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Editorial

Perspectives in narcolepsy and hypocretin (orexin) research

The recent discovery that hypocretins (orexins) are likely to be of clinical importance to narcolepsy caps a whirlwind of scientific development. In an initial study of human patients, our group has found that hypocretin-1 levels were undetectable (<40 pg/ml) in seven of nine narcoleptic patients but present (250–285 pg/ml) in all eight control subjects tested [1]. This result follows the reports last year that canine narcolepsy is caused by mutations in the hypocretin receptor-2 (Hcrtr2) gene [2] and that preprohypocretin knockout mouse display narcolepsy-like symptoms [3]. The findings of hypocretin abnormalities in animal models of narcolepsy are therefore not merely a neurological curiosity but a significant clue to the nature of the human disorder. The availability of modern sophisticated molecular techniques led the way to the discovery of the hypocretins, the initial exploration of their functional role and implications as causal factors in narcolepsy.

The hypocretin (orexin) peptides were described by de Lecea et al. [4] and Sakurai et al. [5] in 1998. de Lecea et al. first isolated the rat preprohypocretin transcript using a directional tag polymerase chain reaction (PCR) subtraction technique aiming at the identification of genes preferentially expressed in the hypothalamus. An mRNA clone selectively present within the posterior hypothalamus (clone 35) was isolated and shown to encode a precursor polypeptide. Based on likely cleavage sites for the polypeptide, de Lecea et al. [4] predicted the existence of two related processed products they called hypocretin-1 and 2. 'Hypocretin' was coined from 'hypothalamus' and 'secretin' based on a weak homology between these peptides and secretin (this homology is contested by others). Immunocytochemical staining of the peptide confirmed discrete posterior hypothalamic localization and staining within secretory synaptic vesicles. Neuroexcitatory properties for hypocretin-2 were also demonstrated in rat hypothalamic neuronal cultures and a potential role in the regulation of feeding suggested, based on its preferential expression within the lateral hypothalamus, a region known to be involved in appetite control.

A few weeks later, Sakurai et al. [5] using a panel of cell lines expressing orphan G-protein coupled receptors (genes encoding receptors having unknown natural ligands), independently identified the same neuropeptides. These authors chemically isolated the peptides by purifying brain extracts producing calcium transients in a transfectant cell line expressing the orphan receptor HFGAN72 (later shown to be the human hypocretin receptor-1). The identified peptides were called orexin-A and B by these authors, based on reported 'orexigenic' effects in vivo (i.e. stimulation of feeding). Orexin-A and orexin-B are similar to hypocretin-1 and hypocretin-2, respectively. Two G-protein coupled receptors for these neuropeptides (OXR1 or HCRTR1 and OXR2 or HCRTR2) were also described and mapped onto human chromosome 1p33 and 6cen respectively. The human hypocretin receptor-1 (officially HCRTR1 in GenBank) has a high (20nM) affinity for hypocretin-1 and a 10–100-fold lower affinity for hypocretin-2 while human hypocretin receptor-2 (officially HCRTR2) has an equally high affinity for both hypocretin-1 and 2. In this study, hypocretins were also shown to stimulate feeding after intracerebroventricular injections in rats and a primary role in energy homeostasis suggested [5].

Further neuroanatomical work by Peyron et al. [6] and Nambu et al. [7], confirmed discrete intrahypothalamic localization for hypocretin-containing neurons but reported widespread central nervous system projections. Dense projections to the amygdala, the

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nucleus accumbens, the septum, the diagonal band and to all monoaminergic cell groups – locus coeruleus (NE), tuberomammillary nucleus (Histamine), raphe nucleus (5-HT) and ventral tegmental area/substantia nigra (DA) – were noted. Neuroexcitatory effects were reported in all cell types studied, including those of the locus coeruleus [8–10]. Further work also demonstrated direct synaptic contact for hypocretin containing fibers with monoaminergic cell groups [3,9] and cholinergic cells of the pedunculopontine and Meynert nuclei [3]. The diffuse projections suggested other regulatory effects than the regulation of feeding [6]. Other authors reported variable feeding effects for these peptides and a broader physiological role for hypocretins was confirmed [2,6].

The respective localization of the two hypocretin receptors has been studied in rats by Trivedi et al. [11]. Several other investigations are ongoing and data available to date suggest preferential expression of Hcrtr1 in monoaminergic cell groups of the locus coeruleus, raphe nucleus, and subregions of the hypothalamus, thalamus, amygdala and hippocampus ([11], X. Lu, pers. commun.). Hcrtr2, the receptor mutated in canine narcoleptic animals, was initially reported to be expressed preferentially in cortex, nucleus accumbens, and subregions of the hypothalamus, thalamus, amygdala and hippocampus but not in brain stem nuclei [11]. More recent studies suggest that Hcrtr2 is expressed in the tuberomammillary, ventral tegmental/substantia nigra nuclei, raphe magnus and expressed at low levels in many brainstem regions (X. Lu, pers. commun.).

How could hypocretin/orexin neurons be involved in sleep regulation and narcolepsy? Developmental effects are indicated by the observation that hypocretin knockout or Hcrtr2 mutated animals develop narcolepsy early in life and increase disease severity until early adulthood [3,12]. Hypocretin mRNA levels have been reported to present at low levels as early as embryonic day 18 but dramatically increase after the third postnatal week in rats (sexual maturity is 40 days in rats). This pattern of developmental effects could explain peripubertal onset in many cases of human narcolepsy. Recent studies have shown that the intracerebroventricular administration of hypocretin-1 but not hypocretin-2 induces arousal in adult rats [10]. This last result demonstrates that hypocretins regulate sleep and wakefulness in the adult brain. The lack of effects of hypocretin-2 might be due to peptide instability in vivo, an hypothesis consistent with lower hypocretin-1 levels in human CSF [1].

The sleep cycle is believed to be primarily coordinated by reciprocal monoaminergic-cholinergic interactions in the brainstem [12–14]. Hypocretin projections to monoaminergic cell groups have been suggested to mediate the sleep/wake regulatory effects for these peptides. Locus coeruleus (NE), tuberomammillary (histamine), and raphe (5-HT) nuclei cells are active during wakefulness, decrease firing during non-REM sleep and are almost silent during REM sleep. Excitatory hypocretin projections to the locus coeruleus, the raphe nuclei and tuberomammillary neurons may thus be driving monoaminergic activity across the sleep cycle. In vivo recordings of hypocretin cell activity will be needed to answer this question. A primary role for projections to the locus coeruleus in sleep regulation has also been suggested. The fact that Hcrtr1 but not Hcrtr2 receptors (the receptor mutated in canine narcolepsy) are located in the locus coeruleus [11] rather suggests this site is not primarily important in the expression of the narcolepsy phenotype. The preferential localization of Hcrtr2 in tuberomammillary cells and dopaminergic cells may be important in the regulation of wakefulness, based on the role of these systems in the pharmacology of wake-promoting drugs [15] but functional data is lacking. Finally, the dense hypocretin projections and Hcrtr2 location in limbic structures and the nucleus accumbens might explain cataplexy, a symptom generally triggered by pleasurable emotions. In view of the widespread projection sites of hypocretin neurons, uncertain receptor distribution patterns, and lack of published data demonstrating functional sleep effects for specific projections makes any further speculation premature.

In contrast to autosomal recessive canine narcolepsy and hypocretin knockout mice, human narcolepsy is not a simple genetic disorder [16]. The disorder is tightly associated with HLA-DR2 and DQB1*0602, suggesting a possible autoimmune mechanism. Twin and family studies indicate multigenic and environmental influences [16]. Highly penetrant hypocretin gene mutations are thus unlikely to be involved in narcolepsy predisposition in most cases. Further genetic studies in the HLA complex region indicate that HLA-DQB1 itself is a major

susceptibility factor but all attempts to demonstrate an autoimmune process in narcolepsy have failed. The discovery that human narcolepsy is associated with low levels of hypocretins in the CSF suggests that this hypothesis may have to be reconsidered. Only a few thousands cells in the lateral hypothalamus contain hypocretins in animals. A rapid and complete destruction of these cells by an autoimmune process could easily explain clinical features of the disorder, such as peripubertal onset of narcolepsy (when cells may become normally most active), undetectable levels of hypocretins (due to lack of cells to synthesize the peptides). Because these cells make up a small and otherwise indistinct population, their absence would have escaped detection in pathological studies performed before the present investigation.

The observation that CSF hypocretin-1 levels are undetectable in most patients with narcolepsy has potential diagnostic applications. This test may be especially useful earlier in the course of the disease, in subjects with ill-defined excessive daytime sleepiness and/or to predict treatment response to various medications. Additional studies in a larger number of control individuals and patients with other sleep and neuropsychiatric disorders are needed to address disease specificity. Peripheral measures of hypocretin transmission may also one day be developed to facilitate diagnosis. Hypocretin-containing cells have been recently reported in the gut [17] and very low plasma peptide levels may be present in normal individuals. Studies in other sleep disorders such as in idiopathic hypersomnia, insomnia and Kleine–Levin syndrome would be of great interest. It will also be important to study hypocretin levels in narcoleptic subjects without cataplexy or DQB1*0602 to provide a more complete picture of the biological spectrum of the disorder.

This discovery also opens new therapeutic avenues for narcolepsy and other sleep disorders. Human narcolepsy is associated with decreased hypocretin levels and not hypocretin receptor mutations like canine narcolepsy. Hypocretin receptors are thus most likely functional in most patients and represent obvious targets for drug development. Supplementing hypocretin neurotransmission may provide symptomatic relief in narcoleptic patients more effectively than amphetamine-like stimulants and antidepressant compounds. All the currently available narcolepsy treatments are acting downstream on monoaminergic transmission [16]. Hypocretins themselves do not penetrate well in the central nervous system but centrally acting hypocretin receptor agonists may be developed, providing these compounds do not have unacceptable side effects. Hypocretin-related compounds might also find other therapeutic applications, for example hypocretin receptor antagonists as hypnotic compounds or hypocretin receptor-1 agonists as antidepressants.

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