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Research paper

Age-dependent effects on sensory axonal excitability in normal mice

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HIGHLIGHTS

- Age-dependent changes occur on axonal excitability of both motor and sensory neurons. These changes may reflect alterations in passive membrane properties.
- Alterations of an ion conductance (e.g., H conductance) and cable properties (e.g., Barrett–Barrett conductance) explain the interval changes of sensory axonal excitability with maturation and aging.
- Dynamic changes of sensory axonal excitability may explain age-dependent sensory symptoms.

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ABSTRACT

Serial recordings were performed to measure sensory excitability in peripheral nerves and elucidate age-dependent changes in neuronal ion currents in the peripheral sensory nervous system. The threshold tracking technique was used to measure multiple excitability indices in the tail sensory nerves of five normal male mice at four time points (6, 10, 14, and 19 weeks of age). A separate group of four mice was also measured at 43 weeks and at 60 weeks of age. Maturation was accompanied by an increase in early hyperpolarization and superexcitability at 10 weeks. At 60 weeks, the hyperpolarizing electrotonus shifted downward, while superexcitability became greater and subexcitability (double stimuli) decreased. Computer modeling showed that the most notable age-related interval changes in excitability parameters were Barrett–Barrett, H, and slow K^+ conductances. Understanding age-related changes in the excitability of sensory axons may provide a platform for understanding age-dependent sensory symptoms and developing age-specific channel-targeting therapies.

1. Introduction

Increasing age affects the structure and function of the peripheral nervous system. During maturation, the axonal diameter of human peripheral nerves increases during the first 5 years, the myelin thickens until 14 years of age, and the internodal length increases until the second decade [9,20]. Histopathological changes consistent with aging become evident by the fifth decade, such as the loss of myelinated and unmyelinated fibers [1,5,20]. Animal studies have shown a decrease in axon diameter, disruption of the myelin sheath, a pronounced increase in collagen fibers in the endoneurium and perineurium, the disruption of axoglial junctions, and separation of the myelin loops from the paranodal axolemma, which widens the nodes of Ranvier of peripheral nerves in old animals [1,5].

Studies using threshold tracking to assess axonal membrane excitability *in-vivo* have provided insight into the properties of axonal membranes under normal conditions and in many peripheral-nerve disorders [4,7,12,14,16]. The establishment of a threshold tracking model of animal sensory nerves and a mathematical model of human sensory axons provides detailed information about the molecular mechanisms underlying axonal membrane function, and these models have enabled the study of axonal excitability in sensory nerves [7].

Previous studies have uncovered differences between motor and sensory nerve function [19]. A recent study reported that there is an increased hyperpolarization-activated current (Ih) and a smaller nodal slow K^+ current (IKs) in human sensory axons than in motor axons.

Therefore, we hypothesized that there are age-related changes to neuronal ion currents in the sensory nervous system that may be

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different from those in the motor system. The present study utilized axonal excitability in normal mice to determine the age-related effects on sensory axons, describing the underlying molecular mechanisms with mathematical modeling.

2. Methods

2.1. Study protocol

The experiment was approved by the local animal facility at the Tokushima University. ICR normal male mice (SLC, Hamamatsu, Japan) were tested. Serial electrophysiological testing was performed in five mice at four time points, i.e., juvenile, adolescent, young-adult, and mature (6, 10, 14, 19 weeks of age, respectively). A different group of four male mice was tested at 43 weeks of age (adult) and 60 weeks (aged).

2.2. Axonal excitability study

Electrophysiological studies were performed on the tail under 1.5% isoflurane anesthesia. Sensory nerve action potentials were recorded orthodromically and the setup of the electrodes, temperature maintenance, and neuronal excitability testing were performed as previously described in detail [18]. In brief, stimulation was controlled by a PC running the QtracS program (Institute of Neurology, London, UK), and the TRONDNF multiple excitability recording protocol was used for excitability tests. A set of excitability parameters was derived from the recordings, as previously described [17]. One cycle of multiple excitability tests took approximately 20 min.

2.3. Data analysis

Axonal excitability data from serial recordings were compared using one-way repeated measures ANOVA with Bonferroni corrections where appropriate, and data from three different time points (19, 43, and 60 weeks old) were compared using one-way ANOVA with *Post Hoc* tests (SPSS version 22: IBM, New York, NY, USA). The level of significance was $P < 0.05$. All data are presented as means ± SD.

2.4. Modeling of the excitability data

The commercially available Bostock model of the human motor axon was used in the simulation of axonal excitability (MEM-Fit, QtracP version 17/10/2014), as previously explained in detail [8,13]. Parameter adjustments were made to improve the fit to the normal human recovery cycle (RC), strength-duration time constant (SDTC), current-threshold relationship (I/V), and threshold electrotonus (TE). To reflect better the characteristic waveform changes (see Results section), the weighting factors were set as follows: TE, 2; RC, 1; SDTC, 0.5; and I/V, 1. The tested parameters were as follows: nodal and internodal resting potentials (ENR and EIR, respectively), nodal Na^+ permeability (PNa), percent persistent Na^+ channels (PNap), nodal and internodal slow K^+ conductance (GKs), nodal and internodal fast K^+ conductance (GKf), internodal H conductance (GH), nodal and internodal leak conductance (GLk), Barrett–Barrett conductance (GBB), and total pump currents (IPump).

First, the recording of the 14-week-old mice was taken as a reference and best fits were obtained by changing the aforementioned parameters. The recordings for the other ages were then fitted by changing single parameters to reduce the discrepancy. If that did not yield a satisfactory reduction (i.e., arbitrarily less than 65%), another fitting was performed by first changing two parameters, and then up to three parameters.

3. Results

3.1. Sensory nerve excitability changes with maturation

To examine the sequential changes to sensory nerve excitability with maturation, serial recordings were tested at four time points (Table 1, Figs. 1 and 2A and B). The peak latency decreased gradually from 1.87 ± 0.1 ms for the 6 week-old mice to 1.31 ± 0.08 ms for 19 week-old mice, suggesting greater conduction velocities with

Table 1
Changes in sensory axon excitability parameters with maturation in mice (depolarizing threshold electrotonus, TEd; hyperpolarizing threshold electrotonus, TEh; #: $P < 0.05$; *: $P < 0.01$).

	6 weeks old (A)	10 weeks old (B)	14 weeksold (C)	19 weeks old (D)	ANOVA *P* values	With Bonferroni correction *P* values
Amplitude (μV)	61.7 ± 25.3	112.3 ± 45.2	128.3 ± 62.7	135.7 ± 46.5	0.9	
Rheobase	0.42 ± 0.1	0.35± 0.05	0.31 ± 0.06	0.31 ± 0.06	0.06	
Peak latency (ms)	1.87 ± 0.1	1.49 ± 0.09	1.39 ± 0.1	1.31 ± 0.08	0.001*	0.02# (A/B);0.032# (A/C);0.032# (A/D)
Threshold electrotonus (TE)						
TEd (10–20 ms)	45.2 ± 2.4	42.2 ± 2.7	44.1 ± 3.4	45.3 ± 1.8	0.07	
TEd (40–60 ms)	43.3 ± 1.4	40.5 ± 1.7	42.2 ± 3.3	41.1 ± 1.2	0.13	
TEd (90–100 ms)	41.9 ± 2.2	39.1 ± 1.3	40.6 ± 1.1	40.2 ± 0.4	0.1	
TEh (10–20 ms)	−61.9 ± 2.9	−58.0 ± 3.3	−56.7 ± 5.9	58.5 ± 2.0	0.2	
TEh (20–40 ms)	−72.1 ± 3.8	−58.8 ± 4.9	−60.0 ± 7.2	−64.9 ± 2.9	0.005*	0.003* (A/B)
TEh (90–100 ms)	−59.87 ± 9.9	−48.09 ± 5.5	−53.21 ± 5.0	−58.9 ± 4.3	0.051	
S2 accommodation	3.5 ± 2.6	3.2 ± 1.4	3.7 ± 2.2	4.8 ± 1.9	0.4	
TEh (peak: −70%)	−140.0 ± 21.4	−119.1 ± 9.2	−122.9 ± 14.0	−137.0 ± 4.9	0.6	
S3 accommodation (−70%)	41.8 ± 5.3	46.2 ± 7.3	39.8 ± 14.5	46.4 ± 12.1	0.1	
Recovery cycle (RC)						
Refractoriness at 2 ms	2.9 ± 5.9	2.3 ± 2.9	−1.5 ± 3.7	−2.5 ± 4.1	0.013#	
Superexcitability (%)	−5.62 ± 2.8	−1.82 ± 0.8	−5.32 ± 2.4	−7.1 ± 2.4	0.023#	
Superexcitability at 7 ms	−1.3 ± 2.7	2.0 ± 1.1	−1.0 ± 2.4	−2.5 ± 0.6	0.037#	0.017# (B/D)
Late subexcitability (%)	1.8 ±1.6	1.5 ±1.3	2.5 ±1.0	1.8 ±1.1	0.57	
RC2-subexcitability (%)	1.6 ± 1.3	2.5 ± 0.4	4.5 ± 0.5	3.7 ± 1.1	0.01#	
Current/threshold relationship (I/V)						
Resting I/V slope	0.85 ± 0.04	0.99 ± 0.1	0.92 ± 0.07	0.78 ± 0.1	0.03	
Minimum I/V slope	0.58 ± 0.1	0.81 ± 0.03	0.67 ± 0.07	0.65 ± 0.06	0.007*	0.027# (B/D)
Hyperpolarizing I/V slope	0.83 ± 0.2	1.0 ± 0.06	1.2 ± 1.0	1.3 ± 0.8	0.7	
Stimulus-response (SR) relationship						
Stimulus for 50% max response	0.59 ± 0.2	0.46 ± 0.07	0.43 ± 0.07	0.42 ± 0.09	0.07	
Strength-durationtime constant (SDTC)	0.15 ± 0.03	0.15 ± 0.04	0.17 ± 0.02	0.16 ± 0.03	0.6	