Immunocytochemical Studies on Synaptophysin in the Anterior Horn of Lower Motor Neuron Disease

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Abstract. This report concerns the study of synaptophysin (SP) expression in the anterior horn of three cases of lower motor neuron disease (L-MND). All patients studied had anterior horn cell degeneration without neuropathological evidence of corticospinal tract degeneration. Spinal cords from six patients with no neurological disease served as controls. Immunocytochemical techniques were used. The results show that in L-MND there is decreased SP immunoreactivity of the anterior horn neurons, but preservation of immunoreactive dots around the cell body and proximal processes, and the presence of some residual neurons in the affected gray matter that are surrounded by a dense accumulation of immunoreactive products. These findings resemble those of classical amyotrophic lateral sclerosis (ALS), indicating similarities in the distribution patterns of presynaptic terminals in the anterior horn of L-MND and classical ALS.

Key Words: Anterior horn cell; Dendrite; Immunocytochemistry; Motor neuron disease, lower; Neuropil; Spinal cord, lumbar; Synaptophysin.

INTRODUCTION

The pathology of the anterior horn in motor neuron disease (MND), especially amyotrophic lateral sclerosis (ALS), has been the subject of continuous interest, with most studies focusing on the cell body (1–3) and proximal processes (4–8). However, other than ultrastructural studies (9–11), almost nothing is known about the morphological alterations of anterior horn synapses in MND.

Synaptophysin (SP), a 38 kilodalton glycoprotein, is a constituent of the membrane of synaptic vesicles in presynaptic terminals (12–14). Since immunocytochemical procedures with antibodies to SP allow the visualization of presynaptic terminals (15), it is possible to detect synaptic alterations in neurological disorders.

This approach was used recently by Kawanami et al (16, 17) to examine anterior horn cells of ALS patients. Here we present the results of applying immunocytochemical techniques to determine SP expression in the anterior horn of patients with lower motor neuron disease (L-MND).

MATERIALS AND METHODS

Materials

This study was carried out on three autopsied cases of L-MND. None had corticospinal tract degeneration as determined by routine neuropathological examination, including staining for myelin and fat (Table 1). Six autopsied cases of non-neurological disorders with normal spinal cords were used as controls (one male, five females). Their ages at the time of death ranged from 26 to 85 years (mean: 63.3 years). Postmortem interval before necropsy was from 7 to 23 hours (mean: 15 hours).

Immunocytochemical Procedure

The lumbar segments of the spinal cords of the nine cases were fixed in 10% phosphate-buffered formalin, embedded in paraffin, and cut into 10 μm sections which were used in the immunocytochemical studies. Deparaffinized sections were incubated with 3% H2O2 for 10 minutes (min), treated with 1.5% normal horse serum in phosphate-buffered saline (PBS), pH 7.2, for 30 min, and then incubated with the monoclonal antibody to SP (clone SY38, ready-to-use, BioGenex Laboratory, San Ramon, CA) overnight at room temperature. Sections incubated with PBS containing 1% bovine serum albumin (BSA) served as reaction controls. The sections were subsequently stained by the avidin-biotin peroxidase complex (ABC) method (Vectorstain ABC kit, Vector Co., Burlingame, CA), using 3,3'-diaminobenzidine tetrahydrochloride (DAB; DAKO, Carpinteria, CA) as chromogen and hematoxylin as counterstain.

RESULTS

Transverse sections of spinal cords of normal controls showed SP immunoreactivity in the entire gray matter area, except for the area around the central canal, while none was seen in the white matter (Fig. 1A). Immunoreactivity was symmetric and especially prominent in the substantia gelatinosa, the intermediate gray matter, and the anterior horn. These results were identical to those previously reported (15–18). By contrast, in the spinal cord sections of L-MND patients, SP immunoreactivity was decreased in the anterior horn, particularly in the lateral portion, whereas in the other gray matter areas, immunoreactivity was similar to that of normal spinal cords (Fig. 1B, C).

In the normal controls, the neuropil of the entire anterior horn had numerous uniformly scattered fine granules that were positively stained (Fig. 2A). By comparison, in L-MND, immunoreactivity was diffusely...
TABLE I
Clinical and Pathological Features of the Cases of L-MND

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>Clinical course</th>
<th>Post-mortem interval</th>
<th>Neuropathological findings in the lumbar anterior horns</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44/F</td>
<td>20 yr</td>
<td>14 hr</td>
<td>Severe loss and atrophy of neurons with obviously rarefied neuropil</td>
</tr>
<tr>
<td>2</td>
<td>65/M</td>
<td>5 mo</td>
<td>3.5 hr</td>
<td>Relative preservation of number and appearance of neurons</td>
</tr>
<tr>
<td>3</td>
<td>61/M</td>
<td>5 yr</td>
<td>&lt;24 hr</td>
<td>Mild to moderate degeneration of anterior horn cells</td>
</tr>
</tbody>
</table>

decreased, predominantly in the lateral portion of the anterior horn neuropil (Fig. 2B).

Individual anterior horn cells of normal controls had relatively large immunoreactive dots on the surface of the cell body and proximal processes, as well as many fine dots that were positively stained (Fig. 3A). Many remaining normal-appearing anterior horn cells of the L-MND patients had similar immunoreactivity patterns as the neurons in normal spinal cords (Fig. 3B). The staining intensity of residual cells with atrophic cell bodies was usually normal, or somewhat increased at the surface of the cell body and proximal processes (Fig. 3C). Other remaining neurons with abundant cytoplasm had dense accumulations of immunoreaction products around the cell body and proximal processes (Fig. 3D).

The anterior horn of the lumbar segment of case 1 showed severe neuronal loss and residual atrophic neurons, associated with diffuse rarefaction of the neuropil. On the other hand, case 2 had relatively well-preserved anterior horn cells, both in number and in appearance, and the neuropil was not affected. Synaptophysin immunoreactivity in the anterior horn neuropil was correlated with the degree of anterior horn degeneration. This is clearly evident by comparing SP expression in case 1 (Fig. 1C) and case 2 (Fig. 1B), even though immunoreactivity around the cell bodies and proximal processes was similar.

Fig. 1. SP immunoreactivity in the gray matter of the lumbar segment of the spinal cord. A. Normal spinal cord. SP immunoreactivity is distributed throughout the entire gray matter, except for the area around the central canal. B. L-MND. Immunoreactivity is slightly diminished in the anterior horn (case 2). C. L-MND. Immunoreactivity is strikingly decreased, predominantly in the lateral portion of the anterior horn (case 1). The rectangle corresponds to the area shown at higher magnification in Figure 2B. ×10.
Fig. 2. SP immunoreactivity in the anterior horn neuropil. A. Normal spinal cord. Fine immunoreactive granules are scattered uniformly throughout the neuropil of the anterior horn. A higher magnification of the neuron indicated by the arrow is shown in Figure 3A. B. L-MND. Higher magnification of area in rectangle in Figure 1C. There is a decrease of immunoreactive granules in the lateral portion of the anterior horn neuropil (on the right half of the photograph) of case 1. SP immunoreactivity is preserved in the other area of the gray matter. One neuron is intensely immunostained (arrow). For higher magnification of this neuron, see Figure 3D. × 50.
Sections incubated with PBS-BSA instead of the antibody to SP did not show any immunoreactivity.

DISCUSSION

The present studies were undertaken to determine SP immunoreactivity patterns in the anterior horn of patients with L-MND and to compare these patterns with those recently described in classical ALS (16-18). Our results indicate that in L-MND there is a decrease of SP immunoreactivity in the anterior horn neuropil, while it is relatively well preserved around the cell body and the proximal processes, with some residual neurons being surrounded by a dense accumulation of immunoreaction products. These findings resemble those observed in clas-
sical ALS (16–18), suggesting that in L-MND as in classical ALS there is a decrease of presynaptic terminals attached to distal dendrites, whereas those attached to the cell body and proximal processes of anterior horn cells are relatively intact.

It is noteworthy that despite the preservation of corticospinal tracts, SP immunoreactivity is diffusely decreased in the anterior horn neuropil in L-MND as it is in classical ALS (16–18). In the anterior horn neuropil, the axo-dendritic synapse is more common than the axo-axonal or dendro-dendritic synapse (19). Hence, the neuropil could be considered as a place where dendrites of anterior horn cells form synaptic complexes with presynaptic terminals of afferent fibers that terminate in an anterior horn. The degeneration of both anterior horn cells and afferent fibers are possible factors contributing to the reduction of SP immunoreactivity in the neuropil.

As shown in ALS (20), degeneration of anterior horn cells is associated with morphological dendrite alterations, consisting of thinning of dendritic trunks and shrinkage of dendritic trees. It was previously suggested that the loss and atrophy of distal dendrites would precede perikaryon alterations and cause the reduction of afferent presynaptic terminals (21). The demonstrated loss of SP immunoreactivity in the neuropil would substantiate this hypothesis. The reduction of SP immunoreactivity in the neuropil seen in classical ALS and in L-MND seems to be related to the severity of the degeneration of anterior horn cells, but not with corticospinal tract degeneration.

However, we cannot exclude the possible influence of afferent fibers on the diminished SP immunoreactivity in the neuropil. In classical ALS, corticospinal tract degeneration would contribute to the reduction of presynaptic terminals in the neuropil, since in humans its axons terminate directly at the anterior horn cells (22, 23). The loss of interneurons, which has been documented in ALS (24–28), may also contribute to the alteration of presynaptic terminals in L-MND, as well as the loss of the primary sensory afferents (group Ia and group II) (29). In addition, other afferents might be affected by the tract degeneration which is considered to be associated with primary degeneration of spinal gray matter, including that of MND (2, 30). These afferents could be a possible common cause responsible for the reduction of SP immunoreactivity in the neuropil in both classical ALS and L-MND.

As in classical ALS (16–18), the surface of the cell body and proximal processes of some remaining anterior horn cells of L-MND patients exhibits dense accumulations of immunoreaction products. It is possible that to a certain extent this may be due to atrophy of these cells (31, 32) and the proximal processes (3) (Fig. 3C). On the other hand, in the case of neurons with abundant cytoplasm which display prominent SP immunoreactivity (Fig. 3D), it could represent a compensatory mechanism of presynaptic vesicles for reduced synaptic function. Previous findings in certain human disorders (33–37) and in experimental animals (38, 39) are precedents for such a possibility. The enlargement of synapse size seems to be a compensatory process for a diminution in the number of synapses. Hence, the accumulation of SP immunoreactivity may represent synaptic plasticity or a synaptic rearrangement associated with the enlargement of presynaptic terminals and dendritic degeneration.

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