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Morphometric Analysis of Neuronal Changes During Chronic Relapsing Experimental Allergic Encephalomyelitis

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In the present study we used morphometric method to investigate the early changes of hypoglossal nerve neurons of Lewis rats during the first clinical episode of chronic relapsing experimental allergic encephalomyelitis (CREAE). The surface area of the hypoglossal nerve neurons increased statistically significant during the first clinical episode of CREAE in comparison to control rats. These morphometric changes reflect the early degeneration of the neurons and support the notion that neuronal degeneration can begin very early in the pathogenesis of the disease. Our findings provide another evidence of the concept of multiple sclerosis (MS) as a neuronal disease.

Key words: chronic relapsing experimental allergic encephalomyelitis, Lewis rat, hypoglossal nerve, neurons, morphometry.

Introduction

Chronic relapsing experimental allergic encephalomyelitis (CREAE) shares histological, immunological and clinical parameters with multiple sclerosis (MS), a human central nervous system (CNS) demyelinating disease of not elucidated etiology. MS is considered to be prototype of primary demyelinating disease in the CNS in every textbook of neurology. However, in the past few years a considerable body of evidence indicates that neurons are also targets of the disease process [6]. Furthermore, in 2000 Editorial paper of W a x m a n [8] in Archives of Neurology was entitled "Multiple Sclerosis as a Neuronal Disease".

Early neuronal damage in patients with MS has been observed in vivo by magnetic resonance spectroscopy which showed decreased levels of the neuronal specific marker N-acetylaspartate (NAA) in early stages of MS [1]. By assessing central brain NAA in MS patients with a wide range of disability and disease duration De S t e f a n o et al. [1] showed that diffuse cerebral axonal damage begins in the early stage of relapsing-remitting MS and develops more rapidly in the earlier clinical stage of the disease. Pathological studies of F e r g u s o n et al. [2] and T r a p p et al. [7], applying morphological techniques, have provided evidence of axonal injury throughout active MS lesions.

In order to obtain more information concerning the neuronal damage in early MS we performed morphometric investigations on the hypoglossal nerve neurons of Lewis rats during the first clinical episode of CREAE.

Material and Methods

Chronic relapsing experimental allergic encephalomyelitis (CREAE) was induced in Lewis rats by inoculation with purified guinea-pig myelin and complete Freund's adjuvant followed by treatment with low-dose cyclosporin A, as previosly described in detail [9]. Control rats were inoculated and treated as above except that the inoculum did not contain guinea-pig myelin. The animals were weighed and examined daily from the seventh day post-inoculum (DPI) and killed during the first clinical episode of CREAE.

The morphometric studies were performed on paraffin sections (6 μm) stained with Nissl of the medulla oblongata at the level of the hypoglossal nerve nucleus of the experimental animals during the first clinical episode of CREAE as well as of control healthy rats. The surface area of the hypoglossal nerve neurons was measured by using the point-counting method of Автандилов [11] under the magnification of 630. The Student's test was used to determine statistical differences between the rats with CREAE and control animals.

Results

Clinical findings

The majority of the Lewis rats inoculated with myelin and complete Freund's adjuvant and given CsA developed neurological signs commencing 11-16 DPI (first clinical episode). Most of the affected animals recovered fully by 18-27 DPI (first remission).

Morphometric findings

The surface area of hypoglossal nerve neurons of Lewis rats increased significantly during the first clinical episode of CREAE in comparison with the control Lewis rats (Table 1 and 2).

TABLE 1. SURFACE AREA OF THE HYPOGLOSSAL NERVE NUCLEUS NEURONS (in μm^2) OF THE LEWIS RATS WITH CREAE IN COMPARISON WITH CONTROL ANIMALS

RATS WITH CREAE	CONTROL RATS
274.22 ± 7.75	226.15 ± 8.0

TABLE 2. NUMBER OF MEASURED HYPOGLOSSAL NERVE NUCLEUS NEURONS OF THE LEWIS RATS WITH CREAE AND OF CONTROL ANIMALS

RATS WITH CREAE	CONTROL RATS
520	494

Discussion

Our morphometric study revealed a significant increase of the surface area of hypoglossal nerve neurons during the first clinical episode of CREAE. This finding reflects the first sign of primary degeneration of the neuron — the swelling of neuronal cell body. In this investigation using a suitable model of MS [10] we present for the first time data concerning the early degeneration of the neurons of a motor nerve in CREAE. They are in concordance with the results of Hobom et al. [3] on neuronal degeneration of the sensory optic nerve in experimental autoimmune encephalomyelitis (EAE) induced by myelin olygodendrocyte glycoprotein. The retinal ganglion cell death occurred before the onset of clinical symptoms of the disease. Early neuronal dysfunction in EAE has been also revealed in the studies of Nicot et al. [4] on the regulation of gene expression in the Lewis rats lumbar spinal cord during the clinical course of acute EAE.

A degenerative component to MS was always apparent, but was underestimated until recently. It is widely accepted now that the degenerative response is an integral and early component of MS [5]. The fact that neuronal damage occurs very early in the pathogenesis of MS has important implications for therapeutic strategies, which aim at preventing neuronal loss.

In conclusion, we have shown in chronic relapsing EAE, a suitable animal model for the study of MS pathogenesis, using morphometric method, a degeneretion of hypoglossal nerve neurons during the first clinical episode of the disease. This finding, supports the notion that neuronal degeneration can begin very early in MS. It provides another evidence of the concept of MS as a neuronal disease.

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