Atrophic Cell Processes of Large Motor Neurons in the Anterior Horn in Amyotrophic Lateral Sclerosis: Observation with Silver Impregnation Method

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Abstract. Investigation of silver-stained lumbar anterior horns in four autopsied cases of sporadic amyotrophic lateral sclerosis (ALS) revealed frequent extremely small cell processes originating from large motor neurons. Their perikarya were usually smaller in size than those of normal-looking ones and almost invariably had central chromatolysis-like changes, suggesting an intimate pathomorphological relationship between the perikarya and their processes. Although it was difficult to determine whether these small processes were atrophic dendrites or shrunken axons, some were recognized as dendrites from their multiple branchings and some were identified as axons from their tapering configuration followed by widening of the distal portion. Aggregates of lipofuscin were almost always present in the perikaryal portion from which an atrophic process arose. In addition, the somewhat argentophilic slender cytoplasm which in normal neurons separates lipofuscin from the cell surface and merges with the proximal part of a process, was prominently attenuated. The small processes were more frequently observed in cases with many spheroids and chromatolytic neurons. The change in the proximal portion of the processes may implicate some disturbance of functional connection between the soma and the cell processes in ALS.

Key Words: Amyotrophic lateral sclerosis; Neurons, lower motor; Spinal cord, lumbar; Stains and staining, Bielschowsky, modified.

INTRODUCTION

The neuropathology of the spinal anterior horns in amyotrophic lateral sclerosis (ALS) has been described repeatedly (1–9). The attention of the previous researchers, however, has been almost exclusively focused on changes in the perikarya of large motor neurons. Alterations of the cell processes which arise from the soma of these cells have been relatively neglected.

In ALS examination of the anterior horns stained with the silver impregnation technique frequently reveals abnormally small processes originating from the perikaryon of the large motor neurons. This report describes the pathological changes in the proximal segments of cell processes arising from large motor neurons in ALS.

MATERIALS AND METHODS

It is easier to observe processes arising from the soma of large motor neurons in the lumbar segments than in the cervical ones; in ALS the lumbar anterior horns show a tendency to
have more remaining large motor neurons (9). These two points led us to use lumbar segments as material in this study. We chose four ALS cases in which cell processes were clearly stained with the silver impregnation method (Table 1). The silver staining technique is a modified Bielschowsky method which is routinely used in our laboratory (10, 11). The investigation by this method was supplemented by observation of the sections stained with hematoxylin and eosin (H&E), luxol fast-blue–periodic acid Schiff (LFB-PAS), and cresyl violet.

Case one, which showed many atrophic processes, had been autopsied only three hours after death and had excellent staining. The autopsies of the other ALS and control cases were delayed longer (about six hours after death). The brains were fixed with 10% neutral buffered formalin for two weeks.

The first and second cases of ALS have been reported as cases with a large number of spheroids and central chromatolysis (12, 13).

As controls we used six cases (60–85 years old) with no lesions in the lumbar segments and which showed well-stained processes of the large motor neurons in the same segments.

RESULTS

The H&E, LFB-PAS, and Nissl (cresyl violet) sections were useful in observing perikaryal changes in large motor neurons, but were not contributory to a study of the pathological processes. Therefore, this study mainly concentrated on the silver impregnation method.

Normal axons have a conical portion named the axon hillock, followed by a tapered initial segment without a myelin sheath, and then a re-enlarged myelinated portion (14–17). With the silver impregnation method, the initial segment of the large lower motor neurons in the lumbar anterior horns was approximately 1 μm in diameter, and the following myelinated portion was 2–3 μm in diameter. The length of the axon hillock and initial segment was 50–70 μm (17) (Fig. 1). In appropriately stained sections, the myelinated part of the axons was much more darkly stained than the initial segment (Fig. 1). There was a thin cytoplasmic rim, containing argentophilic filaments, presumably 10 nm neurofilaments, around aggregated lipofuscin granules (Fig. 1B). This structure merged into the axon.

Normal dendrites usually had a larger proximal portion than the axons, did not display the unique configuration of the proximal segments of axons, and frequently exhibited branching in a single section. As a rule, the branchings were at acute angles and the branching portion always appeared larger than the more proximal segment. Thus, it was usually easy to distinguish axons from dendrites.

However, some dendrites (Fig. 1A, d*) started with a proximal portion as small as that of axons and showed no branching for a fairly long distance, so that they could easily be mistaken for axons. Nevertheless, careful observation enabled us to distinguish the dendrites from axons because these small dendrites stained well in all parts and did not taper in contrast to the light staining and tapering configuration of the proximal segment of axons. The lumbar motor neurons in control cases usually showed four to five processes, resulting in an angulated cell body (Fig. 1). Neurons in the control cases did not show the thread-like atrophic processes described below in ALS cases.

In ALS, many of the lower motor neurons appeared round with fewer processes (Figs. 2, 3). These neurons frequently had single (Fig. 2A, C), or multiple (Fig. 2B) unusually small, thread-like, thin processes arising from the round soma. These atrophic processes were observed in all four cases of ALS with some variation in frequency among the cases. Some neurons showed normal-looking processes in addition to the atrophic ones (Fig. 2A) and some did not exhibit normal-looking processes.
Fig. 1. A, B. Normal large motor neurons in the anterior horn of a control case. The long processes (a in A and B) are the normal axons with a thick axon hillock and tapering initial segment followed by dense enlarged myelinated portion. A slim argentophilic belt of cytoplasm separating lipofuscin from the cell surface connects the axon (a in B) or dendrites (d and d* in A) with the rest of the cell body. Bielschowsky. × 400.

Whether the individual abnormal processes were atrophic axons or dendrites was impossible to determine in many cases (Fig. 2). But in neurons showing two or more abnormal processes (Fig. 2B), at least one of them could be regarded as a shrunken dendrite. Some atrophic processes had a distal portion thicker than the proximal one (Fig. 2C).

Most of the neurons with the thread-like abnormal processes showed a round cell body with homogeneous cytoplasm and eccentric nucleus, features compatible with central chromatolysis (Figs. 2–4). The neurons with this change, however, were almost always smaller than normal-looking ones, a difference from the central chromatolysis caused by axon injury. The abnormal neurons usually contained noticeable aggregates of lipofuscin (Figs. 2, 3). A small number of normal-looking neurons which were constantly observed in the anterior horns of each ALS case showed no atrophic processes.

The somatic portion from which the atrophic process originated almost invariably contained a fairly large amount of lipofuscin (Figs. 2–4). The neurons without obvious
lipofuscin aggregate in that region showed rarefied and poorly stained cytoplasm. When a normal-looking thick dendrite arose from the somatic part containing lipofuscin, slender (2–3 μm in thickness), moderately argentophilic cytoplasm, which frequently showed a fine filamentous configuration, separated lipofuscin from the cell surface and connected the process with the rest of the cell body (Figs. 1, 5). In contrast, when an atrophic process arises from the region adjacent to lipofuscin, the slender cytoplasmic belt was not found (Figs. 2, 3).

Although it was difficult to tell the origin of the individual atrophic processes, some of them demonstrated their dendritic nature by multiple branchings in the more distal portion (Fig. 3).

In Case one, processes considered to be atrophic axons arose from the neuronal soma. They had a small poorly stained proximal part and a larger, strongly stained distal portion, without branching (Fig. 4). The proximal segment of normal axons (axon hillock) was 3–5 μm in diameter and exhibited a smooth tapering and a longitudinal fibrous configuration (Figs. 1, 5), whereas the atrophic processes started
Fig. 3. An atrophic process (curved arrow) which can be recognized as a shrunken dendrite with branches (open arrow). Bielschowsky. ×400.

with a very small (ca 1 μm in diameter) irregular and rarefied proximal segment without a smooth tapering configuration (Fig. 4).

The cell bodies with the small processes which were considered to be atrophic axons were round with an eccentric nucleus and homogeneous cytoplasm. As in other instances, lipofuscin usually aggregated in the part of the cell body where the atrophic process arose, and the dense moderately stained cytoplasm of the normal axon hillock was markedly attenuated (Fig. 4). The processes shown in Figure 4 had a very small weakly stained proximal segment and a markedly (Fig. 4A, A') or moderately (Fig. 4B) enlarged and strongly stained distal portion.

In ALS cases, in addition to the abnormal processes and cell bodies, large motor neurons with normal soma, normal axons, and normal dendrites were occasionally observed (Fig. 5).

The frequency of these atrophic cell processes and other changes such as central chromatolysis and spheroids are shown in Table 1. The abnormal processes appeared more frequently in cases with many spheroids and central chromatolysis. There was no obvious relationship between the frequency of atrophic processes and amount of lipofuscin or the frequency of Bunina bodies.

DISCUSSION

The intra-axonal or dendritic hyaline inclusion bodies observed in familial ALS (18) and the spheroids composed of neurofilamentous accumulation in classical ALS (9, 12, 13, 19–21) have so far received the greatest attention in studies of the spinal motoneuronal proximal segment in ALS. This report describes atrophy of processes which are connected to the motoneuronal cell bodies.

With regard to axonal atrophy in lower motor neurons in ALS, a decreased number of large myelinated axons and an increased number of small and intermediate axons in the spinal anterior roots have been reported (12, 13), with an assumption that large myelinated axons have become small. As far as we know, only Holmes referred to the atrophy of cell processes connected to the soma of large motor neurons in patients with ALS, but his observation was based on sections stained with Nissl's method (1).

Amyotrophic lateral sclerosis (ALS) is characterized by selective loss of upper and lower motor neurons. Atrophy of the cell processes of large motor neurons described in this article is considered to be a phenomenon which occurs in the course of the disappearance of neurons. An intimate pathomorphological relationship between the soma and its processes is evident from the fact that almost all the neurons with atrophic processes are smaller in size than normal, have much lipofuscin and exhibit features of central chromatolysis. Not all the processes from one cell body atrophy

Fig. 4. A. An atrophic process (curved arrow) in Case one which is considered a shrunken axon with a rod-like enlarged dense distal portion (arrow). The cell body shows chromatolytic features with an eccentric nucleus; the cytoplasmic belt at the base of the process is not evident. × 200. A'. Higher magnification of A. × 400. B. Another process considered an atrophic axon arises from a chromatolytic cell body. Bielschowsky. × 400.

simultaneously, but normal-looking thick processes are observed adjacent to markedly atrophic ones (Fig. 2).

Another finding of note is that the neuronal somatic segment adjacent to atrophic processes—axons or dendrites—almost constantly contains a considerable amount of lipofuscin. Lipofuscin is considered a residual substance in lysosomes with a noxious effect on the cell (22). However, the biological effect of lipofuscin accumulation in neurons remains a controversial subject (22–25), and the relationship
between the atrophic processes and adjacent lipofuscin aggregation remains to be elucidated.

When large normal processes arise from a part of the perikaryon containing aggregated lipofuscin, a thin belt of cytoplasm separates the lipofuscin from the cell surface and connects the major portion of the cytoplasm with the processes (Fig. 1). On the other hand, this cytoplasmic belt is absent or very scanty when atrophic processes are present. Therefore, it is presumed that the cytoplasmic belt atrophies along with shrinkage of the processes and that it is the main route between the processes and the major cytoplasmic area. The moderate argyrophilia and the filamentous configuration of the cytoplasmic belt suggest that 10 nm neurofilaments are its major structural components.

The initial segment of axons is functionally important because impulses transmitted through the axon first originate there (26). Atrophy and degeneration of the proximal portion of the probable axons observed in Case one may signify some functional damage to the axons. The cell bodies having these atrophic processes show degenerative changes, but even in ALS, the perikarya of neurons with normal-

Fig. 5. Normal-looking axons arise from normal-looking large motor neurons in ALS cases. The distal ends of the initial segments are indicated by arrows. Both neurons show a clear cytoplasmic belt at the base of the axons. A, B: Bielschowsky. ×400.
<table>
<thead>
<tr>
<th>Case</th>
<th>Age at onset (years)</th>
<th>Sex</th>
<th>Duration (months)</th>
<th>Atrophic cell processes*</th>
<th>Neuronal loss</th>
<th>Spheroids†</th>
<th>Central chromatolysis†</th>
<th>Bunina bodies*</th>
<th>Amount of lipofuscin</th>
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<tr>
<td>1</td>
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<td>F</td>
<td>10</td>
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<td>+++</td>
<td>+++</td>
<td>++</td>
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</tr>
<tr>
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<td>F</td>
<td>24</td>
<td>++</td>
<td>mild</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>abundant</td>
</tr>
<tr>
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<td>53</td>
<td>M</td>
<td>19</td>
<td>+</td>
<td>mild-moderate</td>
<td>+</td>
<td>+</td>
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<td>abundant</td>
</tr>
<tr>
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<td>77</td>
<td>F</td>
<td>60</td>
<td>+</td>
<td>mild</td>
<td>−</td>
<td>++</td>
<td>+</td>
<td>abundant</td>
</tr>
</tbody>
</table>

* ++++, frequent; ++, intermediate; +, occasional.
† ++++, 10 or more/section; ++, 9–4; +, 3–1; −, not observed.
looking thick axons exhibit no obvious abnormality. These observations imply an intimate pathomorphological association between the soma and its axon.

The frequency of the atrophic processes discussed in this report varied among cases. They were more common in cases with frequent spheroids and chromatolytic neurons, and not necessarily in cases with many remaining anterior horn neurons. Both the spheroids and chromatolysis may be regarded as early changes of ALS (9, 12, 13, 27) and indicate an active pathological process in the anterior horns. This again indicates a close pathological relationship between the neuronal cell body and its processes.

Another condition to consider with regard to the number of the atrophic processes is the state of fixation of the specimens. In Case one, small processes were frequently observed radiating from the soma. Performance of the autopsy only three hours after death may have been important in disclosing this phenomenon.

Further investigation of these atrophic changes in the proximal segments of processes, especially axons of large motor neurons may help in understanding the mechanism of neuronal loss in ALS.

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REFERENCES


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