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Orexin deficiency and narcolepsy

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Orexin deficiency results in the sleep disorder narcolepsy in many mammalian species, including mice, dogs, and humans, suggesting that the orexin system is particularly important for normal regulation of sleep/wakefulness states, and especially for maintenance of wakefulness. This review discusses animal models of narcolepsy; the contribution of each orexin receptor subtype to the narcoleptic phenotypes; and the etiology of orexin neuronal death. It also raises the possibility of novel therapies targeting the orexin system for sleep disorders including insomia and narcolepsy–cataplexy.

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Introduction

A series of studies have suggested that loss of the hypothalamic neurons producing the orexin (hypocretin) neuropeptides causes narcolepsy in humans and other mammalian species, highlighting roles of this neuropeptide in the regulation of sleep and wakefulness [1]. The deficiency of orexin signaling in narcolepsy–cataplexy unequivocally shows that this neuropeptide system plays a physiologically essential role in the regulation of sleep and wakefulness, especially in the maintenance of long, consolidated waking periods. This chapter discusses the relationship between orexin-deficiency and narcolepsy; why orexin deficiency causes narcolepsy; and the therapeutic potential of drugs that target orexin receptors for treating insomnia, narcolepsy, and other sleep disorders.

What is narcolepsy?

Narcolepsy is a debilitating neurological disorder, characterized by instability of sleep/wakefulness states and pathological intrusions of REM sleep-related events into wakefulness. It affects approximately 1 in 2000 individuals in the United States [2]. Males and females are equally affected. The onset of the disease usually occurs during adolescence, suggesting that narcolepsy is an acquired, not an innate, condition. However, although most cases of narcolepsy occur sporadically, familial clustering may be observed; the risk of a first-degree relative of a narcoleptic developing narcolepsy is 10–40 times higher than in the general population [3]. The development of the disease seems to involve both environmental and genetic factors. 25–31% of monozygotic twins were reported to be concordant for narcolepsy [2].

The most disruptive symptom of the disorder is excessive daytime sleepiness, or daytime hypersomnia (an insurmountable urge to sleep), which often results in falling asleep at inappropriate times and situations ('sleep attacks'). Patients with narcolepsy have a three-fold increased risk of motor vehicle accidents from lapses in attention, lack of alertness, and dozing off. The latency for rapid eye movement (REM) sleep is markedly reduced in narcolepsy patients, and REM sleep is sometimes observed shortly after sleep onset ('sleep-onset REM periods'). Nocturnal sleep is often disturbed by sleep fragmentation and premature awakenings. Other symptoms include hypnagogic hallucinations, vivid dreaming, and sleep paralysis which occurs as patients fall asleep or upon awakening.

Narcolepsy patients often suffer from attacks of 'cataplexy' — sudden episodes of muscle weakness, ranging from facial weakness and slurred speech to complete collapse from widespread weakness. Cataplexy is usually triggered by strong emotional stimuli. Unlike sleep attacks, consciousness is preserved during cataplexy. In the International Classification of Sleep Disorders, narcolepsy accompanied by cataplexy is referred to as 'narcolepsy with cataplexy', while that without cataplexy is termed 'narcolepsy without cataplexy' [4].

Dog and rodent models of narcolepsy

Animal models first suggested the involvement of orexin-dysfunction in narcolepsy. Using a forward genetics approach, Mignot *et al.* found that dogs with a mutation in the orexin 2 receptor are remarkably similar to human narcolepsy patients [5••]. As in human narcolepsy, narcoleptic dogs exhibit cataplexy (elicited by the presentation of food), sleepiness (i.e. reduced sleep latency), and SOREMPs [6]. These findings suggested that loss of orexin-2 receptor-mediated signaling can produce a narcolepsy phenotype.

Mouse models also showed a relationship between narcolepsy and orexin system abnormalities (Table 2). At

first, Yanagisawa's group found that *Orexin*$^{-/-}$ mice showed a phenotype remarkably similar to human narcolepsy [7••]. Subsequently, orexin neuron-ablated (*orexin/ataxin-3*-transgenic) mice or *Oxr-1*$^{-/-}$; *Oxr-2*$^{-/-}$ (double-receptor-deficient) mice were shown to have very similar phenotypes that have strong parallels to the human narcolepsy with behavioral arrests very similar to cataplexy, direct transitions from wakefulness to REM sleep, and highly fragmented sleep–wake cycles [8•,9,10], all of which are important features of narcolepsy. *Oxr-2*$^{-/-}$ mice also show a narcolepsy phenotype, though it is milder than that of *orexin*$^{-/-}$ mice, orexin neuron-ablated (*orexin/ataxin-3*-transgenic) mice, *Oxr-1*$^{-/-}$; *Oxr-2*$^{-/-}$ mice [9].

Human narcolepsy and orexin deficiency

The link between orexin dysfunction and narcolepsy has been subsequently confirmed and established by studies on human narcolepsy patients. First, nine human narcolepsy patients were shown to have very low levels of orexin A in their cerebrospinal fluid (CSF) as compared with healthy controls [11••]. Postmortem brain studies of human narcolepsy patients subsequently showed no detectable levels of orexin peptides in the cortex and pons, in which orexinergic projections are normally found (Figure 1a), and an 80–100% reduction in the number of neurons containing detectable *prepro-orexin* mRNA or orexin-like immunoreactivity in the hypothalamus [12•,13•] (Figure 1b).

Approximately 90% of patients with narcolepsy with cataplexy have decreased orexin A levels in CSF [14] (Figure 1a). Accordingly, a low CSF level of orexin A (less than 110 pg/ml) is now one of the diagnostic criteria for narcolepsy–cataplexy according to the 2nd edition of the International Classification of Sleep Disorders (ICSD-2) [4]. Especially, narcolepsy with cataplexy is thought to be more closely related to orexin deficiency as compared with narcolepsy without cataplexy.

Because of its strong association with certain human leukocyte antigen (HLA) alleles [15], it has long been speculated that narcolepsy results from an autoimmune-mediated mechanism. Recently, Tribbles homolog 2 (Trib2) was reported as a candidate antigen involved in the destruction of orexin neurons [16]. Trib2 was shown to be abundantly expressed in orexin neurons, and levels of Trib2-specific antibodies were much higher in patients with narcolepsy, especially shortly after the disease onset, although it is still unknown if Trib2-specific antibodies are directly involved in cell death, or if the antibody production is a consequence of cell damage by other unknown mechanisms [17].

Recent large-scale genome wide association studies (GWAS) showed that susceptibility to narcolepsy is associated with single nucleotide polymorphisms (SNPs) in the T-cell receptor alpha gene locus [18]. The SNPs are located between *carnitine palmitoyl-transferase 1B* and *choline kinase beta* [19] and SNPs of *purinergic receptor P2Y11* [20]. These genes may be involved in either cell death of orexin neurons or enhancing narcolepsy symptoms. Of note, the association with the T-cell receptor alpha locus might be important, as the interactions between HLA molecules on antigen presenting cells and T cell receptors on T cells play critical roles in self/non-self discrimination by the immune system [21]. Recently, association between narcolepsy and seasonal streptococcus, H1N1 infections and AS03-adjuvanted pH1N1 influenza vaccination was reported in Northern Europe and China [21]. These observations further suggest the involvement of immunological mechanisms responsible for the loss of orexin-producing neurons.

Each receptor in narcolepsy

Detailed characterization of behavioral, pharmacological, and electrophysiological features of *orexin*$^{-/-}$ and *OX2R*$^{-/-}$ mice showed that these mice exhibited two types of behavioral arrests. One is 'abrupt arrests': a sudden loss of muscle tone during various active behaviors such as grooming and ambulation [9]. Detailed observations of behaviors during EEG/EMG recordings found that abrupt arrests in *orexin*$^{-/-}$ and *OX2R*$^{-/-}$ mice are associated with EEG changes suggestive of unusual direct transitions from wakefulness to REM sleep. The other type is 'gradual arrests', which typically begin during quiet wakefulness and can be easily distinguished from the normal onset of resting behavior by the absence of stereotypic preparation for sleep (e.g. nesting and/or assumption of a curled or hunched posture, with limbs drawn under the body) and the presence of ratchet-like 'nodding' of the head over a period of several seconds, with a transition to a collapsed posture. EEG/EMG correlates of the gradual arrests in both *orexin*$^{-/-}$ and *OX2R*$^{-/-}$ mice resemble transitions from wakefulness to non-REM sleep, suggesting that this type is a counterpart of 'sleep attacks' in human narcolepsy patients.

In accordance with these similarities to clinical narcolepsy symptoms, 'abrupt arrests' in *orexin*$^{-/-}$ mice were suppressed by systemic administration of clomipramine, a tricyclic anti-depressant drug used for the treatment of cataplexy, while administration of caffeine, a psychostimulant used to treat excessive sleepiness in human narcolepsy, tends to slightly increased abrupt arrest frequency. In clear contrast, systemic administration of caffeine dose-dependently suppressed gradual arrests, while administration of clomipramine did not affect the frequency of gradual arrests in both *orexin*$^{-/-}$ and *OX2R*$^{-/-}$ mice. These observations suggest that the abrupt and gradual arrests are the presumptive mouse correlates of cataplexy and sleep attacks in human narcolepsy–cataplexy, respectively.

The International Working Group on Rodent Models of Narcolepsy proposed a consensus definition of murine