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MUSCLE FIBER TYPES AND THEIR ELECTRON MICROSCOPIC FEATURES IN THE INTRINSIC LINGUAL MUSCLES OF THE RABBIT AND THE MOUSE

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In this study we investigated the muscle fiber types and their ultrastructural features in the intrinsic lingual muscles of the mouse and the rabbit. Four albino mice and four rabbits were used. Pieces of the tongues were fixed in gluteraldehyde and postfixed in osmium tetroxide. Ultrathin sections stained with uranyl acetate and lead citrate were examined in JEOL-100SX electron microscope. In the rabbit, two types of muscle fibers that varied in ultrastructural features were observed. One of these fibers had numerous mitochondrial columns of large diameter. Tubules of sarcoplasmic reticulum were large and prominent. The other type had fewer numbers of mitochondrial columns of small diameter. But tubules of sarcoplasmic reticulum were fewer and small in diameter. The muscle fibers that make up the mouse lingual muscles were not all identical also. Some of the muscle fibers consisted parallel rows of mitochondria of large diameter. The elements of sarcoplasmic reticulum were small. Some of them consisted prominent and large sarcoplasmic reticulum tubules. Mitochondria were fewer than that of the other fiber type, and these were not arranged as long rows. In some of them mitochondria were fewer and sarcoplasmic reticulum tubules were small. It is concluded that the organization pattern and development degree of mitochondria and sarcoplasmic reticulum may be variable in different muscle fiber types in different mammals.

Key words: *Red Fiber, White Fiber, Mouse, Rabbit, Electron Microscopy*

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Tavşan ve Farede İntrensik Lingual Kas Lifi Tipleri Ve Bu Liflerin Elektron Mikroskopik Özellikleri

Çalışmamızda fare ve tavşanın intrinsik dil kaslarının kas lifi tipleri ve bu liflerin elektron mikroskopik özellikleri incelendi. Bu çalışmada dört adet beyaz fare ve dört adet tavşan kullanıldı. Dilden alınan parçalar gluteraldehidle ve osmium tetroksitle fikse edildi. Uranil asetat ve kurşun sitrat ile boyanan ince kesitler JEOL-100SX elektron mikroskopta incelendi. Tavşanda elektron mikroskopik özellikleri farklı olan iki tip kas lifi gözlendi. Bunlardan biri myofibriller arasında geniş çaplı mitokondri kolonları içermekteydi. Sarkoplazmik retikulum tubulleri geniş ve belirgindi. Diğer lif tipinde ise küçük çaplı az miktarda mitokondri kolonu bulunmaktaydı. Ancak sarkoplazmik retikulum tubulleri de az miktarda ve küçük çaplıydı. Farenin dil kaslarını oluşturan kas lifleri de birbirlerinden farklı özellikteydi. Bazı lifler myofibriller arasında paralel uzanan geniş çaplı mitokondri kolonlarını içermekteydi. Sarkoplazmik retikulum elemanları küçüktü. Bazıları ise belirgin ve geniş çaplı sarkoplazmik retikulum tubullerine sahipti. Mitokondrileri diğer lif tipinden daha azdı ve bunlar uzun diziler şeklinde organize olmamışlardı. Bazı liflerde mitokondri daha azdı, sarkoplamik retikulum tubulleri küçük ve belirsizdi. Mitokondri ve sarkoplazmik retikulumunun gelişim derecesinin ve organizasyon örneğinin farklı memelilerin farklı kas liflerinde değişken olabileceği sonucuna varıldı.

Anahtar kelimeler: *Kırmızı Lif, Beyaz Lif, Fare, Tavşan, Elektron Mikroskopi.*

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The striated voluntary or skeletal muscles are composed of bundles of muscle fibers, which are long, cylindrical, and multinucleated. Muscle fibers are composed of bundles of myofibrils. Myofibrils are bundles of thick filaments of myosin and thin filament of actin organized into chains of repeating units called sarcomers. Sarcomers give skeletal muscle its striated appearance.[1-4] The fibers that make up a muscle are not identical. They vary in color, diameter and cytochemical and physiological properties. Traditionally three types have been described: red fibers, white fibers and intermediate fibers. The red fibers have large and abundant mitochondria and a complex sarcoplasmic reticulum. The sarcolemmal mitochondria of white fibers are smaller than those of the red fibers, and mitochondria between myofibrils are relatively few. As their name implies, the intermediate fibers have characteristics intermediate between the red and white fibers. The disposition of their mitochondria is similar to that of the red fibers, except that thick interfibrillar columns of mitochondria are seldom found.[1,2]

In the present study we investigated the muscle fiber types and their ultrastructural features in the intrinsic lingual muscles (musculus longitudinalis linguae, musculus transversus linguae, musculus verticalis linguae) of the mouse and the rabbit.

MATERIALS AND METHODS

Four albino mice(two males and two females) and four rabbits(two males and two females) were used in the present study. The animals were killed by decapitation and their tongues were removed and cut into pieces. These are fixed in 3% gluteraldehyde buffered with 0.2 M NaH2PO4+NaHPO4 (pH=7.2-7.3), and postfixed in 0.1% osmium tetroxide buffered with 0.2 M NaH2PO4+NaHPO4 (pH=7.2-7.3). Specimens were dehydrated in acetone and embedded in Araldite CY 212. Semithin sections were studied with toluidin blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in an JEOL-100SX electron microscope.

RESULTS

Myofibrils, individual sarcomers, I bands, A bands, H zone, Z and M lines were evident in both rabbit and mouse muscle fibers (Figures 1, 2).

Nuclei were located directly beneath the sarcolemma. Perinuclear areas were devoid of myofibrils. Instead, these areas contained many mitochondria and glycogen particles (Figure 3). The fibers that make up the lingual muscles in the rabbit and in the mouse were not identical. In the rabbit, two types of muscle fibers that varied in their ultrastructural features were observed (Figure 4). The sarcoplasm of one of them was observed more electron dense. These fibers had numerous mitochondrial columns of large diameter between the myofibrils. Z line was thick and tubules of sarcoplasmic reticulum were large and prominent (Figures 4, 5). The other type had fewer numbers of mitochondrial columns of small diameter. Z line was thinner than that of the other fiber type. The tubules of sarcoplasmic reticulum were fewer and small in diameter (Figures 1, 4). (Table 1).

The muscle fibers that make up the mouse lingual muscles were not all identical also. Some of the muscle fibers consisted parallel rows of mitochondria of large diameter between the myofibrils. The elements of sarcoplasmic reticulum were small and not prominent (Figures 2,6). Some of them consisted prominent and large sarcoplasmic reticulum tubules. Mitochondria were fewer than that of the other fiber type, and these were not arranged as long parallel rows (Figures 7, 8). In some of them, mitochondria were fewer, but sarcoplasmic reticulum tubules were small and not prominent (Figure 9). (Table 2). Triads were located at A-I junctions in both mouse and rabbit lingual muscle fibers (Figures 1, 9). In transverse sections, thick

Table 1. The organization pattern and development degree of the mitochondria and sarcoplasmic reticulum in three types of the fibers in the intrinsic lingual muscle of the rabbit

Type 1	mitochondria are large	tubules of sarcoplasmic reticulum are large and prominent
Type 2	mitochondria are fewer and small	tubules of sarcoplasmic reticulum are small and not prominent

Table 2. The organization pattern and development degree of the mitochondria and sarcoplasmic reticulum in three types of the fibers in the intrinsic lingual muscle of the mouse

Type 1	mitochondria are large	tubules of sarcoplasmic reticulum are small and not prominent
Type 2	mitochondria are fewer and small	tubules of sarcoplasmic reticulum are large and prominent
Type 3	mitochondria are fewer and small	tubules of sarcoplasmic reticulum are small and not prominent

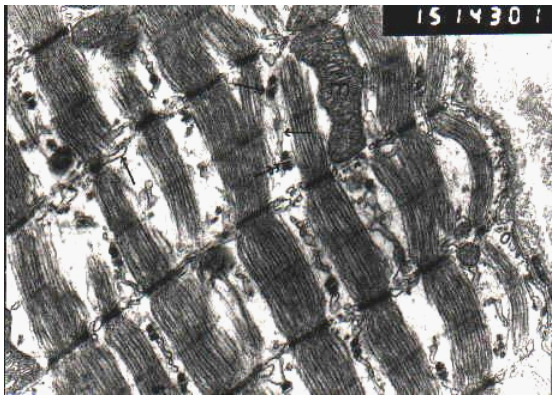

Figure 1. Intrinsic lingual muscle of the rabbit. Myofibrils, individual sarcomeres, H zone, Z and M lines are evident. Mitochondria(m) are thin and mitochondrial columns are rare. The tubules of sarcoplasmic reticulum(arrows) are fewer and small in diamater. Triads are located at A-I junctions(double arrows). Uranyl acetate and lead citrateX15.000.

Figure 2. Intrinsic lingual muscle of the mouse. Myofibrils, individual sarcomeres, I bands, A bands, H zone, Z and M lines are evident. This fiber has numerous mitochondrial(m) columns of large diameter between the myofibrils. The tubules of sarcoplasmic reticulum(arrows) are fewer and small in diamater. Uranyl acetate and lead citrateX6.000.

Figure 3. Intrinsic lingual muscle of the rabbit. Nuclei(N) are located directly beneath the sarcolemma. Perinuclear area is devoid of myofibrils. Instead, there are many mitochondria(m) there. A capillary(c) containing many pynocytotic vesicles is observed beneath the muscle fiber. Uranyl acetate and lead citrateX6.000.

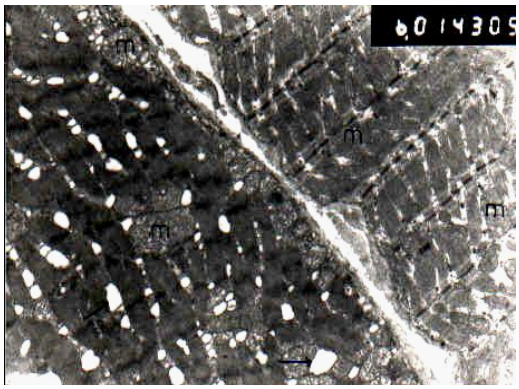

Figure 4. Intrinsic lingual muscle of the rabbit. Two types of muscle fibers that varried in ultrastructural features are observed. The sarcoplasm of one of them is observed more electron dense. There are mitochondrial columns(m) of large diamater and tubules of sarcoplasmic reticulum are large and prominent(arrow). The other fiber type has fewer mitochondria(m) of small in dimater. Tubules of sarcoplasmic reticulum is not prominent. Uranyl acetate and lead citrateX6.000.

and thin myofilaments in a hexagonal arrangement around them, mitochondria and tubules of sarcoplasmic reticulum were observed(Figure 10). Many blood vessels and nerve fibers were observed in the connective tissue surrounding muscle fibers (Figures 3, 8, 11). A myelin figure was observed within the sarcoplasm of one muscle fiber in the rabbit (Figure 12). Many mast cells were located in the connective tissue surrounding intrinsic lingual muscle fibers of the mouse (Figure 6).

DISCUSSION

The muscle fibers that make up the intrinsic lingual muscles of the mouse and the rabbit were composed of bundles of myofibrils. Light I bands, dark A bands, H zone, Z and M lines

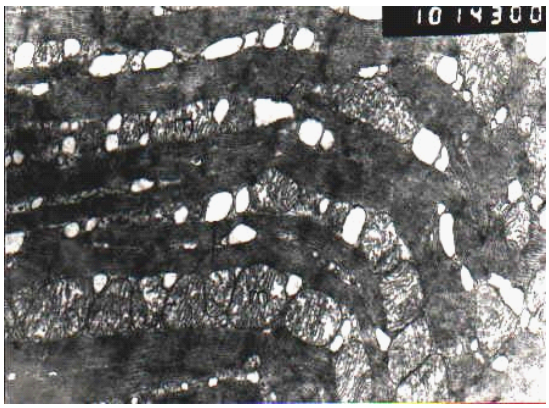

Figure 5.Intrinsic lingual muscle of the rabbit. Many mitochonria columns of large diamater(m) and large tubules of sarcoplasmic reticulum(arrow) are observed in the electron dense sarcoplasm o the muscle fiber. Uranyl acetate and lead citrateX10.000.

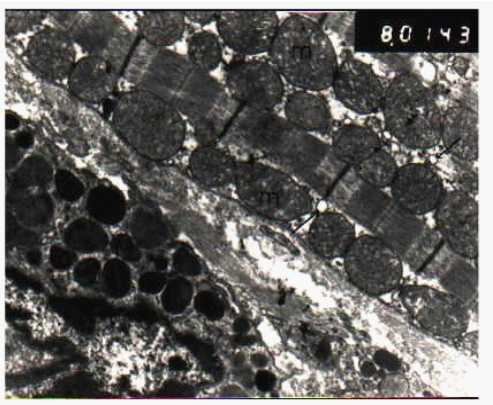

Figure 6. Intrinsic lingual muscle of the mouse. There are mitochondrial columns(m) of large diamater but tubules of sarcoplasmic reticulum are small in diamater and not prominent(arrow). A mast cell(M) is observed in the connective tissue surrounding the muscle fiber. Urany acetate and lead citrateX8.000.

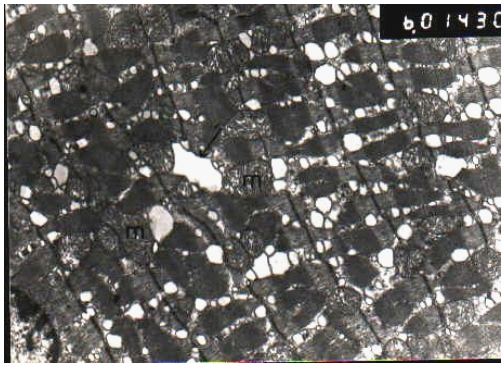

Figure 7. Intrinsic lingual muscle of the mouse. Mitochondial columns(m) are rare. Tubules of sarcoplasmic reticulum(arrow) are large and prominent. Uranyl acetate and lead citrateX6.000.

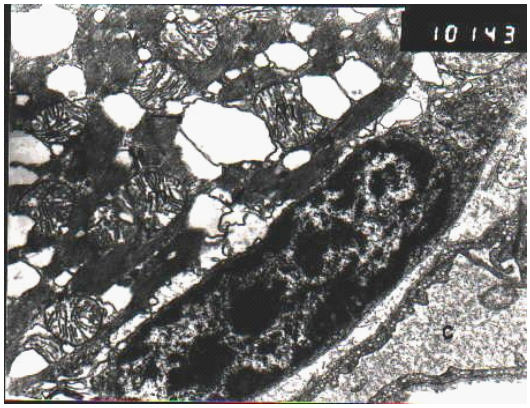

Figure 8. Intrinsic lingual muscle of the mouse. Mitochondial columns(m) are rare. Tubules of sarcoplasmic reticulum(arrow) are large and prominent. Nuclei(N) are located beneath the plasma membrane. A capillary(c) containing many pynocytotic vesicles is observed beneath the muscle fiber. Uranyl acetate and lead citrateX10.000.

were prominent. Sarcomers defined by Z lines were regularly present. Nuclei were located beneath the plasma membrane. Perinuclear areas containing many mitochondria and glycogen particles were devoid of myofibrils. Smooth surfaced endoplasmic reticulum was well-developed. Triads composed of two parallel terminal cisterna and the intervening T-tubule were located in A-I junction. These electron microscopic features of the intrinsic lingual muscles of the are usual characteristic of the striated muscle fibers of the mammals. Traditionally three muscle fiber types have been described: red fibers, white fibers and intermediate fibers. The red fibers are smaller in diameter and have a dark color. The deeper color is attributable to their greater content of myoglobin and cytochroms in their usually large and abundant mitochondria. They have thick Z discs and more complex sarcoplasmic reticulum. White fibers are largest of the fiber types. Their sarcolemmal mitochondria are smaller than those of the red fibers, and mitochondria between myofibrils are relatively few. The intermediate fibers have characteristics intermediate between the red and white fibers. The disposition of their mitochondria is similar to that of the red fibers,

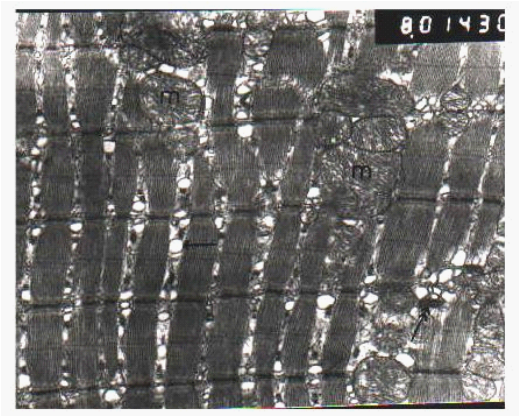

Figure 9. Intrinsic lingual muscle of the mouse. Mitochondrial columns(m) are fewer. Tubules of endoplasmic reticulum are small in diamater(arrow). Triads are located at A-I junction(double arrow). Uranyl acetate and lead citrateX8.000.

Figure 10. Intrinsic lingual muscle of the rabbit. Transverse section of muscle fiber. Mitochondria(m) and transverse section of myofibrils(my) are observed. Thick and thin myoflaments are clearly observed. Tubules of sarcoplasmic reticulum(arrow) are small in diamater. Uranyl acetate and lead citrateX25.000.

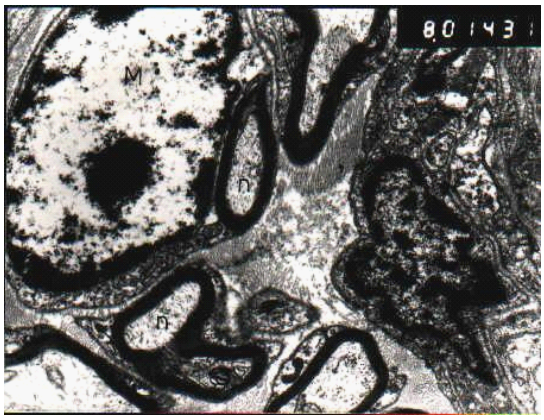

Figure 11. Intrinsic lingual muscle of the mouse. There are many myelinated nerve fibers(n) and capillaries(c) within the connective tissue surrounding muscle fibers. Uranyl acetate and lead citrateX8.000.

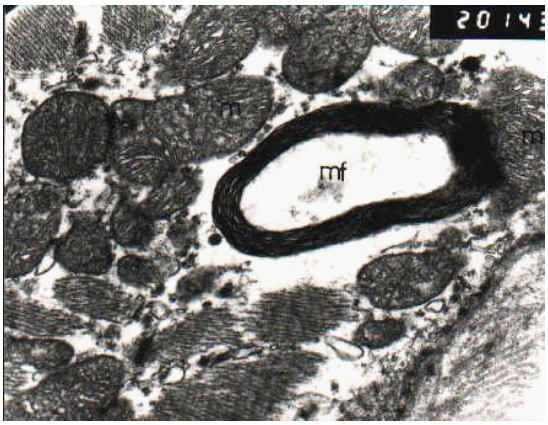

Figure 12. Intrinsic lingual muscle of the rabbit. A myeline figure(mf) associated with a mitochondria(m) is observed. Uranyl acetate and lead citrateX20.000.

except that thick interfibrillar columns of mitochondria are seldom found.[1-7]

The fibers that make up the lingual muscles of the rabbit and mouse were not identical. In the rabbit, two types of muscle fibers that varied in their ultrastructural features were observed. The sarcoplasm of one of them was observed more electron dense. These fibers had numerous mitochondrial columns of large diameter between the myofibrils. Z line was thick and tubules of reticulum were large and prominent. We think that these types of muscle fibers were red muscle fibers because of the characteristics of the mitochondria and sarcoplasmic reticulum. The other type that had fewer numbers of mitochondrial columns of small diameter was white muscle fiber in type. The elements of sarcoplasmic reticulum of these fibers were small in diameter and not prominent. Traditionally it is accepted that sarcoplasmic reticulum of the red muscle fibers is more complex than that of the white muscle fibers[1,2] The muscle fibers that make up the mouse lingual muscles were not all identical also. Some of the fibers consisted parallel rows of mitochondria of large diameter between the myofibrils. The elements of sarcoplasmic reticulum were small and not prominent. Distribution pattern of the mitochondria of this fiber type were similar to that of the red fibers, but development degree of sarcoplasmic reticulum were not suitable. In some of the fibers mitochondria were fewer

than that of the other fiber type, and these were not arranged as long parallel rows. These fibers consisted prominent and large sarcoplasmic reticulum tubules. Distribution pattern of the mitochondria of this fiber type were similar to that of the white fibers, but development degree of sarcoplasmic reticulum were unexpected. We think that mitochondria-rich fibers were red muscle fibers in type and mitochondria-poor muscle fibers were white muscle fibers in type. But the organization pattern of the sarcoplasmic reticulum was different. Because tubules of the white muscle fibers were larger and more prominent than those of the red muscle fibers. Additionally, in some of the fibers mitochondria were fewer and sarcoplasmic reticulum tubules were small and not prominent. These were white fibers in type because of ordinary distribution pattern of the mitochondria and the sarcoplasmic reticulum. In human, skeletal muscle fibers are red, white and intermediate fibers in type. Intermyofibrillar mitochondrial columns are most common in red fibers. Mitochondrial shape and configuration are distinctive for each human skeletal fiber type, but the sarcoplasmic reticulum is similar in all muscles[8] In contrast, we observed that the organization of mitochondria and sarcoplasmic reticulum was variable in red and white muscle fibers. Ogata et al[9] reported that mitochondria rich fiber has a much smaller total volume of sarcoplasmic reticulum than the mitochondria poor white fiber. We also observed that mitochondria-rich red muscle fibers of the intrinsic lingual muscles of the rabbit consisted large sarcoplasmic reticulum tubules whereas white muscle fibers consisted small sarcoplasmic reticulum tubules. Bu this was invalid for the lingual muscle fibers of the mouse.

Three-dimensional structure and arrangement of mitochondria in the red, intermediate and white striated muscle fibers of the rat are examined. The mitochondria are numerous and large in size in the red fibers, intermediate in the intermediate fibers, and few and small in the white fibers. In the red fibers mitochondrial columns are abundant, the white fibers display rare, very thin columns[9,10] In the present study, in the rabbit lingual muscle, red muscle fibers consisted many mitochondrial columns of large diameter and prominent sarcoplasmic reticulum tubules and white muscle fibers consisted few mitochondrial columns and tubules of sarcoplasmic reticulum of small diameter. In mouse lingual muscle, red muscle fibers consisted many mitochondrial columns of large diameter but tubules of sarcoplasmic reticulum were small, whereas white muscle fibers consisted few mitochondrial columns of small diameter but tubules of sarcoplasmic reticulum were large and prominent. Additionally ýn some fibers mitochondria were fewer and tubules of sarcoplasmic reticulum were small in diameter and not prominent. This type of fiber was usual white fiber in type. As a conclusion, we think that determination of the muscle fiber type may be difficult because the organization pattern of the mitochondria and sarcoplasmicreticulum may not be as traditionally expected. We observed that the organization pattern and development degree of mitochondria and sarcoplasmic reticulum are variable in different fiber types in different mammals.

We observed many mast cells in the connective tissue surrounding the muscle fibers of the lingual muscle of mouse. Mast cells, singly or in small groups, are observed in the connective tissue interstitium that normally separates skeletal muscle into fascicles. The close proximity of mast cells to muscle spindles and nerve fascicles suggests that these cells may play a role in modulating their activities.[11]

Myelin figure that we observed in one muscle fiber in the rabbit may be a degenerative change. Degenerative changes such as myelin figures are observed in the myocardium of patients undergoing open cardiac surgery with more than 10 years follow-up and in patients with congenital heart diseases.[12,13]

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