Hypocretin/orexin, sleep and narcolepsy

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Summary

The discovery that hypocretins are involved in narcolepsy, a disorder associated with excessive daytime sleepiness, cataplexy and unusually rapid transitions to rapid-eye-movement sleep, opens a new field of investigation in the area of sleep control physiology. Hypocretin-1 and -2 (also called orexin-A and -B) are newly discovered neuropeptides processed from a common precursor, preprohypocretin. Hypocretin-containing cells are located exclusively in the lateral hypothalamus, with widespread projections to the entire neuroaxis. Two known receptors, Hcrtr1 and Hcrtr2, have been reported. The functional significance of the hypocretin system is rapidly emerging in both animals and humans. Hypocretin abnormalities cause narcolepsy in dogs, human and mice. The role of the hypocretin system in normal sleep regulation is more uncertain. We believe hypocretin cells drive cholinergic and monoaminergic activity across the sleep cycle. Input from the suprachiasmatic nucleus to hypocretin-containing neurons may explain the occurrence of clock-dependent alertness. Other functions are suggested by pharmacological and neurochemical experiments. These include regulation of food intake, neuroendocrine function, autonomic nervous system activity and energy balance. *BioEssays* 23:397–408, 2001. © 2001 John Wiley & Sons, Inc.

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Abbreviations: CSF, cerebrospinal fluid; DB, diagonal band of Brocca; EEG, electroencephalogram; Hcrtr1, hypocretin-receptor-1; Hcrtr2, hypocretin-receptor-2; HLA, Human Leukocyte Antigen; icv, intracerebroventricular; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; MCH, Major Histocompatibility Complex; NPY, neuropeptide Y; OX1R, orexin-receptor-1; OX2R, orexin-receptor-2; PAG, periaqueductal gray; PPT, pedunculopontine tegmental nucleus; PVN, paraventricular nucleus; REM, rapid eye movement; SCN, suprachiasmatic nucleus; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic area; VMN, ventromedial nucleus; VTA/SN, ventral tegmental area and substantia nigra pars compacta

Introduction

The function of sleep and why this behavior was selected by natural evolution is one of the remaining mysteries in physiology today. Sleep is a vital behavior that consumes one third of any given human life; animals die if totally deprived of sleep.[1,2] Electrophysiological studies have long shown that sleep is a heterogeneous state, most classically separated into rapid eye movement (REM) and non-REM sleep. Non-REM sleep can also be subdivided into light non-REM sleep (stage I and II) and slow wave sleep (SWS, stage III and IV). Independent of this organization by sleep stages, the propensity to sleep or to stay awake is regulated independently by homeostatic (sleep debt-dependent) and circadian (clock-dependent) processes. Finally, sleep is associated with a host of peripheral changes that have a physiological impact. These include established sleep-state-specific or circadian-controlled changes in endocrine release, convulsive thresholds, regulation of breathing, cardiovascular control, gastrointestinal physiology and muscle tone.

There has been spectacular progress in the last decade in our understanding of how the brain generates circadian rhythms.[3] In mammals, circadian rhythmicity is generated by the suprachiasmatic nucleus (SCN), a discrete hypothalamic region. At the genetic level, key circadian factors and genes generating circadian rhythmicity have now been isolated.[3] In contrast, there has been little progress in understanding the regulation of sleep. The most accepted neuroanatomical model involves reciprocal inhibitory interactions between cholinergic [e.g. laterodorsal tegmental (LDT) area and pedunculopontine (PPT) nuclei] and monoaminergic [e.g. locus coeruleus (LC), raphe and tuberomamillary (TMN) nuclei] cells groups of the brainstem.[4,5] In this model, ascending cholinergic and monoaminergic projections mediate EEG changes by modulating thalamocortical loops.[4,5] Monoaminergic tone is high during wakefulness (generating EEG desynchronization) and decreases across the sleep cycle (promoting EEG synchronization). Decreased monoaminergic tone desinhibits cholinergic neurons during the later part of the sleep cycle, resulting in REM sleep and associated EEG desynchronization. Little progress has been achieved in elucidating the genetics of sleep control. The two major limitations in the area have been the lack of single gene mutant models with sleep abnormalities and the difficulties of defining "sleep" in non-mammalian species

(e.g. *Drosophila*) that may be more amendable to genetic studies.

This situation is now changing rapidly. Investigators are now trying to equate sleep and locomotion activity control in lower organisms.[6] Our own work has focussed on the only known single gene mammalian mutant in the field, canine narcolepsy. Narcolepsy is a unique model as it is one of the few disorders with a well-defined and dramatic sleep phenotype. A positional cloning project identified two exon skipping Hcrtr2 mutations causing canine narcolepsy in Dobermans and Labradors.[7] This discovery was followed by the observation of narcolepsy-like symptoms in preprohypocretin knockout mice[8] and by the more recent demonstration of generalized hypocretin deficiency in human narcolepsy.[9–11] In this review, we will briefly summarize the emerging knowledge regarding this newly described neurotransmitter system and argue that hypocretins may complement monoaminergic and cholinergic systems as major contributors for the generation of the sleep cycle.

Hypocretins/orexins and their discovery

Hypocretins/orexins were discovered by two independent groups only two years ago.[12,13] In 1998, de Lecea and colleagues selectively enriched a cDNA library for hypothalamic-specific transcripts and identified a clone selectively expressed in the lateral hypothalamus called H35.[12] Their goal was to find novel genes expressed primarily in the hypothalamus, a structure well known to be a major regulatory center of autonomic and endocrine homeostasis. The H35 clone was sequenced and shown to contain the precursor of two related neuropeptides, hypocretin-1 and -2 (Fig. 1). Neuroanatomical characterizations as well as electron microscopy and electrophysiological studies indicated that the hypocretins were genuine neurotransmitters. Of note, high concentrations of hypocretin-2 were found to be generally excitatory in hypothalamic cultures. Based on the neurotransmitters' hypothalamic origin and their similarity to the gut hormone secretin (contested by others) de Lecea et al. named these peptides hypocretin-1 and hypocretin-2.

Figure 1. Preprohypocretin peptide sequences in selected mammalian species[12,13,38,101] and *Xenopus*.[102] Variable residues are marked in light blue letters. Hypocretin-1 and hypocretin-2 peptide hormone domains are indicated in gray. Note that hypocretin-1 is identical across all mammalian species studied while hypocretin-2 differs by two residues. The prohormone contains a signal peptide sequence where a dominant narcolepsy mutation (16L → R) has been described.[10] Note dibasic (RR) prohormone convertase site between the two hypocretin peptide domains and at the C-terminal end of hypocretin-2. The glycine residue located in the C-terminal end of both peptides is converted to an amide indispensable to biological activity.[13,103] The C-terminal regions of hypocretin-1 and hypocretin-2 have high homology areas that are the most critical for biological activity based on recent studies.[103] Hypocretin-1 is also transaminated in the N-terminal region to create a cyclic pyroglutamyl residue and has two disulfide bridges.

The same neuropeptide system was discovered concomitantly by Sakurai et al. using a different approach.[13] The focus of their investigation was on discovering novel ligands for orphan G-protein-coupled receptors. Two peptides (Orexin-A, Orexin-B) and two receptors (OX1R and OX2R) were biochemically characterized in this study. Importantly, the exact location of proteolytic cleavage sites for the precursor and relevant post-translational modifications (dilsufide bonds, N-pyroglutamyl cyclization and C-terminal amidation) were also reported (see Fig. 1 for description). Intrahypothalamic localization of the peptides was demonstrated. The observation that preprohypocretin transcripts were upregulated by fasting while central administration of these peptides stimulated feeding led these authors to suggest a primary role in feeding regulation.[13] Hcrtr1 has a significantly higher affinity for hypocretin-1 while Hcrtr2 binds hypocretin-1 and -2 with similar affinity. Hypocretin-1/orexin-A, hypocretin-2/orexin-B, Hcrtr1/OX1R and Hcrtr2/OX2R refer to the same biological entities.

Neuroanatomical studies of the hypocretin/orexin system

The hypothalamus is one of the most complex brain regions at both the functional and neuroanatomical level.[14] This brain structure has long been recognized as a vegetative center regulating body homeostasis. This regulation involves the integration of functions as diverse as energy balance and body fluid homeostasis, cardiovascular and autonomic regulation, circadian rhythms and sleep, and reproduction.[14,15] The intrahypothalamic circuitry underlying these functions is rapidly emerging, thanks to functional studies and improved neurochemical characterization of hypothalamic subgroups.

The discovery that hypocretin-containing cell bodies were exclusively located in the lateral hypothalamus, an area where lesions are known to dramatically reduce food intake,[14] initially suggested a role for these peptides in the regulation of food intake.[12,13] Further neuroanatomical studies using immunocytochemical and neurochemical techniques indicated that, while cells bodies were restricted to the hypothalamus, projections were widespread, suggesting more complex functions (Fig. 2).[16–23] Intrahypothalamic projections were dense and included arcuate nucleus (ARC), paraventricular nucleus (PVN) and ventromedial nucleus (VMN), structures known to integrate feeding. Limbic system and associated structures (nucleus accumbens, amygdala, septum, basal forebrain area, bed nucleus of the stria terminalis), specific area of the thalamus (paraventricular and reticular thalamus) and the brainstem also received dense fibers (Fig. 2). These contrasted with a lack of projections to motor nuclei such as caudate nucleus, putamen, globus pallidus and sensory thalamic nuclei. Dense projections to all monoaminergic cell groups (locus coeruleus, raphé nucleus, substantia nigra, ventral tegmental area and tuberomamillary nucleus) were also noted. Studies measuring hypocretin content in various nuclei indicated a similar profile, with higher hypocretin-2 versus hypocretin-1 levels noted in all brain regions examined.[10,22–26]

Hypocretin receptor localization has been examined using in situ hybridization by three groups.[27–29] Both receptor subtypes were generally expressed differentially in the central nervous system. Intrahypothalamically, Hcrtr1 was preferentially expressed in the VMN, while Hcrtr2 was mostly expressed in the PVN and arcuate nucleus. Both hypocretin receptors were thus expressed in hypothalamic regions known

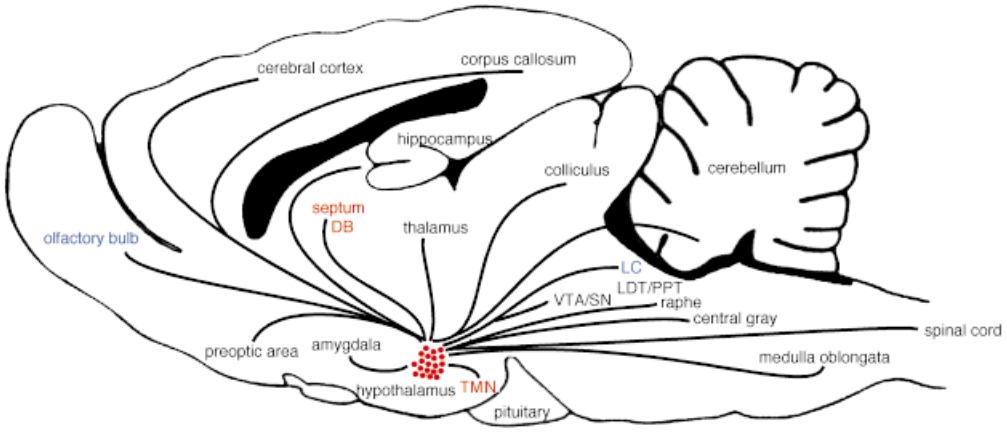

Figure 2. Hypocretin-containing neurons are exclusively located in the posterior hypothalamus, in a discrete region including and surrounding the perifornical nucleus. In contrast, hypocretin-positive fibers and terminals are widely distributed in the brain. Selected projections with corresponding hypocretin receptor subtypes are indicated. Intrahypothalamic projections are not displayed in details. Projections with predominantly Hcrtr2 and Hcrtr1 receptors are denoted in red and blue, respectively. Regions containing both receptors and/or without any information are displayed in black.

to be important for feeding regulation. Another important finding was the preferential localization of Hcrtr1 in the adrenergic locus coeruleus while Hcrtr2 was densely located on tuberomamillary histaminergic cells and within the diagonal band and septum, possibly on cholinergic cells.[29]

Human and canine narcolepsy

Narcolepsy has fascinated clinicians and researcher alike for more than 100 years.[30] A canine model was first described in the 1970s and has been the subject of intense pharmacological and neurochemical investigations.[31] Narcolepsy is the only frequent neurological disorder characterized by a primary disorganization of sleep and wakefulness.[32] The disorder affects approximately 0.05% of the general population in Western Europe and North American countries. In normal subjects, REM sleep occurs in the middle of the night and is associated with dreaming and muscle paralysis. Patients with narcolepsy are chronically sleepy and have an abnormal tendency to enter (REM) sleep prematurely. Nocturnal sleep is also frequently disturbed by insomnia, sleep paralysis and vivid dreaming. The pathognomonic symptom of narcolepsy is cataplexy, a sudden onset of muscle atonia trigged by emotions such as laughing and anger.[33] Cataplexy is believed to represent abnormal REM sleep paralysis in reaction to emotions.[33]

Human narcolepsy is a disabling disorder that necessitates life-long therapy.[4] It usually starts during adolescence and is treated symptomatically using amphetamine-like stimulants and antidepressants. These drugs have been shown to act by stimulating dopaminergic and adrenergic transmission, respectively.[31] Disturbed nocturnal sleep may be treated with sedative antidepressants, benzodiazepine-like hypnotic medications or gamma-hydroxybutyrate. Studies in a well-established animal model, canine narcolepsy, have shown that the disorder is associated with cholinergic and monoaminergic abnormalities.[31] These results are generally consistent with decreased monoaminergic tone, cholinergic hyperactivity and cholinergic hypersensitivity, a result that may explain daytime sleepiness and REM abnormalities in narcolepsy.[31]

Genetic aspects of human narcolepsy

Human narcolepsy is primarily a sporadically occurring disorder but familial clustering has been observed since its initial description.[34] The disorder is tightly associated with HLA-DQB1*0602 and HLA-DQA1*0102, suggesting the involvement of the immune system in the pathophysiology of the disorder.[34] HLA-DQB1*0602 is present in a large portion of the general population (e.g. 25% in North European Caucasians) who do not have narcolepsy-cataplexy.[34] Interestingly, HLA-DQB1*0602-positive healthy controls have shorter REM sleep latency implicating an increased susceptibility to narcolepsy in subjects carrying this antigen.[35]

The low penetrance of HLA-DQB1*0602 in producing clinically significant symptoms suggests that factors other than HLA genes are involved in triggering narcolepsy. Family and twin studies have shown that both environmental factors and non-HLA genes are important.[34] The familial risk of a first degree relative of a narcoleptic patient developing the disorder is approximately 20–40 times higher than in the general population, a result that cannot be explained by the sharing of HLA alone.[34,36] Finally, familial cases are more frequently HLA-DQB1*0602 negative than sporadic cases (e.g. 30% DQB1*0602-negative in probands of multiplex families), suggesting that these cases might involve highly penetrant non-HLA genes. These data suggest that human narcolepsy is both genetically complex and etiologically heterogeneous.[10,34]

Genetic alteration of the hypocretin system causes narcolepsy in dogs and mice

The first clue suggesting hypocretin involvement in narcolepsy came from studies in a canine model of the disorder.[7] As in humans, most cases of canine narcolepsy are sporadic but autosomal recessive narcolepsy was also observed in some breeds (*canarc-1*).[30,37] A ten year long positional cloning project led to the isolation of the canine narcolepsy gene in 1999.[7] Three Hcrtr2 mutations causing narcolepsy in Labradors, Dobermans and Dachshunds have been identified (Fig. 3).[7,38] Two of these mutations are exon skipping alterations while the third is a single base pair change in the N-terminal part of the Hcrtr2 gene.[7,38] Functional analysis of the three mutations indicate complete loss of function.[38] Exon skipping resulted in truncated Hcrtr2 receptors that are not normally translocated on the plasma membrane.[38] The point mutation changes a glutamic acid to a lysine, and produces a loss of ligand binding.[38] Hcrtr2-mutated narcoleptic canines have normal hypocretin levels and neurons, suggesting a primary role of Hcrtr2-mediated transmission in generating the narcolepsy phenotype.[39,40]

These results have now been extended to other animal models. The positional cloning of *canarc-1* was independently followed by the observation that preprohypocretin knockout mice have a narcolepsy-like phenotype.[8] These animals exhibit episodes of behavioral arrest similar to cataplexy and have disrupted sleep/wake during their activity period.[8] The phenotype is more difficult to study and cataplexy is hard to quantify in mice.[41] Further developments of mouse models revealed that Hcrtr2 knockout mouse also have cataplexy but the phenotype may be less severe than that of prepro-hypocretin knockout mice.[42,43] In contrast, Hcrtr1 knockout mice have almost no obvious behavioral changes, with the exception of a possible milder sleep fragmentation.[42,44] This suggests that Hcrtr2 may be critical to the narcolepsy phenotype, while Hcrtr1 acts as a modifying gene enhancing the severity of the phenotype. Additional studies in Hcrtr1/

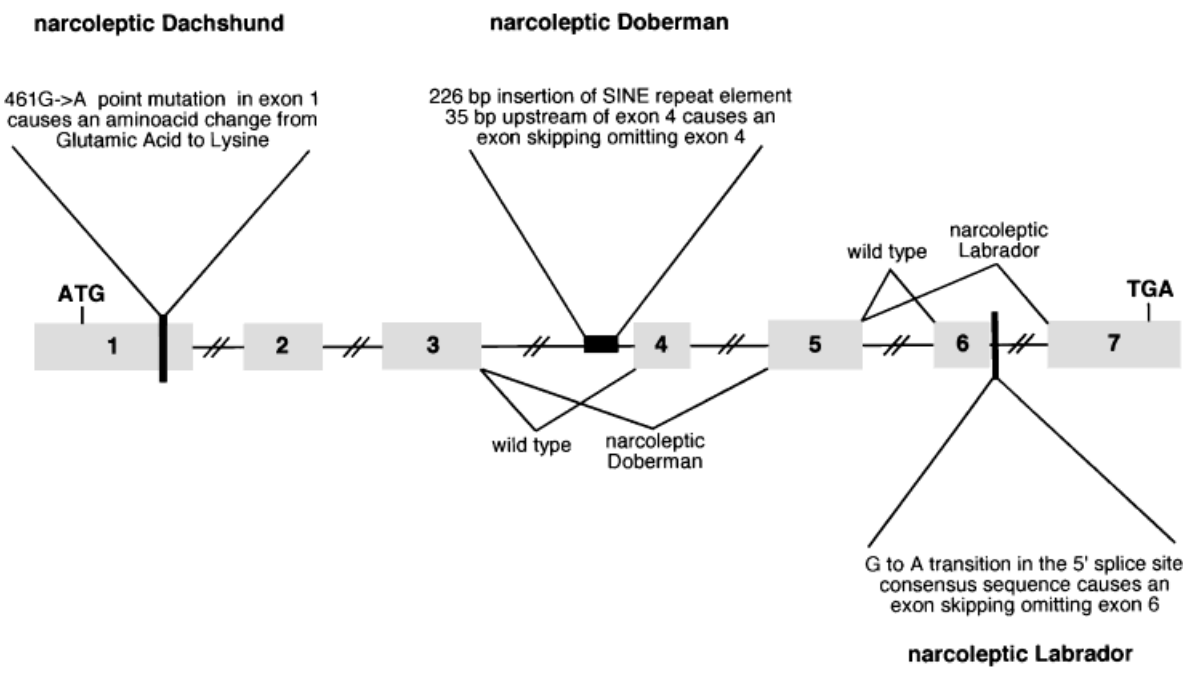

Figure 3. Genomic organization of the canine hypocretin receptor 2 gene (Hcrtr2) and identified narcolepsy-causing mutations. The gene encodes a 7 transmembrane G-protein coupled receptor. Sequence analysis revealed exon skipping mutations in narcoleptic Dobermans (exon 4) and Labradors (exon 6). Deleted exons result in frameshifts and premature stop codons. In Dachshunds, a point mutation in exon 1 results in an aminoacid change from glutamic acid to lysine at the transition point from the N-terminal domain to the first transmembrane region. Functional analysis indicates that all these mutations result in a complete loss of Hcrtr2 function.[38]

Hcrtr2 double knockout mice are currently in progress and seem to confirm this hypothesis.[42]

Interestingly, hypocretin gene mutations were not observed in sporadic cases of canine narcolepsy.[38] This result does not necessarily mean that abnormalities in the hypocretin system are not involved in these cases. In fact, hypocretin levels were found to be dramatically decreased or absent in the cerebrospinal fluid (CSF) and the brain of sporadic canine narcolepsy cases.[39] The fact that hypocretin-ligand-deficient, sporadic cases of canine narcolepsy are generally more severely affected with narcolepsy than Hcrtr2 mutated familial cases[37] also agree with the suggestion that Hcrtr1 has an enhancing role.

Hypocretin abnormalities in human narcolepsy

Mutation screening of the hypocretin, Hcrtr1 and Hcrtr2 genes was first carried out in a large number of human patients.[10] These cases included unusual patients with familial occurrence and/or HLA-DQB1*0602 negativity.[10] Surprisingly, only one mutation was identified in an HLA-negative patient with atypical onset at 6 months of age (Fig. 1). This result indicates etiologic and genetic heterogeneity in familial narcolepsy-cataplexy.[10] The mutation with early onset narcolepsy is a T to G transversion causing a leucine to arginine change in the hydrophobic core of the signal peptide of the hypocretin peptide (Fig. 1). Functional analysis indicates impaired hypocretin trafficking and processing for the mutant polypeptide, with accumulation in the smooth endoplasmic reticulum and most probably cell death in vivo.[10]

As in canines, however, the absence of hypocretin mutations in most human cases does not indicate a lack of involvement of this system in human narcolepsy. A study in nine narcoleptic patients showed low CSF hypocretin levels in seven narcoleptic patients (Fig. 4).[9] More recently, postmortem studies in ten narcoleptic subjects indicated undetectable hypocretin-1 and -2 peptides in projection sites such as cortex and pons and a 80–100% reduction of hypocretin-containing cells in the hypothalamus as measured by in situ hybridization and immunocytochemistry (Fig. 4).[10,11] Six subjects in one study were all HLA*0602 positive, had definitive cataplexy and included a case with familial occurrence.[10] Taken together, the CSF and postmortem studies indicate that hypocretin deficiency causes narcolepsy in most human cases with cataplexy.[9–11]

Hypocretin and sleep regulation

Pharmacological studies indicate potent wake-promoting and REM sleep reduction effects after central administration of

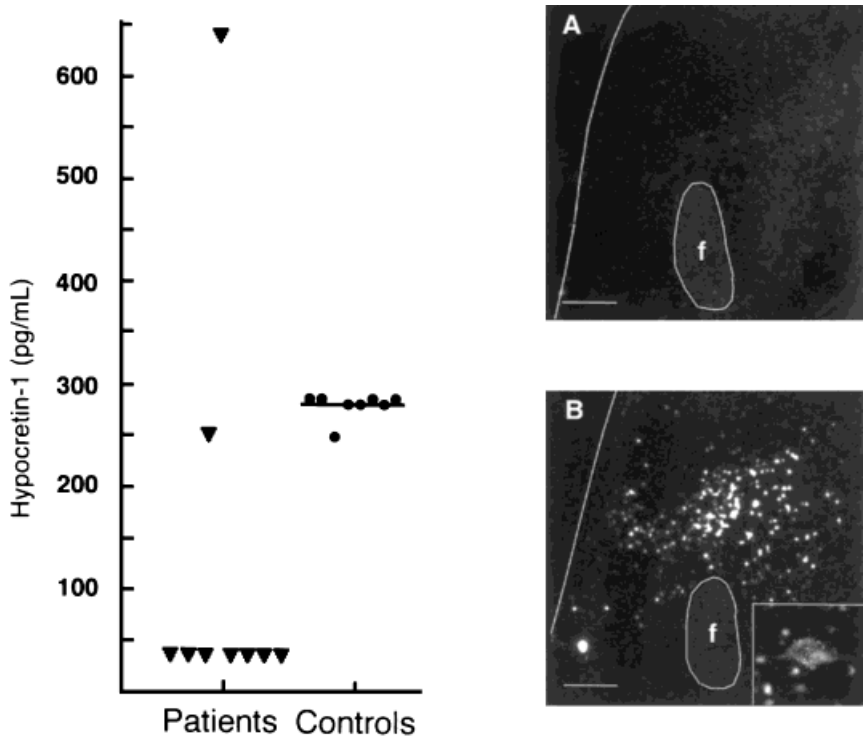

Figure 4. Most cases of human narcolepsy are associated with undetectable CSF hypocretin-1 levels (left). Measuring CSF hypocretin levels may become an established diagnostic procedure.[9] In situ hybridization studies revealed a greatly reduced level or absence of hypocretin neurons in the hypothalamus (right).[10] These results indicate that human narcolepsy is caused by an hypocretin deficiency. The loss of hypocretin neurons is generally believed to be secondary to an autoimmune process but this hypothesis has not been formally proven.

hypocretins.[45] Intracerebroventricular (icv) administration of hypocretin-1 but not hypocretin-2 induces wakefulness and reduces REM sleep.[45] At high doses, increased locomotion, grooming and stereotypes are even observed.[45,46] The lack of effects of hypocretin-2 in vivo is generally believed to be due to biological instability for this peptide.

The wake-promoting effects of hypocretins are hypothesized to be mediated by a stimulation of monoaminergic transmission via excitatory hypocretin receptors. In favor of this hypothesis, administration of hypocretins directly onto the locus coeruleus increases wakefulness and reduces REM sleep in rats.[47] This, together with the observation that hypocretin application strongly stimulates firing rate in the locus coeruleus (LC, Fig. 2) suggests a primary role for this structure in the wake-promoting effects of hypocretins.[18,45] Other authors have, however, shown similar in vitro excitatory effects of hypocretins on dopaminergic VTA,[46] serotoninergic raphe magnus[48] and histaminergic TMN cells (Haas, personal communication). Hypocretin-1 effects on locomotion are also blocked by haloperidol and sulpiride, suggesting secondary dopaminergic effects.[46] These results are thus more consistent with a global stimulatory effect of hypocretins on monoaminergic tone than preferential adrenergic effects.

Interestingly, hypocretin-1 is often more effective than hypocretin-2 in stimulating monoaminergic activity in vitro, suggesting Hcrtr1 mediation.[45,46] This last result is surprising considering the primary importance of Hcrtr2 in expressing the narcolepsy phenotype in genetic studies. Hcrtr2 is mostly located in dopaminergic, histaminergic and, possibly, cholinergic neurons.[27–29] Hcrtr1 is coupled with Gq and known to be excitatory. In contrast, Hcrtr2 has been suggested to be both excitatory and inhibitory and may coupled to either Gq or Gi/o.[42] Hypocretins have excitatory effects on laterodorsal tegmental cholinergic[50] and tuberomamillary histaminergic neurons (Haas, personal communication) in slices, consistent with Hcrtr2-mediated excitation rather than inhibition.

While a role for hypocretins in narcolepsy is well established, very little data to date suggest the involvement of hypocretins in normal sleep or circadian regulation. Based on the results indicated above, we hypothesize that hypocretin cells are driving monoaminergic and cholinergic tone during the sleep cycle, with maximal and minimal activity during

wakefulness and REM sleep, respectively (Fig. 5). In this model, the reversal of cholinergic tone observed during REM might be secondary to changes in monoaminergic activity, as predicted by the reciprocal interaction model[4,5] and various pharmacological experiments.[49,51] In favor of this hypothesis, electrophysiological studies have indicated the existence of wake-on, REM-off cells in the perifornical area[52] but this finding is not a very specific without associated neurochemical characterization. Similarly, double-staining studies have shown increased *c*-fos production in hypocretin-containing neurons with wakefulness but other neurons in the region display similar, but weaker, fluctuations.[53,54]

In our model, high hypocretin tone during wakefulness activates both cholinergic and monoaminergic tone. In non-REM sleep, decreased hypocretin activity reduces monoaminergic and cholinergic tone. In REM sleep, the decreased monoaminergic tone reaches such a low level that a desinhibition of cholinergic systems occur. Cholinergic activation in REM would thus occur in spite of a depressed excitatory hypocretin tone on these cells (Fig. 5). The recent report of direct projections from the suprachiasmatic nucleus, onto hypocretin cells[55] also suggest that hypocretin activity may be modulated by the biological clock. This projection could provide a biological substrate for the concept of SCN-dependent alertness, a system that has been suggested to be abnormal in hypocretin-deficient narcolepsy.[56]

An alternative hypothesis proposed by Kilduff and Peyron postulates increased hypocretin tone during both REM and wakefulness.[57] In this model, inhibitory GABAergic projections from the preoptic hypothalamus and periaqueductal gray (PAG) to monoamine- and hypocretin-containing cells plays a complementary role in the driving of the sleep cycle.[57] We believe that the REM sleep desinhibition observed in hypocretin-deficient narcolepsy is more compatible with the hypothesis that hypocretin tone is depressed during REM sleep. Other investigators have also speculated that hypocretin may inhibit cholinergic tone during REM sleep as the result of direct inhibitory Hcrtr2 effects.[42] Recent studies indicating excitatory effects of hypocretins on laterodorsal tegmental cholinergic neurons in slices,[50] are also inconsistent with this last hypothesis.

The concept that hypocretin neurotransmission is excitatory to both cholinergic and monoaminergic systems parallels neurochemical data reported in narcolepsy. In the sleep disorder, symptoms are best explained by baseline monoaminergic hyperactivity, hyperactivity of cholinergic systems and cholinergic receptor hypersensitivity.[31,49] Monoaminergic hypoactivity and hypersensitivity to cholinergic stimulation are consistent with a reduced cholinergic and monoaminergic tone across the 24 hour day. The depressed monoaminergic tone would also have the tendency to deactivate the cholinergic system more easily, especially at sleep onset. At this time, monoaminergic cell activity may be decreased by GABAergic inhibition from the VLPO and the PAG, a mechanism that has been suggested to also regulate monoaminergic activity across the sleep cycle.[58] A small additional decrease in monoaminergic tone on a depressed baseline could lead to cholinergic desinhibition and acetylcholine release on supersensitive receptors, thus promoting REM-sleep-like transitions at sleep onset. Most strikingly, canine narcolepsy is associated with decreased dopamine[59] and histamine[60] levels in the brain, two systems with known Hcrtr2 localization.

These models are still speculative. Hypocretin projections to non-monoaminergic, non-cholinergic cell groups are also likely to play a role. For example, hypocretin-1 injection in the lateral preoptic hypothalamus increases wakefulness.[61] Unknown projections, for example to the limbic system/basal forebrain area may be involved in the triggering of cataplexy by emotions.[31] Other experiments indicate surprisingly small state- and circadian-associated changes in hypocretin stores and mRNA.[62] Sleep deprivation does not affect hypocretin mRNA levels in rats.[63] Hypocretin mRNA level fluctuates with circadian time, but the highest levels are observed during the inactive period, in opposition with the model proposed above.[62] Similarly, hypocretin-1 peptide content does not exhibit circadian variations in most brain regions examined, with the possible exception of preoptic/anterior hypothalamic nuclei (maximal levels at 9 am, when rats are mostly sleeping) and pons (maximal levels at 1 am, when rats are most active).[62] Finally, in our clinical CSF studies, we also did not observe any significant change in CSF hypocretin-1 levels between early morning and late afternoon (unpublished results). These results suggest minimal changes in global hypocretin transmission but do not exclude more localized but relevant changes in neurotransmission. Of note, hypocretin-2, the most abundant of the two hypocretin peptides, was generally not studied. In vivo dialysis studies of the local release of these neurotransmitters in functionally significant sites of projections is now critically needed. Additional electrophysiological studies of fully characterized hypocretin neurons across the sleep cycle will also in time answer if electrical or metabolic changes in hypocretin neurons are functionally relevant to sleep regulation.

Hypocretins as orexinogenic agents

Hypocretins were initially described as neuromodulator of food intake. Central administration of hypocretin-1 stimulates food intake, while data are more inconsistent after hypocretin-2 administration.[13,64–75] Recent pharmacological studies using a Hcrtr1 antagonist, SB-334867-A, suggest a Hcrtr1 mediation for this effect.[76] A reduced food intake was also observed when an anti-orexin polyclonal antibody was applied intracisternally in fasted rats.[77] Hypocretin-1 microinjections in the perifornical hypothalamus, the lateral hypothalamus, the dorsomedial nucleus and, possibly, the paraventricular

nucleus but not in the ventral tegmental area, the arcuatus nucleus, central nucleus of the amygdala, the preoptic area or the nucleus tractus solitarius induce feeding.[64,71] Hypocretin-1 administration in the lateral hypothalamus also stimulates *c*-fos expression in projection areas involved in appetite and body weight regulation.[78] Recent data showed that NPY receptor antagonist block partially the hypocretin-induced feeding behavior, suggesting that the orexinogenic effect of hypocretins may be mediated secondarily via NPY activation.[69,74] Several recent experiments, however, suggest more minor pharmacological effects of hypocretins on food intake. First, the orexigenic effects of hypocretin-1 are far

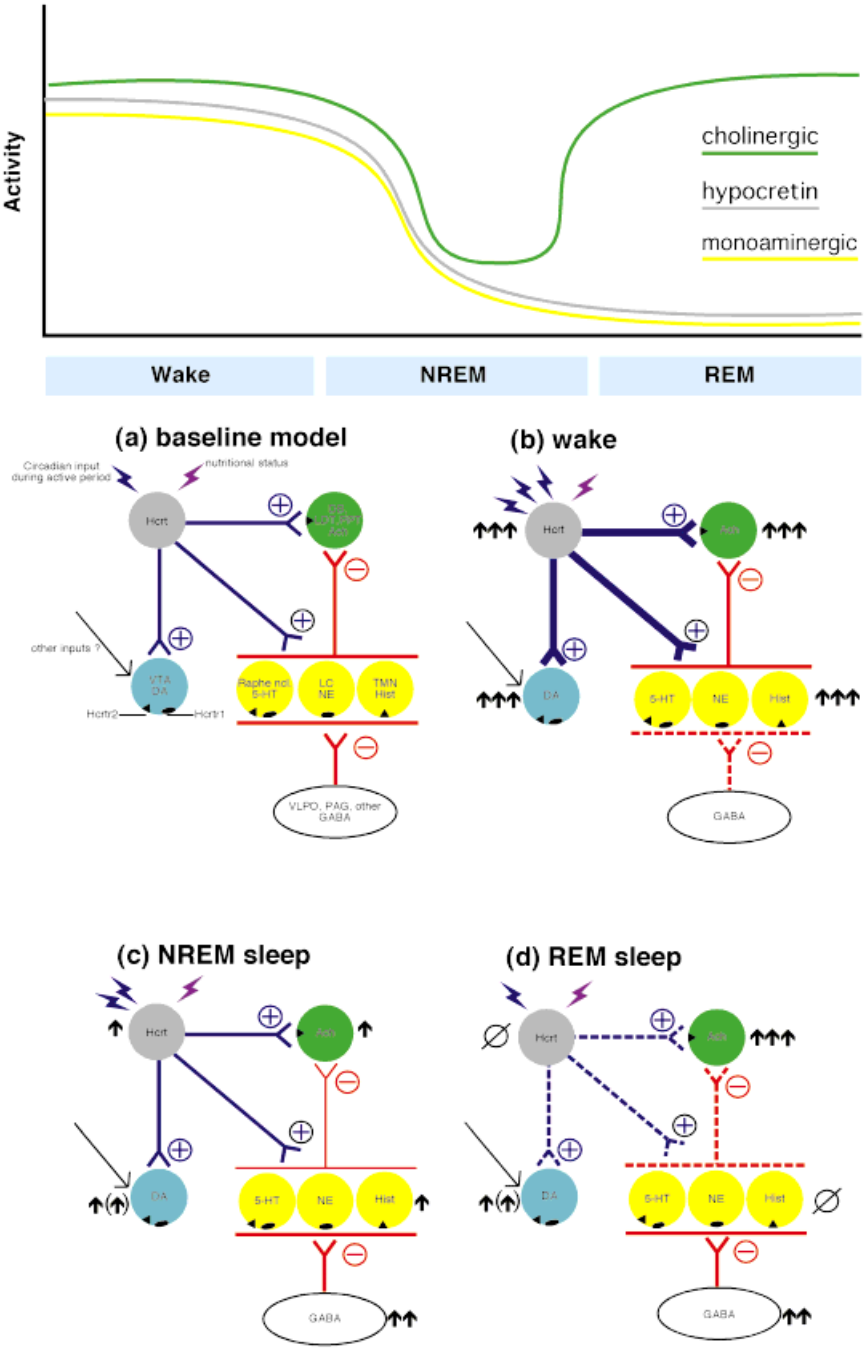

Figure 5.

weaker than those of NPY, and more similar to those of MCH and galanin.[66,70] Chronic administration of hypocretins also never leads to obesity.[67,73] Second, these effects are more pronounced when hypocretins are administered during the rest period.[67,73,75] a result that may partially reflect effects of wakefulness that would secondarily affect food intake. Third, preprohypocretin knockout mice are not lean as would be expected if a purely orexinogenic signal was removed.[42] In fact, recent data suggest that preprohypocretin knockout mice can have normal body weight with reduced food intake.[42] This last result, together with the observation of increased obesity in human narcolepsy,[79] suggest the existence of other downstream metabolic effects quantitatively more important to general body metabolism than the control of food intake.

Food intake and metabolic status regulates hypocretin systems

In contrast with these results, many experiments suggest that food and energy balance may regulate the hypocretin system under physiological conditions. Preprohypocretin mRNA transcript levels are upregulated 2.4-fold in the lateral hypothalamus during fasting or hypoglycemia.[13,80,81] Inhibitory leptin receptors have been reported on hypocretin-containing cells.[82] Hypocretin neurons are also activated during hypoglycemia.[83,84] Not only hypocretin but also hypocretin receptors expression is modulated by food deprivation.[28] Quantitative in situ hybridization after fasting revealed a time-dependent and region-specific Hcrtr1 and Hcrtr2 mRNA expression.[28] These results are consistent with the idea that hypocretin systems are activated by starvation while their activity may be reduced by satiety.

Hypocretin and other functions

Other experiments also suggest a broader role for hypocretins in the homeostatic regulation of energy metabolism, autonomic function, hormonal balance and the regulation of body fluids.[42,85] A modulation effect on luteinizing hormone-releasing hormone secretion also suggests a more minor contribution to the regulation of reproductive functions.[86,87] These effects generally result in increasing global energy consumption. In mice, an icv injection of low doses of hypocretin-1 induces an increase in the respiratory quotient without increasing activity or feeding, indicating an increased metabolic rate.[70]

Central administration of hypocretin increases drinking and preprohypocretin mRNA is upregulated when rats are deprived of water.[88] The presence of hypocretin-immunoreactive fibers in the medulla of the brainstem also indicates cardiovascular regulatory effects.[89,90] Hypocretin injections intracisternally or into the ventrolateral medulla increase mean arterial blood pressure in rats suggesting strong influences on sympathetic outflow.[91–93] Interestingly, the hypocretin system also modulates vagal tone, stimulating gastric acid secretion after icv administration.[94] Finally, icv injection of hypocretin stimulates corticosterone and adrenergic secretion while decreasing plasma growth hormone and prolactin levels.[45,95,96] Some of these effects occur after direct application onto peripheral organs, for example with hypocretin-1 stimulation of corticosterone release.[97] Subcutaneously injected hypocretin-1 also increase blood insulin and blood glucose in rats, an effect partially reconstituted by direct application onto the pancreas.[98]

Perspectives

The finding that hypocretins are absent and/or greatly diminished in the brain and CSF of narcoleptic patients opens a new area of investigation for this disabling condition. Measuring hypocretin levels in the CSF may become a standard diagnostic procedure. Of note, however, our current investigation indicates that narcolepsy can occur occasionally with normal or elevated hypocretin levels, independently of HLA status and family history, suggesting disease heterogeneity.[9,10] Finding the cause of these other rarer cases may in time further our understanding of the molecular pathway implicated in narcolepsy.

Figure 5. Hypothetical model illustrating the influence of hypocretins on the regulation of sleep. The top panel displays hypothesized changes in monoaminergic, cholinergic and hypocretinergic activity across the sleep cycle. Red and blue lines indicate inhibitory and excitatory projections, respectively. In this simplified model (a), hypocretin systems are strongly excitatory to both monoaminergic and cholinergic system but have stronger effects on monoaminergic tone. During wakefulness (b), high hypocretin tone drives high cholinergic and monoaminergic tone. During non-REM sleep (c), decreased hypocretin tone reduces monoaminergic and cholinergic activity. During REM sleep (d), the depressed monoaminergic tone is so complete that cholinergic systems are desinhibited. Note that, in our model, hypocretin activity is mostly depressed during REM sleep, differentially from the Kilduff and Peyron's model.[57] Electrophysiological studies are needed to differentiate between these hypotheses. The influence of dopamine in this model might be more complex and could involve other inputs as the role of this system in the regulation of the sleep cycle is more uncertain. Differential effects of hypocretin/monoamines on brainstem and basal forebrain cholinergic systems may also explain a differential activation of these forebrain versus brainstem cholinergic systems across the sleep cycle, as suggested by some authors. Note that the presence of Hcrtr2 on cholinergic cells has not been finally established but high Hcrtr2 concentrations are observed in regions containing cholinergic neurons (e.g. basal forebrain). Inhibitory GABAergic projections from the preoptic hypothalamus or the periaqueductal gray to monoaminergic cell groups are also likely to be critical.[58]

Most human brains have no preprohypocretin transcripts and reduced immunocytochemical staining in the perifornical area.[10,11] These results suggest either a lack of transcription or a selective loss of hypocretin-containing cells. Our histochemical studies did not detect any sign of acute inflammation using HLA-DR immunostaining[10] while another study indicated residual gliosis in four brains examined in the perifornical area.[11] This, together with the well-established HLA association in narcolepsy, suggests an autoimmune mediation of human narcolepsy with selective destruction of hypocretin-containing cells early in the course of the disorder. This hypothesis would be consistent with the complex genetic inheritance of human narcolepsy and associated peripubertal onset.[34] Postmortem studies and examination of the peripheral immune system closer to disease onset will probably be needed to establish autoimmunity. This difficult line of investigation will now be pursued with renewed enthusiasm.

Whether or not autoimmunity is involved in cell loss in narcolepsy, the finding opens direct therapeutic opportunities. All currently available treatments act downstream of hypocretins, on monoaminergic systems.[31] Supplementing hypocretins in narcolepsy using agonists with central penetration should be a better and more specific treatment for this disabling condition. Hypocretin receptors are classical G-protein-coupled receptors and are thus ideal drug targets for the pharmaceutical industry. Replacing hypocretins will, however, only treat the condition, and other approaches, for example transplantation of hypocretin-producing neurons may provide better long-term cure. This strategy is already under exploration in other disorders such as Parkinson disease and Type I diabetes.[99,100]

These discoveries have other far reaching implications in the field of sleep disorder medicine. For example, would hypocretin antagonists be effective hypnotic agents? Are hypocretins involved in other sleep disorders such as insomnia or sleep apnea (a disorder associated with obesity)? Similarly, the role of hypocretins in regulating normal sleep and body metabolism is still uncertain and worthy of further exploration. Are excitatory projections to monoaminergic cell groups truly mediating the sleep effects of hypocretins? Metabolic effects are suggested by the fact that most narcoleptic patients gain weight around disease onset.[79] Considering the stimulatory effect of hypocretins on food intake, removing an orexinogenic signal in narcolepsy should reduce body weight. Obesity in hypocretin deficiency thus most likely reflects decreased metabolism and increased sleepiness, a result consistent with the strong metabolic and sympathomimetic effects observed after hypocretin administration. This, together with the fact leptins and glucose may have effects on hypocretin cell activity, suggest complex interactions between body metabolism and sleep, as suggested by many early theories regarding the function of sleep.

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