Beneficial effect of locomotor training on the structure of the denervated rat soleus muscle

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Abstract
In order to study mechanisms of muscle denervation atrophy, a locomotor training on a treadmill was applied to denervated rat *soleus* muscle. Ultrastructure of the treated muscles were investigated two months after denervation. It has been found that pathological changes caused by denervation were less pronounced in trained muscle comparing to untrained muscle. For example, the number of capillaries was increased, accumulation of collagen fibrils was reduced, typical degenerated myofibers were hardly seen, and the structure of the contractile apparatus was much better preserved. Thus, locomotor training seems to attenuate some of the pathological processes within the denervated *soleus* muscle.
Key Words: attenuation of atrophy, denervation, locomotor training, muscle ultrastructure, skeletal muscle.

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Early after denervation (*i.e.* up to about one month in the case of rat leg muscles) progress of atrophy is the fastest and the symptoms of the so-called simple atrophy prevail, which are mainly manifested as the loss of muscle weight and diminishing of the fibers diameter. Some moderate increase of the amount of collagen between muscle fibers can be visible during this stage of atrophy, while the seriously damaged or necrotic and regenerating fibers are absent. Simple atrophy is reversible when the muscle becomes reinnervated [7, 8, 9].

On the contrary, in the long-term denervated muscle (*i.e*. several months or years after denervation) when process of atrophy has already advanced but its progress is negligible, the number of muscle fibers is reduced and their diameter is significantly diminished. Simultaneously, degenerating and/or dying fibers can be easily found and the amount of connective tissue considerably increases.

In the long-term-denervated muscle, the number of satellite cells and vessels is evidently reduced and the restorative capacity after reinnervation becomes poorer. Such muscle is not able to fully restore its structure and function, despite that individual fibers retain ability to be reinnervated even for years. Interestingly, formation of new muscle fibers remains possible even in the very long-time-denervated muscle [1, 3, 4, 5, 14].

The reduced number of satellite cells and blood vessels as well as the increased amount of collagen are believed to be the main reasons of irreversibility of the denervation atrophy [4, 12, 14].

After two months, the progress of atrophy in denervated rat leg muscles slows down and the muscle enters in some stabilized stage. The total number of fibers per muscle remains constant for couple next months, and muscle still retains its restorative capacity. However, fiber death/regeneration processes begin [2, 13, 14]. Thus, the two-month denervated rat leg muscles seem to serve as a convenient model in studies addressing the problem of irreversibility of the process of denervation atrophy.

In the present work, a locomotor training on a treadmill was applied to the rat soleus muscle during the second month after denervation. Passive locomotion was applied in order to answer the question whether it may prevent the irreversible changes within denervated atrophying muscle. Morphology of the isolated muscle were examined by the electron microscopy.

Material and Methods

Female Wistar 3-month old rats were used in the studies. Slow (*soleus*) rat leg muscle was denervated by cutting the sciatic nerve as described in detail earlier [7, 8]. Both nerve stumps were strongly ligated, and the

proximal stump was implanted into subcutaneous dorsal region. Rats were then trained to walk on a treadmill; the training begun one month after denervation. Each training session consisted of 30-min walk, during which the rats were passing a distance of about 300 m with a speed within a range 15-25 cm/s. Both, 2-month denervated untrained rats and intact animals of the same population (6-7 animals in each group) served as the control groups. Two months after denervation the animals were sacrificed. All procedures of sample preparation for morphological and ultrastructural studies were performed as previously described [6, 7, 9].

Ultrathin sections were prepared on an LKB Ultratome RIII and inspected using JEM 1200 EX electron microscope.

Results and Discussion

Two months after denervation the weight of rat soleus muscle decreased to about 27% of the weight of the control and/or contralateral muscle, and muscle fibers were significantly reduced in size. After training, the muscle weight and fiber diameter remained in the same range as parameters observed for muscle isolated from untrained animals.

In muscles of untrained animals changes in the ultrastructure of muscle fibers, characteristic to denervation atrophy, were observed [see introduction]. In the majority of muscle fibers, the structure of contractile apparatuses was preserved, but exhibited several anomalies. Myofibrils had smaller diameter, were separated from each other and were irregularly positioned. The Z-line was thinner and irregular. The A-band, I-band and Z-line were often hardly recognizable and the appearance of the contractile structures differed between the neighboring muscle fibers (Fig. 1).

Contrary to that, in the majority of muscle fibres of trained animals the contractile structure look as regular and the A-band and I-band are well recognizable (Fig. 2). Myofibrils became larger and were more regularly situated; they often presented the hexagonal arrangement of myosin filaments, characteristic for healthy muscle (not shown). Connections between neighboring myofibrils seemed to be more stable than in untrained muscles, resulting in preserving the structure of Z-line, that looked almost regular throughout the entire muscle fiber. While in the denervated untrained muscle displacement of myofibrils and contraction bands were locally seen [7, 10], such anomalies were practically not occurring in trained muscle. Similarly, degenerating mitochondria, myelin figures, lipofuscin bodies or folds of sarcolemma and basement lamina were less frequently observed in denervated trained muscle than in untrained ones [7, 10]. Accumulation of collagen fibrils between muscle fibers decreased following the training as well (Figures 3, 4). Also, the number of seriously damaged fibers looking as necrotic and/or apoptotic was smaller in trained muscle, when compared to untrained one. In the untrained muscle about 1.4% of muscle fibers were seriously damaged [10]. Moreover, myotubes, that constituted about 2% of muscle fibers in denervated untrained soleus [10], were hardly seen in the muscle after training. The number of satellite cells was increased in denervated muscle from both trained and untrained animals, when compared to controls. However, their increase at this stage of muscle denervation was already documented previously [14]. Interestingly, another evident difference between trained and untrained muscle was observed in the number of capillary vessels among muscle fibres.

***Fig. 1**. The untrained denervated* soleus *muscle, longitudinal section. Muscle fibres are decreased in size, with the recognizable but irregular contractile structure. Myofibrils with smaller diameter are separated from each other and irregularly positioned, the Z-line is thin and irregular. In the right-hand fiber, the Z-line, A-band and I-band are hardly recognizable. Groups of small and dark mitochondria seen within spaces devoid of contractile structure. Bar is 4 mμ̃*

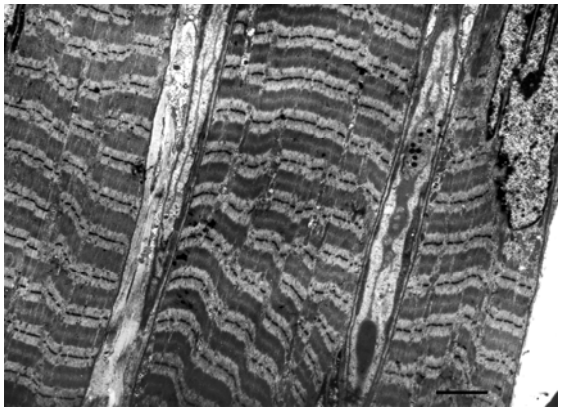

***Fig. 2**. The trained denervated soleus muscle, longitudinal section. Three neighbouring myofibers with well preserved contractile structure; the Z-line, A-band and I-band are regular; the Z-line continues throughout the entire width of myofibers. Capillary vessels are seen between myofibers. Bar is 4 mμ̃.*

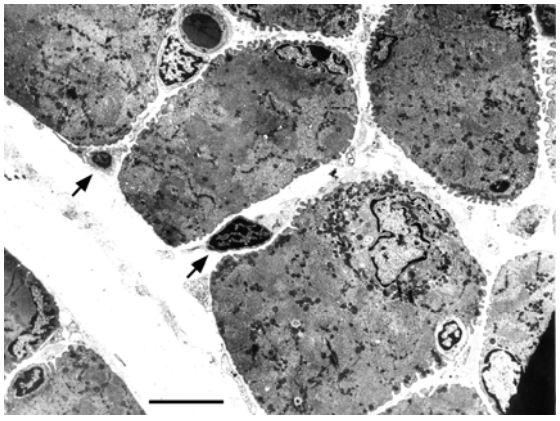

***Fig. 3**: The untrained denervated* soleus *muscle, transverse section. Myofibers reduced in size and with folded sarcolemma are surrounded with large amounts of collagen. Irregular muscle nuclei; two satellite cells. Capillary vessel among myofibers; remnants of capillary (arrows). Bar is 4 mμ̃.*

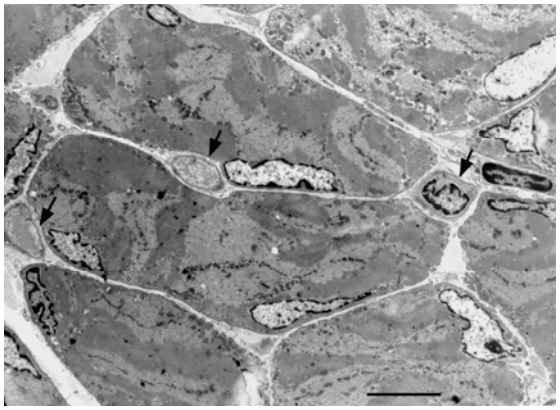

***Fig. 4**: The trained denervated soleus muscle, transverse (partially oblique) section. Less folded sarcolemma and less collagen surounding myofibres as compared with untrained muscle (see Fig. 3). Three capillary vessels are marked with arrows. Bar is 4 mμ̃.*

In untrained denervated muscle their number was considerably decreased, when compared to control muscle, and the lumen of the vessels was mostly empty or only some vessel remnants were recognizable (Fig. 3). Contrary to that, in the trained muscle capillary vessels were numerous with the filled lumen (Figures 2, 4).

Our results report the evident improvement of the structure of denervated soleus muscle subjected to the treadmill training, thus indicating that passive locomotion positively affects the denervated atrophying *soleus* muscle. It is plausible that passive training attenuates some of the pathological processes and diminishes the changes, which are believed to be the cause of irreversible damage of denervated muscle.

Preliminary results were presented [11].

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