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HYPEREXCITABILITY IN SYNAPTIC AND FIRING ACTIVITIES OF SPINAL MOTONEURONS IN AN ADULT MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Abstract—Hyperexcitability is hypothesized to contribute to the degeneration of spinal motoneurons (MNs) in amyotrophic lateral sclerosis (ALS). Studies, thus far, have not linked hyperexcitability to the intrinsic properties of MNs in the adult ALS mouse model with the G93A-mutated SOD1 protein (mSOD1^{G93A}). In this study, we obtained two types of measurements: ventral root recordings to assess motor output and intracellular recordings to assess synaptic properties of individual MNs. All studies were carried out in an *in vitro* preparation of the sacral spinal cords of mSOD1^{G93A} mice and their non-transgenic (NT) littermates, both in the age range of 50–90 days. Ventral root recordings revealed that maximum compound action potentials (coAPs) evoked by a short-train stimulation of corresponding dorsal roots were similar between the two types of mice. Although the progressive depression of coAPs was present during the train stimulation in all recordings, the coAP depression in mSOD1^{G93A} mice was to a lesser extent, which suggests an increased firing tendency in mSOD1^{G93A} MNs. Intracellular recordings showed no changes in fast excitatory postsynaptic potentials (EPSPs) in mSOD1^{G93A} MNs. However, recording did show that oscillating EPSPs (oEPSPs) were induced by poly-EPSPs at a higher frequency and by less-intense electrical stimulation in mSOD1^{G93A} MNs. These oEPSPs were dependent upon the activities of spinal network and N-methyl-D-aspartate receptors (NMDARs), and were subjected to riluzole modulation. Taken together, these findings revealed abnormal electrophysiology in mSOD1^{G93A} MNs that could underlie ALS excitotoxicity.

Key words: amyotrophic lateral sclerosis, spinal motoneurons, hyperexcitability, synaptic transmission, short-term plasticity, NMDA receptor.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a massive loss of motoneurons (MNs). Several pathogenic mechanisms, such as glutamate-induced excitotoxicity, are recognized in this disease (Van Den Bosch et al., 2006; Ferraiuolo et al., 2011). Excitotoxicity could emerge either from changes in intrinsic electrical properties of MNs or from an increase in excitatory synaptic properties. Remarkably, the intrinsic excitability of MNs increases at a very early stage in mSOD1^{G93A} mice (Pieri et al., 2003; Kuo et al., 2004, 2005) and even in the embryonic state (Martin et al., 2013). Once an animal is born, however, this intrinsic excitability tends to subside to near-normal levels. In mSOD1^{G93A} neonates, persistent sodium (Na^+) currents continue to increase with age, but are offset by increases in cell input conductance (Quinlan et al., 2011). Consequently, net excitability stays normal. However, there is some variation in MN excitability among mice of different SOD1 mutations (Amendola et al., 2007). As an animal matures, normal excitability prevails, and hypo-excitability in firing properties begins to develop (Delestree et al., 2014). These findings suggest that excitotoxicity in adult mSOD1^{G93A} mice may primarily occur via synaptic activity.

Indeed, several lines of evidence support the involvement of synaptic mechanisms in excitotoxicity. Extracellular glutamate can be enhanced by the loss of glutamate transporters in glial cells, which has been found in both ALS patients (Rothstein et al., 1995) and mouse models (Bruijn et al., 1997; Bendotti et al., 2001). An increase in synaptic transmission was reported

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Abbreviations: ACSF, artificial cerebrospinal fluid; ALS, amyotrophic lateral sclerosis; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; AP, action potential; APV, (2*R*)-amino-5-phosphonovaleric acid; Ca^{2+}, calcium; CICR, calcium-induced calcium release; coAP, compound action potential; DNQX, 6,7-Dinitroquinoxaline-2,3-dione; EPSP, excitatory postsynaptic potential; ER, endoplasmic reticulum; IPSP, inhibitory postsynaptic potential; I–V, current–voltage; mACSF, modified artificial cerebrospinal fluid; MN, motoneuron; mSOD1^{G93A}, G93A mutated SOD1 protein; MT, mitochondria; Na^+, sodium; NMDAR, N-methyl-D-aspartate receptors; NT, non-transgenic; oEPSP, oscillating excitatory postsynaptic potential; sSTD, system short-term depression; STP, short-term plasticity; VGCC, voltage-gated calcium channel.

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in hypoglossal MNs of neonatal mSOD1^{G93A} mice and in cortical MNs of young adult mSOD1^{G93A} mice (van Zundert et al., 2008; Saba et al., 2015). A disinhibitory synaptic mechanism may be involved in ALS due to breakdowns in glycinergic transmission (Chang and Martin, 2009; McGown et al., 2013) and the synaptic connections between MNs and Renshaw cells (Wootz et al., 2013). For instance, data from an *in vitro* sacral cord preparation of an mSOD1^{G93A} mouse, exhibiting overt symptoms in late ALS, confirmed an increased tendency for spontaneous motor output due to increased excitatory synaptic inputs to mSOD1^{G93A} MNs (Jiang et al., 2009). In MNs, inefficient RNA editing of the GluR2 mRNA is found in both ALS patients and mSOD1^{G93A} mice (Tortarolo et al., 2006; Kwak et al., 2010), which results in increased calcium (Ca^{2+}) permeability of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs). NMDARs also play an important role in ALS excitotoxicity based on their Ca^{2+} permeability and altered levels of their endogenous co-agonists/inhibitors (Tokuda et al., 2007; Paul and de Belleroche, 2014, 2015). Although these findings imply that ALS hyperexcitability is imparted by synaptic properties, detailed measurements of these changes in these adult MNs are lacking.

In this study, we applied two recording techniques to investigate possible malfunctions involving synaptic activities in adult mSOD1^{G93A} MNs. With ventral root recordings to analyze overall motor output, we found enhancements in repetitive motor firings during train stimuli. Conversely, in intracellular recordings, we observed normal fast excitatory postsynaptic potentials (EPSPs) and hyperexcitable oscillating EPSPs (oEPSPs) in mSOD1^{G93A} MNs. Such findings further suggest the involvement of several electrical activities in ALS excitotoxicity.

EXPERIMENTAL PROCEDURES

Transgenic mSOD1^{G93A} mice with B6SJL background and their non-transgenic (NT) littermates were bred and maintained at the Center for Comparative Medicine of Northwestern University Medical Center. All experimental procedures were reviewed and approved by the University Animal Research Committee and were in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. Excitability of spinal MNs was studied at both system and single neuronal levels. Bipolar, stainless-steel wire electrodes were used to investigate motor output (i.e. the evoked coAPs) on ventral roots at sacral segments 2–3 (S2–3) while sharp, glass electrodes were used to record synaptic and intrinsic properties of single MNs in the same segments. In total, 61 adult mice of ages between P50 and P90 were tested in the experiments. Of those 61 adult mice, 33 were mSOD1^{G93A} mice, and 28 were NT littermates. The age range was determined by pathological changes in the mSOD1^{G93A} mouse model (Pun et al., 2006; Jiang et al., 2009).

Sacral cord preparation

The spinal cord at S2–3 was chosen in this study because ALS-resistant MNs are not located in this area (Hamson et al., 2002). Furthermore, the response of S2–3 ventral roots can be readily evoked by stimulating the corresponding dorsal roots. The surgical procedure was similar to previous publications (Jiang and Heckman, 2006; Jiang et al., 2009) with some modifications. Briefly, animals were deeply anesthetized with intraperitoneal injections of urethane at 0.18 g/100 g for NT mice and at 0.15 g/100 g for mSOD1^{G93A} mice. Supplemental anesthesia (0.01–0.05 g/100 g) was determined by each animal's response to foot pinching with forceps. After the vertebral column was opened below thoracic region, the spinal cord was immediately superfused with modified artificial cerebrospinal fluid (mACSF) at a flow rate of 5–7 ml/min. The mACSF was composed of the following (in mM): 120 NaCl, 3 KCl, 1 NaH_2PO_4, 5$MgSO_4$, 1 $CaCl_2$, 26 $NaHCO_3$, 10 glucose, and 1 kynurenic acid. Also, the mACSF was adjusted to a pH value of 7.4 by carefully adding NaHCO3 and bubbled with 95% O_2–5% CO_2. The mouse was then decapitated, and the cord was transected at the rostral edge of lumbar enlargement. Dorsal and ventral roots of the caudal region of the cord were cut at their spinal outlets. The transected spinal cord with its attached roots was quickly transferred to a 100-mm Petri dish filled with the mACSF, bubbled with 95%O_2/5% CO_2. After separating the S2–3 ventral roots and the dorsal roots that enter at the S3 segment, the cord was cut at the caudal edge of the lumbar enlargement and transferred to a recording chamber comprised of a 55-mm Petri dish filled with silicone elastomer (Sylgard) at the bottom. The cord was pinned at its rostral/caudal edges and positioned with its ventral side facing up at the center of the plate. Two homemade electrode plates, containing six bipolar, stainless-steel wire electrodes (three on each side), were placed on the lateral sides of the cord (Jiang et al., 2015). Electrodes, at a distal distance of about 1.5 mm from the cord, were mounted on the dorsal and ventral roots, which were covered with a mineral oil/petroleum jelly (2:1) mixture. It should be noted that the quality of extracellular recording depends on both the electrical properties of the electrode plate and the contacts between the electrodes and roots. To ensure quality of the recording, the electrodes were newly made for this study and the same surgery protocol was used. The cord was submerged in ~ 0.2 ml normal artificial cerebrospinal fluid (ACSF), which was circulated at a flow rate of 2.5–3 ml/min and bubbled with 95% O_2/5% CO_2. The normal ACSF was composed of the following (in mM): 126 NaCl, 3 KCl, 1 NaH_2PO_4, 1.5 $MgSO_4$, 2.5 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose. Also, the normal ACSF was bubbled with 95% O_2/5% CO_2 and adjusted to a pH value of 7.4. For the ACSF containing low Ca^{2+} (1 mM), supplemental NaCl was added to compensate osmolality.

Ventral root recording and protocols

Compound action potentials (CoAPs) were produced by stimulating dorsal roots and recording from their corresponding ventral roots. The stimulating pulse was