Ultrastructure of the 2-months denervated rat leg muscle

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Abstract
Ultrastructure of slow (soleus, SOL) and fast (extensor digitorum longus, EDL) rat leg muscles were examined two months after denervation when progress of atrophy slow down, and it enters in some more stable stage. In the experimental muscles the mass decreased to about 25% of the control value. Muscle fibres were decreased in size considerably, but not uniformly. The most muscle fibres were preserved showing features of denervation atrophy. However about 1.4% of muscle fibres, both in SOL and EDL, were seriously damaged, that is, they underwent severe atrophy, necrosis and/or programmed cell death. They showed generally increased electron density, disorganised contractile structure with unrecognisable Z-line, damaged or not recognisable mitochondria and large vacuoles. Such fibres were grouped or situated individually close to preserved ones or to myotubes. Myotubes constituted about 2% of muscle fibres. Some of them were degenerating or dying. Large accumulation of collagen fibrils was common among muscle fibres, surrounding often blood vessels or myotubes. Thus, in addition to "simple" muscle atrophy, as early as 2 months after denervation, both slow and fast muscles show "degenerating and dying/regenerating myofibers.
Key words: denervation atrophy, muscle ultrastructure, programmed cell death, skeletal muscle.

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Introduction

Progress of denervation atrophy is not fully clear, especially in some later stages. In the early stage of denervation atrophy loss of muscle mass and diminishing of fibre diameters are the highest. Several changes, as diminishing of the contractile structure, progressive loss of mitochondria, anomalies of nuclei, formation of myelin figures and lipofuscin granules and folds of sarcolemma are the main features of muscle fibres in first month after denervation. Some moderate increase of collagen among muscle fibres is also observed. In this stage of atrophy the seriously damaged or necrotic muscle fibres and signs of regeneration were hardly seen [6-8], Those characteristics of "simple" atrophy are reversible when the muscle is reinnervated [1,6,7, and unpublished observations].

In the long-term denervated muscle (several months) atrophy is very advanced and its progress is negligible. The number of muscle fibres is reduced and their cross-section areas highly decreased. The number of satellite cells and vessels is evidently reduced. Simultaneously, degenerating and dying fibres are observed and connective tissue is considerably increased [2,3,4,5,10,11]. In the long-term-denervated muscle the restorative capacity by reinnervation becomes progressively poorer [11] and the muscle, contrary to the early stages, is not able to fully recover its structural and functional state. Reason(s) of this is not clear. Especially that muscle fibre keeps ability to be reinnervated for years and formation of new muscle fibres takes place even in the very long-time-denervated muscle [2,5,11].

In the present work muscle ultrastructure was examined two months after denervation, when progress of atrophy slow down, and the atrophy enters in some stabled stage. In the 2-month-denervated muscle the total number of fibres per muscle remains constant and muscle keeps its restorative capacity, however fiber death/regeneration begins [3,10,11]

Materials and methods

Female Wistar rats 3-months of age were used. Slow (soleus, SOL) and fast (extensor digitorum longus, EDL) rat leg muscles were denervated by cutting the

sciatic nerve as described earlier [6,7]. Both nerve stumps were strongly ligated. The proximal stump was implanted in subcutaneous dorsal region. All procedures

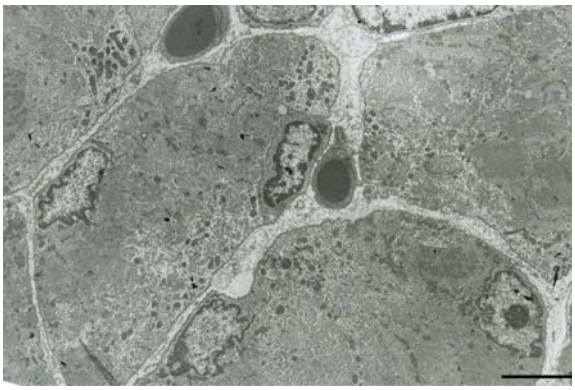

Figure 1. SOL, transverse section. Myofibres decreased in size, with the recognisable contractile structure. Myofiber nuclei are of irregular shape; satellite cell seen in the central region, close to a vessel. Groups of mitochondria within spaces empty of myofibrils (left-hand and centrally situated fibres). Folds of sarcolemma are hardly seen. Bar is 4 μm

of sample preparation for study of ultrastructure were performed as previously [8,9].

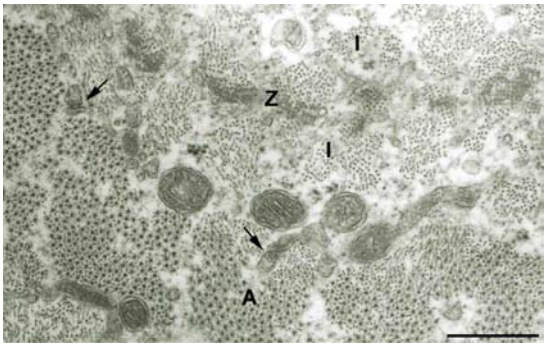

Figure 2. SOL, transverse section. The contractile apparatus with recognisable sarcomeric structure: A-band (A), I-band (I) and the Z-line (Z). Myofibrils are considerably decreased in size and the hexagonal array of myosin filaments is disturbed, but still the tetragonal arrangement of the Z-line is seen. Irregularly situated triads are recognizable (arrows). Bar is 0.5 mm

Results and discussion

Two months after denervation the muscle mass decreased to about 25% of the control/contralateral values in both SOL and EDL muscles. Muscle fibres were considerably reduced in size, but not uniformly – some large fibres were present. Angulated and split fibres were frequent. On the other hand, in the most muscle fibres the contractile structure was preserved, but with some anomalies. Myofibrils were small and separated,

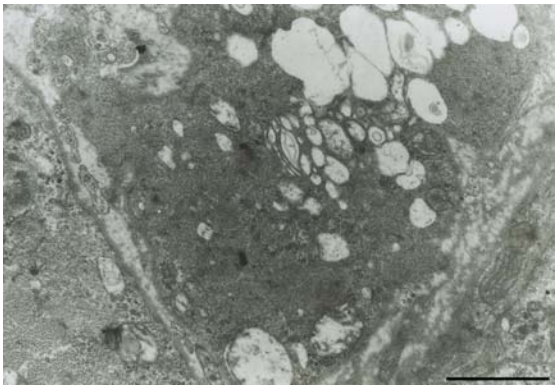

Figure 3. SOL, transverse section. A muscle fibre of high electron density, containing some contractile structures and numerous vacuoles, some of which look as remnants of mitochondria (central down region) or dilated Golgi apparatus (central upper region). Collagen fibrils are present among muscle fibres. They look as negatively stained. Bar is 1 mm

devoid of hexagonal arrangement of filaments, but keeping tetragonal order of the Z-line (Figs. 1,2). Disorganization of myofibrils and contraction bands appeared occasionally. Regularity of the contractile structure was more disturbed in SOL than in EDL muscle. Triads were properly arranged within regions of the regular contractile structure. Otherwise they were abnormally situated where the myofibrils were disorganized (Fig. 2). Nuclei, normal looking or of abnormal morphology (Fig. 1), were often situated in central fibre regions or in rows. Sparse mitochondria, small and dark, were grouped in areas free of myofibrils (Fig. 1). Ultrastructure of muscle fibres in general resembled that in the first month after denervation, but fibre organelles and structures seemed to be more stabilised. Degenerating mitochondria, myelin figures, lipofuscin bodies or folds of sarcolemma and basement lamina were much less frequent than in the first month after denervation [6,8].

Among the majority of atrophying muscle fibres, some seriously damaged fibers were seen. Some of them look as "necrotic", with myofibrils devoid of the Z-line and considerably swollen and disrupted mitochondria. Several muscle fibres resembled cells undergoing "programmed cell death". Those fibres showed generally increased electron-density, disorganised contractile structure with the Z-line hardly seen, damaged or unrecognisable mitochondria and large vacuoles

(Figs. 3,4), Those "degenerative" or "necrotic" muscle fibres constituted 1.4% of about 1800 fibres randomly

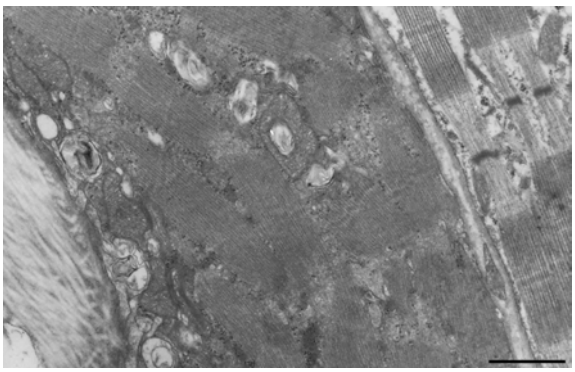

Figure 4. EDL, longitudinal section. The centrally situated fibre, of high electron density, contains irregular myofibrillar structures devoid of the Z-line. Vacuoles contain myelin figures, perhaps remnants of mitochondrial materials. The right-hand fibre looks normal. Numerous crowded collagen fibrils seen on the left-hand side. Bar is 1 mm

taken for observation (from SOL and >EDL muscles of 6 rats). Heavily damaged or dying muscle fibres were situated close to other damaged ones or intermixed to preserved muscle fibres (Fig. 4) and to some myotubes. Individual TUNEL-positive nuclei present within a few myofibres (data not shown) suggest that some of those damaged myofibres could enter on the apoptotic way of degradation. Myotubes appeared beneath to basal lamina in viable muscle fibres or developed individually resembling those in muscle regeneration. Myotubes constituted about 2% of muscle fibres. They appeared evidently more frequent in regions containing damaged fibres. Additionally, about 2% of muscle fibres were of very small size. Those fibres perhaps develop from myotubes or they appear as an effect of muscle fibre splitting. Some of myotubes and small fibres were degenerating or dying. Large accumulation of collagen fibrils were common among muscle fibres, especially in regions of damaged fibres (Figs. 3,4) where fat cells were occasionally observed as well. Often collagen fibrils surrounded blood vessels or myotubes. Those anomalies were observed both in SOL and EDL muscles.

The results allow to conclude, that as early as 2-month after denervation, muscle tissue shows some degeneration. Dying and regenerating fibres are present in addition to "simple" muscle atrophy. It seems that some irreversible damage of muscle tissue begins already during the second month of denervation.

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