Muscle fiber and motor end plate involvement in the extraocular muscles of the myotonic mouse*

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The extraocular muscles of the C57BL/6dy²j myotonic mouse were studied by phase and electron microscopy. The most susceptible ocular muscle was the levator palpebrae; the other muscles manifested scattered abnormalities to varying degrees. Central nucleation and fiber splitting were prominent. Junctional abnormalities consisted of a reduction in postjunctional folding, excessive numbers of axonal terminals on hypertrophic fibers, and the presence of dense granules between axon and muscle. This study demonstrates the affection of both muscle fiber and motor end plate in mouse myotonic dystrophy.

Key words: extraocular muscle, mouse myotonic dystrophy, muscle fiber alterations, motor end plate alterations.

In myotonic dystrophy the facial and extraocular muscles typically become involved in addition to the limb musculature.1-3 The morphology of extraocular muscles in myotonic dystrophy has rarely been examined, and then only by light microscope.1-5 These studies utilized human biopsy and autopsy specimens and described changes compatible to those encountered in the skeletal musculature.

An animal model for the study of myotonic dystrophy has been suggested in mouse Strain Re-129 dy²j/dy²j insofar as these animals show myotonic physiologic responses in addition to progressive muscle weakness and atrophy.6 Previous studies of animals with this myotonic (dy²j) gene have been limited to the peripheral musculature.6-7 In the present study, the extraocular muscles of another strain of mouse (Bar Harbor C57BL/6Jdy²j) carrying this same myotonic (dy²j) gene were examined by light and electron microscopy. A variety of abnormal changes were seen in the muscle fibers and motor end plates of these muscles.

Materials and methods

Five myotonic and five nonmyotonic littermate controls of the Bar Harbor Mouse Strain C57BL/
Fig. 1. Two fibers, the result of fiber splitting, as verified in sequential sections, are seen by electron microscopy. In one fiber, deep sarcolemmal invaginations (arrow) are observed, some of which completely isolate an incipient daughter fiber (arrowhead). Levator palpebrae, x4,500.

6Jdy2J, age 3 to 5 months, were examined. The affected animals manifested atrophic hind limbs which were extended and dragging. The upper extremities were little affected and the eyes appeared to be normal. The limb musculature of these animals was clinically examined by electromyography and with tension measurements. These muscles exhibited myotonic electrophysiologic and tension responses typical of this disorder. The control animals were normal in this respect.8 The animals were perfused through the heart with 1 per cent paraformaldehyde-1 per cent glutaral-
Fig. 2. Abnormal mitochondria with paracrystalline inclusions (arrow). Superior rectus, ×18,000.

Fig. 3. Higher magnification of bracketed area in Fig. 2, as seen in a nearby section. A triplicate lamellar structure is apparent. Its total thickness is about 208 Å, with electron transparent layers of about 104 Å between the three dense layers. The inclusion appears between the outer and inner mitochondrial membranes (arrows). Superior rectus, ×80,000.

dehydro in phosphate buffer. The extraocular muscles, including the levator palpebræ, were freed under a dissecting microscope, fixed in cold 4 per cent glutaraldehyde for 10 hours, postfixed in 1 per cent osmium tetroxide for two hours, dehydrated in graded alcohols, and embedded in Epon 812. The embedded muscles were serially sectioned transversely at 15 microns by steel knife on a sliding microtome. These thick Epon sections were cleared for light microscopy by curing a layer of Epone onto them within a sandwich of polystyrene film; the complete muscles could thus be initially surveyed by phase contrast. Sections of interest were freed from the plastic sandwich and remounted for further one micron and ultrathin sectioning.

Results

In the nonmyotonic littermate control animals, the muscles examined appeared normal by light and electron microscopy. In the myotonic animals, morphologic alterations were observed only in the muscle cells and innervation of fibers characterized by well delineated myofibrils separated by abundant sarcoplasmic reticulum in both the I and A bands. These cells have relatively complex myoneural junctions, and vary widely in size and mitochondrial content. Such fibers would cover the range encompassed by the so-
Fig. 4. Paracrystalline inclusions (arrows) in the intracristae space of mitochondria. Such abnormal mitochondria were found near the end plate of a fiber which contained dense granules. Superior rectus, ×48,000.

called Type I and Type II fibers described in the skeletal musculature. The former appeared to be much more susceptible to morphologic change. In a given section, approximately 5 to 10 per cent gave evidence of being affected. The "morphologically slow" fibers, which are also found in extraocular muscle, are not affected at this stage of the disease process. Such "slow" fibers have a sparse sarcoplasmic reticulum in the A band, large or irregularly shaped myofibrils in the I band, and relatively simple myoneural junctions wherein the postjunctional folding is minimal or altogether absent. The levator palpebrae muscle was the most susceptible to morphologic alteration; "slow" fibers were not observed in this muscle.

Fiber splitting was a prominent finding; the complexity of this process ranged from that of simple focal separation and rejoining to cases where the daughter fibers atrophied and terminated. Splitting fibers often present an otherwise essentially normal ultrastructure (Fig. 1).

The mitochondria of occasional fibers contained triplicate (Figs. 2 and 3) or duplicate (Fig. 4) lamellar paracrystalline inclusions. These were located in the outer mitochondrial chamber, which includes the space between the outer and inner mitochondrial membranes and the connecting intracristae space.

Muscle cells of irregular shape and bizarre ultrastructure were sometimes seen. The fiber in Fig. 5 contains numerous large vacuoles, the limiting membranes of which are in close apposition thereby forming extended membrane pairs. Patches of disoriented myofibrillar material and several dense bodies are also seen in this cell.

Additional intracellular alterations included distention of the sarcoplasmic reticulum, vacuolization, excessive lipid accumulations, and myofibrillar breakdown.

In the myotonic animals, junctional areas with an atypical reduction or absence of postjunctional folds were observed on both abnormal and apparently normal "fast" fibers (Fig. 6). This conclusion of pathologic deviations from the normal state was based on a large sampling of end plates, many of which were studied in serial section. Such density of sampling was necessitated by the variability in postjunctional folding evidenced by normal fibers of this
Fig. 5. In this irregular shaped fiber, an abundance of large vacuoles (V) are apparent. Several dense bodies (DB) and disoriented myofibrils (arrow) are also seen. Medial rectus, ×18,000.
Fig. 6. Several terminal axons (Ax) are smoothly apposed to the sarcomlemma of this fiber, the myofibrils of which are clearly delineated in both the I (I) and A (A) bands. The axons and the fiber appear to be otherwise normal. Levator palpebrae, ×9,000.

Fig. 7. Normal end plate of a typical morphologically "fast" fiber. There are numerous axonal terminals (Ax) apparent, one of which (arrow) makes a simple apposition to the muscle fiber. The other terminals have well developed postsynaptic membranes with complex junctional folding. Superior rectus, ×6,000.

type. Extraocular muscle end plates are characterized by separate small axonal terminals which vary in number from one to approximately six within a given plane of section. The complexity of postsynaptic folding appears to vary from one to another of such junctional components. Some axonal terminals may show numerous deep folds, while other terminals of the same end plate may be virtually flat within a given plane of section (Fig. 7). In sequential sections of a given axonal terminal, essentially flat junctions (Fig. 8, A) were sometimes seen to develop folding (Fig. 8, B), or conversely, the junctional folding of some terminals was seen to gradually disappear. Thus, flat terminals in themselves can not be considered as indicative of abnormality. In the abnormal tissue, however, a reduction or absence of post-
junctional folding was seen with much higher frequency as compared to the normal, and flat terminals were seen to persist in their simplicity in serial sectioning.

Another variety of apparently abnormal end plates in this tissue were those showing a profuse elaboration of the end plate region with excessive numbers of terminals (Fig. 9). Occasionally, dense granules were seen between the axonal terminal and postsynaptic membrane of otherwise apparently normal end plates (Fig. 10).

Discussion

Most of the alterations which were seen in this study to affect the muscle fibers themselves, i.e., dilation of the sarcoplasmic reticulum, fiber splitting, central nuclei, excessive lipid accumulations, and myofibrillar breakdown were previously described in the limb musculature of this and other strains carrying the dy2j gene. Some of these changes were also observed in human myotonic peripheral and extraocular muscle.

Paracrystalline mitochondrial inclusions appear not to have been previously described in any of the animal strains inbred for a neuromuscular disorder. Such inclusions have been observed in a variety of human muscle diseases, though not in myotonic dystrophy. These distinctive structures are apparently nonspecific, though they would seem to be the dominant finding in some pathologies. In extraocular muscles, such mitochondrial inclusions were observed in a case of human muscular dystrophy and in human chronic progressive ophthalmoplegia.

The bizarre formations of numerous very large vesicles seen here (Fig. 5) would seem not to have been previously described in dystrophic disorders, but have been observed in muscle fibers after long periods of denervation, and in primary hypokalemic periodic paralysis. It is unclear whether these are true vacuoles or represent excessive membrane proliferation.

The most prominent alterations affecting the motor end plate were a reduction in the number of postjunctional folds, and an increase in the number of axonal terminals in hypertrophic fibers. The reduction of postjunctional folding has been observed in dystrophic mice of the Re-129 strain, and in mice paralyzed by local injection of tetanus toxin. Excessive numbers of axonal terminals on hypertrophic fibers have been reported in myotonic and other dystrophies. Conceivably, such increase in area of contact between axonal terminals...
Fig. 9. Enlarged end plate region on hypertrophic fiber containing an atypical abundance of axonal terminals (Ax). Superior rectus, ×9,000.

Fig. 10. Motor end plate showing two axonal terminals (Ax). Dense granules (arrowhead) are seen interposed between each terminal and sarcolemma of the muscle fiber. Superior rectus, ×39,000.
and the muscle surface may be a structural compensatory response to a reduced efficiency of impulse transmission or a partial functional denervation induced by the decreased available area of postjunctional synaptic contact.

The presence of dense granules between axon and muscle has been reported in mice paralyzed by tetanus toxin. It was suggested that they might have originated from lysosomes of muscle fibers which had become atrophic due to prolonged denervation. In the present case, however, these granules were associated with junctions on nonatrophied fibers.

A prior ultrastructural study of a different strain of the Bar Harbor mouse, carrying this myotonic (dy2j) gene1 emphasized abnormalities of the end plate in the limb musculature, the most prominent of which was a decrease in the number of synaptic vesicles in the axonal terminals. A comparable reduction in the number of synaptic vesicles was found by us in the limb musculature of the presently studied strain of animals. This alteration of the axonal terminal did not seem to be a significant factor in their extraocular muscles, however. It would seem that alterations of the synaptic vesicles is not a critical modification of the end plate.

In this study of extraocular muscle, (1) the reduction of postjunctional folding, (2) the splitting of fibers, and (3) the presence of central nuclei were considered to be abnormal only when they occurred in relation to "fast" fibers, as defined above. These three structural characteristics are an apparently normal aspect of the morphology of the well known "slow" fibers of these muscles. Insofar as structural changes which were unequivocally abnormal were not observed in these "slow" fibers, it would seem that they are the most resistant of the fiber varieties observed.

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REFERENCES


