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EDITORIAL

The orexins/hypocretins: hypothalamic peptides linked to sleep and appetite[1]

The orexins/hypocretins are novel neuropeptides synthesized by neurons whose cell bodies are located in the lateral hypothalamus. Although these neurons are few in number, they send projections widely throughout the central nervous system (Kilduff & Peyron, 2000). There has been great excitement about the orexins/hypocretins from both the scientific and medical community. These peptides are remarkable in that they were discovered using state-of-the-art molecular techniques before their physiological actions were studied. Furthermore, there has been an exponential progress in our scientific knowledge of these peptides culminating in the orexins/hypocretins being linked to the sleep disorder, narcolepsy. With the importance of the orexins/hypocretins in sleep and arousal being increasingly recognized, it is likely that these peptides are altered by or contribute to several medical and psychiatric disorders.

There has been much confusion regarding the name of this family of neuropeptides. This is because the peptides were independently discovered by two groups. Using directional tag polymerase chain reaction subtraction, de Lecea *et al.* (1998) isolated a messenger RNA that was exclusively expressed in the lateral hypothalamus and encoded a novel precursor peptide (prepro-hypocretin). The two possible peptide products of this precursor were named hypocretin-1 (Hcrt-1) and hypocretin 2 (Hcrt-2). Independently, Sakurai *et al.* (1998), in search of endogenous ligands for orphan G protein-coupled receptors (receptors having unknown natural ligands), isolated two peptides, derived from the same precursor (prepro-orexin), which they named orexin A and orexin B based on observations suggesting that these peptides have a role in the regulation of appetite (orexin is derived from the Greek word for appetite, *orexis*). It is now known that prepro-hypocretin and prepro-orexin are the same with Hcrt-1 corresponding to orexin A and Hcrt-2 corresponding to orexin B. Until a universally accepted name for these peptides is agreed, they will be referred to as the orexins/hypocretins.

Orexin A/Hcrt-1 is composed of 33 amino acids with two intrachain disulphide bonds, while orexin B/hyocretin-2 is a 28 amino acid linear peptide. Orexin/hypocretin immunoreactivity and immunoreactive fibres are widely distributed throughout the central nervous system (Peyron *et al.* 1998; Taheri *et al.* 1999) but are particularly concentrated in the hypothalamus, amygdala, nucleus accumbens, the septum and monoaminergic centres such as the noradrenergic locus coeruleus, histaminergic tuberomammillary nucleus, serotonergic raphe nucleus and dopaminergic ventral tegmental area. Two G protein-coupled receptors for these peptides have been reported: OX_1R/Hcrtr-1 and OX_2R/Hcrtr-2. Orexin A/Hcrt-1 has greater affinity than orexin B/Hcrt-2 for the human OX_1R/Hcrtr-1 receptor, but both peptides have similar affinities for the human OX_2R/Hcrtr-2 receptor. OX_1R/Hcrtr-1 messenger RNA (mRNA) has been detected in the ventromedial hypothalamus, the tenia tecta, hippocampus, dorsal raphe and locus coeruleus. OX_2R/Hcrtr-2 mRNA is expressed in the paraventricular hypothalamic nucleus, the subthalamic and thalamic nuclei, the septum, the cerebral cortex, nucleus accumbens, anterior pretectal nucleus and several regions in the medulla oblongata (Trivedi *et al.* 1998).

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The location of orexin/hypocretin neurons in the lateral hypothalamus, classically known as the 'feeding centre', and early experiments with the orexins/hypocretins suggested that they have a role in feeding. When administered into the cerebral ventricle of rats, they dose-dependently stimulated food intake and prepro-orexin/hypocretin mRNA was shown to be upregulated with fasting (Sakurai *et al*. 1998). There is also evidence for the inhibition of food intake by the OX_1R antagonist, l-(2-methylbenzoxazol-6-yl)-3-[1,5] naphthyridin-4-yl urea hydrochloride (SB-334867-A), (Haynes *et al*. 2000). Prepro-orexin/hypocretin gene knockout mice (Chemelli *et al*. 1999) are hypophagic, but are not lean; this could be due to a reduction in energy expenditure. Despite the above evidence, the importance of orexins/hypocretins in food intake is not entirely clear. Edwards *et al*. (1999) observed that intracerebroventricular (ICV) administration orexin A/Hcrt-1 stimulated food intake in rats; but not as potently as other appetite-stimulating (orexigenic) peptides such as neuropeptide Y (NPY). Orexin B/Hcrt-2 is only occasionally orexigenic and less potent than orexin A/Hcrt-1. It is possible that the observed orexigenic effects of orexin A/Hcrt-1 are more related to an increase in activity and arousal that is also observed when the peptide is administered to animals. It is also possible that orexins/hypocretins are important in food intake only in particular circumstances, such as in response to hypoglycaemia (Griffond *et al*. 1999) and/or in the regulation of circadian food intake.

There is cumulating evidence that the orexins/hypocretins are involved in the regulation of the sleep–wake cycle and CNS arousal mechanisms with orexin/hypocretin deficiency linked to narcolepsy. Narcolepsy is a debilitating disorder associated with excessive daytime sleepiness, cataplexy (loss of muscle tone triggered by emotional stimuli) and abnormalities in rapid eye movement (REM) sleep. Unexpectedly, night-time sleep is often disturbed with frequent awakening. Narcolepsy is a much more common disorder than generally believed with a prevalence of up to 1 in 2000 (Thorpy, 2001). The onset of the disease is usually in adolescence. Since this condition is closely associated with the human leucocyte antigen (HLA) system (HLA-DR2 and HLA-DQB1*602), there have been suggestions that it is an autoimmune disease (Mignot *et al*. 1997).

The link between the orexins/hypocretins and narcolepsy was again a triumph for modem molecular biology. Simultaneously, it was reported that canine narcolepsy, a condition that is inherited as an autosomal recessive trait with full penetrance, is associated with mutations in the orexin/hypocretin receptor-2 gene (Lin *et al*. 1999) and that prepro-orexin/hypocretin gene knockout mice exhibit periods of 'behavioural arrest' that resemble cataplexy and sleep-onset REM periods characteristic of narcolepsy in humans (Chemelli *et al*. 1999).

To characterize the feeding phenotype of prepro-orexin/hypocretin knockout mice, these mice were observed with infrared cameras at night. Unexpectedly, it was observed that prepro-orexin/hypocretin gene knockout mice suffered from episodes of behavioural arrest, 'totter' and atonia. Electroencephalographic studies of these mice revealed that they have an altered sleep structure with reduced latency to REM sleep. Sleep fragmentation was most pronounced in the dark phase, when mice are normally most active (Chemelli *et al*. 1999). In mice, orexin/Hcrt neurons have been shown to be activated in response to the anti-narcolepsy drug modafinil (Chemelli *et al*. 1999). Further animal studies have shown that intracerebroventricular administration of orexin A/Hcrt-1 increases arousal and locomotor activity, while reducing REM sleep (Hagan *et al*. 1999). Orexin A/Hcrt-1 immunoreactivity shows diurnal variation in areas of the brain involved in the regulation of sleep and arousal (Taheri *et al*. 2000). It is not surprising that the orexins/hypocretins alter the release of hormones whose secretion is intimately linked with the sleep–wake cycle (growth hormone, prolactin and corticosterone). The pontine locus coeruleus, a major site for monoaminergic neurons, has been identified as a major target for orexin/hypocretin activity, but other brain targets for these peptides are also likely to be important.

Animal studies pointed strongly to a possible role for the orexins/hypocretins in human narcolepsy. In a study measuring orexin A/Hcrt-1 immunoreactivity in the cerebrospinal fluid (CSF) of patients with narcolepsy, it was reported that orexin A/Hcrt-1 was undetectable in CSF from seven out of nine patients, but was detectable in all control subjects, indicating an abnormality in orexin/hypocretin neurotransmission in narcolepsy (Nishino *et al*. 2000). In a recent study,

narcolepsy patients were screened for mutations in prepro-orexin/hypocretin and the orexin/hypocretin receptor genes. Unlike canine models of narcolepsy, no orexin/hypocretin receptor mutations were observed. One severely affected patient with very early onset narcolepsy (cataplexy at the age of 6 months) had a prepro-orexin/hypocretin gene mutation resulting in abnormal trafficking of the mutant peptide precursor. It was also shown that orexin/hypocretin mRNA was undetectable in the post-mortem hypothalami of narcoleptic subjects (Peyron *et al*. 2000). These findings were confirmed by another post-mortem study (Thannickal *et al*. 2000). Together, these studies suggest that even in the absence of a specific mutation, narcolepsy is still associated with a deficiency in the orexin/hypocretin system. It is unclear how the selective loss of orexin/hypocretin neurons occurs in narcolepsy. In the above studies, neighbouring neurons synthesizing melanin-concentrating hormone, another lateral hypothalamic neuropeptide with multiple functions including stimulation of appetite, were shown to be intact. There was little evidence of an autoimmune inflammatory insult, as suspected from the HLA association, to orexin/hypocretin neurons. It is possible that this is because of few and/or older patients. The autoimmune insult and any associated degeneration may have occurred years before the death of the patients studied.

Hypotheses about the pathophysiology of depression have traditionally focused on the serotoninergic and noradrenergic systems. Recently, there has been a growing interest in the role of hypothalamic neuropeptides in affective disorders, both through a direct action and through interactions with classical neurotransmitters. Depressed patients are often characterized as having abnormal levels of 'arousal', be it hypo- or hyper-arousal. Disturbances in both central neuroendocrine function and in sleep have been used to support the notion that some depressed patients have abnormal central 'arousal' in depression (Nofzinger *et al*. 2000). Also, depressed patients often exhibit disturbances in endocrine circadian rhythms as well as the sleep–wake cycle. Alterations in the sleep-wake cycle of depressed patients include a shortened latency to the first REM sleep episode, an increase in the amount and frequency of REM sleep during the first part of the night, and increased sleep fragmentation. The Wistar-Kyoto (WKY) rat is an animal model of depression with behavioural passivity and abnormal sleep–wake patterns resembling depression (Dugovic *et al*. 2000). When orexin/hypocretin immunoreactivity was measured in brain regions of this rat model and compared with the Wistar parent strain, it was found that the WKY rat has significantly lower levels of orexin/hypocretin immunoreactivity in brain regions involved in the regulation of the sleep–wake cycle and emotion (Taheri *et al*. 2001). It is possible that reduced orexin/hypocretin levels play a role in the pathophysiology of mood disorders and it would be interesting to investigate the role of these peptides in different animal models of depression to ascertain whether abnormalities in orexins/hypocretins are seen consistently in all animal models or whether they are related only to certain symptoms of depression.

There is now sufficient evidence to link orexin/hypocretin deficiency to narcolepsy. However, more studies are needed to establish the role of the orexins/hypocretins fully in this condition and to identify whether there are different patient subsets. Measurement of orexin A/Hcrt-1 immunoreactivity in the CSF may be used as a diagnostic test and also for identification of patient subsets. Since most current treatments for narcolepsy appear to act downstream of the orexins/hypocretins and have considerable side effects, the development of orexin/hypocretin receptor agonists may be useful in the alleviation of symptoms. The importance of the orexins/hypocretins in other sleep disorders and in depression remains to be determined. There may be a role for orexin/hypocretin receptor agonists in the treatment of depression. Orexin/hypocretin receptor antagonists may find a role in the treatment of insomnia and possibly obesity. There is much more to be learned about the orexins/hypocretins in normal physiology and neuropsychiatric disorders; the great momentum in the study of these fascinating peptides is likely to continue.

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REFERENCES

Chemelli, R. M., Willie, J. T., Sinton, C. M., Elmquist, J. K., Scammell, T., Lee, C., Richardson, J. A., Williams, S. C., Xiong, Y., Kisanuki, Y., Fitch, T. E., Nakazato, M., Hammer, R. E., Saper, C. B. & Yanagisawa, M. (1999). Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* **98**, 437–451.

De Lecea, L., Kilduff, T. S., Peyron, C., Gao, X., Foye, P. E., Danielson, P. E., Fukuhara, C., Battenberg, E. L., Gautvik, V. T., Bartlett, F. S. 2nd, Frankel, W. N., van den Pol, A. N., Bloom, F. E., Gautvik, K. M. & Sutcliffe, J. G. (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proceedings of the National Academy of Sciences USA* **95**, 322–327.

Dugovic, C., Solberg, L. C., Redei, E., Van Reeth, O. & Turek, F. W. (2000). Sleep in the Wistar-Kyoto rat, a putative genetic animal model for depression. *Neuroreport* **11**, 627–631.

Edwards, C. M., Abusnana, S., Sunter, D., Murphy, K. G., Ghatei, M. A. & Bloom, S. R. (1999). The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. *Journal of Endocrinology* **160**, R7–12.

Griffond, B., Risold, P. Y., Jacquemard, C., Colard, C. & Fellmann, D. (1999). Insulin-induced hypoglycemia increases preprohypocretin (orexin) mRNA in the rat laternal hypothalamic area. *Neuroscience Letters* **262**, 77–80.

Hagan, J. J., Leslie, R. A., Patel, S., Evans, M. L., Wattam, T. A., Holmes, S., Benham, C. D., Taylor, S. G., Routledge, C., Hemmati, P., Munton, R. P., Ashmeade, T. E., Shah, A. S., Hatcher, J. P., Hatcher, P. D., Jones, D. N., Smith, M. I., Piper, D. C., Hunter, A. J., Porter, R. A. & Upton, N. (1999). Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proceedings of the National Academy of Sciences USA* **96**, 10911–10916.

Haynes, A. C., Jackson, B., Chapman, H., Tadayyon, M., Johns, A., Porter, R. A. & Arch, J. R. (2000). A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regulatory Peptides* **96**, 45–51.

Kilduff, T. S. & Peyron, C. (2000). The hypocretin/orexin ligand-receptor system: implications for sleep and sleep disorders. *Trends in Neurosciences* **23**, 359–365.

Lin, L., Faraco, J., Li, R., Kadotani, H., Rogers, W., Lin, X., Qui, X., de Jong, P. J., Nishino, S. & Mignot, E. (1999). The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* **98**, 365–376.

Mignot, E., Hayduk, R., Black, J., Grumet, F. C. & Guilleminault, C. (1997). HLA DQB1*0602 is associated with cataplexy in 509 narcoleptic patients. *Sleep* **20**, 1012–1020.

Nishino, S., Ripley, B., Overeem, S., Lammers, G. J. & Mignot, E. (2000). Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* **355**, 39–40.

Nofzinger, E. A., Price, J. C., Meltzer, C. C., Buysse, D. J., Villemagne, V. L., Miewald, J. M., Sembrat, R. C., Steppe, D. A. & Kupfer, D. J. (2000). Towards a neurobiology of dysfunctional arousal in depression: the relationship between beta EEG power and regional cerebral glucose metabolism during NREM sleep. *Psychiatry Research* **98**, 71–91.

Peyron, C., Tighe, D. K., van den Pol, A. N., de Lecea, L., Heller, H. C., Sutcliffe, J. G. & Kilduff, T. S. (1998). Neurons containing hypocretin (orexin) project to multiple neuronal systems. *Journal of Neuroscience* **18**, 9996–10015.

Peyron, C., Faraco, J., Rogers, W., Ripley, B., Overeem, S., Charnay, Y., Nevsimalova, S., Aldrich, M., Reynolds, D., Albin, R., Li, R., Hungs, M., Pedrazzoli, M., Padigaru, M., Kucherlapati, M., Fan, J., Maki, R., Lammers, G. J., Bouras, C., Kucherlapati, R., Nishino, S. & Mignot, E. (2000). A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nature Medicine* **6**, 991–997.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., Williams, S. C., Richarson, J. A., Kozlowski, G. P., Wilson, S., Arch, J. R., Buckingham, R. E., Haynes, A. C., Carr, S. A., Annan, R. S., McNulty, D. E., Liu, W. S., Terrett, J. A., Elshourbagy, N. A., Bergsma, D. J. & Yanagisawa, M. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* **92**, 573–585.

Taheri, S., Mahmoodi, M., Opacka-Juffry, J., Ghatei, M. A. & Bloom, S. R. (1999). Distribution and quantification of immunoreactive orexin A in rat tissues. *FEBS Letters* **457**, 157–161.

Taheri, S., Sunter, D., Dakin, C., Moyes, S., Seal, L., Gardiner, J., Rossi, M., Ghatei, M. & Bloom, S. (2000). Diurnal variation in orexin A immunoreactivity and prepro-orexin mRNA in the rat central nervous system. *Neuroscience Letters* **279**, 109–112.

Taheri, S., Gardiner, J., Hafizi, S., Murphy, K., Dakin, C., Seal, L., Small, C., Ghatei, M. & Bloom, S. (2001). Orexin A immunoreactivity and prepro-orexin mRNA in the brain of Zucker and WKY rats. *Neuroreport* **12**, 459–464.

Thannickal, T. C., Moore, R. Y., Nienhuis, R., Ramanathan, L., Gulyani, S., Aldrich, M., Cornford, M. & Siegel, J. M. (2000). Reduced number of hypocretin neurons in human narcolepsy. *Neuron* **27**, 469–474.

Thorpy, M. (2001). Current concepts in the etiology, diagnosis and treatment of narcolepsy. *Sleep Medicine* **2**, 5–17.

Trivedi, P., Yu, H., MacNeil, D. J., Van der Ploeg, L. H. & Guan, X. M. (1998). Distribution of orexin receptor mRNA in the rat brain. *FEBS Letters* **438**, 71–75.