Neuroscience 241 (2013) 239–249

PERIPHERAL MOTOR AXONS OF SOD1^{G127X} MUTANT MICE ARE SUSCEPTIBLE TO ACTIVITY-DEPENDENT DEGENERATION

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Abstract—Motor neuron disorders may be associated with mitochondrial dysfunction, and repetitive electrical impulse conduction during energy restriction has been found to cause neuronal degeneration. The aim of this study was to investigate the vulnerability of motor axons of a presymptomatic late-onset, fast-progression SOD1^{G127X} mouse model of amyotrophic lateral sclerosis to long-lasting, high-frequency repetitive activity. Tibial nerves were stimulated at ankle in 7 to 8-month-old SOD1^{G127X} mice when they were clinically indistinguishable from wild-type (WT) mice. The evoked compound muscle action potentials and ascending compound nerve action potentials were recorded from plantar muscles and from the sciatic nerve, respectively. Repetitive stimulation (RS) was carried out in interrupted trains of 200-Hz for 3 h. During the stimulation-sequence there was progressive conduction failure in WT and, to a lesser extent, in the SOD1^{G127X}. By contrast, 3 days after RS the electrophysiological responses remained reduced in the SOD1^{G127X} but recovered completely in WT. Additionally, morphological studies showed Wallerian degeneration in the disease model. Nerve excitability testing by ''threshold-tracking'' showed that axons recovering from RS had changes in excitability suggestive of membrane hyperpolarization, which was smaller in the SOD1^{G127X} than in WT. Our data provide proof-of-principle that SOD1^{G127X} axons are less resistant to activity-induced changes in ion-concentrations. It is possible that in SOD1^{G127X} there is inadequate energy-dependent Na^+/K^+ pumping, which may lead to a lethal Na^+ overload. © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: nerve activity, ion channels, excitability, node of Ranvier, internode, motor neuron disease.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder caused by degeneration of motor neurons in the central and peripheral nervous system, which leads to progressive weakness and fatal respiratory failure (Brown, 1995). Besides motor neuron degeneration, ALS is associated with general defects in energy metabolism, including weight loss, hypermetabolism, and hyperlipidemia (Dupuis et al., 2011). Furthermore, accumulating evidence suggests that mitochondrial dysfunction occurs early in both sporadic and familial ALS (Cozzolino and Carri, 2012; Cozzolino et al., 2012). To which extent mitochondrial dysfunction and deficient energy metabolism have a pathogenic contribution to motor neuron degeneration in ALS remains poorly understood.

Cu/Zn superoxide dismutase (SOD1) gene is mutated in 20% of the familial ALS cases (Rosen et al., 1993). Transgenic SOD1 mice were found to develop a motor neuron disease phenotypically similar to ALS (Rosen et al., 1993; Gurney et al., 1994) due to a toxic gain-of-function rather than loss of SOD1 activity (Reaume et al., 1996; Andersen et al., 1997).

In the most explored SOD1 rodent model, the SOD1^{G93A} mouse, it was recognized that the initial pathogenic process may involve the distal axon rather than the motor neuron cell body (Fischer et al., 2004) leading to the hypothesis that neuronal degeneration in ALS may be a 'dying-back' phenomenon (Dadon-Nachum et al., 2011). Different mechanisms have been proposed to account for axonal degeneration including impaired axonal transport of mitochondria (Zhang et al., 1997). Recent studies suggested, however, that deficits of axonal transport may not directly lead to degeneration in mouse models of ALS (Marinkovic et al., 2012), thus leaving the relationship between transport of mitochondria and neuronal degeneration uncertain.

In response to Na^+ entry during an action potential, the energy-dependent Na^+/K^+ pumps restore ionic equilibrium (Skou, 1965). In conditions of mitochondrial energy restriction, strenuous repetitive electrical nerve stimulation can induce conduction failure and even trigger axonal degeneration in normal rats (Kapoor et al., 2003) and mice (Alvarez et al., 2008). While Na^+ ions are not toxic per se, insufficient energy-dependent Na^+ pumping during impulse conduction can lead to accumulation of Na^+ that reverses the Na^+/Ca^{++} exchanger (Stys et al., 1991; Tatsumi and Katayama, 1995) triggering the Ca^{++} degeneration cascade (Smith and Hall, 2001; Waxman, 2005) in peripheral axons.

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Abbreviations: ALS, amyotrophic lateral sclerosis; CMAP, compound muscle action potential; CNAP, compound nerve action potential; RS, repetitive stimulation; SOD1, Cu/Zn superoxide dismutase; PBS, phosphate buffered saline; WT, wild-type.

0306-4522/13 $36.00 © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuroscience.2013.03.017

Although it was reported that long-lasting electrical hyper-stimulation of peripheral nerve at the motor endpoint hastened motor neuron loss in SOD1^{G93A} rats (Lepore et al., 2010), the role of "Na^+-mediated axonal degeneration" (Bechtold and Smith, 2005) was not addressed. The aim of our study was to investigate the acute susceptibility of peripheral motor axons to activity-dependent degeneration in transgenic mice expressing the G127insTGGG (G127X) mutant human SOD1 (Jonsson et al., 2004), referred to hereafter as SOD1^{G127X}. At rest, presymptomatic SOD1^{G127X} motor axons are slightly depolarized, consistent with deficient energy-dependent Na^+/K^+ pumping (Moldovan et al., 2012) similar to early symptomatic SOD1^{G93A} mice (Boërio et al., 2010). Nevertheless, the SOD1^{G127X} mouse has a longer presymptomatic phase with minimal motor axon loss than other SOD1 models, which makes it particularly suitable to investigate procedures affecting motor axon survival (Jonsson et al., 2004).

EXPERIMENTAL PROCEDURES

Animals and experimental design

Mice transgenically expressing G127X mutant human SOD1 were backcrossed on C57BL/6J mice for more than 25 generations (Jonsson et al., 2004). Homozygotes from the original line 716 overexpressing 19 copies of a human SOD1^{G127X} (Jonsson et al., 2004), were bred at our institution in Copenhagen.

Experiments included eight SOD1^{G127X} mice 7–8 months old. For controls we used eight age-matched C57BL/6J mice referred to hereafter as wild-type (WT). The mean survival duration for the SOD1^{G127X} is 8 months (Moldovan et al., 2012), and the presymptomatic mice included in this study were clinically normal. Mice of either gender were used, as gender was not expected to play a decisive role in motor neuron loss in SOD1 mutants (Hegedus et al., 2009).

The G127X mutation, first found in a Danish ALS pedigree (Andersen et al., 1997), results in the synthesis of a SOD1 with a long C-terminal truncation. This defective mutant SOD1 lacks SOD1 activity and is rapidly degraded resulting in very low levels in the spinal cord (Jonsson et al., 2004). Compared with most other ALS models, in which enzymatically active mutant SOD1s are present at high levels, the risk of overexpression artifacts not related to ALS pathogenesis was reduced (Jonsson et al., 2006). Since the transgenic SOD1^{G127X} has no enzymatic activity (Jonsson et al., 2004) we did not consider it relevant to use transgenic WT mice with enzymatically active normal human SOD1 as additional controls (Forsberg et al., 2010, 2011).

Electrophysiological investigations were carried out under anesthesia using a 1:1 mixture of Hypnorm/Midazolam (5 mg/ml). A volume of 0.1 ml/10 g from the mixture was injected subcutaneously for induction, and then maintained with 50% hourly for up to 4 h as needed.

Following a complete tibial nerve injury, loss of compound muscle action potential (CMAP) occurs within 18 h, whereas morphological signs of Wallerian degeneration are clearly identifiable only after 3 days (Beirowski et al., 2005; Alvarez et al., 2008; Moldovan et al., 2009). Therefore, following the repetitive stimulation (RS), tibial nerve function was investigated electrophysiologically at 1 h, 1 day and 3 days. At the completion of the experiments, tibial nerves at ankle were harvested for histology. The mice were sacrificed by cervical dislocation. Experimental procedures were approved by the Danish National Animal Experiment Committee.

Motor performance

Estimation of motor performance was accomplished using an Ugo Basile 7650 accelerating RotaRod (Ugo Basile Srl, 21025 Comerio, VA, Italy) as previously described in detail (Moldovan et al., 2012). Briefly, the mice were placed on an accelerating rod from 4 to 40 rpm over a period of 300 s. Any mice remaining on the apparatus after 600 s were removed. Each determination represents the longest endurance time of three consecutive measurements at ~10-min intervals.

Electrophysiological setup

Through a minimal incision under a stereomicroscope (MZ6, Leica Microsystems, Wetzlar, Germany) the right sciatic nerve was exposed and freed from surrounding connective tissue and proximal deep branches. The mouse was fixed in a stereotaxic frame (dual manipulator with mouse adaptor 51624, Stoelting Wood Dale, IL, USA) on a temperature controlled pad (HB 101/2, LSI Letica Barcelona, Spain) set to 37 °C as previously described in detail (Moldovan and Krarup, 2006). The right leg was placed on a piece of hydrophobic cotton to reduce the stimulus artifact.

For stimulation and recording we used custom made platinum electrodes. Stimulation of the right tibial nerve was carried out at ankle using needle electrodes inserted perpendicularly through the skin close to the tibial nerve. The descending CMAP from the plantar muscles was recorded using needle electrodes inserted into the foot ~0.5 cm apart. The exposed sciatic nerve was lifted on a hook electrode referenced to a needle electrode placed subcutaneously over the abdomen (Alvarez et al., 2008) to record the ascending compound nerve action potential (CNAP). A ground electrode was inserted subcutaneously in the left thigh.

Electrical stimuli were delivered from a constant current stimulator (DS4, Digitimer Co. Ltd, London, UK). The evoked responses were amplified (10C02, Dantec Copenhagen, Denmark) at 10 Hz–10 kHz for CMAPs and 200 Hz–6 kHz for CNAPs. Amplitudes were evaluated peak-to-peak and latencies were measured at the peak of the first positive phase (CNAP) and at the first deflection from baseline (CMAP).

Prolonged repetitive activity

The stimulation protocol has previously been described in detail (Alvarez et al., 2008). Stimulation and recording were controlled by MTRACK (Moldovan and Krarup, 2006) developed in MATLAB (Mathworks Inc., Natick, MA, USA). Baseline recordings were carried out at 1.3-Hz frequency in phosphate buffered saline (PBS). To ensure supramaximal stimulation, the stimulation current was determined as 1.5× the largest current (~1 mA) that elicited a maximal CMAP and CNAP. Eight-minute trains of stimuli at 200 Hz alternating with 2-min trains of 1.3 Hz were delivered for 3 h. During the 200-Hz trains, the sciatic nerve was immersed in PBS. During the 2-min trains the CNAPs and the CMAPs were recorded to ascertain recovery of the responses.

Multiple measures of tibial nerve excitability

Excitability studies are used to investigate the membrane function of the myelinated motor axon population at the site of stimulation by tracking the evoked CMAP, and they provide information about the membrane potential and the activity of ion channels and energy-dependent ion exchange pumps. Peripheral nerve excitability was assessed using QtracS stimulation software (© Institute of Neurology, London, UK) using the TRONDH multiple excitability protocol designed for