Formation of Unique Vacuoles in Tenotomized Rat Soleus Muscle Fibers*

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Received January 9, 2001

Summary. The formation of unique vacuoles in tenotomized rat soleus muscle fibers was examined by light and electron microscopy. After tenotomy at both proximal and distal tendons, virtually all muscle fibers underwent characteristic degenerative changes with a disorganization of myofibrils called the central core lesion, but eventually recovered. At 3 days after tenotomy, some muscle fibers showed small vacuoles in the sarcoplasm of the end segments, which were larger in diameter and paler in staining than those of the control fibers in light microscopy. At 5 days, more fibers formed larger vacuoles together with the extensive disorganization of myofibrils. Such vacuole formation was more conspicuous in the distal end than in the proximal end. At 1 week the myofibrillar disorganization was most extensive in the central areas, and vacuoles were considerably enlarged in some fibers to occupy most of the sarcoplasm near the fiber ends. Vacuoles decreased in number and size with time and could rarely be seen at 4 weeks postoperative. In thin-section electron microscopy, the early forms of vacuoles were often connected with the T-system tubules. The limiting membrane of such vacuoles possessed many caveolae, some of which appeared to be continuous with the T-system networks. The vacuole membrane was closely associated with the sarcoplasmic reticulum to form dyadic connections. In later stages, the vacuole membrane was lined in part with the basal lamina. From these findings, it can be concluded that the vacuoles are sarcolemmal in nature and derived from the T-system. The significances of the vacuole formation are discussed with special reference to the mechanism and fate of the vacuoles and their clinical implications.

Tenotomy induces characteristic central core lesions in the rat soleus muscle. The central core lesion reaches its maximum extent at 1 week, and then recovers until no trace of the lesion can be seen by 6 weeks (Karpati et al., 1972; Baker and Hall-Craggs, 1980b; Abou Salem et al., 2001). Because of the unique pattern of myofibrillar degeneration in muscle fibers, experimental tenotomy has served as a model system for studies of pathological changes that occur in various muscle diseases.

Interestingly, the muscle belly retracts and remains at one half or two thirds of its original length in the tenotomized soleus muscle (Baker and Hall-Craggs, 1978). Indeed, there is a 33-50% reduction in the number of sarcomeres in the series at the point of recovery in order to adjust to the new functional length of the muscle (Baker and Hall-Craggs, 1980a). We have previously demonstrated that tenotomized muscle fibers form many surface grooves and folds along their entire length, completely recovering their smooth surface by the 6th week (Abou Salem et al., 1993a). The ends of muscle fibers also undergo drastic morphological changes after tenotomy, as has been revealed by thin-section electron microscopy (Abou Salem et al., 1993b). Most previous studies focused on the disorganization and reorganization of myofibrils with little attention to interfibrillar membranous systems such as the sarcoplasmic reticulum and T-system tubules (see review, Jamali et al., 2000).

During a study of morphological changes in tenotomized muscle fibers, we found unique vacuoles which were formed in the central area near the fiber ends (Abou Salem et al., 1993b). Apparently such

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*This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan and from the National Center for Nervous, Mental and Muscular Disorder of the Ministry of Health and Welfare, Japan.
Fig. 1. Light micrographs of semi-thin sections of soleus muscles fibers. 

a. Control muscle. Transversely cut muscle fibers (MF) are uniformly stained with toluidine blue showing a smooth surface contour with characteristic parallel stripes.

b. 3 days after tenotomy. Muscle fibers (MF) are larger in diameter and paler in staining without parallel stripes as compared with the control. Note vacuoles of varying sizes formed in some muscle fibers which were cut at the level of the end segments.

c. 5 days after tenotomy. The end segments of muscle fibers are still larger in diameter than the control. Some muscle fibers showed formation of multiple vacuoles together with an extensive disorganization of myofibrils.

d. 1 week after tenotomy. The majority of muscle fibers show extensive degenerative changes of myofibrils with larger vacuoles (asterisks). Note two adjacent vacuoles positioned close to each other with a very thin septum (arrows).

e. Longitudinal section of muscle fibers at 1 week after tenotomy. A muscle fiber shows large longitudinally elongated vacuoles (asterisks) which occupy most of the sarcoplasm, while the other shows a typical central core lesion (CCL).
vacuole formation at the end segments of muscle fibers has escaped the attention of researchers, for their observations have concentrated on typical degenerative changes in the middle segments. The present paper describes the formation of vacuoles in the end segments of tenotomized rat soleus muscle fibers, and discusses the nature of such vacuoles in view of not only their pathogenesis but also the possible involvement of interfibrillar membranous systems in their adjustment to the new length of the muscle — besides the reduced serial sarcomere number during recovery from the extensive degenerative changes after tenotomy.

**MATERIALS AND METHODS**

**Animals**

Adult, male Wistar rats 230–270 g body weight were used for this study. All animals were kept in an animal house under the same environmental conditions, and allowed to move freely in their cages. Food and water were continuously available. For tenotomy, animals were intraperitoneally anesthetized with sodium pentobarbital (45 mg/kg) and both proximal and distal ends of the soleus were transected (BAKER and HALL-CRAGGS, 1980b) with standard antiseptic precautions. Through a small posterio-lateral skin incision at the knee, the proximal tendon of the soleus was cut with a scalpel while the lateral head of the gastrocnemius was pulled medially, so as to avoid damage. Subsequently, the distal tendon was transected through a small midline skin incision by cutting the whole tendon Achilles. Special care was taken to avoid any damage to the nerve and blood supply for the muscle.

**Light and electron microscopy**

Three animals were used for each group of different time intervals after tenotomy: 3 and 5 days, 1, 2, 3, and 4 weeks. Each animal was anesthetized as described above. The hind limb was fixed by perfusion through the descending thoracic aorta with 200 ml of 1/2 Karnovosky’s fixative containing 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3). Then both proximal and distal ends of the soleus muscle were excised and cut into 1 mm-thick strips. Tissue strips were immersed in fresh fixative for additional 2 h at room temperature and then overnight at 4°C. After washing with 10%
sucrose in 0.01 M cacodylate buffer at 4°C, the tissue strips were postfixed with 1% OsO₄ in the same buffer for 2 h at 4°C, stained en bloc with 0.5% aqueous uranyl acetate for 2 h at room temperature, dehydrated in graded concentrations of ethanol, and then embedded in Epon 812. The Epon-embedded samples prepared for electron microscopy were used for light microscopic observations on semi-thin sections stained with 1% toluidine blue. Thin sections were cut, stained doubly with uranyl acetate and lead citrate, and examined with a Hitachi H-800 type electron microscope at an accelerating voltage of 100 kV.
RESULTS

Light microscopic observations

Semi-thin sections of the soleus muscles before and after tenotomy were stained with toluidine blue and observed with a light microscope (Fig. 1). The control muscle fibers were seen to be uniformly stained with smooth surface contours. In transversely cut fibers, characteristic parallel stripes were conspicuous, representing the lateral arrangement in register of myofibrils (Fig. 1a). At 3 days after tenotomy, muscle fibers (MF) appeared to be larger in diameter and paler in staining without any parallel stripes in the end segments as well as in the middle segments indicating the irregular arrangement of myofibrils (Fig. 1b). Vacuoles were found in the end segments of some muscle fibers. At 5 days after tenotomy, the end
segments of muscle were still larger in diameter than the control, and more fibers showed the formation of multiple vacuoles together with the extensive disorganization of myofibrils (Fig. 1c). It should be stressed that the vacuoles occurred only in the end segments, especially in the distal ends of muscle fibers. Vacuoles varied in size with more or less elongated forms. At 1 week after tenotomy, the majority of muscle fibers showed extensive degenerative changes of myofibrils with large vacuoles occurring in the end segments (Fig. 1d). Adjacent vacuoles were positioned close to each other, suggesting that they were about to fuse to form larger ones. In some fibers, longitudinally elongated, large vacuoles were seen to occupy most of the sarcoplasm near the fiber ends (Fig. 1e). At 2 and 3 weeks after tenotomy, muscle fibers were reduced in size and vacuoles decreased in number, to be rarely seen by 4 weeks.

**Electron microscopic observations**

At 3 days after tenotomy, myofibrils were seen to be disorganized with disintegrated Z-discs along the entire length of the muscle fibers, especially in the central areas initiating the central core lesion. Some muscle fibers showed vacuoles of varying sizes at the end segment of muscle fibers. The limiting membrane of the vacuoles appeared to be sharp, similar to the sarcolemma and T-system tubules. The lumen of such vacuoles was usually filled with homogeneous substances of various electron densities (Figs. 2, 3a). Vacuoles were found only in the end segments of muscle fibers. However, they were clearly distinguishable from the invaginations of myotendinous junctions because of their lack of a basal lamina along the limiting membrane. Vacuoles often contained membranous structures such as membrane-bound bulb, membranous fragments, and myelin-like structures (Figs. 2, 3a). The vacuoles formed many
caveolae along the limiting membrane (Fig. 3b).

At 5 days after tenotomy, the central areas of the very end segments of muscle fibers showed a conspicuous disorganization of myofibrils with disintegrated Z-discs and a loss of thick filaments. Interestingly, such membrane-bound bulbs were continuous with the sarcoplasm in such a way that the limiting membrane of the vacuoles was reflected to be continuous with the membrane of the bulbs (Fig. 4). This feature suggests that the membrane-bound bulbs in the vacuole may represent bulla-like bulges of the sarcoplasmic matrix. The end segment contained an increased number of vacuoles of variable shapes and sizes (Fig. 5a). Two adjacent vacuoles were often positioned close to each other, separated by a very thin septum (Fig. 5a, b).

At 1 week, vacuoles increased in size (Fig. 6), showing various profiles (Fig. 7a). In some fibers vacuoles occupied the most of the sarcoplasm. The limiting membrane of the vacuoles possessed more caveolae and were associated with more T-system networks (Figs. 7b, 8).

At 2 weeks after tenotomy, the vacuoles were reduced in number and size as compared with those found at 1 week after tenotomy. One of the most interesting features was that the lumen of vacuoles acquired the basal lamina in an intermittent manner (Fig. 8). The areas around the vacuoles still showed characteristic features of the disorganization of myofibrils with disintegrated Z-discs. The nemaline-like dense bodies could also be seen. The limiting membrane of the vacuoles formed more caveolae and T-system networks (Fig. 8). The sarcoplasmic reticulum (SR) appeared dilated with an irregular outline (Fig. 9a) and showed a tendency to be rough-surfaced (Fig. 9b).

At 4 weeks after tenotomy, vacuoles only appeared rarely seen — if at all — in the end segments of muscle fibers which were under recovery process.
DISCUSSION

The present study os the first yo demonstrate the occurrence of unique vacuoles in the end segments of muscle fibers of the rat soleus muscle when both proximal and distal tendons has been transected. Such vacuoles were more conspicuous in the distal end segments, where the disorganization of myofibrils was also more extensive in the proximal ends. Careful electron microscopic examination has proved that such vacuoles are derived from the T-system tubules, which appeared to be distended as the earliest forms of vacuole formation. Furthermore, the vacuoles possessed caveolae along the limiting membrane, which was also associated with the sarcoplasmic reticulum to form dyadic connections. Clearly, the limiting membrane of vacuoles is sarcolemmal in nature as is the T-system.

There are several types of sarcoplasmic vacuoles of different origins. The vacuoles of sarcolemmal origin including T-system tubules are formed in various experimental and pathological conditions such as fatigue, hypertonic treatment, injury, and certain myopathies (Kroenke, 1969; Gonzalez-Serratos et al., 1978; Caseademont et al., 1988; Carpenter and Carpenter, 1989; Lannergren et al., 2000). Fraser et al. (1998) morphologically distinguished between “open” and “closed” tubular vacuoles, considering that the vacuolation process was limited solely by the osmotic event; extracellular glycerol causes an initial water efflux, a decrease in muscle fiber volume and
Fig. 7. Electron micrographs of transversely cut muscle fibers at 1 week after tenotomy. a. Transverse section of a muscle fiber. Vacuoles (v) show various profiles. Some vacuoles are connected to T-system tubules (arrows). b. Longitudinal section of a muscle fiber. The vacuole (v) possesses caveolae (arrows) which are associated with proliferating T-system networks (TN).

Fig. 8. Electron micrograph of a transversely cut muscle fiber at 2 weeks after tenotomy. Vacuoles (v) in the very end segment close to the myotendinous junction are connected to proliferating T-system networks (TN). Note that the limiting membrane of the vacuole is partly lined with the basal lamina (arrows). ECM extracellular matrix.
tubular luminal dilatation owing to water movement from the sarcoplasm to hypertonic extracellular space. Similar conditions may occur in the tenotomized muscle fibers, though the same mechanism may not be easily applicable to vacuole formation. In this connection, it is interesting to note that the vacuoles contained some homogeneous materials which are not found in the T-system tubules in the control fibers. Although such contents can not be characterized in the present study, the complex features of the contents may be explained by bulla formation from the limiting membrane of vacuoles as seen in the surface sarcolemma in some degenerative conditions of muscle fibers. Elaborate T-system networks were formed in close association with the vacuoles (ANDREWS and WASSILEV, 1994), suggesting that the contents of the vacuoles may stimulate the proliferation of caveolae to form such networks (ISHIKAWA, 1968).

One question that naturally arises is why the vacuoles are formed only in the end segments but not in the middle segments of muscle fibers. Our previous study showed that the myotendinous junctions underwent a series of morphological changes after tenotomy as revealed by scanning and thin-section electron microscopy (ABOU SALEM et al., 1993a, b). The ends of muscle fibers showed highly marked changes much more than originally supposed; the sarcoplasmic processes collapsed, becoming shorter and less pointed, and the number of the processes drastically decreased. Deep cavities were formed between the stocky processes. In the present light microscopic observations, the fiber ends were seen to be enlarged with a pale-stained sarcoplasm at 3 days after tenotomy. The areas of vacuole formation also showed extensive myofibrillar disorganization. These findings indicate that the end segments — including myotendinous junctions — were highly affected by tenotomy, probably more than the middle segments. In addition, the morphological changes were more extensive in the distal end segments than in the proximal ones when both tendons were transected. These findings suggest that the vacuole formation may be another form of destructive change induced by tenotomy that parallels in time course with the myofibrillar disorganization and disintegration in the middle segments of muscle fibers (ABOU SALEM et al., 2001).

It is interesting to consider the fate and significance of vacuoles. Vacuoles appeared first as small vacuoles at 3 days after tenotomy, became larger in size with time until 2 weeks, and then were reduced in number, eventually becoming a rare occurrence at 4 weeks. Electron microscopy clearly indicated that such large vacuoles were formed by the fusion of smaller vacuoles. In later stages after tenotomy, the limiting membrane of vacuoles was seen to be lined in part with the basal lamina. It is possible that the vacuoles may eventually become part of the surface sarcolemma at
the fiber end to form new sarcoplasmic processes and invaginations as reorganized into elaborate surface specializations of the myotendinous junction (ABOU SALEM et al., 1993a, b).

The rat soleus muscle shortened to half to two thirds of the original length at 1 week after tenotomy, and recovered while keeping its shortened length. During degeneration and subsequent recovery, muscle fibers have to adjust their length to a new state, removing many sarcomeres. Membranous systems may also be reorganized in some way. BAKER and HALL-CRAGGS (1980a) attempted to explain the mechanism of sarcomere removal and noted the preferential degeneration of myofibrils at both ends of muscle fibers. It is interesting to speculate that vacuoles may also be formed to reserve the excess membrane of T-system tubules during the sarcomere removal and to supply the sarcosomal membrane for accommodation to form sarcoplasmic processes and invaginations at the fiber ends. Further study is needed to elucidate the exact mechanism of readjustment for muscle fibers to their new length during recovery from tenotomy.

REFERENCES


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