N-CAM and isomyosins of long term denervated human muscle

Katia Rossini, Donatella Biral, Ugo Carraro, Winfried Mayr (1), Helmut Kern (2)

Translational Myology Lab of the Interdepartmental Research Center of Myology, Department of Biomedical Science, University of Padua, Italy; (1) Center for Biomedical Engineering and Physics, Medical University of Vienna, Austria; (2) Ludwig Boltzmann Institute of Electrostimulation and Physical Rehabilitation, Department of Physical Medicine, Wilhelminenspital, Vienna, Austria.
E-mail: ugo.carraro@unipd.it

Abstract

Histochemical and immunohistochemical analyses show that the checkerboard appearance of the human denervated tissue is maintained at least up to 2 year post-SCI, but generally a fast-like ATPase staining prevails. At longer time the severely atrophic myofibers show if any, a very light staining. About 1% of the myofibers present in the cryosections are anti-MHCemb positive. These regenerated myofibers are also stained with an antibody recognizing N-CAM, an adhesion molecule restricted to the synapse in innervated myofibers, but expressed along all atrophic fibers early after denervation in rodents and humans. Since during aneural myogenesis transition from MHCemb to a fast-like isoform(s) occurs, while N-CAM restriction to synapse is inhibited, we believe that myofiber regeneration accounts for much more of the long term denervated human myofibers than now accepted. On the other hand, beside in the regenerated myofibers, the expression of N-CAM seems to be lost in the atrophic myofibers of the long-term SCI subjects in both large and small size myofibers.

Key Words: human muscle, permanent denervation of the lower extremity, FES, muscle recovery, N-CAM, myofibrillar ATPase.

Basic Appl Myol 16 (3&4): 108-110, 2006

In rodents 1-year of permanent denervation ends in lipodystrophy of the muscle tissue. Indeed the normal fascicular architecture of the muscle is lost and interstitial tissue, which includes adipocytes and sheets of collagen, dramatically increases at the expenses of the myofibers. The ATPase stainings of the residual myofibers demonstrate expression of a fast-like type of myosin, suggesting that fiber types differentiation is lost.

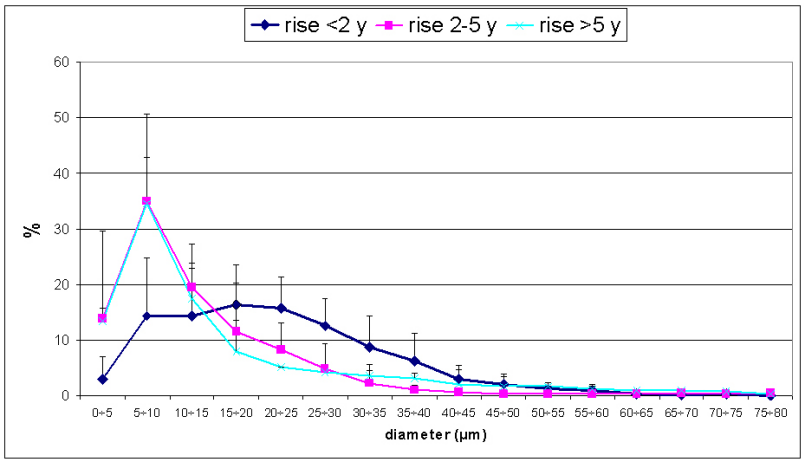

Figure 1. *Fiber size distribution at different times of denervation.*

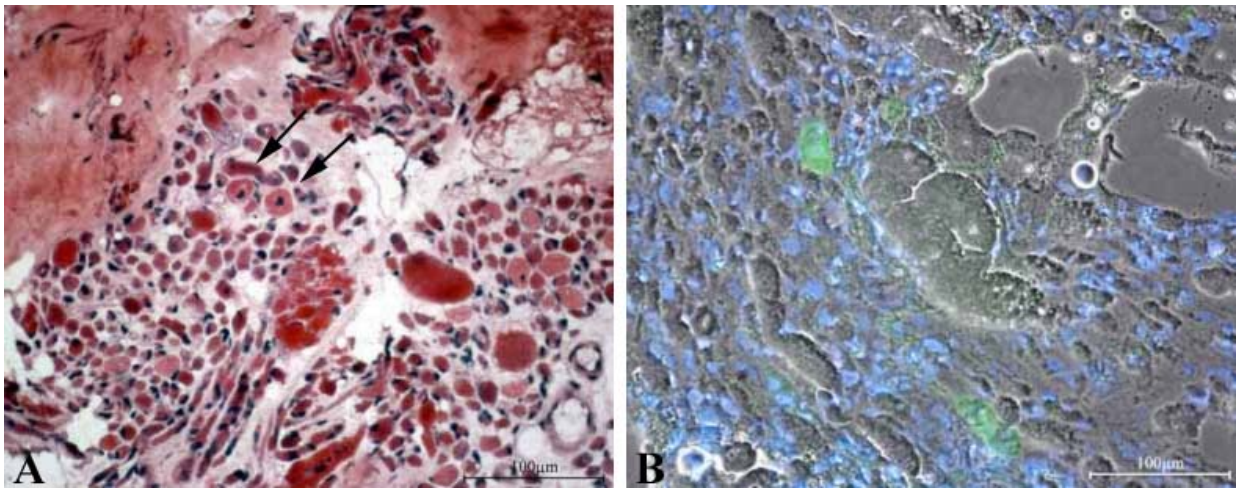

Figure 2. *Markers of myogenic events in long-term denervation of human muscle. A, Hematoxilin and eosin stain;, B, anti-MHCemb positive myofibers in skeletal muscle biopsies of 9-year human flaccid paralysis. Arrows point to small centrally nucleated myofibers.*

In particular slow isomyosin disappears and only a fast-like myosin is present in the surviving fibers. Histochemical SDH, a marker of mitochondria of oxidative myofibers, decreases and finally disappears in both slow and fast myofibers. The biopsy bank of the European Project RISE (contract n. n. QLG5-CT-2001-02191) provides for the first time the chance to study long term effects of permanent denervation on human muscle tissue after spinal cord injury (SCI).

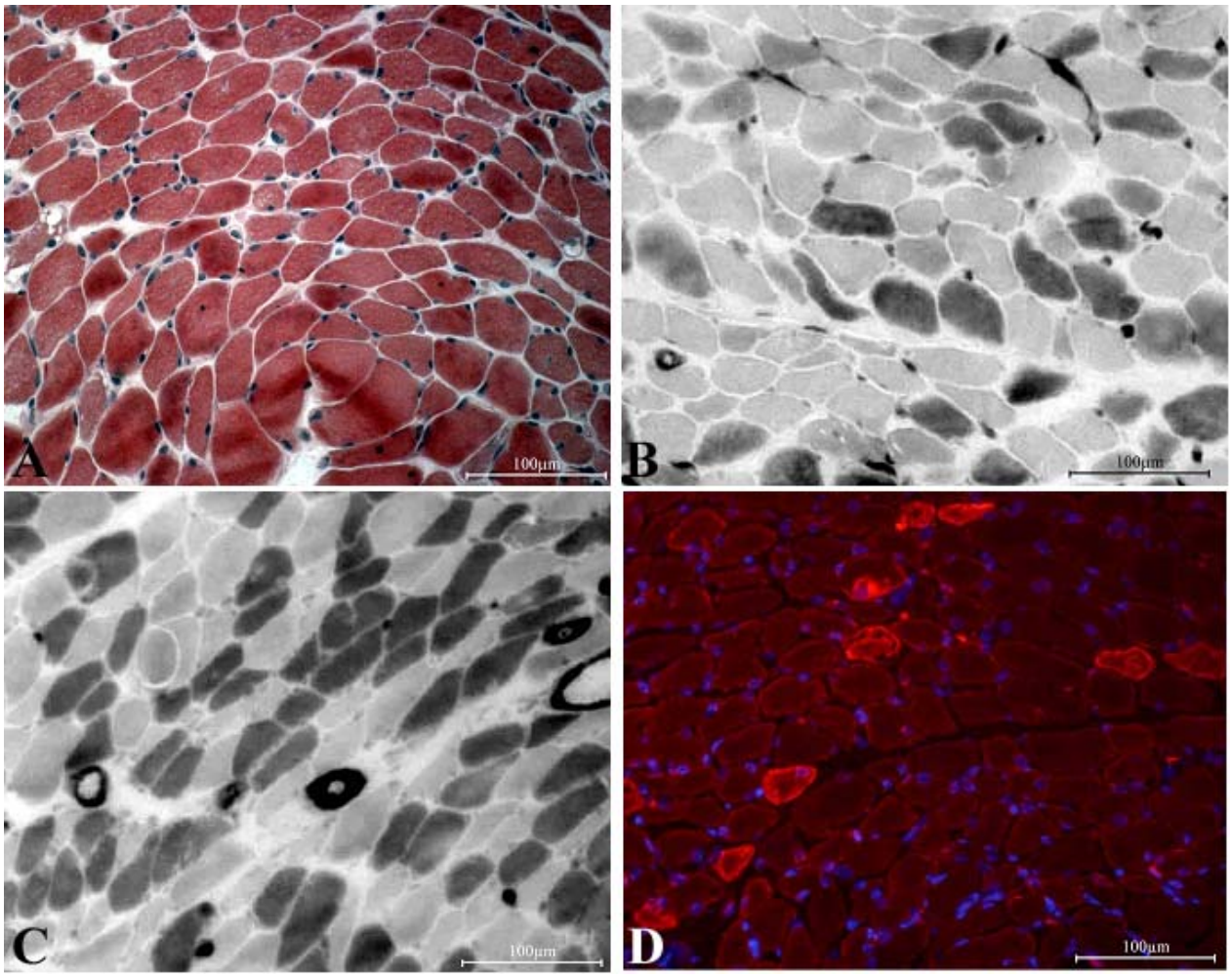

Figure 3 *Muscle biopsies of 0.8-year human flaccid paralysis. A, Hematoxilin and Eosin stain; B, ATPase pH 10.4 stain; C, ATPase pH 4.35 stain; D anti-NCAM positive myofibers.*

Main results [1,2,3] at light microscopy level are: 1. Human skeletal muscle tissue undergoes three phases during long-term lower motor neuron denervation: i) Atrophy (up to 18 months post-injury). Figure 1 shows the fiber size distribution at different times of denervation. ii) Lipodystrophy (3 – 10 year post-injury); iii) Fibrosis (after 10-year post-injury). Besides the three phases change over time at a much slower rate than in rodents (year vs month); 2. Small or large regenerating myofibers, that is, those positive to an anti-embryonic myosin monoclonal antibody (anti MHC-emb) are rare (about 1% of the myofibers present in the cryosection), but present in almost all the biopsies also many years after SCI as shown in figure 2.

ATPase staining confirm that after 1 year SCI, human muscles continue to show differentiated fibers, either fast than slow (figure 3 B and C). Histochemical analyses show that the chekboard appearance of the human denervated tissue is maintained at least up to 2 year post-SCI, but generally a fast-like ATPase staining prevail. At longer time the severely atrophic myofibers show if any, a very light staining. A marker of denervated muscle are NCAM proteins. These are membrane proteins and are normally situated in central and peripheric nervous system. They are also present during the embryonic muscle development and in adult muscle in post synaptic membrane along all the sarcolemma. Some studies show NCAM proteins are express in denervated muscle in order to create a synaptic connection [4]. In human muscle biopsies that we have analyzed the expression of NCAM seems to be lost in the atrophic myofibers of the long-term SCI subjects in both large and small size myofibers, suggesting that the wide-long re-expression of this molecule is possibly restricted to the early periods of SCI indeed in denervated human muscle NCAM is present about until 1 year (figure 3D).

Acknowledgements

This research was undertaken with the financial support of EU Commission Shared Cost Project RISE (Contract n. QLG5-CT-2001-02191).

References

[1] Kern H, Rossini K, Carraro U, Mayr W, Vogelauer M, Hoellwarth U, Hofer C: Muscle biopsies show that FES of denervated muscles reverses human muscle degeneration from permanent spinal motoneuron lesion. *J Rehabil Res Dev* 2005; 42(3 Suppl 1): 43-53.

[2] Carraro U, Rossini K, Mayr W, Kern H: Muscle fiber regeneration in human permanent lower motoneuron denervation: relevance to safety and effectiveness of FES-training, which induces muscle recovery in SCI subjects. *Artif Organs* 2005; 29(3):187-191.

[3] Kern H, Boncompagni S, Rossini K, Mayr W, Fano G, Zanin ME, Podhorska-Okolow M, Protasi F, Carraro U; Long-term denervation in humans causes degeneration of both contractile and excitation-contraction coupling apparatus, which is reversible by functional electrical stimulation (FES): a role for myofiber regeneration? *J Neuropathol Exp Neurol* 2004; 63(9): 919-931.

[4] Urbanchek MG, Ganz DE, Aydin MA, van der Meulen JH, Kuzon WM Jr: Muscle-nerve-muscle neurotization for the reinnervation of denervated somatic muscle. Neurol Res. 2004; 26(4): 388-394.