

Denervation-related changes in acetylcholine receptor density and distribution in the rat *flexor digitorum sublimis* muscle

Matteo Guzzini¹, Salvatore Raffa², Stefano Geuna³, Silvia Nicolino⁴,
Maria Rosaria Torrisi², Pierluigi Tos⁵, Bruno Battiston⁵,
Francesca Grassi⁶ and Andrea Ferretti¹

¹Dipartimento di Ortopedia e Traumatologia, Ospedale S. Andrea,
Università La Sapienza, Roma, Italy.

²Unità Operativa di Diagnostica Cellulare,
Ospedale S. Andrea, Università La Sapienza, Roma, Italy.

³Dipartimento di Scienze Cliniche e Biologiche, Università di Torino,
Ospedale San Luigi Gonzaga, Orbassano (TO) Italy.

⁴Dipartimento di Biologia Animale e dell'Uomo, Università di Torino, Italy

⁵Unità Operativa di Microchirurgia Ricostruttiva, Ospedale C.T.O, Torino, Italy.

⁶Dipartimento di Fisiologia Umana e Farmacologia, Università La Sapienza, Roma,
Italy and IRCCS San Raffaele Pisana, Rome Italy.

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SUMMARY

Reorganization of the muscle endplate structures is an important parameter for the study of posttraumatic neuromuscular recovery. The aim of this study was to investigate the changes and the distribution of the acetylcholine receptors (AChRs) in *flexor digitorum sublimis* muscle after 30 days of denervation in the rat forelimb experimental model. In young male rats, the median and ulnar nerves of the right forelimb were surgically transected and a 1-cm-long segment was removed to avoid spontaneous regeneration. Along the postoperative, the presence of complete functional loss was assessed by the grasping test. After 30 days, rats were sacrificed and *flexor digitorum sublimis* muscles of both limbs were explanted. The muscles were analysed by light microscopy, to assess the degree of muscle atrophy, and by immunofluorescence after rodamine-conjugated alpha-bungarotoxin incubation to investigate the reorganization of endplates.

The occurrence of muscle denervation was established, prior to sacrifice, by complete loss of the grip function. Light microscopy showed that 30-day denervation is sufficient to induce severe muscle fiber atrophy. Fluorescence analysis at low resolution showed that background fluorescence was higher in denervated muscles possibly because of the presence of extrajunctional AChR. At higher resolution, the endplates were clearly visible as ribbon-like structures. In control fibres, AChR formed a compact and bright structure while in denervated samples it appeared more diffuse and dimmer. Quantitative analysis showed that endplate area was larger in denervated muscles than in control samples. A corresponding decrease in fluorescence intensity was observed after subtracting the basal fluorescence.

In conclusion, results of the present study demonstrate that 30 days of denervation induce severe atrophy in rat *flexor digitorum sublimis* muscle that is accompanied by significant changes in