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Hypocretin/Orexin Pathology in Human Narcolepsy with and Without Cataplexy

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Abstract Hypocretin (Hcrt, also called orexin) neurons have been implicated in the pathology underlying narcolepsy. The number of Hcrt cells in normal humans ranges from 51,000–83,000. Human narcolepsy is correlated with a greatly reduced number of Hcrt containing neurons and axons, and an elevated level of hypothalamic gliosis. Narcolepsy with cataplexy is characterized by a loss of approximately 90 % of Hcrt neurons. However, more than a quarter of narcoleptics do not have cataplexy and have normal levels of Hcrt in their cerebrospinal fluid. Narcolepsy without cataplexy has an overall a loss of 33 % of Hcrt cells compared to normal, with maximal cell loss in the posterior hypothalamus. A better understanding of the pattern of damage to Hcrt containing somas and axons and of the gliosis occurring in narcolepsy should clarify the nature of the pathological process responsible for this disorder.

Keywords Hypocretin · Orexin · Narcolepsy · Cataplexy · Neurodegeneration

1 Introduction

As often happens in medical sciences, it is research on the possible treatment for another disease, obesity, which led to the discovery of two peptides expressed in the hypothalamus and named "hypocretins" or "orexins". The discovery of these peptides and of their receptors opened the door to the most current understanding of narcolepsy (de Lecea et al. 1998; Sakurai et al. 1988). In 1999, the positional

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cloning of the canine narcolepsy gene and its identification as the Hcrt receptor 2 gene was another milestone in the field (Lin et al. 1999). A mouse knockout model for the Hcrt gene was also found to display narcolepsy–like symptoms (Chemelli et al. 1999). In 2000, these discoveries were followed by the report that most cases of human narcolepsy-cataplexy are associated with hypocretin deficiency (Nishino et al. 2000; Peyron et al. 2000; Thannickal et al. 2000).

The hypocretins (Hcrt) are two peptides, Hcrt-1 (orexin-A) and Hcrt-2 (orexin-B), generated from a single preprohypocretin molecule and synthesized by a small number of neurons restricted to the lateral, dorsomedial and perifornical hypothalamus (de Lecea et al. 1998; Sakurai et al. 1988). In contrast, Hcrt axons are found throughout the CNS, with innervations of the hypothalamus, locus coeruleus, raphe, midline thalamus, all levels of spinal cord, sympathetic and parasympathetic centers, and many other brain regions (Peyron et al. 1998). Two G protein–coupled receptors that respond to the hypocretins have been identified (Sakurai et al. 1988). In parallel to the wide distribution of axons, the two Hcrt receptors show a widespread and heterogeneous pattern of expression throughout the CNS (Trivedi et al. 1998).

2 Narcolepsy and Hypocretin System

Human narcolepsy is a lifelong sleep disorder characterized by excessive daytime sleepiness, disrupted nocturnal sleep, rapid eye movement (REM) sleep occurring at the onset of sleep, and cataplexy. The presence of cataplexy is distinctively characteristic for narcolepsy. Narcolepsy affects approximately 1 in 2000 individuals in the United States. Importantly, however, the prevalence of the milder form of narcolepsy, narcolepsy without cataplexy, could be substantially higher.

The potential importance of Hcrt neurons in preventing narcolepsy was suggested by the finding that narcoleptic dogs have mutations in Hcrt receptor 2 (Lin et al. 1999). Although different mutations were found in each of two narcoleptic breeds of dogs, Dobermans and Labradors, in each breed the mutation was localized to the Hcrt receptor 2, rendering it non-functional. Parallel studies in Hcrt knockout mice revealed a similar narcoleptic phenotype (Sakurai et al. 1988) indicating that loss of either the peptide or one of the two peptide receptors results in narcolepsy. These findings became even more exciting and relevant when hypocretin was found in the cerebrospinal fluid of eight normal humans but could not be detected in seven of nine narcoleptics (Nishino et al. 2000).

3 Hypocretin Cell Loss in Narcolepsy with Cataplexy

Hcrt immunoreactive cells were found in anterior (AH), dorsal (DH), dorsomedial (DMH), posterior (PH) and lateral (LH) hypothalamic nuclei of both normal and narcoleptic human brains. (Figs. 1, 2 and 3). Cell number was significantly reduced in narcoleptics compared to normals. The percentage cell loss was significantly more severe in certain nuclei (Fig. 3). In the normal brain, Hcrt cell density was highest in DMH and lowest in AH. In narcoleptics, the maximum percentage loss of Hcrt cells occurred in the posterior hypothalamus (97 %). The minimum percent loss of Hcrt cells was seen in AH (74 %). Overall, narcoleptics had a mean 90 % reduction in Hcrt cell number compared to the average number seen in normals (Thannickal et al. 2003).

The axon density reduction in Hcrt innervated nuclei in narcoleptics was positively correlated with the density of Hcrt axons in normal humans. The total number of Hcrt axons in all the structures analyzed was reduced by 67 % compared to an 89 % reduction of Hcrt soma count in the same brains, suggesting that Hcrt cells

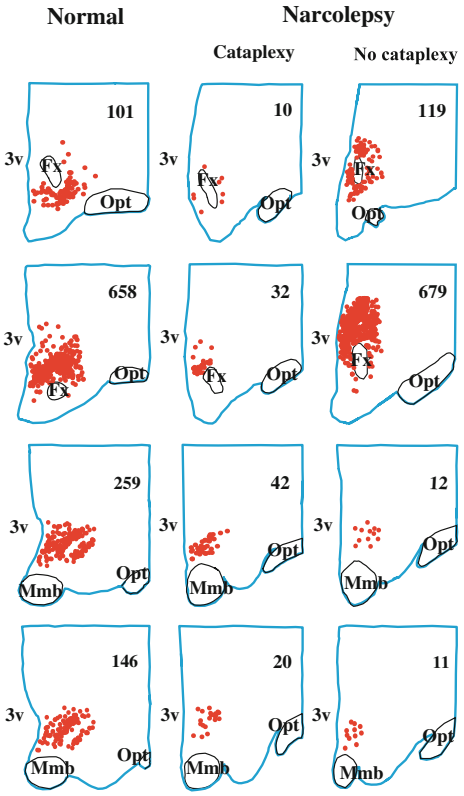

Fig. 1 Neurolucida mapping of Hcrt cells in normal and narcolepsy. The cell counts are listed in each section. *3v* third ventricle, *Fx* fornix, *Mmb* mammillary body, *Opt* optic tract

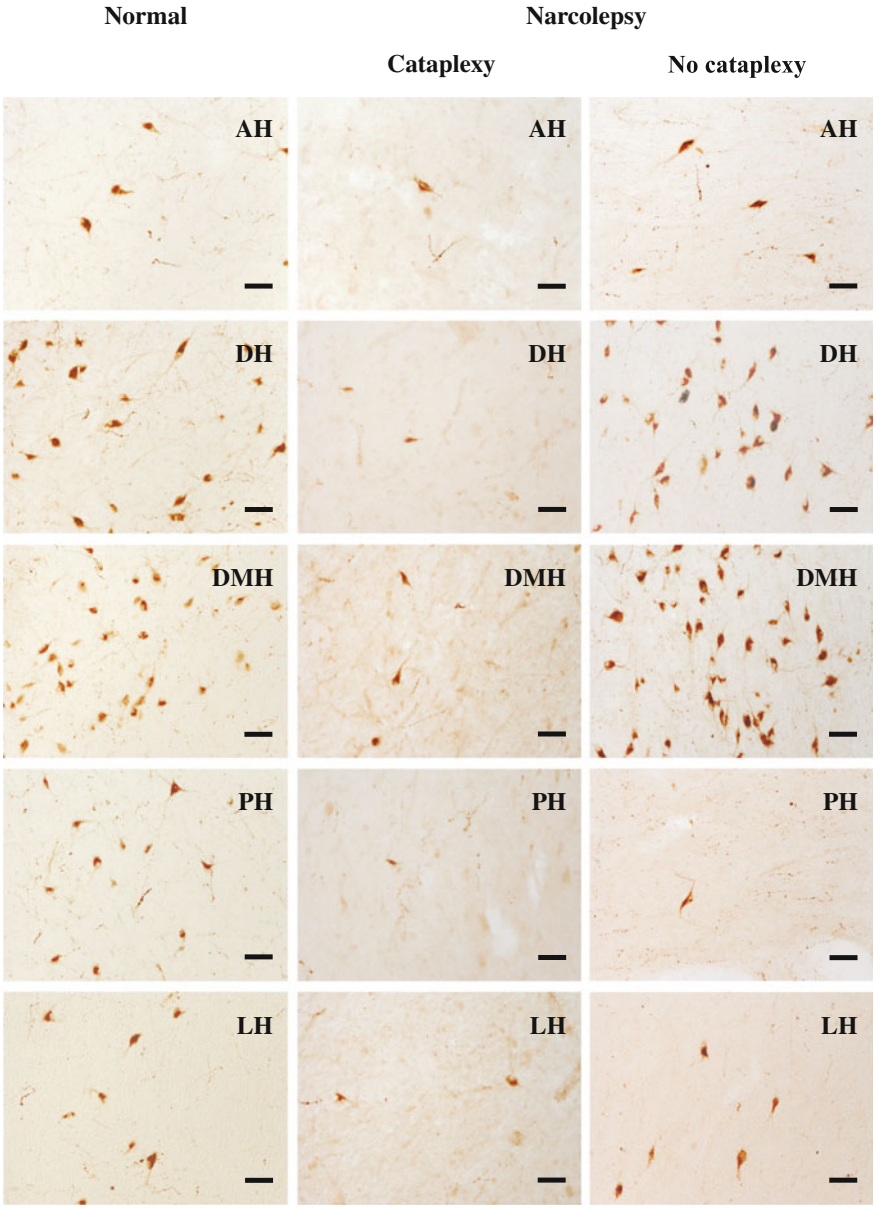

Fig. 2 Hcrt cells in the hypothalamic nuclei of normal and narcolepsy with and without cataplexy. *AH* anterior hypothalamus, *DH* dorsal hypothalamus, *DMH* dorsomedial hypothalamus, *PH* posterior hypothalamus, *LH* lateral hypothalamus, Scale bar–50 μm

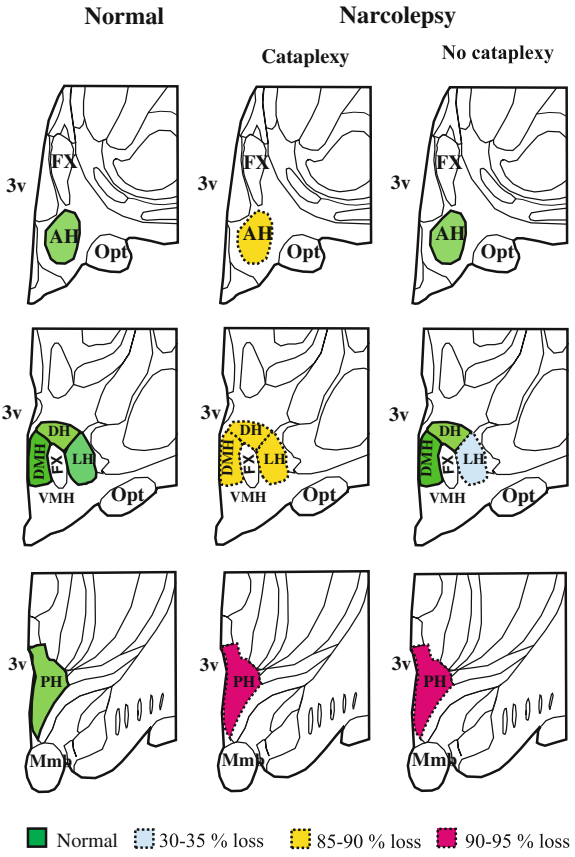

Fig. 3 Diagrammatic representation of the location of hcrt cells in the hypothalmus of normal brain and differential loss of Hcrt cells in narcolepsy with and without cataplexy. In normal, Hcrt cell somas are localized in AH, DH, DMH, PH and LH nuclei. In narcolepsy with cataplexy, cell loss was found in AH, DH, DMH, PH and LH nuclei, whereas, in narcolepsy without cataplexy cell loss was limited to LH and PH nuclei. *AH* anterior hypothalamus, *DH* dorsal hypothalamus, *DMH* dorsomedial hypothalamus, *PH* posterior hypothalamus, *LH* lateral hypothalamus, *3v* third ventricle, *Fx* fornix, *Mmb* mammillary body, *Opt* optic tract, *VMH* ventromedial hypothalamus

with smaller axonal fields are lost to a greater extent in narcolepsy or that axonal sprouting occures in surviving Hcrt cells. (Thannickal et al. 2003). 99 % of Hcrt neurons are Narp positive (Reti et al. 2002).The number of Narp-positive neurons was reduced by 89 % in these areas of the narcoleptic hypothalamus (Blouin et al. 2005; Crocker et al. 2005).

4 Hypocretin Cell Loss in Narcolepsy Without Cataplexy

The narcolepsy without cataplexy patient whose complete brain was available for study had an overall loss of 33 % of Hcrt cells compared to normals, with maximal cell loss in the posterior hypothalamus. The maximum percentage reduction occurred in the posterior hypothalamic nucleus (95 % loss). There was no Hcrt cell loss in anterior, dorsal and dorsomedial nuclei of the narcolepsy without cataplexy patient (Figs. 1, 2 and 3). There was no reduction in the number of MCH neurons in either type of narcolepsy (Thannickal et al. 2009).

Work in animals (Gerashchenko et al. 2003) has shown that cerebrospinal Hcrt levels can be normal even when there is a substantial loss of Hcrt cells. CSF levels may be a function not only of the percentage of Hcrt cells lost, but also the activity and the mean distance of surviving Hcrt cells from the ventricles. Thus, patients with loss of posterior hypothalamic Hcrt cells may still have normal CSF levels of the peptide, even though it is not being synaptically delivered to the cells normally receiving hcrt axonal projections (Oka et al. 2006). Parkinson disease patients have a loss of Hcrt neurons, although to a lesser extent than in narcolepsy with cataplexy (Thannickal et al. 2007; Fronczek et al. 2007). These patients have many of the symptoms of narcolepsy; however, distinct episodes of cataplexy have not been reported. This is consistent with the current observations of narcolepsy without cataplexy with partial loss of Hcrt neurons.

4.1 Elevated Glial Fibrillary Acidic Protein Levels in Narcolepsy

Elevated Glial fibrillary acidic protein (GFAP) levels is an established indicator of astrogliosis. This process is characterized by rapid synthesis of GFAP and is demonstrated by an increase in protein content (Eng et al. 2000). GFAP levels in the CSF of narcoleptics (Feneberg et al. 2013) may represent hypothalamic gliosis and support the hypothesis of a neurodegenerative process (Thannickal et al. 2000). There was a significant increase in gliosis indicated by GFAP staining and a significant difference in the amount of gliosis across hypothalamic nuclei in narcoleptics (Fig. 4). Narcoleptics with cataplexy had increased GFAP staining throughout the hypothalamus, with a maximum percentage GFAP increase in the posterior hypothalamic nucleus. The number of Hcrt axons in the anterior hypothalamus was normal in narcolepsy without cataplexy (Thannickal et al. 2009). We speculate that high GFAP levels may be a differential biomarker in some sleep diseases such as secondary hypersomnia in Prader–Willi syndrome or neurologic

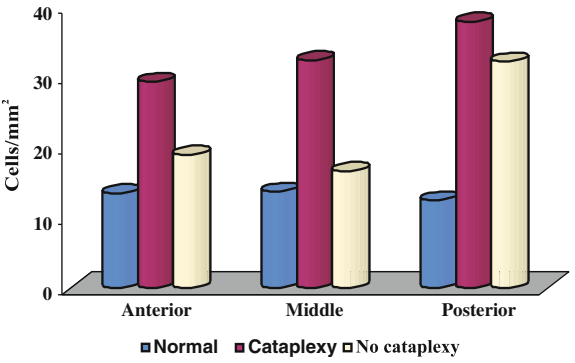

Fig. 4 Gliosis in narcolepsy. Glial fibrillary acidic protein labeled astrocytes (GFAP) density (cells/mm^2) in normal and narcoleptic brains of the hypothalamus

disorders such as in certain cases of Guillain–Barré syndrome, both of which also may present with low CSF hcrt-1 levels (Mignot et al. 2002). In conclusion, GFAP may be useful as an additional disease biomarker in patients with narcolepsy.

4.2 Changes in Hypocretin Neuronal Expression with Normal Aging in the Human Hypothalamus

It has been found that Hcrt concentration in the cerebral spinal fluid (CSF) of infants increased from birth until 2–4 postnatal months and then decreases throughout childhood and puberty (Feneberg et al. 2013). However, Aran et al. (2012) found no change in CSF Hcrt concentration between birth and 4 years of age. In older humans, the number of Hcrt neurons was unchanged between 50 and 90 of age (Kanbayashi et al. 2002; Fronczek et al. 2005). There was 24 % decrease in the number of Hcrt neurons in adults compared with infants and children (Fronczek et al. 2012). This may contribute to changes in sleep regulation during development and with aging. Animal studies have shown that decreased Hcrt expression is correlated with changes in sleep regulation with aging (Hunt et al. 2015; Brownell and Conti 2010).

4.3 Molecules Co-expressed in Hypocretin Neurons

Hcrt deficiency is directly involved in several neurological disorders. Dynorphin, glutamate and secretogranin II are found co-localized within Hcrt cells (Sawai et al. 2010; Chou et al. 2001; Bayer et al. 2002). There are several receptors and transporters that are expressed in Hcrt cells such as 5HT1-A, adenosine A1-R, GABA A alpha 3, GABA A epsilon, GABA B, group III metabotropic glutamate receptors, leptin R, Y4-R, vGlut1 and vGlut2 (Torrealba et al. 2003; Collin et al. 2002; Thakkar et al. 2002; Bäckberg et al. 2003, 2004; Moragues et al. 2003; Acuna-Goycolea et al. 2004; Håkansson et al. 1999; Rosin et al. 2003; Campbell et al. 2003). Acetylcholinesterase E, STAT-3, Narp and neuroglobin (Chou et al. 2004; Reti et al. 2002; Håkansson et al. 1999; Hundahl et al. 2008) are also found in Hcrt cells. Hcrt cell loss also causes the deficiency of these molecules. Their role in the symptoms of narcolepsy is not known.

5 Conclusions

The identification of hypocretin/orexin deficiency as the cause of human narcolepsy and the potential role of hypocretin peptides in other neurological disorders has sparked interest in the pathophysiology of the Hcrt system. As narcolepsy is found

in about 1 of 2000 humans, it is the third most prevalent type of neurodegenerative disease, behind Alzheimer's (the most common) and Parkinson's (which affects about 1 in 1000 humans) (Dorsey et al. 2005), but more prevalent than Huntington's or amyotropic lateral sclerosis (each about 1 in 5000).

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