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Bunina bodies in dendrites of patients with amyotrophic lateral sclerosis.

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Abstract

We studied the brains of two cases of amyotrophic lateral sclerosis with dementia. Bunina bodies were found in the motor neurons of cranial nerve nuclei (trigeminal, facial and hypoglossal nerves) as well as in the spinal motoneurons. They appeared mostly in the cytoplasm and occasionally in the neuronal processes. However, the present electron microscopic study disclosed clearly that Bunina bodies were present not only in the cell body but also in the dendrites. No Bunina bodies were observed in the axons. It is inferred that the Bunina bodies were degenerative products formed as a result of a protein metabolism disorder.

KEYWORDS: intradendritic Bunina body, amyotrophic lateral sclerosis

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Bunina Bodies in Dendrites of Patients with Amyotrophic Lateral Sclerosis

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We studied the brains of two cases of amyotrophic lateral sclerosis with dementia. Bunina bodies were found in the motor neurons of cranial nerve nuclei (trigeminal, facial and hypoglossal nerves) as well as in the spinal motoneurons. They appeared mostly in the cytoplasm and occasionally in the neuronal processes. However, the present electron microscopic study disclosed clearly that Bunina bodies were present not only in the cell body but also in the dendrites. No Bunina bodies were observed in the axons. It is inferred that the Bunina bodies were degenerative products formed as a result of a protein metabolism disorder.

Key words : **intradendritic Bunina body, amyotrophic lateral sclerosis**

The Bunina body is an inclusion body that is observed in lower motor neurons of patients with amyotrophic lateral sclerosis (ALS) and is supposed to be an early change of ALS (1-3). Bunina bodies are mainly observed in the cytoplasm of neurons and rarely in the neuronal processes. We experienced two cases of ALS which had abundant Bunina bodies. This report is a light and electron microscopic description of Bunina bodies in the neuronal processes.

Materials and Methods

Case 1. The patient was a 54-year-old man. At age 52, he made mistakes at his job and exaggerated about himself and his family. He was referred to our clinic, and at that time he was diagnosed as having Alzheimer's disease. Three months after discharge he noticed weakness in his upper extremities bilaterally, and the weakness progressed to all four extremities. He died due to pneumonia. The duration of illness was 2 years and 4 months.

Case 2. The patient was a 60-year-old man. He recognized hoarseness and dysarthria at age 58 followed by weakness of left upper extremity. Neurologic examination 5 months later revealed dementia, with personality changes as a dominant feature, and atrophy of the four extremities and facial muscles. He became progressively weaker and expired due to respiratory failure at the age of 60.

The brains of the two cases were fixed in 10% formalin, and sections were taken from the pons, medulla and lumbar cord and embedded in paraffin. Histochemical stains included hematoxylin and eosin (HE), phosphotungstic acid hematoxylin (PTAH) and silver stain (Bodian). Tissue for electron microscopy was obtained from the anterior horn of the lumbar cord, fixed in 10% formalin, embedded in epon, and stained with uranyl acetate and bismuth subnitrate.

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Results

Case 1. The brain weighed 1,340 g. There was mild atrophy of the frontal and temporal lobes. Coronal sections revealed the lateral ventricles to be enlarged. The left ventricle was larger. Microscopic examination revealed some sponginess in the superficial layers of the frontal and temporal lobes, accompanied by glial proliferation. No neurofibrillary changes or senile plaques were recognized anywhere in the cerebral cortices, including Ammon's horn. In the precentral area, Betz cells seemed to be decreased in number, but the glial reaction was minimal and there was no pallor or gliosis in the subcortical white matter. There were few motoneurons in nuclei of the seventh and twelfth cranial nerves and in the spinal cord, and glial cells proliferated. A mild pallor was seen in the lateral cortico-spinal tract in the spinal cord. Bunina bodies are described later.

Case 2. The brain weighed 1,120 g. There was obvious, but circumscribed area of atrophy in the temporal poles. The left side was more atrophic. In the atrophic area, a diffuse neuronal depletion was found, but it was more severe in the superficial layers. There were rare neurofibrillary tangles and senile plaques in the cerebral cortices. There were few motor neurons in the seventh and twelfth cranial nerves and in the spinal cord. Degeneration of the lateral columns of the spinal cord was minimal.

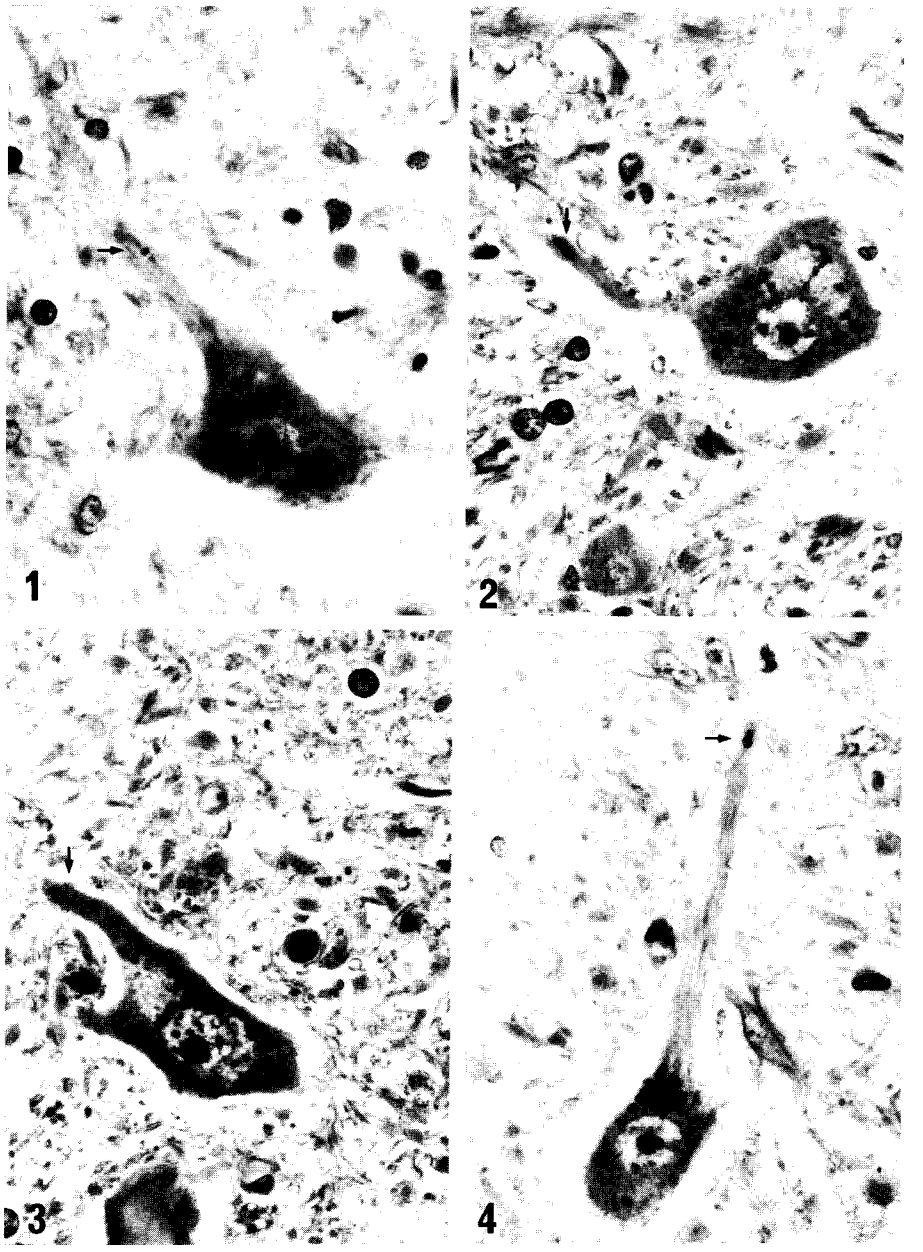
In these two cases, Bunina bodies were frequently seen in the remaining motor neurons of the cranial nerves (trigeminal, facial and hypoglossal) and spinal cord. They were round or oval and often moniliform. They measured from one to several μm. They stained eosinophilic with HE and blue with PTAH, and were negative for periodic acid Schiff (PAS) and silver stain. They were mainly observed in the soma and rarely in the neuronal processes (Figs. 1–4). Among neurons microscopically examined, eleven neurons contained Bunina bodies in the neuronal processes as well as in the cytoplasm. Eight neurons appeared normal, while three were atrophic. Light microscopy suggested that the processes were dendrites. Electron microscopy confirmed the presence of Bunina bodies in the processes. Bunina bodies in the soma were present adjacent to the processes. Processes continuous with the cell body were not covered with a myelin sheath and contained debris of microtubules, neurofilaments, mitochondria, and fragments of rough endoplasmic reticulum. These structural findings were consistent with the characteristics of dendrites (Fig. 5). Bunina bodies observed in the dendrites were composed of an amorphous electron-dense substance, had many clear areas, and contained filaments, lamellar structures, and vesicular structures (Figs 5, 6). Depending on the site, vesicular structures were continuous with lamellar structures. The external surfaces of the bodies were continuous with ribosomes at some sites.

Discussion

Early changes observed in ALS (1–3) are central chromatolysis, spheroids (axonal swelling), and Bunina bodies. Bunina bodies were described in 1962 by Bunina (4) as spherical inclusion bodies which gather in the soma like a string of beads. They are eosinophilic and stain dark blue with PTAH stain, but are negative for PAS and silver stain. These staining characteristics suggest that they contain proteins. Bunina bodies are primarily observed in patients with ALS, especially in those with a rapid course, those accompanied by dementia, and those with only symptoms of lower motor neurons. The only disease other than ALS in which these bodies have been observed is atypical polyradiculoneuritis, in a case reported by Sato *et al.* (5). The incidence of Bunina bodies in patients with ALS is 47–91 % (1). If a greater number of specimens were to be examined, the incidence may be found to be higher. Bunina bodies are contained primarily in lower motor neurons ; only Tomonaga *et*

al. (6) reported the presence of these bodies in Betz cells, which are upper motor neurons.

In ALS, both lower and upper motor neurons are damaged. Previous pathological studies have evaluated mainly spinal anterior horn cells, and there are only a few reports on the ventral root and peripheral nerves. As to upper motor neurons, though the pyramidal tract of the spinal cord has been recently studied (7), there are few reports on cerebral peduncles of the midbrain, or



Figs. 1-4 Bunina bodies in a neuronal process (arrows) as well as in the cell body. The somas in Figs. 1-3 look normal. Hematoxylin-eosin, × 300.

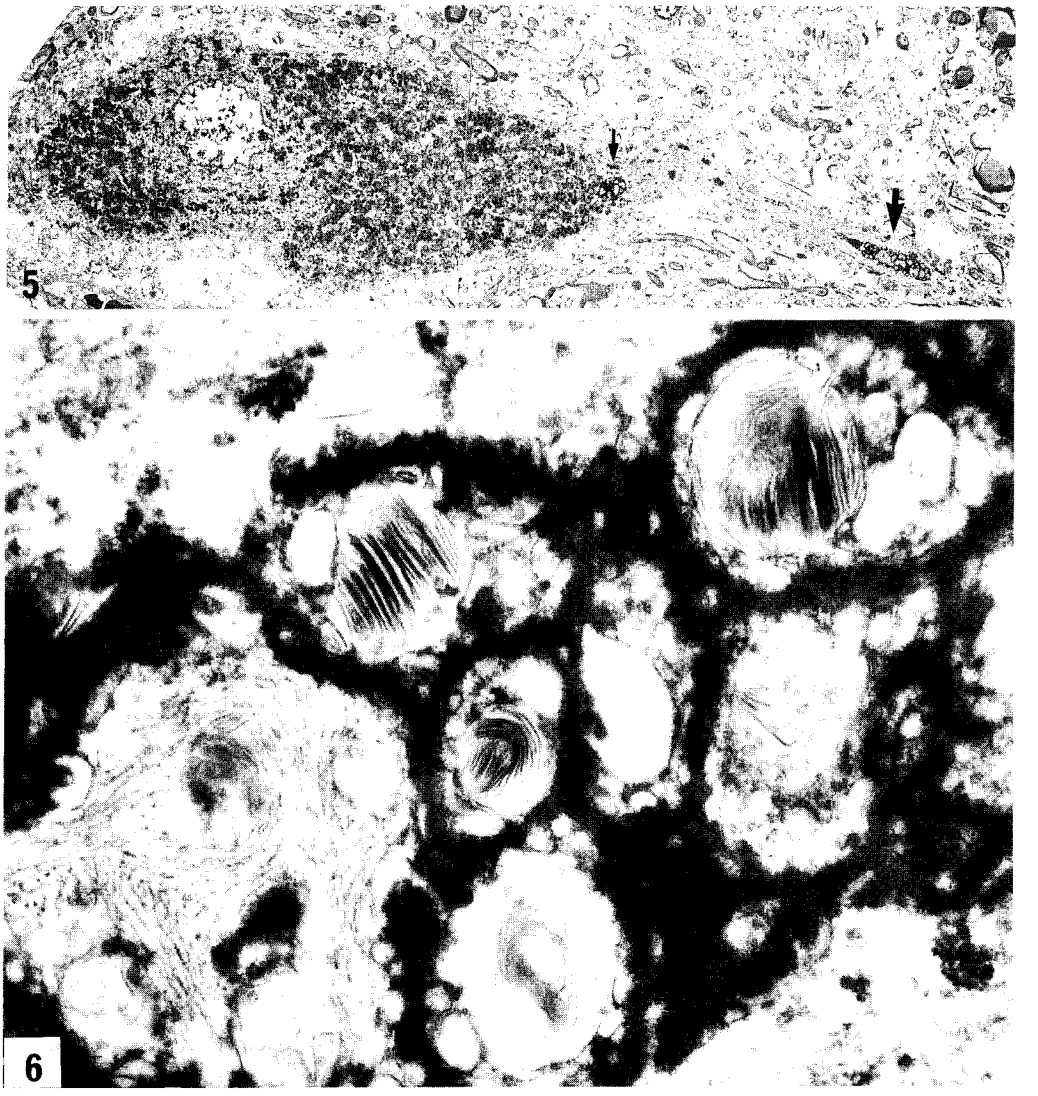


Fig. 5 Bunina bodies at the edge of the soma (↓) and in a dendrite (⬇). The cell appears atrophic and has much lipofuscin. × 1,050.

Fig. 6 Electron microscopic picture of a Bunina body in a dendrite. It consists of amorphous electron dense material, filaments, and lamellar and vesicular structures. × 36,700.

changes in Betz cells. In addition, whether the pathological mechanism is similar between upper and lower motor neurons remains unclear. Bunina bodies are, as mentioned above, inclusion bodies considerably specific to ALS and frequently observed in lower motor neurons (8–12). Spheroids, which are an early change in ALS, are also frequently observed near or continuous with the soma of lower motor neurons (13), but have not been reported in upper motor neurons.

In the present study, Bunina bodies were observed in eleven dendrites ; eight somas of which were normal, but three somas of which were atrophied. Since these bodies were observed in the soma, they may be produced there. However, the organelles that produce these bodies are unclear. Bunina bodies were continuous with the rough endoplasmic reticulum and

lipofuscin, but their association was obscure. Tomonaga *et al.* (6), Okamoto *et al.* (8, 9, 11), and Sasaki *et al.* (12) also reported the presence of Bunina bodies in processes. Tomonaga *et al.* suggested that the processes were axons ; Okamoto *et al.* and Sasaki *et al.* considered the processes to be dendrites, which was confirmed by electron microscopy in the present study. The soma was rich in lipofuscin and atrophied, but these findings are non-specific. The presence of Bunina bodies in the dendrites indicates two possibilities. One is that these bodies are produced in dendrites, and the other is that they are produced in the soma and transferred to the dendrites. We suspect that they are produced in dendrites because the site from which dendrites arise is morphologically similar to the soma, and Bunina bodies are rather large to be transferred from the soma to dendrites. The derivation of Bunina bodies is obscure. Hart *et al.* (14) suggested that they are autophagic corpuscles derived from altered mitochondria, while Tomonaga *et al.* (6) suggested that they are a manifestation of impaired protein metabolism in the lower motor neuron. Iwata and Hirano (15) regarded them as non-specific alterations common to some degenerative processes of motor neurons, Okamoto *et al.* (8) regarded them as deposition of some protein metabolite, and Sasaki (1) regarded them as a secondary degenerative product. These bodies were not present in the normal control group and olivo-ponto-cerebellar atrophy patients (16). We also consider these bodies to be a degenerative product associated with cell degeneration, especially protein metabolism in lower motor neurons.

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