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An electron microscopic study on differences in the fine structures of motor endplate in red, white and intermediate muscle fibers of rat intercostal muscle. A preliminary study

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# An electron microscopic study on differences in the fine structures of motor endplate in red, white and intermediate muscle fibers of rat intercostal muscle. A preliminary study\*

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## Abstract

An electron microscopic study on the fine structural differences of motor endplates among the red, white and intermediate muscle fibers of the rat intercostal muscles was made and the following results were obtained. 1. In the motor endplate of the red fiber, the junctional folds were poorly developed and their number was small. 2. In the motor endplate of the white fiber, the junctional folds were well developed and their number was far more numerous than those in the red fiber. 3. The fine structure of the motor endplate of the intermediate fiber was of an intermediate character between the red and white fiber.

**AN ELECTRON MICROSCOPIC STUDY ON DIFFERENCES  
IN THE FINE STRUCTURES OF MOTOR ENDPLATE  
IN RED, WHITE AND INTERMEDIATE MUSCLE  
FIBERS OF RAT INTERCOSTAL MUSCLE.  
A PRELIMINARY STUDY**

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In the previous histochemical study<sup>1</sup> on the mouse motor endplate, it was shown that different type of striated muscle fibers had a motor endplate of different structure; namely, the motor endplate of the red muscle fiber was small and compact, while that of the white muscle fiber large and complicated. The motor endplate of the intermediate fiber was intermediate in size and structure between that of the red and white fiber.

In this preliminary study, fine structural differences of the motor endplate in red, white and intermediate muscle fibers of the rat intercostal muscle were observed by electron microscopy.

MATERIALS AND METHODS

Male Wistar rats weighing 200—300 g, of 100 days old were used. Thin strips of the intercostal muscle were fixed in 1% osmium tetroxide buffered to pH 7.4 with veronal acetate, rapidly dehydrated, and embedded in epon. Ultrathin sections were cut on a Porter-Blum ultramicrotome MT-I, stained with uranyl acetate and lead hydroxide, and then examined with a Hitachi model HU-11A. Thicker sections (about 2.0  $\mu$ ) were stained with toluidine blue and examined with the light microscope.

RESULTS

In all the three types of muscle fibers of rat intercostal muscle, both sides of Z-line, bracelet-like mitochondria encircled the myofibril at the I-band level. In the red fiber, a heavy accumulation of large mitochondria occurred in subsarcolemmal space. Another feature of the red fiber was the presence of the chain of large mitochondria which ran longitudinally among myofibrils.

In the white fiber, subsarcolemmal aggregation and interfibrillar chains of mitochondria were usually absent.

In the intermediate fiber, a moderate amount of mitochondria was located in the subsarcolemmal space. In the interfibrillar space, the number of the chains of interfibrillar mitochondria was less numerous per area than that of the red fiber and the size of interfibrillar mitochondria was smaller than that of the red fiber.

These differences in three types of fibers of the rat intercostal muscle are similar to those in the rat diaphragm reported by STEIN and PADYKULA<sup>2</sup>, and GAUTHIER and PADYKULA<sup>3</sup>.

With a motor endplate under the electron microscope observation, the entire muscle fiber including the motor endplate was photographed at low magnification. From these pictures, the fiber type of each motor endplate was identified.

*The motor endplate of the red fiber:* The most prominent difference of the fine structure of the motor endplate in three types of fibers was the degree of the development of the junctional folds. In the red fiber, the size of the motor endplate was small. The junctional folds were poorly developed and their number was small (Figs. 1 and 2). The space between each junctional fold was wide. Generally between two terminal nerve endings (or terminal axon branchlets) with the cluster of junctional folds, there was discontinuous zone of junctional folds: namely, each terminal nerve ending was isolated by the junctional sarcoplasm which numerous mitochondria occupied, although in the white fiber this space was usually filled with continuous arrangement of numerous junctional folds. The depth of junctional folds were relatively shallow, and the branches or their anastomoses were not so conspicuous as those in the white one. The intensity of aggregation of mitochondria in the junctional sarcoplasm was more marked than that in white one. Thus the fine structure of the red fiber motor endplate was simpler in appearance.

*The motor endplate of the white muscle fiber:* The motor endplate of the white muscle fiber was large and the junctional folds were well developed (Figs. 3, 4 and 5). The junctional folds were far more numerous than those in the red fiber and the space between two terminal nerve endings was usually occupied by the continuous arrangement of the numerous junctional folds. The depth of the junctional folds were deeper, more branched, and anastomosed than that of the red one. The junctional folds were so numerous and packed in the white fiber, so that the distance between each junctional fold was narrow and in some part they

were arranged in parallel. Generally, the aggregation of mitochondria in the junctional sarcoplasm was less numerous than the red one and the motor endplate of the white fiber was more complicated in appearance than that of the red one.

*The motor endplate of the intermediate muscle fiber:* On the whole, the fine structure of the motor endplate of the intermediate fiber was of an intermediate character between that of the red and white fiber. Namely, its size was intermediate between that of the red and white fibers, and the junctional folds were better developed than those in red one but less than the white one (Figs. 6, 7 and 8). The number of the junctional folds were moderate and the distance between individual junctional folds was intermediate between that in the red and the white one. The average depth of the junctional folds were intermediate between the red and white one, and the degree of their branch or anastomosis was also intermediate between the red and the white one. Some junctional folds were relatively shallow, but others were considerably deep, branched and anastomosed. Between the two terminal nerve endings, the junctional folds were usually contiguous, but their number were less numerous. Generally, the number of aggregated mitochondria in the junctional sarcoplasm were moderate.

The narrow neck was observed in the junctional folds in all the three types of motor endplates.

The contents of so-called "synaptic vesicles" in the terminal axon were variable, so there was no distinct difference in the contents of synaptic vesicles in the three types of motor endplates.

#### DISCUSSION

It has been generally accepted that in the amphibian or reptile striated muscles<sup>4,5</sup>, the different types of the motor endplates are observed in different types of muscle fibers; namely, the slow fiber has the multiple "en grappe type" motor endplate, while the fast fiber the "en plaque type" motor endplate.

In the mammalian muscle, only the extraocular muscle or cryothyroid muscle has different type of motor endplate<sup>4,6</sup>; namely, the slow fiber has the "en grappe" type and the fast fiber the "en plaque" type motor endplate. However, in other muscles of mammals, for example, the limb muscles, it is generally accepted that there is no difference in motor endplate of different types of muscle fibers.

Recent histochemical<sup>7,2</sup> or electron microscopic<sup>8,3</sup> studies reveal that

three different types of muscle fibers, the red, white and intermediate muscle fibers can be distinguished from the difference in their size and the content of mitochondria in mammalian striated muscle fibers.

In the previous paper<sup>1</sup>, the structural differences in the red, white and intermediate muscle fibers were demonstrated by double staining of choline esterase and succinoxidase. Namely, the motor endplate of the red fiber had a small, simple and compact structure, while that of the white fiber a large and complicated interlacing structure. And the endplate of the intermediate fiber was of a medium size and moderately developed structure. From the present study, the fine structural differences in the motor endplate in the red, white and intermediate fibers were elucidated at ultrastructural level. The striking difference in them was the degree of the development of the junctional folds.

In the white fiber, the total surface area of the junctional folds in endplate was much wider than that of the red one, because the endplate was larger, the junctional folds occupied wider area, the number of junctional folds were more numerous, and the junctional fold was deeper and more branched than that of the red fiber. Possibly, such an extremely wide surface area of the junctional folds was necessary to transmit the rapid impulse from the motor nerve to fast contracting larger white fiber. And much smaller in the total surface might be sufficient for slow contracting red fiber. The function of intermediate fiber is unknown. However, if the development of the junctional folds is closely correlated to the physiological function of muscle fiber, possibly this fiber has the intermediate function between the red and the white fibers, because the structure of its endplate is intermediate between them.

According to KATZ<sup>2</sup>, the synaptic vesicles are thought to represent the storage form of acetylcholine. The contents of them are variable in terminal nerve endings of each type of motor endplates. The most closely packed with synaptic vesicles is the nerve ending of white fiber (Fig. 4), but not so closely packed with other endings of white fiber, so there is no distinct difference in the contents of synaptic vesicles in the three types of motor endplate.

#### SUMMARY

An electron microscopic study on the fine structural differences of motor endplates among the red, white and intermediate muscle fibers of the rat intercostal muscles was made and the following results were obtained.

1. In the motor endplate of the red fiber, the junctional folds were poorly developed and their number was small.
2. In the motor endplate of the white fiber, the junctional folds were well developed and their number was far more numerous than those in the red fiber.
3. The fine structure of the motor endplate of the intermediate fiber was of an intermediate character between the red and white fiber.

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#### REFERENCES

1. OGATA, T.: A histochemical study on the structural differences of motor endplate in the red, white and intermediate muscle fibers of mouse limb muscle. *Acta Med. Okayama*. 19, 149, 1965
2. STEIN, J. M., and PADYKULA, H. A.: Histochemical classification of individual skeletal muscle fibers of the rat. *Am. J. Anat.* 110, 103, 1962
3. GAUTHIER, J. G., and PADYKULA, H. A.: Cytological studies of fiber types in skeletal muscle. A comparative study of the mammalian diaphragm. *J. Cell Biol.* 28, 333, 1966
4. ZACKS, S. I.: *The Motor Endplate*. W. B. Saunders Company, Philadelphia and London, 1964
5. ANDERSON-CEDERGREN, E.: Ultrastructure of motor endplate and sarcoplasmic components of mouse skeletal muscle fiber as revealed by three-dimensional reconstructions from serial sections. *J. Ultrastruct. Res. Supplement* 1, 1959
6. HESS, A.: The structure of slow and fast extrafusal muscle fibers in the extraocular muscles and their nerve endings in guinea pigs. *J. Cell. and Comp. Physiol.* 58, 63, 1961
7. OGATA, T.: A histochemical study of the red and white muscle fibers. Part I. Activity of the succinoxidase system in muscle fibers. *Acta Med. Okayama* 12, 216, 1958
8. OGATA, T.: An electron microscopic study on the red, white and intermediate muscle fibers of mouse. *Acta Med. Okayama* 18, 271, 1964
9. KATZ, B.: Microphysiology of the neuromuscular junction. *Bull. Johns Hopkins Hosp.* 102, 275, 1958

#### EXPLANATIONS

- Fig. 1 The motor endplate of the red muscle fiber. Note the number of junctional folds are scarce and their depth was relatively shallow. Between the two terminal nerve endings with cluster of junctional folds, the uncontinuous zone of junctional folds and it is filled with numerous mitochondria and junctional sarcoplasm.  $\times 13,500$
- Fig. 2 The motor endplate of the red muscle fiber. Note the junctional folds are scarce and they are generally shallower than that of the white one. The neck (N) of the junctional folds is narrow and these folds are less branched or anastomosed than in the white one. The space between individual junctional folds is much wider than that of the white one. The small vesicles are filled with the terminal nerve cell endings.  $\times 30,000$

- Fig. 3 The motor endplate of the white muscle fiber. Note numerous junctional folds. Between two terminal nerve endings the junctional folds are contiguous. The mitochondria in junctional sarcoplasm are not so numerous as the red fiber.  $\times 9,600$
- Fig. 4 The motor endplate of the white fiber. Note the numerous deep junctional folds. Many synaptic vesicles are closely packed in this terminal nerve ending.  $\times 10,000$
- Fig. 5 The motor endplate of the white fiber. The junctional folds are so numerous that the space between the junctional folds is narrow. The depth of the junctional folds is deep and these folds are more branched or anastomosed than those of the red one. Some junctional folds are arranged in parallel.  $\times 26,000$
- Fig. 6 The motor endplate of the intermediate muscle fiber. The number of the junctional folds are moderate. The space between the two terminal nerve endings are filled with continuous junctional folds.  $\times 8,000$
- Fig. 7 The motor endplate of the intermediate fiber. The depth of the junctional folds is relatively deep and the folds are moderately branched or anastomosed.  $\times 8,000$
- Fig. 8 The motor endplate of the intermediate fiber. Note that the degree of the development of the junctional folds is intermediate between that of the red and white fibers. The space between the two junctional folds is wider than that of the white one but narrower than that of the red one. The aggregation of mitochondria in junctional sarcoplasm is moderate.  $\times 30,000$

JF: Junctional fold, M: Mitochondria, MC: Muscle fiber, Nu: Nucleus, S: Small vesicles, SC: Schwann cell, TB: Terminal axon branchlet, NU: Neck of the junctional fold













