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CASE REPORT

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Disaggregation of polyribosomes in the spinal anterior horn cells in a patient with X-linked spinal and bulbar muscular atrophy

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Abstract The spinal anterior horn cells (AHCs) in a patient with X-linked spinal and bulbar muscular atrophy (SBMA) were examined by light and electron microscopy, giving special attention to alterations in the rough endoplasmic reticulum (ER). Seven age-matched subjects were used as controls. The patient with SBMA showed a severe decrease of AHCs, but the Nissl substance in the remaining AHCs appeared well preserved on light microscopy. Electron microscopy revealed a relatively well preserved parallel lamellar pattern of ER and marked disaggregation of the polyribosomes surrounding the ER in the remaining AHCs. These findings indicate that the Nissl substance was affected in spite of its light microscopic appearance in SBMA, and that the AHCs degenerate through disaggregation of the polyribosomes of the ER.

Key words Anterior horn cells · Ribosome · Rough endoplasmic reticulum · Spinal and bulbar muscular atrophy · Kennedy-Alter-Sung syndrome

Introduction

Spinal and bulbar muscular atrophy (SBMA, Kennedy-Alter-Sung syndrome) is an X-linked recessive disease characterized by adult onset, slowly progressive bulbospinal muscle weakness and atrophy, and endocrine disturbances including gynecomastia, testicular atrophy, and mildly increased serum creatine kinase (CK) [6, 8–11, 16]. Recently, an increased number of CAG repeats was found in the first exon of the androgen receptor gene [7].

The major neuropathological finding in SBMA is a loss of lower motor neurons, with a topographic distribution quite similar to that observed in classic amyotrophic lateral sclerosis (ALS) [6, 8–11, 13, 16, 17]. However, it has been reported that the Nissl substance in the remaining anterior horn cells (AHCs) of SBMA appears quite well preserved on light microscopy [11, 16], although Nissl substance is usually decreased in the remaining AHCs in classic ALS [4].

In the present study we performed light and electron microscopy to elucidate the degenerative process of the AHCs in SBMA, giving special reference to the Nissl substance, i.e., rough endoplasmic reticulum (ER).

Case report

The patient was a 64-year-old male with SBMA. His parents had died of apoplexy, and the patient had also suffered from a stroke and right hemiplegia at the age of 31 years. At 10 months prior to death (ptd), dysphagia and weight loss were noticed. Motor weakness progressed subacutely. Gower's sign was seen at 3 months ptd, and he could only lift his head with his hands at 1 month ptd. He was admitted to Shinrakuen Hospital suffering mainly from severe dyspnea and dysphagia in May 1991. His tongue was found to be mildly atrophic with fasciculation. Moderate muscle weakness (values of 3–4 on the Medical Research Council scale) and muscular atrophy of the four extremities were noted. Deep tendon reflexes of the limbs were diminished and he had difficulty in walking. Results of sensory examination were normal. The chest X-ray film revealed lung cancer with an old tuberculous lesion in the left lung. Serum CK and lactic dehydrogenase levels were elevated to 1819 IU/l (normal range 24–195 IU/l) and 701 IU/l (normal range 180–460 IU/l), respectively. Paraneoplastic polymyositis was suspected, but electromyography showed typical neuropathic changes. At 12 days after admission, the patient died of acute respiratory failure. General autopsy, performed 6 h after death, revealed adenocarcinoma of the right lung with lymph node metastasis. Gene analysis using frozen brain tissue after death disclosed an increased number (n = 43) of CAG repeats in the first exon of the androgen receptor gene (normal repeat range 17–26).

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Materials and methods

Seven subjects were used as controls (age range 53–83 years). None of the patient or control subjects had suffered from ischemic or hypoglycemic episodes, severe liver dysfunction, intracranial masses or cerebral infarcts, nor had they taken anticonvulsants or received anticancer chemotherapy.

General autopsy was performed on each control subject within 3–6 h after death. For light microscopic examination of the spinal cord, the seventh cervical, eighth thoracic, fourth lumbar and second sacral segments in each case were cut transversely and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3), and embedded in paraffin. Sections were stained with hematoxylin-eosin, Klüver-Barrera, phosphotungstic acid-hematoxylin, Masson's trichrome or Bodian, and examined with a light microscope.

Paraformaldehyde (4%)-fixed paraffin-embedded 6-µm-thick sections were subjected to immunohistochemical studies using the avidin-biotin-peroxidase complex (ABC) method with a Vectastain ABC kit (Vector, Burlingame, Calif.). The following primary antibodies were used: rabbit anti-cow ubiquitin polyclonal antibody (dilution 1:150; Dakopatts, Glostrup, Denmark); monoclonal antibodies against two neurofilament proteins, phosphorylated (SMI31, dilution 1:1,000) and non-phosphorylated (SMI38, dilution 1:600) determinants (both from Sternberger Monoclonals, Baltimore, Md.). For ubiquitin immunohistochemistry, sections were pretreated with 0.025% trypsin for 15 min at room temperature to increase the antigenicity of the ubiquitin. Nonspecific binding of the biotin/avidin system reagents was blocked by pretreating the sections using a Blocking Kit (Vector), then incubating them with the required primary antibody overnight at 4°C. They were then incubated with the secondary reagent containing biotinylated anti-rabbit or anti-mouse IgG (diluted 1:200) for 30 min, and finally with ABC for 30 min. The tissues were subjected to the peroxidase reaction using freshly prepared 0.02% 3,3´-diaminobenzidine-tetrachloride and 0.005% hydrogen peroxide in 0.05 M TRIS-HCl buffer, pH 7.6, for 10 min at room temperature. As antibody controls, the primary antisera were either omitted or replaced with normal rabbit or mouse serum. Several specimens of neural and non-neural tissue from the patients served as positive or negative tissue controls. The immunostained sections were counterstained with hematoxylin for 1 min, mounted on glass slides and examined using a light microscope.

For electron microscopy, the fourth, fifth or sixth cervical segment and/or the third or fifth lumbar segment of the spinal cord in each case were fixed in 3% glutaraldehyde-1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). The tissue blocks were post-fixed in 1% osmium tetroxide, followed by dehydration through a graded ethanol series, and embedded in Epon 812. Sections, 1 µm thick, were stained with toluidine blue. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined with an electron microscope (Hitachi H-7100). AHCs were examined in controls (n = 70) and in SBMA (n = 21).

Results

The Nissl substance in the AHCs from the control subjects was abundant with a rough granular appearance (Fig. 1 a).

In the spinal cord of the patient with SBMA, the number of AHCs was severely decreased, but the Clarke's column and intermediolateral and Onuf's nuclei were well preserved. No Bunina bodies, ubiquitinated inclusions, spheroids, or intraneuronal accumulation of lipofuscin and neurofilaments were observed. Although the remaining AHCs were atrophic, the Nissl substance looked well preserved, and no chromatolytic changes were observed (Fig. 1 b).

Electron microscopically, the rough ER in the AHCs of the control subjects showed a parallel and lamellar arrangement, and the surrounding ribosomes were present in small groups forming polyribosomes (Fig. 2a).

On the other hand, in the patient with SBMA, the parallel lamellar pattern of the ER and number of ribosomes attached to the ER (granulation) were relatively well preserved, while marked disaggregation of the polyribosomes and an increase of free ribosomes surrounding the ER in the examined AHCs were observed (Fig. 2b).

Discussion

The present study revealed that in the SBMA patient, the parallel lamellar pattern of the ER was relatively well preserved; the granulation of the ER appeared to be sustained, but the polyribosomes were markedly disaggregated. The free ribosomes surrounding the ER looked increased.

The ribosomes are believed to be attached to specific sites on a strand of messenger RNA that has the encoded information which determines the amino acid sequence for the specific protein being synthesized. The ribosomes are thought to move along the length of the messenger RNA and to be released when the specific polypeptide chain has been completed. In these steps, aggregated ribosomes (polyribosomes) are essential [1, 3]. These processes might be interrupted by disaggregation of polyribosomes on ER in AHCs in the SBMA patient. Disaggregated polyribosomes indicate depressed or arrested pro-

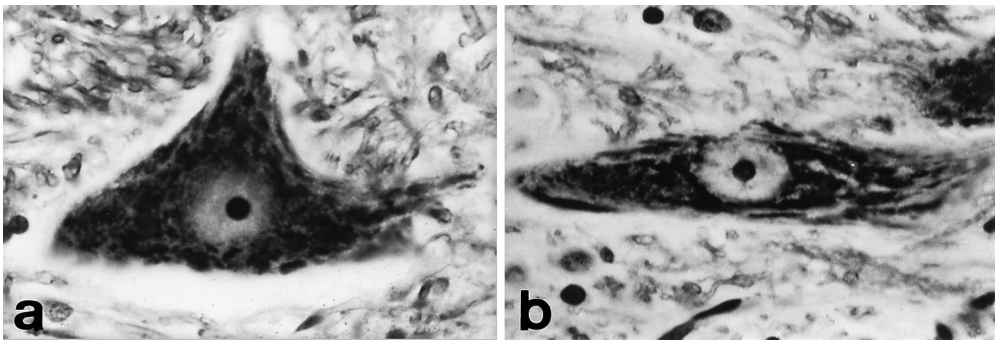

Fig. 1 a, b In the spinal cord of the patient with spinal and bulbar muscular atrophy, the number of anterior horn cells is severely decreased. Although the remaining anterior horn cells are atrophic, the Nissl substance looks well preserved, as compared with that of a control case (**a**), and no chromatolytic changes are observed (**b**). Anterior horn of the cervical segment, Klüver-Barrera preparation, **a, b** × 610

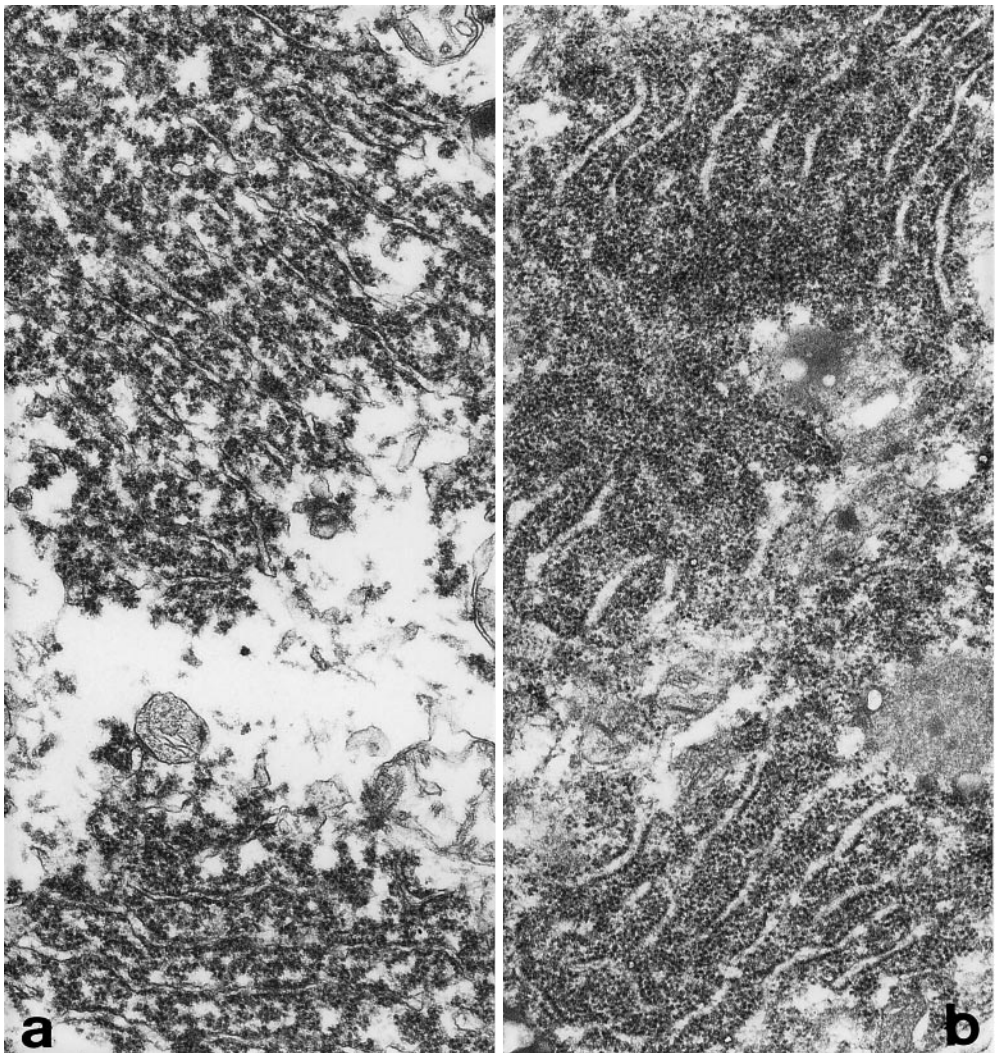

Fig. 2 a, b Rough endoplasmic reticulum (ER) in anterior horn cells. The rough ER of a control subject shows a parallel and lamellar arrangement of ER, and the surrounding ribosomes are present in small groups forming polyribosomes (**a**). In the patient with spinal and bulbar muscular atrophy, the parallel lamellar pattern of the ER, and number of ribosomes attached to the ER (granulation) are relatively well preserved, while marked disaggregation of the polyribosomes, and an increase of free ribosomes surrounding the ER are observed (**b**). Uranyl acetate-lead citrate, **a, b** × 28000

tein synthesis [3]. Thus, in SBMA, protein synthesis may be decreased in the remaining AHCs, although the Nissl substance appears preserved on light microscopy. The AHCs of SBMA may degenerate through this mechanism.

It has been reported that there is a strong correlation between the number of CAG repeats in the androgen receptor gene and the clinical onset of SBMA [2, 5]. The present patient showed 43 CAG repeats on this gene. The question of whether alterations of polyribosomes and rough ER in the AHCs vary as the number of repeats of the CAG changes in SBMA patients needs to be examined.

It has been discovered that androgen receptor-containing neurons are widely distributed, also in areas other than those usually involved in SBMA [12, 14, 15]. Further study is necessary to ascertain whether neurons other than the AHCs show disaggregation of the ribosomes, and to clarify the pathological processes from the expansion of CAG repeats in the androgen receptor gene to the loss of motor neurons in the selected areas in SBMA.

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