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Chromogranin peptides in amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder which primarily affects motor neurons. Eight cases of ALS and seven control cases were studied with semiquantitative immunocytochemistry for chromogranin A, chromogranin B and secretogranin II that are soluble constituents of large dense core vesicles, synaptophysin as a membrane protein of small synaptic vesicles and superoxide dismutase 1.
Among the chromogranin peptides, the number and staining intensity of motor neurons was highest for chromogranin A. In ALS, the staining intensity for chromogranin peptides and synaptophysin was significantly lower in the ventral horn of ALS patients due to a loss in immunoreactive motor neurons, varicose fibers and varicosities. For all chromogranins, the remaining motor neurons displayed a characteristic staining pattern consisting of an intracellular accumulation of immunoreactivity with a high staining intensity. Confocal microscopy of motor neurons revealed that superoxide dismutase 1-immunopositive intracellular aggregates also contained chromogranin A, chromogranin B and secretogranin II.
These findings indicate that there is a loss of small and large dense core vesicles in presynaptic terminals. The intracellular co-occurrence of superoxide dismutase 1 and chromogranins may suggest a functional interaction between these proteins. This study should prompt further experiments to elucidate the role of chromogranins in ALS patients.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common adult motor neuron disease, affecting three to five in every 100 000 individuals [1]. It typically affects individuals in their mid-50s and is characterized by rapidly progressive degeneration of motor neurons in the cerebral cortex, brainstem and spinal cord. ALS exists in both sporadic and familial forms. Multigenic, somatic mutation, and gene–environment models may all contribute to the genetic [1]. In approximately 20% of all familial cases mutations in the gene for the cytosolic free radical-scavenging enzyme superoxide dismutase-1 (SOD1) are involved [2].

The chromogranins A (CgA), chromogranin B (CgB) and secretogranin II (SgII) comprise a family of acidic, soluble proteins. They are characterized by numerous pairs of basic amino acids as potential cleavage sites for processing by the co-stored prohormone converting enzymes PC 1/3 and PC2 [3].

They are found in large dense core vesicles (LDCV) throughout the endocrine and neuroendocrine system [4,5], and in neurons from the central and peripheral nervous system [6]. Each of these proteins has a distinct localization in the human brain [7–9]. CgB- and SgII-LI has been detected in the fetal human vagal/nucleus solitary complex at prenatal week 11 [10]. Chromogranin peptides are stored and secreted together with a variety of peptide hormones and neuropeptides, and have not been found to be associated with small synaptic vesicles (SSV). Like synaptophysin is an appropriate marker for SSV [11], chromogranin peptides are useful markers for LDCV [12] and can be therefore used to analyze specific synaptic alterations in ALS.

In a recent paper, it has been shown that chromogranins interact with mutant forms of superoxide dismutase that are linked to ALS, but not with wild-type SOD1 in transgenic mice harbouring the G39A mutant of human SOD1 [13]. Chromogranins have been partially colocalized with SOD1 in ventral horn motor neurons. Moreover, experimental evidence has been found that chromogranins act as chaperone-like proteins that promote secretion of SOD1 mutants [13].

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Table 1

Sex	Age	Duration of disease (years)		Cause of death
Controls				
M	71			Myocardial infarction
M	71			Aspiration pneumonia
F	67			Liver cancer
F	70			Traffic accident
M	57			Pulmonary embolism
F	68			Myocardial infarction
F	59			Myocardial infarction
	66±5.4			
ALS				
M	62	5	ALS (UL, LL)	Pulmonary embolism
F	57	3	ALS (UL, LL, B)	Myocardial infarction
F	75	7	ALS (UL, LL, B)	Pneumonia
M	72	6	ALS (UL, LL, B)	Rupture of aorta
M	87	7	ALS (UL, LL)	Pneumonia
M	69	6	ALS (UL, LL, B)	Myocardial infarction
F	55	7	ALS (UL, LL, B)	Pneumonia
F	62	6	ALS (UL, LL, B)	Rupture of aorta
	67±9.9	5.9±1.3		

Predominant clinical features of ALS are shown: UL = upper limbs; LL = lower limbs; B = bulbar. F, female; M, male.

In ALS patients, one study has been performed for chromogranin A reporting that staining pattern of chromogranin A is altered in motor neurons of sporadic ALS patients [14]. To our knowledge, no studies have been presented for chromogranin B and secretogranin II in ALS.

In the present study, we investigate the alterations in the localization and expression of chromogranins, and examine whether these changes comprise CgA, CgB and SgII, or whether they are specific for chromogranin A. The comparison between synaptophysin and chromogranin peptides will reveal how presynaptic terminals are affected in ALS as chromogranin peptides are useful markers for LDCV and synaptophysin is an appropriate marker for SSV.

2. Materials and methods

2.1. Tissue preparation

Eight cases of sporadic ALS and seven control cases were studied. Patients' characteristics are presented in Table 1. Neuropathological examination did not reveal any neuropathological lesions that were not likely to be related to ALS, as for example vascular pathology.

Tissue blocks containing the spinal cord were dissected. They were immediately fixed by immersion in cold 4% paraformaldehyde in sodium phosphate buffer (PBS), pH 7.2, for 1 week. One block was dehydrated in graded ethanols, embedded in paraffin and cut serially in 5 µm thin coronal sections. Paraffinized sections were mounted on poly-L-lysine-coated slides and for cytoarchitectural orientation every tenth section was stained with cresyl violet. The research was with regular clinical autopsies and was centered on diagnostic fields according to the Tyrolean Authority Permission.

2.2. Antisera

For chromogranin A, two antibodies were applied. The catestatin antibody was characterized [15] and provided by Dr. Sushil K. Mahata, Department of Medicine and Center for Molecular Genetics, University of California, San Diego, CA, USA. This polyclonal antibody was generated in rabbit. Catestatin is contained in the human CgA, amino acid sequence human CgA340–372: The antibody is raised against human CgA352–372 (SSMKLSFRARAYGFRGPGP). The second one, was a mouse monoclonal, commercially purchased from LAB VISION, Fremont CA, that reacts with the 68–75 kDa band in western blot according to manufacturer's information.

For chromogranin B, a polyclonal antibody was used and described in detail [16]. Briefly, it was generated against a synthetic peptide (PE-11) corresponding to rat CgB 552–562 which is identical with the human

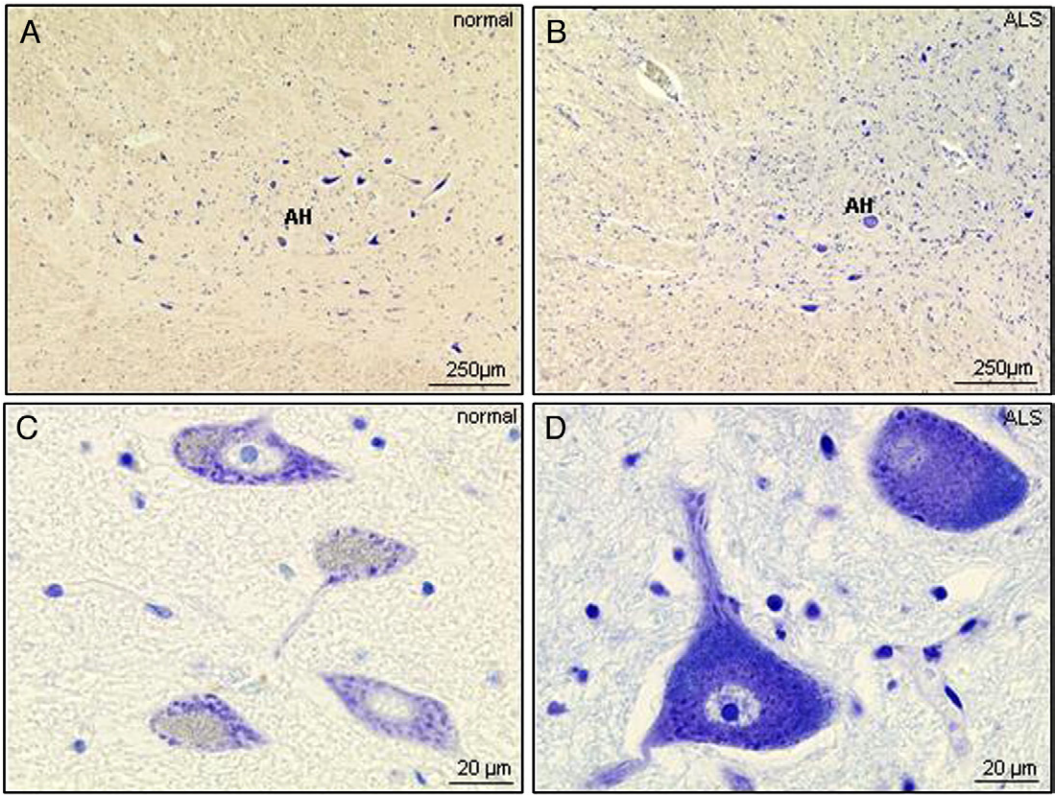

Fig. 1. Cresyl violet staining in healthy subjects (A) and ALS patients (B) illustrating a neuronal loss in the anterior horn (AH). A higher magnification of an aspect of the anterior horn of a control subject (C) and ALS patient (D) shows that in ALS patients the remaining motor neurons are generally larger with a more intense staining of the cytoplasma.