Marked depletion of dorsal spinal cord substance P and calcitonin gene-related peptide with intact skin flare responses in multiple system atrophy

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SUMMARY In view of the presence of neuropeptides in spinal cord autonomic pathways, their regional concentration was studied in post mortem thoracic cord from four cases of multiple system atrophy with progressive autonomic failure (MSA). A marked depletion was observed of substance P, its related peptide substance K, and of calcitonin gene-related peptide (CGRP), particularly in dorsal regions where peptide-containing sensory fibres terminate. As substance P and CGRP in primary sensory fibres are considered mediators of skin flares in Lewis' triple response, histamine-induced skin flares were measured in 12 MSA patients and were found to be preserved. These results provide a new key to the classification and aetiology of autonomic and multiple system degenerations, as well as a model to study the role of sensory neuropeptides in man.

Although a number of diseases may cause secondary damage to autonomic fibres,¹ primary progressive autonomic failure (PAF) results from an unexplained selective neuronal degeneration. It may occur either alone or in association with two different degenerations of the nervous system, Parkinson's disease and multiple system atrophy (MSA). Progressive autonomic failure with multiple system atrophy was first described by Shy and Drager in 1960:² "The full syndrome comprises the following features: orthostatic hypotension, urinary and rectal incontinence, loss of sweating, iris atrophy, external ocular palsies, rigidity, tremor, loss of associated movements, impotence, the findings of an atonic bladder and loss of rectal sphincter tone . . . The date of onset is usually in the 5th to 7th decade of life". The best classification of PAF syndromes is pathological, but is still controversial on crucial points such as whether the loss of spinal cord intermediolateral column cells, which form the final common pathway of thoracic auto-

nomic outflow, occurs in all cases of PAF.³ A major difficulty has been the quantitative assessment of autonomic neurons, even in normal subjects.

The discovery that neuropeptides are present in autonomic pathways in the spinal cord thus provided a new approach to pathological studies of MSA. Various neuropeptides are selectively present in autonomic pathways,⁴ for example vasoactive intestinal polypeptide (VIP) selectively marks pelvic nerve afferent fibres in human sacral spinal cord.⁵ In addition, they may act as trophic agents.⁶

A study was therefore undertaken of post mortem spinal cord peptide concentrations in four clinically established cases of MSA. The peptides studied were substance P, somatostatin, VIP, calcitonin gene-related peptide (CGRP) as well as newly discovered peptides not previously examined regionally in human spinal cord: galanin,⁷ and substance K,⁸ which belongs to the tachykinin family as does substance P.

The neuropeptide changes found in MSA dorsal spinal cord, and the current view that substance P,^{9,10} and CGRP in unmyelinated afferent fibres in human skin may mediate the flare component in Lewis' triple response, led to the measurement of skin flares in MSA patients.

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Cases

Thoracic spinal cords were collected from the following four cases of the MSA. There was no evidence in any case of diabetes, amyloidosis or abnormal serum protein electrophoresis. Post mortem histological examinations were performed by Dr D Oppenheimer at the Radcliffe Infirmary, Oxford, and Dr S Love at the National Hospital, Queen Square, London.

Case 1: This patient died aged 60 years, following a 6 year history of postural dizziness and fainting, sexual impotence, urinary incontinence and laryngeal stridor. Formal tests confirmed autonomic dysfunction and electrophysiological studies showed normal sensory and motor nerve conduction. He was treated at various times with fludrocortisone, indomethacin, tyramine and an elective tracheostomy. He died following acute laryngeal obstruction. Post mortem histological examination showed multiple system atrophy involving substantia nigra, Purkinje cells and inferior olives. Cells in the lateral horns were depleted to a third of the normal number. The Gasserian, spinal sensory and sympathetic ganglia showed no histological abnormalities.

Case 2: The patient died of bronchopneumonia aged 53 years after a progressive illness lasting 5 years, comprising tremor, rigidity, akinesia, ataxia, dysarthria, urinary incontinence and postural hypotension. Autonomic function tests showed cardiac denervation. She was prescribed fludrocortisone, Sinemet, amantadine and probanthine. Post mortem examination revealed atrophic grey sympathetic trunks and splanchnic nerves. Macroscopic brain examination showed abnormalities of the body of putamen,

substantia gelatinosa, pons, locus coeruleus and inferior olive. The spinal cord was not examined histologically.

Case 3: The patient died of bronchopneumonia aged 76 years after a 2 year history of progressive ataxia, weakness, frequency of micturition and dysarthria. He had markedly abnormal autonomic function tests. He was treated with fludrocortisone. Post mortem histological studies showed loss of neurons from the putamen, substantia nigra, Purkinje cells, dorsal vagal nucleus and about 50% loss of cells in lateral columns of the lower part of the thoracic cord.

Case 4: This patient died suddenly aged 62 years after 3 year history of weakness and tremor, dysarthria, sexual impotence, urinary and faecal incontinence and postural faintness with blackouts. He had cardiac autonomic neuropathy on formal testing. He was treated with Sinemet, methylphenidate and cimetidine. Post mortem studies showed loss of neurons in the putamen, substantia nigra, cerebellum and marked loss in lateral horns of thoracic spinal cord. Although the spinal roots and the femoral nerve appeared normal there was a mild to moderate depletion of cells in the dorsal root and superior cervical sympathetic ganglia.

Control cases

Thoracic spinal cords were collected from five cases with no known neurological abnormality either clinically or at post mortem examination (mean age 60 years, range 51–68). Two patients died of bronchopneumonia, two of acute left ventricular failure and one of aspiration of vomitus. None of the control of MSA cases had received any treatment that is known to affect neuropeptide levels.

Fig 1 (a) Transverse section of human thoracic spinal cord showing the regions microdissected for peptide analysis. (b) A diagrammatic representation of some autonomic pathways in human spinal cord.

Methods

Spinal cord processing

The thoracic spinal cords had been removed post mortem and frozen at -20°C prior to storage at -70°C at the MRC Brain Bank, Cambridge. The post mortem delay for MSA cases was 31 hours (range 5–124) and controls 41 hours (range 6–145). Transverse thin slices of frozen cord were microdissected on a low temperature plate with a cold scalpel or punched with a glass micropipette with care to avoid tissue thawing. The regions dissected, shown in fig 1A, were the dorsal, ventral and lateral horns, the dorsal columns, and dorsolateral white matter. Peptides were extracted in boiling 0.5M acetic acid, and assayed as previously described for substance P,⁵ somatostatin,⁵ VIP,⁵ CGRP¹¹ and galanin.⁷ The neurokinin radioimmunoassay used a rabbit antiserum raised to synthetic substance K. It fully cross-reacted with substance K and neurokinin beta but to less than 0.5% with substance P and physalaemin. On chromatography the immunoreactivity eluted predominantly in the position of synthetic substance K.

The specimens were processed within a period and manner generally established for stability of neuropeptides in post mortem specimens,¹² and control specimens were treated in identical fashion.

Skin flares

Skin wheal and flares were performed as previously described:¹⁰ 0.03 ml of histamine acid phosphate BP (1 mg/ml) was injected intradermally on forearm (seven cases) and thoracic (five cases) skin of MSA patients. The control patients also attended the National Hospital outpatient department, for follow up with intracranial neurological disorders. Neither the MSA patients (mean age 63 years $\pm$ 6) nor the controls (mean age 59 years $\pm$ 5) were taking drugs known to affect skin flare responses.

Results

The thoracic cord results are shown in fig 2, with the exception of VIP whose levels were below the detection limit (less than 0.8 pmol/g), in accord with a previous study.⁵ The depletion appeared most marked in MSA dorsal cord regions, particularly for substance P and substance K. There appeared to be almost complete loss of substance P and substance K immunoreactivity in the dorsal and dorsolateral columns. All neuropeptides measured were depleted in the dorsal horn, and substance P was significantly reduced in ventral horn as well.

No difference was observed between MSA and control subjects in the area or intensity of flare, size of wheal, or of subjective reports of itch sensation on the injection of histamine. Flare areas (cm^2) in thoracic skin ($n = 5$): controls 12 ± 2.2 , MSA 10.8 ± 2.6 ; in forearm skin ($n = 7$): controls 16.5 ± 3.0 , MSA 17.5 ± 3.3 .

Discussion

These results show marked parallel depletion of sub-

Fig 2 Regional concentrations of neuropeptides (pmol/g) in control and MSA (hatched columns) spinal cord.

Asterisk (*) denotes a statistically significant difference in concentration between control and MSA specimens of a particular region ($p < 0.05$, Student's unpaired t test).

stance P and substance K in MSA dorsal spinal cord, and in the lateral horns. The thoracic autonomic efferents originate in the lateral horns, where significant cell loss was observed in all the MSA cases examined histologically. The fibres terminating in the lateral horn project from a number of sources: primary afferents, local interneurons, cell bodies in the dorsolateral funiculus and intermediomedial nucleus, and supraspinal regions. It is remarkable that substance P containing fibres have been shown to be present in all these sources in mammalian cord (fig 1b).⁴ However, substance P immunostaining is not apparent in cell bodies of thoracic autonomic efferents. The depletion of substance P thus provides a new marker to study neuronal fibre loss or dysfunction in the dorsal and lateral horns in syndromes

of PAF. In agreement with the general depletion of substance P, a 50% reduction of substance P has been found in the CSF of MSA patients, with normal levels in Parkinsonian subjects.¹³

The depletion of substance P, substance K and CGRP in dorsal regions in MSA spinal cord was both unexpected and intriguing, and further studies are necessary to establish its significance. None of the cases had any sensory symptoms or clinical evidence of somatic sensory neuropathy. In the two cases where dorsal root ganglia were examined histologically, only case 4 showed some loss of cell bodies but normal dorsal roots. Could it be that the peptide depletion occurred exclusively in visceral afferents? Or even in non-sensory pathways in dorsal cord?

The findings in the dorsal column made it unlikely the peptide depletion was restricted to non-sensory pathways. Previous studies have found that substance P is confined to unmyelinated afferents, and tracing studies show these to terminate in superficial layers of the rat dorsal horn.¹⁴ The decrease in substance P immunostaining seen in human cord ipsilateral to limb amputation¹⁵ and bilaterally in the Riley-Day syndrome¹⁶ is also located within the substantia gelatinosa. However, there is an abundance (even majority) of unmyelinated fibres in the rat dorsal column and there is evidence that they may be primary afferents.¹⁷ The peptide loss we observed in the dorsal columns may thus occur in these fibres. In support, Otsuka *et al*¹⁸ found decreased concentrations of substance P in the dorsal horn in motoneuron disease (which spares sensory and autonomic pathways), but they reported normal levels in the dorsal columns. Whereas substance K and CGRP may co-exist with substance P in some primary sensory neurons, somatostatin and galanin are confined to different primary afferents: this may be relevant to their preservation in the dorsal column of MSA spinal cord.

There is evidence that skin flares are produced by release of substance P or CGRP (or indeed both) from sensory unmyelinated fibre terminals in skin. As skin flares were found to be intact in MSA patients, it may again be argued that the substance P and CGRP depletion may occur mainly or exclusively in visceral afferents. On the other hand, it is possible that substance P and CGRP are sufficient but not essential in the production of flares in human skin, or that there is much redundancy in the system. Whatever the correct interpretation, we have discovered in MSA a human model of depletion of thoracic dorsal cord neuropeptides which may be of use in demonstrating their functional and pathological roles.

It is now feasible and necessary to examine other cord regions, and measure neuropeptides (such as vasopressin¹⁹) selectively present in central autonomic pathways or in preganglionic sympathetic and

parasympathetic neurons: combined with peptide immunocytochemistry, this would help clarify the syndromes associated with progressive autonomic failure. A comparison with changes in classical transmitters would also be of interest. Although no studies appear to have reported levels of classical neurotransmitters in MSA spinal cord, widespread depletion of dopamine, noradrenaline and choline acetyltransferase activity have been found in the brain regions of cell loss and fibre termination in MSA cases.²⁰

It may be postulated that defects of neurotrophic agent synthesis, transport or release (and thereby of post synaptic effects) may be responsible for the "chain" pattern of cell loss in autonomic and multiple system atrophy. In trophic neuropeptides we have a new key to investigate the pathogenesis of multiple system degenerations.

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