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Utility of recovery cycle with two conditioning pulses for detection of impaired axonal slow potassium current in ALS

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ABSTRACT

Objective: Slow potassium current (I_{Ks}) is important in controlling nerve excitability and its impairment is known in various neurological diseases, including amyotrophic lateral sclerosis (ALS). I_{Ks} gives rise to the late subexcitability phase of the recovery cycle, which can be amplified by the use of multiple conditioning pulses. The clinical utility of this technique has not previously been explored.
Methods: Nerve excitability tests, including recovery cycles with single and double conditioning pulses 4 ms apart (RC and RC2, respectively) were performed in patients with ALS and control subjects. Late subexcitability values obtained by RC and RC2 were compared in both groups.
Results: RC2 was well tolerated in all the subjects. The threshold changes in late subexcitability by RC2 were greater than those by RC in both groups (mean (%): RC, 16.0/13.3; RC2, 34.9/29.4 (Control/ALS)). The ALS group showed lower threshold changes than controls by both methods. Statistical analysis between the ALS and control groups provided smaller P value by RC2 ($P = 0.018$) than by RC ($P = 0.046$). Also, RC2 provided non-significant, but slightly more distinguishing non-parametric rank analysis and greater Area Under the Curve (AUC) by Receiver Operating Characteristic (ROC). RC2 produced more identifiable single peak for late subexcitability than RC in an ALS patient whose late subexcitability was decreased.
Conclusions: Two conditioning stimuli provide greater threshold change for late subexcitability and possibly clearer identification of a peak threshold change than conventional recovery cycle. The findings obtained by this new protocol reinforce the previously reported impairment of I_{Ks} in ALS.
Significance: Amplification of I_{Ks} by double conditioning pulses is applicable in humans and may help elucidating its clinical significance in pathophysiology in neurological diseases.

1. Introduction

Nerve excitability testing by threshold tracking is an *in vivo*, non-invasive electrophysiologic examination which has shed light on the pathophysiology of various neurological diseases, revealing changes in membrane potential and ion channel function (Bostock et al., 1998; Nodera and Kaji, 2006). Nerve excitability changes following conduction of a single nerve impulse, before return to the resting state, are called the recovery cycle (RC). In a normal human peripheral nerve, RC has three phases: refractory period, superexcitability, and late subexcitability. Late subexcitability appears between approximately 20 and 100 ms following the single nerve impulse, and reaches a maximum value at about 40–50 ms (Kiernan et al., 2000). It reflects transient membrane hyperpolarization due to slow potassium current (I_{Ks}) at the nodes of Ranvier, generated by potassium channels of the K_v7 (formerly KCNQ) family (Schwarz et al., 2006). Due to their membrane stabilizing effects, slow potassium channels have been considered as therapeutic targets for diseases with neuronal hyperexcitability. Retigabine, a slow potassium channel opener, has shown its clinical efficacy in epilepsy (Porter et al., 2007). Recent data in patients with amyotrophic lateral sclerosis (ALS) has suggested that impairment of slow potassium channel function may contribute to the peripheral nerve hyperexcitability manifesting as fasciculations (Kanai et al., 2006; Vucic and Kiernan, 2006). Late subexcitability can be amplified

Abbreviations: ALS, amyotrophic lateral sclerosis; AUC, Area Under the Curve; CMAP, compound muscle action potential; CNS, central nervous system; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; I/V, current–threshold relationship; PNH, peripheral nerve hyperexcitability; PNS, peripheral nervous system; RC, recovery cycle (single conditioning pulse); RC2, recovery cycle (double conditioning pulses); ROC, Receiver Operating Characteristic; RRP, relative refractory period; SDTC, strength-duration time constant; TE, threshold electrotonus.

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with a train of up to about seven conditioning stimuli, as reported by Bergmans, who referred to it as H1 (first hyperpolarizing afterpotential) (Bergmans, 1970). Schwarz et al. applied a train of seven conditioning stimuli to rats and reported the usefulness of H1 to identify the *in vivo* electrophysiologic effects of XE991, a slow potassium channel inhibitor (Schwarz et al., 2006). However, there have been no reports on the clinical usefulness of H1 for assessment of neurological diseases in humans. Thus, the aim of this study was to elucidate the clinical utility for evaluation of I_{Ks} in ALS patients by RC with multiple conditioning pulses. We used a train of two conditioning stimuli in this study because a longer train of stimuli could not be tolerated in awake subjects.

2. Methods

2.1. Subjects

(1) Normal individuals: The subjects included healthy individuals with no symptoms or signs of a neurological disease. An individual with conditions known to affect nerve excitability was excluded, such as diabetes mellitus (by obtaining fasting serum glucose and hemoglobin A1c) and family history of polyneuropathy among others (Bostock et al., 1998; Nodera and Kaji, 2006). The findings of routine nerve conduction studies of the median (motor, sensory, and F waves), tibial (motor and F waves), and sural (sensory) nerves were normal for all the subjects using the standard techniques of nerve conduction studies (Kimura, 2001). (2) ALS: Among the patients who were referred and evaluated at Tokushima University Hospital, the patients who met either definitive or probable revised El Escorial criteria were studied (Brooks et al., 2000). Of note, all the subjects with ALS were ambulatory, reported no respiratory distress, and required no respiratory support. The pulmonary function tests including forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were normal in all the subjects. Normal results of arterial blood gas was obtained in 17 patients, rest of whom had only mild limb weakness, thus arterial blood gas was not performed at the discretion of the treating physicians. Informed consent was obtained from all the participants before the study was conducted. The study was approved by the Institutional Review Board of Tokushima University and was performed in accordance with the principles embodied in the Declaration of Helsinki.

2.2. Nerve excitability measurements

We examined the excitability of median motor axons of the non-dominant side, by recording over abductor pollicis brevis, with the stimulation electrode placed 3 cm proximal to the wrist crease and the remote electrode at 10 cm proximal to the point of stimulation. Multiple excitability measurements were recorded using commercially available software (QtracW; Digitimer, Hertfordshire, UK), as described previously (Bostock et al., 1998; Kiernan et al., 2000). The recording protocol used was TRONDK, which provided for recovery cycles with 1, 2, or 7 conditioning stimuli. The current required to produce certain percentage of the maximal CMAP was defined as "threshold current" (Bostock et al., 1998). In threshold electrotonus (TE) studies, long subthreshold conditioning currents of 100 ms duration (±40% of the threshold current) were used to alter the membrane potential. Threshold currents for the target CMAPs were examined at different time points during and after the 100 ms polarizing current. Strength-duration time constant (SDTC) was estimated on the basis of the thresholds at different durations of stimulus (0.2, 0.4, 0.6, 0.8, and 1 ms) according to Weiss's law (Bostock et al., 1998). Current–threshold relationship (I/V) was recorded with a 1-ms test stimulus applied 200 ms after the onset of a long-lasting subthreshold polarizing current, the strength of which was altered in steps of 10%, from +50% (depolarizing current) to −100% (hyperpolarizing current) of the control threshold. RC was recorded by delivering the test stimulus at different intervals after the single supramaximal conditioning stimulus. The intervals in the conditioning-test were systematically increased from 2 to 200 ms. Immediately after RC, a similar protocol with two supramaximal conditioning stimuli 4 ms apart (RC2) were applied for H1 (Bergmans, 1970; Schwarz et al., 2006). The intervals between the second conditioning pulse and test stimulus increased from 5 to 200 ms. The greatest percentage reduction in threshold current during the supernormal and late subexcitability periods were defined as supernormality (RC only) and late subexcitability (RC and RC2), respectively. The recording was successfully and comfortably performed in all the subjects. The skin temperature of the hand and forearm was maintained at >32 °C by wrapping in a blanket.

2.3. Data analysis

Mann–Whitney's U tests were used for comparing the data since the distribution of patient subexcitability measurements did not pass the Lilliefors test of normality. Receiver Operating Characteristic (ROC) curve was drawn to calculate Area Under the Curve (AUC) to quantify the overall ability of the test to discriminate between the two subject groups. Statistical analysis was performed using the SPSS version 11.0J software (Tokyo, Japan). $P < 0.05$ was considered statistically significant.

3. Results

3.1. General characteristics

Twenty-two subjects in each of the ALS and control groups were tested. In ALS group, there were 12 men and 10 women (Mean age ± SD: 62.6 ± 8.6 years, range 43–80). In closely age-matched normal controls (NC), there were also 12 men and 10 women (Mean age ± SD: 59.9 ± 8.9 years, range 52–78). In ALS, median motor conduction study revealed that the mean compound muscle action potential (CMAP) amplitude (wrist stimulation) was 4.4 ± 1.6 mV (range 1.5–7.9) and the distal latency was 3.9 ± 0.6 ms (range 3–4.9 ms), reflecting relatively mild-to-moderate degrees of axonal damage in our ALS patients. None of the ALS patients had sensory symptoms and signs (Tinel and Phalen signs) suggestive of carpal tunnel syndrome and their median sensory nerve conduction studies were all within normal limits.

3.2. Threshold tracking tests

Table 1 summarizes the nerve excitability measures between the two groups. As previously reported, there were the following characteristics in the ALS patients: Strength-duration time constant, which reflects persistent sodium currents, showed non-significant tendency towards greater values in ALS than in normal controls (Kanai et al., 2006; Vucic and Kiernan, 2006). Depolarizing threshold electrotonus showed greater threshold changes in ALS than in controls, more noticeably with long conditioning pulse. This may reflect impairment of accommodation due to decreased slow potassium current (Kanai et al., 2006). Hyperpolarizing TE showed no significant difference. I/V showed greater threshold change by depolarizing current, but not by hyperpolarizing current in ALS, consistent with the findings by TE.

Recovery cycles were compared between single (RC) and double (RC2) conditioning pulses (Fig. 1, Table 1). Relative refractory periods (RC only) were similar between ALS and control groups. Supe-