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Increased nodal persistent Na^+ currents in human neuropathy and motor neuron disease estimated by latent addition

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Abstract

Objective: To investigate the changes in nodal persistent Na^+ currents in human neuropathy and motor neuron disease. In human motor axons, approximately 1.0% of total Na^+ channels are active at rest, termed "persistent" Na^+ channels, and the conductance can be non-invasively estimated by the technique of latent addition in vivo.
Methods: Latent addition was performed in median motor axons of 93 patients with axonal neuropathy ($n = 38$), lower motor neuron disorder (LMND; $n = 19$) or amyotrophic lateral sclerosis (ALS; $n = 36$) and in 27 age-matched normal subjects. Brief hyperpolarizing conditioning current pulses were delivered, and threshold change at the conditioning-test interval of 0.2 ms was measured as an estimator of the magnitude of persistent Na^+ currents. Threshold electrotonus and supernormality were also measured as indicators of resting membrane potential.
Results: Threshold changes at 0.2 ms were significantly greater in patients with neuropathy or LMND ($p < 0.05$), and tended to be greater in ALS patients ($p = 0.075$) than in normal controls. Threshold electrotonus and supernormality did not differ in each patient group and normal controls, suggesting that membrane potential is not altered in patients. In the recovery phase of axonal neuropathy, the threshold changes increased in parallel with an increase in amplitudes of compound muscle action potential.
Conclusions: Persistent Na^+ currents appear to increase commonly in disorders involving lower motor neurons, possibly associated with axonal regeneration or collateral sprouting or changes in Na^+ channel gating.
Significance: The increased axonal excitability could partly be responsible for positive motor symptoms such as muscle cramping frequently seen in lower motor neuron disorders.

Keywords: Persistent Na^+ channel; Na^+ channel; Latent addition; Axonal regeneration; Threshold tracking

1. Introduction

In disorders involving lower motor neurons/axons such as peripheral neuropathies, amyotrophic lateral sclerosis (ALS), and spinal muscular atrophy (SMA), a loss of motor axons is usually accompanied by collateral innervation of denervated muscles by the remaining motor neurons, or by regeneration of the injured axons. Experimental studies have shown that Na^+ channels are over-expressed on the axolemma when nerves are growing and sprouting, and that local remodeling of Na^+ channels results in altered axonal excitability properties (Devor et al., 1989; Matzner and Devor, 1994). Previous studies have suggested the increased axonal excitability in ALS, SMA, and peripheral neuropathies (Mogyoros et al., 1998; Kanai et al., 2003, 2006).

Recent evidence suggests that a number of subtypes of Na^+ channels are expressed in the peripheral nervous system (Waxman et al., 1999; Sanja et al., 2001), but that only one ($Na_v1.6$) is found at the nodes of Ranvier in adults (Caldwell et al., 2000). However, in each subtype of Na^+ channels, two functionally distinct types of Na^+ channels

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are present; in addition to the classical transient Na^+ channels, 1.0–2.5% of the total Na^+ channels, termed "persistent" Na^+ channels, are active at the resting membrane potential, and one of the major determinants of axonal excitability (Crill, 1996; Baker and Bostock, 1997, 1999). The strength-duration time constant (SDTC) is a classical measure of axonal excitability and partly depends on persistent Na^+ conductance (Mogyoros et al., 1996; Bostock et al., 1998; Kiernan and Bostock, 2000; Kiernan et al., 2002). Previous studies have shown that SDTC is longer in patients with ALS, SMA, or peripheral neuropathy than in normal subjects, which raises the possibility of increased persistent nodal Na^+ conductance (Mogyoros et al., 1998; Kanai et al., 2003). However, SDTC is also affected by passive membrane properties at the nodes of Ranvier (Bostock et al., 1998; Burke et al., 2001), and therefore it has not yet been determined whether the prolonged SDTC in lower motor neuron disorders results from increased Na^+ currents or altered passive membrane properties.

Latent addition using automatic threshold tracking is a new technique and is currently considered the best way to non-invasively estimate nodal persistent Na^+ conductance in vivo (Bostock and Rothwell, 1997; Bostock et al., 1998; Misawa et al., 2006; Kuwabara et al., 2006). The major advantage of latent addition is to separately evaluate passive membrane properties and persistent Na^+ currents, and this information cannot be obtained from SDTC measurements. We applied this technique to patients with peripheral neuropathy and those with motor neuron disease in order to investigate whether nodal persistent Na^+ conductance is altered and whether an increase in this depolarizing conductance is associated with positive motor axonal symptoms such as muscle cramping.

2. Methods

2.1. Subjects

A total of 93 patients with peripheral neuropathy ($n = 38$), lower motor neuron disorders ($n = 19$), or ALS ($n = 36$), referred to the EMG clinic, Chiba University Hospital between May, 2003 and May, 2005, were studied. Patients who received medications to affect Na^+ channels were excluded. Peripheral neuropathies included axonal neuropathy caused by neurotoxic drugs, systemic vasculitis, acute motor axonal neuropathy, and vitamin B1 deficiency. To eliminate the effects of nerve ischemia, edema, or other factors associated with inflammation, patients with vasculitic neuropathy ($n = 17$) and acute motor axonal neuropathy ($n = 8$) were examined in the chronic recovery phase, or at least 6 months after onset. We excluded patients with diabetic neuropathy because hyperglycemia and a resulting altered trans-axonal Na^+ gradient significantly affect axonal excitability properties, including SDTC (Misawa et al., 2004, 2005, 2006; Kitano et al., 2004). We also excluded patients with Charcot-Marie-Tooth disease type 1 or acute/chronic inflammatory demyelinating neuropathy, because demyelination would expose the internodal axonal membrane, therefore resulting in substantial changes in passive membrane properties and axonal excitability properties (Nodera et al., 2004; Sung et al., 2004). Patients with axonal neuropathy ranged in age from 25 to 82 years (mean, 56 years), with a disease duration of 0.5–20 years (mean, 5.2 years). Lower motor neuron disorders included Kugelberg-Wielander disease (KWD; spinal muscular atrophy type 3; $n = 8$), bulbo-spinal muscular atrophy (BSMA; $n = 8$), and Machado-Joseph disease (MJD; $n = 3$). MJD patients were included because the spinal pathology and electrophysiology showed loss of anterior horn cells and therefore this disorder can be

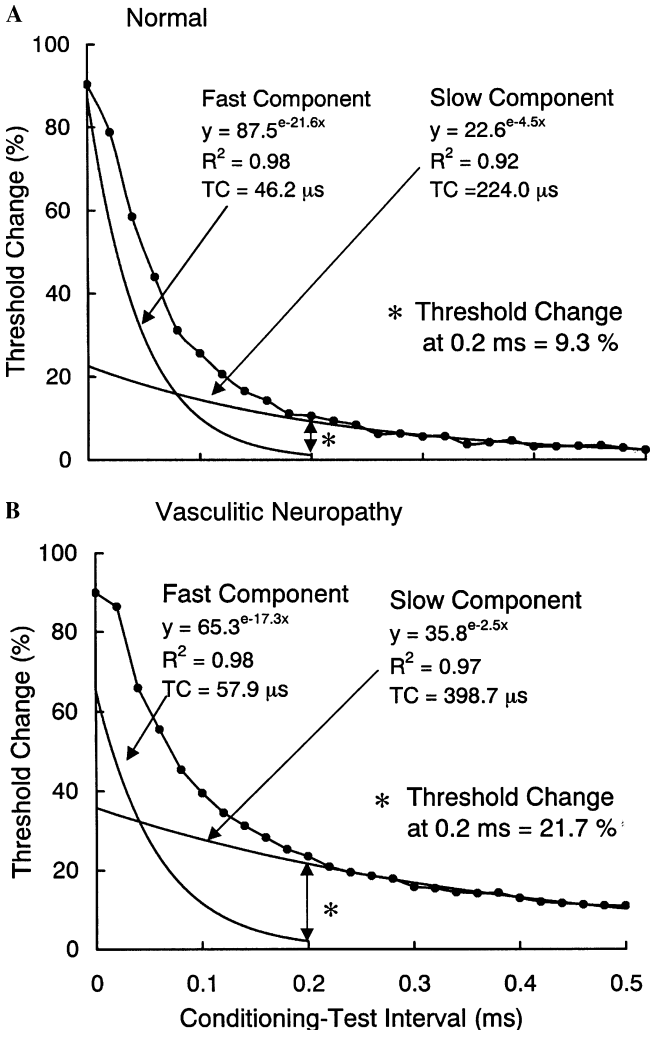

Fig. 1. Latent addition recorded from a normal subject (A, 55-year-old man) and a patient with vasculitic neuropathy (B, 64-year-old man): the test stimulus was conditioned by a hyperpolarizing stimulus fixed at −90% of the current required to produce the test response. At the wrist of median motor axons, the decay of the threshold increases fitted accurately by two exponential curves. The fast component (0.02–0.2 ms) is dependent on a passive input membrane constant, and the slow component (0.2–0.5 ms) is associated with a current active at the resting potential. The increase in threshold measured at the 0.2-ms interval (a time when the first exponential had decayed almost to zero) can be used to estimate persistent Na^+ conductance (Bostock and Rothwell, 1997); TC, time constant.