Narcolepsy: Pathophysiology and Pharmacology

Seiji Nishino, M.D., Ph.D.

Narcolepsy, which affects 1 in 2000 people in the general population, is characterized by excessive daytime sleepiness (EDS), cataplexy, and other dissociated manifestations of rapid eye movement sleep (hypnagogic hallucinations and sleep paralysis). The disease is currently treated with amphetamine-like central nervous system stimulants (for EDS) and antidepressants (for cataplexy). Some compounds from other classes, such as modafinil (a non-amphetamine wake-promoting compound for EDS) and sodium oxybate (a short-acting sedative for EDS and cataplexy, administered at night), are also employed. The major pathophysiology of human narcolepsy has recently been revealed by the extension of discoveries of narcolepsy genes in animal models: hypocretin/orexin ligand deficiency has been shown in about 90% of human narcolepsy-cataplexy. This finding led directly to the development of new diagnostic tests (i.e., cerebrospinal fluid hypocretin measures). Hypocretin replacement is also likely to be a new therapeutic option for hypocretin-deficient narcolepsy, but is still not available in humans. In this review, the pharmacologic and pathophysiologic aspects of narcolepsy are discussed.

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narcolepsy entered REM sleep more rapidly when falling asleep than those without narcolepsy. This phenomenon is called a reduced REM sleep latency or a sleep onset REM period (SOREMP). Sometimes this transition to REM sleep occurs immediately upon falling asleep, without an entry into NREM sleep. The cataplexy, sleep paralysis, and hypnagogic hallucinations associated with narcolepsy may all result from intrusion of REM sleep into wakefulness. However, it should also be noted that cataplexy is pathognomonic for narcolepsy, whereas SOREMPs, sleep paralysis, and hypnagogic hallucinations often occur in other sleep disorders, such as sleep apnea syndromes, or even in normal populations when their sleep patterns are disturbed.

The next major discovery came in the 1980s, when it was found that many people with narcolepsy have the tissue type human leukocyte antigen (HLA) DR2. High-resolution typing revealed that narcolepsy has the closest association with HLA DQB1*0602, which is found in 95% of narcoleptic patients with cataplexy and 41% of patients with narcolepsy without cataplexy, but only 18% to 35% of the general population. Whereas the significance of this association with immune system antigens is not well understood, it may suggest that autoimmune processes play a role in narcolepsy because many autoimmune diseases exhibit tight associations with HLA haplotypes. There is, however, no strong evidence of inflammatory processes or immune abnormalities associated with narcolepsy, and studies have found neither classical autoantibodies nor an increase in oligoclonal cerebrospinal fluid (CSF) bands in narcoleptics.

In 1998, 2 groups of researchers independently discovered a novel hypothalamic peptide neurotransmitter, named orexin by one group and hypocretin by the other. Hypocretins 1 and 2 are produced exclusively by a group of several thousand neurons localized in the lateral hypothalamus. These neurons project widely to the olfactory bulb, cerebral cortex, thalamus, hypothalamus, and brainstem, and more densely to the locus ceruleus, tuberomammillary nucleus, raphe nucleus, and bulbar reticular formation.

The following year, using positional cloning of a naturally occurring familial canine narcolepsy model, the Stanford researchers identified an autosomal recessive mutation responsible for narcolepsy in dogs. Narcoleptic dogs have cataplexy (primarily elicited by the presentation of food), sleepiness (i.e., reduced sleep latency), and SOREMPs. The authors determined that canine narcolepsy is caused by disruption of the hypocretin (orexin) receptor 2 gene (HCRTR2). This result identified hypocretins as major sleep-modulating neurotransmitters.

Following the discovery of the hypocretin gene–narcolepsy association in dogs, the role of the hypocretin system in human narcolepsy was examined. Systematic screening of mutations in the hypocretin system in patients with cataplexy has so far identified only 1 patient with a mutation in hypocretin-related genes, and this patient was atypical, with very early disease onset (6 months old). Hence, most human cases of narcolepsy are not caused by gene mutations.

Whereas gene mutations were not identified as a major cause of narcolepsy in almost all humans, narcoleptic patients were found to have low levels of hypocretin. Experiments using in situ hybridization, immunohistochemistry, and radioimmunologic assays of peptides in the postmortem brain tissue of narcoleptic patients found undetectable levels of pre-hypocretin RNA, loss of hypocretin peptides, and a selective loss of hypocretin neurons, all representing significant disparities from people without narcolepsy. The loss of hypocretin function did not result from a generalized neuronal dysfunction in these brain regions, because melanin-concentrating hormone neurons that are normally located within the same region as the hypocretin neurons were intact. Together, these results indicated that hypocretin neurons and function were selectively damaged in narcoleptic patients.

The finding of low concentrations of hypocretin in the central nervous system (CNS) is now used to help diagnose narcolepsy, and this can be clinically detected by CSF hypocretin-1 measures (Table 1). CSF hypocretin-1 concentrations lower than 110 ng/L have a high positive predictive value (94%) for narcolepsy with cataplexy. In controls and in individuals with other sleep and neurologic disorders, hypocretin-1 concentrations in the CSF were almost always above 200 ng/L (Figure 1). In rare instances, low CSF hypocretin concentrations in the absence of narcolepsy were indicative of subsets of Guillain-Barre syndrome, brain tumors, vascular diseases, or head trauma.

### Table 1. Diagnostic Criteria for Narcolepsy

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Narcolepsy with cataplexy</th>
<th>Narcolepsy without cataplexy</th>
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<tr>
<td>A. Excessive daytime sleepiness</td>
<td>A. Excessive daytime sleepiness</td>
<td>A. Excessive daytime sleepiness</td>
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<tr>
<td>B. Definite history of cataplexy</td>
<td>B. Typical cataplexy not present</td>
<td>B. Typical cataplexy not present</td>
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<tr>
<td>C. MSLT (mean sleep latency ≤ 8 minutes and 2 or more SOREMPs) required</td>
<td>C. Abnormal MSLT (mean sleep latency ≤ 8 minutes and 2 or more SOREMPs) optional but advised. Alternatively, CSF hypocretin-1 level less than or equal to 110 pg/mL, or one third of mean normal control values</td>
<td>C. Underlying medical or neurological disorder accounts for daytime sleepiness</td>
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</table>

*Adapted with permission from Longstreth et al. Abbreviations: CSF = cerebrospinal fluid, MSLT = Multiple Sleep Latency Test, SOREMP = sleep onset REM period.

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Seiji Nishino
These results, along with other evidence, suggest that hypocretin deficiency causes most cases of narcolepsy with cataplexy, although the cause of the hypocretin cell death is unknown.12 No evidence has also been found indicating a role for an autoimmune reaction in the loss of the hypocretin neurons; however, this does not exclude the possibility that a transient autoimmune reaction restricted to the CNS could have occurred around the disease onset, but disappeared later.12

NEUROBIOLOGY OF NARCOLEPSY

After the discovery of hypocretin, anatomic and electrophysiologic studies demonstrated that the hypocretin system is a major excitatory system with neuronal connections on the monoaminergic (dopamine, norepinephrine, serotonin, and histamine) and cholinergic systems, thereby having a major effect on vigilance states.18–21 Hypocretin neurons project to the olfactory bulb, cerebral cortex, thalamus, hypothalamus, and brainstem (Figure 2). In the brainstem, hypocretin neurons innervate regions that are believed to be important in sleep regulation and/or behavioral activation, including the adrenergic locus ceruleus, dopaminergic ventral tegmental area, and serotonergic raphe nucleus, as well as the cholinergic nuclei and cholinceptive sites such as the laterodorsal tegmentum and pontine reticular formation, respectively.18,22 Hypocretin neurons also innervate important feeding centers in the hypothalamus, such as the arcuate nucleus.18

Figure 1. CSF Hypocretin-1 Concentrations in People With Narcolepsy With and Without Cataplexy, in Those With Idiopathic Hypersomnia, and in Controls* *

*Reprinted with permission from Dauvilliers et al.12 Each point represents the crude concentration of CSF hypocretin-1 in an individual. Cutoffs for normal (>200 ng/L) and low (<110 ng/L) hypocretin-1 concentrations are represented by horizontal lines. Median values are represented as a thick horizontal bar in each group. Abbreviation: CSF = cerebrospinal fluid.

Figure 2. Hypocretin Structure and Location in the Rat Brain*

A. Structures of Mature Hypocretin-1 (orexin-A) and Hypocretin-2 (orexin-B) Peptides

B. Schematic of the Hypocretin (orexin) System

C. Projections of Hypocretin Neurons in the Rat Brain and Relative Abundances of Hcrtr1 and Hcrtr2

*Reprinted with permission from Nishino.3

A. The shaded areas indicate the amino acid identities. Asterisks indicate that human and mouse sequences were deduced from the respective cDNA sequences and not from purified peptides. Hypocretin-1 (orexin-A) and hypocretin-2 (orexin-B) are derived from a common precursor peptide, prepro-hypocretin (prepro-orexin).

B. The actions of hypocretins are mediated via 2 G protein–coupled receptors named hypocretin receptor 1 (Hcrtr1) and hypocretin receptor 2 (Hcrtr2), also known as orexin-1 (OX1R) and orexin-2 (OX2R) receptors, respectively. Hcrtr1 is selective for hypocretin-1, whereas Hcrtr2 is nonselective for both hypocretin-1 and hypocretin-2. Hcrtr1 is coupled exclusively to the Gq subclass of heterotrimeric G proteins, whereas in vitro experiments suggest that Hcrtr2 couples with Gi/Go and/or Gq.

C. Hypocretin-containing neurons project to these previously identified monoaminergic and cholinergic and cholinceptive regions where hypocretin receptors are enriched. Impairments of hypocretin input may, thus, result in cholinergic and monoaminergic imbalance and generation of narcoleptic symptoms. Abbreviations: BF = basal forebrain, DR = dorsal raphe, LC = locus ceruleus, LDT = laterodorsal tegmental nucleus, LHA = lateral hypothalamic area, PPT = pedunculopontine tegmental nucleus, PRF = pontine reticular formation, SN = substantia nigra, TMN = tuberomammillary nucleus, VLPO = ventrolateral preoptic nucleus, VTA = ventral tegmental area.
Recent studies have supported the role of hypocretin neurons in maintaining wakefulness. Hypocretin release in the CSF or in the extracellular fluid fluctuates significantly across 24 hours and is high during the active period and low during the inactive period. Interestingly, the hypocretin levels gradually build up during active periods and are highest at the end of active period.

Hypocretin release is also increased by forced wakefulness (i.e., sleep deprivation). In vivo electrophysiological single unit recordings of hypocretin neurons were made across the sleep-wake cycle in head-fixed rats. Hypocretin neurons fired during active waking, a period when postural muscle tone is highly associated with movement, and had decreased discharge during quiet waking in the absence of movement. The neurons virtually ceased firing during sleep when postural muscle tone was low or absent. Using in vivo single-unit measures in freely behaving rats, another research group reported that hypocretin cells discharged during active waking and had moderate levels of activity during grooming and eating; maximal activity was recorded during exploratory behavior. Together, these findings suggest that hypocretin cells are inactive during sleep, but are activated during emotional and sensorimotor conditions similar to those that trigger cataplexy in narcoleptic animals.

In vitro electrophysiologic studies have provided evidence that hypocretin neurons are regulated by monoamines and acetylcholine as well as peripheral metabolic hormones such as leptin, glucose, and ghrelin. Thus, hypocretin neurons have functional interactions with hypothalamic feeding pathways and monoaminergic/cholinergic centers, and provide a critical link between peripheral energy balance and the central mechanisms that coordinate sleep/wakefulness and motivated behavior, such as food seeking.

On the basis of these and other studies, it has been hypothesized that a buildup or an acute increase of hypocretin levels may counteract the homeostatic sleep propensity that typically increases during the daytime and forced wakefulness. Due to the lack of increase in hypocretin tone, narcoleptic subjects may not be able to stay awake for a prolonged period and do not respond to various alerting stimuli.

PHARMACOLOGY OF NARCOLEPSY: ANIMAL STUDIES

To understand the neuronal mechanisms that underlie narcolepsy, the pharmacologic control of its 2 primary symptoms, EDS and cataplexy, has been examined using animal models, especially canine models. Cholinergic interactions, which play a role in triggering REM sleep or REM sleep atonia, were identified as investigatory targets, as was monoaminergic transmission. Although cholinergic blockades, such as by muscarinic antagonists, significantly reduce cataplexy in the canine models, this class of compound has not been used in humans as anticaataplactics due to significant peripheral side effects. All therapeutic agents currently used to treat cataplexy are known to act on monoaminergic systems (e.g., antidepressants and monoamine oxidase inhibitors).

CATAPLEXY

The anticaataplectic effects of a large number of uptake blockers/release enhancers specific for the adrenergic, serotonergic, or dopaminergic systems were examined in the canine models. The results pointed to adrenergic uptake inhibition as the key property involved in the anticaataplectic effect. Serotonergic uptake blockers at high doses were found to be only marginally effective at countering cataplexy, and dopaminergic uptake blockers were completely ineffective.

The anticaataplectic effects of several antidepressants that act on the serotonergic and adrenergic systems were investigated and compared with their demethylated metabolites (Figure 3). It is known that many antidepressants have demethylated active metabolites, including
imipramine demethylated to desipramine and clomipramine demethylated to desmethylclomipramine. Demethylation occurs rapidly at the hepatic first pass, and active metabolites cumulate during chronic drug administration.\(^2\) In general, these active metabolites are more selective for norepinephrine uptake inhibition than their parent compounds and have longer half-lives.\(^3\) The canine study clearly demonstrated that the demethylated metabolites were found to be more active in preventing cataplexy than the parent compounds. Further, the active dose of all antcataplectic compounds correlated with the in vitro potency of each compound for the adrenergic transporter, but not for the serotonergic transporter.\(^2\) These results supported the strong involvement of adrenergic neurons on the cataplectic state.

To further identify receptor subtypes that significantly modify cataplexy, more than 200 compounds with various pharmacologic properties, including cholinergic, adrenergic, dopaminergic, serotonergic, prostaglandins, opioids, benzodiazepines, GABAergics, and adenosinergics, were studied using the canine cataplexy model.\(^4\) Whereas many compounds were found to reduce cataplexy, very few compounds exacerbated it. It appears that the cataplexy-exacerbating effects are more specific, because cataplexy, similar to REM sleep, is easily reduced nonspecifically by fear, reduction of appetite, or any other uncomfortable drug side effects. The important receptors involved in aggravating cataplexy include the muscarinic M\(_2\) (non-M\(_1\)) receptors, as well as the postsynaptic adrenergic \(\alpha_{1b}\) receptors and presynaptic adrenergic \(\alpha_2\) receptors.\(^3,4,33,34\) This result is consistent with the adrenergic involvement of the control of cataplexy, and antcataplectic effects of adrenergic uptake inhibitors are thus likely to be mediated by postsynaptic adrenergic \(\alpha_{1b}\) receptors. Sodium oxybate has been shown to improve cataplexy and EDS in human narcolepsy.\(^35–37\) The compound is administered chronically at night and improves cataplexy and EDS during the daytime, but there is a time delay before significant effects appear.\(^35–37\) Sodium oxybate may act on GABA\(_{\beta}\) receptors or its own receptors and may also modulate dopaminergic neurotransmissions.\(^38\) The modes of action of sodium oxybate on cataplexy and EDS are not known, partially due to the lack of data in the animal models.

In addition to the adrenergic and cholinergic influences, cataplexy also has been associated with the loss of hypocretin neurotransmission, as discussed above.\(^3\) It has been hypothesized that persistent cholinergic monoaminergic imbalance due to the loss of hypocretin transmission may be required for cataplexy.\(^2\) Hypocretin replacement was evaluated in hypocretin ligand–deficient narcoleptic dogs (nonfamilial sporadic narcoleptic dogs).\(^39,40\) However, systemic and intrathecal administration of hypocretin-1 did not improve cataplexy in these animals. To treat hypocretin deficiency present in narcolepsy with cataplexy, therefore, more centrally penetrable and longer-lasting hypocretin analogues will need to be developed.\(^3\) It should be noted that EDS is the first symptom of narcolepsy to appear at the disease onset, and cataplexy usually occurs several months after the onset of EDS. In several cases studied at the time of EDS onset, a significant reduction of CSF hypocretin-1 was already observed before the onset of cataplexy, suggesting that chronic loss of hypocretin signaling and possibly secondary changes may be required for the occurrence of cataplexy. If the secondary changes (to the impaired hypocretin signaling) are irreversible processes, hypocretin replacement may not sufficiently resolve cataplexy.

The mechanism for emotional triggering of cataplexy also remains unknown, but multiple functional and anatomic systems are likely to be involved.

### EXCESSIVE DAYTIME SLEEPINESS

Narcoleptic Doberman dogs were also used to examine the modes of action of wake-promoting compounds in treating EDS. To examine these mechanisms, the effects of dopaminergic and adrenergic uptake inhibitors (which act on dopamine and norepinephrine transporters, respectively) were compared with the effects of amphetamine and modafinil, which are used to treat EDS in narcolepsy in humans.\(^41\) These latter agents nonspecifically enhanced presynaptic monoaminergic transmission\(^1\) and were found to enhance wakefulness in both narcoleptic and control dogs, suggesting that wake-promoting mechanisms of these compounds are independent of hypocretin neurotransmission. All of the examined dopaminergic uptake inhibitors, but not adrenergic uptake inhibitors, induced significant EEG arousal as potent as that seen with amphetamines and modafinil. Adrenergic uptake inhibitors instead suppressed REM sleep (Figure 4),\(^3,4,1\) which may be consistent with their antcataplectic effects. Furthermore, it was also found that wake-promoting effects of amphetamine differ among amphetamine analogues and their isomers; the wake-promoting effects of \(d\)-amphetamine are about 4 times more potent than those of \(l\)-amphetamine, and \(l\)-methyl amphetamine had little effect on wakefulness.\(^42\) In vivo microdialysis experiments demonstrated that the wake-promoting effects of these compounds correlated well with enhancements of these compounds on dopamine efflux (through dopamine transporters), but not norepinephrine efflux.\(^4\) Finally, it has been shown that modafinil also binds to dopamine transporters with non-negligible affinity, and the wake-promoting effect of modafinil was totally abolished in mice lacking dopamine transporters (i.e., dopamine transporter knockout mice).\(^43\)

These results were consistent with the hypothesis that the presynaptic modulation of dopaminergic transmission is involved in mediating the EEG arousal effects of these compounds.\(^3\) Other experiments, however, have
Figure 4. Dopamine and EEG Arousal*

A. Effects of Various Dopamine and Norepinephrine Uptake Inhibitors and Amphetamine-Like Stimulants on the EEG Arousal of Narcoleptic Dogs

B. Correlation Between In Vivo EEG Arousal Effects and In Vitro Dopamine Transporter Binding Affinities

*Adapted with permission from Nishino.³

A. The effects of various compounds on daytime sleepiness were studied using 4-hour daytime polygraphic recordings (10:00–14:00) in 4–5 narcoleptic animals. Two doses were studied for each compound. All DA uptake inhibitors and CNS stimulants dose-dependently increased EEG arousal and reduced slow-wave sleep when compared with vehicle treatment. In contrast, nisoxetine and desipramine, 2 potent NE uptake inhibitors, had no significant effect on EEG arousal at doses that completely suppressed cataplexy. Compounds with both adrenergic and dopaminergic effects (nomifensine, mazindol, d-amphetamine) were active on both EEG arousal and cataplexy. The effects of the 2 doses studied for each stimulant were used to approximate a dose-response curve; the drug dose that increased the time spent in wakefulness by 40% above baseline (vehicle session) was estimated for each compound. The order of potency of the compounds obtained was as follows: mazindol > d-amphetamine > nomifensine > GBR 12909 > amineptine > modafinil > bupropion.

B. In vitro DAT binding was performed using [3H]WIN 35428 onto canine caudate membranes. Affinity for the various DA uptake inhibitors tested varied widely between 6.5 nM and 3.3 mM. In addition, it was found that both d-amphetamine and modafinil have a low, but significant, affinity (same range as amineptine) for the DAT. A significant correlation between in vivo and in vitro effects was observed for all 5 DA uptake inhibitors and modafinil. d-Amphetamine, which had potent EEG arousal effects, has a relatively low DAT binding affinity, suggesting that other mechanisms, most probably monoamine-releasing effects, are also involved. In contrast, there was no significant correlation between in vivo EEG arousal effects and in vitro NE transporter binding affinities for DA and NE uptake inhibitors (data not shown). These results suggest that presynaptic enhancement of DA transmission is the key pharmacologic property mediating the EEG arousal effects of most wake-promoting CNS stimulants.

Abbreviations: CNS = central nervous system, DA = dopamine, DAT = dopamine transporter, EEG = electroencephalogram, NE = norepinephrine.

SUMMARY

Narcolepsy is characterized by EDS, cataplexy, and other dissociated manifestations of REM sleep, such as hypnagogic hallucinations and sleep paralysis. The major pathophysiology of human narcolepsy has been learned based on the discovery of narcolepsy genes in animals; these genes are involved in the pathology of the hypocretin/orexin ligand and its receptor. Mutations in hypocretin-related genes are rare in humans, but hypocretin ligand deficiency is found in many cases of narcolepsy with cataplexy.

Current treatments include amphetamine-like CNS stimulants and modafinil for EDS and antidepressants for cataplexy. Nighttime administration of sodium oxybate is also used for the treatment of EDS and cataplexy. Considering the fact that these compounds are effective regardless of hypocretin status, action mechanisms of these compounds are independent from hypocretin neurotransmission and are possibly located downstream of the hypocretin-signaling pathway. Animal experiments suggest anticultaplectic effects of monoamine uptake inhibi-
tors are mediated by noradrenergic transports, whereas wake-promoting effects of amphetamines and modafinil are mediated by enhancement of dopamine neurotransmission by interacting with dopaminergic neurotransmissions. The mode of action of sodium oxybate is largely unknown.

Hypocretin replacements are promising new therapeutic options in humans, but development of highly CNS-penetrant, nonpeptide hypocretin receptor agonists is likely required.

**Drug names:** bupropion (Wellbutrin and others), clomipramine (Anafranil and others), desipramine (Norpramin and others), fluoxetine (Prozac and others), imipramine (Tofranil and others), modafinil (Provigil), nortriptyline (Pamelor and others), sodium oxybate (Xyrem).

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