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Depletion of putative chemosensitive respiratory neurons in the ventral medullary surface in multiple system atrophy

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Multiple system atrophy (MSA) is a disorder that may manifest with reduced respiratory chemosensitivity and central sleep apnoea. Chemosensitive glutamatergic and serotonergic neurons located just beneath the ventral medullary surface, corresponding to the human arcuate nucleus (ArcN), have recently been implicated in control of automatic breathing in response to hypercapnia and hypoxia. We sought to determine whether these neurons were affected in MSA. Medullae were obtained at post-mortem from 11 patients (8 men, 3 women, age 64 ± 3 years) with neuropathologically confirmed MSA and 11 control subjects (6 men and 5 women, age 66 ± 4 years). Fifty micrometre sections obtained throughout the medulla were processed for vesicular glutamate transporter-2 (VGLUT-2), tryptophan-hydroxylase (TrOH), glial fibrillary acid protein (GFAP) and α-synuclein immunoreactivity. Cell counts, GFAP immunoreactivity and presence of glial cytoplasmic inclusions (GCIs) were assessed in the ArcN. In MSA, compared with controls, there was a marked depletion of ArcN neurons immunoreactive for either VGLUT-2 (74 ± 21 versus 342 ± 84 cells/section, $P < 0.004$) or TrOH (5 ± 1 versus 16 ± 2 cells/section, $P < 0.001$). There was also marked astrocytic gliosis and accumulation of α-synuclein immunoreactive GCIs in the ventral medullary surface in all cases. Our results indicate that there is severe loss of putative chemosensitive glutamatergic and serotonergic neurons as well as marked astrocytic gliosis in the ventral medullary surface in MSA. This may provide a possible morphological basis for impaired respiratory chemosensitivity and central sleep apnoea in this disorder.

Keywords: arcuate nucleus; glutamate; serotonin; respiration; apnoea

Abbreviations: ArcN = arcuate nucleus; GCI = glial cytoplasmic inclusion; MSA = multiple system atrophy; OSA = obstructive sleep apnoea; TrOH = tryptophan hydroxylase; VGLUT-2 = vesicular glutamate transporter-2

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Introduction

Automatic breathing depends on activity of chemoreceptive neurons located in the brainstem and responding to changes in O_2, CO_2 and pH in the blood and cerebrospinal fluid (Feldman *et al.*, 2003; Putnam *et al.*, 2004; Nattie and Li, 2006). The brain contains several groups of chemosensitive neurons, including serotonergic groups of the medullary raphe and ventral medullary surface (Richerson, 2004), glutamatergic neurons of the ventral medulla (Guyenet *et al.*, 2005) and neurons in the locus ceruleus, nucleus of the solitary tract (NTS) and fastigial nucleus (Feldman *et al.*, 2003).

The highest sensitivity to local increases in CO_2/H^+ levels is found in serotonergic neurons of the medullary raphe (Richerson *et al.*, 2005) and glutamatergic neurons in the retrotrapezoid nucleus, located just beneath the ventral medullary surface (Guyenet *et al.*, 2005), and therefore these two neuronal groups have been proposed as the prime candidates for central chemoreceptor function. However, there is still controversy as to whether the glutamatergic or serotonergic groups of the ventral medullary surface are primarily responsible for central chemosensitivity. The glutamatergic neurons project to the ventral respiratory group (Weston *et al.*, 2004) and receive inputs from hypoxia-sensitive peripheral chemoreceptors via the NTS (Takakura *et al.*, 2006). The serotonergic neurons of the caudal raphe (Richerson *et al.*, 2005) and ventral medullary surface provide relatively fewer projections to the ventral respiratory group

(Weston *et al.*, 2004) but project to phrenic motoneurons of the spinal cord, where they modulate neuronal plasticity in response to hypoxia (Feldman *et al.*, 2003). These neurons also provide inputs that modulate activity of cranial motor neurons controlling the upper airway (Sun *et al.*, 2002).

Morphological (Filiano *et al.*, 1990) and functional neuroimaging (Gozal *et al.*, 1994) studies in humans suggest that the arcuate nucleus (ArcN), located just beneath the ventral medullary surface, may correspond to the central chemosensitive area described in experimental animals. The human ArcN contains glutamatergic and serotonergic neurons with morphologies that resemble that described in experimental animals (Paterson *et al.*, 2006). Astrocytes in this region contain glutamatergic and serotonergic receptors and may participate in local chemosensitive network interactions. Autopsy studies show that there is loss of serotonin receptors in the ArcN in infant victims of sudden infant death syndrome (SIDS) (Kinney *et al.*, 2001), suggesting a critical role of serotonergic mechanisms in the ArcN in respiratory chemosensitivity and control of automatic ventilation, particularly during sleep.

Multiple system atrophy (MSA) is a sporadic, progressive, adult onset disorder characterized by autonomic dysfunction, parkinsonism and ataxia in various combinations (Gilman *et al.*, 1999; Wenning *et al.*, 2004). The autonomic manifestations include orthostatic hypotension, impaired vagal regulation of heart rate (cardiovagal failure), anhidrosis, impotence, and urinary incontinence or retention, which may precede the motor symptoms (Wenning *et al.*, 2004). Respiratory dysfunction, including sleep-related breathing disorders, is a serious manifestation of MSA (Silber and Levine, 2000; Vetrugno *et al.*, 2004; Ghorayeb *et al.*, 2005). Although obstructive sleep apnoea (OSA) is an important manifestation of this disorder, central sleep apnoea may occur as the presenting feature of MSA (Cormican *et al.*, 2004) or develop in later stages of the disease (Glass *et al.*, 2006). Central sleep apnoea may reflect impaired ventilatory chemosensitivity in MSA, as these patients have been shown to have impaired ventilatory responses to hypercapnia (Chokroverty *et al.*, 1978) or hypoxia (Tsuda *et al.*, 2002). The pathological substrate of impaired respiratory chemosensitivity in MSA is still poorly defined. We have previously shown that there is loss of cholinergic neurons in the ArcN in MSA (Benarroch *et al.*, 2001), but there is no experimental evidence that these neurons have a chemosensitive role. In the present study, we sought to determine whether the putative chemosensitive glutamatergic and serotonergic neurons in the ArcN are affected in MSA. We hypothesize that involvement of any or both of these neurons may contribute to impaired ventilatory responses to hypercapnia and hypoxia in this disorder.

Material and methods

Subjects

We examined the medulla obtained at post-mortem from 11 subjects (8 men, 3 women, age 64 ± 3 years) with neuropathologically confirmed diagnosis of MSA and 11 age-matched controls (6 men, 5 women, age 66 ± 4 years) with no neurological disease (Table 1). Ten MSA cases had predominantly parkinsonism (MSA-P); this was associated with ataxia in five; one patient had ataxia only (MSA-C). All had evidence of severe autonomic failure, manifested by orthostatic hypotension, impaired heart rate response to deep breathing suggesting impaired vagal control of the sinus node (cardiovagal failure, except for one case) and impaired thermoregulatory sweating. Nine had history suggestive of OSA and six had history suggestive of laryngeal stridor. Six of the 11 MSA patients had had polysomnographic studies performed between 1 and 4 years (25 ± 6 months) before the time of death. Of these six cases, two had severe OSA with a respiratory disturbance index (RDI, defined as the number of apnoeas and hypopnoeas per hour divided by 60) of 33 and 113, respectively; 2 had moderate sleep apnoea with RDI of 20 and 25, respectively, and 2 had mild OSA (RDI of 2 and 4, respectively). Four of these six cases (corresponding to the cases with RDI of 33, 25, 2 and 4, respectively) had polysomnographically documented stridor. None of the MSA cases studied had evidence of central sleep apnoea. Unfortunately, ventilatory responses to hypercapnia or hypoxia while awake had not been assessed in any of the subjects. One control had a polysomnogram showing OSA 20 months before the time of death. This patient was obese but had no evidence of congestive heart failure. He had severe OSA with a RDI of 36, but no stridor. Post-mortem delay was similar in MSA cases (15 ± 2 h) and controls (16 ± 2 h).

Tissue processing and immunocytochemistry

The left half of the brain was processed for routine neuropathological studies. All MSA cases showed various degrees of striatonigral and olivopontocerebellar degeneration, associated with accumulation of glial cytoplasmic inclusions (GCIs).

Of the 11 MSA cases studied, the degree of striatonigral degeneration was marked in six, moderate in four and mild in one; and the involvement of the olivopontocerebellar system was prominent in one case, moderate in five, mild in four and absent in one (Table 1). A block containing the whole medulla, from the level just above the pontomedullary junction to just below the pyramidal decussation, was used for this study. Brains were immersion fixed in 5% formalin for 24 h at 4°C and cryoprotected in buffered 30% sucrose for 5–7 days and then frozen in dry ice. Serial 50 μm cryostat sections were obtained throughout the medulla and every eighth section was immunostained to identify glutamatergic or serotonergic neurons. The vesicular glutamate transporter-2 (VGLUT-2, polyclonal guinea pig antibody, 1 : 2000, Chemicon, Temecula, CA) was used as a marker of glutamatergic neurons (Weston *et al.*, 2004; Paterson *et al.*, 2006). The pattern of immunoreactivity found was similar to that described in previous studies on human medulla (Weston *et al.*, 2004; Paterson *et al.*, 2006). Tryptophan-hydroxylase (TrOH, polyclonal sheep antibody, 1 : 1000, Chemicon, Temecula, CA) was used to label serotonergic neurons (Benarroch *et al.*, 2004). Paraffin embedded 6 μm sections obtained from the contralateral medulla at the same levels were immunostained for glial fibrillary acid protein (GFAP, 1 : 4000, Chemicon, Temecula, CA) or α-synuclein (1 : 400, Santa Cruz Biotechnology Inc., Santa Cruz, CA).

Antibodies were diluted in a 0.1 M phosphate buffer, pH 7.4. To block endogenous peroxidase, the sections were preincubated in a 3% hydrogen peroxide solution for 30 min at room temperature. Sections were then incubated for 3 days at 4°C. To block non-specific

Table 1 Patient population

Case	Age/ sex	PMD (h)	Motor/cognitive manifestations	Autonomic manifestations	Disease duration (years)	Sleep disorder	Clinical diagnosis	Pathological diagnosis
Con 1	73/M	18	Mild memory loss	Constipation	N/A	None recorded	CHF	CHF
Con 2	32/F	6	None	None	N/A	None recorded	CHF	CHF
Con 3	74/M	13	Memory loss	None	N/A	None recorded	HTN, DM, vasculopathy	HTN, DM, vasculopathy
Con 4	78/F	9	Memory loss	Constipation	N/A	None recorded	Alzheimer, CAD	Alzheimer, CAD
Con 5	51/M	22	None	DM, HTN	N/A	None recorded	CHF, bronchiolitis	CHF, bronchiolitis
Con 6	64/F	20	None	None	N/A	None recorded	COPD	COPD, cardiac arrest
Con 7	66/M	22	None	None	N/A	OSA, PLMS, EDS*	AAA	AAA
Con 8	66/F	18	None	None	N/A	None recorded	Pulmonary fibrosis	Pulmonary fibrosis
Con 9	68/M	16	None	None	N/A	None recorded	Cholestatic hepatitis	Cholestatic hepatitis
Con 10	84/M	6	Memory loss, gait instability	Constipation	N/A	None recorded	CHD	CHD
Con 11	77/F	22	Memory loss, gait instability	Constipation	N/A	None recorded	Scleroderma, CAD	Scleroderma, CAD
MSA 1	54/F	26	Parkinsonism, ataxia	OH, NB, upper GI, anhidrosis	4	OSA, RBD, PLMS*	MSA	MSA-SND-OPCA
MSA 2	69/F	13	Parkinsonism, dystonia, ataxia	OH, NB, constipation, anhidrosis, NCV	13	OSA, stridor, RBD, PLMS*	MSA	MSA-SND-OPCA
MSA 3	67/M	1	Parkinsonism	OH, NB, constipation, hypertension, cardiovagal failure	2	OSA, stridor	MSA	MSA-SND-
MSA 4	68/M	23	Parkinsonism	OH, NB, impotence, GI/GU	14	OSA, stridor, RBD, hypopnoea*	MSA	MSA SND-OPCA
MSA 5	70/M	24	Parkinsonism	OH, NB, dysphagia, constipation	4	OSA, PLMS	MSA	MSA-SND/OPCA
MSA 6	54/M	13	Ataxia	OH, NB, upper GI, constipation	5	OSA, stridor, RBD	MSA	MSA-OPCA
MSA 7	67/M	8	Parkinsonism	OH, NB, dysphagia, constipation	8	None recorded	MSA	MSA-SND-OPCA
MSA 8	59/M	5	Parkinsonism, ataxia	OH, NB, constipation, hypertension, anhidrosis, cardiovagal failure	7	OSA, RBD*	MSA	MSA-SND-OPCA
MSA 9	81/F	13	Parkinsonism	OH, NB, constipation, anhidrosis, cardiovagal failure	5	OSA, stridor, RBD, PLMS*	MSA	MSA-SND-OPCA LBD (neocortical)
MSA 10	65/M	17	Parkinsonism	OH, NB, impotence, anhidrosis, NCV	6	RBD	MSA	MSA-SND-OPCA
MSA 11	46/M	17	Parkinsonism, ataxia	OH, NB, impotence, constipation, anhidrosis, cardiovagal failure	5	OSA, stridor, RBD, EDS*	MSA	MSA-SND-OPCA

AAA, aortic abdominal aneurysm; CAD, coronary artery disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; Con, control; DM, diabetes mellitus; EDS, excessive daytime sleepiness; HTN, hypertension; MSA, multiple system atrophy; N/A, non-applicable; NB, neurogenic bladder; NCV, normal cardiovagal; OH, orthostatic hypotension; OPCA, olivopontocerebellar atrophy; OSA, obstructive sleep apnoea; PLMS, periodic leg movements of sleep; PMD, post-mortem delay; RBD, REM sleep behaviour disorder; SND, striatonigral degeneration; *documented on polysomnogram.

binding, sections were incubated with a 5% normal serum. Omission of the primary antibody or incubation with normal sera resulted in a lack of immunostaining. Sections were then rinsed and reacted in diaminobenzidine (DAB)/glucose oxidase for 5–10 min. All sections were co-stained with thionin to identify the nucleus and Nissl bodies, in order to determine whether loss of immunoreactive neurons represented actual cell loss rather than functional impairment of expression of VGLUT-2, TrOH or both. Sections were then mounted, dehydrated and coverslipped in DPX (Fluka, Rokonhoma, NY). The paraffin embedded 6 μm sections were prepared and processed using routine methods.

Image analysis and quantification

The sections were examined under bright field microscopy. We focused our study on immunolabelled cells of the ventral medullary

surface, including those located in the medial portion of the ArcN just ventral to the pyramids and the lateral ArcN located just below the ventral medullary surface (Paxinos and Huang, 1995; Paterson *et al.*, 2006). Cells immunolabelled for VGLUT-2 or TrOH and identified by the presence of both nucleus and cell processes were mapped and counted every 400 μm. Twenty sections, spanning an 8 mm rostrocaudal extent that included essentially the whole ArcN (Paxinos and Huang, 1995), were analysed in each case. Sections were viewed on a Diaplan Axiophot microscope (Carl Zeiss, Inc.), equipped with a 2.5×/0.075 na objective lens. Image analysis was performed using the Carl Zeiss Axiocam and Axiovision Interactive Measurement software (Carl Zeiss Microimaging, Inc., Thornwood, NY). Images were digitized and stored. We did not use stereology because there were no differences in the shape or orientation of cells in the control and MSA groups and the main aim of our study was to compare the numbers of VGLUT-2 or TrOH immunoreactive cells between MSA and controls, rather than determine the total numbers of cells in these groups. Furthermore, we counted only cells that could be identified by their immunoreactivity as well as the presence of both nucleus and cytoplasmic processes, in order to avoid counting profiles that may represent fragments of cells. The investigator performing the cell counts was blinded to the clinical and neuropathological diagnosis. Cell numbers (mean ± SEM) were compared using Student's *t*-test. A *P*-value of <0.05 was considered significant.

Results

Glutamatergic neurons in the ArcN

Abundant VGLUT-2 immunoreactive neurons were observed along the medial, ventral and ventrolateral aspects of the ventral medullary surface (Fig. 1). Most of these neurons where of round shape and relatively large, and occupied predominantly the ventromedial portion of the ArcN, consistent with previous findings in human infants (Paterson *et al.*, 2006). There was severe loss of VGLUT-2 immunoreactive neurons in the ArcN in MSA compared to controls (Fig. 1). The size of the few surviving neurons in MSA was similar to that in control brains (23 ± 1 μm in MSA versus 25 ± 2 μm in controls; $n = 20$ each). Cell loss was consistent in all MSA cases (577 ± 157 total cells or 74 ± 21 cells/section in MSA versus 3342 ± 815 total cells or 342 ± 84 cells/section in controls; $P < 0.0004$) (Fig. 2). The mean number of cells in MSA cases with OSA was less than that in the single control case with OSA (65 ± 20 cells/section in MSA versus 320 ± 73 cells/section in the control case). The degree of VGLUT-2 immunoreactive cell loss was not related to disease duration or severity of respiratory manifestations during sleep. Cell loss was similar in cases with different degrees of involvement of the striatonigral system or the pontine or inferior olivary nuclei or cerebellum. The mean number of VGLUT-2 cells in the single MSA patient with pure striatonigral degeneration was similar to that of patients with additional involvement of the pontine nuclei, inferior olivary nucleus and cerebellum, suggesting that ArcN cell loss cannot be attributable solely to involvement of precerebellar nuclei. The mean number of VGLUT-2 cells in the ArcN in the single control patient with OSA (319 cells/section) was similar to that of the rest of the controls.

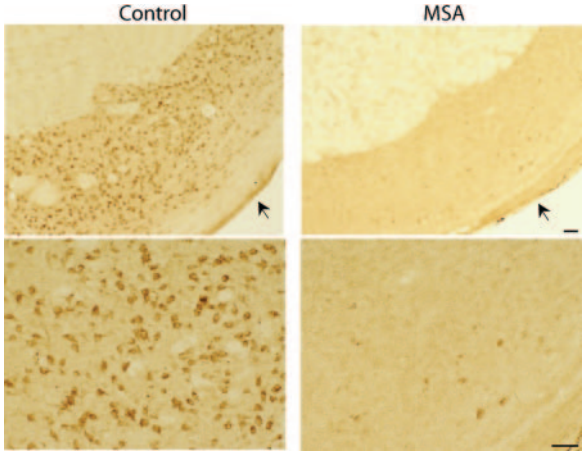

Fig. 1 Fifty micrometre section of the medulla at the level of the ArcN obtained 2 mm rostral to the obex and immunostained for the VGLUT-2 to label glutamatergic neurons. Left: 73-year-old man with no history of neurological disease; post-mortem delay 18 h. Right: 59-year-old man with MSA post-mortem delay 5 h. There was severe depletion of VGLUT-2 immunoreactive cells in the ArcN in the MSA case. Arrow = ventral medullary surface. Bar = 50 μm.

Serotonergic neurons in the ArcN

Elongated TrOH immunoreactive fusiform neurons and fibres were observed extending from the raphe pallidus along the entire ventral medullary surface, particularly in its ventral and lateral aspects of the ArcN (Fig. 3). These findings are similar to those reported in human infants (Paterson *et al.*, 2006). These cells were clearly different from and much less abundant than the VGLUT-2 immunoreactive neurons. Processes of TrOH immunoreactive cells were found to traverse long distances just underneath the pial surface. In MSA, there was a marked loss of TrOH immunoreactive neurons located just below the ventral medullary surface, and the few remaining

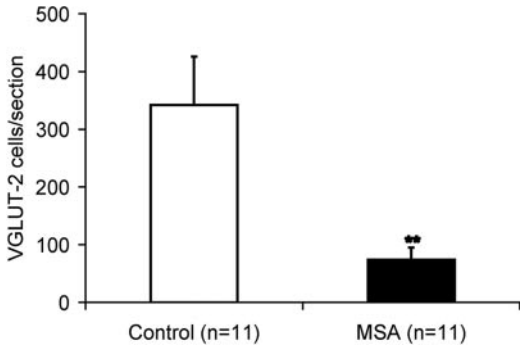

Fig. 2 Number of VGLUT-2 immunoreactive neurons per section of the ArcN in 11 control cases, and 11 cases with pathological diagnosis of MSA. There was severe depletion of VGLUT-2 neurons in all of the MSA cases. **$P < 0.0004$.

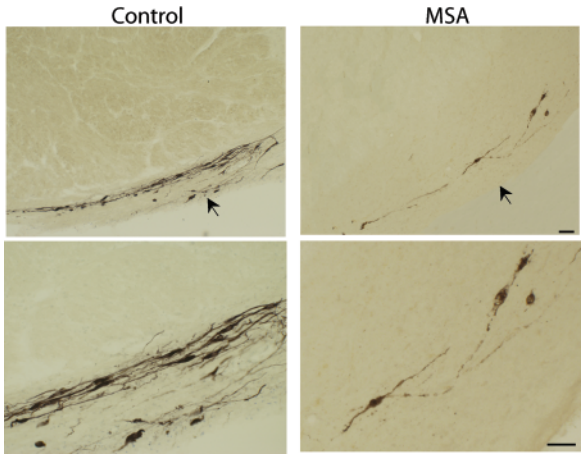

Fig. 3 Fifty micrometre section of the medulla at the level of the ArcN obtained 2 mm rostral to the obex and immunostained for tryptophan hydroxylase (TrOH) to label serotonergic neurons. Left: 73-year-old man with no history of neurological disease; post-mortem delay 18 h. Right: 59-year-old man with MSA post-mortem delay 5 h. There was severe depletion of TrOH immunoreactive cells and processes in the ArcN in the MSA case. Arrow = ventral medullary surface. Bar = 50 μm.

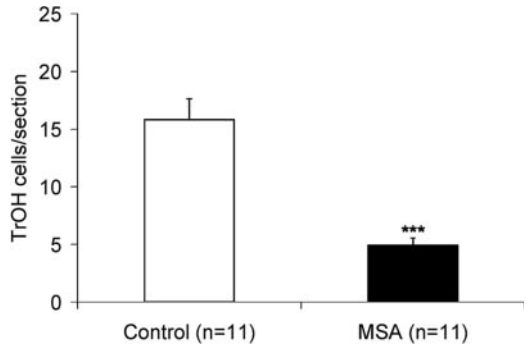

Fig. 4 Number of TrOH immunoreactive neurons per section of the ArcN in 11 control cases, and 11 cases with pathological diagnosis of MSA. There was severe depletion of serotonergic neurons in all of the MSA cases. ***$P < 0.0001$.

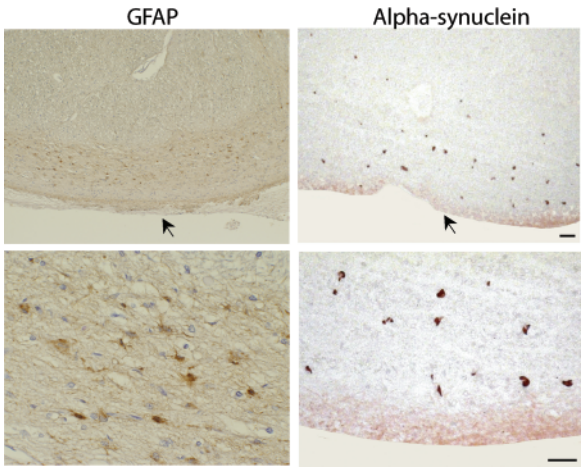

Fig. 5 Six micrometre sections of the ArcN immunostained for glial fibrillary acid protein (GFAP, left) to label astrocytes or alpha-synuclein (right) in a 69-year-old woman with MSA. Note the marked astrocytosis and presence of cytoplasmic inclusions. Arrow = ventral medullary surface. Bar = 50 μm.

neurons showed clear dystrophic changes and loss of processes (Fig. 3). Serotonergic neuron loss in the ArcN was a consistent finding in all MSA cases (57 ± 8 total cells or 5 ± 1 cells/section in MSA versus 140 ± 20 total cells or 16 ± 2 cells/section, in controls) (Fig. 4). Like in the case of VGLUT-2 immunoreactive cells, loss of serotonergic cells in the ArcN was not related with disease duration, severity of respiratory manifestations during sleep, or severity of striatonigral or olivopontocerebellar degeneration. The mean number of serotonergic neurons in the single control patient with OSA (24 cells/section) was similar to that of the other controls.

Other neuropathological findings in the ventral medullary surface

In MSA cases, there was a marked increase in the number of GFAP immunoreactive astrocytes and their processes both in the medial and lateral portions of the ArcN (Fig. 5). Abundant α-synuclein immunoreactive GCIs were also observed just below the ventral medullary surface, not only at the level of the pyramids but also in the medial and lateral ArcN (Fig. 5). Alpha-synuclein immunoreactive neuronal nuclear or cytoplasmic inclusions were not detected in our cases.

Discussion

Our findings indicate, for the first time to our knowledge, that there is severe depletion of putative chemosensitive glutamatergic and serotonergic neurons in the ArcN of the ventral medullary surface in MSA. On the basis of abundant experimental evidence, loss of either or both of these ventromedullary neuronal groups could conceivably contribute to impaired respiratory chemosensitivity in this disorder.

Loss of neurons in the ArcN has previously been reported in MSA (Noda *et al.*, 1997; Benarroch *et al.*, 2001; Braak *et al.*, 2003). However, the availability of the VGLUT-2 antibody, allowing identification of putative chemosensitive glutamatergic neurons in this region (Weston *et al.*, 2004), and the recent characterization of the cytochemical organization of the ArcN (Paterson *et al.*, 2006) allowed us to better define the neurochemical nature of the neurons involved. Although VGLUT-2 can also label astrocytes in the ArcN (Paterson *et al.*, 2006) in our MSA cases there was loss of immunoreactivity in the ArcN despite abundant reactive astrocytosis. It has been suggested that VGLUT-2 immunoreactive neurons in the ArcN may correspond to those described in the ventral medullary surface in rats (Weston *et al.*, 2004; Paterson *et al.*, 2006). In the rat, VGLUT-2 mRNA expressing neurons in the marginal layer and

retrotrapezoid nucleus of the rostral medulla are CO_2 chemosensitive and project to neurons of the ventral respiratory group, including those in the preBötzinger complex involved in respiratory rhythmogenesis (Weston *et al.*, 2004). In addition, neurons of the rat retrotrapezoid nucleus respond to hypoxia, receive inputs from neurons of the NTS receiving afferents from peripheral chemoreceptors (Takakura *et al.*, 2006), and, like these structures, express Phox2B and may be part of a hypoxia chemosensitive network that is affected in congenital alveolar hypoventilation syndrome (Stornetta *et al.*, 2006). Therefore, if the VGLUT-2 neurons in the ArcN are homologous to those in the rat marginal zone and retrotrapezoid nucleus, our results could provide a structural basis for impaired response to both hypercapnia (Chokroverty *et al.*, 1978) and hypoxia (Tsuda *et al.*, 2002) reported in MSA. Our present results support our previous finding of loss of cholinergic neurons in the ArcN in MSA (Benarroch *et al.*, 2001). Since VGLUT-2 may also be expressed in some cholinergic neurons (Herzog *et al.*, 2004), we cannot exclude the possibility that some of the VGLUT-2 neurons may also be cholinergic.

There was also loss of serotonergic neurons and fibres in the ventral medullary surface, particularly in the area corresponding to the lateral ArcN, in all our MSA cases. Serotonergic neurons in the ventral medulla constitute a second population of neurons highly sensitive to CO_2 that may mediate the ventilatory responses to hypercapnia (Richerson *et al.*, 2005). This finding extends our previous observation that there is loss of serotonergic neurons in the medullary raphe in MSA (Benarroch *et al.*, 2004) and supports the contention that serotonergic neurons in the ArcN constitute a ventral extension of the nucleus raphe pallidus and nucleus raphe obscurus (Zec and Kinney, 2001). There is still controversy as to whether the glutamatergic or the serotonergic groups of the ventral medullary surface are the primary chemosensitive neurons (Guyenet *et al.*, 2005; Richerson *et al.*, 2005). Although ventromedullary serotonergic neurons appear to provide less robust input to the medullary respiratory groups than the chemosensitive glutamatergic neurons (Weston *et al.*, 2004), serotonergic neurons of the ventral medullary surface and caudal raphe nuclei project to the spinal cord, including phrenic motoneurons, where they have an important role in mechanisms of plasticity in response to chronic hypoxia (Feldman *et al.*, 2003). Disruption of the serotonergic network at the level of the ArcN, including reduced number of serotonin receptors, has been shown in SIDS (Kinney *et al.*, 2001), which supports the potentially critical role of these neurons in maintaining automatic ventilation during sleep. There is also evidence that serotonergic inputs from the ventral medulla modulate activity of cranial motor neurons controlling the upper airway, facilitating activity of laryngeal abductor motoneurons (Sun *et al.*, 2002). It has been proposed that impaired activity of motoneurons innervating muscles responsible for opening of the upper airway during sleep may have a role in mechanisms of OSA. Thus, loss of serotonergic inputs to the nucleus ambiguus may contribute to the mechanisms of OSA and laryngeal stridor documented polysomnographically in our MSA patients.

The human ArcN also contains astrocytes that express glutamate and serotonin receptors and may participate in local network interactions involved in respiratory chemosensitivity (Paterson *et al.*, 2006). Our results show that there is a profound astrocytic gliosis in the ArcN in MSA, which could potentially disturb these network mechanisms. There is *in vitro* evidence that reactive astrocytes undergo significant phenotype changes, including downregulation of purinergic $P2_{\times 2}$ receptors and potassium channels (Nakagawa *et al.*, 2005). Purinergic $P2_{\times 2}$ receptors are a target of adenosine triphosphate (ATP), which is produced in the ventral medullary surface during hypercapnia and hypoxia and has a critical role in stimulating ventilation in these conditions (Gourine *et al.*, 2005). Thus, reactive astrocytosis is not only a marker of neuronal loss in the ventral medullary surface but may also contribute to impaired ventilatory chemosensitivity in MSA patients.

There were abundant GCIs just below the ventral medullary surface in MSA. We could not detect any α-synuclein immunoreactive neuronal nuclear or cytoplasmic inclusions, which can reflect the profound neuronal loss in this region.

In summary, there is severe loss of glutamatergic and serotonergic neurons, reactive gliosis and GCI accumulation in the ArcN of the ventral medullary surface in MSA. Our findings provide a possible structural basis for impaired ventilatory chemosensitivity and risk of central hypoventilation during sleep in these patients.

The chemosensitive respiratory network in the brain is widespread and includes not only the glutamatergic and serotonergic neurons of the ventral medullary surface and medullary raphe, but also neurons in the preBötzinger complex, NTS, locus coeruleus, C1 area and fastigial nucleus (Feldman *et al.*, 2003). However, the evidence indicating involvement of ventromedullary serotonergic mechanisms in SIDS (Kinney *et al.*, 2001), and recent data suggesting that glutamatergic neurons of the ventral medullary surface may be part of the hypoxia-activated network that is affected in congenital hypoventilation syndrome (Stornetta *et al.*, 2006), support the possibility that loss of these neurons may provide an important, if not primary, basis for impaired control of automatic ventilation during sleep in MSA. Although none of the MSA subjects included in this study had polysomnographic evidence of central sleep apnoea, this may develop late in the course of the disease (Glass *et al.*, 2006). Patients first shown to have OSA on polysomnogram may then develop central sleep apnoea, which may be a cause of death despite management of upper airway obstruction in these patients. One limitation of our study is that none of our cases had been tested for ventilatory responses to hypercapnia or hypoxia during life. Impairment of these responses may constitute an early marker for risk of central sleep apnoea in these patients. Further post-mortem studies

on patients that had undergone longitudinal testing of respiratory chemosensitivity and polysomnography would be necessary to further support the clinical implications of our present findings.

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References

Benarroch EE, Schmeichel AM, Parisi JE. Depletion of cholinergic neurons of the medullary arcuate nucleus in multiple system atrophy. Auton Neurosci 2001; 87: 293–9.

Benarroch EE, Schmeichel AM, Low PA, Parisi JE. Involvement of medullary serotonergic groups in multiple system atrophy. Ann Neurol 2004; 55: 418–22.

Braak H, Rub U, Del Tredici K. Involvement of precerebellar nuclei in multiple system atrophy. Neuropathol Appl Neurobiol 2003; 29: 60–76.

Chokroverty S, Sharp JT, Barron KD. Periodic respiration in erect posture in Shy-Drager syndrome. J Neurol Neurosurg Psychiatry 1978; 41: 980–6.

Cormican LJ, Higgins S, Davidson AC, Howard R, Williams AJ. Multiple system atrophy presenting as central sleep apnoea. Eur Respir J 2004; 24: 323–5.

Feldman JL, Mitchell GS, Nattie EE. Breathing: rhythmicity, plasticity, chemosensitivity. Annu Rev Neurosci 2003; 26: 239–66.

Filiano JJ, Choi JC, Kinney HC. Candidate cell populations for respiratory chemosensitive fields in the human infant medulla. J Comp Neurol 1990; 293: 448–65.

Ghorayeb I, Bioulac B, Tison F. Sleep disorders in multiple system atrophy. J Neural Transm 2005; 112: 1669–75.

Gilman S, Low PA, Quinn N, Albanese A, Ben-Shlomo Y, Fowler CJ, et al. Consensus statement on the diagnosis of multiple system atrophy. J Neurol Sci 1999; 163: 94–8.

Glass GA, Josephs KA, Ahlskog JE. Respiratory insufficiency as the primary presenting symptom of multiple-system atrophy. Arch Neurol 2006; 63: 978–81.

Gourine AV, Llaudet E, Dale N, Spyer KM. ATP is a mediator of chemosensory transduction in the central nervous system. Nature 2005; 436: 108–11.

Gozal D, Hathout GM, Kirlew KAT, Tang H, Woo MS, Zhang J, et al. Localization of putative neural respiratory regions in the human by functional magnetic resonance imaging. J Appl Physiol 1994; 76: 2076–83.

Guyenet PG, Stornetta RL, Bayliss DA, Mulkey DK. Retrotrapezoid nucleus: a litmus test for the identification of central chemoreceptors. Exp Physiol 2005; 90: 247–53; discussion 53–7.

Herzog E, Landry M, Buhler E, Bouali-Benazzouz R, Legay C, Henderson CE, et al. Expression of vesicular glutamate transporters, VGLUT1 and VGLUT2, in cholinergic spinal motoneurons. Eur J Neurosci 2004; 20: 1752–60.

Kinney HC, Filiano JJ, White WF. Medullary serotonergic network deficiency in the sudden infant death syndrome: review of a 15-year study of a single dataset. J Neuropathol Exp Neurol 2001; 60: 228–47.

Nakagawa T, Yabe T, Schwartz JP. Gene expression profiles of reactive astrocytes cultured from dopamine-depleted striatum. Neurobiol Dis 2005; 20: 275–82.

Nattie E, Li A. Central chemoreception 2005: a brief review. Auton Neurosci 2006; 126–127: 332–8.

Noda K, Katayama S, Watanabe C, Yamamura Y, Nakamura S. Decrease of neurons in the medullary arcuate nucleus of multiple system atrophy: quantitative comparison with Parkinson's disease and amyotrophic lateral sclerosis. J Neurol Sci 1997; 151: 89–91.

Paterson DS, Thompson EG, Kinney HC. Serotonergic and glutamatergic neurons at the ventral medullary surface of the human infant: observations relevant to central chemosensitivity in early human life. Auton Neurosci 2006; 124: 112–24.

Paxinos G, Huang XF. Atlas of the human brainstem. San Diego: Academic Press; 1995.

Putnam RW, Filosa JA, Ritucci NA. Cellular mechanisms involved in CO(2) and acid signaling in chemosensitive neurons. Am J Physiol Cell Physiol 2004; 287: C1493–526.

Richerson GB. Serotonergic neurons as carbon dioxide sensors that maintain pH homeostasis. Nat Rev Neurosci 2004; 5: 449–61.

Richerson GB, Wang W, Hodges MR, Dohle CI, Diez-Sampedro A. Homing in on the specific phenotype(s) of central respiratory chemoreceptors. Exp Physiol 2005; 90: 259–66; discussion 66–9.

Silber MH, Levine S. Stridor and death in multiple system atrophy. Mov Disord 2000; 15: 699–704.

Stornetta RL, Moreira TS, Takakura AC, Kang BJ, Chang DA, West GH, et al. Expression of Phox2b by brainstem neurons involved in chemosensory integration in the adult rat. J Neurosci 2006; 26: 10305–14.

Sun QJ, Berkowitz RG, Goodchild AK, Pilowsky PM. Serotonin inputs to inspiratory laryngeal motoneurons in the rat. J Comp Neurol 2002; 451: 91–8.

Takakura AC, Moreira TS, Colombari E, West GH, Stornetta RL, Guyenet PG. Peripheral chemoreceptor inputs to retrotrapezoid nucleus (RTN) CO2-sensitive neurons in rats. J Physiol 2006; 572: 503–23.

Tsuda T, Onodera H, Okabe S, Kikuchi Y, Itoyama Y. Impaired chemosensitivity to hypoxia is a marker of multiple system atrophy. Ann Neurol 2002; 52: 367–71.

Vetrugno R, Provini F, Cortelli P, Plazzi G, Lotti EM, Pierangeli G, et al. Sleep disorders in multiple system atrophy: a correlative video-polysomnographic study. Sleep Med 2004; 5: 21–30.

Wenning GK, Colosimo C, Geser F, Poewe W. Multiple system atrophy. Lancet Neurol 2004; 3: 93–103.

Weston MC, Stornetta RL, Guyenet PG. Glutamatergic neuronal projections from the marginal layer of the rostral ventral medulla to the respiratory centers in rats. J Comp Neurol 2004; 473: 73–85.

Zec N, Kinney HC. Anatomic relationships of the human nucleus paragigantocellularis lateralis: a DiI labeling study. Auton Neurosci 2001; 89: 110–24.