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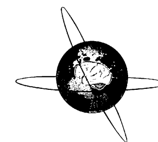

Electrophysiological analysis of a murine model of motoneuron disease

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HIGHLIGHTS

- Electrophysiological tests for peripheral and central conduction are useful to evaluate the early detection and the temporal progression of motor dysfunction in the SOD1 transgenic mouse model.
- Motor evoked potentials revealed early abnormalities in central motor pathways.
- Motor nerve conduction tests revealed very early abnormalities in peripheral motor conduction.

ABSTRACT

Objective: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by loss of motoneurons of the primary motor cortex, the brainstem and the spinal cord, for which there are not effective treatments. Several transgenic mice that mimic motoneuron disease have been used to investigate potential treatments. The objective of this work is to characterize electrophysiologically the $SOD1^{G93A}$ transgenic mouse model of ALS, and to provide useful markers to improve early detection and monitoring of progression of the disease.
Methods: We performed nerve conduction tests, motor unit number estimation (MUNE), H reflex tests and motor evoked potentials (MEPs) in a cohort of transgenic and wild type mice from 4 to 16 weeks of age.
Results: The results revealed dysfunction of spinal motoneurons evidenced by deficits in motor nerve conduction tests starting at 8 weeks of age, earlier in proximal than in distal muscles of the hindlimb. MUNE demonstrated that spinal motoneurons loss muscle innervation and have a deficit in their sprouting capacity. Motor evoked potentials revealed that, coexisting with peripheral deficits, there was a dysfunction of central motor tracts that started also at 8 weeks, indicating progressive dysfunction of upper motoneurons.
Conclusions: These electrophysiological results provide important information about the $SOD1^{G93A}$ mouse model, as they demonstrate by the first time alterations of central motor pathways simultaneously to lower motoneuron dysfunction, well before functional abnormalities appear (by 12 weeks of age).
Significance: The finding of concomitant dysfunction of upper and lower motoneurons contributes to the validation of the $SOD1^{G93A}$ mouse as model of ALS, because this parallel involvement is a diagnostic condition for ALS. Electrophysiological tests can be used as early markers of the disease and to evaluate the potential benefits of new treatments on both upper and lower motoneurons.
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1. Introduction

Motoneuron diseases are progressive neurodegenerative disorders characterized by the loss of upper and/or lower motoneurons (MN). Amyotrophic lateral sclerosis (ALS) is the most common form of MN disease and it differs from others because of the degeneration involves both upper and lower MN. Clinically, ALS is manifested as weakness, muscle atrophy and progressive paralysis and finishes with patient's death 2–5 years after diagnosis (Wijesekera and Leigh 2009; Worms 2001). No satisfactory treatment is presently available for ALS. Despite numerous therapeutic trials have been attempted, the only drug approved by the FDA for the treatment of ALS is riluzole, with limited therapeutic benefits of about 3–4 months survival increase (Ludolph and Jesse 2009). One of the problems of ALS is the delay in diagnosis, mainly because the initial symptoms mimic other spinal cord diseases, neuropathies and neurological syndromes (Kraemer et al., 2010). With the advent of possible new therapies for ALS, there is an increasing need for early diagnosis, because probably early therapies will produce better results.

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The development of transgenic animal models carrying genetic mutations described in familiar ALS cases has facilitated the study of ALS. The most widely used is a transgenic mouse with a glycine to alanine conversion at the 93rd codon of the SOD1 gene in high copy number ($SOD1^{G93A}$) (Ripps et al., 1995). These mice develop a rapidly progressive motoneuron degeneration, which leads to locomotor deficits starting at 12–13 weeks and ending up with hindlimb paralysis at 16 weeks and death around 17–19 weeks of age (Gurney et al., 1994; Miana-Mena et al., 2005, Turner and Talbot 2008). This phenotype recapitulates several clinical and histopathological features of both familial and sporadic forms of the human disease (Ripps et al., 1995). Although animal models carrying SOD1 mutations have been developed based on familial cases of ALS and their validity has been questioned, especially after the development of new models based on mutations in the TDP-43 protein (Wegorzewska et al., 2009), it has been recently found that alterations of SOD1 protein are also related to sporadic ALS cases (Bosco et al., 2010), thus, increasing the interest in the study of these transgenic animals.

Electrophysiological tests are fundamental for diagnosis and progression monitoring of patients suffering MN diseases (Mitsumoto et al., 2007; de Carvalho et al., 2008; Wijesekera and Leigh 2009; Krarup 2010). Several authors have used these techniques on the $SOD1^{G93A}$ model but, in most cases, only focusing on the analysis of lower MN function (Kennel et al., 1996; Azzouz et al., 1997; Shefner et al., 2006) and on several occasions using methods that do not allow a time follow-up of the same animal (Hegedus et al., 2007, 2008). The objective of this work is to provide a detailed electrophysiological characterization of the $SOD1^{G93A}$ transgenic mouse model of ALS. Lower and upper MN function was evaluated from early pre-symptomatic (4 weeks) to end stage of the disease (16 weeks) by means of nerve conduction and evoked potential tests. The advantages of electrophysiological tests compared to behavioral and histological methods are established for the early detection and evaluation of disease progression in this animal model. We have previously used the electrophysiology tests to assess peripheral nerve function in neuropathic diseases (Verdú et al., 1999; Bruna et al., 2010) and after nerve trauma (Navarro et al., 1994; Udina et al., 2003) in small laboratory animals, showing that these methods can reliably help in the early detection and quantitation of loss or recovery of motor and sensory functions (Navarro and Udina 2009).

2. Materials and methods

2.1. Transgenic mice

Transgenic mice with the G93A human SOD1 mutation (B6SJL-Tg[SOD1-G93A]1Gur) were obtained from The Jackson Laboratory (Bar Harbor, ME, USA), and provided from the colony maintained at the Animal Service of the Universidad de Zaragoza. Hemizygotes were maintained by breeding $SOD1^{G93A}$ males with female littermates. The offspring was identified by PCR amplification of DNA extracted from the tail tissue. All experimental procedures were approved by the Ethics Committee of the Universitat Autònoma de Barcelona. Rotarod and nerve conduction tests were performed comparing female SOD1 and wild type mice with 25 animals in each group, while motor unit number estimation (MUNE) was performed in subgroups of 10 mice.

2.2. Rotarod test

Rotarod test was performed to evaluate motor coordination, strength and balance of the animals (Miana-Mena et al., 2005), and to establish the onset of symptomatic disease. Mice were placed onto the rod rotating at a constant speed of 14 rpm (rotating cylinder 3.4 cm in diameter). The time for which each animal could remain on the rotating rod was measured. Each animal was given three trials and the longest latency without falling was recorded; 180 s was chosen as the arbitrary cut-off time. Rotarod was tested weekly from 4 to 16 weeks of age.

2.3. Nerve conduction tests

For motor nerve conduction tests, the sciatic nerve was stimulated percutaneously by means of single pulses of 0.02 ms duration (Grass S88) delivered through a pair of needle electrodes placed at the sciatic notch. The compound muscle action potential (CMAP, M wave) and the reflex H wave were recorded from the tibialis anterior (TA) and the plantar (interossei) muscles with microneedle electrodes (Navarro et al., 1994; Udina et al., 2003; Navarro and Udina 2009). The amplitude of the maximal M and H waves were measured, and the H/M amplitude ratio was calculated for assessment of spinal reflex function (Valero-Cabré and Navarro 2001). For evaluation of the motor central pathways, motor evoked potentials (MEP) were recorded from the TA and plantar muscles in response to transcranial electrical stimulation of the motor cortex by single rectangular pulses of 0.1 ms duration, delivered through needle electrodes inserted subcutaneously, the cathode over the skull overlaying the sensorimotor cortex and the anode at the nose (Garcia-Alias et al., 2003). For sensory nerve conduction tests, the recording electrodes were placed near the digital nerves of the fourth toe to record the compound sensory nerve action potential (CNAP) following stimulation of the sciatic nerve as above.

All potentials were amplified and displayed on a digital oscilloscope (Tektronix 450S) at settings appropriate to measure the amplitude from baseline to the maximal negative peak and the latency from stimulus to the onset of the first negative deflection, to the maximal negative peak and to the end of the wave. To ensure reproducibility, the recording needles were placed under microscope to secure the same placement on all animals guided by anatomical landmarks. During the tests, the mice body temperature was kept constant between 34–36 °C by means of a thermostated heating pad.

2.4. Motor unit number estimation

For motor unit number estimation (MUNE) a subset of 10 female SOD1 mice and their respective wild type littermates were used. The setting was the same as for the motor nerve conduction tests. The protocol used consisted in the incremental technique (Shefner et al., 2002; Lago et al., 2007). Starting from subthreshold intensity, the sciatic nerve was stimulated with single pulses of gradually increased intensity until the first response appeared, representing the first motor unit recruited. With the next stimuli, quantal increases in the response were recorded. Increments >50 μV were considered like the recruitment of an additional motor unit. The amplitude of a single motor unit was calculated as the mean of ~15 consistent increases. The estimated number of motor units results from the equation: MUNE = CMAP maximal amplitude/mean amplitude of single motor unit action potentials.

Inherent to this technique is a problem called "alternation", in which a difference in the amplitude of the CMAP could be due to a different combination of already recruited motor units rather than to the recruitment of a new motor unit. The influence of alternation on the MUNE causes the number of motor units to be overestimated (Arasaki et al., 1997). In order to minimize the effect of the "alternation" phenomenon only the increases >50 μV in the amplitude of the CMAP were considered as representing recruitment of a new motor unit. Moreover, MUNE of SOD1 animals is always referred to age matched control mice, evaluated in parallel,

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