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Behavioral and Cellular Level Changes in the Aging Somatosensory System

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Abstract

A study of the peripheral sensory system of young and aged Blk6 mice obtained from the National Institutes on Aging mouse colony is described. Behavioral assays showed male and female aged mice had decreased sensitivity to thermal stimuli. The receptor for the growth factor artemin (Artn), GFRα3, and the ion channel TRPV1, which is expressed by 95–99% of GFRα3-positive primary sensory neurons, was also decreased. Calcium imaging of isolated dorsal root ganglia neurons grown with nerve growth factor was used to test the *in vitro* effects of Artn on TRPV1 activation by capsaicin in young and old neurons. Artn potentiated initial TRPV1 responses to capsaicin in young and old neurons, but the percent of capsaicin responders following repeated exposure to capsaicin increased only in young neurons. The lack of change in aged neurons suggests that differences in TRPV1 responses occur with aging in the somatosensory system.

Keywords

sensory neuron; artemin; thermal and taste hypersensitivity; calcium imaging

Introduction

Abnormal perception of thermal and mechanical stimuli is common in aged individuals. Change in sensory perception appears to be, in part, the consequence of anatomical, cellular, and molecular level changes in the peripheral nervous system (PNS).1–3 On the anatomical level, deficits in somatosensation may result from a loss of nerve fibers, myelin abnormalities, and changes in connective tissue and vascularization. These types of changes have been documented in studies of human and rodent models and are particularly apparent in very old individuals. Indeed, studies using very old mice (about 24 months) show that impaired perception of thermal and mechanical stimuli applied to the footpad skin correlates with abnormalities in nerve structure and in some cases loss of sensory neurons.4 However, evidence also suggests that reduced sensation does not necessarily reflect neuronal loss since only a slight decrease in neuron number may occur in aged rodents that exhibit increased thresholds to thermal and mechanical stimuli.5–7 In addition, studies of rat show no significant correlation between the degree of neuron loss and extent of behavioral deficit.6 These findings suggest that age-related deficits in thermal and mechanical responses are not simply a result of anatomical changes but might also reflect changes at the cellular and molecular level that alter

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Conflicts of Interest
The authors declare no conflicts of interest.

sensory neuron response properties. Such change could include altered expression of genes encoding trophic factors and their receptors, neuropeptides, cell adhesion molecules, ion channels or genes related to mitochondrial function and calcium handling.[8,9]

Much of our understanding of changes that occur in aging sensory neurons is based on studies of anatomy and behavioral responses carried out in rodents. Sensory nerves of aging rodents exhibit slower nerve conduction velocity and action potential amplitude, and this decrease begins in early adulthood.10،11 Both C-fiber and A-fiber function decrease with age, although impairment of A-fiber function appears greatest. [12] Studies of calcium currents in rat dorsal root ganglia (DRG) neurons found reduction of calcium current and an increase in the percentage of high-threshold calcium currents in 30-month-old rats.[13] Consistent with these findings, a depolarization-induced increase in intracellular calcium transients in old neurons was also lower compared with cells isolated from 7-month-old rats.14 Cultured DRG neurons from old mice exhibit decreased electrical excitability and increased action potential duration. These changes are consistent with an age-induced shift from voltage-sensitive sodium channels to less excitable voltage-dependent calcium channels,15 and suggest that age-induced changes in neuronal sensitivity and excitability may result from altered expression and/or function of ion channels with aging.

Change in the level of target-derived neurotrophic growth factor support of sensory neuron phenotype may also occur in aging systems and underlie, in part, age-related decline in sensation. Neurotrophic support is important for maintaining phenotype and functional properties of adult neurons, and changes of growth factor receptor expression in the aged PNS have been reported.[16] Two growth factor families with central roles in sensory neuron development and maintenance are the neurotrophins and the glial cell line derived growth factor (GDNF) family. Neurotrophins (NGF, NT3, BDNF, and NT4) released from target tissues, such as the skin, exert survival and differentiation effects on sensory neurons by binding to trk receptor tyrosine kinases (trkA, trkB, and trkC) expressed on nerve terminals. Decreases in mRNA and protein levels of each trk were observed in DRG neurons of aged rats, suggesting that reduced growth factor signaling is present in the aging sensory system.[16،17]

The role of GDNF family ligands in adult sensory function and aging is less understood. The GDNF family consists of GDNF, artemin (Artn), neurturin, and persephin, which preferentially bind to the glycophosphatidylinositol-anchored co-receptors GFRα1, GFRα2, GFRα3, and GFRα4, respectively. Ligands bound to GFRs form a functional signaling complex upon association with the Ret receptor tyrosine kinase. Similar to the trk receptors, Ret has been reported to activate several signaling cascades that include PI3K, PLC, and src kinase pathways.[18]

In recent studies using young Blk6 mice, we examined the role of Artn and its effects on GFRα3-positive sensory neurons. GFRα3 neurons have small to medium somal diameters and many (approximately 80%) are trkA- and CGRP-positive.19¯21 Interestingly, nearly all GFRα3 neurons express the transient receptor potential channels TRPV1 and TRPA1.19،22 TRPV1 is activated by capsaicin (CAP), protons, and noxious heat and is required for the thermal hyperalgesia that accompanies inflammatory pain. TRPA1 is sensitive to noxious cold (around 10–15°C), cinnamonaldehyde, and mustard oil.23،24 Transgenic mice that overexpress Artn in the skin and tongue (ART-OE mice) exhibit increased expression of TRPV1 and TRPA1mRNA in sensory ganglia and increased density of TRPV1-positive afferents in the periphery.19،22،25 Calcium imaging of DRG neurons of ART-OE mice (and neurons from wild-type mice acutely treated with Artn) indicate that Artn sensitizes primary sensory neurons to chemical (CAP and mustard oil) stimuli.26 Behaviorally, ART-OE mice were more sensitive to both hot and cold stimuli applied to the skin and exhibited an increase in taste aversion to water containing small amounts of CAP or mustard oil.22،25 Because Artn

appears to have an important role in regulating cutaneous neuron response properties, we investigated the effect of Artn in the aging somatosensory system. We focused our studies on the Artn receptor GFRα3 and the TRPV1 channel.

Results

We began analysis of age-related changes in sensory perception by comparing the thermal response properties of young (6 week) and old (2 year) male and female C57Blk6 mice. Measures of the response latency to a focused thermal stimulus showed that both aged males and aged females (n = 11–12 animals/group) exhibited longer latencies.[27] Comparison of all females (young and old, n = 23) to all males (young and old, n = 24) also showed that, as a group, females had a shorter latency than males, that is, they were more sensitive to thermal stimuli ($P < 0.05$).

The reduced thermal sensitivity exhibited by 2-year-old (and 16-month-old) mice[25] suggested that aged animals are less sensitive to a transient thermal stimulus applied to the footpad skin. To begin to identify the cellular-level changes that could underlie this reduction in thermal sensitivity, we compared expression of the thermosensitive TRPV1 channel in young and aged DRG. In pooled ganglia from the L3/L4/L5 lumbar level, RT-PCR analysis showed no difference in expression on the transcriptional level.27 However, on the translational level, western immunoblotting showed a reduction in TRPV1 in ganglia from aged mice that was present at 15 months of age and further reduced in 2-year-old ganglia. Comparison of TRPV1 protein in tibial nerves of young and old animals using western blotting and immunolabeling also showed a reduction in TRPV1 in nerves of aged animals. Thus, TRPV1 protein expression steadily declines in aged neurons, although no change occurs on the transcriptional level.

Analysis of ART-OE transgenic mice indicated that Artn growth factor modulates expression of TRPV1.22 The decline in TRPV1 in aging neurons therefore suggested that Artn signaling is reduced in ganglia of aged mice. Indeed, relative to 6-week-old animals, GFRα3 receptor mRNA was reduced 32% in lumbar DRGin 15-month-old animals, and protein expression was reduced by 24%.[27] Immunolabeling showed the percentage of GFRα3-positive neurons in lumbar DRG from 2- to 3-month-old mice was not different from the percentage in 16-month-old ganglia (young, 31% ± 2.6%; old, 28% ± 0.7%). These data suggest that there is not a loss of GFRα3-positive neurons in DRG of aged mice but rather a reduction in synthesis on a per cell level.

The overall reduction in TRPV1 protein in sensory afferents may contribute to the reduced thermal sensitivity exhibited by aged animals. However, since TRPV1 is a calcium-permeable channel, the functional properties of TRPV1 may also be altered in the aging system, which is known to exhibit altered calcium handling.[28,29] To determine whether functional properties of TRPV1 in primary sensory neurons change with aging, calcium imaging was used to compare Ca^{2+} transients induced by application of 1 μmol/L CAP, a highly specific TRPV1 agonist. DRG neurons from 2-to 4-month-old and 15- to 18-month-old male mice were compared.

We examined whether Artn modulates TRPV1 responses differentially in DRG neurons from young and old mice by comparing CAP responses of neurons incubated in growth media containing 250 ng/mL Artn. This medium also contained 50 ng/mL NGF since cultures of old neurons were found to require NGF for overnight survival. Thus, all neurons (young and old) were plated with 50 ng/mL NGF. ART plus NGF (A + N) significantly increased the percentage of CAP-responsive cells in both young (45%, $P < 0.01$) and old animals (42%, $P < 0.01$) compared with NGF alone (young, 33%; old, 30%). However, no difference in the percentage of CAP^+ neurons (45% versus 42%) was detected between young and old animals. This

outcome is consistent with prior immunolabeling analysis that showed no difference in the percent of TRPV1 neurons in young and old ganglia.[27]

In cultures of young neurons, Artn alone was found to increase the percentage of neurons that responded to a second application of CAP (CAP1 neurons; 48% versus 90%). In addition, exposure of young neurons to Artn plus NGF significantly increased the percentage of $CAP1^+$ neurons (94%, $P < 0.05$) compared with NGF alone (80%). In contrast to these findings, no significant increase in CAP1-responsive neurons was found in cultures from aged mice (A + N, 87%; NGF, 81%). These studies suggest that Artn has less effect on sustained TRPV1 activation in old neurons compared to young neurons. This reduction may be related to the decreased level of GFRα3 in sensory ganglia and nerves of aged animals.

Discussion

These studies suggest that age-related changes in the expression of the GFRα3 receptor and the TRPV1 ion channel in peripheral cutaneous sensory neurons contribute to decreased thermal sensitivity in aged mice. The GFRα3-positive neurons may have particular importance here since nearly all of these neurons express TRPV1, and Artn signaling through GFRα3 appears to regulate TRPV1 gene expression.[22] Thus, any decrease in GFRα3 is predicted to reduce TRPV1 expression and thereby contribute to impaired perception of a heat stimulus, as measured by foot withdrawal in the mouse.

Although reduction in TRPV1 appears to have an important role in age-related changes in thermal perception, it is important to recognize that changes in other proteins related to transmission of thermal stimuli have been detected. Reduced expression of Nav1.8 and Nav1.3 sodium channel proteins, which are important for generating action potentials and determining the excitability of sensory neurons, was also measured in DRG of aged mice.[27] Similar to TRPV1, these changes in expression may reflect a reduced growth-factor signaling environment due to age-related changes in the target tissue or associated glial support cells. This putative mechanism of control raises the possibility of improving somatosensory function through therapeutic interventions that restore in a specific manner growth-factor signaling to levels present in young adult systems.

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