Modulation of nerve fibers in the rat thyroarytenoid muscle following nerve injury

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ABSTRACT

Oxidative stress: To determine the regeneration process of nerve fibers in the thyroarytenoid (TA) muscle following transection and immediate anastomosis of the recurrent laryngeal nerve (RLN) or vagus nerve (VNH), and to compare the differences between RLN and VNH. Modulation of nerve fibers in the thyroarytenoid (TA) muscle following the same procedure on the peroneal nerve (PN) was also examined. Methods: Three types of animal model were prepared: an RLN-anastomosis model (RLNa), a VN-anastomosis model (VNa), and a PN-anastomosis model (PNa). Animals were sacrificed at five timepoints following the procedure. The modulation of axons, myelin sheaths, Schwann cells (SCs), nerve terminals (NTs), and acetylcholine receptors (AchRs) in the TA muscle was examined by immunohistochemical analysis. The ratios of the expression areas in axons, myelin sheaths, and SCs, and the number of NTs and AchRs between treated (T) and untreated (U) sides (T/U ratio) were evaluated. Results: At 18 weeks, the T/U ratios of expression in RLNa were 53.7, 0, and 96.4% in SCs; 93.6% in VTs; and 99.4, 67.0, and 101.2% in AchRs. Conclusion: Regeneration of intramuscular nerve fibers occurred more actively in RLNa than in VN. Remyelination took place only in RLNa.

INTRODUCTION AND PURPOSE

Few reports have scrutinized changes in intramuscular nerve fibers and the nerve muscle junction, following RLN or VN injury. Dynamic modulation of the regenerative process of RN fibers consisting of axons, myelin sheath, and Schwann cells (SCs) in the intramuscular muscle remains to be examined. The primary purpose of this study was to elucidate the relationship between the location of nerve damage (RLN or VN) and the degree of nerve fiber regeneration and remyelination. We also compared the nerve fiber changes in the TA muscle following the same procedure on the peroneal nerve (PN) and immediate anastomosis of RLN or VN. In addition, the regenerative process of the nerve fibers in the thyroarytenoid (TA) muscle following the same treatment to the peroneal nerve (PN) was examined as a typical skeletal muscle for comparison.

MATERIALS AND METHODS

Seventy-five 8-week-old female Wistar rats weighing 150–180 g were used for three models below.

1. Remyelination nerve anastomosis model (RLNa): The left RLNa was transected at a level of 10 mm distal from the left cricothyroid joint and immediately anastomosed in a silicone tube. Vagus nerve anastomosis model (VNa): The left VNa was transected at a level of 10 mm distal from the left cricothyroid joint and immediately anastomosed in a silicone tube. Peroneal nerve anastomosis model (PNa): The left PN was transected at 10 mm central from the left TA muscle and anastomosed in a silicone tube. Each group was divided into five subgroups (n = 5 each) based on post-treatment observation periods (2, 4, 7, 10, 18 weeks). Animals were sacrificed at observation periods, larvae- and both TA muscles were dissected coronal frozen sections of lateral and longitudinal frozen sections of TA muscle were made for immunohistochemistry.

Axons, myelin sheath, Schwann cells (SCs), nerve terminals (NTs) and acetylcholine receptors (AchRs) were immunolabeled.

RESULTS

In the VNa group at all time points, and in the PNa and RLNa groups 2 weeks post-treatment, no expression was found, with the exception of AchRs. In the VNa group, the T/U ratio increased to 53.7±2.1% at 18 weeks. In the PNa group, the T/U ratio increased to 93.6±6.4% at 18 weeks. Differences in the number of NTs and AchRs were evident in the VNa group at all time points, and in the PNa and RLNa groups 2 weeks post-treatment.

Myelin sheaths: The Ta/Va ratio was lower following the same procedure on the VNa, but was not significant. In the VNa group, the T/U ratio increased to 9.3±2.9 at 18 weeks. In the PN group, the T/U ratio increased to 63.4±4.4% at 10 weeks. Schwann cells: The ratio of AchRs increased in the VNa group at all time points. In the PNa group, the T/U ratio increased to 63.4±6.4% at 18 weeks. In the PN group, the T/U ratio increased to 93.6±2.9 at 18 weeks.

DISCUSSION

Differences of regenerative capacity between RLN and VN

In the present study, regeneration of axons, NTs, and motor receptors was observed in the RNa group 7 weeks post-treatment but not in the VN group. We hypothesized that these factors may contribute to the failure of regeneration following treatment of VNs.

1) Distance from the TA muscle

It is generally accepted that the degree of regeneration depends on the distance from the location of nerve damage to the target muscle (Campbell WN, 2008). After VNs, regenerating RN fibers have to grow down into the tissue with the TA versus the VN back up to the RN.

2) Low percentage of the RN in the VN (Boltehol WR, 2008)

A regenerating nerve may grow into the wrong neural tube in the distal part of the VN and not reach the TA muscle. The percentage of the nerve fibers in the muscle nucleous appears to be higher in the TA muscle than in the VN muscle. Differences in the neuronal responses in the nucleus ambiguous between the RN and VN group were possibly the reason for the differences in regeneration of nerve fibers between RN and VN.

Differences of regenerative capacity between RLN and PN

In the present study, although regeneration was not observed in the RN group, it was observed at 4 weeks in the PNa group and reached almost normal level at 18 weeks. In addition, axons and SCs appeared much earlier in the PNa group than in the RN Na group and reached a normal level (Saito K, 2003). These reports showed that in the rat, the peak diameter of myelinated axons in the normal RN is 2–3 μm (Sahlgren A, 1982), much smaller than in normal PN (4–7 μm) (Koenig-Lampski C, 1980). It is well known that the larger the nerve fiber diameter, the stronger is its regeneration capacity (Singer M, 1952). Based on these reports, we assume that the original difference in diameter resulted in the varying degrees of remyelination of RN and PN.

CONCLUSION

Our results suggest that nerve regeneration and reinnervation ability is much higher in the RN than in the VN. In addition, remyelination occurred only in PNs, not in RNa or VNa after 18 weeks. Because remyelination of the axon is indispensable for functional recovery, remyelination may be key for the development of novel treatments for RN injuries.