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Parabrachial nucleus involvement in multiple system atrophy[☆]

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Abstract

Multiple system atrophy (MSA) is associated with respiratory dysfunction, including sleep apnea, respiratory dysrhythmia, and laryngeal stridor. Neurons of the parabrachial nucleus (PBN) control respiratory rhythmogenesis and airway resistance.

Objectives—The objective of this study is to determine whether there was involvement of putative respiratory regions of the PBN in MSA.

Methods—We examined the pons at autopsy in 10 cases with neuropathologically confirmed MSA and 8 age-matched controls. Sections obtained throughout the pons were processed for calcitonin-gene related peptide (CGRP) and Nissl staining to identify the lateral crescent of the lateral PBN (LPB) and the Kölliker-Fuse nucleus (K-F), which are involved in respiratory control. Cell counts were performed using stereology.

Results—There was loss of CGRP neurons in the PBN in MSA (total estimated cell counts for the external LPB cluster was $12,584 \pm 1146$ in controls and 5917 ± 389 in MSA, $p < 0.0001$); for the external medial PBN (MPB) cluster it was $15,081 \pm 1758$ in controls and 7842 ± 466 in MSA, $p < 0.001$. There was also neuronal loss in putative respiratory regions of the PBN, including the lateral crescent of the LPB ($13,039 \pm 1326$ in controls and 4164 ± 872 in MSA, $p < 0.0001$); and K-F (5120 ± 495 in controls and 999 ± 308 in MSA, $p < 0.0001$).

Conclusions—There is involvement of both CGRP and putative respiratory cell groups in the PBN in MSA. Whereas the clinical implications of CGRP cell loss are still undetermined, involvement of the LPB and K-F may contribute to respiratory dysfunction in this disorder.

Keywords

Medial parabrachial; Lateral parabrachial; CGRP; Stridor; MSA

1. Introduction

Multiple system atrophy (MSA) is a neurodegenerative disorder characterized by autonomic failure combined with parkinsonism, cerebellar ataxia, or both (Gilman et al., 2008).

Respiratory manifestations such as sleep apnea, respiratory dysrhythmia, and laryngeal stridor may be a prominent cause of death in MSA patients (Silber and Levine, 2000;

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Yamaguchi et al., 2003; Vetrugno et al., 2007; Schwarzacher et al., 2011). Whereas involvement of the preBötzinger complex (Schwarzacher et al., 2011) and putative chemosensitive neurons of the ventral medullary surface (Benarroch et al., 2007) including serotonergic neurons of the medullary raphe (Tada et al., 2009) may contribute to sleep apnea and respiratory dysrhythmia in MSA, abnormal premotor control of laryngeal motoneurons leading to paradoxical laryngeal adductor muscle activation during inspiration may contribute to laryngeal stridor (Simpson et al., 1992; Isono et al., 2001; Shiba et al., 2007; Vetrugno et al., 2007). The parabrachial nucleus (PBN) and adjacent Kölliker-Fuse (K-F) nucleus are involved in respiratory rhythmogenesis and control of upper airway resistance (Smith et al., 1989; Chamberlin and Saper, 1994; Ellenberger and Feldman, 1994; Gang et al., 1998; Mutolo et al., 1998; Lara et al., 2002; Chamberlin, 2004). The lateral crescents of the PBN and the K-F are interconnected with the medullary respiratory network (Smith et al., 1989; Ellenberger and Feldman, 1994; Tan et al., 2010) and provide direct or indirect inputs to laryngeal motoneurons of the nucleus ambiguus controlling the laryngeal muscles (Nunez-Abades et al., 1990; Jordan, 2001; Waldbaum et al., 2001; Kunibe et al., 2003; Ono et al., 2006). These respiratory groups are located laterally to clusters of calcitonin-gene related peptide (CGRP) immunoreactive neurons that relay viscerosensory information to the forebrain (de Lacalle and Saper, 2000). We sought to determine whether there was involvement of the PBN, including the CGRP groups and the putative respiratory cell groups, in MSA.

2. Methods

2.1. Subjects and methods

Brains were obtained at autopsy from 10 patients (6 men, 4 women; age 63 ± 3 years) with clinically probable MSA according to current criteria (Gilman et al., 2008), and 8 controls (3 men, 5 women, age 68 ± 3 years) with no history of neurologic disease (Table 1). All subjects had given informed consent for autopsy according to the guidelines of the Institutional Review Board. For the MSA cases, symptom duration was 5 ± 0.4 years (range 2–6); parkinsonism was the presenting feature in 6 cases (MSA-P) and cerebellar ataxia in 4 cases (MSA-C). All MSA cases had history of orthostatic hypotension and neurogenic bladder, and 4 of the 6 men had erectile dysfunction (see Table 2). All MSA patients had undergone a standard polysomnogram (PSG) within 1–3 years of disease onset. Polysomnography and scoring of all PSG data were performed according to the guidelines of the American Association of Sleep Medicine Manual for the Scoring of Sleep and Associated Events; Rule, Terminology and Technical Specifications (American Academy of Sleep Medicine, Westchester, IL, 2007). Stridor was diagnosed by both auditory identification and PSG documentation.

Five (3 MSA-P, 2 MSA-C) of the 10 patients had laryngeal stridor. The timing of the PSG was not different between patients with and those without stridor. There were no significant differences in age or postmortem delay between controls and MSA cases; within MSA cases, age and disease duration were similar between cases with and those without stridor (Table 1). Follow-up information was available from 4 MSA patients, 2 with and 2 without documented stridor on PSG. One patient with stridor died within 1 year and the second within 2 years of the PSG; the 2 patients without documented stridor died within 2 years of evaluation. One patient with documented stridor died during sleep; there was no information available about the other 3 patients.

2.2. Tissue processing and immunocytochemistry

One half of the brain was separated for routine neuropathological examination and the other half for the present study. All cases fulfilled neuropathological criteria for MSA

(Trojanowski and Revesz, 2007). Of the 10 MSA cases, all had severe involvement of the putamen and substantia nigra pars compacta and 6 had moderate to severe involvement of the pons, inferior olivary nucleus, and cerebellum. All cases had moderate to marked accumulation of α -synuclein immunoreactive glial cytoplasmic inclusions (GCI) in these regions. One MSA case had mild to moderate and two had only rare associated Lewy body pathology. There was no difference on disease duration or clinical course between the 3 MSA cases with associated Lewy body pathology and the remaining 7 MSA cases without associated Lewy body pathology. The presence of neurofibrillary tangles and senile plaques was classified in each case according to the stages of Alzheimer disease as described by Braak and Braak (B&B) and defined by the Consortium to Establish a Registry for Alzheimer's disease (CERAD). Eight MSA cases had B&B staging 0–II; 2 had stage III; and none fulfilled CERAD criteria for Alzheimer disease.

A block containing the pons from 18 to 32 mm rostral to the obex to include the whole extent of the PBN according to the atlas of Paxinos and Huang (1995) was separated, immersion fixed in 5% formalin for 24 h at 4 °C and cryoprotected in buffered 30% sucrose for 5 to 7 days prior to processing. Serial transverse 50 μ m cryostat sections were obtained and every eighth section was immunostained for calcitonin-gene related peptide (CGRP, rabbit polyclonal, Millipore, Temecula, CA.). Diaminobenzidine/glucose oxidase solution with nickel enhancement (SIGMA, St. Louis, MO.) was used for the substrate reaction. Immunoreactive neurons were identified under bright-field microscopy. Omission of the primary antibody, use of appropriate blocking peptide, or incubation with normal sera resulted in a lack of immunostaining. All sections were co-stained with thionin to identify the different areas of the PBN, including the putative respiratory regions, and surrounding structures, and to determine whether loss of immunoreactivity reflected neuronal loss or lack of expression of the antigen. Selected paraffin embedded 12 μ m sections were processed for α -synuclein (mouse monoclonal, Invitrogen, Camarillo, CA.), glial fibrillary acidic protein (GFAP) (rabbit polyclonal, Dako, Glostrup, Denmark) and HLA-DR, a marker of activated microglia (mouse monoclonal, ICN Biomedicals, Aurora, Ohio).

2.3. Image analysis and quantitation

The sections were examined under bright-field microscopy using a modified light microscope (Zeiss Axioimager A-1; Zeiss, Thornwood, NY) equipped with a motorized specimen stage for automated sampling (Ludl Electronics; Hawthorne, NY, USA), CCD color video camera (Microfire; Optronics, Goleta, CA, USA) and stereology software (Stereo Investigator, v10.0; MBF Bioscience, Williston, VT, USA). The medial and lateral PBN were identified in Nissl-stained sections according for the atlas of Paxinos and Huang. CGRP immunoreactivity was used to define external lateral (eLPB) and external medial (eMPB) subnuclei of the PBN, respectively, as described by de Lacalle and Saper (2000). The location of the putative respiratory regions of the PBN, including the lateral crescent of the lateral PBN (LPBlc) and the K-F was determined by using the distribution of CGRP neurons as a reference; cell counts were performed in Nissl-stained sections as there is no specific immunocytochemical marker available to identify these putative respiratory neurons in post-mortem human brain tissue. In all MSA and control cases, we analyzed 14–18 sections obtained 800 μ m apart to span the length of the PBN.

2.4. Statistical analysis

All results are expressed as mean $\pm$ SEM. Statistical analysis was performed using the JMP software for Windows. Multivariate analysis of variance was used to determine if age, gender, disease phenotype, symptom duration, or post-mortem delay had any effect on cell counts. For cell counts in the lateral or medial PBN, comparison between control and MSA cases as a group was performed using unpaired *t*-test; comparison among controls, MSA

cases without, and MSA cases with stridor was performed using univariate analysis of variance. Post-hoc analysis was then conducted using Dunnett's and Bonferroni's formulas. A p value of <0.05 was considered significant.

3. Results

3.1. CGRP immunoreactive neurons

There were two distinct clusters of CGRP immunostained neurons, corresponding to the eLPB and eMPB, respectively (de Lacalle and Saper, 2000) (Fig. 1). In control cases, CGRP immunoreactive neurons constituted approximately 32% of all Nissl-stained neurons in the eLPB and 55% of all Nissl immunostained neurons in the eMPB. There was loss of CGRP immunoreactive neurons in both the eLPB and eMPB in MSA patients compared to controls (Fig. 2). For the eLPB cluster, total estimated cell number was $12,584 \pm 1146$ in controls and 5917 ± 389 in MSA ($p < 0.0001$); for the eMPB cluster, total estimated CGRP cell number was $15,081 \pm 1758$ in controls and 7842 ± 466 in MSA ($p < 0.001$). There were no differences in CGRP cell counts in MSA cases with documented laryngeal stridor compared with those without stridor (total cell counts for the eLPB 5396 ± 287 in MSA cases with and 6437 ± 681 in cases without stridor; for the eMPB 7395 ± 487 in MSA cases with and 8288 ± 800 in cases without stridor; ($n = 5$)).

3.2. Cell counts in putative respiratory regions of the PBN

The location of the lateral crescent of the PBN (LPBlc) and K-F was determined by using the distribution of CGRP neurons as a reference (Fig. 1). There was a significant neuronal loss in the LPBlc in MSA compared to that in the control cases (Fig. 3). Total estimated cell count was $13,039 \pm 1326$ in controls and 4164 ± 872 in MSA ($p < 0.0001$). Although there was a tendency for lower cell counts in cases with history of stridor (2581 ± 1141) than in those without stridor (5746 ± 762) the difference was not statistically significant. There was also neuronal loss in the area corresponding to the K-F in MSA compared to control cases. Total estimated cell counts were 5120 ± 495 in controls and 999 ± 308 in MSA ($p < 0.0001$). Like in LPBlc, there was a tendency to lower total estimated cell counts in the K-F in cases with documented stridor (666 ± 136) than in those without stridor (1332 ± 593) but the difference did not reach statistical significance.

There was no effect of age, gender, disease duration or postmortem delay in the CGRP or Nissl cell counts in either the lateral or medial PBN areas evaluated in this study.

3.3. MSA-related neuropathology in the PBN

In both the lateral and medial PBN, there was accumulation of GCIs, astrogliosis, and microglial inflammation. These changes occurred with no apparent regional selectivity (Fig. 4).

4. Discussion

Our findings indicate that the PBN is affected in MSA. The PBN is a complex structure that includes several regions with specific connections and functions (Fulwiler and Saper, 1984; Chamberlin and Saper, 1994; Chamberlin, 2004). Our results indicate that both the CGRP immunoreactive neurons and neurons in the putative respiratory PBN regions, including the LPBlc and K-F are affected in this disorder. Although there is one previous report of PBN involvement in MSA (Nomura et al., 2001), this previous study was limited to 4 cases and only showed decreased density of substance P fibers and astrogliosis.

CGRP immunoreactive neurons in the lateral and the medial PBN are sites of integration and relay of interoceptive inputs (de Lacalle and Saper, 2000). The relationship of loss of

CGRP neurons in the PBN and the clinical manifestations of MSA are yet to be determined. Loss of neurons in both LPB_{lc} and K-F may contribute to the respiratory manifestations of MSA. Both regions have reciprocal connections both with medullary respiratory neurons and with motoneurons controlling the caliber of the upper airways, including vagal motoneurons of the nucleus ambiguus innervating the larynx (Herbert et al., 1990; Chamberlin and Saper, 1994; Hopkins et al., 1996; Lara et al., 2002; Chamberlin, 2004; Dutschmann and Herbert, 2006; Yokota et al., 2007). Microstimulation of different portions of the lateral PBN and K-F elicits site-specific changes in respiration and airway resistance (Chamberlin and Saper, 1994; Lara et al., 2002; Chamberlin, 2004). Chemical stimulation of the lateral PBN elicits inspiration and hyperpnea (Chamberlin and Saper, 1994; Chamberlin, 2004); as well as laryngeal dilatation (Lara et al., 2002); activation of the medial K-F region elicits variable changes on respiration and airway resistance (Chamberlin, 2004). Bilateral lesions of the K-F elicit apnea in anesthetized adult rats (Song et al., 2010).

Laryngeal stridor is a typical and life-threatening manifestation of MSA (Silber and Levine, 2000), but its mechanism is still poorly understood. Initial studies showed vocal cord abduction failure associated with atrophy of the posterior cricoarytenoid muscles suggestive of denervation, but without evidence of motor cell loss in the nucleus ambiguus (Bannister et al., 1981). Several later reports indicate the presence of bilateral vocal cord paralysis in MSA patients with laryngeal stridor (Hanson et al., 1983; Isozaki et al., 1995; Egami et al., 2007; de Mello et al., 2010; Louter et al., 2011).

However, other studies show that electrophysiological evidence indicates that laryngeal stridor in MSA is associated with paradoxical vocal cord motion during inspiration (Simpson et al., 1992; Isono et al., 2001; Shiba et al., 2007; Vetrugno et al., 2007). Thus, the mechanism of stridor in MSA is probably multifactorial and variable among patients. Some neuropathological studies show preservation of branchiomotor neurons in the compact portion of the nucleus ambiguus, but reduced numbers of neurons in the ventrolateral portion of the nucleus (Benarroch et al., 2003); others show reduced numbers of nucleus ambiguus motoneurons and vagal myelinated axons as well as atrophy in laryngeal abductor muscle in MSA patients with, but not in those without, stridor (Ikeda et al., 2003). There is a differential distribution of laryngeal abductor and adductor motoneurons in the nucleus ambiguus (Gacek et al., 1977; Kitamura et al., 1993) indicating the possibility of a selective vulnerability within this nucleus in MSA. However, the paradoxical vocal cord motion during inspiration, with increased activity of laryngeal adductors (Isono et al., 2001) also suggests that abnormal premotor control of laryngeal motoneurons by the brainstem respiratory network, including the lateral PBN and K-F, may also have a role in the development of stridor. In this regard, the relationship between our present findings and the development of laryngeal stridor remains to be established. Although our results showed a tendency for more severe cell loss in lateral PBN and K-F in MSA cases with polysomnographically demonstrated laryngeal stridor than in cases without stridor, this difference was not statistically significant. This could in part reflect the relatively small number of cases in each group included in this study. Furthermore, whereas disease duration was similar in patients with compared to those without documented laryngeal stridor, we cannot exclude that this complication developed later in the disease course and contributed to death in the patients who had no documented stridor during their PSG evaluation. Therefore, further studies in larger groups of MSA cases with or without stridor that had undergone long-term follow-up may help confirm the possibility that PBN involvement may contribute to this complication. These studies may also provide further insight into the topographical distribution and neurochemical identity of the PBN neurons contributing to this life-threatening respiratory manifestation of MSA.

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Fig. 1. Distribution of calcitonin gene related peptide (CGRP) immunoreactive neurons (upper panel) in the external lateral (eLPB) and the external medial parabrachial nucleus (eMPB); and Nissl stained neurons (lower panel) in the lateral crescent of the PBN (LPB(lc)) and the Kölliker-Fuse (K-F). Comparison between 68 year old man with no history of neurologic disease (post mortem delay 16 h), and a 46 year old man with clinical and pathological diagnosis of MSA and laryngeal stridor (post mortem delay 17 h).

Fig. 2.

Total estimated number of calcitonin gene related peptide (CGRP) immunoreactive neurons in the external lateral (eLPB) and the external medial parabrachial nucleus (eMPB) in control and MSA cases. There was a significant loss of CGRP neurons in both the external lateral and the external medial PBN in MSA cases compared to controls. (*** $p < 0.0001$, ** $p < 0.001$).

Fig. 3. Total estimated Nissl stained neurons in the lateral crescent of the PBN (LPB(lc)) and the Kölliker-Fuse (K-F) in control and MSA cases. There was significant loss of neurons in both the LPB(lc) and the K-F in MSA compared to controls. (***) $p < 0.0001$.

Fig. 4. Representative 12 μm sections immunostained for A, α -synuclein showing glial cytoplasmic inclusions (GCIs), B, GFAP showing astrocytic gliosis, and C, HLA-DR showing activated microglia. Bar = 50 μm .

Table 1

Demographics.

Group (n)	Controls (8) (Mean $\pm$ SEM)	MSA, no stridor (5) (Mean $\pm$ SEM)	MSA stridor (5) (Mean $\pm$ SEM)
Age (years)	68 $\pm$ 3	63 $\pm$ 3	63 $\pm$ 6
Disease duration (years)	N/A	5 $\pm$ 1	5 $\pm$ 1
Postmortem delay (h)	17 $\pm$ 2	11 $\pm$ 3	10 $\pm$ 4

Table 2

Clinical features of the individual cases.

Case	clinical dx	Age/sex	DD (years)	PMD (h)	Stridor	Sleep disorders	Autonomic disorders	Diagnosis
Control 1		58/M	N/A	8.5	No	None	None	Lymphoma
Control 2		75/F	N/A	25	No	None	None	ARDS
Control 3		67/F	N/A	15	No	None	None	GI bleed
Control 4		68/M	N/A	16	No	None	None	Cholestatic hepatitis
Control 5		57/F	N/A	8.5	No	None	None	Ovarian carcinoma
Control 6		77/F	N/A	22	No	None	None	Scleroderma
Control 7		64/F	N/A	20	No	None	None	COPD, cardiac arrest
Control 8		75/M	N/A	18	No	None	None	Renal failure
MSA 1		53/M	6	10	No	RBD	OH, NB, ED, GI, Anhidrosis	MSA-SND/OPCA
MSA 2		66/M	4	5	No	None	OH, NB, ED, Anhidrosis	MSA-SND/OPCA
MSA 3		63/F	6	11	No	RBD	OH, NB, GI, Dysarthria, Anhidrosis	MSA-SND/OPCA
MSA 4		63/F	4	6	No	None	OH, NB, GI, Anhidrosis	MSA-SND/OPCA
MSA 5		70/M	4	24	No	RLS, RBD	OH, NB, GI	MSA-SND/OPCA
MSA 6		81/F	5	13	Yes	PLMS, RBD	OH, NB, ED, GI, Anhidrosis	MSA-SND/OPCA
MSA 7		67/M	2	1	Yes	OSA	OH, NB, GI, Anhidrosis	MSA-SND
MSA 8		59/M	5	26	Yes	RBD, OSA, EDS	OH, NB, GI, hypertension	MSA-SND/OPCA
MSA 9		63/F	6	6	Yes	OSA*	OH, NB, ED, GI, Anhidrosis,	MSA-SND/OPCA
MSA 10		46/M	5	17	Yes	PLMS, RBD, OSA, EDS	OH, NB, GI, Anhidrosis,	MSA-SND/OPCA

ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disorder; DD, disease duration; ED, erectile dysfunction; EDS, excessive daytime sleepiness; GI, gastrointestinal; MSA, multiple system atrophy; NB, neurogenic bladder; OH, orthostatic hypotension; OPCA, olivopontocerebellar atrophy; OSA, obstructive sleep apnea; PLMS, periodic leg movements of sleep; PMD, post-mortem delay; RBD, REM behavior sleep disorder; RLS, restless leg syndrome; SND, striatonigral degeneration.