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Aging-associated changes in motor axon voltage-gated Na^+ channel function in mice

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ABSTRACT

Accumulating myelin abnormalities and conduction slowing occur in peripheral nerves during aging. In mice deficient of myelin protein P_0, severe peripheral nervous system myelin damage is associated with ectopic expression of $Na_v1.8$ voltage-gated Na^+ channels on motor axons aggravating the functional impairment. The aim of the present study was to investigate the effect of regular aging on motor axon function with particular emphasis on $Na_v1.8$. We compared tibial nerve conduction and excitability measures by threshold tracking in 12 months (mature) and 20 months (aged) wild-type (WT) mice. With aging, deviations during threshold electrotonus were attenuated and the resting current-threshold slope and early refractoriness were increased. Modeling indicated that, in addition to changes in passive membrane properties, motor fibers in aged WT mice were depolarized. An increased $Na_v1.8$ isoform expression was found by immunohistochemistry. The depolarizing excitability features were absent in $Na_v1.8$ null mice, and they were counteracted in WT mice by a $Na_v1.8$ blocker. Our data suggest that alteration in voltage-gated Na^+ channel isoform expression contributes to changes in motor axon function during aging.

1. Introduction

The aim of this study was to investigate the influence of age on voltage-dependent ion channels in motor fibers with particular emphasis on ectopic $Na_v1.8$ channels. We hypothesize that dysregulation of voltage-gated sodium channels (VGSC), similar to that observed in demyelinating neuropathy (Moldovan et al., 2011), could contribute to motor axon dysfunction during regular aging. Accumulating degenerative changes in myelin (Adinolfi et al., 1991) and signs of demyelination-remyelination (Lascelles and Thomas, 1966) were found during regular aging in both the central (Guttmann et al., 1998) and the peripheral nervous system (Verdú et al., 2000). In central axons, these changes are thought to perturb conduction and disrupt the timing in neuronal circuits to an extent that aggravates the cognitive decline independently of neuronal loss (Peters, 2002). In peripheral axons, the age-related slowing of conduction is well known (LaFratta and Canestrari, 1966). Nevertheless, the aging-associated changes in myelinated axon function could not be solely accounted for by changed passive cable properties resulting from myelin alterations (Bae et al., 2008; Jankelowitz et al., 2007; McHugh et al., 2011).

Longitudinal morphological studies in peripheral nerve in rodents showed slowly accumulating degenerative changes in myelin and reduced axonal caliber up to around 20 months of age and subsequent further acceleration toward the end of life (Ceballos et al., 1999; Verdú et al., 1996, 2000). These histological changes in mouse were associated with altered levels of several myelin proteins including the myelin protein zero (P_0), which showed reduced expression at both the mRNA and protein levels (Melcangi et al., 1998; Shen et al., 2011). Intriguingly, in the protein P_0-null mice, an experimental model of an extremely severe hereditary neuropathy, the congenital myelin damage was associated with VGSC dysregulation with an ectopic coexpression of the "sensory-neuron-specific" (SNS) $Na_v1.8$ channel isoform along with the $Na_v1.6$ isoform commonly expressed at the nodes of Ranvier of motor axons (Ulzheimer et al., 2004). This acquired channelopathy aggravated the membrane dysfunction, precipitating conduction failure in motor axons (Moldovan et al., 2011).

To distinguish the age-associated changes in active, voltage-dependent, membrane properties from changes in passive cable

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properties of axons in vivo, we combined conventional motor nerve conduction studies with nerve excitability studies by "threshold tracking" (Bostock et al., 1998; Krarup and Moldovan, 2009). The clinically available multiple nerve excitability protocol (Kiernan et al., 2000) was adapted to the study of mouse tibial nerve (Moldovan and Krarup, 2006; Moldovan et al., 2009) and was able to detect axonal ion channel dysfunction in P_0-null mice (Moldovan et al., 2011). Previous longitudinal nerve excitability studies in rodents focused on maturational changes during the first months of life (Boërio et al., 2009; Yang et al., 2000). In mice, fully mature properties of peripheral nerves are reached at 1 year (Ceballos et al., 1999; Verdú et al., 1996). To study aging independently of maturation, we therefore compared motor axons in ~12 months (mature) versus ~20 months (aged) wild type (WT). The contribution of $Na_v1.8$ was distinguished using SNS null mice (Akopian et al., 1999) and subtype-selective $Na_v1.8$ pharmacological block (Kort et al., 2010).

2. Methods

2.1. Animals and experimental design

Investigations in WT mice were carried out in 51 female black mice (C57BL/6J, Harlan) aged ~12 months (n = 33) and ~20 months (n = 18) weighing 30 ± 1 g (mean ± SEM) and 34 ± 2 g, respectively. These age groups are referred to hereafter as "mature" and "aged," respectively. Additional electrophysiological measurements were obtained from 6 older WT mice at 24 months. Three of these old mice were surviving at 28 months and were subjected to additional tests.

Mice deficient for $Na_v1.8$ were generated by targeting the S4 voltage sensor of domain I of the SNS Na^+ channel α subunit (Akopian et al., 1999). The null mutants expressing a nonfunctional SNS transcript are fertile and clinically healthy. We established in Copenhagen our own colony of SNS deficient mice (Moldovan et al., 2011). Genotypes of the SNS mice were verified by conventional PCR using oligonucleotides 5′-GACTGATGCATATGATGTCATGTGTGG-3′, 5′-GCCTTCACTGTTGTTTACACCTCCGAGG-3′, and 5′-GCAGCGCATCGCCTTCTATC-3′ (Sigma-Aldrich Denmark A/S, Brøndby, DK) leading to amplicons of 900 bp or 1100 bp for the SNS null mutation or WT allele, respectively. In this study, we investigated tibial nerves from mature SNS-null mice (n = 16) and aged SNS-null mice (n = 9). At both these age groups, SNS-null mice had similar body weights and behavioral motor performance on a Rotarod compared to WT mice (Ugo Basile Srl 7650 accelerating Rotor-Rod, Comerio, VA, Italy).

Electrophysiological investigations were carried out under anesthesia using a 1:1 mixture of Hypnorm/Midazolam (5 mg/mL). A volume of 0.1 mL/10 g from the mixture was injected subcutaneously for induction. This was enough to ensure surgical anesthesia for the 30–45 minutes required for most of the experimental procedures, and it was further supplemented if required. We have previously established that nerve conduction and excitability recordings remain stable during repeat administration over several hours (Alvarez et al., 2008; Moldovan et al., 2009, 2011). Of note, the need for repeating of anesthesia was less in the old mice than expected from their body weight. At the completion of the experiments, some of the WT mice were assigned to serve as control for additional terminal experiments investigating the effects of strenuous repetitive stimulation, as reported elsewhere (Alvarez et al., 2013). The other mice were killed by cervical dislocation, and tibial nerves were harvested for morphological studies.

All experimental procedures were approved by the Danish National Experimental Animal Inspectorate.

2.2. Nerve conduction and excitability studies

The mouse was fixed on a temperature-controlled pad (HB 101/2, LSI Letica) set to 37 °C as previously described in details (Moldovan and Krarup, 2006). The investigated leg was placed on a piece of hydrophobic cotton to reduce the stimulus artefact.

Stimulation of the tibial nerve was carried out at ankle using platinum needle electrodes inserted perpendicularly through the skin close to the tibial nerve. The descending compound muscle action potential (CMAP) from the plantar muscles was recorded using platinum needle electrodes inserted into the foot ~0.5 cm apart. A ground electrode was inserted subcutaneously in the thigh.

Electrical stimuli were delivered from a constant current stimulator (DS4, Digitimer Ltd). The amplified signal (10 Hz–6 kHz, 10C02, Dantec) was digitized by computer (PC) with an analogue-to-digital board (NI-6221, National Instruments Inc) at a sampling rate of 10 kHz.

The plantar CMAP amplitudes were measured peak to peak. The latencies were evaluated at half-peak. This measurement, although yielding absolute values about 1 ms longer than the latencies measured at the "first deflection from baseline" on the same model, is in reasonably good correlation with the latency of the fastest fibers (unpublished methodological controls).

Peripheral nerve excitability was assessed using QtracS stimulation software (Institute of Neurology, London, UK) using the TRONDH multiple excitability protocol designed for clinical use (Kiernan et al., 2000). These methods can be directly used for excitability testing of the mouse tibial nerve motor axons via platinum electrodes because the low currents required do not cause significant electrode polarization (Moldovan et al., 2009, 2011) indicated by the similarity with measurements using surface nonpolarizable Ag/AgCl electrodes (Boërio et al., 2009; Moldovan et al., 2009).

The stimulus-response curves were obtained using test stimuli of 1-ms duration to establish the maximal CMAP (100%) to supramaximal nerve stimulation (Fig. 1A). Then, the "threshold" current necessary to evoke a submaximal target potential set to 40% of maximum CMAP could be tracked automatically by trial-and-error computer feedback allowing for the investigation of several measures of excitability as previously described in detail (Bostock et al., 1998; Kiernan et al., 2000): stimulus-response relationship, threshold electrotonus, current-threshold relationship, and the recovery of excitability following conduction of the action potential.

The strength-duration properties (Fig. 1B) reflecting primarily passive membrane properties as well as active nodal conductances at the resting membrane potential (Mogyoros et al., 1996, 1997) were determined by measuring the changes in thresholds for test stimuli of 0.2, 0.4, 0.6, 0.8, and 1.0-ms duration. Rheobase and the strength-duration time constant (SDTC) were estimated from the corresponding linear charge (current strength × duration)-duration relationship using Weiss law (Bostock, 1983): rheobase from the slope and SDTC from the negative intercept on the duration axis.

Paranodal-internodal membrane function was ascertained by investigating changes in threshold in response to prolonged depolarization and/or hyperpolarization comprised in threshold electrotonus and current-threshold relationship. Threshold electrotonus (Fig. 1C) was measured for 100-ms depolarizing and hyperpolarizing currents set to 40% of the control threshold current. The maximal threshold change during depolarizing threshold electrotonus was quantified at the end of depolarization, measured as TEd (90–100 ms) as well as the peak change quantified as TEd (Peak). Threshold changes during hyperpolarizing threshold electrotonus were quantified at the end of hyperpolarization, measured as TEh (90–100 ms). Excitability changes after 200-ms current