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Research Report

Effects on sleep of melanin-concentrating hormone (MCH) microinjections into the dorsal raphe nucleus

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ABSTRACT

Neurons that utilize melanin-concentrating hormone (MCH) as a neuromodulator are located in the lateral hypothalamus and incerto-hypothalamic area, and project diffusely throughout the central nervous system, including areas that participate in the generation and maintenance of the states of sleep and wakefulness. Recent studies have shown that the hypothalamic MCHergic neurons are active during rapid eye movements (REM) sleep, and that intraventricular microinjections of MCH induce slow wave sleep (SWS) and REM sleep. There are particular dense MCHergic projections to the dorsal raphe nucleus (DR), a neuroanatomical structure involved in several functions during wakefulness, and in the regulation of sleep variables. Because of this fact, we analyzed the effect of microinjections of MCH into this nucleus on sleep and waking states in the rat. Compared to control microinjections, MCH (100 ng) produced a moderate increase in SWS (243.7±6.0 vs. 223.2±8.8 min, $p<0.05$) and an important increment in REM sleep (35.5±2.5 vs. 20.8±3.4 min, $p<0.01$) due to an increase in the number of REM sleep episodes. The increase of REM sleep was accompanied by a reduction in the time spent in light sleep and wakefulness. We therefore conclude that the hypothalamic MCHergic system, via its action in the DR, plays an important role in the generation and/or maintenance of the states of sleep.

1. Introduction

The melanin-concentrating hormone (MCH) was initially characterized as a circulating factor mediating color change in teleost fish (Kawauchi et al., 1983). Thereafter, MCH was identified as a 19 amino acid cyclic neuropeptide that functions as a neuromodulator in the rat; MCH was found to be fully conserved in mammals, including humans (Forray, 2003; Shi, 2004; Nahon, 2006; Saito and Nagasaki, 2008). The biological function of MCH is mediated by two G-protein coupled receptors known as MCHR-1 and MCHR-2. Although the MCHR-2 gene was found to be a pseudo-gene in rodents, this receptor is functional in humans, monkeys, ferrets and dogs (An et al., 2001; Tan et al., 2002).

Neurons that synthesize MCH are located mainly in the lateral hypothalamus and incerto-hypothalamic area, and project throughout the central nervous system (CNS) (Bittencourt et al., 1992; Mouri et al., 1993; Torterolo et al., 2006). They

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Abbreviations: CNScentral nervous system; CSFcerebrospinal fluid; DRdorsal raphe nucleus; EEGelectroencephalogram; EMGelectromyogram; LDTlaterodorsal tegmental nuclei; MCHmelanin-concentrating hormone; MCHR-1MCH receptor type 1; MCHR-2MCH receptor type 2; NPOnucleus pontis oralis; PPTpedunculopontine tegmental nuclei; REMrapid eye movements; SWSslow wave sleep

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send relatively dense projections to specific areas of the brainstem that have been involved in sleep and waking functions such as the dorsal raphe nucleus (DR), the locus coeruleus, the ventral periaqueductal gray and the laterodorsal and pedunculopontine tegmental nuclei (LDT and PPT); high densities of MCHR-1 are also present in these areas (Bittencourt et al., 1992; Hervieu et al., 2000; Kilduff and De Lecea, 2001; Saito et al., 2001; Jones, 2005; Vanini et al., 2007).

MCH has been involved in the central regulation of feeding and energy homeostasis (Nahon, 2006; Saito and Nagasaki, 2008). However, based upon the expression of the immediate-early gene *c-fos*, which is used as a marker of neuronal activity, recent reports have shown that MCHergic neurons in the rat are active during sleep, predominantly during rapid eye movement (REM) sleep (Verret et al., 2003; Modirrousta et al., 2005; Hanriot et al., 2007). In addition, there is a large increase in REM sleep after the intraventricular microinjections of MCH; consequently, it has been suggested that MCH plays an important role in the control of REM sleep (Verret et al., 2003). It was recently demonstrated in rats, that systemically applied MCHR-1 antagonists results in a dose-dependent decrease in slow wave sleep (SWS) and REM sleep, together with an equivalent increase in wakefulness (Ahnaou et al., 2008). In accordance with the preceding results, MCH knock-out mice sleep less than wild-type animals, and in response to fasting exhibited hyperactivity and an exaggerated decrease in REM sleep (Willie et al., 2008). However, these results are difficult to reconcile with the finding that MCHR-1 knock-out mice present larger amounts of REM sleep in comparison to wild type animals (Adamantidis et al., 2008).

In the cat, studies that employed Fos immunoreactivity did not replicate previous findings that were obtained in rats (Torterolo et al., 2006). However, microinjections of MCH into the nucleus pontis oralis (NPO), an executive area for REM sleep generation, result in an increment in REM sleep (Torterolo et al., in press).

Since the DR is strongly innervated by the MCHergic system and plays a key role in waking-related activities (Jacobs and Azmitia, 1992; Dringenberg and Vanderwolf, 1998; Jacobs and Fornal, 1999; Monti and Monti, 2000), in the present study we explored the effects of microinjections of MCH into the DR on wakefulness and sleep in the rat. The administration of MCH into the DR produced a large increase in the time spent in REM sleep as well as a modest increase in SWS accompanied by a decrease in wakefulness and light sleep. This results support the hypothesis that MCHergic neurons promote the sleep states by modulating the activity of the DR neurons.

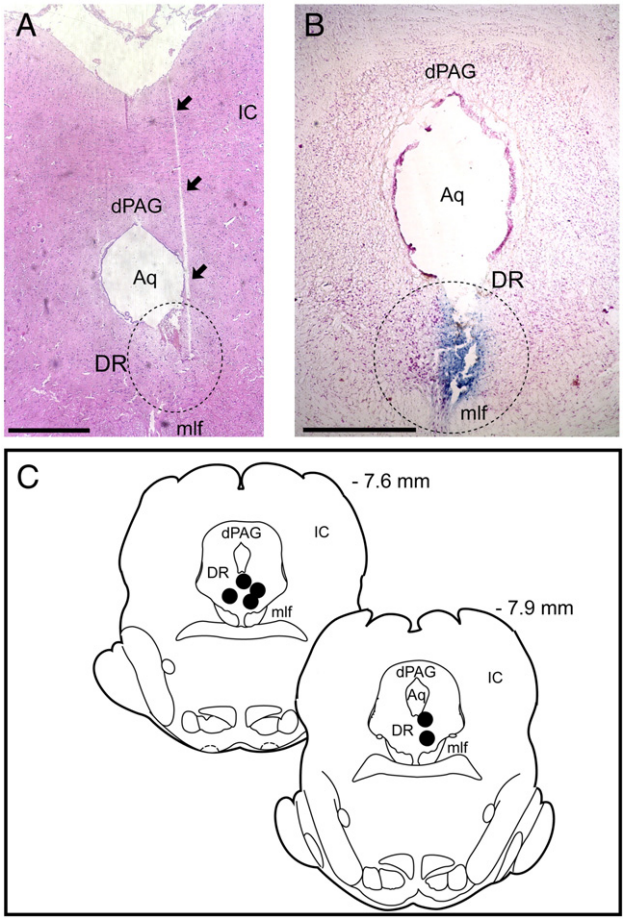

Fig. 1 – Microinjections were localized in the dorsal raphe nucleus. (A) Photomicrograph of a coronal section of a representative rat brain at the level of the DR. The microinjection site is recognizable by the cannula track and lesion. This 5 μm thick section was stained with hematoxilin and eosin. (B) Microinjection site localized by Pontamine Sky Blue microinjection. The blue stain is readily observed in the DR. This 40 μm thick coronal section was counterstained with Pyronin-y. (C) Schematic showing the location of the microinjection sites. Each spot corresponds approximately to the center of the cannula lesion or of the dye deposit, and its position was adjusted to the prototypical coronal sections of Paxinos and Watson (2005). Aq, aqueduct; IC, inferior colliculus; dPAG, dorsal periaqueductal gray; DR, dorsal raphe nucleus; mlf, medial longitudinal fascicle. Calibration bar, 0.5 mm.

2. Results

MCH (50 and 100 ng) and vehicle (as a control) were microinjected into the DR in six rats. In all animals, the tip of the cannula was localized in the DR. Photomicrographs showing examples of the cannula track and lesion, the dye deposit after the end of the microinjections procedures, as well as a schematic of the locations of the microinjections sites are presented in Fig. 1.

The results obtained after recording sessions of 6 h following microinjections of MCH or vehicle into the DR are summarized in Table 1. Compared with the control vehicle, MCH 100 ng significantly modified the values corresponding to a number of sleep variables. Accordingly, MCH 100 ng increased significantly REM sleep time from a control value of 20.8±3.4 min (5.8% of the total recording time) to 35.5±2.5 min (9.7% of the total recording time). The increment of REM sleep time amounted to 70.7% of the control value, and was related to a greater number of REM episodes (Table 1). The increased number of REM episodes after MCH 100 ng administration is