Histochemical and Biochemical Changes in Fast and Slow Rabbit Muscle Following Denervation and Electrical Stimulation With Long Bidirectional Impulses (LIB-Stimulation)
T. Mokrusch, B. Neundörfer, H. Reichmann, U. Carraro

Hedon-Klinik Lingen
University of Erlangen-Nürnberg, University of Dresden, University of Padua
Hedon-Allee 1   D-49811 Lingen (Germany)
Mokrusch@geset.de

Abstract:
LIB-stimulation has proved to be effective in maintaining and restoring contraction force of fast rabbit muscle following denervation. The present study in white New Zealand rabbits was done to investigate the influence of LIB-stimulation on the changes of contractile proteins and energy metabolism following denervation.

Without stimulation, TA showed a clear reduction of fibre diameter (19.9 µ) as compared to 41.1 µ after stimulation; (Normal: 52.7 µ). While, after denervation alone, no major changes were found in fibre type distribution, stimulation induced a shift towards type IIB (2.2 % I, 3.2 % IIA, 94.6 % IIB). - In SOL, fibre diameter was 23.5 µ without and 34.3 µ with stimulation; (62.0 µ). Without stimulation, 53.9 % type I fibres were found, and 45.8 % after stimulation; (97.1 %). In TA, denervation alone induced no significant changes in MHC distribution (0 [3.2] % I, 14.8 [17.6] % IIA, 80.3 [79.2] % IIB, 4.9 % undefined isoforms). Following stimulation, we found an increase of MHC IIB (96.8 %). - In SOL, type I decreased after denervation (61.0 [94.8] %) as well as after stimulation (51.4 %). Following denervation, we found an overall decrease of enzymes, except for hexokinase and G6PDH. In TA, stimulation induced a severalfold increase of mitochondrial enzymes (citrate synthase 7x, cytochrome-c-oxidase 2-4x and ketoacid-CoA- transferase 2x), while the glycolytic enzymes (phosphorylase, phosphofructokinase) showed only small changes. In SOL, these enzymes increased (6x, 4x), while the mitochondrial enzymes tended to decrease.

The present results show that electrical stimulation with long bidirectional rectangular balanced impulses (LIB-stimulation) has a clear beneficial effect on histochemical and biochemical features of denervated fast contracting skeletal muscle of rabbit, and only a small effect on the slow muscle. For the different reactions of small and fast muscles, stimulation parameters are thought to be responsive.

1. Introduction

Background
In previous studies of our group (Ref. 1-3), electrical stimulation with balanced bidirectional rectangular impulses of high intensity and long impulse duration (LIB-stimulation) has proved to be effective in maintaining and restoring muscle contraction force in fast muscles of rabbit. Additionally, the morphological sequelae of denervation atrophy was stopped and muscle bulk was restored.

LIB-Stimulation has also proved its efficacy in a reasonable number of patients with complete and irreversible states of denervation following destruction of brachial and/or lumbosacral plexus. Tetanic contraction force was increased up to 400% and more, at maximum up to 30% of normal). Additionally, it was shown that the histological signs of denervation atrophy could be avoided and that muscle bulk even could be restored after a long time course of denervation.

The response of a fast skeletal rabbit muscle to different stimulus patterns, varying frequency, impulse duration and total amount of stimulation, has shown some unexpected findings before (same author, ifess 2002): A shorter impulse duration resulted in a slower muscle than a longer impulse width (20 ms vs. 10 ms) three months after denervation, following daily electrical stimulation for several minutes. Stimulation with a higher frequency (50 Hz vs. 25 Hz) resulted in a slower contracting muscle.

Additionally, the stimulated muscle proved to be fast contracting but at the same time fatigue resistant.

The question was whether these unexpected findings of contractile properties correspond to morphological and biochemical findings. The present study in white New Zealand rabbits was done to investigate the influence of LIB-stimulation with variation of stimulus patterns on the changes of contractile proteins and energy metabolism following denervation.
1.1. Previous Work

Design/Methods
Animals/Denervation

Thirty adult white New Zealand rabbits were denervated reaching a complete sensory-motor loss of the right hindlimb by transection of the sciatic nerve, femoral nerve, obturator nerve and the lateral cutaneous nerve of the thigh. By means of electrophysiology, care was taken that no reinnervation occurred during the time of the overall experiment and the lack of reinnervation then was verified in a final histological evaluation.

Electrical stimulation

A painless electrical stimulation was performed twice daily with a total stimulation time of 9 minutes each, using surface electrodes over a period of three months. The usual training regime included a tetanic contraction time of 30 seconds, followed by a break of 2.5 minutes. The impulse was of a bidirectional rectangular shape which was balanced in charge, with a duration of 20 ms, followed by a break of the same length, from which resulted a frequency of 25 Hz.

Three months after denervation, histochemical investigation of tibialis anterior muscle (TA) and soleus muscle (SOL) included NADH-TR and ATPase stainings (following preincubation at pH 4.3, 4.6 and 9.4). Biochemical investigation included SDS-PAGE of myosin heavy chains and activities of mitochondrial (citrate synthase {CS}, cytochrome-c-oxidase {CCO} and β-oxidation of fatty acids: ketoacid-CoA-transferase {KCT}) and glycolytic or glycogenolytic (phosphorylase {PHO} and phosphofructokinase {PFK}) enzymes.

Results

Histochemical findings

Without stimulation, TA showed a clear reduction of fibre diameter (19.9 µ) as compared to 41.1 µ after stimulation; {Normal: 52.7 µ}. While, after denervation alone, no major changes were found in fibre type distribution, stimulation induced a shift towards type IIB (2.2 % I, 3.2 % IIA, 94.6 % IIB). - In SOL, fibre diameter was 23.5 µ without and 34.3 µ with stimulation; {62.0 µ}.

Without stimulation, 53.9 % type I fibres were found, and 45.8 % after stimulation; {97.1 %}.

Biochemical findings

In TA, denervation alone induced no significant changes in MHC distribution {0 (3.2) % I, 14.8 (17.6) % IIA, 80.3 (79.2) % IIB, 4.9 % undefined isoforms}. Following stimulation, we found an increase of MHC IIB (96.8 %). - In SOL, type I decreased after denervation (61.0 (94.8) %) as well as after stimulation (51.4 %). See Fig. 1

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Following denervation, we found an overall decrease of enzymes, except for hexokinase and G6PDH. In TA, stimulation induced a severalfold increase of mitochondrial enzymes (citrate synthase 7x, cytochrome-c-oxidase 2-4x and ketoacid-CoA-transferase 2x), while the glycolytic enzymes (phosphorylase, phosphofructokinase) showed only small changes. In SOL, these enzymes increased (6x, 4x), while the mitochondrial enzymes tended to decrease (Fig. 2).
Fig. 2: Changes of mitochondrial and glycolytic enzyme patterns in fast and slow rabbit muscle following denervation and different types of electrical stimulation

2. Summary and Conclusions

This type of stimulation (LIB) has a clear beneficial effect on the fast contracting muscle, and only a small effect on the slow muscle concerning contraction force. There are, however, major changes in the enzyme patterns, different in fast and slow muscles, that parallel the changes of contractile properties. For the different reactions of small and fast muscles, these variations of stimulation parameters are thought to be responsible. The clinical meaning of these findings might be to be able to chose whatever type of stimulation pattern to influence the contractile patterns of the stimulated muscle. During therapeutic stimulation, sometimes it might be of some clinical importance to be able to chose a particular stimulation pattern to receive a more or less fast contracting muscle (as there are, for example, fast contracting finger muscles), or to have a more fatigue resistant muscle group. Further studies have to show to what extent this choice can be realized.

Acknowledgment

The author wishes to acknowledge the financial support of the Deutsche Gesellschaft für Elektrostimulation und Elektrotherapie e.V. (GESET)

References