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Neuronal cell loss in the dorsal raphe nucleus and the superior central nucleus in myotonic dystrophy: a clinicopathological correlation

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Abstract A quantitative study of neurons in the dorsal raphe nucleus (DRN) and the superior central nucleus (SCN) was performed in seven patients with myotonic dystrophy (MyD), five of whom showed hypersomnia, and in eight age-matched controls. The densities of neurons in the DRN and the SCN were significantly lower in MyD patients with hypersomnia than in MyD patients without hypersomnia and control subjects. There was an appreciable positive correlation in the density of neurons between the DRN and the SCN in all MyD patients. These data suggest that the neuronal loss of the DRN and the SCN is associated with the presence of hypersomnia in MyD.

Key words Myotonic dystrophy · Dorsal raphe nucleus Superior central nucleus · Hypersomnia

Introduction

Although hypersomnia is a prominent feature of many patients with myotonic dystrophy (MyD) [7], its pathogenesis in MyD patients has not been fully clarified. Recent data support the hypothesis that hypersomnia in MyD is a primary central nervous system disturbance [3, 6].

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The raphe nuclei are the main serotonin-containing neurons in the brain stem and play an important role in the regulation of the sleep-wake cycle [8]. However, studies in patients with MyD that consider possible relationships between the extent of involvement of these systems and the presence of hypersomnia have not been previously reported. The present report is the first morphometric study of the dorsal raphe nucleus (DRN) and the superior central nucleus (SCN) in patients with MyD and in age-matched controls.

Patients and methods

Patients

Seven patients with MyD (mean age, 62 years; range, 55–71 years; necropsy delay, 8 h) and eight control subjects without neurological diseases (mean age, 67 years; range, 61–74 years; necropsy delay, 10 h) were studied (Table 1). The main diagnostic criteria of

Table 1 Clinical summary

Patient no.	Age	Sex	Diagnosis	Hypersomnia	Hypoventilation
Patients with mytonic dystrophy					
1	62	F		+	+
2	58	M		+	+
3	55	M		+	+
4	71	F		+	–
5	66	F		+	–
6	63	M		–	–
7	61	M		–	–
Control subject					
1	70	M	Lung cancer	–	–
2	64	M	Heart failure	–	–
3	74	M	Liver cirrhosis	–	–
4	66	F	Breast cancer	–	–
5	69	F	Pneumonia	–	–
6	65	F	Leukemia	–	–
7	61	F	Renal Failure	–	–
8	67	M	Hepatoma	–	–

MyD were clinical, electrophysiological, and biopsy findings. The age, sex distribution and necropsy delay of the two groups were comparable.

Tissue processing and staining

From each case one or more blocks from brain stem containing the DRN and/or the CNS cut in the transverse plane were dehydrated and embedded in paraffin. Sections of 6-μm thickness were cut and stained with hematoxylin and eosin (H&E), Nissl, and Klüver-Barrera's staining methods.

Selection of slides and definition of boundaries of nuclear groups

The DRN was examined at a level half way through the trochlear nucleus. With reference to Olszewski and Baxter [13] we defined the DRN as the area bordered ventromedially by the trochlear nuclei, the medial longitudinal fascicules, and their thin median connection; laterally by the mesencephalic tracts and nuclei of the trigeminal nerves; and dorsally by a horizontal line across the center of the cerebral aqueduct.

A section of the pons, 2 mm under the inferior colliculus, was taken to enclose the largest part of the SCN. The area of the SCN was determined according to Olszewski and Baxter [13]; the SCN extended ventrally from the medial longitudinal fasciculus and was located dorsal to the superior cerebellar peduncle decussation. Its lateral borders were the predorsal fasciculus.

Counts of neurons

All nucleolus-containing neurons (> 25 μm in greatest diameter) within areas designated as the DRN and the SCN were counted under 400-fold magnification, using an ocular micrometer. The areas of the DRN and the SCN were calculated by counting the number of 1-mm squares in a transparency placed over a photograph enlarged 20-fold. The cell densities of neurons in the DRN and the SCN were calculated by dividing the number of neurons into the areas of the DRN and the SCN previously obtained.

Statistical comparisons were made by unpaired Student's *t*-test and $P < 0.05$ as the significance level. Correlation coefficients were calculated by the least squares methods.

Results

Significant raphe pathology was found in certain MyD cases. When MyD patients were divided into those with hypersomnia (cases 1–5) and those without hypersomnia (cases 6 and 7), severe neuronal loss and gliosis were observed in the DRN and the SCN in MyD patients with hypersomnia, but were not observed in MyD patients without hypersomnia or in control subjects.

The density of neurons in the DRN in MyD patients with hypersomnia (mean ± SD, 7.00 ± 2.12/mm^2; range, 4.9–10.1/mm^2) was significantly lower ($P < 0.02$ and $P <$

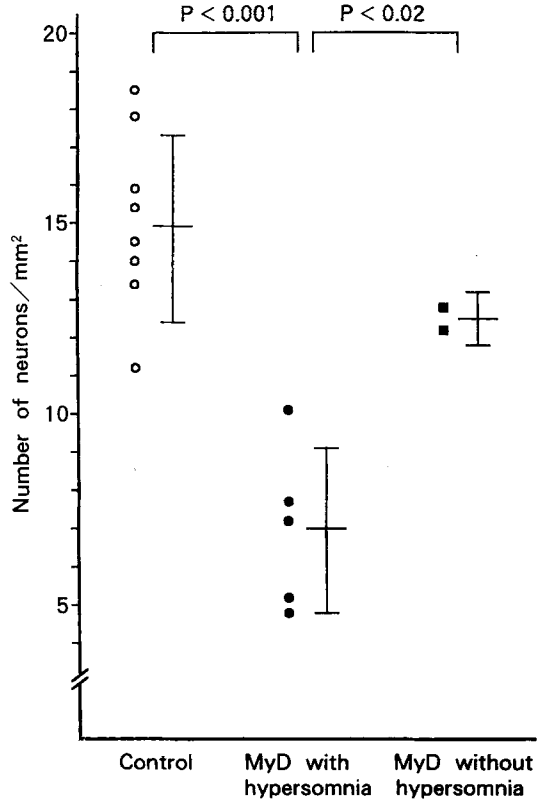

Fig. 1 The density of neurons in the dorsal raphe nucleus in patients with myotonic dystrophy (MyD) with hypersomnia, MyD patients without hypersomnia, and control subjects. The density of neurons was significantly lower in MyD patients with hypersomnia than in MyD patients without hypersomnia ($P < 0.02$) and control subjects ($P < 0.001$). *Bar* shows mean ± SD

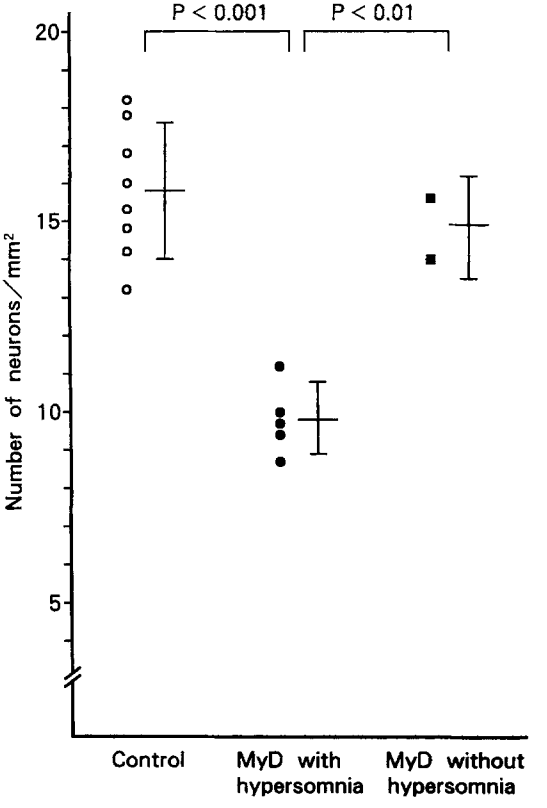

Fig. 2 The density of neurons in the superior central nucleus in patients with myotonic dystrophy (MyD) with hypersomnia, MyD patients without hypersomnia, and control subjects. The density of neurons was significantly lower in MyD patients with hypersomnia than in MyD patients without hypersomnia ($P < 0.01$) and control subjects ($P < 0.001$). *Bar* shows mean ± SD

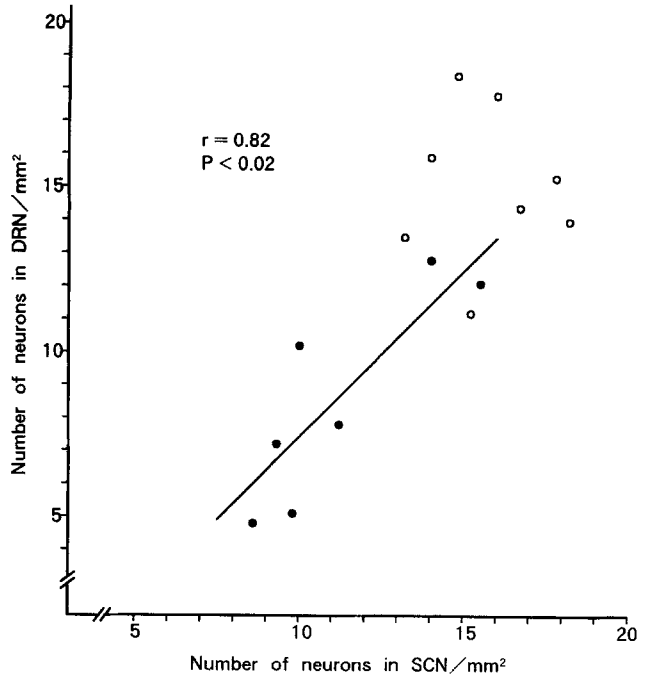

Fig. 3 Correlation of density of neurons between the dorsal raphe nucleus and the superior central nucleus in patients with myotonic dystrophy *(closed circles)* and control subjects *(open circles)*. There was a significant positive relationship between the dorsal raphe nucleus and the superior central nucleus in patients with myotonic dystrophy ($r = 0.82$, $P < 0.02$)

0.001) than in MyD without hypersomnia (mean ± SD, 12.52 ± 0.71/mm^2; range, 12.1–12.9/mm^2) and control subjects (mean ± SD, 14.91 ± 2.42/mm^2; range, 11.1–18.4/mm^2) (Fig. 1).

The density of neurons in the SCN in MyD patients with hypersomnia (mean ± SD, 9.78 ± 0.87/mm^2; range, 8.6–11.1/mm^2) was significantly lower ($P < 0.01$ and $P < 0.001$) than in MyD without hypersomnia (mean ± SD, 14.85 ± 1.41/mm^2; range, 14.0–15.7/mm^2) and control subjects (mean ± SD, 15.75 ± 1.81/mm^2; range, 13.3–18.2/mm^2) (Fig. 2).

There was an appreciable positive correlation in the density of neurons between the DRN and the SCN in all MyD patients ($r = 0.82$, $P < 0.02$), but there was no such correlation in control subjects (Fig. 3).

Discussion

There is controversy as to the etiology of hypersomnia in MyD. Respiratory insufficiency secondary to involvement of respiratory muscles and impaired respiratory center sensitivity have been suggested [10]. However, several studies provided strong evidence against a respiratory cause of hypersomnia in MyD [3, 14, 17, 18]. It is reported that hypersomnia persisted in spite of normalized blood gas values in MyD patients treated with mechanical ventilation [3, 18], and hypersomnia improved dramatically after treatment with methylphenidate [17]. The tendency to oversleep in some patients with MyD suggests a regulatory disturbance of the sleep-wake cycle [14]. Among our five MyD patients with hypersomnia, hypersomnia preceeded manifestations of muscular weakness by several years in one patient and two showed no hypoventilation (Table 1). Many other progressive muscle diseases may lead to similar physical deficits but are not associated with hypersomnia. These findings show that hypersomnia in MyD could be attributed to a central dysfunction.

The DRN is the midline nucleus from the caudal mesencephalon to rostal pons and the SCN occupies the central part of the pontine tegmentum and extends into the midbrain up to the trochlear nucleus [13]. The DRN and the SCN, which are included in the raphe nuclei, contain the largest aggregation of serotonin-containing neurons in the brain stem [20], provide the bulk of telencephalic and diencephalic serotonin [1, 16], and diffusely project to the cerebral cortex [12]. The demonstration of neuronal degeneration in the raphe nuclei correlates well with the loss of serotonin in the structures innervated by the mesotelencephalic serotonergic pathways [4].

The raphe nuclei play an important role in the regulation of the awake-sleep rhythm and are considered to be related to nonREM sleep [8]. Kitamura et al. [9] reported five patients who showed sleep disturbance and the central type of sleep apnea, and pointed out the relationships between the sleep apnea and abnormalities in monoamine metabolism of the raphe nuclei. The role of the serotonergic system in sleep regulation has long been established [4] and alterations in sleep are frequently observed in patients with degeneration of the serotonergic system [11, 15].

It is remarkable that the neuronal loss in the DRN and the SCN was significantly greater in MyD patients with hypersomnia than in MyD without hypersomnia and control subjects, and that there was an appreciable positive correlation in the density of neurons between the DRN and the SCN in all MyD patients. Several studies have revealed a lack of age-related changes in the raphe [2, 5]. Thus, our data suggest that the decreased densities of neurons of the DRN and the SCN are not an incidental finding but may have an intimate and important relationship with hypersomnia of MyD, and may be a conspicuous and diagnostically important feature of MyD.

There have been several physiological studies of hypersomnia in MyD [3, 14, 17–19]. However, hypersomnia that accompanies MyD has rarely been studied neuropathologically; the relationship between hypersomnia and the pathological findings of the brain remains unknown. Therefore, based on our neuropathological findings, we conclude that extensive neuronal loss within the DRN and the SCN may correspond to the presence of hypersomnia in MyD.

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