An Electron Microscope Study of the Ring Fibers and Its Neuromuscular Junction in Human Progressive Muscle Dystrophy

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It is known that ring fibers are often observed in some human muscle diseases, especially in myotonic dystrophy (Heidenhain, 1918; Perry et al., 1956; Schotland et al., 1966), and in normal ocular muscles (Wohlfart, 1932; Mori, 1953; Rubinstein, 1960; Bethlem and Van Wijngaarden, 1963; Inke and Sitka, 1965 and others). Although there have been a few reports dealing with the fine structure of these ring fibers (Schotland et al., 1966), very little is known about the structural details of their neuromuscular junction. The present paper describes the fine structure of the ring fiber and its neuromuscular junction in human muscle disease.

Materials and Methods

The material was taken at surgical biopsy from the biceps brachial muscle of a 32 years old male with limb-girdle type of muscle dystrophy. The material was put into a drop of fixative on a piece of dental wax and then cut into small bits. The specimens were fixed in glutaraldehyde-formaldehyde solution containing CaCl₂ buffered with 0.1M phosphate at pH 7.4 for 2 hrs at room temperature (according to Karnovsky, 1965), and followed by usual osmium tetroxide fixation for 2 hrs. The specimens were dehydrated in a series of graded ethanol and then embedded in Epon epoxy resin (Luft, 1961). Thick sections for light microscopy were cut with glass knives on a Porter-Blum microtome and stained with toluidine blue in order identify the ring fibers. Thin sections for electron microscopy were made in the same way as mentioned above, stained with 2% uranyl acetate and lead citrate solutions, and examined in a Hitachi HS-7 electron microscope.

Observations

The ring muscle fibers appear to occur singly apart from the group of regular muscle fibers. These are elliptical in cross section and the surface is invested continuously with a thin layer of basement membrane material. In most cases each ring fiber is surrounded by one or more thin cytoplasmic process of the fibroblasts (Fig. 1). The nuclei with irregular outline are located at the peripheral sarcoplasm close to the sarcolemma.

The myofibrils are divided into two groups according to their arrangement; one consists of longitudinal fibrils occupying the central column of muscle fiber and the other consists of circular ones located around the longitudinal fibrils (Fig. 1). A few longitudinal fibrils, however, are also observed to occur sporadically in
between circular ones (Fig. 1, 2). The longitudinal fibrils are about 1 \( \mu \) in diameter and often fuse with adjacent ones, whereas the circular ones are variable in size, mainly less than 1 \( \mu \) in diameter, and tend to branch. The basic structure of myofibrils is the same as that of regular skeletal muscle fibers.

The sarcoplasmic reticulum which usually closely invests the myofibril is not so well developed (Fig. 1, 2). Although triadic structures are encountered in this fiber, these do not occur in definite relation to the banding of myofibrils (Fig. 2).

![Fig. 1. A cross section of a ring fiber from a patient muscle dystrophy. The myofibrils are arranged longitudinally and circularly. A few mitochondria are scattered mainly in the peripheral sarcoplasm. A ring fiber is ensheathed by thin processes of fibroblasts. \( \times 7,500 \) (K. Suzuki)]
A few granular endoplasmic reticula and the Golgi apparatus are seen in the subsarcolemmal cytoplasm where myofibrils are absent (Fig. 1).

Numerous mitochondria varying in size and form are also observed in the peripheral sarcoplasm, without relation to the myofibrils. Particular concentration of mitochondria is found in the sarcoplasm at the neuromuscular junction (Fig. 3). Glycogen particles are distributed homogeneously throughout the sarcoplasmic matrix, especially abundant at the area of the neuromuscular junction (Fig. 4).

The motor nerve ending of this muscle fitted into the concavity of the sarcolemma to make the neuromuscular junction. The sarcolemma facing the nerve ending shows complex infoldings forming the secondary synaptic clefts (Fig. 3, 4). Both the primary and secondary clefts are filled with a basement membrane material.

Fig. 2. A high magnification electron micrograph of the juxtanuclear sarcoplasm. Note the characteristic arrangement of myofibrils (MF). The sarcoplasmic reticulum (SR) investing each myofibril is relatively scanty. N nucleus, M mitochondria, T triad. ×18,000
Synaptic vesicles are classified into two types: the clear and the cored (Fig. 4). The majority are clear vesicles which are about 500 Å in diameter and circular or elliptical in profile. The cored ones are about 1,000–1,500 Å in diameter, very few in number and contain a dense material of about 700–1,000 Å. Besides synaptic vesicles a few coated vesicles occur, implying possible pinocytosis. In relation to these vesicles, small invaginations in the axolemma

Fig. 3. A cross section of a ring fiber showing the motor nerve ending (MNE). The subsynaptic folds are developed in a complicated form. Mitochondria are concentrated in the subsynaptic sarcoplasm. × 7,200
are seen coated with bristle-like structures (arrow; Fig. 4)

Fig. 4. A neuromuscular junction at higher magnification to the numerous clear synaptic vesicles, a few cored and several coated vesicles are contained in the nerve terminal (SV). The arrow indicates that small invaginations in the axolemma are seen coated with bristle-like structures. MF myofibrils, PC primary synaptic cleft, SSC secondary synaptic cleft. ×22,000
Discussion

In earlier light microscope studies HEIDENHAIN (1918) described the occurrence of striated, circular myofibrils, so-called “Ringbinden,” in the muscle fibers of myotonic dystrophy. Later these muscle fibers were found frequently in the normal ocular muscles (WOHLFART, 1932; MORI, 1953; RUBINSTEIN, 1960; BETHLEM AND VAN WIJNGAARDEN, 1963; INKE AND SITKA, 1965).

The present study has revealed the fine structure of the ring muscle fibers and of its neuromuscular junction. It is generally known that the sarcoplasmic reticulum is poorly developed in the slow fiber, whereas it is well developed in the fast one. Thus the relative paucity of sarcoplasmic reticulum in the ring fiber, coupled with the irregular occurrence of triads, may suggest that this fiber likely shows physiological properties similar to those of the slow fiber. Since, however, an electrophysiological study of this type muscle fiber appears to be lacking, further work is required to clarify its real functional nature.

The structural organization of the neuromuscular junction in this ring fiber is similar to that in the fast muscle. In the normal ocular muscles, WOHLFART (1932) reported that most ring fibers are localized at the entrance zone of the nerve. From his observations, WOHLFART (1932) assumed that rich innervation is required for the ring fiber with morphological characteristics. In this case of myotonic dystrophy, however, such characteristic localization of ring fibers as is found in ocular muscle was not recognized. Furthermore, the ring fiber does not appear to be abundantly innervated. The functional significance of ring fibers in this disease is not clear at present. Judging from the structural features observed in present study and taking into account that the ocular muscles exert that exquisite and complex movement of eyeball, the ring fiber is believed to serve in the unusually complex contraction of muscles. The existence of coated vesicles in the nerve ending may suggest an active uptake of some materials from its surrounding, though the physiological implication of these vesicles at the nerve ending is not known at present.

Summary

The material was taken at surgical biopsy from the biceps brachial muscle of a 32 years old male with limb-girdle type of muscle dystrophy. The fine structure of the ring fibers in human myotonic dystrophy were studied by electron microscopy. The myofibrils of this fiber were arranged in two different directions: an inner longitudinal and an outer circular group. The circular myofibrils were also interplaced irregularly with a few longitudinal myofibrils. The sarcoplasmic reticulum seemed to be less developed than in normal fibers. Triads occurred irregularly without a definite relationship to the bandings of myofibrils. Mitochondria were seen exclusively in the peripheral sarcoplasm. The neuromuscular junction of the ring fiber corresponded in fine structure to that in the usual skeletal muscle fibers of the fast type, though the secondary synaptic clefts of the muscle fiber appeared more complex.
進性筋ジストロフィー症にみられたリングファイバーと
その神経筋接合部の微細構造（内容自抄）

進性筋ジストロフィー症患者（32才男）の筋生検を行い、リングファイバーとその
神経筋接合部を観察した。リングファイバーの筋線維は、中心部は長軸方向に、また周
辺部はそれをとりかこむ様に走り、一部はみだれた配列をしめしていた。筋小胞体は、
多少、少ない様にみえた。トライアドは筋線維の横断に関係なく不規則に分布していた。
ミトコンドリアは、筋精の近くに特に多くみられた。

リングファイバーの神経筋接合部は、骨格筋のそれと似た像を呈するが、結合ヒダは、
もっと複雑な像を観察した。

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