Desmin and Vimentin in Tenotomized Rat Soleus Muscle

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Abstract

The distribution of intermediate filament proteins desmin and vimentin was studied in tenotomized rat soleus muscle. Our results indicate that tenotomy influenced significantly the expression of desmin in soleus muscle fibers. Vimentin, which occurs in high concentration only in fetal and regenerating muscle cells, did not reappear in tenotomized muscle fibers in our experiment. It seems that the changes in intracellular organisation of desmin parallel the morphological alterations in myofibrillar structure. The similarity in the pattern of accumulation of both desmin and acridine orange (AO) - RNA fluorescence in the area of intensive myofibrillar formation and hypertrophy indicates that desmin plays a role in the organisation of newly formed myofibrills.

Key words: desmin, vimentin, tenotomy, rat soleus muscle.

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The main component of intermediate filaments (IF) in the adult muscle fiber is desmin [1, 2] whereas vimentin is found in fetal myoblasts and myotubes [3, 4, 5]. The function of these cytoskeletal proteins is to maintain the lateral registry of the Z band and to organise within the intracellular space all the muscle organelles [1, 6]. The composition and structural organization of IFs present in skeletal muscle fibers have been shown to alter in various neuromuscular disorders [2, 6, 7, 8, 9, 10] as well as in experimental conditions [11, 12, 13, 14, 15, 16].

It is well known that experimental tenotomy produces extensive disorganisation of rat soelus muscle fibers. Within 4 weeks the affected fibers undergo a complete morphological reorganization. Among the earliest histologic anomalies of muscle fiber observed after tenotomy was the formation of a central core lesion in which the orderly arrangement of sarcomeres no longer existed [19, 21]. The aim of the current study is to investigate the possible involvement of IF proteins in the morphological reorganization of tenotomized soleus muscle fibers.

Material and Methods

Twenty five male Wistar rats (weighing about 250 g) were used for the experiments. After ether anesthesia, the right soleus tendon was exposed by a lateral approach and cut. Five animals were sacrificed at each of the following times after tenotomy: 5 days and 1, 2, 3, and 6 weeks. The soleus muscles were removed and rapidly frozen in isopentane cooled in liquid nitrogen. Cross sections cut at 8 µm intervals in cryostat. The sections were stained with hematoxylin and eosin, modified Gomori trichrome and NADH was demonstrated by tetrazolinum reductase activity [24]. Further 5 µm cryostat sections were cut and stained by an indirect immunofluorescence technique using monoclonal antibodies to vimentin (DAKO VIMENTIN V9) and desmin (DAKO DESMIN D 33). In brief, anti-vimentin (diluted 1:10 with phosphate buffer saline - PBS) and anti-desmin (diluted 1:50 with PBS) were applied to tissue sections for 30 min. Sections were rinsed with PBS and incubated for a further 30 min with the appropriate fluorescein - conjugated secondary antibody (diluted 1:50 with PBS). After washing with PBS the sections were mounted in glycerol and viewed with an Opton Zeiss standard LAB 16 microscope equipped with epifluorescence optics. Controls consisted of sections incubated with nonimmune goat serum as the first incubating solution. In addition, acridine orange (AO) fluorochrome was used to detect increased levels of sarcoplasmic RNA in freshly cut 5 µm cryostat sections [25].

Results

Five days after tenotomy

The majority of fibers showed definite lesions in all examined muscles. In HE stained material, lesions appeared as a discrete basophilic areas located in the center. Occasionally basophilia was located under the sarcolemma or distributed irregularly in a snake-like pattern. Such lesions were also identified by Gomori trichrome stain and showed a somewhat pale and amorphous appearance but purple staining was never observed. The system of muscle

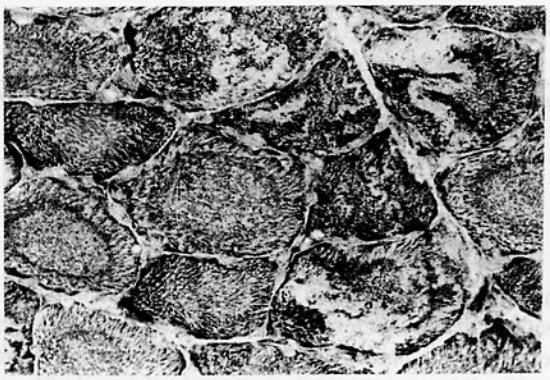

Figure 1. Five days after tenotomy. NADH-tetrazolium reductase x 500. Multiple changes in NADH activity of diverse character are seen on cross section in both types of fibers.

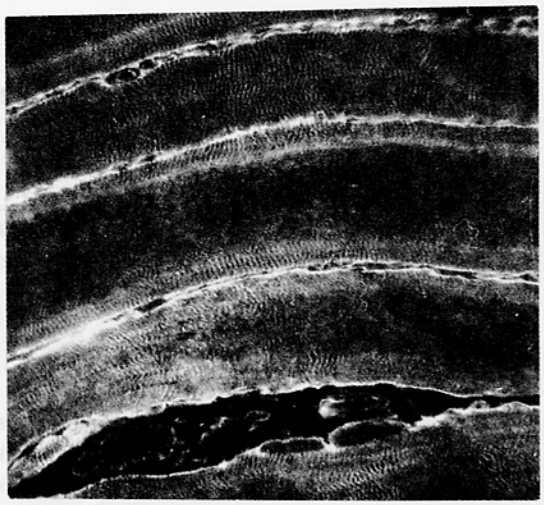

Figure 3. Five days after tenotomy. Anti-desmin x 400. Longitudinal section shows sharply demarcated areas of decreased desmin fluorescence located in the center of the fibers. Pattern of cross striation is preserved at the periphery of the fibers.

fiber typing was based on Dubowitz criteria [24] with type I muscle fibers dark and type II light in NADH-TR reaction. NADH staining demonstrated that many type I fibers but also some type II fibers contained large central areas of decreased activity with an intermediate thin ring of high activity between the central and peripheral zones (Fig. 1). Moreover, muscle fibers with normal or even increased activity in the center were also observed. In some fibers irregular snake-like lesions devoid of oxidative activity were present. Similar lesions were easily identified in anti-desmin staining. Areas of lower stainability for desmin were seen in both transverse (Fig. 2) and longitudinal sections (Fig. 3). They corresponded to areas of intense fluorescence in AO stain (Fig. 4). The bright AO-RNA fluorescence was, however, observed in many fibers not only in the center of the fibers, but also at the periphery (Fig. 4). Central lesions were vimentin negative.

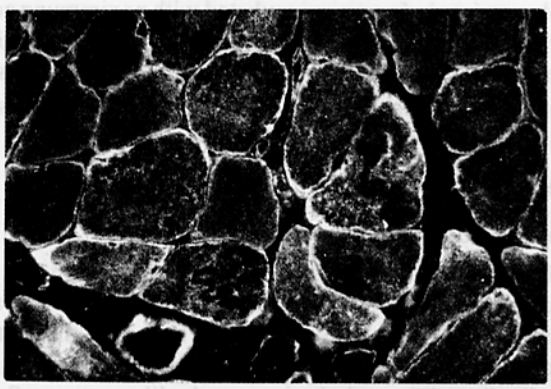

Figure 2. Five days after tenotomy. Anti-desmin x 400. Irregularly distributed areas of lower stainability for desmin are visible in many fibers.

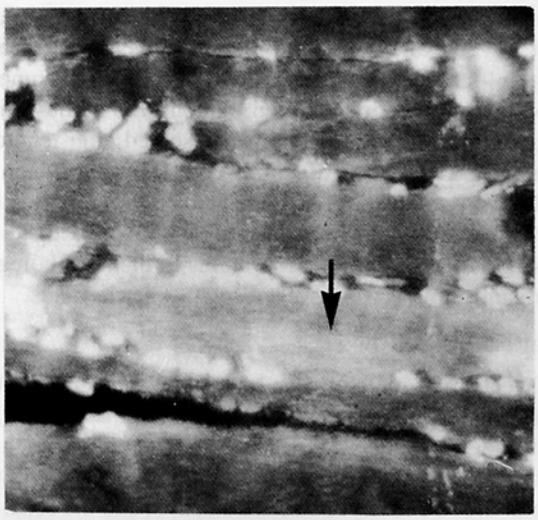

Figure 4. Five days after tenotomy. AO staining x 400. Area of the bright AO-RNA fluorescence located in the center of the fiber (arrow).

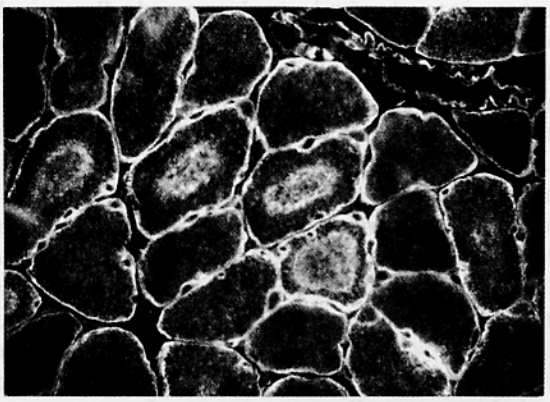

Figure 5. One week after tenotomy. Anti-desmin x 400. Numerous fibers with increased central desmin fluorescence.

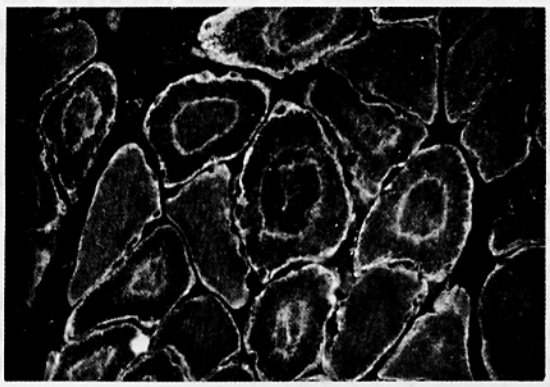

Figure 6. One week after tenotomy. Anti-desmin x 500. Large central zones of desmin activity with intermediate ring of high activity between central and peripheral zone as well as fibers with increased central fluorescence are seen.

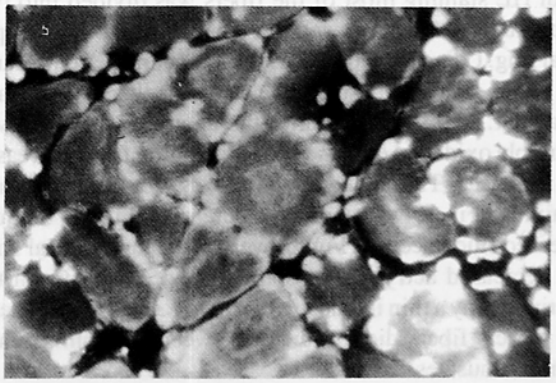

Figure 7. One week after tenotomy. AO staining x 400. Areas of bright fluorescence in the center of many fibers.

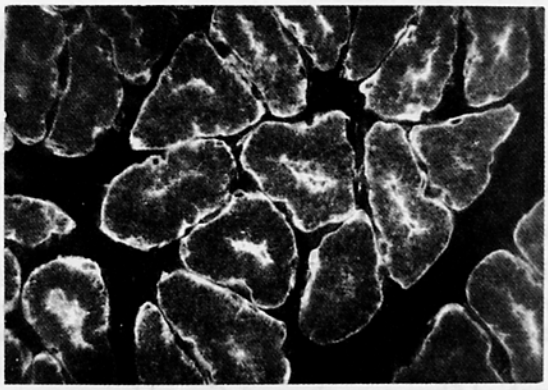

Figure 8. Two weeks after tenotomy. Anti-desmin x 400. Stellate areas of intense desmin fluorescence are present in many fibers.

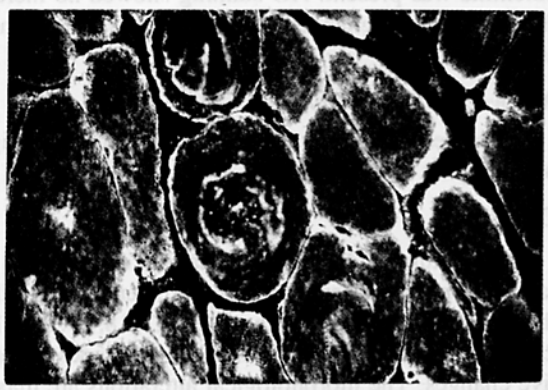

Figure 9. Four weeks after tenotomy. Anti-desmin x 500. Irregular disperse or small round accumulation of desmin is still recognizable in many fibers. Note the variability in fiber size.

One week after tenotomy

In HE staining most of the fibers was divided into two parts: a large central basophilic zone and an outer peripheral ring. Using the Gomori trichrome stain, the central area in some fields appeared pale and in others darker and granular. In NADH staining the central lesions showed variable oxidative enzyme activity ranging from decreased to increased. The same variability was seen in anti-desmin staining. Fibers with normal, increased (Fig. 5) and decreased fluorescence (Fig. 6) were present. A central zone of decreased activity was sometimes separated from the periphery by a thin intensely fluorescent ring. The diameter of muscle fibers did not differ between tenotomized and control muscles.

Their shape, however, was irregular and angulated. In addition to the central lesions many fibers showed accumulation of fluorescence under the sarcolemma (Fig. 6). When stained for AO, the central as well as subsarcolemmal areas in many fibers showed intense orange fluorescence (Fig. 7). Anti-vimentin staining was negative.

Two weeks after tenotomy

A stellate basophilic region located in the center were seen in most fibers in HE staining. In Gomori trichrome such lesions stained darker. The lesions showed high NADH activity and were also intensely stained with anti-desmin. (Fig. 8). The AO-RNA fluorescence became progressively weaker and anti-vimentin staining was negative.

Four weeks after tenotomy

The affected fibers still showed discrete central basophilia in HE and slightly increased NADH activity, although the lesions were much smaller and fewer. The variability in fiber size increased and both slightly hypertrophied and atrophied fibers were observed. Central lesions were still recognizable in anti-desmin staining (Fig. 9). Affected fibers showed either small round accumulation of desmin fluorescence in the center or wider, irregular and dispersed fluorescence.

Occasionally the concentration of desmin under the sarcolemma was present. AO staining showed no increased fluorescence. No changes could be found in anti-vimentin staining.

At 6 weeks after tenotomy

Central lesions were rarely seen and limited to slightly hypertrophic fibers.

Discussion

The structural and enzymatic changes observed by us in rat soleus muscle after tenotomy were generally similar to those described by other authors [16, 17, 18, 19, 20, 21, 22, 23]. The tenotomized soleus muscle undergoes degenerative changes. The morphological alterations are maximal one week after the injury, after which follows a period of recovery. The degenerative changes include the disappearance of mitochondria, the breakdown of myofibrils, disorganisation of sarcomeres and Z-line disintegration [18, 19]. Shafiq et al. [17] and Karpati et al. [18] reported rod-like structures in tenotomized rat soleus muscle. But as in this study many other workers found no evidence of rod-like structures in tenotomized rat soleus muscle [19, 20, 21, 22]. It has been suggested by Karpati et al. [18] that the rapidity of the degenerative process indicated the enzymatic mechanism. It has also been observed that the regression of central lesions was accompanied by the proliferation of ribosomes at the periphery of lesion [18] as well as between myofibrils and under the sarcolemma [19]. Further observations were the aggregation of mitochondria at the junction between the lesion and the normal part of the fiber and the rearrangement of Z lines and the formation of new myofibrils [18, 19]. Six weeks after tenotomy, muscle fiber morphology eventually returns to normal.

The nature of the central lesions has became a subject of some controversy in the literature, nevertheless, most authors refer to those changes as a "central core" or "core-like" lesions [18, 19, 20, 21, 23]. Indeed, by light microscopy the lesions resemble the morphological alterations found in human central core disease [26, 27] as well as target and targetoid fibers observed in human denervated muscle [28, 29, 30]. It is known from experimental studies [31] that target fibers occur frequently in the course of reinnervation. The basic alteration in all those abnormalities is a decrease in mitochondria in the center of muscle fiber. The central cores and targetoid fibers contain only two concentric zones, whereas the targets contain three. A similar pattern of ultrastructural changes can be observed in cores and targets.

Besides the decrease or disappearance of mitochondria from a central region, the focal myofibrillar degeneration which begins with streaming and disintegration of the Z-line is most characteristic.

Thornell et al in 1983 [2] first described the involvement of intermediate filaments in formation of central core lesions in human central core disease. The authors observed the absence of the regular desmin network in the center of many fibers, sometimes with intensely fluorescing demarcation between the core and the rest of the fiber. In many instances, strong fluorescence was revealed in the core, indicating the presence of intermediate filament protein in a disorganized filament form. Very few desmin abnormalities, however, were observed by the same authors in targetoid fibers in 2 cases of Parkinson' s disease as well as in minicores in multicore disease. The authors concluded that central core was due to a different pathological process than targetoid fibers and multicore disease.

To our knowledge intermediate filaments in tenotomized rat muscle have not previously been studied. Our results indicate that the expression of desmin in soleus muscle fibers was significantly altered by tenotomy. Vimentin, which occurs in high concentration only in immature fetal and regenerating muscle cells [5, 13] did not reappear in tenotomized muscle fibers in our experiment. It is known that in normal mature muscle fibers, desmin is involved in the mechanical integration and stability of the myofibrils

[32]. In tenotomy such integration is profoundly disturbed. It does not appear to us that the formation of central lesions in the tenotomized muscle fibers is precipitated by the breakdown and/or accumulation of desmin in the center of the fiber. The time course and comparable intensity of morphological and immunofluorescent alteration in muscle fibers indicates rather that these changes are concomitant.

Basophilia and increased AO fluorescence which suggests increased RNA content in the tissue [33, 34] indicates a restorative effort of muscle fibers following tenotomy. Proliferation of ribosomes in tenotomized muscle has been reported by Karpati et al [18] and Baker and Hall-Craggs [19]. Indeed, Baker and Hall-Craggs have also observed intensive de novo myofibrillar formation within areas of degeneration. Such changes can be interpreted as the processes initiating repair. The similar pattern of accumulation of desmin and AO-RNA fluorescence in such areas may indicate that desmin plays a role in the organisation of newly formed myofibrils. The same pattern with intense desmin staining, and negative [35] or much weaker [13] vimentin staining were observed in later stages of muscle fiber regeneration.

In summary we conclude that the examination of desmin expression is an easy and useful method for visualizing different stages of focal myofibrillar rearrangement in experimentally tenotomized muscle fibers.

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