

Short communication

Growth-associated proteins and regeneration-induced gene expression in the aging neuron

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Abstract

Axonal elongation and sprouting during regeneration are retarded with aging but the etiology of this is unclear. We investigated whether this age-associated decline is related to a decline in expression of three different growth-associated proteins (GAPs): α_1 -tubulin, neurofilament (NF) light subunit (NF-L) and GAP-43. Northern analysis was performed on L4–L5 dorsal root ganglia (DRG) of young (3 months) and aged (23 months) rats following a sciatic nerve crush and compared to their age-matched controls. The results show that initial mRNA levels of α_1 -tubulin and NF-L in the control aged rat DRG were half those of the control young adults, whereas expression of GAP-43 was unchanged. Two weeks after axotomy, the expression of α_1 -tubulin and GAP-43 in the aged DRG was induced to the same levels as in the axotomized young adult, and the expression of NF-L decreased proportionately in both age groups. These results indicate that certain neuronal mRNAs, such as α_1 -tubulin and NF-L may be maintained at lower levels in aging DRG neurons, whereas others, such as GAP-43 appear to be unaltered. However, during regeneration, the aging DRG neuron appears capable of inducing α_1 -tubulin, NF-L and GAP-43 as well as the young adult. © 2004 Elsevier Ireland Ltd. All rights reserved.

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1. Introduction

Axotomy of peripheral mammalian axons induces a variety of metabolic changes in the neuronal cell body, which support the process of axonal regeneration (Griffin and Hoffman, 1993). During this process daughter axons sprout, elongate distally, restore functional connections of their peripheral axons, and finally mature via radial growth. The neuronal cytoskeleton plays an essential role throughout regeneration by providing the basis of motility, structure, and stabilization of the newly formed axons. In order to accommodate the intense demands placed upon a regenerative neuron, major changes in the biosynthesis and export of cytoskeletal and membranal components, such as microtubules, growth-associated proteins (GAPs), and neurofilaments (NFs) must occur (Fu and Gordon, 1997; Chao, 2003; Goldberg, 2003).

Regeneration in young animals is associated with a characteristic profile of gene expression that, in general, is similar to the profile seen during development. In both of these instances, substances that are abundant in elongating axons, such as tubulin and GAPs, have their synthesis enhanced (Oblinger and Lasek, 1988; Fu and Gordon, 1997; Gervasi et al., 2003; Goldberg, 2003). In contrast, substances that are less important for axonal elongation, such as certain NF and neuronal intermediate filament (nIFs) proteins, which regulate axonal caliber, have their synthesis downregulated until the target tissue is reached (Hoffman et al., 1987; Goldstein et al., 1988; Oblinger and Lasek, 1988; Scott et al., 1991; Fu and Gordon, 1997). At the time of reinnervation the NF mRNA and protein levels recover and contribute to the maturation and increase in axon diameter (Hoffman et al., 1987; Scott et al., 1991; Fu and Gordon, 1997).

The age of the animal influences both the ability of the neuron to survive after axotomy and the rate of regenerative outgrowth (Tanaka and Webster, 1991; Zhou et al., 2002; Goettl et al., 2003). Axotomy during embryonic and early post-natal development generally results in neuronal death,

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whereas axotomy after that period is more often associated with neuronal survival and effective axon regeneration (Griffin and Hoffman, 1993). However, following axotomy in aged animals, the process of peripheral regeneration and recovery of function is delayed, although it may eventually be as complete as in younger animals (Vaughan, 1992). The reason for this delay is uncertain but may be related to factors extrinsic to the neuron, including the interactions with supporting (Vaughan, 1992; Fu and Gordon, 1997; Chao, 2003) and target cells (Zhou et al., 2002; Chao, 2003), as well as the presence of required growth-stimulating signals (Bomze et al., 2001; Dechant and Barde, 2002; Chao, 2003); or factors intrinsic to the neuron such as alterations in normal cellular function or in the control of gene transcription (Vaughan, 1992; Namaka et al., 2001; Mori et al., 2002; Mori and Morii, 2002). The present study examines the effects of advancing age on neuronal mRNA expression of three different GAPS in dorsal root ganglion (DRG) neurons during peripheral nerve regeneration.

2. Materials and methods

Three young adult male Fischer 344 rats and three controls (Harlan Sprague–Dawley, Indianapolis, IN), aged 3 months and weighing on average 197 ± 12 g were compared with three adult rats aged 23 months and three controls, weighing on average 395 ± 11 g. Rats were anesthetized (chloral hydrate; 400 mg/kg, intraperitoneally) and the sciatic nerves crushed bilaterally at midhigh level as previously described (Scott et al., 1991). Fourteen days later the rats were killed (chloral hydrate; 800 mg/kg, intraperitoneally) and the L4–L5 DRG removed, weighed, and frozen at -80°C for Northern analysis.

Three probes were used: a 2 kb mouse NF-L cDNA; a 1.1 kb rat GAP-43 cDNA encompassing the full coding region; and a 192 bp MboII fragment of a mouse α_1 -tubulin cDNA consisting exclusively of the 3' untranslated region. This region is specific for α_1 -tubulin and shows extensive homology among species. The cDNAs were amplified, purified, and labeled with [α - ^{32}P] dCTP as previously described (Scott et al., 1991).

Total RNA was isolated using the acid guanidinium thiocyanate–phenol–chloroform method (Chomczynski and Sacchi, 1987). Gel fractionation transfer to nylon membrane and Northern analysis were performed as previously described (Scott et al., 1991). Hybridization was performed at high stringency (hybridized at 60°C and washed at 65 – 70°C , 0.1 standard saline citrate (SSC); IXSCC = 0.15 M sodium chloride and 0.15 M sodium citrate), and the membranes exposed to radiographic film for 16–24 h.

The density and area of specific bands on autoradiograms were quantitated by video densitometry (Bioquant System IV; R&M Biometrics, Inc., Nashville, TN) as previously described (Parhad et al., 1995). The ethidium bromide-stained 28S and 18S RNA bands on the filters were similarly quan-

titated, and the sum of the two bands was used as the denominator for the Northern blots. For each mRNA, the data were normalized to the value of the 3-month DRG sample. An additional filter containing mRNA from all 3-month DRG samples was used to normalize the samples to their mean. Therefore, all values were normalized to the mean of 3-month DRG samples. The data were expressed as mean and standard error of the mean (S.E.), and analyzed with a nonparametric test for comparison of multiple samples (Mood median test) using the Minitab statistics package (Minitab, State College, PA). A *P*-value of 0.05 was considered statistically significant.

3. Results

The amount of RNA recovered from the control and axotomized L4–L5 DRG did not differ for the 3 month or 23 month animals (3 month control, 4.62 ± 0.44 ug/DRG; experimental, 4.59 ± 0.16 ug/DRG; 23 month control, 4.69 ± 0.49 ug/DRG; experimental 4.32 ± 0.19 ug/DRG; Mood median test, $P > 0.05$).

Northern analysis showed marked reductions in α -tubulin and NF-L mRNA levels between 3 and 23 months, decreasing by 40 and 60%, respectively (Figs. 1 and 2). In contrast, the mRNA levels of GAP-43 were not altered with aging (Figs. 1 and 2).

In young adult rats, there was an induction of α_1 -tubulin and GAP-43 mRNA expression, and a decline in the NF-L mRNA level following axotomy (Figs. 1 and 2). This regenerative expression profile was similar to that seen in the aged DRG. Following axotomy in aged rats, α_1 -tubulin and GAP-43 mRNA levels were induced to the maximum levels seen in young rats, even though the baseline level of α -tubulin began much lower in the aged DRG (Figs. 1 and 2). The NF-L mRNA levels declined proportionately in aged and young rats after axotomy, despite the fact that the baseline levels were lower in the aged animal (Figs. 1 and 2). Thus, the final absolute level of NF-L mRNA was lower in aged than young adult axotomized rats.

4. Discussion

The present study evaluated the neuronal gene expression of cytoskeletal and membranal components in DRG sensory neurons of young adult and aged adult rats following axotomy. We have shown that the aged mammalian peripheral neuron can respond to the metabolic challenge that follows axotomy by appropriately regulating the expression of several of its genes necessary for successful nerve regeneration. In particular, the results indicate that at 14 days post-axotomy, the induced response in aged animals of α -1 tubulin and GAP-43 mRNA levels is identical to that seen in the young adult. The degree of decrement in NF-L mRNA levels post-axotomy is also similar between the

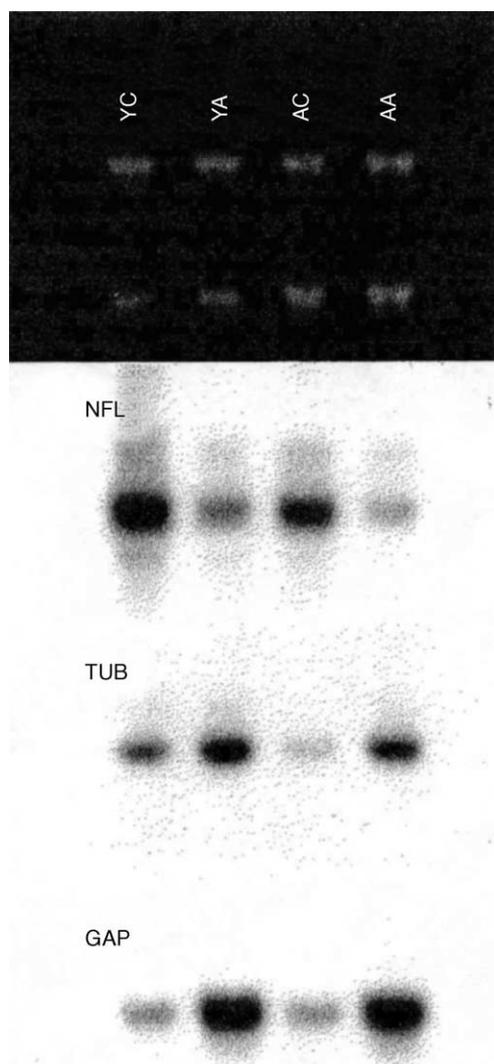


Fig. 1. Northern analysis of young adult and aged rat DRG 14 days following axotomy. Top panel: total RNA (5 µg) from young (3 month) control (YC), young axotomized (YA), aged (23 month) control (AC), aged axotomized (AA) DRG, separated on an agarose/formaldehyde gel with ethidium bromide stain. Note that the 28S and 18S RNA ribosomal bands reflect equal loading of total RNA in each lane. Bottom panel: This gel was sequentially hybridized to α_1 -tubulin (TUB, 1.8 kb mRNA), GAP-43 (GAP, 1.4 kb mRNA, and N-FL (N-FL, 4.0 and 2.5 kb mRNAs).

different age groups, although initial levels are lower in aged animals.

These results suggest that, although differences may exist in the constitutive expression of these genes with advancing age, the aged neuron is as capable as the young adult in regulating its gene expression following axotomy with respect to these particular genes in this animal model. Weaknesses of this study include lack of corroborating evidence from Western blot analysis and the small number of genes studied. Microarray studies may also help further define regeneration-induced gene expression for multiple genes in the aging rat (Kubo et al., 2002; Vijg and Suh, 2003).

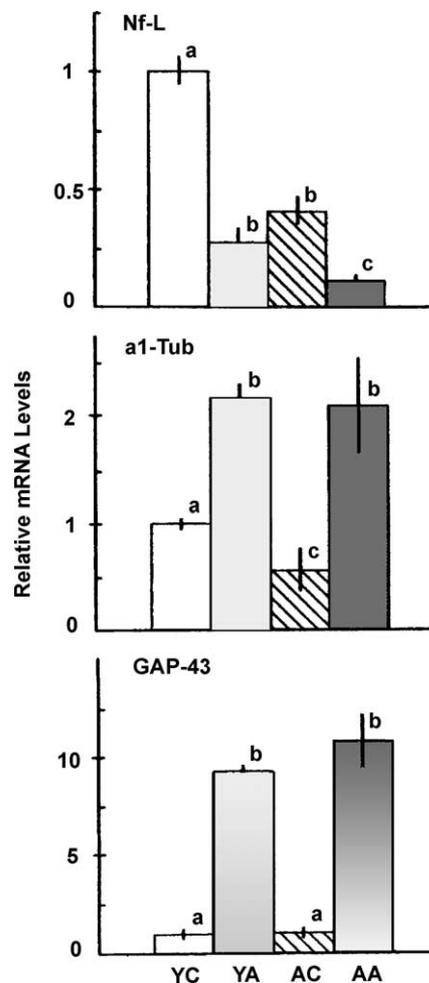


Fig. 2. Quantitative Northern analysis of control and axotomized DRG. Autoradiograms of α_1 -tubulin, GAP-43, and NF-L were quantitated; the levels for young axotomized (YA), aged control (AC), and aged axotomized (AA) rats were normalized to the young control (YC). The vertical lines are the SE. In each graph, the letters (a–c) express statistical significance (Mood median test, $P < 0.05$) such that values that do not share a letter are significantly different.

Several other factors may contribute to the diminished rate of axonal regeneration observed with aging. These include: gene silencing (Burzynski, 2003), other programmed senescent decreases in gene transcription (Roy et al., 2002; Hasty et al., 2003), changes in chromatin/DNA structure (Ikura and Ogryzko, 2003; Vaquero et al., 2003), diminished levels of RNA polymerase (Venugopal and Roa, 1991; da Silva et al., 2000), accumulation of metabolic byproducts (Thomas et al., 1993; Bendiske et al., 2002), problems with axonal transport (Galbraith and Gallant, 2000), lack of trophic factors (Verdu et al., 2000; Hall et al., 2001; Chao, 2003), or other mechanisms.

From a medical perspective the mechanisms of aging nerve regeneration are of immense importance. However, knowledge of age-related effects on regeneration of diseased and injured peripheral nerves is lacking. Studies are needed which examine the specific cellular processes that enable

neural systems to maintain or enhance function following injury. These studies may further our ability to understand and possibly intervene in the capacity of the nervous system to compensate for age-associated neural deficits. It is clear that aged neurons regenerate at a slower rate than young adult neurons but the mechanisms are likely to be complex.

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