The effects of age and nerve transection on mRNA expression of the nicotinic acetylcholinergic receptor subtypes and muscle regulatory factors

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Introduction: During aging, the neuromuscular junction (NMJ) undergoes degenerative changes typified by denervation, reinnervation and remodeling. This denervation and reinnervation of the muscle leads to permanent changes in the NMJ and muscle. Following trauma, denervation greater than 1 month leads to profound impairments of neuromuscular recovery. One theory of this impairment is that there are permanent changes at the NMJ after 1 month that precludes successful innervation (1). Immediately, following denervation, there is a de-clustering of the nAChRs, a shift to the embryonic pattern of nAChR subtype expression and spreading out of the area of acetylcholine sensitivity. This allows reinnervating axons to induce creation of a NMJ upon contact with the muscle and contributes to successful neuromuscular reinnervation. Myogenic regulator factors (MRFs) stimulate myoblast formation, satellite cell proliferation and regulate NMJ subtype expression. The purpose of the current study was to examine nAChR subtype and MRF expression profile due to aging and to characterize the response to transient denervation. The hypothesis was that aging would significantly impair the acute neuroprotective and myogenic response following transection.

Materials and Methods: Sixty-eight male Fischer x Brown Norway rats were obtained from the NIA at 4 or 24 months of age. Under anesthesia, the left tibial nerve was transected 1 cm proximal to its insertion into the gastrocnemius muscle. The nerve was immediately repaired using 9-0 nylon. At 1, 2, 4, 8 or 16 weeks following transection and repair, the animal was anesthetized and the gastrocnemius muscle was harvested, frozen in liquid nitrogen, homogenized and total RNA was extracted. mRNA was transcribed to cDNA using Superscript II (Invitrogen). Taqman® gene expression assays were used to perform qRTPCR. Muscle creatine kinase and GAPDH were used as endogenous controls. Two-way ANOVA was performed with transformations to ranked sums when appropriate. Post-hoc pairwise comparisons were made with Student-Newman-Keuls test.

Results: For normal muscle, there was a significant increase in alpha, gamma and delta-nAChR mRNA expression in aged animals compared to young animals. For the MRFs, there was a significant increase in MRF4 and myogenin in aged animals compared to young animals. At 2 weeks following injury the young animals had significantly more alpha, delta and epsilon—nAChR expression. At 4 weeks, the trend shifted, with the aged animals showing significantly more expression of alpha, beta, gamma and epsilon. By 16 weeks following injury, expression levels in young animals declined but there is a persistent elevation of these factors in aged animals. The persistent elevation at 4 weeks may be due to slower regeneration in the aged animals, thus persistence of the denervation phenotype. However, the lesser response to transection at 2 weeks clearly indicates an impaired acute response to injury. Thus, age significantly impacts the neuromuscular response to injury by inhibiting full upregulation of genes that are critical for NMJ stabilization, muscle repair and muscle regeneration.

Discussion: This study found that during normal aging, there is a slight elevation in alpha, gamma and delta-nAChR mRNA expression in aged animals compared to young animals. For the MRFs, there was a significant increase in MRF4 and myogenin in aged animals compared to young animals. At 2 weeks following injury the young animals had significantly more alpha, delta and epsilon—nAChR expression. At 4 weeks, the trend shifted, with the aged animals showing significantly more expression of alpha, beta, gamma and epsilon. By 16 weeks following injury and repair, there was return to the pre-injury phenotype. For the MRFs at 2 weeks post injury, young animals had significantly higher expression of MRF4, Myf5, myogenin and MyoD. At 4 weeks post injury, this pattern was reversed. At the 8 and 16 week time point, there was no difference between young and aged animals.

Discussion: This study found that during normal aging, there is a slight elevation in alpha, gamma and delta-nAChR, as well as MRF4 and myogenin. This is most likely due to a low level of denervation and reinnervation in aged muscle. Immediately following transection there is a dramatic increase in expression of all nAChR subunits and the MRFs. However in aged muscle, there is less of a response. At 4 weeks following injury, expression levels in young animals declines but there is a persistent elevation of these factors in aged animals. The persistent elevation at 4 weeks may be due to slower regeneration in the aged animals, thus persistence of the denervation phenotype. However, the lesser response to transection at 2 weeks clearly indicates an impaired acute response to injury. Thus, age significantly impacts the neuromuscular response to injury by inhibiting full upregulation of genes that are critical for NMJ stabilization, muscle repair and muscle regeneration.


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