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An Electron Microscopic Study of the Myofilament of the Mammalian Cardiac Muscular Tissue*

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The authors continued the electron microscopic observation of the cardiac muscular tissue to know the fine structure of the myofilament.

The myosinfilament showed a hole in the center in the cross section and electron dense part in both sides and the light part in the center in the longitudinal section. This finding meant each myosinfilament has a central canal.

This central canal was found in the cardiac muscle of mouse and rat, also in skeletal muscle of the rat and human in both n-butyl methacrylate and Epon embedding. The authors concluded that this central canal is a proper structure of the myosinfilament and not an artefact.

HUXLEY¹⁾ published an excellent paper on the fine structure of the skeletal muscular tissue. In his paper he described that myosinfilaments and actinfilaments were arrayed in double hexagonal system, one myosinfilament was surrounded by six actinfilaments. BATTIG²⁾, MOORE³⁾ and STENGER⁴⁾ observed the cardiac muscular tissue on the electron microscope and found that the cardiac muscular tissue was similar to the skeletal muscular tissue.

In previous paper, MIZUNO, ARAYA and TAKAHASHI⁵⁾ reported the electron microscopic structure of the cardiac muscular tissue. We found that myosinfilaments were arrayed in equal distance one another. Three myosinfilaments made one regular triangle and one myosinfilament was surrounded by six myosinfilaments, seven myosinfilaments made one hexagonal system. Concerning actinfilaments, we had an opinion that the number of actinfilaments around one myosinfilament was

more than six. HUXLEY said this difference might be due to the difference of the material (insect, cold-blooded and mammal) and the condition of the contraction of myofibrils. He also said, it might be chiefly originated from the condition of the contraction, that was, the shrinkage of the actinfilaments. This opinion looks reasonable.

We have been trying further observation. Until now, we can not make conclusion. Although, concerning the fine structure of myosinfilaments, we have got an interesting finding. We should like to publish our results.

This study was performed under the kind direction of Professor YASABURO OIKE and with cooperation of all crews of Department of Anatomy of Medical School and the Electron Microscopy Section of Hirosaki University.

MATERIALS AND METHODE

We used chiefly the heart of the mouse and rat. We also studied the skeletal muscle of the rat and human. These tissues were fixed in 1 or 2 per

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cent osmium tetroxide solution with phosphate buffer for 1 or 3 hours at a temperature of 0°C to 3°C. Then, blocks of tissues were dehydrated in the graded solution of ethyl alcohol as usual and embedded in n-butyl methacrylate or Epon. The polymerization of n-butyl methacrylate completed in about 24 hours at a temperature of 45°C and the polymerization of Epon in 3 hours at 37°C, one night at 45°C and 15 hours at 60°C. Embedded tissues were cut into ultrathin sections on the Porter-Blum ultramicrotome. Sections were double stained in lead tartrate solution and then in the uranyl acetate solution. In n-butyl methacrylate, sections were carboned in the evaporator to prevent the evaporation of methacrylate and to protect the specimen from the beam damage. In Epon, carbonation was not necessary.

Then sections were ready for electron microscopy. We used Hitachi Electron Microscope type HU-11A.

RESULTS

1. The myosinfilament of the cardiac muscle of the mouse and rat in the n-butyl methacrylate embedding.

In the cross section of the myofibrils of the mouse heart, myosinfilaments were arrayed in hexagonal system same as described in previous paper⁵⁾. We had got very important finding, that was, each myosinfilament had a hole in the center of the cross section (plate 1). This ring-like figure was also seen in the cross section of the cardiac muscular tissue of the rat (plate 2).

In the longitudinal section of the myosinfilament, we found that the both sides of a filament were electron dense, and the center of the myosinfilament had a light space (plate 3). This finding was same in the longitudinal section of the cardiac muscle of the rat (plate 4).

There was no difference between the cardiac muscular tissues of the auricle and that of the ventricle.

2. The myosinfilament of the cardiac muscle of the mouse and rat in the Epon embedding.

The cross section of the myosinfilament of the heart showed the same shape with central hole as in the n-butyl methacrylate embedding (plate 5). In the longitudinal section, both sides of the myosinfilament were dark and the inside was light (plate 6). This finding was same as the n-butyl methacrylate embedding.

3. The myosinfilament of the skeletal muscle of the rat and human in the n-butyl methacrylate embedding.

The cross section of the skeletal muscle of the rat is shown in plate 7. This finding is same as the cross section of the cardiac muscle of the mouse, that is, each myosinfilament has a hole in the center. In the human skeletal muscular tissue the same shape is found (plate 8).

The longitudinal section of the skeletal muscle of the mouse and human embedded in n-butyl methacrylate showed the electron density of the side walls and the light space in the center (plate 9 and 10).

DISCUSSION

We found a hole in each cross section of the myosinfilament and the electron dense side wall in the longitudinal section of the myosinfilament of the cardiac muscular tissue of the mouse embedded in n-butyl methacrylate. This means that myosinfilaments of the cardiac muscle of the mouse have central canals under this condition.

We wondered, 1) was this finding seen in both cardiac and skeletal muscle? 2) was this finding seen in other kinds of animals? 3) was this a proper structure of the myosinfilament of the cardiac muscular tissue or an artefact? To resolve these questions, we tried some experiments. We observed the skeletal muscle of the rat and human, and we had got the same results. We also tried the cardiac muscle of the rat, and the result was same. We could not find any difference in the fine structure of the myosinfilament between two kinds of tissues and between some kinds of animals. We tried Epon as the embedding material to know this finding is due to the embedding, and we got the same result. This is not an artefact originated from the embedding. Judging from these findings, we must conclude that myosinfilaments of the cardiac and skeletal muscular tissue of these mammals have central canals, in other words, myosinfilaments are tubes. HUXLEY said that he found the central canal in the negative stained myosinfilaments, and he thought the central canal of the myosinfilament actually existed in insects and cold bloodeds, and this

Plate 1. The cross section of the cardiac muscular tissue of the mouse ventricle embedded in n-butyl methacrylate. See the ring form of the cross section of myosinfilaments in the left bottom, the center and the right top of this picture (arrows).

Mi: mitochondria Magnification: $\times 77,000$

Plate 2. The cross section of the cardiac muscular tissue of the rat ventricle embedded in n-butyl methacrylate. Ring form cross section of myosinfilaments in the left bottom, and pipe form longitudinal section in the left top of the picture.

Mi: mitochondria Magnification: $\times 77,000$

Plate 3. The longitudinal section of the cardiac muscular tissue of the mouse auricle embedded in n-butyl methacrylate. See the electron dense side walls and the electron light central canal of the myosinfilament (arrows).

Mi: mitochondria Magnification: $\times 77,000$

Plate 4. The longitudinal section of the cardiac muscle of the rat ventricle embedded in n-butyl methacrylate. Central canals of myosinfilaments are clear (arrows).

Mi: mitochondria Magnification: $\times 77,000$

Plate 5. The cross section of the cardiac muscular tissue of the mouse ventricle embedded in Epon. See the central canal of the myosinfilament in the left and right bottom field of the picture (arrows).
Magnification: $\times 77,000$

Plate 6. The longitudinal section of the cardiac muscular tissue of the mouse ventricle embedded in Epon. See the central canal of the myosinfilament in the right top and bottom field of the picture (arrows).

id : intercalated disk Magnification: $\times 77,000$

Plate 7. The cross section of the skeletal muscular tissue of the rat embedded in n-butyl methacrylate. See the ring form cross section of the myosinfilaments in the left bottom and the right top field of the picture (arrows).

Magnification: $\times 77,000$

Plate 8. The oblique section of the skeletal muscular tissue of the rat embedded in n-butyl methacrylate. See the central canal of myosinfilaments all over the picture.

Mi: mitochondria Magnification: $\times 77,000$

Plate 9. The cross section of the skeletal muscular tissue of the human embedded in n-butyl methacrylate. Ring form cross sections of myosinfilaments all over the picture.
Magnification : $\times 77,000$

Plate 10. The longitudinal section of the skeletal muscle of the human embedded in n-butyl methacrylate. Central canals of myosinfilaments are clear.
Z: Z Band Magnification : $\times 77,000$

meant that myosin molecules were arranged denser in the periphery than in the center of the myosinfilament.

This opinion looks very reasonable. We don't know now the meaning of this central canal, however, we think this finding is very interesting one.

SUMMARY

We looked the cardiac muscle of the mouse and rat, and the skeletal muscle of the rat and human on the electron microscope. We found a hole in the center of the myosinfilament in the cross section, electron dense side walls and electron light central canal in the longitudinal section of the myosinfilament. We consider that the myosinfilament of the cardiac muscle

of mammals has a central canal, in other words, a myosinfilament is a tube electron microscopically. This is also true in the skeletal muscle.

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