LETTER TO THE EDITOR

CSF Hypocretin Levels are Normal in Huntington’s Disease Patients

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DEAR EDITOR,

HUNTINGTON DISEASE (HD) IS A FATAL, AUTOSOMAL-DOMINANT DISORDER ASSOCIATED WITH INCREASES IN THE LENGTH OF A CAG REPEAT IN A GENE called ‘huntingtin’ located on chromosome 4p16.3. Onset is variable but typically occurs in midlife and gives rise to progressive, selective (localized) neural cell death associated with choreic movements and dementia. Sleep disturbances have been reported and include sleep fragmentation and decreased slow wave sleep. The occurrence of sleep disordered breathing and periodic leg movements during sleep in HD have also been reported, but it is uncertain at this point if this is coincidental.5

Low hypocretin-1 (hcrt-1), as measured in lumbar sac CSF (cerebrospinal fluid), is highly predictive of narcolepsy-cataplexy.6 Recently, Petersen et al.7 reported reduced hypocretin (hcrt; also known as orexin) transmission in HD. In this study, a transgenic mouse model of HD (R6/2) displayed 72% fewer hcrt neurons compared to wild-type littermates (at 12.5 weeks), and hcrt-1 measured in the CSF was 72% lower in the HD mouse model. Post-mortem human hypothalami were also examined, and 27% fewer hcrt neurons were observed when compared to controls. The authors hypothesized that hcrt neurons are sensitive to HD-mediated neurodegeneration via mutant huntingtin expression and/or excitotoxicity. Further, hcrt deficiency could explain sleep disturbances in HD, although these subjects do not have cataplexy.

These results suggested to us that in HD patients, hcrt levels may be reduced in the CSF. Such a finding would implicate CSF hcrt as a novel biomarker for HD progression, with potential application in therapeutic trials. To test this hypothesis, we measured hcrt-1 levels in post-mortem CSF from 10 HD patients diagnosed both clinically and neuropathologically (age 43-72, mean 61 years old, 2 females), and 10 controls (age 53-93, mean 78 years old, 5 female, with normal neuropathology). The selection of postmortem samples was because these patients are more likely to be at a late stage of the disease, thus allowing a conservative test of our hypothesis of preferential and early hcrt neurodegeneration in HD.

Hcrt-1 was measured using 125I radioimmunoassay (Orexin A RIA Kit, Phoenix Pharmaceuticals, Belmont, CA) of 25uL nonextracted CSF samples, diluted to 100uL. Measurements were taken blind to diagnosis, in duplicate, in a single assay, with a detection limit of 4 pg/tube. Inter-assay variation was adjusted by including a reference CSF sample (134 pg/mL); intra-assay variability was 2.9%. Our previous data indicates no differences in lumbar CSF hcrt-1 with regard to age, gender, time of collection, freeze-thawing, room-temperature incubation up to 4d, or long-term frozen storage, and autolysis (3-29.5 hours) did not correlate with hcrt-1 levels (p>0.43). Of note, normal values in postmortem CSF have not been previously evaluated.

The mean level of hcrt-1 in CSF from HD patients was 249.2 pg/mL (range 147-432), and that of controls was 254.7 pg/mL (range 98-384). Control values in postmortem CSF were slightly lower than in healthy living volunteers, possibly as the result of dilution, time and location of collection, and/or disease status/agonal state prior to death. CSF hcrt-1 levels were not significantly different in HD patients compared to controls (p>0.9 using parametric or nonparametric statistics; see Figure 1). We also attempted to correlate CSF hcrt-1 levels with age of disease onset (range: 31-58 years old, n=9) or disease duration (9-21 years old, n=9); neither correlation was significant (p>0.24). We conclude that CSF hcrt-1 levels would not serve as an appropriate biomarker for HD in humans.

It is possible that the hcrt reduction observed in a mouse model is specific to that model or to that species. In human tissue, the reduction in hcrt-positive cell number was not as striking as in the mouse model (27% vs 72%). This modest reduction in number appears not to be reflected in the CSF of HD patients.

Disclosure Statement
Drs. Gaus, Lin, and Mignot have indicated no financial conflicts of interest.

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possibly because of compensatory mechanisms. This result is in agreement with rat studies indicating a need for a 73% decrease in neuronal population to decrease CSF hcrt-1 levels by 50%.\textsuperscript{9} Whether sleep disturbances in HD may result from this modest loss of hcrt-expressing cells is unknown; these disturbances are also not necessarily related to dyskinesia,\textsuperscript{11} thus, their etiology is remains unclear.

In narcolepsy, loss of hcrt is attributed to loss of cell bodies.\textsuperscript{10} Specificity of hcrt cell death still needs to be established in HD, although the presence of atrophic hcrt-containing cell bodies\textsuperscript{7} suggests active degeneration. Further studies in mice and other models may shed light on the question of preferential sensitivity of hcrt cells to the HD process in some species or experimental conditions. This question is of interest not only for HD specialists, but also to the field of narcolepsy, as hcrt cells also preferentially degenerate in this disorder.

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REFERENCES