Hypocretin (orexin) deficiency in narcolepsy and primary hypersomnia

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The discovery that hypocretins are involved in narcolepsy, a disorder associated with excessive daytime sleepiness, cataplexy, and unusually rapid transitions to rapid eye movement sleep, opens a new field of investigation in the area of disorders of sleep and activation. Hypocretin-1 (hcrt-1) and hypocretin-2 (hcrt-2) are newly discovered neuropeptides present in a common precursor. Hypocretin containing cells are located exclusively in the lateral hypothalamus, with widespread projections within the central nervous system. The role of the hypocretin system in other disorders causing excessive daytime sleepiness is more uncertain. This study reports the findings of a prospective study measuring cerebrospinal fluid concentrations of hypocretin-1 and hypocretin-2 in HLA DQB1*0602 positive narcolepsy with cataplexy, monosymptomatic narcolepsy, and primary hypersomnia. The results confirmed the previous observations, that hcrt-1 is deficient in narcolepsy and for the first time report very low levels of hcrt-1 in primary hypersomnia. It is also reported for the first time that there is a generalised defect in hcrt-2 transmission in all three of these clinical entities compared with controls.

The hypocretins (orexins) are recently described hypothalamic neuropeptides thought to play a significant part in the regulation of sleep and arousal states. Their discovery was reported by two groups, independently, and using different techniques. The terms, orexin and hypocretin used by the two groups are synonymous and for the purposes of this article we will use the term hypocretin (Hcrt). The finding that cerebrospinal fluid (CSF) concentrations of hypocretin-1 and hypocretin-2 in HLA DQB1*0602 positive narcolepsy with cataplexy, monosymptomatic narcolepsy, and primary hypersomnia. The results confirmed the previous observations, that hcrt-1 is deficient in narcolepsy and for the first time report very low levels of hcrt-1 in primary hypersomnia. It is also reported for the first time that there is a generalised defect in hcrt-2 transmission in all three of these clinical entities compared with controls.

Narcolepsy is a sleep disorder with a prevalence of 1 in 2000 adults, it usually appears between the ages of 15 and 30 years and shows a mixture of four characteristic symptoms: (1) excessive daytime sleepiness (EDS), with irresistible sleep attacks during the day; (2) cataplexy (brief episodes of muscle weakness or paralysis precipitated by strong emotion, such as laughter or surprise); (3) sleep paralysis, the persistence of REM atonia in wakefulness; (4) hypnagogic hallucinations or dream-like images, which characteristically occur at sleep onset. EDS is a prerequisite for the diagnosis and the presence of cataplexy secures the clinical diagnosis. Cataplexy usually develops within two years of the onset of EDS but may take up to two decades to manifest.

HLA typing reveals the DQB1*0602 subtype in 90% of people with narcolepsy compared with 12%-38% of controls, but the possession of this allele is neither necessary nor sufficient for the disorder. Overnight polysomnography helps to rule out other causes of excessive sleep disorder, such as obstructive sleep apnoea. The multiple sleep latency test (MSLT) generally shows reduced mean sleep latency and the occurrence of episodes of sleep onset rapid eye movement within 15 minutes of sleep onset.

The presence of the symptoms of narcolepsy in the absence of cataplexy is referred to as monosymptomatic narcolepsy (MN) (narcolepsy without cataplexy). It is thought that up to 60% of patients with MN progress to narcolepsy-cataplexy (NC). It has been suggested that disruption of hypocretin neurotransmission causes human narcolepsy and that CSF hcrt-1 measures could be used as a diagnostic tool for narcolepsy. The initial report, and subsequent replication study, found most HLA positive patients with NC had undetectable concentrations of CSF hcrt-1.

Primary hypersomnia (PH, also known as idiopathic hypersomnia) is characterised by EDS, but without cataplexy or nocturnal sleep disruption. It usually starts in adolescence and is of unknown aetiology. Patients with this disorder report increased total sleep times. No amount of sleep improves the EDS and naps are frequently unrefreshing (unlike narcolepsy where naps are reported to be refreshing). Polysomnography usually reveals a shortened sleep latency, increased total sleep time, and, reduced sleep latency on the multiple sleep latency test. There is an absence of sleep onset REM periods. Much less is known about the potential role of hypocretin and its neurotransmission in PH.

To date, there have been no published reports on CSF hcrt-2 concentrations in humans.

This study was undertaken firstly to measure CSF hcrt-1 in a sample of patients with NC in our population and to confirm that abnormal hypocretin neurotransmission is a characteristic feature of NC. Secondly, to measure CSF hcrt-1 concentrations, in MN and PH, and, thirdly, to evaluate the possible role of hcrt-2 in these disorders of sleep and activation.

Methods

Volunteers for the study were recruited from the outpatient clinic list of the Sleep Disorders Centre at St Thomas's Hospital, London. All subjects gave informed consent for the study, which had been approved by our local ethics committee.

Patient assessments included the following: Epworth Sleepiness Score (ESS); clinical evaluation by a senior sleep physician (AJW) and HLA typing; conventional nocturnal polysomnography; MSLT.

Diagnostic criteria

NC was diagnosed by the presence of EDS >6 months, an ESS >10, and the presence of cataplexy. MN was diagnosed using overnight polysomnography.
the following criteria: EDS >6 months; ESS >10; no cataplexy; sleep latency <10 minutes; MSLT with >1 sleep onset REMs; no other evident cause(s) of EDS and Apnea-Hypopnea Index (AHI) <10; no improvement in EDS after sleep extension. PH was diagnosed in patients who fulfilled the criteria for MN but who had no sleep onset REMs.

CSF samples were collected between 10 am and 11.30 am, immediately frozen, coded, and transported to the laboratory. Radioimmunoassay kits (Phoenix Pharmaceuticals, Mountain View, CA) were used to measure hcrt-1 and hcrt-2 concentrations as previously reported.

4 Statistical analysis
In addition to a standard descriptive statistics, we used two way analysis of variance and paired t test to examine data relations.

RESULTS
Control group
CSF samples were obtained from stocked samples previously taken for research purposes. The control group of 24 was subdivided as follows: 19 women, five men; median age 36.4 years, range 21.2–47.3. Thirteen of the 24 control CSF samples were drawn from epidural taps of women in labour. The remaining 11 were obtained from patients presenting with neurological symptoms without any organic basis (conversion disorders). In keeping with previously reported data in control groups, hcrt-1 was detectable in all the control samples with a mean (SD) of 210 (46) pg/ml.

Hcrt-2 was detectable in all the CSF samples of the control group (mean (SD) of 9.4 (7.9) pg/ml; range: 2–43 pg/ml).

Study group
The study group comprised 24 patients (17 women, seven men (M), median age 36.5 years, range 19.1–67.5). NC was diagnosed in 14 patients (12 women, two men), MN was diagnosed in four patients (three women, two men), and in six patients (two women, four men) a diagnosis of PH was made.

Narcolepsy-cataplexy
Sleep study assessments in this group revealed ESS results broadly in keeping with that expected with a mean score of 19.6. MSLT analysis (n=13, one patient was unable to complete a sleep study) revealed a mean sleep latency of 3.9 minutes. All the patients with NC were HLA DQB1*0602 positive. The group’s mean body mass index (BMI) was 31.1 (6.2) kg/m², similar to previous published findings. CSF hcrt-1 was below the detection limit (40 pg/ml) in all the patients with NC and the mean CSF hcrt-2 concentration was 4.32 (1.93) pg/ml.

Monosymptomatic narcolepsy
This group scored a mean of 18 on the ESS with the MSLT analysis revealing a mean sleep latency of 5.4 minutes. Three of four patients were HLA DQB1*0602 positive. The mean BMI was 25.6 (5.2) kg/m². Very low values of CSF hcrt-1 were found in patients with MN (n=4; mean=48 (3) pg/ml) and CSF hcrt-2 concentrations were mean 2.86 pg/ml (2.33) pg/ml. Hcrt-2 was completely undetectable in a single patient with MN who was indistinguishable from the others on clinical and NPSG criteria.

Primary hypersomnia
Patients in this group had a mean ESS score of 18 and a mean sleep latency of 5.7 minutes. A single patient with PH was also HLA DQB1*0602 positive. The mean BMI was 22.7 (1.2) kg/m². The mean CSF hcrt-1 was 67.9 (12.4) pg/ml whereas the mean CSF hcrt-2 concentration was 71.3 (3) pg/ml.

Analysis for the entire sample revealed a mean sleep latency (n=23) of 4.6 minutes. BMI measures showed a mean (SD) of 28.1 (6.3) kg/m². There was no demonstrable correlation between CSF hcrt-1 concentrations and ESS or sleep latency in the group as a whole and in each subgroup. The mean CSF hcrt-2 was 4.8 (2.6) pg/ml and a range of 0–11 pg/ml.

Statistical analysis using two way analysis of variance and paired t test for means reveals that the differences observed in CSF hcrt-2 concentrations between the control and study

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<th>Diagnosis</th>
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<th>HCRT-2 (pg/ml)</th>
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<td>210 (46)*</td>
<td>9.4 (7.9)*</td>
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ND, not detectable. + mean (SD).
groups is highly significant ($p<0.01; r=2.80$). In addition, Pearson correlation coefficients were calculated for the groups comparing hcrt-1 and hcrt-2 concentrations. There was no significant correlation between the control group’s hcrt-1 and hcrt-2. There was however a significant correlation ($r=0.44$) between hcrt-1 and hcrt-2 in the study group. There was no correlation between time since onset of symptoms and CSF hcrt concentrations.

Table 1 gives an outline of the individual results.

**DISCUSSION**

There has been substantial data gathering of CSF hcrt-1 concentrations in a variety of disorders and from control populations. The initial study reported a comparatively narrow range of hcrt-1 (250–280 pg/ml) in healthy controls. Subsequent studies in larger samples have examined CSF hcrt-1 across sex and age and, in all these studies there has been no significant difference with respect to sex or generations. The present view is that undetectable and low CSF hcrt-1 concentrations are an abnormal finding at any age.

The normal range as measured in healthy controls, has consistently been reported as being in the range 160 pg/ml to 600 pg/ml. Values above 600 pg/ml are considered increased. Hcrt-1 concentrations may be described as undetectable if they are below the detection limit (40–50 pg/ml) of the assay, low values, according to most researchers, are hcrt concentrations below 200 pg/ml, and very low values are below 100 pg/ml.

**Narcolepsy and primary hypersomnia**

The initial study found seven of nine HLA DQB1*0602 positive patients with NC had undetectable concentrations (<40 pg/ml) of hcrt-1 in their CSF. Of the two patients with detectable levels of hcrt-1, one was within the control range and the other had increased values. More recently, the same authors report that in a sample of 38 patients with NC, CSF hcrt-1 was dramatically decreased (<100 pg/ml) in 32 HLA DQB1*0602 positive patients, four patients had normal values, two HLA negative patients had high values (609 and 637 pg/ml) and two HLA positive patients had high values (609 and 637 pg/ml).

Our findings for CSF hcrt-1 values in NC are consistent with the hypothesis that NC characteristically displays a deficiency of CSF hcrt-1 and that this may be the cause of narcolepsy. We have not replicated the finding that there may be a variant of narcolepsy with normal or high concentrations of CSF hcrt-1. There has not been, to our knowledge, any data published on hcrt-1 concentrations in patients with MN and IH and we report, for the first time, very low values (<100 pg/ml) of CSF hcrt-1 concentrations in MN and PH in a small sample of patients.

The finding that hcrt-2 concentrations are uniformly and significantly decreased in all these conditions suggests that defective hypocretin neurotransmission is generalised and affects both forms of hypocretin. The primary clinical manifestations of this defect are EDS, and in a significant proportion, the presence of cataplexy and a positive test for HLA DQB1*0602. We have confirmed the recent finding that patients with NC have a significantly raised BMI.

One possible implication of these and previous studies is that hypocretin deficiency gives rise to a clinical spectrum that covers the current diagnostic groups of NC, MN, and PH and that these may form a continuum of severity progressing from PH through to MN and finally NC. This is reflected in our findings showing a progressive increase in hcrt-1 concentrations passing from NC to MN and then to PH.

Figure 1 summarises these findings in graphic form.

Another explanation, conforming to HLA associations is that there may be two diagnostic entities; one, a hypocretin deficiency syndrome associated with HLA DQB1*0602 and another group that is not. The first group would include most patients with NC and MN whereas the second group would include most patients with PH and a significant proportion of patients with MN. These findings may shed light on the longstanding debate about the clinical heterogeneity of the diagnoses of MN and PH.

Studies in animals have shown that hcrt-1 and hcrt-2 have similar functionality but with hcrt-1 thought to play a part in energy metabolism and sleep regulation and hcrt-2 to have a more sleep specific role. The analysis of the CSF hcrt-2 concentrations seems to add impetus to this opinion, showing a pattern of deficiency that is generalised in all the diagnostic groups and statistically significant when compared with the control population.

The precise mechanism of hypocretin deficiency in NC is unknown. There is some debate on the autoimmune versus neurodegenerative mechanisms. There is some clinical and anecdotal evidence that narcolepsy is a progressive disorder in a significant proportion of patients.

In conclusion, we have replicated the finding that hcrt-1 is deficient in NC and have demonstrated hcrt-1 and hcrt-2 deficiency in NC, MN and PH. We have also shown, albeit in a small sample, a graded deficiency of hcrt-1 in these three clinical entities. We believe that these findings will have important implications for the diagnosis of pathological sleepiness and suggest that further studies are warranted to clarify these results.

**References**