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MINIREVIEW

The hypocretins and sleep

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The hypocretins (also called the orexins) are two neuropeptides derived from the same precursor whose expression is restricted to a few thousand neurons of the lateral hypothalamus. Two G-protein coupled receptors for the hypocretins have been identified, and these show different distributions within the central nervous system and differential affinities for the two hypocretins. Hypocretin fibers project throughout the brain, including several areas implicated in regulation of the sleep/wakefulness cycle. Central administration of synthetic hypocretin-1 affects blood pressure, hormone secretion and locomotor activity, and increases wakefulness while suppressing rapid eye movement sleep. Most human patients with narcolepsy have greatly reduced levels of hypocretin peptides in their cerebral spinal fluid and no or barely detectable hypocretin-containing neurons in their hypothalamus. Multiple lines of evidence suggest that the hypocretinergic system integrates homeostatic, metabolic and limbic information and provides a coherent output that results in stability of the states of vigilance.

Discovery of the hypocretins

Observations on humans and experimental animals with localized hypothalamic lesions led to the earliest notions about the role of the lateral hypothalamus (LH). From studying patients with encephalitis lethargica, von Economo [1] proposed that the posterior hypothalamus (including the LH) was required for maintaining the awake state. The signaling molecules and circuitry responsible for this observation remained unknown until the discoveries of the hypocretin (Hcrt) and melanin-concentrating hormone (MCH) systems.

Gautvik and colleagues [2] conducted a systematic subtractive hybridization survey aimed at identifying mRNA species whose expression was restricted to discrete nuclei within the rat hypothalamus. Among these was a species whose expression, as detected by *in situ* hybridization analyses, was restricted to the perifornical area in the dorsolateral hypothalamus [2,3] (Fig. 1). The 569 nucleotide sequence of the corresponding cDNA revealed that it encoded a 130 residue putative secretory protein with an apparent signal sequence and two additional phylogenically conserved sites for potential proteolytic maturation followed by modification of the carboxy-terminal glycines by peptidylglycine α-amidating monooxygenase [3]. These features suggested that the product of this hypothalamic mRNA served as a preprohormone for two C-terminally amidated, secreted peptides. These two peptides, 28 and 33 amino acids in length showed some similarity between each other at the C-terminus. The 33 residue peptide displayed a sequence of seven amino acids which is identical within the peptide secretin. Thus, we named the peptides hypocretins for their strict

Abbreviations
CRF, corticotropin-releasing factor; CSF, cerebral spinal fluid; DMH, dorsomedial hypothalamus; EDS, excessive daytime sleepiness; EEG, electroencephalogram; GABA, 4-aminobutyrate; GPCR, G-protein coupled receptor; Hcrt, hypocretin; HD, Huntington disease; HLA, human leukocyte antigen; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; LH, lateral hypothalamus; MCH, melanin-concentrating hormone; NREM, nonrapid eye movement; PPT, pedunculopontine tegmental nucleus; REM, rapid eye movement; SCN, suprachiasmatic nucleus, TMN, tuberomammilary nucleus.

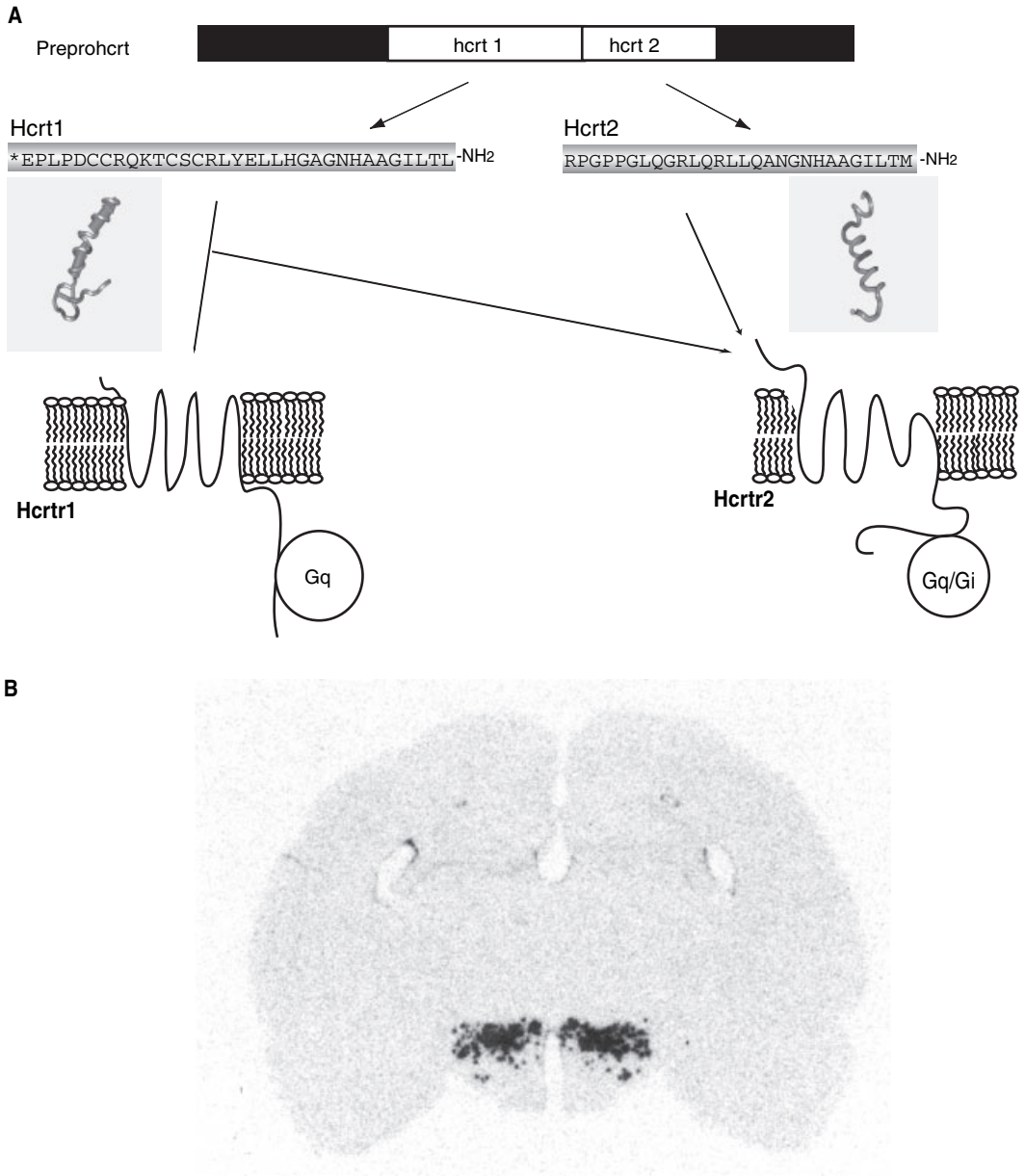

Fig. 1. (A) The hypocretins are two neuropeptides derived from the same precursor. Hcrt1 binds with similar affinity to Hcrtr1 and Hcrtr2, whereas Hcrt2 binds to Hcrtr2 with 10–100-fold higher affinity than to Hcrtr1. (B) Preprohypocretin is expressed by a few thousand neurons in the lateral hypothalamus, a brain region known to be important for homeostatic regulation.

hypothalamic expression and their similarity to the incretin neuropeptide family.

A large collaborative study to identify endogenous ligands for orphan G-protein coupled receptors (GPCRs) discovered the peptides independently [4]. This group referred to the peptides as orexins because they stimulated acute food intake when administered to rats during the daytime. In this minireview, we will refer to the peptides by their first-used name, the hypocretins, but the terms are interchangeable and are both used extensively in the large literature that has grown up around the peptides.

The detection of the two hypocretin peptides within the brain allowed the exact structures of these endogenous peptides to be determined by mass spectroscopy [4]. The sequence of endogenous Hcrt2, RPGPPG LQGRLQRLLQANGNHAAGILTM-amide, was the same as that predicted from the cDNA sequence. The N-terminus of Hcrt1 was found to correspond to a genetically encoded glutamine that was derivatized as

pyroglutamate. Hcrt1 (33 residues; *EPLPDCCRQK TCSCRLYELLHGAGNHAAGILTL-amide) contains two intrachain disulfide bonds. Human Hcrt1 is identical to the rodent peptide, whereas human Hcrt2 differs from rodent Hcrt2 at two residues [4].

Hypocretin cell bodies

A few thousand neurons highly positive for Hcrt mRNA and immunoreactivity are located between the rat fornix and the mammillothalamic tracts [2,3,5–7]. These are first detected at embryonic day E18 [8]. Beginning at E20, hypocretin antisera detect a prominent network of axons that project from these cells to other neurons in the perifornical and posterior hypothalamus. Both mRNA and peptide expression diminish after 1 year of age [9]. The human lateral hypothalamus contains 50 000–80 000 hypocretin neurons [10]. Hcrt neurons with a similar restricted hypothalamic distribution have been detected in monkey, hamster, cat, sheep, pig, chicken, various amphibians and zebrafish.

The LH contains a collection of neurons that express MCH, a peptide that has been implicated in feeding-related behavior [11]. MCH and hypocretin neurons are distinct but spatially intermingled, each set with a different topological distribution [5–7,12]. There is a nearly one-to-one correspondence between LH neurons that express the opioid receptor agonist dynorphin and the hypocretin neurons [13], and nearly all Hcrt neurons express secretogranin II [14]. Glutamate, the excitatory amino acid transporter EAAT3, and the vesicular glutamate transporters VGLUT1 and VGLUT2 are expressed by Hcrt neurons [15–19], thus, Hcrt neurons are likely to be glutamatergic. Other proteins detected in Hcrt neurons include the 4-aminobutyrate $(GABA)_A$ receptor epsilon subunit, $5\text{-}HT_{1A}$ receptor, mu opioid receptor, pancreatic polypeptide Y4 receptor, adenosine A1 receptor, leptin receptor, precursor-protein convertase, transcription factor Stat-3, and the neuronal pentraxin Narp, implicated in clustering of ionotropic glutamate receptors [12,20–27].

Hcrt projections

Projections from Hcrt-immunoreactive cell bodies are detected throughout the brain, with the highest density of terminal fields seen in the hypothalamus [3,6,7]. Hypothalamic regions receiving projections include the LH and posterior hypothalamic areas (regions of Hcrt and MCH neuronal populations), the dorsomedial hypothalamus (DMH), the paraventricular hypothalamic nucleus, and arcuate nucleus. Hcrt is reciprocally connected with neuropeptide Y (NPY) and leptin receptor-positive neurons in the arcuate nucleus [28], an area important in feeding behaviors and endocrine regulation. Hcrt neurons also make reciprocal synaptic contact with neighboring MCH neurons [29,30].

Prominent Hcrt fibers project from the LH to apparent terminal fields in many areas of the brain. Peyron and colleagues [7] referred to four Hcrt efferent pathways; dorsal and ventral ascending pathways and dorsal and ventral descending pathways. The dorsal ascending pathway projects through the zona incerta to the paraventricular nucleus of the thalamus, central medial nucleus of the thalamus, lateral habenula, substantia innominata, bed nucleus of the stria terminalis, septal nuclei, dorsal anterior nucleus of the olfactory bulb, and cerebral cortex. The ventral ascending pathway projects to the ventral pallidum, vertical and horizontal limb of the diagonal band of Broca, medial part of the accumbens nucleus, and olfactory bulb. The dorsal descending pathway projects through the mesencephalic central gray to the superior and inferior colliculi and the pontine central gray, locus coeruleus (LC), dorsal raphe nucleus, and laterodorsal tegmental nucleus. A second bundle of fibers projects through the dorsal tegmental area to the pedunculopontine nucleus, parabrachial nucleus, subcoeruleus area, nucleus of the solitary tract, parvocellular reticular area, dorsal medullary region and the caudal spinal trigeminal nucleus. This tract continues to all levels of the spinal cord [31]. The ventral descending pathway runs through the interpeduncular nucleus, ventral tegmental area, substantia nigra pars compacta, raphe nuclei and the reticular formation, gigantocellular reticular nuclei, ventral medullary area, raphe magnus, lateral paragigantocellular nucleus, and ventral subcoeruleus. The cumulative set of projections is consistent with the combined patterns of expression of the two hypocretin GPCRs. Although a large proportion of Hcrt neurons contribute projections to multiple terminal fields, various subgroups of cells make preferential contributions to particular fields [32,33]. The projection fields in humans are comparable to those in rodents [10]. The diffuse nature of Hcrt projections provided the first evidence of the potential for multiple physiological roles for the peptides.

Two hypocretin receptors

Sakurai and collaborators [4] prepared transfected cell lines stably expressing each of 50 orphan GPCRs, and then measured calcium fluxes in these cell lines in response to fractions from tissue extracts. One of these transfected cell lines responded to a substance in a

brain extract. Mass spectroscopy showed that this substance was a peptide whose sequence was later identified as that of endogenous Hcrt1. The initial orphan GPCR, Hcrtr1 (also referred to as OX1R), bound Hcrt1 with high affinity, but Hcrt2 with 100–1000-fold lower affinity. A related GPCR, Hcrtr2 (OX2R), sharing 64% identity with Hcrtr1, which was identified by searching database entries with the Hcrtr1 sequence, had a high affinity for both Hcrt2 and Hcrt1 [4]. These two receptors are highly conserved (95%) across species. Radioligand-binding studies and calcium flux measurements have shown Hcrt1 to have equal affinity for Hcrtr1 and Hcrtr2, whereas Hcrt2 has $\approx$10-fold greater affinity for Hcrtr2 than Hcrtr1 [34].

Narcolepsy is a disease of the hypocretin system

Sleep is characterized by complex patterns of neuronal activity in thalamocortical systems [35–37]. The fast, low-amplitude electroencephalogram (EEG) activity of the aroused state is replaced by synchronized high-amplitude waves that characterize slow wave sleep. This pattern develops further into high-frequency waves that define paradoxical, or (rapid eye movement) REM, sleep. Switching among these states is controlled in part by the activities of neurons in the hypothalamic ventrolateral preoptic nucleus and a series of areas referred to as the ascending reticular activating system, which is distributed among the pedunculopontine and laterodorsal tegmental nuclei (PPT–LDT), LC, dorsal raphe nucleus and tuberomammilary nucleus (TMN), and regulates cortical activity and arousal [38]. The balance struck among the various phases of sleep and the rapid transitions from one phase to the next are determined by requirements for wakeful activities, homeostatic pressures for sleep and circadian influences [39,40].

The first case of human narcolepsy was reported in 1877 by Westphal, and the sleep disorder acquired its name from Gélineau in 1880. Narcolepsy affects around 1 in 2000 adults, appears between the ages of 15–30 years, and shows four characteristic symptoms: (a) excessive daytime sleepiness with irresistible sleep attacks during the day; (b) cataplexy (brief episodes of muscle weakness or paralysis precipitated by strong emotions such as laughter or surprise); (c) sleep paralysis, a symptom considered to be an abnormal episode of REM sleep atonia, in which the patient suddenly finds himself unable to move for a few minutes, most often upon falling asleep or waking up; and (d) hypnagogic hallucinations, or dream-like images that occur at sleep onset. These latter symptoms have been proposed as pathological equivalents of REM sleep. The disorder is considered to represent a disturbed distribution of sleep states rather than an excessive amount of sleep.

Studies with monozygotic twins have shown that narcolepsy is weakly penetrant; in only 25% of cases does the monozygotic twin of an affected individual also develop the disorder. Sporadic narcolepsy (which accounts for 95% of human cases) is highly correlated with particular class II human leukocyte antigen (HLA)-DR and -DQ histocompatibility haplotypes in about 90% of patients, but most people with these haplotypes are not narcoleptic [41]. Because many autoimmune disorders are HLA-linked and because of the late and variable age of disease onset, narcolepsy has long been considered a probable autoimmune disorder, but the targets of the immune attack were not known (see below).

Both sporadic narcolepsy and heritable narcolepsy are observed in dogs, and the symptoms resemble those exhibited by human narcoleptics. The first link between the hypocretins and narcolepsy came from genetic linkage studies in a colony of Doberman Pinschers, in which narcolepsy was inherited as an autosomal recessive, fully penetrant phenotype. Fine mapping and cloning of the defective canine narcolepsy gene showed it to be the gene that encodes the hypocretin receptor, *HCRTR2* [42]. The mutation in the Doberman lineage is an insertion of a short interspersed repeat (SINE element) into the third intron of *HCRTR2*, which causes aberrant splicing of the Hcrtr2 mRNA (exon 4 is skipped) and results in a truncated receptor protein. In cells that have been transfected with the mutant gene, the truncated Hcrtr2 protein does not properly localize to the membrane and therefore does not bind its ligands [43]. Analysis of a colony of narcoleptic Labradors revealed that their *HCRTR2* gene contained a distinct mutation that resulted in the skipping of exon 6, also leading to a truncated receptor protein. A third family of narcoleptic Dachshunds carries a point mutation in *HCRTR2*, which results in a receptor protein that reaches the membrane but cannot bind the hypocretins. Genetically narcoleptic dogs have increased cerebral spinal fluid (CSF) levels of Hcrt, which diminishes until symptoms appear at 4 weeks, then increases [44]. Administration of immunoglobulins or immunosuppressive/anti-inflammatory drugs doubles time to symptom onset and severity of symptoms, suggesting that the *HCRTR2* deficits alone are not sufficient to elicit all of the symptomology initiated by the loss-of-function mutations [45,46].

In knockout mice in which the hypocretin gene was inactivated by homologous recombination in embryonic stem cells, continuous recording of behavior revealed periods of ataxia, which were especially frequent during the dark period [47]. EEG recordings showed that these episodes were not related to epilepsy, and that the mice suffered from cataplectic attacks, a hallmark of narcolepsy. In addition, the mutant mice exhibited increased REM sleep during the dark period as did their wildtype littermates, and their EEGs showed episodes of direct transition from wakefulness to REM sleep, another event that is unique to narcolepsy. Waking and non-REM sleep bouts were brief, with more transitions among all three states, suggestive of a behavioral state instability with low state transition thresholds [48]. Mice with an inactivated *HCRTR2* gene have a milder narcoleptic phenotype than the *HCRT* knockouts; *HCRTR1* knockouts exhibit only a sleep fragmentation phenotype, whereas double *HCRTR1* and *HCRTR2* mutants recapitulate the full *HCRT* knockout phenotype [49], suggesting that signaling through both receptors contributes to normal arousal, although the role of *HCRTR2* is greater than that of *HCRTR1*. Similar observations were made in rats in which the hypocretin neurons of the lateral hypothalamus were inactivated by saporin targeting [50], although in this model, cataplexy was not observed. However, in mice [51] or rats in which the hypocretin neurons are ablated due to the expression of the toxic ataxin-3 fragment from the Hcrt promoter, Hcrt neurons are lost at 17 weeks, and the hallmarks of narcolepsy ensue, including episodes of muscle atonia and loss of posture resembling cataplexy [52].

Nishino and colleagues [53] studied hypocretin concentrations in the CSF of healthy controls and patients with narcolepsy by radioimmunoassay. In control CSF, hypocretin concentrations were highly clustered, suggesting that tight regulation of the substance is important. However, of nine patients with narcolepsy, only one had a hypocretin concentration within the normal range. One patient had a greatly elevated concentration, while seven patients had no detectable circulating hypocretin. In an expanded study, hypocretin was undetectable in 37 of 42 narcoleptics and in a few cases of Guillain–Barré syndrome [54]. CSF hypocretin was in the normal range for most neurological diseases, but was low, although detectable, in some cases of central nervous system infections, brain trauma and brain tumors. Low CSF hypocretin concentrations have also been measured in a patient with acute disseminated encephalomyelitis presenting similarities to von Economo's encephalitis lethargica, which returned to the normal range as daytime sleepiness was reduced [55], and in two patients with Prader–Willi syndrome accompanied by excessive daytime sleepiness (EDS) [56].

Peyron, Thannikal and their teams of collaborators [57,58] found that, in the brains of narcolepsy patients, they could detect few or no hypocretin-producing neurons. Whether the hypocretin neurons are selectively depleted, as is most likely, or only no longer expressing hypocretin, is not yet known, although one report showed some indications of gliosis [58]. The codistributed MCH neurons were unaffected. Furthermore, a single patient with a non-HLA-linked narcolepsy carries a mutation within the hypocretin gene itself. The mutation results in a dominant negative amino acid substitution in the secretion signal sequence that sequesters both the mutant and heterozygous wildtype hypocretin nonproductively to the smooth endoplasmic reticulum [57]. Amino acid substitutions in Hcrtr2 have been found in two EDS patients and one Tourette's syndrome patient; in each case the variant receptor exhibited reduced response to high concentrations of Hcrt [59].

These findings leave no doubt as to the central role of the hypocretin system in this sleep disorder. Because most cases are sporadic, mutations in the hypocretin gene or those for its receptors can account for no more than a small subset of the human narcolepsies. The HLA association, loss of neurons with signs of gliosis, and age of disease onset are consistent with autoimmune destruction of the hypocretin neurons accounting for the majority of narcolepsy [60], although a nonimmune-mediated degenerative process has not been ruled out. For example, studies of hypothalamic slice cultures have revealed that Hcrt neurons are more sensitive to excitotoxic injury elicited by quinolonic acid than are neighboring MCH neurons, suggesting that glutamatergic signaling could contribute to their selective loss [61]. Interestingly, hypocretin cell loss has recently been described in Huntington disease (HD) patients [62] and in R6/2 mice, which expresses exon 1 of the human mutant *HD* gene with 150 CAG repeats [63]. In advanced stages, these mice display several clinical features reminiscent of HD but relatively little cell death. Thus, Hcrt neurons may have a very low threshold for neuronal apoptosis caused by a variety of environmental stimuli. The narcolepsies as a group are probably a collection of disorders that are caused by defects in the production or secretion of the hypocretins or in their signaling, and these could have numerous genetic, traumatic, viral and/or autoimmune causes.

Measurement of Hcrt1 in human CSF provides a reliable diagnostic for sporadic narcolepsy. Although

local release of Hcrt at its targets within the brain varies during the 24 h day, CSF Hcrt1 levels are relatively stable [64,65]. In a study of 274 patients with various sleep disorders (171 with narcolepsy) and 296 controls, a cutoff value of 110 $pg{\cdot}mL^{-1}$ (30% of the mean control values) was the most predictive of narcolepsy [66]. Most narcolepsy patients had undetectable levels, while a few had detectable, but very reduced levels. The assay was 99% specific for narcolepsy.

Hcrt1 has also been detected in plasma, although its origin remains to be demonstrated, and high nonspecific background immunoreactivities partially mask its detection. Decreased levels of plasma Hcrt1 were measured in narcoleptic patients using high performance liquid chromatography separation to confirm that the signal included genuine Hcrt1 [67]. Reductions in daytime plasma Hcrt have been detected in patients with obstructive sleep apnea hypopnea syndrome [68,69].

Is narcolepsy an autoimmune disorder?

Multiple etiologies may cause narcolepsy. When with typical cataplexy (induced by laughter), the vast majority of narcolepsy patients are HLA-DQB1*0602 positive, have no detectable Hcrt1 in their CSF, and a disease onset between 10 and 30 years of age [70]. A selective autoimmune destruction of the hypocretin neurons is the most likely cause in these patients. This hypothesis is supported by the tight HLA association and the postmortem findings as presented by Thannickal *et al*. [58], but direct evidence for this theory is lacking as of yet. For these patients the development of narcolepsy seems to involve environmental factors acting on a specific genetic (HLA) predisposition. This is supported by the $\approx 30\%$ concordance among monozygotic twins, and the higher risk for narcolepsy and EDS in first-degree family members of these patients. First degree family members have a risk of $\approx 2\%$ for narcolepsy and 2–4% for atypical EDS.

A definite autoimmune cause, with undetectable CSF Hcrt1, has been identified in only one uncommon disorder; the anti-Ma paraneoplastic syndrome [71]. Patients with this disorder develop autoantibodies against Ma proteins and, consequently, encephalitis that predominates in the limbic system, hypothalamus and brainstem [72]. Importantly, these patients always have additional neurological symptoms. Other evidence that an autoimmune process can lead to hypocretin deficiency comes from patients with acute disseminated encephalomyelitis and patients with steroid-responsive encephalopathy associated with Hashimoto's thyroiditis who showed a decrease in CSF Hcrt1 during their disease [73,74].

Recent data also support an autoimmune origin for narcolepsy. Sera from nine narcoleptic patients were transferred to mice and the effect was monitored on the response of smooth muscle contraction to cholinergic stimulation. IgG from all narcolepsy patients enhanced the bladder contractile responses to charbachol, compared with control IgG [75].

Together, the wealth of experimental and clinical data on narcolepsy support the concept that narcolepsy-cataplexy is generally a disease of the hypocretinergic system.

Given that most human narcolepsy is sporadic and results from depletion of Hcrt-producing neurons, replacement therapies can be envisioned. Small molecule agonists of the hypocretin receptors might have therapeutic potential for human sleep disorders and might be preferable to the traditionally prescribed amphetamines. Intracerebroventricular administration of Hcrt1 to normal mice and dogs strongly promotes wakefulness [76,77]. The effect is predominantly mediated by Hcrtr2, because the same dose of Hcrt1 has no effect in Hcrtr2-mutated narcoleptic dogs [76,77]. Transgenic expression of preprohypocretin in the brains of mice in which the Hcrt neurons were ablated prevented cataplexy and REM abnormalities, and central administration of Hcrt1 to Hcrt neuron-ablated mice prevented cataplexy and increase wakefulness for 3 h [78]. Hcrt1 has low penetrance of the blood–brain barrier, so a centrally penetrable agonist will need to be devised.

Hypocretin and arousal circuity

Because narcolepsy is the consequence of a defective hypocretin system, it follows that the dominant role of the system is in maintenance of the waking state and suppression of REM entry, and data about the hypocretins give insights as to how this is accomplished. The hypocretin neurons project to various brainstem structures of the ascending reticular activating system, which express one or both of the hypocretin receptors and have been implicated in regulating arousal (Fig. 2). The noradrenergic neurons of the LC, the serotonergic neurons of the dorsal raphe and the histaminergic neurons of the TMN are all so called REM-off cells; each group fires rapidly during wakefulness, slowly during slow wave sleep, and hardly at all during REM [38,79]. Each of these structures sends projections to a diverse array of targets in the forebrain, and their firing stimulates cortical arousal. The activity state of these groups of aminergic neurons is one of the features that distinguishes wakefulness from REM. Additionally, and importantly, the hypocretin neurons

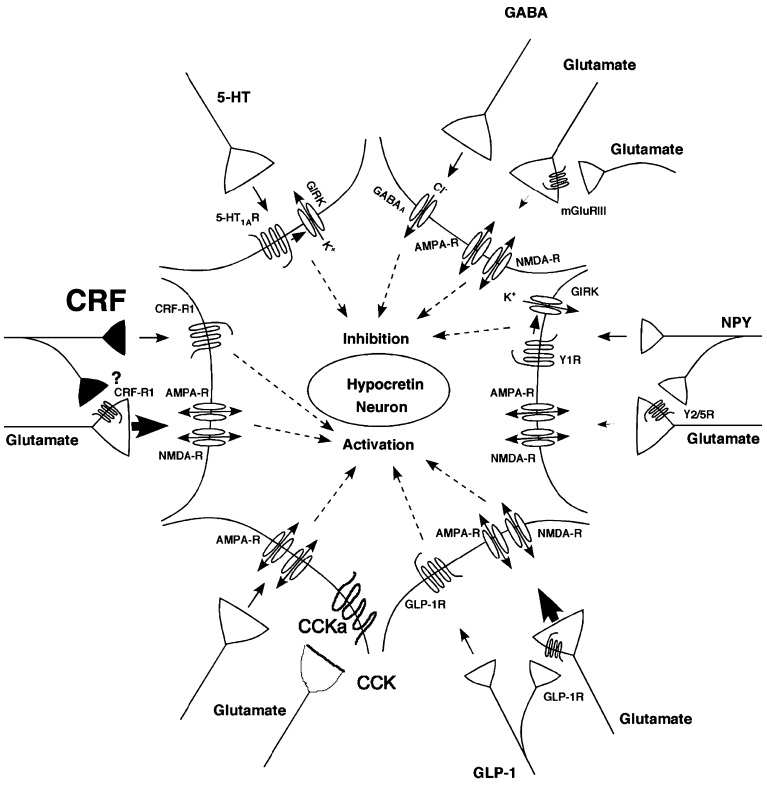

Fig. 2. Multiple inputs exert excitatory and inhibitory action on hypocretin neurons (modified from [33]). Electrophysiologically identified signals that depolarize or hyperpolarize Hcrt cells include glucose, leptin, neuropeptide Y (NPY), peptide YY (PYY), corticotropin-releasing factor (CRF), melanin-concentrating hormone (MCH), nociceptin and cholecytokinin (CCK). Hypocretin neurons integrate this information to provide a coherent output that result in the stability of arousal networks.

project to other brain areas that have been implicated in arousal. For instance, the hypocretins, acting through Hcrtr2, excite cholinergic neurons of the basal forebrain, which produce the cortical acetylcholine characteristic of the desynchronized EEG that is associated with wakefulness and REM [80]. Direct infusion of the hypocretins into the basal forebrain produces dramatic increases in wakefulness [81–83].

Among the neurons of the perifornical lateral hypothalamus, 53% increase their firing rates during both wakefulness and REM, but decrease their activities during slow wave sleep [84]. An additional 38% of the neurons in this area are activated only during the awake phase recordings of hypocretin neurons. Recent *in vivo* electrophysiological studies with electrophysiologically [85] and anatomically [86] identified neurons effectively demonstrate that Hcrt cells belong to the latter group; that is, they are REM-off. Hcrt cells discharge during active waking, when postural muscle tone is high in association with movement, decrease discharge during quiet waking in the absence of movement, and virtually cease firing during sleep, when postural muscle tone is low or absent. Increased discharge of Hcrt cells is observed immediately before waking [85,86]. The off state is most likely established and maintained by inhibition by GABA interneurons, as infusion of the $GABA_A$ antagonist bicuculline into the LH of spontaneously sleeping rats increased both wakefulness and c-*fos* expression by Hcrt neurons [87].

Output of hypocretin neurons

The noradrenergic loop

The densest projection of Hcrt fibers terminates in the locus coeruleus area, the main site of noradrenergic transmission. Thus, this system was one of the first targets of the hypocretinergic system to be analyzed. Noradrenergic neurons of the locus coeruleus are active during wakefulness, display low activity during slow wave sleep, are silent during REM sleep, and are thought to be critical for the alternation of the REM-nonrapid eye movement (NREM) sleep [79]. Most of the LC neurons express Hcrtr1 but not Hcrt2 [88]. Local administration of Hcrt1 in the LC increases wakefulness and suppresses REM sleep in a dose-dependent manner, and this effect can be blocked by antisera that prevent binding of Hcrt to its receptors [88]. Application of Hcrt to slices of the locus coeruleus increased the firing rate of noradrenergic neurons, possibly by decreasing the after-hyperpolarization current [27]. Interestingly, recent data using retrograde tracing has recently shown that the suprachiasmatic nucleus (SCN) of the hypothalamus is a target of

noradrenergic LC neurons, via the DMH. In addition, lesion studies confirmed that the DMH is a relay in this circuit [89]. This noradrenergic loop connects the circadian output of the suprachiasmatic nucleus to the lateral hypothalamus via the DMH. Also, direct connections between SCN and Hcrt neurons have been described [15]. The LC controls the activity of Hcrt neurons directly by inhibiting Hcrt firing [17], and indirectly via the DMH.

Brainstem cholinergic nuclei

The major cholinergic input to the thalamus is from the laterodorsal tegmental nucleus (LDT) and the adjacent pedunculopontine tegmental nucleus (PPT). These neurons act on the thalamocortical network to provoke the tonic activation subtending both sensory transmission and cortical activation during arousal [90]. Considerable evidence has also indicated that mesopontine cholinergic nuclei also play a role in generating REM sleep, notably by stimulating the medial pontine reticular formation. Thus, cholinergic neurons in LDT and PPT, by promoting either EEG desynchronization and wakefulness or REM sleep, play a key role in regulating the vigilance state [91]. Interestingly, the wide projection of the hypocretinergic system throughout the brain includes the locus coeruleus, the raphe nuclei, the basal forebrain and the mesopontine cholinergic system [7]. Moreover, Hcrt receptor mRNAs have been found in these mesopontine cholinergic nuclei [92–94]. Hcrt peptides excite cholinergic neurons in the LDT [95,96], an effect already described in both locus coeruleus noradrenergic neurons [27] and dorsal raphe nucleus [97]. Injection of Hcrt1 into the rat LDT increases wakefulness at the expense of NREM sleep [80].

Histamine

The histaminergic system resides in the TMN [98] and commands general states of metabolism and consciousness, including the sedative component of anesthesia (reviewed in [99]). Histaminergic terminals project throughout the brain, with dense fibers innervating the cerebral cortex, amygdala, substantia nigra, striatum and other monoaminergic nuclei [100]. Lesions of the TMN cause hypersomnia and H1 receptor antagonists increase slow wave sleep. Moreover, mice lacking histidine decarboxylase, the biosynthetic enzyme of histamine, show deficits in attention and waking [101]. H3-deficient knockout mice show deficits in sleep architecture and exhibit excessive muscle activity reminiscent of REM behavior disorder.

Interestingly, Hcrt-containing neurons densely innervate and excite histaminergic neurons in the TMN, most likely via Hcrtr2 receptors [102–104]. Hcrt-induced depolarization of TMN neurons seems to be associated with a small decrease in input resistance and was probably caused by activation of both the electrogenic Na^+/Ca^{2+} exchanger and a Ca^{2+} current [103]. Also, histaminergic cells project back to Hcrt neurons. However, the type of histamine receptors expressed in Hcrt neurons and the effect of histamine on the excitability of Hcrt neurons are unknown.

Cerebral cortex

Hypocretin neurons extend projections throughout the cerebral cortex [7]. Hypocretin directly stimulates thalamocortical synapses in the prefrontal cortex [105]. However, Hcrt1 can only depolarize cortical neurons postsynaptically in layer VIb [106]. This depolarization results from an interaction with Hcrtr2 receptors and depends on the closure of a potassium conductance. In addition to the thalamocortical projection, hypocretin projections may thus be involved in modulating corticocortical projections to promote widespread cortical activation. Hypocretins may also enhance cortical activation indirectly by increasing norepinephrin release [107]. Interestingly, *in vitro* recordings have demonstrated that Hcrt1 can induce hippocampal longterm potentiation [108]. Pharmacological analysis revealed that Hcrt-induced hippocampal longterm potentiation requires coactivation of ionotropic and metabotropic glutamatergic, GABAergic, as well as noradrenergic and cholinergic receptors. Hcrt may thus be involved in regulating the threshold and weight of synaptic connectivity, providing a mechanism for integration of multiple transmitter systems [108].

Afferents to Hcrt neurons

Which signals then regulate the activity of hypocretin neurons? Electrophysiological studies on Hcrt neurons, identified in slice culture by their selective transgenic expression of green fluorescent protein and confirmed by appropriate agonists and antagonists, demonstrate that they are hyperpolarized via the action of glutamate (probably originating from local glutamatergic interneurons) [17] acting at group III metabotropic receptors [109].

Multiple peptidergic systems appear to interact with hypocretin cells in the lateral hypothalamus. NPY (from arcuate neurons) acting at Y1 receptors depolarize Hcrt cells coupled to an inwardly rectifying potassium channel [110]. Hcrt cells are depolarized by glucagon-

like peptide (from the brainstem) acting through the GLP-1 receptor via a nonselective cation conductance [111]. Hcrt neurons also respond to norepinephrin, although it is unclear whether this response is depolarizing or hyperpolarizing [112]. Corticotropin-releasing factor (CRF) has been shown to depolarize Hcrt neurons through CRF receptor 1 (CRFR1) receptors and hypocretin neurons in CRFR1 deficient animals fail to get activated upon stress [33] (Fig. 2). Recently, cholecystokinin (CCK) has been shown to activate Hcrt neurons through CCK A receptors [113]. Other wake-promoting peptides, such as the newly described neuropeptide S [114], may also interact with Hcrt cells.

In addition to these inputs, demonstrated electrophysiologically, other stimuli have been shown to modulate the activity of hypocretin cells. Hypocretin levels fluctuate circadianly, being highest during waking, and peptide concentrations increase as a consequence of forced sleep deprivation [64,65,115], suggesting that the hypocretins and the activity of the hypocretin neurons serve as pressures that oppose sleep. Interestingly, the amplitude of the circadian oscillation of hypocretin levels is decreased in patients with clinical depression, and treatment with the antidepressant sertraline partially restores the circadian oscillation observed in control subjects [65]. In the absence of environmental light cues, circadian cycling of Hcrt persists, but ablation of the SCN abolished cycling and reduced Hcrt in CSF [116,117].

Multiple forms of stress, including restraint stress and food deprivation, have been shown to stimulate the activity of hypocretin-containing cells [118]. This increase in Hcrt activity may be mediated through direct activation of the CRF system [33].

Hypocretins integrate arousal, feeding behavior and motivation

Hcrt neurons receive inputs from diverse neurotransmitter systems, including noradrenergic, serotonergic, histaminergic and cholinergic afferents. These cells also receive information from other peptidergic systems (e.g. melanin concentrating hormone (MCH), proopiomelanocortin (POMC), NPY, CRF, glucagon-like peptide (GLP)) and from metabolic signals (glucose, ghrelin and leptin). All these, possibly conflicting, signals may be integrated in Hcrt cells to provide a coherent output that results in the stability of arousal networks. The activity of hypocretin cells may define the state of vigilance by providing the appropriate cues to the main transmitters that drive cortical excitability. Lack of hypocretin cells in patients with narcolepsy results in uncoordinated and uninvited sleep episodes.

The hypocretin peptides also have diverse effects on brain reward and autonomic systems related to stress that serve to increase motivated behaviors, among these feeding. Recent studies in mice depleted of Hcrt neurons demonstrate that the hypocretinergic system is important for the increased arousal associated with food deprivation. Numerous other studies provide evidence that the hypocretins modulate different aspects of the consummatory behaviors. The effect of the hypocretin peptides on these behaviors is probably counterbalanced by other peptidergic systems, such as MCH.

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