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Research Report

Inactivation of median preoptic nucleus causes c-Fos expression in hypocretin- and serotonin-containing neurons in anesthetized rat

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ABSTRACT

The median preoptic nucleus (MnPN) of the hypothalamus contains sleep-active neurons including sleep-active GABAergic neurons and is involved in the regulation of nonREM/REM sleep. The hypocretinergic (HCRT) neurons of the perifornical-lateral hypothalamic area (PF-LHA) and serotonergic (5-HT) neurons of the dorsal raphe nucleus (DRN) are mostly active during waking and have been implicated in the regulation of arousal. MnPN GABAergic neurons project to the PF-LHA and DRN. It is hypothesized that MnPN promotes sleep by inhibiting multiple arousal systems including HCRT and other wake-active neurons within the PF-LHA and 5-HT neurons in the DRN. We examined the effects of inactivation of MnPN neurons by locally microinjecting 0.2 μl of 1 mM or 10 mM solutions of a $GABA_A$ receptor agonist, muscimol, into the MnPN on Fos expression (Fos-IR) in the PF-LHA neurons including HCRT neurons and 5-HT neurons in the DRN in anesthetized rats. Compared to artificial cerebrospinal fluid control, microinjection of muscimol into the MnPN resulted in significantly higher percentages of HCRT and non-HCRT neurons in the PF-LHA and 5-HT neurons in the DRN that exhibited Fos-IR. The percentage of melanin-concentrating hormone (MCH)+/Fos+ neurons in the PF-LHA did not change after muscimol treatments. These results support a hypothesis that the activation of MnPN neurons contributes to the suppression of wake-promoting systems including HCRT and other unidentified neurons in the PF-LHA and 5-HT neurons in the DRN. These results also suggest that MCH neurons may not be under MnPN inhibitory control. These findings are consistent with a hypothesized role of MnPN in sleep regulation.

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Abbreviations: aCSF, Artificial cerebrospinal fluid; DRN, Dorsal raphe nucleus; Fos-IR, c-fos protein immunoreactivity; GABA, Gamma-aminobutyric acid; GAD, Glutamic acid decarboxylase, a marker for GABAergic neurons; HCRT, Hypocretin; MCH, Melanin-concentrating hormone; MnPN, Median preoptic nucleus; PF-LHA, Perifornical-lateral hypothalamic area; POA, Preoptic area; TBS, Tris buffer saline; VLPO, Ventrolateral preoptic area; 5-HT, serotonin (5-hydroxytryptamine)

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1. Introduction

Various lines of evidence support that the median preoptic nucleus (MnPN) of the hypothalamus is involved in the regulation of non-rapid eye movement (non-REM) and REM sleep (Datta and Maclean, 2007; McGinty and Szymusiak, 2003; Saper et al., 2005). Many MnPN neurons exhibit sleep-associated discharge activity, i.e., the lowest discharge during waking, which increases with the onset of sleep, and the highest discharge during non-REM and REM sleep (Suntsova et al., 2002). The number of MnPN neurons exhibiting c-Fos protein immunoreactivity (Fos-IR), a marker of neuronal activation, increases with the amount of preceding sleep and with increasing homeostatic pressure for REM sleep (Gong et al., 2000; Gvilia et al., 2006a,b).

The perifornical-lateral hypothalamic area (PF-LHA) has been implicated in the promotion and/or maintenance of behavioral arousal (Jones, 2005; Kilduff and Peyron, 2000; Sakurai, 2007; Salin-Pascual et al., 2001; Siegel, 2004). Evidence suggests that hypocretin (HCRT), also called as orexin, containing neurons within PF-LHA are wake-active, i.e., exhibit wake-associated discharge as well as Fos-IR (Espana et al., 2003; Estabrooke et al., 2001; Lee et al., 2005; Mileykovskiy et al., 2005). A loss of HCRT neurons is associated with the pathogenesis of the disease narcolepsy, characterized by excessive sleepiness (Peyron et al., 2000; Thannickal et al., 2000). The HCRT level in cerebrospinal fluid is higher during active-waking and applications of HCRT into various brain regions, e.g., preoptic area, basal forebrain, tuberomammillary nucleus and locus coeruleus, promote waking (Bourgin et al., 2000; Espana et al., 2001; Hagan et al., 1999; Huang et al., 2001; Kiyashchenko et al., 2002; Methippara et al., 2000; Thakkar et al., 2001). On the other hand, the PF-LHA contains other cell types including melanin-concentrating hormone (MCH) and GABAergic neurons, that have been implicated in the regulation of sleep (Kumar et al., 2005; Modirrousta et al., 2005; Verret et al., 2003).

Evidence supports that serotonergic (5-HT) system of the dorsal raphe nucleus (DRN) constitutes an important component of the brainstem arousal system (McGinty and Szymusiak, 2003; Ursin, 2002). Putative 5-HT neurons exhibit wake-related discharge, become less active during non-REM and nearly cease firing during REM sleep (Guzman-Marin et al., 2000; McGinty and Harper, 1976; Trulson and Jacobs, 1983). The firing rates of DRN 5-HT neurons show a strong positive relationship with tonic level of motor activity and 5-HT levels in the DRN as well as at projection sites parallel the 5-HT neuronal discharge, i.e., are higher during waking and lower during sleep, particularly REM sleep (Auerbach et al., 1989; Portas and McCarley, 1994).

Although it is well documented that most of the PF-LHA neurons including HCRT neurons as well as 5-HT neurons in the DRN are active during waking and quiescent in non-REM/REM sleep, the anatomical regions or factors that cause the suppression/inhibition of those neurons during sleep remains poorly understood (Alam et al., 2002; Koyama et al., 2003; Kumar et al., 2007; Lee et al., 2005; McGinty and Harper, 1976; Mileykovskiy et al., 2005). Recent anatomical, immunohistochemical, and electrophysiological studies suggest that PF-LHA and DRN neurons may be under inhibitory control of the MnPN sleep-promoting system. For example, a) MnPN sleep-active neurons are predominantly GABAergic and exhibit the state-dependent activities that are reciprocal to the PF-LHA and 5-HT neurons (Alam et al., 2002; Gong et al., 2004; McGinty and Harper, 1976; Suntsova et al., 2002; b) MnPN neurons constitute a source of afferents to PF-LHA and DRN (Uschakov et al., 2007; c) a subset of MnPN neurons projecting to PF-LHA are GABAergic and express Fos-IR during sleep (Gong et al., 2004; Sakurai et al., 2005; Uschakov et al., 2006); and d) MnPN electrical and chemical stimulations suppress whereas its chemical inactivation increases the discharge activity of the majority of PF-LHA neurons (Suntsova et al., 2007). However, the neurotransmitter phenotypes of the PF-LHA neurons that are under MnPN inhibitory control are not known. It is also not known if 5-HT neurons are, in part, under MnPN inhibitory control.

In this study we determined the phenotypes of neurons exhibiting Fos-IR in the PF-LHA and in the DRN resulting from inactivation of MnPN neurons by local microinjection of a $GABA_A$ receptor agonist, muscimol, in anesthetized rats. We found that percentages of HCRT neurons in the PF-LHA and 5-HT neurons in the DRN exhibiting Fos-IR increased after inactivation of MnPN neurons by local microinjection of muscimol, whereas MCH neurons remained largely unaffected. These findings seem consistent with the hypothesis that MnPN promotes sleep by inhibiting multiple arousal systems including HCRT and other wake-active neurons within the PF-LHA and 5-HT neurons within the DRN.

2. Results

2.1. *Location of the microinjection sites*

Fig. 1 shows a representative histological section (A) as well as reconstruction diagrams through MnPN (B and C) showing the locations of the microinjection sites. There was typical tissue damage at the site of injections. The placements of injections were within and in close proximity of MnPN encompassing areas that constitute sources of afferents to PF-LHA and DRN and where clusters of sleep-active neurons including sleep-active GABAergic neurons are localized (Gong et al., 2004, 2000; Suntsova et al., 2002).

2.2. *Effects of muscimol microinjection into MnPN on Fos-IR in PF-LHA neurons*

2.2.1. *HCRT neurons*

The effects of artificial cerebrospinal fluid (aCSF) and two doses of muscimol microinjections into the MnPN on HCRT+ neurons exhibiting Fos-IR in anesthetized rats are shown in Figs. 2 and 3 and Table 1. The number of HCRT+ neurons in the PF-LHA as a whole or its three sub-regions, i.e., perifornical area (grid-1), medial area (grid-2) and lateral area (grid-3) in both aCSF and muscimol treated rats were comparable (Table 1, Figs. 1–3). In rats injected with aCSF, small percentages of HCRT+ neurons expressing Fos-IR were observed in the PF-LHA as a whole (9±3%) and each sub-region studied. However, as compared to aCSF treatment, the percentages of HCRT+/Fos+ neurons increased significantly in the PF-LHA as a whole (47±10%) as well as each sub-region after 10 mM muscimol