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Reduced thermal sensitivity and Nav1.8 and TRPV1 channel expression in sensory neurons of aged mice

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Abstract

Sensory neurons in aging mammals undergo changes in anatomy, physiology and gene expression that correlate with reduced sensory perception. In this study we compared young and aged mice to identify proteins that might contribute to this loss of sensation. We first show using behavioral testing that thermal sensitivity in aged male and female mice is reduced. Expression of sodium channel (Nav1.8 and Nav1.9) and transient receptor potential vanilloid (TRPV) channels in DRG and peripheral nerves of young and old male mice was then examined. Immunoblotting and RT-PCR assays showed reduced Nav1.8 levels in aged mice. No change was measured in TRPV1 mRNA levels in DRG though TRPV1 protein appeared reduced in the DRG and peripheral nerves. The GFRα3 receptor, which binds the growth factor artemin and is expressed by TRPV1-positive neurons, was also decreased in the DRG of aged animals. These findings indicate that loss of thermal sensitivity in aging animals may result from a decreased level of TRPV1 and Nav1.8 and decreased trophic support that inhibits efficient transport of channel proteins to peripheral afferents.

Keywords: Sensory neuron; Vanilloid receptor; TRPV1; Sodium channel; Behavior; Thermal sensitivity

1. Introduction

The cutaneous sensory system, comprised of sensory ganglia, cutaneous nerves and sensory receptors in the skin, detects tactile and thermal pain sensations. Functional studies in several species have shown that impairment of cutaneous sensitivity occurs with aging [13,37]. This age-related decrease in sensitivity can reduce tactile perception, detection of noxious stimuli and tissue injury. To identify how these degenerative changes occur, several studies of human and rodent have compared electrophysiological and anatomical properties of young and aged neurons, peripheral nerves and target tissues. In humans, electrophysiological measures have found lower nerve conduction velocity in older individuals, suggesting impairment in axon structure and function [9,37]. Analysis of nerve fibers in aging mice from 20 months of age onward, showed both myelinated and unmyelinated fibers to be reduced in number, which in the very old mouse (33 months) approaches 50% loss [13]. In contrast, mice between 12 and 20 months of age show only mild age-related changes (fiber reduction, myelin loops, membrane irregularity). In the peripheral target, measures in human of intraepidermal nerve fiber density showed no significant change in epidermal fiber density with age, except when comparison of aged samples was made to the highest values in the youngest subjects [25,27] (though see [7,14]). In 18-month old mice, innervation to the epidermis showed only a moderate loss (10–15%) in nerve fiber density [37]. Interestingly, this loss of peripheral nerve fibers in very old rodents does not appear to reflect a loss of neurons in cervical and lumbar DRG. Analysis of neuron number using a dissector counting methodology showed no significant change in sensory neurons of very old (30 months) rats [6]. Collectively, these data suggest age-

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related sensory deficits are not a result of neuronal death, but rather reflect a gradual alteration of both afferent structure and sensitivity.

Little is known of the cellular and molecular changes that occur in aging sensory neurons that lead to impaired perception. It is clear however, that afferent sensitivity is highly dependent on the expression of several classes of membrane channel proteins that regulate ion flow in response to a given stimulus. With this in mind, we investigated in this study whether an age-related decline in afferent sensitivity correlates with altered expression of membrane channel proteins. We focused on the thermosensitive ion channels in the transient receptor potential (TRP) family and the tetrototoxin resistant sodium channels since these channels are involved in the generation and transmission of impulse trains in response to mechanical and thermal stimuli [19,23]. Effects of aging on electrical membrane properties (EMP) of mouse DRG neurons in culture have indicated that neurons of aged animals (22–23-month old) exhibit decreased electrical excitability and increased action potential duration compared to younger animals (2–3.5-month old) [33]. This pattern of altered EMP is consistent with an age-induced shift from voltage-sensitive sodium channels to less excitable voltage-dependent calcium channels [24]. Tetrodotoxin-resistant voltage-dependent sodium channels Nav1.8 (SNS) and Nav1.9 (NaN or SNS2) are selectively expressed in small/medium-sized nociceptive neurons and contribute to the production of an action potential in these neurons. In addition, functional studies reveal that Nav1.8 and Nav1.9 have a specialized role in mediating pain [1,2,15–17]. Thus, the level of gene expression and/or distribution of these channels may be altered in aging neurons that have lowered sensitivity.

Members of the TRP channel family may also play a role in the age-related decline in cutaneous sensitivity [20,22]. The TRPV1 (vanilloid receptor 1) channel was first identified as a receptor for capsaicin that was activated by noxious heat [12]. TRPV1 is predominantly expressed in small, unmyelinated neurons and has a thermal activation threshold of ∼43 °C [10,29,36]. The TRPV2 channel is expressed in medium and large myelinated neurons and mediates high threshold heat response with a threshold of ∼52 °C [11]. TRPV3 is also heat sensitive with an activation threshold between 31 and 39 °C [34,38]. TRPV4 (VRL-2 or VROAC) is expressed in small and medium sized neurons and appears to mediate changes in osmoregulation, mechanical nociception and response to temperatures greater than 25 °C [26,35]. Whether age-related changes in expression and/or function of these ion channels contributes to altered neuronal sensitivity and excitability with aging is unclear.

To test whether changes occur in channel expression and distribution in the aging sensory system, we compared expression of the Nav1.8 and TRP channels in ganglia and nerves of young, middle-aged and old mice. To examine the relationship between trophic factor signaling and the measured neuronal properties, the relative expression level of receptor proteins in the glial cell line-derived neurotrophic factor (GDNF) family were also assayed.

2. Materials and methods

2.1. Animals

Young (6–8 weeks), middle-aged (15 months) and aged (2 years) male and female C57BL/6NIA (B6) mice were obtained from the aging rodent colony supported by the National Institute on Aging at Harlan (Indianapolis, IN, USA). Upon arrival at the University of Pittsburgh Animal Facility, mice were housed in microisolator caging and maintained on a 12-h light/dark cycle in a temperature controlled environment (20.5 °C) with free access to food and water ad libitum. These studies were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee at the University of Pittsburgh and the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Behavioral testing

Mice were placed in individual chambers (10.0 cm long × 10.0 cm wide × 13.0 cm high) of a 16-chamber plexiglas container that was placed on top of a 6.0 mm thick glass surface (Model 390; IITC Inc., Woodland Hills, CA). Mice were acclimated to this environment for 1–2 h prior to testing. Focused radiant heat (setting at 20) was applied to the plantar surface of the mid-hind paw of the mouse and the latency of withdrawal measured to the nearest 0.1 s. The left and right hind paw was tested on each mouse once a day for three consecutive days. Data were analyzed using an analysis of variance test (ANOVA). Mechanical responsiveness was tested by applying von Frey filaments (Stoelting, Wood Dale, IL) of varying thickness to the dorsum of the foot and recording the force needed to elicit a response, e.g., hindpaw withdrawal, biting of the filament. Testing was done twice a day for three consecutive days.

2.3. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

RNA was extracted from either pooled (from cervical, thoracic and lumbar levels) or lumbar (L3/L4/L5) dorsal root ganglia. Mice were deeply anesthetized by injection of Avertin (2-2-2 tribromoethanol in *tert*-amyl alcohol) anesthesic and then killed by transcardial perfusion with approximately 75 ml ice-cold phosphate buffer. DRGs were collected on dry ice and frozen at minus 80 °C until RNA extraction. RNA was isolated by homogenizing frozen tissue in 1 ml of Trizol reagent (Invitrogen, Carlsbad, CA) followed by isopropanol precipitation. Pellets were washed with 70% ethanol, suspended in RNase-free water and the concentration determined using a GeneQuant RNA/DNA calculator (Amersham Biosciences, Piscataway, NJ). Five micrograms of RNA