Ultrastructural Study of the Synapses of Central Chromatolytic Anterior Horn Cells in Motor Neuron Disease

SHOICHI SASAKI, MD, AND MAKOTO IWATA, MD

Abstract. This report deals with an ultrastructural investigation of the synapses on the somata of central chromatolytic anterior horn neurons of seven patients with amyotrophic lateral sclerosis (ALS) and four patients with lower motor neuron disease (LMND) who had no upper motor neuron or corticospinal tract involvement. Specimens from 24 age-matched individuals who died of non-neurological diseases served as controls. We examined a total of 171 anterior horn neurons with central chromatolysis (51 from ALS, 42 from LMND and 78 from controls), and 174 normal-appearing anterior horn neurons as controls. The cross-sectional area, the number of synapses, and the length of active zone were significantly reduced in the chromatolytic neurons of both patients and controls as compared with normal-appearing neurons of the controls (p < 0.0001). However, regarding chromatolytic neurons, no significant differences were seen in the number of synapses, length of each individual synapse, and length of its active zone between patients and controls and also in the frequency of presynaptic alterations on the somata. There was no overall difference between ALS and LMND patients in any of these parameters. Our findings suggest that the flow of electrophysiological information fromafferent fibers to the somata may be greatly impaired in central chromatolytic neurons of both central individuals and patients with motor neuron disease (MND), and that the observed synaptic alterations may reflect pathological events resulting from anterior horn neuron degeneration. It may represent a compensatory mechanism of the synapses for diminished synaptic function that synapses were relatively well preserved on the somata of central chromatolytic neurons of the MND patients as compared with those of the chromatolytic neurons of the controls despite of markedly reduced cross-sectional area in the former. It also suggests that the pathomechanism involved in central chromatolysis differs between normal individuals and patients with MND.

Key Words: Amyotrophic lateral sclerosis; Central chromatolysis; Motor neuron disease; Synapse; Ultrastructure.

INTRODUCTION

Central chromatolysis of anterior horn neurons is one of the early pathological changes of amyotrophic lateral sclerosis (ALS) (1, 2), and it has been reported that the features of the ALS alterations differ from those of other conditions (2–4). Although recent immunochemical and ultrastructural studies have launched out into a series of investigations on synapses of anterior horn cells of patients with motor neuron diseases (MND) (5–13), little is known about the synaptic changes in central chromatolytic neurons of such patients (7, 8). As part of our search for differences in the synaptology between control individuals and MND patients, we performed an ultrastructural investigation of the synapses on the somata of anterior horn neurons with central chromatolysis. To the best of our knowledge, this is the first detailed study of synaptic changes in central chromatolytic neurons of MND patients.

MATERIALS AND METHODS

This study was carried out on the spinal cords of seven patients (ages: 59, 59, 61, 61, 63, 78, and 81; average: 66.0 years) with clinically and neuropathologically proven ALS, four patients (ages: 49, 65, 72 and 75; average: 65.3 years) with similarly confirmed lower motor neuron disease (LMND) who had no upper motor neuron or corticospinal tract involvement, and 24 age-matched control individuals (ages: 44–80, average: 63.2 years) who had no neurological disease. All postmortem investigations were performed within 6 hours (h) after death. In all cases, tissue blocks were obtained at autopsy from the same level of the lower lumbar spinal cord (L4–5), and the anterior horns of each level were fixed immediately with 2% glutaraldehyde in phosphate buffer (pH 7.4). After fixation, the anterior horns were cut transversely into approximately 1 mm-thick pieces, postfixed for 2 h with 1% osmium tetroxide, dehydrated, and then embedded flat in epoxy resin. Each embedded tissue block was subsequently cut into semithin (around 1–μm-thick) sections that were almost large enough to contain an entire anterior horn. The sections thus prepared were stained with toluidine blue.

Central chromatolysis was identified by round somata with the nucleus in an eccentric position and the Nissl substance displaced toward the margins of the cytoplasm by the pale-staining central area. After light microscopic identification of central chromatolytic neurons with a nucleus, appropriate portions of the semithin sections were cut into serial ultrathin sections. These were stained with uranyl acetate and lead citrate for electron microscopy. Photomicrographs of the anterior horn cells with central chromatolysis were taken at a magnification of 1,400 and then enlarged to a magnification of 2,660 (Fig. 1A). A total of 171 chromatolytic motoneurons were analyzed; 51 were from ALS patients, 42 from LMND patients and 78 from control individuals. A total of 174 normal-appearing anterior horn neurons from control individuals served as controls (8).
TABLE 1
Comparison of neuronal size, number of synapses, synapse length and active zone length of central chromatolytic neurons between control individuals and MND patients

<table>
<thead>
<tr>
<th></th>
<th>Cell body area (μm²)</th>
<th>Number of synapses per neuron</th>
<th>Synapse length (μm)</th>
<th>Active zone length (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal-appearing neurons</td>
<td>3,492.6 ± 723.3</td>
<td>15.6 ± 6.2</td>
<td>2.10 ± 0.51</td>
<td>0.52 ± 0.14</td>
</tr>
<tr>
<td>(N = 174)</td>
<td>(n = 2,277)</td>
<td>(n = 2,277)</td>
<td>(n = 2,277)</td>
<td>(n = 2,277)</td>
</tr>
<tr>
<td>chromatolytic neurons</td>
<td>2,844.7 ± 939.8</td>
<td>5.4 ± 5.8</td>
<td>1.94 ± 1.21</td>
<td>0.39 ± 0.26</td>
</tr>
<tr>
<td>(N = 78)</td>
<td>(n = 420)</td>
<td>(n = 420)</td>
<td>(n = 420)</td>
<td>(n = 420)</td>
</tr>
<tr>
<td>MND patients</td>
<td>1,709.3 ± 518.8</td>
<td>4.6 ± 4.3</td>
<td>1.71 ± 0.87</td>
<td>0.37 ± 0.21</td>
</tr>
<tr>
<td>chromatolytic neurons</td>
<td></td>
<td>(n = 426)</td>
<td>(n = 426)</td>
<td>(n = 426)</td>
</tr>
</tbody>
</table>

Unpaired t-test. * p < 0.0001. NS: not significant; N: number of neurons; n: number of synapses; MND: motor neuron disease.

TABLE 2
Quantitative data concerning synaptic alterations in somata of chromatolytic anterior horn neurons between controls and MND patients

<table>
<thead>
<tr>
<th></th>
<th>Normal-appearing neurons</th>
<th>Chromatolytic neurons</th>
<th>MND patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of synapses</td>
<td>Number of synapses</td>
<td>Number of synapses</td>
</tr>
<tr>
<td></td>
<td>(n = 2,277)</td>
<td>(n = 420)</td>
<td>(n = 426)</td>
</tr>
<tr>
<td>Dense conglomerates of</td>
<td>11</td>
<td>10</td>
<td>48</td>
</tr>
<tr>
<td>dark mitochondria and/</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>or vesicles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accumulation of neurofila-</td>
<td>10</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>ments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracrystallin arrays</td>
<td>19</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinule-like formation</td>
<td>9</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

* Contingency table analysis or Fisher’s exact probability test. NS: not significant.

Each individual axosomatic synapse seen on somata was examined by using photomicrographs taken at an initial magnification of 8,000 and subsequently enlarged to a magnification of 15,200. A synaptic complex consists of a presynaptic bouton and a postsynaptic membrane separated by extracellular space. Synapses were identified by the presence of synaptic membrane thickenings that were associated with synaptic vesicles. We determined the cell body area of each motoneuron with central chromatolysis, as well as the number of synapses, the length of individual synapses and the length of the active zone of each synapse. The length of an individual synapse was defined as the entire length of the synaptic contact between the presynaptic and postsynaptic sites, and the length of the synaptic active zone as the length of the postsynaptic density. Discontinuous synapses were counted as a single synapse. The lengths of the synaptic contact and of the active zone were not calculated in those instances in which the distance between the axonal membrane and the presynaptic bouton was greater than 0.1 μm.

The measurements were carried out with the Kontron computerized image analyzer (Munich, Germany), and the results expressed as mean ± standard deviation. The data obtained were analyzed by the unpaired t-test using a computerized statistical program. Quantitative data pertaining to degenerative changes of presynapses were analyzed by the contingency table analysis or Fisher’s exact probability method employing the computerized statistical program.

RESULTS

Control subjects: Intensive search brought us 78 such neurons (Table 1). Among 24 controls, two controls relatively often showed motoneurons with central chromatolysis. One died of a rupture of abdominal aneurysma (age: 80 years), and the other, of diabetes mellitus followed by sepsis with disseminated intravascular coagulation (age: 54 years), respectively. Both patients did not show any focal neurological symptom or sign including the motor system function until the terminal stage and there was no neuropathological abnormality observed except for lacunes in the basal ganglia. The mean cross-sectional area of the normal-appearing and chromatolytic neurons was 3,492.6 ± 723.3 μm² and 2,844.7 ± 939.8
µm$^2$, respectively ($p < 0.0001$). A total of 2,277 and 420 synapses were identified on the somata of normal-appearing and chromatolytic neurons, respectively and the mean number of soma synapses per neuron was 15.6 ± 6.2 and 5.4 ± 5.8, respectively ($p < 0.0001$). The mean length of the individual synaptic contacts of normal-appearing and chromatolytic neurons was 2.10 ± 0.51 µm and 1.94 ± 1.21 µm (not significant) and that of the active synaptic zone, 0.52 ± 0.14 µm, 0.39 ± 0.26 µm, respectively ($p < 0.0001$). Regression analyses revealed no significant correlation between the numerical data and the age of the control individuals. With respect to abnormalities, aggregates of electron-dense vesicles were observed in 37 presynapses of 22 chromatolytic neurons, aggregations of electron-dense mitochondria in 4 presynapses of 4 normal-appearing neurons and 18 presynapses of 13 chromatolytic neurons ($p < 0.0001$), and conglomerates of electron-dense vesicles and mitochondria in 7 presynapses of 6 normal-appearing neurons and 9 presynapses of 9 chromatolytic neurons ($p < 0.0001$) (Table 2). Spinule-like formations were found in 9 presynapses of 9 normal-appearing neurons and 12 presynapses of 10 chromatolytic neurons ($p < 0.0001$). Furthermore, bundles of neurofilaments were present in 10 presynaptic terminals of 10 normal-appearing neurons and 6 presynaptic terminals of 6 chromatolytic cells ($p < 0.05$), and paracrystallin arrays resembling Hirano bodies were observed in 19 presynapses of 15 normal-appearing neurons and 3 presynapses of 3 chromatolytic neurons ($p = 0.234$).

**MND patients:** A total of 93 anterior horn neurons with central chromatolysis were examined (Table 1). They consisted of 51 neurons from ALS patients and 42 from iMND patients. The mean cell body area of the central chromatolytic neurons of the 11 patients with MND was 1,709.3 ± 518.8 µm$^2$. This figure was significantly smaller ($p < 0.0001$) than that of the normal-appearing or chromatolytic neurons of the control individuals. A total of 426 synapses were counted on somata. The mean number of soma synapses per neuron was 4.6 ± 4.3 and the mean lengths of the individual synaptic contacts and of the active zone were 1.71 ± 0.87 µm and 0.37 ± 0.21 µm, respectively. The numerical values of these three parameters were smaller than those of normal-appearing neurons of controls ($p < 0.0001$), but were not significantly different from those of chromatolytic neurons of

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the control subjects. There were no significant differences between the ALS and LMND patients with respect to all four parameters. Most remaining synapses were rather small (Figs. 1B, C), and the postsynaptic membranous thickenings were frequently absent.

From a pathological viewpoint, a variety of presynaptic abnormalities were seen in the specimens of the MND patients (Table 2). We observed degenerated boutons containing aggregations of electron-dense presynaptic vesicles and/or mitochondria in 48 presynapses of 38 neurons.
(p < 0.0001) (Figs. 2A, B), containing bundles of neurofilaments in 12 presynapses of 12 neurons with higher frequency than that in normal-appearing neurons of controls (p < 0.0001) (Fig. 3). Pancreatinin arrays resembling Hirano bodies and spine-like formations were found in 3 presynapses of 3 neurons and 5 presynapses of 5 neurons, respectively, with no significant difference in frequency as compared with normal-appearing neurons of controls. The frequency of all these synaptic alterations among the MND patients was not significantly different from that found with the specimens of chromatolytic neurons of the control individuals. The presynaptic boutons were frequently separated from the cell membrane (Fig. 4A), with astrocytic processes occupying the space between the detached boutons and the postsynaptic membrane (Fig. 4B). The surface area covered by

Fig. 2. A. Aggregation of electron-dense presynaptic vesicles in a presynapse. ×26,600. B. Aggregation of electron-dense mitochondria in a presynaptic bouton. ×26,600.
the astrocytic processes was easily identified in degenerated motoneurons by numerous layers of these processes. The cytoplasm of the chromatolytic anterior horn neurons of the MND patients frequently had an increased number of mitochondria, neurofilaments, and vesicular structures. In addition, cytoplasmic inclusions, such as Bunina bodies, skein-like inclusions, and Lewy body-like inclusions were not uncommonly observed.

DISCUSSION

Lesions of the synaptic complexes that terminate on the cell body could render the neuron dysfunctional by influencing its afferent inputs, and as shown in the present study, a spectrum of synaptic alterations accompanies central chromatolytic changes. Thus, the number of synapses, and the lengths of individual synapses and of the active zone are significantly reduced in the chromatolytic neurons of both controls and MND patients as compared with normal-appearing neurons of the controls. In the pathological condition, the decrease in synaptic contacts is caused by disjunction that is accompanied by a correspondingly significant increase in glial cell coverage of astrocytes. It is conceivable that the synaptic changes on the surface membrane of chromatolytic anterior horn neurons may offer a possible explanation for the functional alterations of these cells. Our previous findings (8, 11) are particularly relevant in the present context: the number of synapses, the total synapse length and the total length of the active zone per anterior horn neuron, and the length of each individual synapse and the length of its active zone increase as cell body area increases in normal-appearing cells of control individuals. As our data show, there were no significant differences in these parameters between the chromatolytic neurons of MND patients and control subjects, despite the very pronounced reduction in size of the cell body in the former, suggesting that the synapses in MND are relatively well preserved disproportionately to the cell body size as compared with those in controls. And, the relatively well-preserved synapses on these abnormal neurons in MND may represent a compensatory mechanism or plasticity of the synapses for diminished synaptic function in the somata, or the neuronal processes of anterior horn cells.

The synaptic alterations could be attributed to multiple factors. These include dying-back of afferent terminals and a cell body response to a direct attack or indirect insult such as axonal transport impairment. With respect to the first factor, it is evident that most of the remaining axosomatic synapses on motoneuron cell bodies appear to be morphologically intact, even though there are some synaptic alterations in a few. Thus, it is rather unlikely that the synaptic changes on the chromatolytic neurons may be due to the dying-back of afferent terminals. With respect to a direct or indirect effect on the cell body as the cause of synaptic alterations, it should be indicated that recent investigations of synapses with an antisynaptophysin antibody showed diminished synaptophysin immunoreactivity in anterior horn neuropils, while the proximal processes of dendrites and the somata of normal-appearing neurons of MND patients were relatively intact.
At the ultrastructural level, most synapses of the remaining normal-appearing anterior horn cells and proximal dendrites of patients with MND display no abnormalities (8, 11–13), even though their number and length are reduced and degenerative changes are evident in a minority of them (8, 9, 11–13). Moreover, as also noted here, the synaptic alterations are more pronounced in neurons with central chromatolysis than in normal-appearing anterior horn cells. These observations point to the possibility that the synaptic changes of the central chromatolytic neurons of both control subjects and MND patients may represent a pathological alteration, probably as a consequence of the degeneration of anterior horn cells. However, the possible influence of afferent fibers,
such as the interneurons (14–16) and primary sensory afferents (group 1a and group 2) (17, 18) on the reduced or altered presynaptic terminals must also be considered. The present observations of dissimilarities between controls and MND patients with respect to cell size, synaptic alterations on the somata, and cytoplasmic constituents of chromatolytic neurons suggest that the pathomechanism involved in the genesis of central chromatolytic changes may differ between MND and other conditions.

REFERENCES


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