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Defining the mechanisms that underlie cortical hyperexcitability in amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis [ALS] is a rapidly progressive neurodegenerative disorder of motor neurons, heralded by the development of cortical hyperexcitability. Reduction of short interval intracortical inhibition [SICI] in ALS, a feature linked to the development of cortical hyperexcitability, may be mediated by degeneration of inhibitory circuits or alternatively activation of high threshold excitatory circuits. As such, determining the mechanisms of SICI reduction in ALS has clear diagnostic and therapeutic significance. Consequently, the present study utilized a novel threshold tracking paired-pulse paradigm to determine whether SICI reduction in ALS represented reduced inhibition or excessive excitation. Using a 90 mm circular coil, SICI was assessed at three different conditioning stimulus intensities: 40%, 70% and 90% of resting motor threshold [RMT]. Motor evoked potential responses were recorded over the abductor pollicis brevis muscle. Short interval intracortical inhibition was uniformly reduced across all three levels of conditioning intensities in ALS [40% RMT, ALS -0.6 ± 0.7%, controls 2.0 ± 0.6%, $P<0.01$; 70% RMT, ALS 0.6 ± 2.7%, controls 12.8 ± 2%, $P<0.001$; 90% RMT, ALS -15.9 ± 1.3%, controls 2.2 ± 4.1%, $P<0.01$]. In addition, the resting motor threshold was reduced, while the motor evoked potential amplitude was increased in ALS patients, in keeping with cortical hyperexcitability. These findings establish that SICI reduction in ALS represents degeneration of inhibitory cortical circuits, combined with excessive excitation of high threshold excitatory pathways. Neuroprotective strategies aimed at preserving the integrity of intracortical inhibitory circuits, in addition to antagonizing excitatory cortical circuits, may provide novel therapeutic targets in ALS.

Introduction

Amyotrophic lateral sclerosis [ALS] is a progressive neurodegenerative disorder of the motor neurons that results in progressive paresis of limb, bulbar and respiratory muscles (Rowland and Shneider, 2001). ALS is universally fatal, with a median survival of 3–5 years (Cudkowicz et al., 2004). Although the mechanisms underlying motor neuron degeneration in ALS remain elusive, cortical hyperexcitability has been proposed as a possible mechanism (Bruijn et al., 1997). Linked to cortical hyperexcitability, a "dying forward" hypothesis was proposed whereby corticomotoneurons mediate anterograde degeneration of anterior horn cells via glutamate-mediated excitotoxicity (Eisen et al., 1992). Such a dying forward hypothesis is further supported by improvement in survival of ALS patients treated with riluzole, a central inhibitor of glutamate release (Bensimon et al., 1994; Lacomblez et al., 1996).

Cortical excitability may be identified in vivo using non-invasive transcranial magnetic stimulation [TMS] techniques (Chen et al., 2008). Specifically, the functioning of inhibitory and excitatory intracortical circuits that influence the output from the motor cortex, and thereby regulate cortical excitability, can be investigated by paired-pulse TMS techniques, whereby a subthreshold conditioning stimulus [CS] suppresses a response produced by a subsequent test stimulus. This process has been termed short interval intracortical inhibition [SICI] and is mediated by $GABA_A$ secreting cortical interneurons (Ziemann, 2004a,b).

Cortical hyperexcitability, as heralded by a reduction of SICI, appears to be intrinsic to ALS (Vucic and Kiernan, 2006, 2008; Vucic et al., 2008; Zanette et al., 2004; Ziemann et al., 1997). The development of cortical hyperexcitability was identified as an early feature in ALS that correlated with markers of motor neuron degeneration (Vucic and Kiernan, 2006). Furthermore, in individuals who expressed mutations of the superoxide dismutase-1 gene, cortical hyperexcitability preceded the clinical onset of disease (Vucic et al., 2008). Although these findings provide further support for an excitotoxic process in ALS, the mechanisms underlying reduction of SICI have not been elucidated. Notably, reduction of SICI may represent either a loss of inhibitory intracortical circuits or, alternatively, lowering in threshold of excitatory circuits. While pathological studies have suggested that SICI reduction is predominantly mediated by

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degeneration of inhibitory cortical interneurons (Nihei et al., 1993), the fact that SICI is partially normalized by riluzole in ALS patients suggests a role for activation of excitatory circuits (Stefan et al., 2001). Determining whether SICI reduction represents a loss of inhibition or activation of excitatory circuits would be of critical diagnostic and therapeutic significance.

The relative contributions of inhibitory and excitatory circuitry may be assessed by varying the conditioning stimulus intensity (Vucic et al., 2009). Short interval intracortical inhibition becomes apparent when a conditioning impulse is set to 40% of resting motor threshold [RMT], with maximal SICI occurring when the conditioning intensity is set to 70% of RMT (Kujirai et al., 1993; Vucic et al., 2009). Increasing the conditioning intensity to threshold results in activation of high threshold intracortical excitatory circuits (Kujirai et al., 1993; Vucic et al., 2009). Consequently, to clarify the pathophysiological mechanisms underlying the development of cortical hyperexcitability in ALS, novel paired-pulse threshold tracking techniques were utilized in the present series to address whether reduction of SICI represented a true loss of inhibition or alternatively excessive excitation of intracortical circuitry.

Materials and methods

Studies were undertaken on 15 patients with clinically probable or definite ALS patients [11 male, 4 female; age range, 38–77 years; mean age, 56 years] as defined by the revised El Escorial criteria (Brooks et al., 2000). All patients were clinically staged using the amyotrophic lateral sclerosis functional rating scale-revised [ALSFRS-R] (Cedarbaum et al., 1999) hand strength using the Medical Research Council [MRC] rating scale (Medical Research Council, 1976), forced vital capacity [FVC], sniff nasal inspiratory pressure [SNIP] and upper motor neuron [UMN] score (Turner et al., 2004). Subjects gave written informed consent to the procedures, which had been approved by the South Eastern Sydney Area Health Service Human Research Ethics Committee [Eastern Section].

Peripheral nerve studies

Prior to assessment of cortical excitability with TMS, the median nerve was stimulated electrically at the wrist using a stimulus of 1 ms duration delivered via 5-mm Ag–AgCl surface electrodes [ConMed, Utica]. The resultant compound muscle action potential [CMAP] was recorded from the abductor pollicis brevis [APB] using surface electrodes. Peak-to-peak amplitude and onset latency for the CMAP were determined. In addition to calculating the CMAP amplitude, the neurophysiological index [NI], a measure of peripheral disease burden, was derived according to a previously reported formula (de Carvalho and Swash, 2000).

Cortical excitability

Cortical excitability was assessed by applying TMS to the motor cortex by means of a 90 mm circular coil oriented to induce current flow in a posterior–anterior direction. Currents were generated by two high-power magnetic stimulators which were connected via a BiStim module [Magstim Co., Whitland, South West Wales, UK] such that conditioning and test stimuli could be independently set and delivered through the one coil. A circular coil was chosen over a focal [figure-of-eight] coil as the former was easier to use with less frequent overheating of the coil. Previous studies established no difference in the pattern of inhibition and facilitation when using either coil (Abbruzzese et al., 1999). A previous study that incorporated threshold tracking transcranial magnetic stimulation used a focal coil and reported similar SICI results when compared to our own study using a circular coil (Fisher et al., 2002; Vucic et al., 2006).

TMS threshold tracking

The threshold tracking TMS technique was used to assess SICI according to previously reported techniques (Vucic et al., 2009, 2006). Specifically, in the present study the target MEP was of predetermined, fixed amplitude and changes in the test TMS intensity required to generate this target response, when preceded by a subthreshold conditioning stimulus, were measured (Vucic et al., 2006). The threshold tracking strategy used a target response of 0.2 mV as previously established (Fisher et al., 2002; Vucic et al., 2006). Resting motor threshold [RMT] was defined as the stimulus intensity required to produce and maintain this target MEP response.

Initially, the maximal MEP amplitude [mV] and onset latency [ms] were determined by delivering a supramaximal TMS intensity set to 150% RMT. Central motor conduction time [CMCT, ms] was calculated using the F-wave method (Mills and Murray, 1986).

Paired-pulse stimuli

In order to assess short interval intracortical inhibition [SICI] a paired-pulse paradigm was used according to a previously devised protocol (Vucic et al., 2009, 2006). SICI was determined using three different levels of subthreshold conditioning stimuli [conditioning stimulus, at 40%, 70% and 90% of RMT], in three different experiments performed in the same sitting. These three conditioning stimulus intensity levels were chosen to assess low threshold [conditioning stimulus 40% RMT] and high threshold [conditioning stimulus 90% RMT] cortical circuits as well as those mediating maximal SICI [conditioning stimulus 70% RMT] (Vucic et al., 2009). SICI was determined over increasing interstimulus intervals [ISIs] delivered in a sequential order as follows: 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7. Stimuli were delivered in a recurring sequence of three: (1) tracked the stimulus intensity required to produce the unconditioned test response [i.e., RMT], (2) monitored the response to the subthreshold conditioning stimulus, and (3) tracked the stimulus required to produce the target MEP when conditioned by a stimulus equal in intensity to that on channel 2. Stimuli were delivered every 5–10 s and the computer was advanced to the next ISI when the tracked MEP was stable.

Recordings of CMAPs and MEPs were amplified and filtered [3 Hz–3 kHz] using a Grass ICP511 AC amplifier [Grass-Telefactor, Astro-Med, Inc., West Warwick, RI] and sampled at 10 kHz using a 12-bit data acquisition card [National Instruments PCI-MIO-16E-4]. Data acquisition and stimulus delivery [both electrical and magnetic] were controlled by QTRACS software [© Professor Hugh Bostock, Institute of Neurology, Queen Square, London, UK].

Data analysis

Cortical excitability in ALS patients was compared to control data (Vucic et al., 2009) obtained from 14 right-handed healthy volunteers [7 men and 7 women; mean, 44 years; age range 24–73 years]. It is noted that in our control data for SICI for conditioning stimulus 70% (Vucic et al., 2006), no age-related changes were evident between younger and older subjects [SICI age<40 years, $8.3 \pm 1.3\%$; SICI>40 years, $9.7 \pm 1.1\%$, $P = 0.2$]. Intracortical inhibition induced by a conditioning stimulus was measured as the increase in the test stimulus intensity required to evoke the target MEP. Inhibition was calculated off-line using a previously reported formula (Fisher et al., 2002; Vucic et al., 2006).

Each data point was weighted [by the QTRACS software] such that any measures recorded outside the threshold target window, defined as values within 20% of the tracking target of 0.2 mV [peak-to-peak], contributed least to the data analysis. All results are expressed as mean ± standard error of the mean. Student's t-test was used for