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Partial resistance of ataxin-2-containing olivary and pontine neurons to axotomy-induced degeneration

M.T. Viscomi [a], F. Florenzano [a], S. Amadio [a,b], G. Bernardi [a,b], M. Molinari [a,c,*]

[a] *Experimental Neurorehabilitation Laboratory, Santa Lucia Foundation I.R.C.C.S., Via del Fosso di Fiorano 65, 00143 Rome, Italy*
[b] *Department of Neuroscience, University of Rome "Tor Vergata", Viale Oxford 81, 00133 Rome, Italy*
[c] *Institute of Neurology, Catholic University Medical School, L.go A. Gemelli 8, 00168 Rome, Italy*

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Abstract

Spinocerebellar ataxia type 2 (SCA2) is caused by the expansion of a polyglutamine tract in ataxin-2, the SCA2 gene product. In spite of the identification of the genetic defect and the coded protein, the function of wild-type ataxin-2 has not been clarified. In order to identify the possible resistance of ataxin-2-containing neurons to degeneration, we investigated in this study the distribution and the characteristics of cell reaction to axotomy in ataxin-2-positive olivary and pontine neurons in a model of cerebellar damage represented by hemicerebellectomy. We also performed double immunofluorescence studies of ataxin-2 and purinergic receptors to characterize ataxin-2-positive surviving neurons. The present data demonstrated that after axotomy olivary and pontine ataxin-2-expressing neurons survived longer than the ataxin-2-negative cell population. Cell counting performed in the different olivary subdivisions failed to reveal any topographical prevalence in the distribution of ataxin-2-positive neurons. Therefore, the relative resistance to axotomy appears to be an intrinsic property of the ataxin-2 cell population. In addition, the capacity to modify the pattern of purinergic receptor expression in response to damage was present in only one subset of ataxin-2-positive surviving neurons. These data suggest that ataxin-2 is involved in resistance to degeneration phenomena which may be lost after mutation.

Keywords: Spinocerebellar ataxia type 2; Poly-Q diseases; Purinergic receptors; Inferior olive; Pontine nuclei; Axotomy

1. Introduction

Autosomal dominant cerebellar ataxias are hereditary neurodegenerative disorders, known as spinocerebellar ataxias (SCAs), caused by degeneration of the cerebellum and its afferent and efferent connections. At least six of these SCAs represent a subgroup of the polyglutamine (polyGln) diseases, characterized as slow progressive and late onset neurodegenerative disorders [26]. Huntington's disease and Kennedy disease, dentatorubro-pallido-Luysian and spinal bulbar muscular atrophies are among the polyGln diseases [31]. The latter are caused by mutations in different genes, with no homologies except for the CAG repeat encoding the polyGln tracts. The expansion of the CAG trinucleotide repeat in the mutated gene produces expansion of the polyGln tract in proteins. This triggers a cascade of events, which are toxic only for a subset of neurons [31]. Although genetic defects and coded proteins have been identified, little is known about pathogenetic mechanisms. Thus, various hypotheses have been suggested [18,22–24]. The issue is still controversial and the importance of studies on normal protein function, to better investigate the role of the mutated protein in degeneration, has often been stressed [15,25].

Expanded polyGln truncated tracts are reported to be more toxic than the entire mutated protein. This suggests that other factors besides the expansion of the polyGln tract intervene in determining the course and the features of polyGln diseases [6,30].

* Corresponding author. Tel.: +39 06 51501600; fax: +39 06 51501679.
E-mail address: m.molinari@hsantalucia.it (M. Molinari).

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One of the most intriguing characteristics of expanded polyGln disorders, which supports the involvement of different pathogenetic mechanisms, is the specific regional neuronal degeneration exhibited by each individual disease despite the ubiquitous expression of many polyGln proteins [26]. A clear example of the importance of regional selectivity is the specific and primary degeneration of Purkinje cells in almost all SCAs in spite of differences among the various mutated proteins [31]. The presence of different degrees of secondary degeneration phenomena in precerebellar structures further stresses the importance of factors other than protein mutation.

Spinocerebellar ataxia type 2 (SCA2) is caused by expansion of a polyGln tract in ataxin-2 (ax-2). The clinical features of SCA2 include ataxia and dysarthria, slow saccades, tremor, hypo- or areflexia, peripheral neuropathy and dementia [1]. SCA2 histopathological picture reveals severe primary degeneration of Purkinje neurons followed by conspicuous neuronal loss and gliosis in the inferior olive and pons [11]. Despite widespread ax-2 distribution in neural and non-neural tissues [20], the protein is not expressed by all classes of degenerating neurons [12,9]. Indeed, in the cerebellum ax-2 is expressed by less than 60% of Purkinje neurons in a scattered and uneven distribution [9]. In addition, almost all granule, Golgi and basket cells, but not stellate and Lugaro cells, express ax-2. In SCA2, while almost all Purkinje cells degenerate without any indication of regional prevalence, granule cells, in spite of their ax-2 content, tend to be spared [12]. Similarly, pontine and olivary degeneration occurs irrespective of the ax-2 content. Consequently, it can be inferred that cells in the cerebellar circuits degenerate independently of ax-2 content and that interactions between the mutated protein or its products and specific cellular metabolic pathways may trigger cell death. For instance, Purkinje and olivary neurons present intrinsic differences in their capacity to support axonal regeneration and to survive different insults [4]. These differences have been related to the capacity to express specific proteins, such as growth associated protein-43 (GAP-43) and neuronal nitric oxide synthase (nNOS) [3,27], or to modify the pattern of purinergic receptor subtypes [7]. In order to identify any possible specificities of ax-2-containing neurons in surviving insults, we analyzed the characteristics of cell reaction to axotomy in ax-2-positive precerebellar neurons in a model of cerebellar damage [5,19].

This model, which is represented by ablation of one cerebellar hemisphere, one half of the vermis, subcortical white matter and deep cerebellar nuclei, results not only in target ablation, but also in the transection of incoming axons.

2. Materials and methods

2.1. Animals and surgical procedures

A total of 25 adult male Wistar rats (body weight 200–250 g; Harlan, San Pietro al Natisone, Udine, Italy) were used in this study: 18 for cerebellar lesion (three for each postlesional survival time, plus three at 21 days) and seven unlesioned rats as controls (six for immunohistochemistry and one for western blotting). The experimental protocol used in this study was approved by the Italian Ministry of Health and was in agreement with the guidelines of the European Communities Council Directive of November 24, 1986 (86/609/EEC) for the care and use of laboratory animals. All efforts were made to minimize the number of animals used and their suffering. For surgical procedures, the rats were deeply anesthetized by i.p. injections of sodium pentobarbital (60 mg/kg) and secured in a stereotaxic apparatus. The skin covering the skull was incised and the occipital bone drilled and removed. Subsequently, the dura was incised to expose the cerebellum and the right cerebellar hemisphere was removed by suction. Then, the wound was sutured and the animals were returned to their cages. For histological procedures, groups of three specimens for control and three for experimental purposes for each time point considered (7, 14, 21, 28 and 35 days after the lesion) were deeply anesthetized as described above and perfused transcardially with 250 ml of saline followed by 250 ml of 4% paraformaldehyde in phosphate buffer (PB, 0.1 M pH 7.4).

2.2. Total protein extraction and Western blot analysis

Punching was performed on three different cerebellar cortex regions. The tissues were extracted in RIPA buffer (PBS supplemented with 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.5 μM PMSF, 10 μg/ml leupeptin, all from Sigma, Milan, Italy) and homogenized. They were maintained for one hour on ice and centrifuged at 4 °C at 10,000 × *g* for 10 min. Protein concentration was performed by Bradford colorimetric assay (Biorad, Milan, Italy). Equal amount of total protein from each sample (30 μg) was separated by SDS-PAGE on a 10% polyacrylamide gel and transferred overnight onto a nitrocellulose membrane Hybond™-C extra (Amersham Biosciences, Milan, Italy). The filter was prewetted in 5% non-fat milk in TBS-T (10 mM Tris pH 8, 150 mM NaCl, 0.1% Tween 20) and hybridized for 24 h with monoclonal antiserum for ataxin-2 (1:100; Imochem, Christchurch, New Zealand). The antiserum was immunodetected with an anti-mouse horseradish peroxidase-conjugated antibody (1:2000) and analyzed using ECL chemiluminescence (Amersham Biosciences). Quantification was performed by Kodak Image Station (KDS IS440CF 1.1).

2.3. Histology and immunohistochemistry

Each brain was immediately removed, post-fixed in the same fixative for 2 h and then transferred to 30% sucrose in PB at 4 °C until it sank. Cerebellum and brainstem were cut into three series of 40 μm-thick transverse sections using a freezing microtome and collected in PB. For single immunohistochemistry, one series was processed with a monoclonal