NERVE REGENERATION EFFECTS ON MUSCLE FUNCTION

A. Stefanovska, S. Ribarič*, L. Vodovnik, M. Kogovšek

Faculty of Electrical Engineering and
*Pathophysiological Institute, Medical Faculty
Edvard Kardelj University, Ljubljana, YUGOSLAVIA

Abstract

A method for evaluating muscle function during nerve regeneration is proposed. The sciatic nerve of a rat was crushed, and isometric tetanic tension measured during ten weeks, thus recording the progress of peripheral nerve regeneration. Complete degeneration of nerve endings was noticed two weeks after axonothmesis. After the third week, the new endings which had reached the muscle, became functional and enabled minimal muscle force as a response to tetanic stimuli. Recovery of muscle function to a normal level was found 35 days after crushing the nerve. The introduced method is non-invasive, sensitive and quantitative, reliable for assessing the effects of different treatments (i.e. electrical stimulation) on functional state of the regenerated motor units.

Key words: peripheral nerve, regeneration, muscle function

INTRODUCTION

The electrical nature of nerve tissue, both central and peripheral, easily suggests the application of electrical stimulation to repair processes (1). Whilst a large evidence that electromagnetic fields or electric currents might stimulate electrochemical processes disturbed due to the injury of nerves, bones or soft tissues already exists (1,2,3,4), there is still a lack of quantitative data.

The ability to regenerate is related to that of repair and remodelling. While repair produces restoration of function in a damaged structure, regeneration requires replacement of structure as a prerequisite to functional restoration. The model how the structure is replacing during nerve regeneration is well established (5,6,7,8), while there is still a lack of quantitative methods for measuring the functional restoration.

Morever, the fact that tissue growth does not necessarily lead to functional improvements, implies its careful evaluation.
The toe-pinch and toe-spreading reflexes have been used extensively to evaluate sensory and motor nerve regeneration (5,9), however they are highly subjective.
Non invasive recording of action potentials requires implanted stimulation electrodes (10).
Biomechanical methods for measuring muscle contraction have been widely used, but either a terminal or in vitro preparation was necessary (11). In vitro measurements are oriented to measure selective contraction of particular muscle or even muscle fiber. Less selective, but not invasive is the technique when muscle contraction force is measured between two externally fixed joints. Stimulating the nerve might be performed using either imp (10) or surface electrodes (12). In this study direct intramuscular stimulation was applied to study the rate of functional reinervation of muscle soleus, after axonothmesis of sciatic nerve.

METHODS

Nerve injury

A group of six male Wistar rats was included in the study. Aseptic surgery under pentobarbital anaesthesia (50 mg/kg body wt., injected intraperitoneally) was performed. Via an insection over trochanter, the left sciatic nerve was exposed and crushed for one minute with a fine forceps. The proximal part of the lesion was approximately 5 mm appart from femoral trochanter. The skin incision was closed with wound clips.

Tetanic isometric contractions

Isometric contraction of soleus muscle was recorded as a measure of functional state of reinervated muscle (13). The measurement set-up is presented on Fig. 1. Intramuscular electrodes, with constant distance of 7 mm were used. The stimulation frequency was 33 Hz and pulse durration 0.3 ms. Stimulation sequences of 5s stimulation and 5s pause were repeated five times. Both, affected and unaffected limbs were controlled once a week at the amplitudes of 5 and 10 mA. The fatigue index I_f was defined to evaluate the possible changes in muscle contraction characteristics:

$$I_f = F_5/F,$$

where:

F is the average value of muscle force of five stimulation sequences,
and F_5 is the average value of muscle force during the last stimulation sequence.

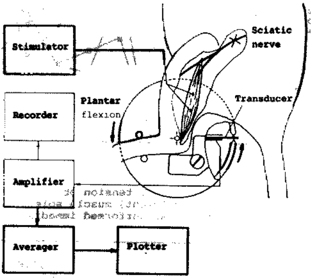

Fig. 1: Illustration of measurement method. The foot of anaesthetized rat is fixed and muscle force as a response to intramuscular stimulation recorded.

RESULTS

Complete denervation of soleus muscle was found two weeks after axonothmesis of sciatic nerve. After the third week, the new nerve endings that had reached the muscle became functional,and enabled minimal muscle force as a response to tetanic stimuli. Recovery of the muscle function to a normal level was found 35 days after crushing the nerve. Typical results are shown on Fig. 2. There are no changes in muscle fatiguability after reinervation comparing to control i.e. unaffected side (Fig. 3).

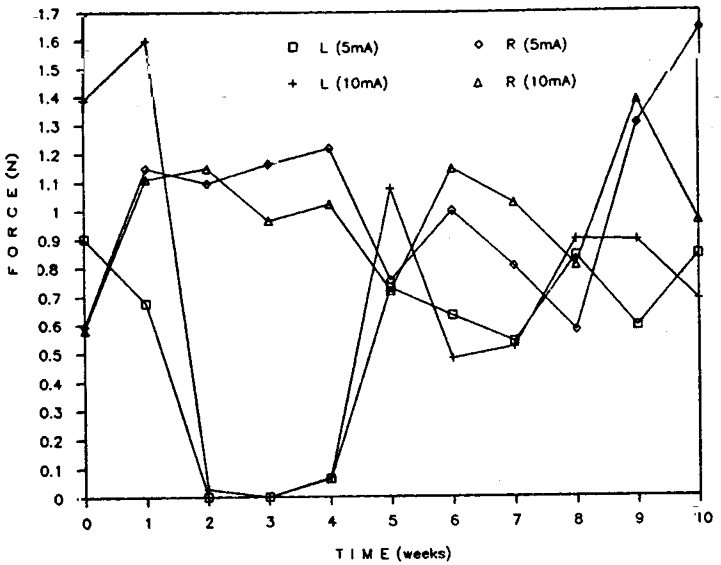

Fig. 2: Time-histories of tetanic tension obtained in affected (left) and unaffected (right) muscle soleus. The crush of left sciatic nerve was performed immediately after the first measurement.

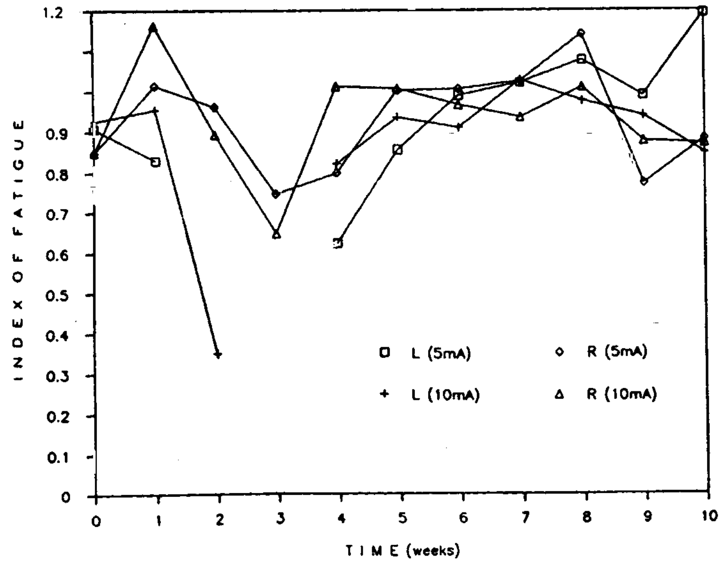

Fig. 3: Fatigue index in muscle soleus during regeneration of crushed left sciatic nerve. Right side serves as a control

DISCUSSION

The data obtained regarding the recovery of muscle function to a normal level are in agreement with the results of previous studies (10,12). There are some discrepances regarding the results obtained in the first three weeks of nerve crush. Kerns et al (12) reported that there is no muscle response when the sciatic nerve was stimulated one week after the axonothmeses. Nix and Hopf (10) have obtained some muscle force, and have ascribed it as a sistematic error, to the response due to costimulation of gastrocnemius nerve by the cuff (only motor branches for the soleus muscle had been crushed). However, in our study, by direct muscle stimulation, muscle force was obtained one week after axonothmesis showing that the nerve endings did not degenerate completely by that time. Such response cannot be obtained by nerve stimulation due to the loss of structural and/or electrochemical continuity between the injury site and the muscle fibers. Further differences obtained by nerve or muscle stimulation are related to the time when the new nerve endings that reach the muscle became functional. Results obtained in our study show recovery of muscle function one week later compared to the results of previous studies. Possible explanation is again the site of stimulation. Nerve electrodes are positioned close to the normaly innervated gluteus muscle whose contraction is superimposed to the observed muscle contraction. However, the obtained differences are explicable, and they confirm the sensitivity of biomechanical evaluation of functional reinervation. The introduced method is non-invasive, and it is reliable for measuring the effects of different treatments (i.e. electrical stimulation) on the functional state of regenerated motor units.

References

Black J.: Electrical Stimulation. Its Role in Growth, Repair, and Remodeling of the Musculoskeletal System. Praeger Publishers, New York, 1987.

Borgens R.B., Vanable J.W., Jaffe L.F.: Bioelectricity and regeneration, J. Exp. Zool., 200:403-416, 1977.

3. Borgens R.B.: What is the role of naturally produced electric current in vertebrate regeneration and healing?. Int. Rev. Cytol. 76: 245-298, 1982.
4. Pockett S., Gavin R.M.: Acceleration of peripheral nerve regeneration after crush injury in rat. Neuroscience letters, 59:221-224, 1985.
5. Gutmann E., Gutmann L., Madawar P.B., Young J.Z.: The rate of regeneration of the nerve, J. exp. Biol., 19:14-44, 1942.
6. Speidel C.C.: In vivo studies of myelinated nerve fibers, Int. Rev. Cytol., 16:173-231, 1964.
7. Jacobson S., Guth L.: An electrophysiological study of the early stages of peripheral nerve regeneration, Experimental Neurology, 11:48-60, 1965.

8. Albani M., Vrbová G.: Physiological study of innervation of regenerated muscle in the rat, Neuroscience, 15:489-498, 1985.
9. Sebille A., Bondoux-Jahan M.: Effects of electric stimulation and previous nerve injury on motor function recovery in rats, Brian Res., 193:562-565, 1980.
10. Nix W.A., Hope C.: Electrical stimulation of regenerating nerve and its effects on motor recovery, Brian Reserch, 271:21-25, 1983.
11. Hie H.B., Van Nie C.J., Vermeulen-van der Zee E.: Twitch tension, muscle weight, and fiber area of exercised reinnervating rat sceletal muskle, Arch. Phys. Med. Rehabil., 63:608-612, 1982.
12. Kerns J.M., Fakhouri A., Pavkovic I.: Evaluating the functional progress of nerve regeneration by a twitch tension method, Journal of Neuroscience Methods (in press).
13. Juričić Đ., Kogovšek M., Stefanovska A., Vodovnik L.: Biomechanical and myoelectrical method for nerve regeneration level measurement, (In slovene) Proc. ETAN, XI85-XI91, Hercegnovi, 1986.

Acknowledgements

This work was supported by the Slovene Research Community, Ljubljana, Yugoslavia. The authors are grateful to M. Brzin, J. Sketelj and D. Križaj for useful discussions.