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Short communication

Reduced orexin immunoreactivity in Perry syndrome and multiple system atrophy

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ABSTRACT

Introduction: Orexin is a neuropeptide that plays a key role in maintaining a state of arousal, and possibly associates with sleep apnea syndrome (SAS). Reduced orexin immunoreactivity has been reported in various neurologic conditions such as narcolepsy, Alzheimer's disease, Lewy body disease and multiple system atrophy (MSA); however, there has been no report investigating orexin in Perry syndrome, a rare hereditary neurodegenerative disease characterized by four clinical cardinal signs (parkinsonism, depression/apathy, weight loss, and central hypoventilation). Perry syndrome patients frequently have sleep disturbances, including SAS and insomnia.

Methods: We evaluated orexin immunoreactivity in Perry syndrome. Using imaging analysis, we quantitatively assessed orexin immunoreactivity in the nucleus basalis of Meynert in three Perry syndrome cases, as well as five cases of frontotemporal lobar degeneration with motor neuron disease, five cases of MSA and five age-matched controls. For these cases, antemortem clinical information on sleep disturbances has been reviewed.

Results: In Perry syndrome and MSA, there was reduction of orexin immunoreactivity compared with controls (Perry syndrome: $p = 0.020$, MSA: $p < 0.001$). In contrast, FTLD-MND did not have significant reduction of orexin immunoreactivity. Two out of three cases of Perry syndrome had SAS confirmed by polysomnography.

Conclusions: This is the first report assessing orexin immunoreactivity in Perry syndrome, and it showed significant reduction, similar to select neurodegenerative diseases, such as MSA. Further analysis with more cases will be needed to elucidate the specific mechanism of orexin loss in these disorders.

1. Introduction

Perry syndrome is a rare autosomal dominant disorder clinically characterized by four cardinal signs – parkinsonism, apathy/depression, weight loss, and hypoventilation [1–5]. Genetically, nine point mutations have been identified: F52L, K56R, G67D, G71A, G71E, G71R, T72P, Q74P, and Y78C located in exon 2 of *DCTN1*, encoding $p150^{glued}$, the large subunit of the dynactin complex [1,3,4]. Perry syndrome is a neurodegenerative disorder that has severe neuronal loss and gliosis in the substantia nigra and few or no Lewy bodies or neurofibrillarytangles [2,5]. In addition, all autopsy proven cases of Perry syndrome have had TAR DNA-binding protein of 43 kDa (TDP-43) pathology. Therefore, Perry syndrome is categorized as a TDP-43 proteinopathy along with amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) with TDP-43 inclusions (FTLD-TDP) [2]. Perry syndrome shows various morphological lesions of TDP-43: neuronal cytoplasmic inclusions (NCIs), dystrophic neurites (DNs), perivascular astrocytic inclusions (PVIs), oligodendroglial cytoplasmic inclusions (GCIs), neuronal intranuclear inclusions (NIIs), and axonal spheroids [2].

Orexin is neuropeptide synthesized in the lateral hypothalamus, and its neurons send extensive projections to the basal forebrain, brainstem, and cerebral neocortex [6–8]. It is well known that orexin plays a role in regulating state of arousal by modifying

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ascending arousal systems, including basal forebrain cholinergic neurons in the nucleus basalis of Meynert (nbM) [6]. Reduction of orexin immunoreactivity has been reported in various neurologic conditions with impaired wakefulness, including narcolepsy, Alzheimer's disease (AD), Lewy body disease (LBD) and multiple system atrophy (MSA) [7,8] although the relationship of neurodegenerative accumulation of pathologic proteins and orexin remains unclear. To date no studies have been published on orexin immunoreactivity and its relation to TDP-43 pathology in Perry syndrome.

Therefore, in this study, we assessed orexin immunoreactivity in the nbM of Perry syndrome. We also evaluated orexin immunoreactivity in frontotemporal lobar degeneration with motor neuron disease (FTLD-MND), a form of TDP-43 proteinopathy, and MSA for comparison. Furthermore, we investigated correlations between orexin immunoreactivity and TDP-43 pathology.

2. Methods

2.1. Subject selection and sleep evaluation

We examined brain samples from three Perry syndrome cases (Case 1; West Virginia family, Case 2; Canada family, and Case 3; Columbia family). We also included five age-matched control, five FTLD-MND, and five MSA cases with available paraffin-embedded tissue and medical documentation with respect to age, sex, and clinical diagnosis in the Mayo Clinic brain bank. The detailed clinical history and sleep evaluation of patients with Perry syndrome have been previously reported [1,2]. Available clinical information on sleep disturbances in medical records was evaluated.

All brain autopsies were carried out after consent of the families or individual with power-of-attorney. Studies on autopsy tissues are considered exempt from human subject research by the Mayo Clinic Institutional Review Board.

2.2. Immunohistochemistry

Most of the brains were obtained with the left hemibrain fixed in 10% formalin and the right hemibrain frozen at −80 °C. Formalin-fixed brains underwent systematic and standardized sampling and neuropathological diagnosis by a single, experienced neuropathologist (D.W.D) [9]. Regions sampled on all cases included six areas of neocortex; two levels of hippocampus; a basal forebrain section that includes the nbM, amygdala, globus pallidus, putamen, and anterior hypothalamus; caudate nucleus; thalamus at the level of the subthalamic nucleus; midbrain; pons; medulla, and two sections of cerebellum, one with the deep cerebellar nuclei [9]. Paraffin-embedded 5-μm thick sections mounted on glass slides were stained with hematoxylin and eosin. Two Perry syndrome cases (Case 1 and Case 2) were included in a previously published pathologic study [2]. Immunohistochemistry for α-synuclein (NACP, 1:3000, Mayo Clinic, FL) was examined to determine neuropathological diagnosis of MSA. MSA cases were pathologically divided into MSA with predominant striatonigral involvement, MSA with predominant olivopontocerebellar involvement, and MSA with equally severe involvement of striatonigral and olivopontocerebellar systems [10].

To evaluate orexin immunoreactivity, we performed immunohistochemistry using orexin (1:200, Phoenix Pharmaceuticals Inc) in the nbM as previously reported [9]. Due to previous tissue sampling for routine diagnostic neuropathology and the retrospective nature of the pathologic evaluation, the lateral hypothalamus where orexin neurons are produced was not available for analysis. Therefore, data analyses were performed with specific emphasis on available regions of the brain known to be involved in the nbM. Orexin immunoreactivity was quantified using a positive pixel count algorithm that uniquely identifies neuritic burden, based on morphology (Aperio Technologies, Inc, Vista, CA) [9].

To assess TDP-43 pathology in the nbM, we also performed immunohistochemistry with TDP-43 antibody (MC2085, 1:3,000, Dr. L Petrucelli, Mayo Clinic, FL) and phospho TDP-43 (1:5,000, Cosmo Bio, CA). Slides were reviewed simultaneously by two observers (D.W.D., T.M.) who agreed on the presence of TDP-43 lesions, defined as NCIs, DNs, PVIs, GCIs, NIIs, and spheroids in the nbM. The severity of TDP-43 pathology was scored on a five-point scale as 0 = absent; 0.5 = rarely observed; 1 = sparse; 2 = moderate and 3 = frequent in each lesion. A total TDP-43 pathology score was calculated as the summation for each score of six morphologic lesions of TDP-43 (NCIs, DNs, PVIs, GCIs, NIIs, and spheroids).

2.3. Statistical analysis

All statistical analyses were performed using SigmaPlot 12.0 (Systat Software Inc.). Shapiro-Wilk test was carried out to assess if the data were normality distributed. The mean orexin immunoreactivity of the group was assessed with ANOVA on ranks followed by the Tukey Kramer post hoc test. Spearman correlation analysis was used to test the correlation between orexin immunoreactivity and total TDP-43 pathology scores. P values < 0.05 were considered statistically significant.

3. Results

3.1. Clinical findings

Characteristics of the subjects are shown in Table 1. The mean ages at death were as follows: control, 52.8 ± 5.1 years; Perry syndrome, 52.7 ± 8.1 years; FTLD-MND, 53.2 ± 6.6 years; MSA, 60.0 ± 4.8 years ($p = 0.214$). Two of the Perry syndrome patients (Case 1 and Case 2) were confirmed as having SAS by polysomnography (PSG). One FTLD-MND patient (Case 6) had unspecified sleep disturbances. All MSA patients showed sleep disturbances, which was documented on PSG. Two of MSA patients (Case 11 and Case 12) showed SAS. Eight patients (44.4%) had clinical documentation of sleep disturbances.

3.2. Orexin immunoreactivity

In the nbM, orexin immunoreactivity was detected in beaded neurites in the control cases (Fig. 1A and B). In contrast, the Perry syndrome and MSA had decreased orexin immunoreactivity, which was significantly reduced compared to controls (Perry syndrome: $p = 0.020$, MSA: $p < 0.001$). FTLD-MND showed some reduction of orexin immunoreactivity, but it did not reach statistical significance ($p = 0.405$) (Fig. 1C).

3.3. TDP-43 pathology

The density of each morphological type of TDP-43 lesion was assessed in Perry syndrome, FTLD-MND, MSA, and controls and summarized in Supplementary Table 1. The range of total TDP-43 pathology score was 0–3.5. All Perry syndrome cases had TDP-43 lesions in the nbM, with NCIs, DNs, PVIs, and NIIs detected in Perry syndrome. Four FTLD-MND cases had TDP-43 lesions in the nbM, including NCIs, PVIs, DNs, and GCIs. One case (Case 4) did not have TDP-43 lesions in the nbM. MSA and control cases did not have TDP-43 pathology in the nbM. Reduction of orexin immunoreactivity was not correlated with total TDP-43 pathology scores in the nbM ($r = -0.554$, $p = 0.139$).

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