The Role of the Hypothalamic Neuropeptides Hypocretin/Orexin in the Sleep-Wake Cycle

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Abstract

The novel neuropeptides hypocretin/orexin have recently been located on the lateral hypothalamus cells. This system has been linked to the regulation of both feeding and sleep, and recent studies have found an association between a defect in these neuropeptides and narcolepsy. We conducted a MEDLINE review of all the articles published since the discovery of hypocretin/orexin peptides, narrowing the field to the relationship between these neuropeptides and sleep. The finding of a deletion in the transcription of the hypocretin receptor 2 gene in narcoleptic Doberman pinschers and the development of a knockout of the hypocretin gene in mice pointed to the relevance of this system in the sleep-wake cycle. We provide further evidence of the role of the hypocretin/orexin system in narcolepsy and in sleep regulation and present an integrative model of the pathophysiology of narcolepsy. The discovery of the link between these peptides and narcolepsy opens new avenues to both the understanding of sleep mechanisms and therapeutic implications for sleep disorders.

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Hypothalamic structures have became relevant for understanding chronobiology as well as sleep-regulatory mechanisms. Early research in this area was launched by Nauta's finding [1] that lesions in the preoptic area of hypothalamus produced insomnia, which led him to conclude that there was a sleep center in this area. Before that, von Economo [2] contended that posterior hypothalamus contained a "wake center," based on his observations that patients suffering from viral encephalitis were excessively sleepy and that postmortem analysis revealed damage in the posterior hypothalamus.

Sleep active neurons were found in the preoptic area and adjacent basal forebrain in rats, cats and rabbits [3]. These neurons begin to fire during drowsiness, with peak activity occurring during non-rapid eye movement sleep. Sleep active neurons were identified using c-Fos and were located in the ventral lateral preoptic region [4]. The VLPO Fos-ir (c-Fosimmune reactive) cells project to the histaminergic cells in the tuberomammillary area and the brainstem monoaminergic neuron group. The VLPO cells contain GABA and galanin and inhibit the wake-active cell population to which they project. Electrophysiology studies have shown that VLPO cells are sleep active [5], and *in vitro* slice studies have shown that these cells are inhibited by acetylcholine, serotonin and norepinephrine, all of them wake-active neurotransmitters [6]. The finding that excitoxic lesions of the VLPO produce insomnia is further evidence of the role of this area in sleep [7].

Histamine has been linked to waking mechanisms, given the well-known sedative action of antihistaminergic drugs. Intraventricular administration of histamine was found to produce an arousing effect [8]. Neurotoxic lesions, selective to cell bodies, have been shown to produce a decrease in wakefulness and an increase in both slow wave and paradoxical sleep [6].

Hypocretins/orexins

Two research groups independently described the same type of neuropeptides. Sakurai et al. [9] described the orexins and orexin receptors as a family of neuropeptides and G protein-coupled receptors, respectively, that regulated feeding behavior. At the same time De Lecea et al. [10] found what they named hypocretins, two neuropeptides with excitatory effects. Hypocretin and orexin peptides were the same molecules but no agreement has been reached as to which of the two names will be used in the future.

Pre-prohypocretin is cleaved by proteolytic processing into two smaller peptides: hypocretin-1/orexin-A (33 amino acids) and hypocretin-2/orexin-B (28 amino acids). The distribution of hypocretin-containing neurons has been determined in the mouse [11], cat [12], rat [9], and in humans [10].

Two hypocretin receptors have been identified and the distribution of the receptor mRNA levels has been determined [13]. Orexin-1 (hypocretin-1) receptor mRNA is more abundant in ventromedial hypothalamic nucleus, hippocampal formation, dorsal raphe and locus coeruleus. In the rat, OX2R mRNA is mainly expressed in cerebral cortex, nucleus accumbens, subthalamic and paraventricular thalamic nuclei, and posterior pretectal nuclei [13,14]. The locus coeruleus receives the heaviest projections of orexin-containing terminals, and intraventricular administration of hypocretins excites locus coeruleus neurons [15,16]. Hypocretin terminals are also found in areas implicated in wakefulness such as locus coeruleus, tuberomammillary

VLPO = ventral lateral preoptic region

nucleus, the dorsal raphe, and basal forebrain [11]. Because of these projections to neuronal populations implicated in wakefulness, it is believed that orexin promotes wakefulness [11].

Hypocretins and narcolepsy

Narcolepsy is a lifelong neurological disorder characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations and sleep paralysis. Narcolepsy has also been reported to occur in animals and has been most intensively studied in dogs [17]. Although familial cases of narcolepsy have been reported, most human occurrences are sporadic, and the disorder is generally believed to be multigenic and environmentally influenced [18]. One predisposing genetic factor is a specific HLA-DQ allele, HLA-DQB1*62 [19]. Because of the tight HLA association the disorder in humans has been suggested to be autoimmune in nature, however all attempts to verify this hypothesis have failed [19]. In Doberman pinschers, the disorder is transmitted as a single autosomal recessive trait with full penetrance, canarc-1 [20]. The cloning of the canine narcolepsy gene led the Stanford group [21] to identify a mutation in the gene encoding the receptor for hypocretin-2 or orexin-2 receptor, which they believed to be the cause of canine narcolepsy.

Almost simultaneously, Chemelli and co-workers [22] created a constitutive knockout mouse that lacked the orexin gene. These orexin knockout mice (-/-) had increased REM sleep as well as cataplexy-like episodes that were entered directly from states of active movement. The hypothesis that hypocretin suppressed the appearance of REM sleep, whereas the absence or reduction of these neuropeptides or receptors enhanced REM sleep and REM sleep-related phenomena, was suggested by these two early works. Takkar et al. [23] perfused orexin type II receptor antisense in the pontine reticular formation in rats, and observed that 10–24 hours later, REM sleep increased two to threefold during both the light and the dark period. On the other hand [24], the systemic administration of hypocretin-1 reduced cataplexy and improved the sleep and waking cycle in narcoleptic dogs. The sleep changes were longer waking periods and a decrease in REM sleep, but no change in non-REM sleep [24]. Also, the administration of orexin-A into the lateral preoptic area revealed that compared to saline vehicle control, orexin-A induced arousal and REM sleep suppression, but brain temperature was not differentially affected [submitted for publication].

To specifically test the hypothesis that lesions of the lateral hypothalamus, which result in loss of hypocretin-containing neurons, produce symptoms of narcolepsy, Shiromani et al. created a neurotoxin by conjugating the ribosomal inactivating protein saporin to the hypocretin/orexin receptor-binding ligand hypocretin-2/orexin-B [submitted for publication]. Since the hypocretin-containing neurons have an autoreceptor [15], the conjugate produced lesions in hypocretin neurons. It was also found that when the neurotoxin was administered to the lateral hypothalamus, the hypocretin immunoreactive neurons were lesioned and the rats had sleep fragmentation, excessive sleepiness, increase in REM sleep, and sleep-onset REM sleep periods – symptoms characteristic of narcolepsy in humans, dogs and hypocretin null mice. This finding demonstrates that a defect in the lateral hypothalamus, which also involves the hypocretin neurons, produces narcoleptic-like sleep behavior in rats.

A malfunction of the hypocretin/orexin system has been observed in humans with narcolepsy. In seven of nine people with narcolepsy hypocretin levels in the cerebral spinal fluid were undetected. *In situ* hybridization for hypocretin receptors and radio immunoanalysis in six narcolepetic brains demonstrated a loss of hypocretin signal in perifornical area, and the peptides were undetected; there were no signs of gliosis or signs of inflammation in any of the human brains examined. Moreover, one hypocretin mutation, impairing peptide trafficking and processing, was found in a single case with early-onset narcolepsy [27].

Conclusions

The way in which an integrative model can link hypocretin gene defects to the symptoms of narcolepsy is in preliminary stages. Although more work is needed in this field, the identification of hypocretins/orexins is a cornerstone in the pathophysiology of narcolepsy, which will open the way for new approaches to understanding its neurobiology and devising appropriate therapy.

REM = rapid eye movement

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