptides to identify the possible differential distribution of MCH or Hcrt neurons within medial and lateral subdivisions of the LH and to assess the degree of MCH/Hcrt projection to the brainstem aminergic or cholinergic nuclei.

2. Results

A total of 42 Sprague-Dawley rats were utilized to examine several aspects of MCH projections to the LC core or DR. First, the possibility was assessed whether MCH-immunoreactive axon terminals exist within the brainstem aminergic nuclei and, if any, whether they make contact with DR or LC somata. Second, following the unilateral injection of Red Retrobeads into the LC or DR, LH sections were double-immunostained for MCH and Hcrt to examine the regional distribution of retrogradely-labeled MCH or Hcrt neurons within medial and lateral subdivisions of the LH. Finally, the quantitative analysis was performed to assess the MCH/Hcrt ratios of LH neurons projecting to the brainstem monoaminergic nuclei.

2.1. MCH- and Hcrt-immunoreactive axon terminals within the LC or DR

Without involving any tracer injection ($n=4$), LC or DR sections were double-immunostained for MCH and Hcrt (Fig. 1). In the caudal (principal) LC, both MCH (green-labeled) and Hcrt (red-labeled) axon terminals were observed in the medial dendritic zone (Fig. 1A–B_1) as well as the nuclear core (Fig. 1C–D_1). MCH- or Hcrt-immunoreactive boutons were also observed at rostral (Fig. 1,E–F_1) to caudal (Fig. 1,G–H_1) extent of the DR nucleus.

Based on the observation that MCH neurons sent axon terminals to the LC or DR (Fig. 1), additional experiments were performed to examine whether the boutons made contact with neuronal profiles of the LC or DR ($n=5$). At the rostral (Fig. 2A–B_2) as well as the caudal, principal (Fig. 2C–D_2) LC levels, MCH (green-labeled) boutons formed close appositions to LC somata (red-labeled). Likewise, MCH (red-labeled) axon terminals made contact with DR somata (green-labeled) at both rostral (Fig. 2E–F_2) and caudal (Fig. 2G–H_2) levels.

2.2. MCH- or Hcrt-ergic LH neurons projecting to the LC

Red retrobeads were unilaterally injected into various LC regions ($n=17$). The confinement of tracer within the nucleus was assessed by the dopamine-beta-hydroxylase (DBH) immunostaining (Fig. 3). In a rostral LC injection case (Fig. 3A), the tracer diffusion into the medial parabrachial nucleus was minimal (Fig. 3B and C). In a couple of middle LC injection cases (Fig. 3D and E), the tracer diffusion into the medial dendritic zone was negligible (Fig. 3F). In a caudal injection case (Fig. 3G and I), the tracer diffusion into the medial parabrachial nucleus (Fig. 3H) or the ventral dendritic zone (Fig. 3I) was minimal.

In cases involving the tracer injections into the caudal, principal (Fig. 4A–E_4) or middle (Fig. 4F–J_4) LC levels, retrogradely-labeled (retro-) MCH as well as retro-Hcrt neurons were observed in the LH subdivision medial or lateral to the line across the fornix. In LH sections, retro-MCH cells were far fewer in number than retro-Hcrt ones (Fig. 4C_4 and J_4). The size of retro-MCH cells (diameter, 10–20 μm) was relatively smaller (Fig. 4C_4 and J_4) than that of retro-Hcrt cells (diameter, 15–25 μm). The morphology of retro-MCH neurons was round or irregular (Fig. 4C_4 and J_4), while that of retro-Hcrt cells was round or multipolar (Fig. 4C_4, E_4, H_4, and J_4).

2.3. MCH- or Hcrt-ergic LH cells projecting to the DR

Red retrobeads were unilaterally injected into the midline ($n=11$) or lateral wing ($n=5$) subdivisions of the DR. The confinement of the tracer within the nucleus was assessed by the tryptophan hydroxylase (TPH) immunostaining (Fig. 5). At rostral DR levels, the tracer was injected into either midline (Fig. 5A) or lateral wing (Fig. 5B) subdivision. The midline-DR injection was often deep enough to involve the interfascicular region (Fig. 5C). At caudal DR levels, the injection was confined within the midline DR (Fig. 5D).