Neurobiology of Disease 54 (2013) 116–126

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Embryonic alteration of motoneuronal morphology induces hyperexcitability in the mouse model of amyotrophic lateral sclerosis

Elodie Martin, William Cazenave, Daniel Cattaert, Pascal Branchereau *

Univ. Bordeaux, INCIA, UMR 5287, F-33400 Talence, France
CNRS, INCIA, UMR 5287, F-33400 Talence, France

ARTICLE INFO

Article history:
Received 21 November 2012
Revised 31 January 2013
Accepted 22 February 2013
Available online 4 March 2013

Keywords:
Amyotrophic lateral sclerosis
SOD1 mouse
Embryonic spinal cord
Motoneuron
Hyperexcitability
Morphology
Electrophysiology
3D reconstruction
Computer simulation

ABSTRACT

Although amyotrophic lateral sclerosis (ALS) is an age-dependent fatal neurodegenerative disease in which upper and lower motoneurons (MNs) are targeted for death in adults, increasing lines of evidence indicate that MNs display physiological and morphological abnormalities during postnatal development, long before disease onset. Here, using transgenic mice overexpressing the G93A mutation of the human Cu/Zn superoxide dismutase gene (*SOD1*), we show that SOD1^{G93A} embryonic lumbar E17.5 MNs already expressed abnormal morphometric parameters, including a deep reduction of their terminal segments length. Whole-cell patch-clamp recordings from acute spinal cord preparations were made to characterize functional changes in neuronal activity. SOD1^{G93A} E17.5 MNs displayed hyperexcitability compared to wild-type MNs. Finally, we performed realistic simulations in order to correlate morphometric and electrophysiological changes observed in embryonic SOD1^{G93A} MNs. We found that the reduced dendritic elongation mainly accounted for the hyperexcitability observed in SOD1^{G93A} MNs. Altogether, our results emphasize the remarkable early onset of abnormal neural activity in the commonly used animal model for ALS, and suggest that embryonic morphological changes are the primary compensatory mechanisms, the physiological adjustments being only secondary to morphological alterations.

Introduction

Amyotrophic lateral sclerosis (ALS) also known as Lou Gehrig's disease is an age-dependent fatal paralytic disease that results from the degeneration of upper descending cortical neurons and lower motoneurons (MNs) located in the brainstem and spinal cord (Boillee et al., 2006). ALS is the third neurodegenerative cause of adult death after Alzheimer's disease and Parkinson's disease, with an incidence of 1–2 per 100,000 humans that peaks in the sixth decade of life, and a lifetime risk at about 1 in 1000 (Boillee et al., 2006; Pasinelli and Brown, 2006). Usually, respiratory paralysis leads to death 2 to 5 years after onset of the disease. To date, causes of ALS remain elusive. Many hypothesis have been explored to explain ALS pathogenesis including mitochondrial dysfunction (Higgins et al., 2003), axonal transport (De Vos et al., 2007), protein misfolding and endoplasmic reticulum stress (Atkin et al., 2006), and glutamate excitotoxicity (Van Den Bosch et al., 2006).

The majority of the ALS cases are sporadic with unknown etiology, but ~10% of ALS patients suffer from a familial form of ALS. If mutations in TAR DNA-binding protein (TDP-43, encoded by the *TARDBP* gene) (Kabashi et al., 2008; Sreedharan et al., 2008), fused in sarcoma (encoded by the *FUS/TLS* gene) (Kwiatkowski et al., 2009; Vance et al., 2009) and Cu/Zn superoxide dismutase gene (*SOD1*) that encodes an antioxidant enzyme (Deng et al., 1993; Rosen et al., 1993) account for approximately 30% of classical inherited ALS, *SOD1* mutations represent the most common cases. More than 100 mutations of the *SOD1* gene are known in ALS patients (Boillee et al., 2006).

Among these mutations, SOD1^{G93A}, in which glycine is substituted by alanine at residue 93, has been particularly studied, and a transgenic mouse line overexpressing this mutant human SOD1^{G93A} has been produced. Interestingly, SOD1^{G93A} mouse expresses phenotypic and pathological symptoms resembling ALS in humans (Cleveland and Rothstein, 2001; Gurney et al., 1994).

Because a phenotype becomes apparent at quite late stages in the SOD1^{G93A} mouse ALS model, i.e. abnormal gait at P50 (Wooley et al., 2005), most studies performed on this mouse line have focused at late post-natal asymptomatic stages (Kanning et al., 2010). However, in spite of an absence of clear phenotype, it is possible that early changes accompanied by compensatory mechanisms occur in SOD1 mice as suggested by studies performed during the first two post-natal weeks. Changes include alteration of MN morphology and excitability (Amendola and Durand, 2008; Bories et al., 2007; Pambo-Pambo et al., 2009) as well as transient delays in development of gross locomotor abilities (van Zundert et al., 2008). In addition, data collected from

* Corresponding author at: Institut de Neurosciences Cognitives et Intégratives d'Aquitaine (INCIA), Université de Bordeaux et CNRS - UMR 5287, Av. des Facultés, 4ème étage Est, 33405 Talence, France. Fax: +33 5 40 00 25 61.
E-mail address: pascal.branchereau@u-bordeaux1.fr (P. Branchereau).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.nbd.2013.02.011

cultured SOD1^{G93A} embryonic (E13.5) MNs demonstrate defects in axonal transport (De Vos et al., 2007; Kieran et al., 2005), enhanced sensitivity of MNs to Fas- or NO-triggered cell death (Raoul et al., 2002) and impaired mitochondrial dynamics (Magrane et al., 2012), highlighting early developmental pathological features in the SOD1 rodent model (Kanning et al., 2010).

There is increasing evidence that the toxicity of mutant SOD1 is linked to its propensity to misfold and to aggregate (Liu et al., 2012). Based on the use of an antibody (C4F6) specifically recognizing a "toxic" form of the mutant SOD1 protein, a recent study (Brotherton et al., 2012) suggests that a subset of SOD1 protein (misfolded form) accumulates in MNs when they become sick. This study also indicates that C4F6 staining is observed throughout the disease course (presymptomatic through end stage), and even shows staining in certain MNs at early stages (P15). This indicates that toxic form of mutated SOD1 may be present at perinatal stages.

In the present study, by analyzing SOD1^{G93A} MNs at the late embryonic stage E17.5 when they undergo major developmental changes and when motor spinal networks become functional (Branchereau et al., 2000), we show that SOD1^{G93A} MNs are hyperexcitable and exhibit a reduced dendritic arborization. Interestingly, using computer simulations, we show that the reduced growth of embryonic SOD1^{G93A} MNs accounts, for the most part, for their hyperexcitability.

Material and methods

Animals and spinal cord preparation

All procedures were carried out in accordance with the French Directive (87 / 148, Ministère de l'Agriculture et de la Pêche), the European Communities Council Directive (86 / 609 / EEC), and local French legislation for care and use of laboratory animals. B6SJL-TgN(SOD1-G93A)/1Gur/J mice expressing the human G93A Cu/Zn superoxide dismutase (SOD1) mutation (glycine substituted for alanine at position 93) were obtained from The Jackson Laboratory (http://jaxmice.jax.org/strain/002726.html). These transgenic mice had an abbreviated life span (50% survive at 128.9 ± 9.1 days). Mice were bred in our animal facilities. Hemizygous B6SJL-TgN(SOD1-G93A)/1Gur/J were maintained by crossing transgene-positive male mice with B6SJL F1 hybrid females (Janvier SAS, France). All experiments were carried out on embryos at Embryonic (E) stage 17.5 (E17.5), the embryonic day 0.5 corresponding to the day following the mating night. Embryos were surgically removed from pregnant mice previously killed by cervical dislocation. Embryos were decapitated and the brainstem-spinal cord preparation was dissected out. The spinal cord was dorsally opened and meninges were removed. The preparation was placed in a recording chamber, maintained opened under a nylon mesh, and superfused (~1.5 mL·min^{-1}) with an artificial cerebrospinal fluid (aCSF) containing (in mM): 111.5 NaCl, 6 KCl, 2 $CaCl_2$-$2H_2O$, 1 $MgCl_2$-$6H_2O$, 25 $NaHCO_3$, 1 NaH_2PO4-H_20, 11 D-Glucose, pH 7.4 (296 mosmol·l^{-1}), oxygenated with a 95% O_2 and 5% CO_2 mixture. All experiments were carried out at constant room temperature (~23 °C). All experiments were blindly performed. Indeed, each embryo was genotyped after electrophysiological experiments by standard PCR from tail biopsies using established primers and protocol as stated by The Jackson Laboratory (http://jaxmice.jax.org/protocolsdb/f?p=116:2:3006631337136840::NO:2:P2_MASTER_PROTOCOL_ID,P2_JRS_CODE:523,002726).

Whole-cell patch-clamp recordings and motoneuron injection

We performed whole-cell patch-clamp recordings from MNs identified according to their morphological features (pear-shaped large cell body), their disposition in ventral column (Delpy et al., 2008) and their input resistance (5 to 10 times lower than interneurons). An Olympus BX51 WI microscope equipped with differential interference contrast (DIC) and a CCD camera (SPOT RT-SE6, Diagnostic Instruments, Sterling Heights, MI, USA) was used to visualize MNs. Patchclamp electrodes were constructed from thin-walled single-filamented borosilicate glass (1.5 mm outer diameter, Harvard Apparatus, Les Ulis, France) using a two-stage vertical microelectrode puller (PP-830, Narishige, Tokyo, Japan). Patch electrodes resistances ranging from 3 to 5 MΩ were filled with the following intracellular medium (in mM): 130 potassium gluconate, 10 Hepes, 10 EGTA, 5 NaCl, 2 MgATP and 1 $CaCl_2$, pH 7.4 (296 mosmol·l^{-1}). Motorized micromanipulators (Luigs & Neumann, Ratingen, Germany) were used to position patch-clamp electrode on a visually identified MN.

For each brainstem-spinal cord preparation, we recorded only 1 to 3 MNs. Hence, 10 SOD1^{G93A} MNs (7 embryos) and 32 WT MNs (15 embryos) were analyzed from a total of 12 pregnant mice. All WT and SOD1^{G93A} embryos were harvested from B6SJL F1 hybrid females crossed with SOD1^{G93A} male mice. Six SOD1^{G93A} and 6 WT MNs were stained during whole-cell recordings with pipettes containing Neurobiotin (0.4%, CliniSciences, Montrouge, France) diluted in the intracellular medium (see above). All recordings were made with an Axon Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA). Data were low-pass filtered (2 kHz) and acquired at 20 kHz on a computer *via* an analog-to-digital converter (Digidata 1322A, Molecular Devices) and a data acquisition software (Clampex 10.3, Molecular Devices). Measurements were corrected for liquid junction potentials (13.7 mV, calculated using the Clampex tool).

Membrane properties and data analysis

All data analysis were performed off-line using Clampfit 9.0 (Axon Instruments). MN input resistance was determined in current-clamp mode by injecting current pulses of different intensity (I) and polarity (duration: 1.5 s) through the recording pipette. MN voltage (V) responses were measured and V/I curves were constructed. Input resistance R_{in} was measured as the slope of the linear portion of the V/I relationship. For positive pulses evoking action potentials (APs), the instantaneous frequency (F) was measured and plotted against the current intensity (I) in order to build F/I curves. The MN capacitance c_m was collected immediately after establishing whole-cell patch-clamp. The rheobase current was defined as the minimal constant current intensity necessary to evoke an AP. Spike characteristics (time to peak, half-width, amplitude and after-hyperpolarisation — AHP) were determined on the first evoked AP during over-threshold current injection.

Immunohistochemistry

After the recording session, during which MNs were injected with Neurobiotin, the entire brainstem-spinal cord preparation was fixed in 4% paraformaldehyde (PFA) for 2 h at room temperature. It was then rinsed three times with 0.1 M Phosphate Buffer Saline (PBS), and incubated with streptavidin-Cy3 (1:400, Invitrogen) overnight at 4 °C in 0.1 M PBS containing 0.2% bovine serum albumin (BSA, Sigma, St Louis, MO, USA) and 0.1% Triton X-100 (Sigma). To confirm that injected neurons were MNs, a mouse monoclonal antibody directed against Islet-1/2 (1/100, Developmental Studies Hybridoma Bank), a marker of developing MNs (Ericson et al., 1992), was added to the incubating medium (Fig. 1). Then the preparation was incubated with Alexa Fluor488 goat anti-mouse IgG(H + L) (1/400, Invitrogen SARL, Cergy Pontoise, France) for 2 h, at room temperature, abundantly rinsed in 0.1 M PBS, and finally mounted with anti-fade reagent (Fluoromount, Electron Microscopy Sciences). Fig. 1 illustrates a representative MN immunopositive for Islet-1/2.

Confocal microscopy

Preparations were imaged with a BX51 Olympus Fluoview 500 confocal microscope. Serial optical sections of 0.2 μm were obtained