Acta Neuropathol (1993) 86: 190–192

Acta Neuropathologica

The intermediolateral nucleus in sporadic amyotrophic lateral sclerosis*

H. Takahashi[1], K. Oyanagi[2], and F. Ikuta[1]

[1]Department of Pathology and [2]Center for Materials of Brain Diseases, Brain Research Institute, Niigata University,
1 Asahimachi, Niigata 951, Japan

Received March 3, 1993/Revised, accepted April 27, 1993

Summary. Histological and morphometrical observations of the intermediolateral nucleus (IML) at the levels of the upper and lower thoracic segments (T2 and T9) were carried out in 18 patients with sporadic amyotrophic lateral sclerosis (ALS) and 15 age-matched control subjects. Of the 18 ALS patients 6 had been on a respirator before death. No Bunina bodies were found in the IML neurons in either the ALS patients or the control subjects. Only a small number of spheroids were encountered rarely in the IML in both the patients and controls. The number of neurons in the IML in the non-respirator-supported ALS patients were reduced at T2, but well preserved at T9 compared with the control subjects. In the respirator-supported ALS patients, there was a marked reduction of IML neurons at both T2 and T9. Considering the absence of direct synaptic contacts with anterior horn cells, these neurons, without the formation of Bunina bodies, appeared to be involved primarily in the disease process in sporadic ALS.

Key words: Amyotrophic lateral sclerosis – Intermediolateral nucleus – Quantitative examination – Bunina body – Neuronal loss

On the basis of histological and morphometrical findings, we have recently suggested that Clarke's column neurons are involved primarily in the disease process in sporadic amyotrophic lateral sclerosis (ALS), although they may start to disappear after the patients have begun to require respiratory support [7]. In the present study, we extended similar observations to the intermediolateral nucleus (IML) of the thoracic spinal cord, which contains the preganglionic neurons of the sympathetic nervous system [4].

* Supported in part by a research grant for CNS degenerative diseases from the Ministry of Health and Welfare, Japan

Correspondence to: H. Takahashi (address see above)

Materials and methods

The examined cases were the same as those reported previously [7]. Eighteen patients with ALS showing typical clinical histories and neuropathological findings were studied. The patients ranged in age from 35 to 73 years (average = 56.8 years), and the duration of the illness ranged from 1 to 7 years. Twelve of the ALS patients, aged between 35 and 73 years (average = 56.6 years), died of respiratory failure without artificial respiratory support. The rest, aged between 48 and 69 years (average = 57.2 years), had been on a respirator for various periods ranging from 7 months to 4 years and 10 months before death. We also examined 15 age-matched control subjects, aged between 36 and 75 years (average = 57.5 years).

Three serial transverse slices, 5 mm thick, were made at the levels of both the 2nd and 9th thoracic segments of the formalin-fixed spinal cord, and embedded in paraffin. The IML was defined as a triangular area of gray matter according to the method described by Oppenheimer [6] and Nakajima et al. [5]. Serial 4-μm-thick sections were cut, and the 1st, 5th and 9th sections were stained with hematoxylin and eosin for examination of Bunina bodies, and the 4th, 8th and 12th were stained by Bodian's method for investigation of spheroids, which were defined as round to oval argyrophilic structures with a diameter of more than 20 μm.

For quantification of the IML neurons, serial 10-μm-thick sections were cut, and 10 sections 100 μm apart were subjected to Klüver-Barrera (KB) staining. Thus, a total 30 sections of T2 and T9 from each individual were examined. Neurons were identified by the presence of Nissl substance and prominent nucleoli. The numbers of these neurons in the bilateral IML were counted.

Statistical evaluation was done using the Mann-Whitney U-test to compare the numbers of neurons in the IML in non-respirator-supported ALS patients, respirator-supported ALS patients and control subjects.

Results

No Bunina bodies were found in the IML neurons in the 18 ALS patients or in the 15 control subjects. Only a small number of spheroids were encountered rarely in the IMLs in both the patients and controls. In this connection, Bunina bodies were found in the remaining anterior horn cells in the patients (13/18 at T2, 13/18 at T9 and 15/18 at T2 or T9). No such bodies were found in

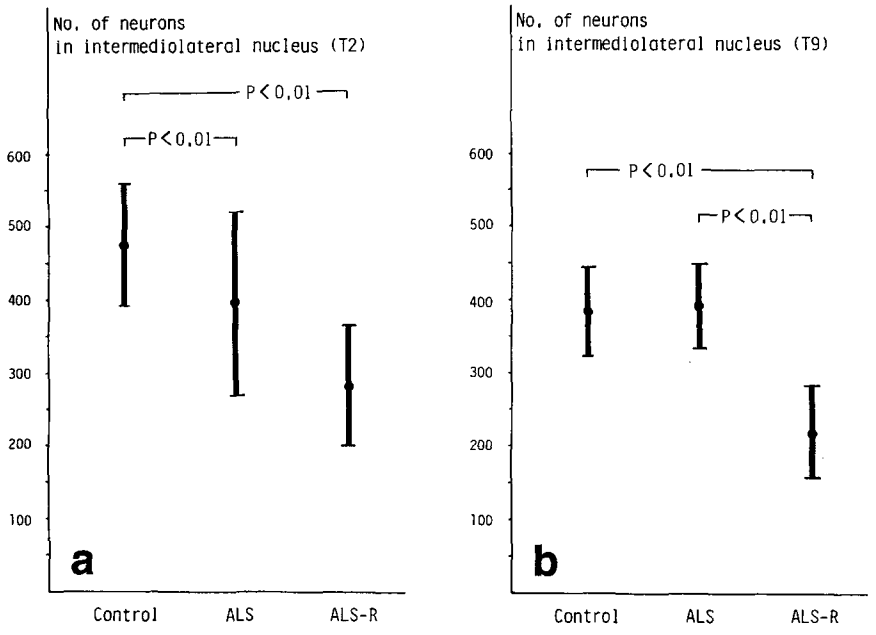

Fig. 1. The numbers of intermediolateral nucleus (IML) neurons at T2 (**a**) and T9 (**b**) in non-respirator-supported amyotrophic lateral sclerosis (ALS) and respirator-supported ALS (ALS-R) patients, and controls. *Bars* indicate SD. The differences were significant

the controls. A small number of spheroids were observed in the anterior horns at T2 and T9 in both the patients and controls; however, it was obvious that severe anterior horn cell loss was present in the former.

The average numbers of neurons in the IML at T2 in the non-respirator-supported ALS patients and respirator-supported ALS patients were 84 % ($P < 0.01$) and 60 % ($P < 0.01$) of those in the controls, respectively (Fig. 1a). On the other hand, there was no significant difference in the average number of neurons in the IML at T9 between the non-respirator-supported ALS patients and the controls, although the average number of neurons in the IML at T9 in the respirator-supported ALS patients was 57 % ($P < 0.01$) of that in the controls (Fig. 1b).

In the respirator-supported patients, neurons in the IML tended to be lost with time after the start of respiratory support, although no significant correlation was evident between the degree of neuronal loss and the duration of respiratory support because of the small number of cases examined (Fig. 2).

Discussion

Early histopathological studies showed that the IML was apparently normal in ALS [1]. However, subsequent quantitative studies suggested that this nucleus may be involved in the disease process in ALS [3, 5]. Nakajima et al. [5] studied the thoracic IML in five cases of ALS and five control subjects and reported an average 47 % reduction of the neuronal population in the disease group. Kennedy and Duchen [3] examined the upper (T2 and T4), middle (T6 and T8) and lower (T10 and T12) thirds of the thoracic cord in five cases of ALS, one of which was familial, and in three control subjects and reported that there was slight reduction in the number of IML neurons in ALS cases, the difference being greatest in the upper third, although not to a significant extent. These studies dealt with relatively small numbers of ALS cases and did not pay attention to Bunina bodies or spheroids, which are also known to be essential features in the anterior horns in this disease.

In the present study, we examined the IML at two distinct levels of the thoracic cord (T2 and T9) in 18 patients with sporadic ALS and 15 age-matched control subjects. To perform neuron counts, we prepared sufficient numbers of sections at equally spaced intervals. It

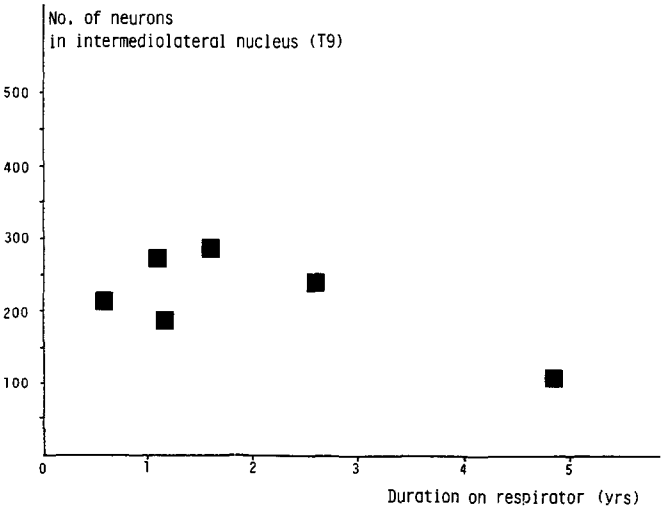

Fig. 2. Scatter plot of the number of IML neurons at T9 against the duration of respiratory support

was found that neither Bunina bodies nor spheroids were a feature of the IML in ALS patients, irrespective of the presence or absence of respiratory support, and that the number of IML neurons was reduced only at T2 in non-respirator-supported ALS patients, and reduced more markedly at both T2 and T9 in respirator-supported ALS patients.

From the present quantitative findings, it was considered that neuronal loss in the IML in ALS starts from the upper thoracic segment and extends to the lower thoracic segment, without Bunina bodies and only rarely with spheroids. At the lower thoracic segment (T9), the IML neurons may start to disappear only after the patients have begun to require respiratory support.

The findings that IML neuron loss was more evident in the upper thoracic segment (T2) in ALS patients appeared to correspond to the well-known fact that in this disease, loss of the anterior horn cells is usually more severe in the upper spinal segments, implying that the former is secondary to the latter. However, a literature search failed to reveal any reports about direct synaptic contact of IML neurons with anterior horn cells (alpha motoneurons). Therefore, at present, loss of the IML neurons appears difficult to explain in terms of a change secondary to that in the anterior horn cells. Furthermore, Ito et al. [2] have recently reported that the IML neurons in ALS patients showed a significantly higher incidence of perikaryal immunoreactivity for phosphorylated neurofilament proteins than those in controls. Such a phenomenon was also observed in the anterior horn cells, but not in the peripheral sensory and sympathetic ganglion cells. The latter authors concluded that both IML neurons and anterior horn cells are affected by a derangement of phosphorylated neurofilament metabolism, and that a degenerative process similar to that in motoneurons may take place in IML neurons of ALS patients [2].

In conclusion, we believe that the IML neurons are involved primarily in the disease process in sporadic ALS. However, as shown previously in Clarke's column neurons [7], the rate of degeneration of these neurons is much slower than that of lower motor neurons; loss of IML neurons in the lower thoracic segments may become apparent only after the patients have begun to require respiratory support. The absence of Bunina bodies and the only rare occurrence of spheroids in the IML of ALS patients are of interest. It can be speculated that their appearance depends upon certain intrinsic factors peculiar to the IML neurons themselves.

Acknowledgements. We express our appreciation to Mr. K. Kobayashi, Mrs. H. Kobayashi, Mr. K. Egawa and Ms. Y. Ota for their technical assistance, and to Ms. K. Murayama and Ms. M. Kainuma for their help in preparing the manuscript.

References

1. Hughes JT (1982) Pathology of amyotrophic lateral sclerosis. In: Rowland LP (ed) Human motor neuron disease. Raven Press, New York, pp 61–74
2. Itoh T, Sobue G, Ken E, Mitsuma T, Takahashi A, Trojanowski JQ (1992) Phosphorylated high molecular weight neurofilament protein in the peripheral motor, sensory and sympathetic neuronal perikarya: system-dependent normal variations and changes in amyotrophic lateral sclerosis and multiple system atrophy. Acta Neuropathol 83:240–245
3. Kennedy PGE, Duchen LW (1985) A quantitative study of intermediolateral column cells in motor neuron disease and the Shy-Drager syndrome. J Neurol Neurosurg Psychiatry 48:1103–1106
4. Low PA, Okazaki H, Dyck PJ (1977) Splanchnic preganglionic neurons in man. I. Morphometry of preganglionic cytons. Acta Neuropathol (Berl) 40:55–61
5. Nakajima R, Takahashi K, Nakamura H, Otomo E, Kameyama M (1981) A quantitative study of the intermediolateral cells of the thoracic cord in degenertive disease of the nervous system. Clin Neurol (Tokyo) 21:581–586
6. Oppenheimer DR (1980) Lateral horn cells in progressive autonomic failure. J Neurol Sci 46:393–404
7. Takahashi H, Oyanagi K, Ohama E, Ikuta F (1992) Clarke's column in sporadic amyotropic lateral sclerosis. Acta Neuropathol 84:465–470