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Narcolepsy: A Neurodegenerative Disease of the Hypocretin System?

Minireview

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In the 2½ years since hypocretin (also called orexin) was first described (de Lecea et al., 1998; Sakurai et al., 1998), more than 120 papers on the peptide have already been published. A pair of current contributions to this rapidly expanding literature adds a new dimension to our understanding of this new peptide neurotransmitter/neuromodulator. Although additional confirming work is required, these new findings suggest that narcolepsy, a condition characterized by serious sleep disturbances, may be due to selective neurodegeneration of the hypothalamic hypocretin system. This would raise a disease that not too long ago could find itself on the couch of psychoanalysis onto the stage of the more common neurodegenerative diseases.

The hypocretins are two peptides, hypocretin 1 (orexin-A) and hypocretin 2 (orexin-B), generated from a single preprohypocretin and synthesized by a small number of neurons restricted to the lateral hypothalamus and perifornical area (de Lecea et al., 1998; Sakurai et al., 1998). In contrast, hypocretin axons are found throughout the CNS, with innervation of the hypothalamus, locus coeruleus, raphe, midline thalamus, all levels of spinal cord, sympathetic and parasympathetic centers, and many other brain regions (Peyron et al., 1998; van den Pol, 1999). Two G protein–coupled receptors that respond to the hypocretins have been identified (Sakurai et al., 1998). In parallel to the wide distribution of axons, the two hypocretin receptors show a widespread and heterogeneous pattern of expression throughout the CNS (Trivedi et al., 1998). Hypocretin raises synaptic activity in neurons of the hypothalamus (de Lecea et al., 1998) and locus coeruleus (Hagan et al., 1999; Horvath et al., 1999). Hypocretin can act on postsynaptic receptors to increase cytosolic calcium and can act at presynaptic receptors on axon terminals to enhance release of glutamate and GABA (van den Pol et al., 1998). The fact that hypocretin can enhance activity of either excitatory or inhibitory neurons suggests that the peptide could ultimately increase or decrease the activity of innervated brain circuits.

Dysfunction of Hypocretin System Causes Narcolepsy

Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness and unusual patterns of REM sleep. Narcolepsy is often associated with cataplexy, a sudden loss of muscle tone evoked by strong emotion. During attacks of cataplexy that last a few seconds or minutes, narcoleptics are conscious of their environment but unable to move. This condition appears to be related to muscle atonia that occurs during REM sleep. Narcoleptics may also have hypnogogic hallucinations, a complex state most likely to occur at the transition between wake and sleep. Sleep problems are not restricted to the daytime; at night, narcoleptics often show disturbed sleep patterns and may wake several times.

The potential importance of hypocretin neurons in preventing narcolepsy was first suggested by the finding that narcoleptic dogs have mutations in one of the hypocretin receptors, the hypocretin receptor 2 (orexin 2 receptor) (Lin et al., 1999). Although different mutations were found in each of two narcoleptic breeds of dogs, Dobermans and Labradors, in each breed the mutation was localized to the hypocretin receptor 2, rendering it nonfunctional. Detection of this mutation culminated a 10 year search for a defective gene using positional cloning. Parallel studies in hypocretin knockout mice revealed a similar narcoleptic phenotype (Chemelli et al., 1999), indicating that loss of either the peptide or one of the two peptide receptors results in narcolepsy. These findings became even more exciting and relevant when hypocretin was found in the cerebrospinal fluid of eight normal humans but could not be detected in seven of nine narcoleptics (Nishino et al., 2000).

Absence of Hypocretin Neurons in Narcoleptic Brain

New data based on immunocytochemistry and in situ hybridization indicate that there is a substantially decreased number of neurons producing hypocretin in the hypothalamus of human narcoleptics (Peyron et al., 2000; Thannickal et al., 2000 [this issue of *Neuron*]). Thannickal et al. show that the number of hypocretin immunoreactive neurons is decreased by about 90% in four narcoleptic human brains. In parallel, Peyron et al. show an absence of hypocretin mRNA from the hypothalamus, and the loss of hypocretin 1 and 2 peptides from extrahypothalamic hypocretin target regions of six narcoleptic brains. Although each study is based on a relatively small number of narcoleptic brain specimens, and each lab had only a few brain regions available, the combined brain number of both studies is ten, with six total hypothalami, although a possibility exists that one or two brains might have been shared by both groups. Both studies of narcoleptic brains report no decreased number of neurons containing melanin-concentrating hormone, a neuroactive peptide found in cells in the same general perifornical hypothalamic region where hypocretin neurons normally exist. This finding suggests a very selective absence of hypocretin neurons, rather than a general loss of neurons from the hypothalamus.

The absence of hypocretin neurons could be due to a number of factors, including degeneration of hypocretin neurons, failure of hypocretin neurons to develop, a reduced synthesis or release of the peptide, or some mutation in the DNA sequence coding for hypocretin. Several lines of evidence support the hypothesis that hypocretin neurons may degenerate in narcoleptics. Thannickal et al. find about 10% of the normal number of hypocretin neurons in narcoleptics, suggesting that the peptide was at least synthesized by a small proportion of neurons, arguing against a total absence of hypo-

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cretin neurons from birth. The detection of an increased immunostaining of astrocytes in the hypocretin area of the brain of narcoleptics also argues in favor of some type of postnatal degeneration. That narcolepsy is due to postnatal neuron loss is consistent also with the wide range of ages at which the disease generally is first detected, usually in the middle teenage years. Most narcoleptics appear to have regular sleep–wake cycles in their earlier lives, suggesting that the hypocretin system may be normal for many years prior to the development of narcolepsy. That the absence of releasable hypocretin can precipitate narcolepsy at very early ages is suggested by the detection of narcolepsy in a 2-year-old child (cataplexy found at 6 months of age) with a mutated signal sequence (Peyron et al., 2000). In this patient, a defect in the signal peptide sequence of hypocretin was found, based on a G to T transversion in the DNA sequence, and a resultant arginine substituted for leucine. In this case, hypocretin appeared to be synthesized, but rather than being transported to secretory vesicles for subsequent release, it instead accumulated in branching tubular bodies that were identified as smooth endoplasmic reticulum by morphological overlap with syntaxin-17. With this one exception, examination of the hypocretin gene from a number of narcoleptic patients failed to detect a mutation associated with the disease.

Although the most parsimonious explanation of the combined data is that hypocretin cells degenerate, dying hypocretin cells have not been identified, and the less likely possibility that the neurons do not die, but rather cease making hypocretin, remains. These questions may be addressed by future longitudinal experiments in humans showing that hypocretin is present in CSF prior to the manifestation of narcoleptic symptoms, but then is lost when symptoms appear. These experiments are complicated by the difficulty in predicting who will become narcoleptic. Additional examination of postmortem narcoleptic brain tissue obtained closer to the time of disease onset, potentially examining independent molecular markers of the hypocretin neurons, would also aid our understanding; these may be difficult to obtain given the long life expectancy of narcoleptics. Whereas degeneration of the hypocretin neurons may account for narcolepsy, other less likely possibilities have not been excluded. Even in the absence of a specific mutation in the hypocretin gene, other factors that might influence hypocretin synthesis and detection include mutations in specific transcription factors required for hypocretin transcription or alterations in proteins required for proper splicing of the hypocretin RNA or its stability. Along these lines, a cause of Rett syndrome, a progressive neurodevelopmental disorder, is the mutation of a gene called methyl CpG binding protein 2 (MeCP2). Its protein product is required for successful initiation of transcription of other downstream genes, and it is the absence of these gene products that is thought to lead to symptoms of the disorder.

The two studies differ in the detection of local hypothalamic neuropathology. Based on increased immunostaining for glial fibrillary acidic protein (GFAP), Thannickal et al. suggest gliosis is found selectively in the hypothalamus, but not in the thalamus of narcoleptic brains. Peyron et al. studied a number of potential indicators of neuropathology including HLA-DR microglial labeling, tumor necrosis factor in situ hybridization, thionin, and crystal violet staining and reported no local pathology in narcoleptics. Unlike Thannickal, Peyron did not find enhanced GFAP staining. Together, the data imply that although cells were lost at an earlier time point, suggested by the GFAP staining of some but not all of the brains, no continuing state of degeneration or immune response is found in narcoleptic brains. An inherent complication in these studies is that the brains were obtained many years or decades after the disease was detected, at a period when a local immune response to dying cells would have been obscured by the passage of time. Differences in the two studies could be due to small sample sizes, different patient populations, or different means (frozen or fixed) of tissue preservation.

Earlier reports (Tafti et al., 1996; Siegel et al., 1999) suggested that narcoleptic dogs (lacking the hypocretin receptor 2, but with normal hypocretin levels) show pathological signs of neurodegeneration and microglial influxes in nonhypothalamic brain regions. These canine observations indicate either that activation of the hypocretin receptor 2 may be important in sustaining neuronal survival or that the reports of neuropathology in the canine narcoleptic brains are not directly related to narcolepsy and the hypocretin system.

A central question remains: if hypocretin cells are lost postnatally, what might cause the specific disappearance of these cells? One clue comes from the association of narcolepsy in over 90% of patients with particular alleles coding for class II human leucocyte antigens, HLA DQB1*0602 and HLA-DR2, leading to the suggestion that the loss of hypocretin cells might be due to an autoimmune condition (Honda, 1988; Mignot et al., 1997). HLA class II molecules are expressed on the surfaces of macrophages, dendritic, and B cells that present antigens to CD4-positive T cells. HLA-related autoimmune disease could result in a cell- or antibody-mediated immune attack on restricted populations of cells. Many normal humans (30%) have the same haplotype, yet show no evidence of narcolepsy, indicating that these alleles do not cause the disease but may increase the sensitivity to other factors. The incidence of the disease in first-order family members of a narcoleptic is 1%–2%. Furthermore, the concordance rate among monozygotic twins is not high (about 25%). Together, this suggests there may be some environmental factor (toxin, chemical, virus, etc.) that might initiate an autoimmune imbalance.

One can only speculate at this point what might be the precipitating factor in narcolepsy. One possibility may be related to an infectious agent. An infectious agent could target hypocretin neurons directly or could alter the delicate balance between different classes of T and B cells of the immune system by stimulating or depressing specific cell types. Such an imbalance might lead to the destruction of hypocretin cells due to a particular antigenic determinant on the neuronal surface. Molecular mimicry could play a role, whereby an infectious agent may express some antigenic determinant that is similar to one expressed by a specific cell. An immune response to the foreign molecule results in an autoimmune response against the intrinsic cell sharing that antigen. If hypocretin cells degenerate from an auto-

immune response, it is possible that other neuronal systems that express a similar surface molecule may also be targeted. At this point, there are no substantive data giving direct support for an autoimmune attack on hypocretin neurons in narcoleptics.

Neurodegenerative Disease

If caused by degeneration of the hypocretin system, narcolepsy enters the fold of neurodegenerative diseases that includes Parkinson's (loss of nigral dopamine neurons), Huntington's (loss of striatal neurons), Alzheimer's (widespread loss of neurons), amyotropic lateral sclerosis (motor neuron loss and demyelination), and a number of other less frequently occurring diseases. As narcolepsy is found in about 1 of 2000 humans, a verification of the neurodegeneration hypothesis would establish narcolepsy as the third most prevalent type of neurodegenerative disease, behind Alzheimer's (the most common) and Parkinson's (which affects about 1 in 1000 humans), but more prevalent than Huntington's or amyotropic lateral sclerosis (each about 1 in 5000).

It may be instructive to compare narcolepsy with common neurodegenerative diseases. Most of these diseases, including Parkinson's, Huntington's, and Alzheimer's, generally strike late in life, often in the 50s or later. In contrast, narcolepsy generally begins in the teen years. Parkinson's patients can lose up to 80% of their dopamine cells before the more substantial motor dysfunction is apparent, and dopamine cells may be lost for years prior to symptomatic problems. Similar to Parkinson's disease, hypocretin neurons may begin to die long before the first symptoms of narcolepsy are detected. Nigral dopamine neurons from Parkinson brains show characteristic Lewy bodies, brains from Alzheimer's show plaques and tangles, Huntington brains show neuron loss and gliosis, and amyotropic lateral sclerosis patients show motor neuron loss and demyelination. Whether hypocretin neurons show some neuropathological sign of dysfunction prior to loss remains to be determined. Patients with the other neurodegenerative diseases generally deteriorate over years, in large part due to a continued loss of neurons. Although patients with narcolepsy do not recover, they generally do not show continued decline, and unlike most of the other diseases, narcolepsy is not fatal. Similar to narcolepsy, the specific causes of Parkinson's, amyotropic lateral sclerosis, and Alzheimer's remain to be determined. Although we do not know the cause of most cases of the disease, Parkinson's syndrome can be precipitated by both chemical toxins and viral agents. MPTP can destroy dopamine neurons of the nigra resulting in Parkinson's syndrome. Parkinson symptoms have also been found at long intervals after illness due to von Economo's disease, probably resulting from a neurotropic virus. The other name for this disease is encephalitis lethargica, related to the extended sleepiness caused by the disease. Degeneration in both the nigral and hypothalamic areas has been reported in postmortem examinations. It would be interesting to determine if a virally initiated degeneration had occurred to the hypothalamic hypocretin system potentially contributing to sleep problems including narcolepsy found in some patients as an aftermath of the disease. Although an epidemic of this encephalitis resulted in the death of up to half a million people in the early part of the 20th century, the disease has been rare for a number of years.

Hypocretin and Hypothalamic Function

Hypocretin initially generated interest because of its location in an area of the hypothalamus that plays a role in energy homeostasis and because of its putative role in evoking feeding (Sakurai et al., 1998), generating an alternate name for the peptide, orexin. Modest success in evoking feeding with intracerebral injections of hypocretin 1 or 2 has been reported by some (see Kilduff and Peyron, 2000). Human narcoleptics, rather than being thin, tend to maintain a heavier body weight than controls (Honda, 1988), the opposite of what would be expected from the loss of an orexigenic peptide. Whether this weight gain is due directly to hypocretin loss or to a secondary effect related to decreased activity in narcoleptics is not clear. Although the role of hypocretin in the regulation of body weight remains to be determined, hypocretin neurons do respond to a number of metabolic signals, including insulin, glucose, and leptin, that reflect the state of energy resources. This sensitivity may reflect a metabolically modulated arousal system. For instance, in conditions of caloric deprivation, it might be better to search for food, rather than sleep.

Given the focus of the narcolepsy literature on other brain regions such as the pons and medulla in recent decades, the hypothalamus would not have been the first guess as the neuronal site responsible for narcolepsy, although several decades earlier the hypothalamic region had been suggested as a probable brain locus regulating sleep and narcolepsy (von Economo, 1930; Nauta, 1946). Narcolepsy includes a number of symptoms not generally associated with hypothalamic function. For instance, hypnogogic hallucinations are not uncommon in narcolepsy—these are often of an unpleasant or negative nature; hallucinations have generally been considered the domain of higher brain regions, particularly the cortex. In contrast to the negativity of the hallucinations, the sudden loss of skeletal muscle tone in cataplexy is generally precipitated by strong emotional situations of a positive nature, and less frequently by emotional situations with a negative overtone (Honda, 1988). Regulation of muscle tone has not generally been considered a function of the hypothalamus, yet with the absence of hypothalamic hypocretin, transient paralysis occurs during cataplexy. Thus, the absence of hypocretin neurons reveals new dimensions to hypothalamic function.

Hypocretin Agonists and Antagonists

If the absence of hypocretin leads to narcolepsy, can the symptoms of narcolepsy be alleviated by hypocretin? Possibly. However, global elevation of hypocretin may not stimulate the receptors selectively as would release of the peptide at specific synaptic sites. In rodents, intracerebral injections of hypocretin do increase arousal and alter sleep patterns (Hagan et al., 1999). The hypocretins have poor permeability across the blood brain barrier; if a hypocretin agonist can be designed that would survive the intestinal tract and have enhanced permeability into the brain, it may reduce the symptoms of narcolepsy. The symptoms of narcolepsy are currently treated with amphetamines or related compounds. These can have significant negative side effects, including habituation, dependence, heightened

anxiety, and weight loss. It will be interesting to see what actions hypocretin agonists might have on human behavior and mental function. Given the role hypocretin appears to play in arousal, hypocretin agonists might serve as a safer substitute for amphetamines in a wide range of situations where amphetamines are now employed. Serotonin and norepinephrine systems are key targets for many drugs used to treat mental illness, including depression, anxiety, bipolar, and attention deficit hyperactivity disorders; given the strong hypocretin innervation of the raphe and locus coeruleus, a primary origin of these systems, hypocretin agonists may ameliorate or exacerbate these conditions. A number of intriguing questions emerge. Do sleep problems in aged humans relate to ongoing loss of hypocretin neurons? Does an exaggerated axonal release of hypocretin or sensitized hypocretin receptors underlie insomnia or other problems relating to sleep difficulty, and could hypocretin antagonists be used as an effective treatment? Would hypocretin agonists enhance alertness or facilitate concentration?

The hypocretin story to date is an excellent example of the importance of basic cellular, molecular, and whole animal studies that can serve as a critical substrate for understanding the cause of human brain disease. The rapidity of the hypocretin story is a credit to the National Institute of Health that funded much of the basic and clinically related research leading to the discovery that hypocretin absence is a primary cause of narcolepsy.

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