Fine structure of neuromuscular junctions in myasthenic extraocular muscles

Takashi Sakimoto and Kensei Cheng-Minoda

The fine structure of neuromuscular junctions in extraocular muscles obtained from 10 myasthenic patients was investigated. In the Felder-struktur muscle fibers, all of the junctions appeared normal in their structure, although the number studied was limited. In the Fibrillen-struktur, approximately one fourth of the junctions studied showed structural alterations of various types. The terminal axons were frequently elongated, although their synaptic vesicles and mitochondria appeared normal in structure and number. The primary synaptic cleft was occasionally dilated to form a cystic enlargement. The junctional folds often increased in number, reaching more than 20. An extensive elongation and ramification of the infolds was occasionally observed. Sponge-form structures, not described in previous literature, were encountered in the interior of a few muscle fibers. They seemed to have resulted from an extensive elongation and ramification of the junctional folds.

Key words: myasthenia gravis, extraocular muscles, neuromuscular junction, neuroneuronal synapse, mitochondria, axon, ultrastructure, histopathology, electron microscopy.

The neuromuscular junction of myasthenic muscles has been considered to be the primary lesion of the disease. Histologic and histochemical observations on the myasthenic neuromuscular junctions of the skeletal muscles revealed an elongation, expansion, and complex arborization of the nerve terminals.1-5

However, electron microscopic studies of the neuromuscular junctions of myasthenic skeletal muscles lead to conflicting views. Bickerstaff and associates6 failed to find significant differences between the myasthenic and nonmyasthenic junctions, while Zacks and associates7,8 reported a widening of the primary and secondary synaptic clefts, and the existence of dense granular or lamellar structures in the terminal axons. Woolf9 described paucity of the junctional folds as well as expanded terminal axons, containing large numbers of synaptic vesicles and dense mitochondria.

The purpose of this paper is to describe the fine structure of the neuromuscular junctions of the myasthenic extraocular muscles which have never been the subject of electron microscopic study. Myasthenia gravis frequently begins at the extraocular muscles. The nerve endings in the muscles are more numerous than in the other skeletal muscles. One can therefore, take
advantage of studying a large number of neuromuscular junctions at various stages of the condition so that an extensive survey of the structural alterations can be made.

Materials and methods

A total of 11 extraocular muscles, including 5 levators, were obtained from 10 myasthenic patients. The detailed data for the patients are given in Table I. Seven of the patients were classified into Group I, ocular myasthenia (Osserman),10 and 3 into Group II, generalized myasthenia. The diagnosis of myasthenia gravis was always confirmed with the edrophonium chloride test (Tensilon) and in some instances together with electromyography of skeletal and extraocular muscles. Surgery was performed because all of the muscles failed to respond sufficiently to anticholinesterase drugs, and the muscle specimens were obtained during the operations. The specimens stretched on a dental wax sheet were fixed mostly in 2.5 per cent glutaraldehyde, buffered with Millonig's phosphate (pH 7.3 to 7.4) for approximately 15 minutes. They were cut into smaller pieces and again fixed in the above solution for 1 to 2 hours. After a brief washing with the phosphate buffer, they were again fixed in 1 per cent osmium tetroxide buffered with the same phosphate for 1.5 hours. A few specimens were fixed only with 1 per cent osmium tetroxide. Subsequently they were dehydrated in a series of graded ethanols, and embedded in Epon 812 according to Luft's method. Ultrathin sections were cut with glass or diamond knives in the Porter-Blum MT-1 ultramicrotome, and were stained with uranyl acetate and lead citrate. They were observed with Hitachi's electron microscopes HS-7 and 11.

Results

The numbers of the muscles and the neuromuscular junctions studied are listed in Table I. In the Fibrillen-struktur muscle fibers of the rectus and oblique muscles, 44 out of 58 neuromuscular junctions appeared normal in their fine structure. In the Felder-struktur muscle fibers, all 4 junctions apparently preserved normal fine structure.

The normal end-plate of the Fibrillen-struktur type made a prominent elevation on the muscle fiber. In this elevated area there were clusters of mitochondria, several nuclei, and numerous ribosome particles. Each of the terminal axon branchlets was located on a synaptic groove of the end-plate. The axon contained numerous synaptic vesicles, 450 to 500 A in diameter, and small mitochondria. The post-synaptic membrane of the muscle fiber invaginated regularly into the interior of the muscle, thus forming well-developed junctional folds. On the other hand, the terminal of the Felder-struktur type did not make a conspicuous elevation because the terminal axon was in contact with a flat surface of the muscle fiber. The muscle membrane formed sparse junctional folds. There was scant sarcoplasm in the synaptic region. These findings were essentially in agreement with previous reports on the neuromuscular junction of normal extraocular muscles.11-14

Table I

<table>
<thead>
<tr>
<th>Case No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age and sex</td>
<td>3F</td>
<td>20F</td>
<td>16F</td>
<td>26F</td>
<td>5F</td>
<td>20M</td>
<td>23M</td>
<td>24M</td>
<td>3F</td>
<td>3F</td>
<td>27F</td>
</tr>
<tr>
<td>Duration (mo.)</td>
<td>3</td>
<td>6</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>15</td>
<td>21</td>
<td>23</td>
<td>23</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Osserman type</td>
<td>1F</td>
<td>1I</td>
<td>I</td>
<td>II</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>II</td>
<td>II</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>Number of junction examined</td>
<td>Fibrillen-struktur type</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>26</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Felder-struktur type</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Findings</td>
<td>Increase of junctional folds</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sponge-form structure</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Distinctive changes were found in 14 junctions of the Fibrillen-struktur type. Their terminal axons were often elongated, reaching occasionally up to 20 µ in length (Fig. 1). In those axons were observed a number of synaptic vesicles and numerous small mitochondria (Fig. 1). The synaptic vesicles showed a tendency to aggregate on the synaptic side. The fine structure of those vesicles and mitochondria appeared normal. Contrary to the previous reports, large numbers of synaptic vesicles and dense mitochondria, or dense granular material and myelin bodies in the terminal axons were not observed in this study. The primary synaptic cleft between the plasma membranes of the terminal axon and the muscle fiber was approximately 500 Å in width, and in the middle of the cleft was a continuous layer of basement membrane-like material. Occasionally, the synaptic cleft was dilated to form an enclosed cystic space (Fig. 2). In this space were observed flocculent or granular substances, presumably derivatives of disorganized basement membrane material. No myelin bodies were detected in the clefts. In 4 patients the junctional folds increased in number to more than 20 (Fig. 3). The depth of the infoldings also increased from 2 to 3.5 µ. Occasionally the infolds of the muscle membrane appeared to be extensively elongated and ramified, reaching into the core of the muscle fiber (Fig. 4). Consequently, in transverse section, these infolds were observed as disseminated islands in the interior of the muscle fiber. The width of the infolds was approximately 1,500 Å and their internal surface was always covered with a thin layer of basement membrane-like material. The myofilaments, mitochondria, and sarcoplasmic reticulum of the muscle fiber were normal in their structure.

In a few muscle fibers, extensively developed infolds demonstrated peculiar sponge-form structure (Figs. 5 and 6). This structure consisted of complex ramification of the infolds, approximately 1,800 to 2,100 Å in width, with the sarcoplasm...
interspersed in between. The surface of those folds was covered with a continuous layer of basement membrane-like material. The inside of the folds appeared to be translucent or negatively stained. The interspersed sarcoplasm contained a number of myofilaments, glycogen particles, a few mitochondria, and dilated sarcoplasmic reticulum. The dilated sarcoplasmic reticulum occasionally contained a granular ground substance. No degenerative change was found in the myofilaments and mitochondria. The observation of the serial sections suggested that this sponge-form structure was extended at least 5 to 6 μ along the long axis of the muscle fiber. Although no direct connection of terminal axon with the sponge-form structure was demonstrated, it is most likely that the structure resulted from extensive elongation and ramification of the junctional folds. The structure was observed in 3 patients, with 3, 12, and 23 months' duration of the disease. There seemed to be no close correlation between the neuromuscular changes and the duration of the disease.

The sarcoplasmic organelles in the end-plate region, such as nuclei, clusters of mitochondria, glycogen, and ribosome particles appeared normal in their structure and distribution. In addition, a number of tubules containing an internal granular substance were occasionally observed in the end-plate region (Fig. 2). No myelin bodies were found in the region.

It was more difficult to find neuromuscular junctions in the levators than in the

**Fig. 2.** A dilated synaptic cleft forming a cystic space which contains flocculent substance (arrow). Note the number of tubules with internal granular substance (arrows with bar) in the end-plate sarcoplasm. (x17,000.)
Fig. 3. A neuromuscular junction showing the increased number (more than 20) and elongated depth (2 to 3.5μ in length) of the junctional folds (arrows). (×14,000.)

Fig. 4. A transverse section of the muscle fiber showing extensively elongated and ramified infolds (In). Note a distinct continuity (arrow) of the infolds with the sarcolemma (SL). (×13,000.)
rectus and oblique muscles, since the number of the junctions is relatively less in the former than the latter. The present study detected, however, 5 neuromuscular junctions in the 2 levators and 3 of the junctions showed an increase of the junctional folds.

Discussion

An extensive increase of the junctional folds is of primary interest in the present study. Since the number of junctional folds is relatively less in normal extraocular muscle than in skeletal muscle, the presence of more than 20 junctional folds, as shown in this paper, is quite unusual. It is of interest that a similar increase of the infolds has also been reported in skeletal and extraocular muscles after denervation. The peculiar sponge-form structure, as first described in the present study, is considered to be a result of extensive elongation and ramification of the junctional folds for two reasons: (1) A distinct continuity between the surface membrane of the infolds and the sarcolemma was confirmed in this study, as shown in Fig. 4, and (2) the internal surface of the infolds was always covered with a layer of basement membrane-like material, as is the case with the junctional folds.

Electrophysiological data have pointed to a deficiency of acetylcholine release from myasthenic terminal axons. Accordingly, if the synaptic vesicles are the storage site of this neurohumoral transmitter, the compensation of the disturbed transmission may take the form of increased synaptic vesicles. Nevertheless, most of the electron microscopic studies have failed, so far, to find any obvious increase of the synaptic vesicles. Although Woolf's re-
Fig. 6. The same sponge-torn structure as shown in fig. 5 at higher magnification. The surface of the infolds (In) is constantly covered with a thin layer of basement membrane-like material (arrow) and their inside appears translucent or negatively stained. The network of the sarcoplasm interpersed with the infolds contains apparently normal myofilaments (arrow with bar), mitochondria (Mi), glycogen particles, and a few vesicles (Ve). (×31,000.)
ported increased numbers of synaptic vesicles and dense mitochondria at the expanded terminal axon, the present study did not find any significant increase of the vesicles, thus agreeing with most of the previous reports cited above. The present finding of a widening of the synaptic clefts is in agreement with that of Zacks and associates.\(^7\)\(^8\) The widening of the cleft may prevent the transmitter substance from making normal contact with the postsynaptic membrane. Another form of compensation of the disturbed transmission may be an increase in contact area of the synapse. The elongation of the terminal axon, the increase of the junctional folds in number and depth, and the formation of the sponge-form structure may all indicate compensatory responses of the junctions against disturbance of neuromuscular transmission in myasthenia gravis.

The tubular structures with internal granular substance in the sarcoplasm of the end-plate has not been previously described. The present authors, however, occasionally observed a few of those tubules in the subsarcolemmal area of normal extraocular muscle fibers,\(^20\) so that the significance of the tubules in the myasthenic end-plate has to be further investigated.

Attention should be given to the fact that there was no close correlation between changes in the neuromuscular junctions and the duration of the disease. For instance, the sponge-form structure was also found in the patient with 3 months' duration of the disease. This may suggest rapid changes of the junctional folds following the disturbance of neuromuscular transmission in myasthenia gravis. Such quick change of the infold was also reported in experimentally denervated skeletal and extraocular muscles.\(^17\)\(^18\)

As regards the relationship between the muscle fiber type and myasthenia gravis, it should be noted that 3 of the 5 neuromuscular junctions in the levator, which are known to possess Fibrillen-struktur type exclusively,\(^21\) showed an increased number of junctional folds. Moreover, all junctions of the Felder-struktur type in the extraocular muscles showed apparently normal fine structure. These findings may raise doubts as to whether the Fibrillen-struktur type is more vulnerable in myasthenia gravis than the Felder-struktur, or the former is specifically involved in the disease. However, the numbers of investigated junctions of both levators and Felder-struktur type were quite limited in this study, so that the question remains to be solved by further investigations.

We wish to thank Prof. Shin-ichi Shikano and Assoc. Prof. Saiichi Mishima for their helpful advice and encouragement.

REFERENCES


Meet our new salesmen

They found Soaclens® more comfortable than the other contact lens solutions tested*

Soaclens may well provide the comfort required for the successful use of contact lenses. You should see the complete study—and we'll be happy to send you a reprint on request.


Burton, Parsons & Co., Inc.
Manufacturers of Ethical Pharmaceuticals Since 1885
WASHINGTON, D.C. 20027 • TELEPHONE (301) 336-5700