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Hypocretin Pathology in Human Narcolepsy

Thomas C. Thannickal and Jerome M. Siegel
Neurobiology Research, VA GLAHS Sepulveda, UCLA Dept. of Psychiatry, David Geffen School of Medicine, Los Angeles, CA 90024, USA.

I. Introduction

Most cases of narcolepsy are caused by abnormalities of the hypocretin (Hcrt) system. Human narcolepsy is most likely due to postnatal degeneration of Hcrt cells through an inflammatory process. The most reasonable hypothesis explaining Hcrt cell loss is that it is immune system mediated. Intravenous administration of Hcrt can reverse the symptoms of narcolepsy. Variability in symptom expression among human narcoleptics, and even among animal mutant narcoleptics, remains to be fully explained and most likely involves interactions between Hcrt neurons and brain aminergic and amino acid systems.

In 2000, two papers identified the loss of Hcrt cells as the cause of human narcolepsy.[1,2] Both papers also concluded that melanin concentrating hormone cells were present in approximately normal numbers in the regions in which Hcrt cells are typically found. This suggested that the loss of Hcrt cells was relatively specific; that adjacent melanin concentrating hormone cells were spared by whatever process was responsible for the loss of Hcrt cells. The specificity of cell loss is consistent with the hypothesis that narcolepsy is an autoimmune disease. This hypothesis was first suggested by Honda, who discovered the HLA linkage of narcolepsy.[3] Since HLA linked diseases have been found to have an autoimmune etiology, Honda hypothesized that narcolepsy was caused by immune system damage to the CNS. This hypothesis was given greater specificity by the finding of a discrete loss of Hcrt cells.

Although the general conclusions of the Thannickal et al.[2] and Peyron et al.[1] papers were similar, the differences in the details of the findings of these papers have important implications for understanding the etiology of the disease. The Peyron et al. paper reported that there was no visible gliosis in the narcoleptic brains compared to controls. However, Thannickal et al. reported greatly elevated gliosis in hypothalamic regions, with numbers of astrocytes in the hypothalamus of narcoleptic humans that were double or triple those of control brains. Thannickal et al. also reported that levels of gliosis in thalamic regions did not differ between narcoleptic and normal brains.

The presence of gliosis indicates that Hcrt cells were lost due to an inflammatory process, rather than a developmental or apoptotic process. This would be consistent with the idea that human idiopathic narcolepsy is caused by an autoimmune attack. We compared the intensity of gliosis in narcoleptic brains in the hypothalamic regions in which Hcrt cells had been lost and in the Hcrt projection regions.[4] We found that although gliosis was intense in Hcrt cell loss regions, it was equally or more intense in certain projection regions that were devoid of Hcrt cell somas such as paraventricular, periventricular, arcuate and tuberomammillary nucleus. When we investigated the pattern of gliosis we found that it was most intense in regions which contained

Hcrt receptor 2. It was also particularly intense in regions with high concentrations of Hcrt axons in the normal brain. The number of axons counted with the unbiased counting frames yields an unbiased estimate of the total number of axons. By identifying the axon hillock or by excluding tapering processes each axon could be distinguished from dendritic branches. Dendrites have many processes and their surface is irregular and covered in dendritic spines where as axons have varicosities. The numerical density of hcrt axons whereas calculated as number of axons per unit area (mm^2).[4] Our statistical analysis indicated that gliosis was linked to the density of Hcrt receptor 2 and to axonal density, and that these two correlations were independent. A unifying hypothesis that incorporates these findings states that the type 2 Hcrt receptor or an antigen that is anatomically linked to it is the target for an autoimmune attack that ultimately kills Hcrt cells and that this immune reaction is intensified in regions of high axonal density.

Another difference between the Peyron et al. and the Thannickal et al. papers is that the latter repotted the presence of surviving cells in all narcoleptics, although the overall number of cells was reduced on average by 90%. In contrast, the Peyron et al. paper reported that all Hcrt cells were lost. The latter observation coincided with results from assays of Hcrt levels in the cerebrospinal fluid of narcoleptics.[5] However, both observations have been limited by the sensitivity of the assays used. In situ autoradiography, used for the detection of Hcrt cells is less sensitive than immunohistochemistry. Immunohistochemistry labels the antigen in an all or none process if a specific antigen is used and procedures properly followed. In our experience this procedure produces highly reproducible number of neurons when sampled with unbiased stereology in repeated measures of the same or several normal brains. In contrast, in situ labeling, the labeling is graded and thresholds have to be established ad hoc and be compared with control tissue. Thus although relative numbers can be determined, absolute number and issue of presence or absence of particular cell types can not be made with great confidence. Likewise, the report that Hcrt CSF levels are below detectable limits may be a function of the sensitivity of the assay. We predict that more sensitive assays will show greatly reduced, but still detectable, Hcrt levels in most human narcoleptics. Residual Hcrt function can explain the much less severe cataplexy in humans than is observed in knockout animals, which completely lack Hcrt. The variability in the location and projections of Hcrt cells lost, may also affect the relative intensity of symptoms expressed in human narcolepsy, although this has yet to be documented.

Hcrt levels can affect the intensity of narcoleptic symptoms. We administered Hcrt-1 intravenously to canine narcoleptics and found a significant reduction in symptoms, with increased consolidation of waking activity and reduced cataplexy, as well as a reduction in REM sleep comparable to that seen with intracerebroventricular Hcrt.[6] In recent work these same effects have been seen in the idiopathic narcoleptic dogs with presumed Hcrt cell loss, and also in genetically narcoleptic (Hcrtr-2 mutant) dogs.[7,8] In the latter, a significant suppression of REM sleep was reported after IV administration of Hcrt. Although the latter studies used much higher doses of Hcrt, both studies indicate that systemic Hcrt administration is effective, and that administration of effective doses produces no apparent side effects.

Because increasing Hcrt levels by IV administration reduced narcoleptic symptomatology, and because exercise increased Hcrt levels,[9] we wondered whether exercise would reduce cataplexy levels. We found that this was indeed the case. Exercise decreased cataplexy with a time course mirroring that of exercise induced shifts in Hcrt level.[10] We also find that physostigmine and prazosin given in doses that increase cataplexy greatly reduce Hcrt

level. Conversely, labetalol, phenylephrine and methamphetamine, all of which reduce narcoleptic symptomatology, increase Hcrt levels. However, atropine, which reduces cataplexy, does not affect Hcrt levels, presumably acting "downstream" from Hcrt.[11]

Although these data clearly indicate a tight linkage between Hcrt levels and narcoleptic symptomatology, the level of Hcrt and even the genetic integrity of the Hcrt system is not the sole determinant of the nature and severity of symptomatology. There is a marked developmental variation in symptom intensity in genetically narcoleptic Hcrtr-2 mutant dogs. Dogs are not symptomatic at birth and gradually become symptomatic starting at 1-2 months of age. Symptoms peak in intensity and gradually diminish with age. Some older dogs show no cataplexy by 2-3 years of age, although symptoms can be reinstituted by administration of physostigmine or prazosin. These drugs produce no cataplexy when administered alone or in combination to normal dogs, even at very high doses. A recent study of the developmental changes in Hcrt levels show that adult Hcrt levels are present at birth and although these levels change over the course of development, these changes cannot by themselves explain the changes in symptoms with age.[12]

In an additional study, we found that treatment of narcoleptic dogs with methylprednisolone and methotrexate starting at birth dramatically delayed and greatly reduced symptoms of narcolepsy compared to littermates.[13] This reduction was not a direct drug effect of these immunosuppressant drugs. Transient treatment with these drugs for up to two weeks had no significant effect on cataplexy and other measures of narcoleptic symptomatology. However, chronic treatment from birth to the age of 4-6 months produced an apparently permanent reduction in symptoms in otherwise normally active and healthy animals. This treatment effect may have been mediated by immune system interactions with neural development or by direct long term effects of the administered drugs on the brain degenerative processes in the developing narcoleptic brain.[14] It illustrates that the genetically determined trait of narcolepsy can be strongly modulated by drug administration produced changes in the brain.

These demonstrations of variability in symptom expression in narcoleptic animals are of great relevance to understanding and treating human narcoleptics. Humans with apparently identical Hcrt depletion can have severe cataplexy or no cataplexy at all. One explanation of this variability is that, although overall Hcrt depletion is similar, patients may have a heterogeneous pattern of cell loss, and each pattern of cells loss may be associated with a distinctive set of symptoms. Another explanation is that the primary determinant of the symptomatology resulting from Hcrt depletion depends on indirect effects of Hcrt loss of brain functioning which are distinctive in each individual. Cases of narcolepsy with normal Hcrt level have also been identified.[5] These cases suggest that disruption of systems affected by Hcrt can cause symptoms of narcolepsy even without any Hcrt cell loss. Another explanation of this phenomenon is that there may be other causes of narcolepsy independent of Hcrt pathology. Hypocretin neurons have been shown to powerfully drive aminergic neurons[15-17] and has been shown to produce glutamate release.[18,19] Therefore, the loss of both direct and indirect effects of hypocretin are likely to be responsible for the symptoms of narcolepsy.

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