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A Commentary on the Neurobiology of the Hypocretin/Orexin System

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Hypocretins/orexins are rapidly emerging as functionally important neurotransmitters. Two related neuropeptides (Hcrt-1/OXA, Hcrt-2/OXB) encoded by the same precursor gene and two G-protein coupled receptors (Hcrtr1/OXR1, Hcrtr2/OXR2) are currently known. Hypocretin-containing cells are discretely localized within the perifornical hypothalamus but have widespread projections, with generally excitatory postsynaptic effects. Dense excitatory projections to all monoaminergic cell groups have been reported. A major emerging function for this system is likely to be the regulation of sleep. Alterations in hypocretin neurotransmission causes the sleep disorder narcolepsy in mice, dogs and humans. Effects on appetite, neuroendocrine and energy metabolism regulation are also suggested by other studies. Hypocretins are uniquely positioned to link sleep, appetite and neuroendocrine control, three behaviors of major importance in psychiatry. The potential role of this system in regulating the sleep cycle, modulating wakefulness at selected circadian times and in mediating the deleterious effects of sleep deprivation is discussed.

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Rarely in the history of medicine has scientific discovery moved so quickly from the report of a neurotransmitter system to a disease pathophysiology (Mignot 2001). In 1998, two groups independently identified the same neuropeptide system and called the molecules "hypocretins" and "orexins" respectively (de Lecea et al. 1998; Sakurai et al. 1998). One of the most striking findings of these initial studies was the discrete localization of hypocretin-containing cells within a very discrete region of the lateral hypothalamus. This led one group to call this peptide system "hypocretin", a term derived from *hypo*thalamus and se*cretin* (a weak—and contested—homology with secretin was noted by these authors). The established role of the perifornical region in the regulation of appetite, together with the observation that intracerebroventricular injections of the peptides induced food intake in rats, led the other group to coin the term "orexin" (orexis=appetite) for this system.

The discovery in 1999 that canine narcolepsy was caused by mutations in the Hypocretin receptor 2 (Hcrtr2) gene is shifting the research emphasis from appetite control to sleep regulation (Lin et al. 1999). This finding was followed by the observation that hypocretin knockout mice have sleep and behavioral abnormalities reminiscent of narcolepsy (Chemelli et al. 1999). Other studies have indicated dense projections to all monoaminergic cell groups and wake-promoting effects of hypocretins when administered centrally (Hagan et al. 1999). More recently, clinical studies have shown that most patients with narcolepsy have undetectable hypocretins in the CSF (Nishino et al. 2000) and a striking decrease in hypocretin immunoreactivity and transcript levels in the perifornical hypothalamus (Peyron et al. 2000; Thannickal et al. 2000). The most fre-

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quent cause of human narcolepsy is now known to be hypocretin deficiency, most probably as the result of an autoimmune attack against hypocretin-containing cells. In this commentary, I will briefly review the current knowledge regarding this system, speculate on its possible function in sleep regulation and discuss the potential importance of this system for neuropsychiatry.

HYPOCRETIN/OREXINS: NEURONATOMY AND RECEPTOR SYSTEMS

The description of this system has been the object of multiple recent reviews (Kilduff and Peyron 2000; Sutcliffe and de Lecea 2000; Hungs and Mignot 2001; Overeem et al. 2001; Willie et al. 2001) and will only be briefly outlined. Two biologically active peptides encoded by a single two-exon precursor gene, the preprohypocretin (Hcrt) locus, have been described. The precursor gene contains a signal peptide sequence, followed by a first active peptide, hypocretin-1 (or orexin-A), a second active peptide, hypocretin-2 (or orexin-B) and a C-terminal section of unknown biological activity. Endopeptidic cleavage occurs at typical dibasic residue sequences located between the hypocretin peptides and at the C-terminal of the hypocretin-2 sequence. The hypocretin-1 and hypocretin-2 regions but not the C-terminal region of the precursor are conserved across species, suggesting biological importance. Both Hypocretin-1 and hypocretin-2 are C-terminal amidated, a feature essential to biological activity and receptor binding. Hypocretin-1 is also known to be transamidated in the N-terminal region to create a cyclic pyroglutamyl residue and has two disulfide bounds. These features may explain the higher potency of hypocretin-1 in most pharmacological experiments, generally believed to be due to the better stability *in vivo*.

Neuroanatomical studies indicate that while all hypocretin-containing cells are discretely located within the hypothalamus, projections are widespread and include not only the hypothalamus but also cortex, basal forebrain, various limbic structures, the thalamus, most of the brainstem and the spinal cord. Remarkably dense projections with direct synaptic contact were observed in the locus coeruleus and all monoaminergic cell groups. Two receptors, Hcrtr1 (or OX1R) and Hcrtr2 (OX2R) are known to date for this system. Both are 7-transmembrane G-protein coupled receptors encoded by seven exons. The two receptors have high homology with each other and are more distant relatives of the Y2 neuropeptide Y receptor. Confusingly, Hcrtr1 has 10–100-fold higher affinity for hypocretin-1 versus hypocretin-2 while Hcrtr2 binds both neuropeptides with similar affinity. One of the most interesting features of this two-receptor system is the differential neuroanatomical distribution of Hcrtr1 and Hcrtr2, best summarized in Marcus et al. (2001). Most strikingly, the noradrenergic locus coeruleus is densely packed with Hcrtr1 but does not contain Hcrtr2 while the histaminergic tuberomammillary nucleus contains Hcrtr2 but not Hcrtr1 receptors. The serotoninergic raphe magnus and dopaminergic ventral tegmental area and substantia nigra contain both receptor subtypes.

The downstream molecular pathways mediating hypocretin receptor function are still uncertain. In all direct electrophysiological experiments reported to date, hypocretins are highly excitatory peptides. This is especially well established in the locus coeruleus, a structure with Hcrtr1 (Hagan et al. 1999; Sutcliffe and de Lecea 2000) . Similar excitatory effects have been reported on the raphe nucleus, substantia nigra and tuberomammillary nucleus (Hungs and Mignot 2001; Haas, personal communication). *In vitro*, both Hcrtr1 and Hcrtr2 can couple to Gq and mobilize intracellular calcium. Most investigators thus now believe Hcrtr1 to be excitatory in all cases while the case is still debated for Hcrtr2. Indeed, recent experiments suggest that Hcrtr2 may couple with either Gq or Go/Gi in some cases. Using isolated POMC neurons and fura-2 fluorescence imaging, Yada et al., have shown decreased intracellular calcium after application of hypocretin-1 and hypocretin-2, an effect blocked by pertussis toxin (Yada, personal communication). Other authors have also suggested the existence of Hcrtr2 inhibitory autoreceptors on hypocretin neurons using similar techniques. The finding that tuberomammillary nucleus neurons are strongly excited by hypocretin (Haas, personal communication) indicate probable Hcrtr2 excitatory effects at least on histaminergic transmission.

The differential expression of Hcrtr1 versus Hcrtr2 may explain the primary role of Hcrtr2 in the mediation of the narcolepsy phenotype in narcoleptic dogs. Hypocretin receptor knockout mice have now been generated and studied. In agreement with the canine data, Hcrtr2 $-/-$ animals were shown to have episodes of behavioral arrest reminiscent of cataplexy and/or REM onset sleep. Interestingly however, Hcrtr2 $-/-$ were less affected than hypocretin ligand knockout animals (Tokita et al. 2001). Furthermore, Hcrtr1 $-/-$ mutated animals were found to have mild sleep abnormalities but no behavioral arrest episodes (Kisanuki et al. 2000). This indicates that Hcrtr1 is a strong modifying narcolepsy locus and may have some sleep regulatory effects in mice.

CLINICAL ASPECTS OF THE HUMAN SLEEP DISORDER NARCOLEPSY

Narcolepsy is associated with severe daytime sleepiness and a complete disorganization of the sleep/wake cycle (Overeem et al. 2001). A major abnormality in narcolepsy is the inability to maintain wakefulness or sleep for extended periods of time. Napping typically relieves sleepiness temporarily and patients often have

difficulties maintaining sleep at night. Other symptoms include cataplexy (muscle atonia triggered by emotions, typically laughing and anger; see http://www.med.stanford.edu/school/Psychiatry/narcolepsy/), sleep paralysis (inability to move at sleep onset or upon awakening) and hypnagogic hallucinations (dream-like hallucinations at sleep onset or during sleep attacks). These last symptoms are believed to be abnormal events of dissociated REM sleep. Of those symptoms, only cataplexy is highly specific of narcolepsy. Severity for this symptom varies from very mild (knees buckling when laughing, a few times per month) to disabling (several times per day, with complete atonia). Of note, human narcolepsy is also often associated with periodic limb movements (nocturnal myoclonus) during sleep (Overeem et al. 2001), REM behavior disorder and moderate obesity (Schuld et al. 2000).

DIAGNOSIS OF NARCOLEPSY

Human narcolepsy is not a rare condition. When only cases with cataplexy are considered, 0.05% of the population is affected in the United States (Mignot 1998), a prevalence similar to multiple sclerosis. Diagnosis is generally performed using a 4–5 nap test called the multiple sleep latency test (MSLT). In this test, patients with narcolepsy typically exhibit a short mean sleep latency ($\leqslant$8 min) and more than two transitions into REM sleep. This test is performed after a night in the laboratory where nocturnal sleep is studied to exclude obstructive sleep apnea or other more frequent sleep disorders. The prevalence of milder cases, including unexplained sleepiness documented by the MSLT, without cataplexy (with or without other ancillary symptoms such as sleep paralysis and hypnagogic hallucinations) is unknown. The MSLT is especially useful to objectively document daytime sleepiness and justify future life-time treatment with amphetamine-like stimulants.

PSYCHOSOCIAL IMPACT OF NARCOLEPSY

The psychosocial impact of narcolepsy is severe, especially when the disorder is treated late in the course of the disease. Well-controlled studies have shown that the impact of the disease is as severe as that of epilepsy as a reference condition. Narcolepsy typically starts during adolescence (15–25 years old), at a time critical to normal development, interpersonal relationships and proper schooling. Social isolation and professional difficulties are common but patients have normal intelligence. Patients with narcolepsy are more frequently overweight, an abnormality that probably reflect decreased energy metabolism and activity. They are also frequently clinically depressed, a symptom often masked by the pharmacological treatments of their symptoms. Support groups and encouraging discussions with other patients are often helpful.

GENETIC ASPECTS OF HUMAN NARCOLEPSY

Human narcolepsy is a multigenic, environmentally influenced disorder (Mignot 1998). Approximately 25–35% of reported monozygotic twins are concordant for the disorder. Most cases do not have a familial history but first degree relatives have a 20–40-fold increase risk of developing narcolepsy-cataplexy. Most strikingly, narcolepsy is tightly associated with HLA-DR2 and DQB1*0602, suggesting a possible autoimmune etiology. Complex HLA association in narcolepsy have also been recently reported (Mignot et al. 2001).

TREATMENT OF HUMAN NARCOLEPSY

Current treatments are purely symptomatic and partially effective (Nishino and Mignot 1997; Overeem et al. 2001). Behavioral modifications such as scheduled napping and work adjustments are helpful but rarely sufficient. Pharmacological treatment is needed in over 95% of the cases. Sleepiness, the most disabling symptom in narcolepsy, is treated with amphetamine-like stimulants or the newer compound modafinil. These compounds act by inhibiting dopamine reuptake (e.g. modafinil) and/or stimulating dopamine release (e.g. dextroamphetamine) (Nishino et al. 1998; Wisor et al. 2001). Amphetamine-like stimulants are generally more efficacious than modafinil but are less specific pharmacologically and produce more side effects. Amphetamine-like compounds are more difficult to prescribe (triplicate, scheduled drugs) and are now more often used as second-line therapeutic agents unless cost is an issue. Stimulant therapy is unfortunately not very effective for cataplexy and other symptoms of abnormal REM sleep.

Cataplexy, the second most disabling symptom in narcolepsy, is usually treated using tricyclic medications (e.g. protriptyline, clomipramine) or a higher dosage of serotonin reuptake inhibitors (e.g. fluoxetine). Adrenergic reuptake inhibition has been shown to be most effective in reducing cataplexy in animal models (Mignot et al. 1993a) but few antidepressants are targeting the adrenergic transporter site to date. Clinical experience using reuptake blockers of this system such as reboxetine suggest very positive anticataplectic effects but experience with these newer drugs is more limited.

Parsimonious information is available on the treatment of sleep paralysis, disturbed nocturnal sleep and hypnagogic hallucinations. Sleep paralysis and hypnagogic hallucinations, two symptoms of dissociated REM sleep, respond partially to antidepressive therapy. Disturbed nocturnal sleep can be treated using benzodiazepine hypnotic but this treatment does not alleviate daytime sleepiness.

A novel compound, Gammahydroxybutyrate (GHB), is now in clinical trial for the treatment of narcolepsy (Nishino and Mignot 1997; Bernasconi et al. 1999). This hypnotic, a former anesthetic agent, is unique as it consolidate nocturnal sleep by increasing deep slow wave sleep and to a less extent REM sleep. It also decreases daytime cataplexy and sleepiness, most often 1–3 months after chronic administration. Current experience suggest a favorable side effect/efficacy profile. Unfortunately, however, the compound has a very short half-life and must be administered twice during the night. The margin of safety for the compound is also small and approval for GHB is complicated by its popularity as a drug of abuse. Indeed, because of its promoting effect on slow wave sleep, the compound releases Growth Hormone, a property that has made the drug popular in athletes. Other abusers have used the compound as a rape drug or in association with other drugs as an euphoric agent in rave parties.

Of note, the mode of action of the GHB is still uncertain and may involve specific GHB receptors and/or stimulation of GABA-B receptors (Bernasconi et al. 1999). In electrophysiological studies, GHB administration decreases dramatically the firing rate of dopaminergic cells and the release of this neurotransmitter in awake animals. Interestingly, dopamine synthesis is uncoupled and brain dopamine content increases as a result. It is hypothesized that increased dopamine store could explain improved daytime sleepiness the following day.

PHARMACOLOGICAL STUDIES IN CANINE NARCOLEPSY

Canine narcolepsy was first reported in 1973. Like in humans, most cases of canine narcolepsy are sporadic but in 1975, familial cases were reported, leading Dr. W.C. Dement to establish a stable colony at Stanford University (Baker and Dement 1985). Familial cases are due to Hcrtr2 mutations while sporadic cases are hypocretin deficient but have no hypocretin gene mutations (Lin et al. 1999; Ripley et al. 2001a). The narcolepsy phenotype of these animals is striking. Positive excitation, such as the presentation of food or playing with the animals, results in muscle atonia (see http://www.med.stanford.edu/school/Psychiatry/narcolepsy/). Cataplexy is generally more severe and onset of symptoms more variable in ligand-deficient animals. Sleepiness, the core symptom of human narcolepsy, is more difficult to document in narcoleptic animals, considering the polyphasic nature of sleep in canines. Twenty-four sleep recordings have indicated that narcoleptic animals, whether ligand or receptor deficient, have disrupted sleep patterns. MSLT-like procedures have also been adapted to the canine species and have shown reduced sleep latency and REM latency in narcoleptic canines (Nishino and Mignot 1997).

The effect of more than 200 pharmacological agents have been examined over three decades in these animals (Baker and Dement 1985; Nishino and Mignot 1997). Most of the studies have focused on cataplexy. In humans, this symptom is unpredictable and difficult to quantify. In contrast, canine cataplexy can be reliably evaluated in dogs using the food-elicited cataplexy test. In general, the pharmacological control of cataplexy was found to be similar to that of REM sleep and consistent with the aminergic-cholinergic reciprocal interaction model first proposed by Hobson and McCarley (McCarley and Massaquoi 1992). In this model, wake is associated with high cholinergic and aminergic activity leading to EEG activation. In NREM sleep, cholinergic and monoaminergic activity decreases, in association with EEG synchronization During REM sleep, cholinergic systems are activated while aminergic neuronal activity is almost absent. Of note however, this pattern of activity is most striking for serotoninergic, adrenergic and histaminergic tone; dopaminergic neuronal activity does not change very significantly across the sleep cycle.

Consistent with this model, drugs that either activate cholinergic tone or decrease monoaminergic activity increase cataplexy while anticholinergic and monoaminergic enhancers reduce the symptoms (Baker and Dement 1985; Mignot et al. 1993b; Nishino and Mignot 1997). As mentioned above, however, adrenergic uptake inhibition is more effective than serotonin or dopamine reuptake inhibition in reducing cataplexy (Mignot et al. 1993a). As serotonin reuptake inhibitors also reduce REM sleep, this may suggest a preferential adrenergic control of REM atonia by the adrenergic system. Surprisingly, we also found that D2/D3 dopaminergic drugs have important effects on cataplexy, an effect that contrast with the lack of effect of dopaminergic reuptake blockers on this symptom (Nishino and Mignot 1997). Local perfusion studies have shown this effect to be mediated by dopaminergic autoreceptors, with stimulation and blockade exacerbating and reducing the symptoms respectively (Nishino and Mignot 1997).

Fewer pharmacological studies have been performed to evaluate drug effects on daytime sleepiness. A number of studies have shown that amphetamine-like stimulants and modafinil produces wakefulness by presynaptically enhancing dopaminergic transmission, either via dopamine reuptake inhibition and/or by enhancing dopamine release (Nishino et al. 1998; Wisor et al. 2001).

HYPOCRETIN DEFICIENCY IN HUMAN NARCOLEPSY

The finding that canine narcolepsy was caused by mutations in the Hypocretin receptor-2 gene led us to explore

the role of this system in human narcolepsy. Mutation screening experiments of preprohypocretin, Hcrtr1 and Hcrtr2 only revealed a single mutation in an unusually severe patient with narcolepsy-cataplexy onset at six months of age (Peyron et al. 2000). Polymorphisms were also identified and shown not to be associated with the disease. We next explored the possibility of a functional involvement of the hypocretin system by measuring hypocretin levels in the cerebrospinal fluid of patients with the disorder. Strikingly, hypocretin-1 levels were easily measurable in all control CSF samples while over 90% of patients had undetectable levels (hypocretin-2 could not measured in any sample) (Nishino et al. 2000). Further immunocytochemical and *in situ* hybridization studies have now shown greatly diminished if not absent hypocretin mRNA and peptide immunoreactivity in postmortem narcolepsy samples (Peyron et al. 2000; Thannickal et al. 2000). Together with the previously established HLA association in narcolepsy, these recent findings suggest that human narcolepsy may be an autoimmune disorder associated with peripubertal destruction of hypocretin-containing cells. This line of research is now under intense investigation.

FOOD INTAKE, ENERGY METABOLISM AND NEUROHORMONAL CONTROL OF HYPOCRETINS

The role of hypocretin in the regulation of appetite was first proposed based on the perifornical localization of hypocretin-containing cells and the observation that intracerebroventricular injections of hypocretin-1 and 2 stimulated food intake in rats (de Lecea et al. 1998; Sakurai et al. 1998). Consistent with the findings, hypocretin deficient animals have also been reported to have reduced food intake (Willie et al. 2001). Further experiments have generally confirmed these findings but the appetite stimulating effects were shown to be rather weak and dependent of circadian time of administration (Hungs and Mignot 2001).

More recent studies now indicate a more complex interplay between the regulation of hypocretin-containing cells, neurohormonal release and energy metabolism (Kilduff and Peyron 2000; Sutcliffe and de Lecea 2000; Hungs and Mignot 2001; Overeem et al. 2001; Willie et al. 2001). First, rather than only stimulating food intake, downstream effects of hypocretins on energy consumption might be more important to the regulation of body homeostasis. In fact, hypocretin knockout mice have normal body weight (Willie et al. 2001) and ataxin-3 mutants with absent hypocretin containing-cells have increased body weight (Hara et al. 2001), an effect inconsistent with a decreased orexinogenic signal. Obesity is generally believed to reflect decreased metabolic activity and energy expenditure in hypocretin deficient animals, an effect that would be quantitatively more important than hypophagia. Interestingly, intracerebroventricular injection of hypocretin has potent stimulatory effects on sympathetic outflow (Samson et al., 1999; Shirasaka et al. 1999) and cortisol release but decreases prolactin and growth hormone release (Hagan et al. 1999). Central administration of hypocretin-1 was also shown to increase metabolic rate (Lubkin and Stricker-Krongrad 1998). These findings are not always consistent with human data indicating decreased prolactin and growth hormone release during sleep but relatively unaffected cortisol levels in hypocretin deficient narcolepsy (Higushi et al. 1979).

Second, hypocretin activity is modulated by mediators of energy metabolism. Using dissociated hypocretin-containing cells expressing EGFP, preliminary results indicate that leptin and glucose inhibits hypocretin activity while ghrelin, a gastric peptide, stimulates spontaneous activity (Dr. Sakurai, personal communication). These results contrast with a lack of effects of monoamine or acetylcholine, suggesting important metabolic modulation of hypocretin activity (Dr. Sakurai, personal communication). Together with the findings of hypocretin abnormalities in animal model of obesity and other experiments (see Hungs and Mignot 2001; Willie et al. 2001), data indicate that hypocretin systems may be activated by starvation and inhibited by satiety.

WAKE PROMOTING EFFECTS OF HYPOCRETIN-1

Pharmacological studies indicate potent wake-promoting and REM sleep reduction effects after central administration of hypocretin-1 either locally in specific brain structures (locus coeruleus, preoptic area) or after intracerebroventricular injection. At high doses, increased locomotion, grooming and stereotypies are even observed, an effect blocked by dopaminergic antagonists (Nakamura et al. 2000). Importantly, intracerebroventricular administration of hypocretin-1 on sleep is inactive in Hcrtr2 mutated dogs (Fujiki et al. 2001a,b), suggesting a primary effect of Hcrtr2 in the promotion of wakefulness.

HYPOCRETIN ACTIVITY EFFECTS ON MONOAMINERGIC TONE ACROSS THE SLEEP CYCLE

While a role for hypocretins in narcolepsy is well established, very little data to date suggest the involvement of hypocretins in normal sleep/wake stage regulation. The observation that hypocretin neurons project densely to all monoaminergic cell groups have led to the hypothesis that these cells may be driving monoaminergic activity across the sleep cycle (Kilduff and Peyron 2000; Hungs and Mignot 2001). In agreement with this hypothesis, c-fos studies have shown increased activity during wake and decreased hypocretin activity during NREM sleep (Estabrooke et al. 2001). Effects on

cholinergic transmission and hypocretinergic activity during REM sleep are more debated, and may involve more complex and indirect effects (Kilduff and Peyron 2000; Hungs and Mignot 2001). Of note, dopaminergic activity also receives dense hypocretin projections but does not change activity as dramatically as other monoaminergic cell groups across sleep states. Clearly, a model involving global monoaminergic hypoactivity must be an oversimplification. Electrophysiological studies of fully characterized hypocretin neurons will be needed to answer these important questions.

PROMOTION OF HYPOCRETIN ACTIVITY BY THE CIRCADIAN CLOCK

The recent report of direct projections from the suprachiasmatic nucleus (SCN), onto hypocretin cells (Abrahamson et al. 2001) also suggest that hypocretin activity may be modulated by the biological clock. Hypocretin in the CSF or measured using *in vivo* dialysis have recently been shown to increase during the second half of the active period in rats under entrained conditions (Fujiki et al. 2001; Yoshida et al., in press). Additional studies in constant darkness and in SCN lesioned animals are now needed to establish if the fluctuation is genuinely driven by the SCN. Interestingly, a recent model of sleep regulation, the opponent model of sleep regulation, has proposed the existence of a wake promoting signal generated from the SCN in the second part of the active phase (Edgar et al. 1993). In humans, this signal would oppose an increasing sleep debt that accumulates as the day progresses, thus helping to maintain a constant period of wakefulness. A projection from the SCN to hypocretin cells could thus provide a biological substrate for the concept of SCN dependent alertness, a system that has also been suggested to be abnormal in hypocretin deficient narcolepsy (Dantz et al. 1994).

HYPOCRETIN DEFICIENCY AND SECONDARY NEUROCHEMICAL ABNORMALITIES IN NARCOLEPSY

The concept that hypocretin neurotransmission is excitatory to monoaminergic systems and possibly cholinergic systems parallels neurochemical data reported in narcolepsy. In the sleep disorder, symptoms are best explained by baseline monoaminergic hypoactivity, hyperreactivity of cholinergic systems and cholinergic receptor hypersensitivity (Baker and Dement 1985; Nishino and Mignot 1997). Most strikingly, canine narcolepsy is associated with decreased histamine levels in the brain (Nishino et al. 2001), a systems with almost exclusive Hcrtr2 localization. Other studies have shown increased dopamine and DOPAC content in the amygdala (Miller et al. 1990), a finding that may suggest secondary dysregulated dopaminergic tone. Dopamine dysregulation could either contribute primarily to daytime sleepiness in narcolepsy or represent a compensatory mechanism. Abnormal dopaminergic systems are also likely to explain periodic leg movements in narcolepsy, a symptom currently treated using Dopamine D2/D3 agonists.

HYPOCRETIN OVERACTIVATION DURING SLEEP DEPRIVATION

These models are still speculative. Hypocretin projections to non-monoaminergic, non-cholinergic cell groups are also likely to play a critical role. Most importantly, the role of hypocretins in the regulation of the homeostatic aspects of sleep also remain to be explored. C-fos experiments indicate that hypocretin neurons may be activated during enforced wakefulness (Estabrooke et al. 2001). Short term sleep deprivation does not alter preprohypocretin mRNA levels but transcription may not reflect extracellular release. In fact, a recent study by our group suggest that sleep deprivation increases hypocretin-1 release as measured using *in vivo* dialysis (Yoshida et al., in press). Interestingly, sleep deprivation has established antidepressant effects (Gillin 1983), a property that could reflect activated serotoninergic and adrenergic transmission by increased hypocretin tone. Similarly, long term sleep deprivation is associated with hyperphagia, hyperthermia and a paradoxical increase in metabolic expenditure (Rechtschaffen and Bergmann 1995), symptoms that could all reflect increased hypocretin activity. In this model, the hypocretin signal would oppose sleepiness during sleep deprivation and indirectly increase metabolism, an effect that could even become life threatening during long term sleep deprivation

OTHER PERSPECTIVES IN HYPOCRETIN RESEARCH

The function of this novel neurotransmitter system is rapidly emerging as more information becomes available on a weekly basis. New experimental protocols are now critically needed. Most of the current experiments have studied hypocretin content and/or mRNA transcription in response to often extreme metabolic manipulations. These measures are unlikely to be very relevant physiologically and other techniques such as *in vivo* dialysis and electrophysiological studies in naturally behaving animals are now badly needed. Similarly, pharmacological studies using local injections and/or intracerebroventricular experiments have limitations. The observed effects may be mediated by the strong excitatory effects of this peptide on most neurons and may not represent physiological regulation. For example, hypocretin-1 intracerebroventricular in-

jections probably activate the dopaminergic system, a effect that could explain stereotypes (Nakamura et al. 2000), and decreased prolactin and growth hormone release (Hagan et al. 1999). However, it is not clear if dopaminergic activation is important in the natural circuitry regulating sleep and/or if hypocretin deficient narcolepsy is associated with decreased dopaminergic tone. In fact, narcolepsy is rather associated with disturbed nocturnal sleep, periodic leg movements (Overeem et al. 2001) and decreased prolactin and growth hormone release (Higushi et al. 1979), possibly suggesting a compensatory hyperactivation of dopaminergic systems. Finally, hypocretin agonists and antagonists are also badly needed to assess the potential therapeutic value of this pathway for sleep and other disorders. Only one compound with moderate affinity and bioavailability, SB-334867, a Hcrtr1 antagonist, is available (Rodgers et al. 2001).

The phenotype of the system knockout, narcolepsy, indicate important and non redundant effects of this peptide system in the area of wake regulation, sleep consolidation and the cohesion of REM sleep. Based on current knowledge, hcrtr2 modulation could have significant effects on sleep and wakefulness, possibly via hcrtr2 effects on histaminergic transmission. Cholinergic and dopaminergic effects may also be relevant to sleep abnormalities, cataplexy and periodic leg movements while the role of other monoamines may be important for mood dysregulation. Projections to non monoaminergic, non cholinergic may also be involved. Experiments aiming at locally rescuing hypocretin deficits in animal models are needed to further our understanding of the narcolepsy circuitry.

Metabolic and neuroendocrine abnormalities are also apparent in narcolepsy but it is still difficult to fully dissociate those that are primary versus secondary to sleep abnormalities. Sleep, neuroendocrine release and metabolism are functionally interrelated. Sleep deprivation is associated with dramatic metabolic and neuroendocrine changes. Most likely, the hypocretin system integrates these multiple functions within the hypothalamus. Most convincingly, hypocretin neurons are sensitive to satiety and hunger signals, potentially explaining why alertness is modulated by food intake. As one of the proposed functions of sleep has been suggested to be energy conservation, these relationships are very logical from the point of view of natural evolution. Clearly however, other regulatory inputs relevant to sleep regulation remain to be discovered as sleep is not only regulated by metabolic changes and food intake.

The role of this system in neuropsychiatry also remains to be investigated. Hypocretins are uniquely positioned to link sleep, appetite and neuroendocrine control, three behaviors of major importance in psychiatry. A small study did not find significant changes in CSF hypocretin levels in schizophrenic patients (Ripley et al. 2001b). Based on its strong interactions with the monoaminergic system, this system may be more important for mood regulation. This interaction could explain the high prevalence of depression in human narcolepsy and the antidepressant effect of sleep deprivation. The finding that a genetically complex disorder like human narcolepsy is caused by the destruction of a few thousand cells secreting a neuropeptide that was unknown a few years ago is also encouraging for the future of neuropsychiatry in general. Many other unknown transmitters and peptides are currently been discovered. Phenotyping complex behaviors in knockout models such as sleep, memory or indirect measures of mood and anxiety is becoming more and more routine. As in narcolepsy, the effect of a discrete lesion of these systems may only become apparent before when these new systems are fully mapped and studied across the animal kingdom.

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