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Research Report

Distribution of hypothalamic neurons with orexin (hypocretin) or melanin concentrating hormone (MCH) immunoreactivity and multisynaptic connections with diaphragm motoneurons

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ABSTRACT

Prior work showed that neurons in the lateral, dorsal, and perifornical regions of the tuberal and mammillary levels of the hypothalamus participate in the control of breathing. The same areas also contain large numbers of neurons that produce either orexins (hypocretins) or melanin concentrating hormone (MCH). These peptides have been implicated in regulating energy balance and physiological changes that occur in transitions between sleep and wakefulness, amongst other functions. The goal of this study was to determine if hypothalamic neurons involved in respiratory control, which were identified in cats by the retrograde transneuronal transport of rabies virus from the diaphragm, were immunopositive for either orexin-A or MCH. In animals with limited rabies infection of the hypothalamus (<10 infected cells/section), where the neurons with the most direct influences on diaphragm motoneurons were presumably labeled, a large fraction (28–75%) of the infected hypothalamic neurons contained orexin-A. In the same cases, 6–33% of rabies-infected hypothalamic cells contained MCH. However, in animals with more extensive infection, where rabies had presumably passed transneuronally through more synapses, the fraction of infected cells that contained orexin-A was lower. The findings from these experiments thus support the notion that hypothalamic influences on breathing are substantially mediated through orexins or MCH.

1. Introduction

Two peptides with extensive overlap in their amino acid sequences are localized to neurons in the lateral, dorsal and perifornical hypothalamic regions of mammalian species (Abrahamson and Moore, 2001; de Lecea et al., 1998; Peyron et al., 1998; Zhang et al., 2001). These peptides were initially named hypocretins by one group due to their structural similarity to the gastrointestinal hormone secretin (de Lecea et al., 1998), and orexins by another because they were implicated in regulating appetite (Sakurai et al., 1998). In cats, the two orexin peptides (orexin-A and orexin-B) are co-expressed

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Abbreviation: MCH, Melanin concentrating hormone

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in the same hypothalamic neurons (Zhang et al., 2002). Orexinergic neurons project widely throughout the nervous system (Peyron et al., 1998), although the heaviest concentrations of terminations in felines are present in the nucleus raphe dorsalis, the laterodorsal tegmental nucleus, and the locus coeruleus (Zhang et al., 2004). This pattern of connections is consistent with a now well-established role of the orexins in modulating alterations in physiological activity that occur between sleep and wakefulness (Bourgin et al., 2000; Chemelli et al., 1999; Gerashchenko et al., 2001; Lin et al., 1999; Taheri et al., 2002; Willie et al., 2001; Xi et al., 2002; Zeitzer et al., 2003). The peptides have also been shown to participate in generating arousal, addiction, goal-oriented behaviors, and energy homeostasis, as discussed in a number of recent reviews (Aston-Jones et al., 2009; de Lecea et al., 2002; Martynska et al., 2005; Siegel, 2004; Sutcliffe and de Lecea, 2000, 2002; Taylor and Samson, 2003; Winsky-Sommerer et al., 2003; Zhang et al., 2006).

It has also long been appreciated that lesions of the lateral hypothalamic areas containing orexinergic neurons depress the rate and depth of respiration (Redgate and Gellhorn, 1958). Lateral ventricular administration of orexin elicits increases in both tidal volume and respiratory frequency (Zhang et al., 2005). In addition, knockout mice lacking orexin have attenuated hypercapnic respiratory responses while awake, but not while asleep (Deng et al., 2007; Nakamura et al., 2007), and lack the capacity for respiratory long-term facilitation following hypoxic stimuli (Terada et al., 2008). Orexinergic neurons apparently influence breathing via connections with multiple brainstem regions involved in respiratory control. Orexin receptors are present on neurons in the pre-Bötzinger region of the ventral respiratory group and the phrenic motor nucleus, and microperfusion of orexin at either site produces a dose-dependent increase in diaphragm electromyographic activity (Young et al., 2005). The firing rate of neurons in the retrotrapezoid nucleus (a central chemosensory area) is also modulated by orexinergic inputs (Dias et al., 2009; Fortuna et al., 2009). Stimulation of the perifornical hypothalamic region increases the activity of retrotrapezoid nucleus neurons (Fortuna et al., 2009), and blockade of orexin receptors in this region inhibits the ventilatory response to hypercapnia during wakefulness, but less so during sleep (Dias et al., 2009).

Further evidence that orexinergic neurons participate in the control of breathing comes from our experiments where the transneuronal tracer rabies virus was injected into the diaphragm of cats (Lois et al., 2009). The hypothalamic neurons infected following the multisynaptic passage of virus from diaphragm motoneurons were located in the perifornical region, where orexinergic cells are concentrated. A caveat is that neurons that produce other peptides, particularly melanin concentrating hormone (MCH) (Abrahamson and Moore, 2001; Bayer et al., 2002; Bittencourt et al., 1992; Nahon et al., 1989; Torterolo et al., 2006), are also found in the same region. Although MCH and the orexins are present in separate populations of neurons (Bayer et al., 2002; Broberger et al., 1998; Torterolo et al., 2006), these cells may have synaptic interconnections (Bayer et al., 2002; Guan et al., 2002; Torterolo et al., 2006). It is thus not surprising that MCH-containing neurons have been attributed similar physiological roles as orexinergic cells, including regulation of sleep/wake cycles and energy homeostasis (Georgescu et al., 2005; Goutagny et al., 2005; Modirrousta et al., 2005; Shimada et al., 1998; Tritos and Maratos-Flier, 1999; Verret et al., 2003). Nonetheless, no prior studies have directly implicated MCH-containing neurons in the regulation of breathing.

The goal of the present study was to estimate the fraction of hypothalamic neurons that provide polysynaptic inputs to phrenic motoneurons and contain either orexins or MCH. As in our prior studies (Lois et al., 2009; Rice et al., 2009), neurons that polysynaptically regulate breathing were identified by the transneuronal transport of rabies virus from the diaphragm. Dual-labeling immunohistochemistry was performed to identify rabies-infected cells in the hypothalamus that were immunopositive for either orexin-A or MCH. We tested the hypothesis that a majority of hypothalamic neurons that regulate diaphragm activity are orexinergic.

2. Results

Orexin-A immunopositive neurons were present in the lateral, dorsal and perifornical hypothalamus, and were concentrated in the tuberal and tuberomamillary regions. Orexin-A immunopositive cells were present in each hypothalamic section; the median number of labeled cells/section was 159. When the analysis was limited to sections through the caudal half of the hypothalamus, where orexinergic neurons were most heavily clustered, 267±41 (median of 206) cells/section contained the peptide. The same hypothalamic areas also contained comparable numbers of neurons that were immunopositive for MCH. As such, our observations of the locations of hypothalamic neurons containing orexin-A and MCH were similar to those previously documented in felines (Torterolo et al., 2006; Zhang et al., 2001). Micrographs of neurons that were immunopositive for orexin-A and MCH are illustrated in Figs. 1 and 2, respectively. Plots of the locations of neurons containing orexin-A and MCH in representative sections from two animals (C37 and C39) are shown in Fig. 3.

Cells infected by the transneuronal transport of rabies virus from the diaphragm were present in the same hypothalamic areas containing neurons that were immunopositive for orexin-A and MCH, as indicated in the micrographs in Figs. 1, 2 and the plots of neuronal locations provided in Fig. 3. The distribution of rabies-infected neurons in the brain and spinal cord of four of the animals (C36, C37, C39, and C51) was described in detail in previous publications (Lois et al., 2009; Rice et al., 2009). These studies also reported a variety of controls that have been performed to demonstrate that this pattern of rabies infectivity is due to transport of virus from the diaphragm. Tissue from two additional animals (C93 and C94) was added to this study to increase the number of cases with infected neurons in the diencephalon following rabies injections into the diaphragm. Table 1 indicates the number of rabies-infected neurons in hypothalamic sections from each animal. Only a few infected hypothalamic neurons were present in each brain section of animals C39, C51, and C94, but such cells were somewhat more prevalent in animal C93. Nonetheless, the locations of infected neurons in the brainstem and spinal cord of both animals C93 and C94 were similar to those previously described for animals C39 and C51 (Lois