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Age-Related Loss of Spiral Ganglion Neurons

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Abstract

Spiral ganglion neurons (SGNs) are the relay station for auditory information between hair cells and central nervous system. Age-related decline of auditory function due to SGN loss can not be ameliorated by hearing aids or cochlear implants. Recent findings clearly indicate that survival of SGNs during aging depends on genetic and environmental interactions, which can be demonstrated at the systemic, tissue, cellular, and molecular levels. At the systemic level, both insulin/insulin-like growth factor-1 and lipophilic/steroid hormone pathways influence SGN survival during aging. At the level of organ of the Corti, it is difficult to determine whether age-related SGN loss is primary or secondary degeneration. However, a late stage of SGN degeneration may be independent of age-related loss of hair cells. At the cellular and molecular level, several pathways, particularly free radical and calcium signaling pathways, can influence age-related SGN loss, and further studies should determine how these pathways contribute to SGN loss, such as whether they directly or indirectly act on SGNs. With the advancement of recent genetic and pharmacologic tools, we should not only understand how SGNs die during aging, but also find ways to delay this loss.

Keywords

Spiral ganglion neuron; hair cell; neural presbycusis; aging; glucocorticoid; caloric restriction; calcium

1. Introduction

Functional decline of the nervous system is a cardinal feature of aging. In the central nervous system, loss of neuronal connections rather than loss of neurons may be the major cause of age-related functional decline (Morrison and Hof, 2007; Rapp and Gallagher, 1996; Scheff and Price, 2003). In the peripheral nervous system, however, age-related loss of neurons significantly contributes to functional decline (Coggan et al., 2004; Rattner and Nathans, 2006; Thrasivoulou et al., 2006). In the cochlea, age-related loss of hair cells and SGNs is a major contributor to age-related hearing loss (presbycusis). Presbycusis is the third most common disability of the elderly in our society today, affecting about half of the population over 75 years old (Gates and Mills, 2005). Currently, there is no effective medication to prevent or treat presbycusis. Although cochlear implants can effectively replace the mechanosensory transduction function of lost hair cells by providing direct electrical stimulation of SGNs, this

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technique can only be successful when enough SGNs remain (Roehm and Hansen, 2005). Thus, with the continuous increase of the elderly population around the world, there is an urgency to understand the etiologies of age-related SGN loss.

Age-related loss of SGNs is consistently observed in humans and animals. In humans, age-related loss of SGNs with a relative preservation of the organ of Corti is classified as neural presbycusis (Schuknecht, 1964; Schuknecht and Gacek, 1993). Not all SGN loss is debilitating, or necessarily merits a label of neural presbycusis. We are born with a substantial surfeit of neurons that may be ten times the number needed to detect sounds, and perhaps twice the number needed for fine frequency discrimination (Ohlemiller and Frisina, 2008). A meaningful label of neural presbycusis requires an accelerated loss of SGNs that progressively impairs sound perception at a rate that exceeds the overall `biological age' of the individual. The early manifestations of this may include decreased speech intelligibility (especially in noisy environments), poor signal-to-noise ratios, and impaired frequency resolution (Pauler et al., 1986; Schuknecht and Gacek, 1993). The latter problem may contribute to impaired ability to identify or localize natural sounds, and likely alters the perceptual quality of music. The fact that not everyone exhibits neural presbycusis suggests that both genetic and environmental factors contribute this condition. Thus, the survival of SGNs during aging is largely established by genetic and environmental interactions. These complicate interactions can be manifested at the systemic, organ, and molecular levels.

2. Interactions at the systemic level

Because age is the strongest predictor of SGN survival, signaling pathways impacting aging of the whole organism could influence age-related SGN loss. Two key molecular pathways have been identified by genetic studies in model organisms such as *Caenorhabditis elegans* and *Drosophila* (Broue et al., 2007; Giannakou and Patridge, 2007; Guarente and Kenyon, 2000). These are the insulin/insulin-like growth factor-1 (IGF-1) pathway and lipophilic/steroid hormone pathway.

IGF-1 regulatory pathway and caloric restriction

Extensive studies clearly demonstrate the important role of the insulin/IGF-1 pathway in the control of vertebrate life span (Bartke, 2006; Mair and Dillin, 2008), and its roles in the survival of neurons. One effective way to modulate the IGF-1 pathway is caloric restriction (CR). CR is one major nongenetic manipulation clearly shown to extend life span in various species (Berg and Simms, 1960; McCay et al., 1935; Weindruch and Walford, 1982). CR results in dramatic alterations of the level of IGF-1 and regulates the neuroendocrine axis during aging (Sonntag et al., 1999). CR acts via IGF-1 to enhance plasticity of the brain (Mattson et al., 2002), and delays age-related neuronal loss in the enteric nervous system (Cowen et al., 2002; Thrasivoulou et al., 2006). However, a decrease of synaptic connections under CR was also reported (Shi et al., 2002).

Besides acting on the insulin/IGF-1 pathway to delay age-related functional decline of the nervous system, CR also has effects on a wide variety of genetic components that can be grouped into two general categories: (1) influencing mitochondrial function, leading to decreased production of reactive oxygen species (ROS) and increased energy output; (2) regulating gene expression, resulting in increased levels of neuroprotective factors such as neurotrophins and molecular chaperones, and decreased activity of pro-apoptotic and inflammatory factors.

Early studies showed that CR can delay age-related hearing loss in AU/Ss and CBA/J mice, but not in AKR/J mice (Henry 1986; Sweet et al., 1988), suggesting an interaction between diet and genetic variables. In an extensive study of CR effects on cochlear aging in five inbred

mouse strains and ten F1 hybrid strains (Willott et al., 1995), CR was found to reduce ABR threshold shifts during aging in three of the hybrids, and to ameliorate age-related SGN loss in C57BL/6 mice, consistent with other reports (Park et al., 1990; Someya et al., 2007; Yomasoba et al., 2007). CR also reduced age-related decline of auditory function in rats but not in rhesus monkeys (Seidman, 2000; Torre et al., 2004). These studies suggest that CR can delay age-related hearing loss, but its efficacy may require specific alleles of genes. It will be interesting to test whether CR can delay presbycusis in certain human populations.

Lipophilic/steroid hormones

The role of lipophilic/steroid hormones in age-related degenerative processes of vertebrates is complicated (Russell and Kahn, 2007; Jin et al., 2009). However, it has been shown that one class of vertebrate adrenal steroid hormones, glucocorticoids, has detrimental effects on neuronal function during aging (Lanfield et al., 2007; Miller and O'Callaghan, 2005; Sapolsky et al., 1986). Glucocorticoids have a broad array of biological functions. In the brain, the increase of glucocorticoids contributes to age-related functional decline such as loss of memory (McEwen et al., 1992; McEwen, 2005; McEwen, 2008; Sandi and Pinelo-Nava, 2007). Most studies in this area have focused on the hippocampus, a neural structure important for learning and memory. Three main effects of glucocorticoids are reported: (1) atrophy of neuronal processes such as CA3 apical dendrites; (2) inhibition of adult neurogenesis at the dentate gyrus; and (3) decreased ability of hippocampal neurons to survive further insults. Excessive glucocorticoids may also kill hippocampal neurons, although this finding has been questioned with the emergence of unbiased stereology for neuronal counting (Sandi and Pinelo-Nava, 2007). Nonetheless, the potential loss of hippocampal neurons due to prolonged age-related increases of glucocorticoids raises the possibility of it also contributing to age-related SGN loss.

To date, no direct evidence has linked excess glucocorticoids during aging to neural presbycusis in humans. A high level of aldosterone, a mineralocorticoid, is found to correlate with better hearing during aging, perhaps through an effect on the cochlear lateral wall (Tadros et al., 2005). Accelerated loss of SGNs during aging is found in mice lacking the β2 subunit of nicotinic acetylcholine receptor (Bao et al., 2005). This is noteworthy for the present discussion because age-related increase of serum corticosterone, a major glucocorticoid, has also been found in these mice (Zoli et al., 1999). Recently, a similar acceleration of age-related SGN loss is reported in mice lacking the nuclear factor-κB (NFκB; Lang et al., 2006). Nuclear translocation of NFκB in SGNs appears to be under glucocorticoid control (Tahera et al., 2006). Together these studies suggest a potential role for glucocorticoids in the age-related loss of SGNs (Jin et al., 2009). In consideration of the fact that synthetic glucocorticoids are frequently used therapeutically for many pathological conditions including diseases of the inner ear, possible roles of the glucocorticoid signaling pathway in age-related hearing loss should be studied further.

3. Interactions at the organ level

Studies of aging human and animal cochleae have typically shown mixed pathology in the organ of Corti, SGNs, and lateral wall (Adams and Schulte, 1997; Bohne et al., 1990; Schuknecht, 1964; Schuknecht and Gacek, 1993; Sha et al., 2008; Shimada et al., 1998). The frequent co-degeneration of distinct cochlear cells and structures presents a challenge, as it will ultimately be important to determine whether, and under what conditions, pathology of hair cells, neurons, and lateral wall are causally linked (Ohlemiller, 2004). Loss of hair cells may often be the main cause of age-related SGN loss, and it is imperative to distinguish between neuronal loss as a primary versus secondary degeneration (Schacht and Hawkins, 2005; Ohlemiller and Frisina, 2008). After chemical or mechanical damage to hair cells, SGNs begin to die, albeit at a highly species-dependent rate. That fact the SGNs reliably disappear is

consistent with the notion that hair cells provide SGNs with trophic support (Ernfors et al., 1995; Fritzsch et al., 1997; Takeno et al., 1998).

Loss of SGNs without associated loss of hair cells is common among mammals during aging (Keithley and Feldman, 1979; Keithley et al., 1989; Linthicum and Fayad, 2009; Ryals and Westbrook, 1988; Suzuka and Schuknecht, 1988; White et al., 2000). Moreover, apparent primary and secondary degeneration of SGNs may coincide in the same cochlea (e.g., Hequembourg and Liberman, 2001). It is thus possible that age-related SGN and hair cell loss operate in parallel by independent mechanisms. It is difficult, however, to rule out hair cell causes definitively. For example, C57BL/6 mice carry a mutation (*Cdh23*^{Ahl}) that promotes progressive hair cell loss (Ohlemiller, 2004). Ultrastructural signs of synaptic pathology—a likely precursor to neuronal loss—can be found in these mice prior to overt hair cell loss (Stamacki et el., 2006). This may reflect an early and subtle aspect of *Cdh23*^{Ahl}-related hair cell degeneration. Interestingly, primary degeneration of SGNs has been observed in the cochlea of CBA/CaJ mice after mild noise exposure (Kujawa and Liberman, 2006). This suggests a link between noise exposure—especially early exposure—and later apparent neural presbycusis. Noise may produce slight pathology of hair cells that that interferes with critical trophic support hair cells normally provide. Even if age-related loss of SGNs can occur in the presence of normal hair cells, other cell types in the cochlea may contribute. For example, the primary loss of SGNs in the cochlear apex of mice correlates with degenerative changes in pillar cells and even Reissner's membrane (Ohlemiller and Gagnon, 2004). This was interpreted in terms of multiple abnormal processes operating in the local environment of afferent dendrites, including ion dysregulation and loss of trophic interactions between supporting cells and neurons. In summary, even in the case of clear hair cell loss, true primary versus secondary neuronal loss may be impossible to separate at the early degeneration stage. At the later stages, certain independent mechanisms may contribute to the uncoupling of age-related loss of hair cells and SGNs.

4. Interactions at the cellular and molecular level

Based on the mouse transgenic approaches, several molecular cascades have been implicated in age-related SGN loss (Bao et al., 2005; Keithley et al., 2005; Lang et al., 2006; McFadden et al., 1999; Nelson et al., 2007). Significant loss of SGNs observed in mice lacking copper/zinc superoxide dismutase (Keithley et al., 2005; McFadden et al., 1999). This enzyme is the first-line defense against oxidative damage caused by ROS. Although extensive loss of hair cells is also observed in these mice, age-related loss of SGN fibers occurs prior to the hair cell loss. The finding clearly points to a role for oxidative metabolism in age-related SGN loss. Because age-related changes in the mitochondrial electron transport chain can increase free radical generation, many studies have focused on the role of mitochondria in presbycusis (Pickles, 2004). Generation of ROS by mitochondria can promote injury throughout the entire cell, but may critically promote further injury to the mitochondria themselves as part of an accelerating process. An increase in mutations in mitochondrial DNA is found in samples from people with presbycusis (Bai et al., 1997). In knock-in mice in which base substitutions impair the proofreading ability of mitochondria DNA polymerase, age-related loss of SGNs is more severe than the control mice (Niu et al., 2007; Yamasoba et al., 2007). Notably, CR retards the deterioration of mitochondrial respiratory functions during aging (Feuers, 1998). In addition to ROS generation, mitochondria also play a key role in apoptosis and cell calcium signaling, so that mitochondrial injury may promote presbycusis via multiple pathways at multiple levels including at the systemic level.

As noted above, we observed an accelerated age-related SGN loss in mice lacking the β2 subunit of nicotinic acetylcholine receptor (Bao et al., 2005). While an indirect effect mediated by elevated systemic glucocorticoids may be the primary tie, other causal links are possible.

Because the ion channel containing this subunit is highly permeable to calcium, and calcium dysregulation is linked to neuronal death during aging, altered calcium signaling could contribute. Consistent with these two possibilities, an accelerated loss of SGNs is also observed in mice lacking NFκB, which is a key signaling molecule in both calcium and glucocorticoid signaling pathways (Lang et al., 2006). Recently, a selective loss of support cells, hair cells, and SGNs was found in mice lacking in Fbx2, a ubiquitin ligase F-box protein with specificity for high-mannose glycoprotein (Nelson et al., 2007), which suggests the components for monitoring protein structural integrity are also essential for the survival of SGNs during aging. However, all of these findings are based on transgenic mouse models in which the gene of interest is modified not only in SGNs but also in other cell types. Therefore, we cannot address whether the lack of these genes has direct effects on SGN loss, or acts at the systemic level (such as on the rate of aging) to delay age-related loss of SGNs. Future development of animal models able to conditionally modify gene expression only in SGNs would greatly help to address this issue.

5. Apoptosis and age-related SGN loss

By what process do SGNs die during aging? In general cells may die by either passive or active processes (Kerr et al., 1972). Necrosis is a passive process characterized by swelling and rupture of the cell body and release of intracellular contents. Apoptosis is an active form of cell death characterized by a shrunken cell body and masses of condensed DNA. Recently a third type of cell death has been claimed for outer hair cells (OHCs; Bohne et al., 2007). It can be difficult to distinguish clearly between forms of cell death *in vivo* during aging (Bohne et al., 2007; Choi, 1996; Wood et al., 1993). Neurons may show most hallmarks of apoptosis, but fail to show key properties such as DNA laddering or condensation during death (Cohen et al., 1992; Yuan et al., 2003). Nevertheless, the current view is that most of the cell death during aging occurs via apoptosis, whether in the brain (Mattson, 2002; Pollack and Leeuwenburgh, 2001) or cochlea (Alam et al., 2001, Nevado et al., 2006, Someya et al., 2006).

Active cell death requires synthesis of new proteins and a programmed biochemical cascade. This cascade has been elegantly revealed by studies on *Caenorhabditis elegans*. Genetic analysis in this worm has identified several key cell-death (CED) genes (Hengartner and Horvitz, 1994; Metzstein et al., 1998; Yuan et al., 1993; Yuan and Horvitz, 2004). The mammalian counterpart of CED-9, is Bcl-2. In the auditory system, over-expression of Bcl-2 in transgenic mice prevents apoptosis in afferent deprivation-induced neuronal death of the anteroventral cochlear nucleus and aminoglycoside-induced hair cell death (Cunningham et al., 2004; Mostafapour et al., 2002). In contrast, Bax is a proapoptotic member of the Bcl-2 family (Deckwerth et al., 1996; Sun and Oppenheim, 2003; White et al., 1998), and deletion of the Bax gene reduces the incidence of naturally occurring neuronal apoptosis during development (White et al., 1998). Since it is still uncertain whether there is age-related neuronal loss in the central nervous system, it is difficult to interpret means of changing expression levels of various apoptotic genes in the brain during aging (Pollack and Leeuwenburgh, 2001; Sastry and Rao, 2000). However, in the cochlea, age-related loss of hair cells and SGNs are consistently observed across species (Dazert et al., 1996; White et al, 2000). Several studies implicate the role of apoptosis in age-related loss of hair cells and SGNs. Using the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) method, studies found the presence of the DNA fragmentation in the hair cells and SGNs during aging (Jokay et al., 1998; Someya et al., 2007; Usami et al., 1997). Further evidence to support age-related loss of hair cells and SGNs through apoptosis has come from an association of aging with the expression of apoptosis-related proteins in the cochlea (Alam et al., 2001; Nevado et al., 2006). An further indirect piece of evidence is a significant reduction in the number of TUNEL-positive cells and cleaved caspase-3-positive cells in the cochleae from mice under CR (Someya et al., 2007), and a significant increase in the number of TUNEL-positive cells

and activated caspase-3-positive cells in mice with the mutated mitochondrial DNA polymerase (Niu et al., 2007; Yamasoba et al., 2007). However, TUNEL is not absolutely specific for apoptotic cells; nuclear fragments from necrotic or autolytic cells may also be TUNEL-positive (Ben-Saddon et al., 1995; Kressel and Groscurth, 1994; Nishizaki et al., 1999). Furthermore, loss of hair cells and SGNs is found at one month-old mice lacking caspase-3, a key downstream caspase in the apoptotic cascade, which suggests that activated caspase-3 may not be essential for the death of hair cells and SGNs (Takahashi et al., 2001). Given the technical difficulty of identifying apoptotic cells *in vivo* during aging, it would be informative to examine whether there is a delay of age-related SGN loss by utilizing both Bax knockout (Bax−/−) and Bcl-2 over-expressing mice

6. Conclusions

Although age-related loss of SGNs is consistently observed in humans and animals, its underlying mechanisms are only partially characterized. Recent studies in the field of aging provide a new framework for exploring mechanisms underlying age-related SGN loss at the systemic, organ, and cellular levels. At the systemic level, both insulin/IGF-1 and steroid hormone pathways may play roles. Caloric restriction can effectively delay age-related SGN loss in animals under certain genetic backgrounds, and should be examined in humans. Because of the wide clinical use of synthetic glucocorticoids, it is urgent to determine whether they may exert harmful effects on the survival of SGNs during aging. At the organ level, age-related loss of SGNs can occur without hair cell loss, but in that case, may reflect subtle hair cell or supporting cell pathology. At the cellular/molecular level, overall mitochondrial `health' may set the rate of age-related SGN loss. Other signaling pathways such as calcium, glucocorticoids, and protein `quality controls' (e.g, ubiquitins) signaling pathways have also been implicated in SGN survival. However, new transgenic models with the ability to modify gene expression only in SGNs are needed to address whether these signaling pathways have direct effects on SGNs during aging. Finally, the issue on whether age-related SGN loss occurs via typical apoptotic pathways needs to be further examined.

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Abbreviation List

SGNs	spiral ganglion neurons
IGF1	insulin-like growth factor-1
CR	caloric restriction
ROS	reactive oxygen species
NFκB	nuclear factor-κB
OHCs	outer hair cells
TUNEL	terminal deoxynucleotidyl transferase-dUTP-biotin nick end-labeling

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