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The muscle spindle of the rat: Peculiarities of motor innervation and ultrastructure and effect of increased activity.

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Introduction

The muscle spindle is an encapsulated stretch-receptor, being located among muscle fascicles, parallel to extrafusal fibers and associated with pericapsular connective tissue and blood vessels. The spindle-like shape of multilayered outer capsule is named after this receptor organ. Outer capsule cells are similar to perineural epithelial cells of the nerve fascicle sheath (*Merrillees,* 1960, *Shantha & Bourne* 1968, *Patten & Ovalle* 1991).

The most essential data on the structure and function of muscle spindle are found in cat's spindles (*Boyd* 1962). In recent years investigators have shown that the dynamic and static intrafusal systems of rat's muscle spindle are less sharply demarcated than in the cat (*Walro & Kucera,* 1985a, *Kucere et al.* 1989,1991). At the morphological level it reveals sharing of sensory terminals between the dynamic bag1 and static bag2 intrafusal fibers; in addition fusimotor axons may sometimes coinnervate the dynamic and static intrafusal muscle fibers of a rat.

The comparison of structure and motor innervation of three muscles in rat's hindlimb (*Soleus, Extensor Digitorum Longus, Lumbricalis)* has showed that the spindles of slow- and fast-twitch muscles had similarity, whereas differences were observed between the structure of the proximal and distal muscles (*Kucera et al.* 1991). These authors expect that the structure of muscle spindles in different muscles may be related to the muscle function, with muscle ability to perform delicate motor tasks. It is believed that the ratio of gamma and beta axons may increase with the increase of muscle distance from the spinal cord. Thus, the relative contribution of dynamic and static systems to muscle afferent outflow may differ among spindles located in different segments of rat's hindlimb.

As the structure and innervation of rat's muscle spindle has been investigated only in some small skeletal muscles of rats and the data about the effect of muscular activity on intrafusal fibers are scarce, the present study was undertaken to study the rat's intrafusal muscle fibers and the ultrastructure of their motor endings in ultrathin sections in the biggest hindlimb muscle *Quadriceps femoris* (Q.FEMOR) and in typical slow-twitch *soleus* (SOL) muscle in the usual conditions and increased muscular activity.

Materials and Methods

Male rats of the Wistar strain, 16-17 weeks old, were maintained on a constant diet SDS-RMI (C) 3/8 (SDS, Witham, England). Food and water were given *ad libitum.* The rats, six in both group, were housed for 12/12 hrs light/dark period. The rats were assigned to control and exercised. The exercised animals were running at 35 m/min 5 days a week for 6 weeks. The running time (Table 1) and total work per week was progressively increased. In the first week the total work per week was ~ 4 500 J and in the sixth week of exercise it was 30 000 J. Fast-twitch and slow-twitch muscle fibers from different skeletal muscles of a rat were separated as described earlier (*Seene & Alev* 1991). Muscle samples for ultrastructural studies were fixed in 2,5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in graded alcohol and embedded in Epon-Araldite resin. Ultrathin sections were cut from transversely and longitudinally oriented muscle blocks, stained with uranyl acetate and lead citrate, using 3-5 blocks from each animal. In the red deep part of Q. FEMOR we succeeded to find the whole muscle

spindle which transcended the region of extrafusal muscle fibers' endplates.

The determination of extrafusal muscle fibre types was performed at the ultrastructural level by the number of mitochondria, taking into account the previous data on the content of myoglobin and cytochromes aa_3 in the investigated bundles of fibers (*Umnova & Seene* 1991, *Seene & Umnova* 1992).

Results

The capillaries are usually located inside the external layer of the outer capsule of rat's muscle spindles in Q.FEMOR and SOL. Mast cells are often observed in the vicinity of blood vessels in muscle spindles. Rat's muscle spindle of both Q.FEMOR and SOL involves 4 intrafusal fibers: bag2, its diameter is the largest; bag1 with lesser diameter and two small chain fibers. In juxtaequatorial zone of Q.FEMOR small motor nerve endings with short terminals (length about 1-2 ìm) are located at the distance of about 3-5 ìm from each other. Numerous light spherical synaptic vesicles are concentrated in their axoplasm, dense-core vesicles can often be arranged among them. Within the terminals mitochondria can also be observed. Junctional sarcoplasm contains few small mitochondria. The bag1 intrafusal fiber of Q.FEMOR has four motor endings of this type. The last of them is located at the distance of about 10 ìm from the others. Its postsynaptic membrane forms some finger-like projections, muscle nucleus is present in synaptic sarcoplasm. In the

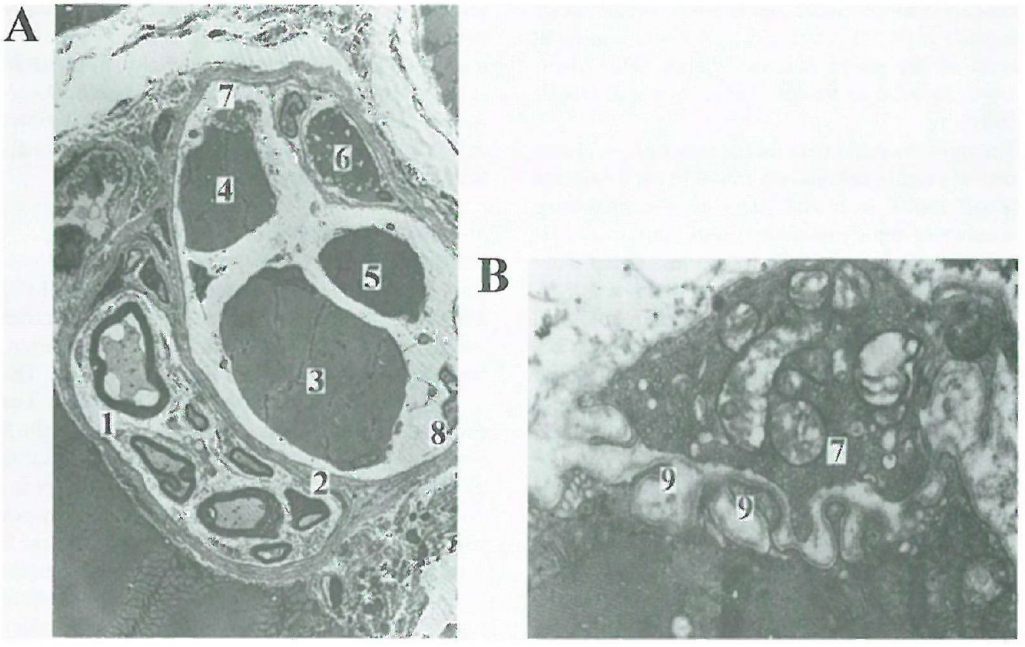

Figure 1. Transversal ultrathin section through intracapsulated distal part of quadriceps femoris mucle spindle pole.

A. Nerve bundle (1) is conected with outer capsule of mucle spindle (2), a few intrafusal fibers are present in periaxial space: bag2 (3), bag1 (4), chain 1 (5), chain 2 (6), separated by inner capsule. Sixth gamma motor ending (7) is on the bag1 fiber. Location of gamma motor nerve (8). Magnification x 2,500.

B. Postsynaptic membrane of the bag1 intrafusal fiber forms finger-like projections (9) in the vicinity of gamma axon terminal (7). Magnification x 20,000.

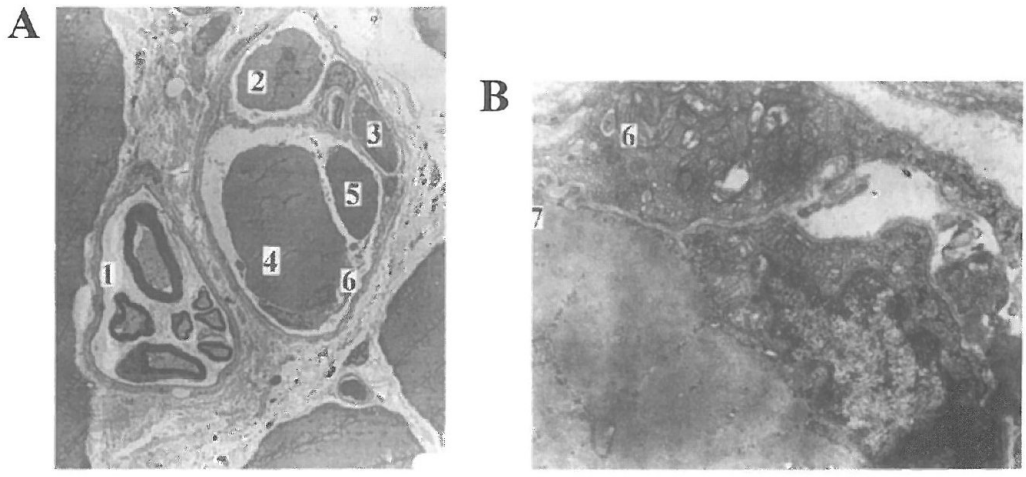

Figure 2. Transversal ultrathin section through intracaptulated distal part of the quadriceps femoris mucle spindle pole.

A. Nerve bundle (1) is separated from the muscle spindle. The bag1 (2) and chain 2 (3) intrafusal fibers are located in different compartments. The bag2 (4) and chain 1 (5) fibers are localized in the same compartment. Gamma axon forms motor ending (6) on the bag2 fiber. Magnification x 1,900

B. Postsynaptic membrane of the bag2 fiber forms two short projections (7) in the vicinity of gamma motor axon terminal (6). Magnification x 15,000.

distal part of intracapsulated region of muscle spindle two other motor endings (5th and 6th) are located on the bag1 fiber. They have a larger size, area of synaptic contact, narrower synaptic cleft and more complicated structure of postsynaptic

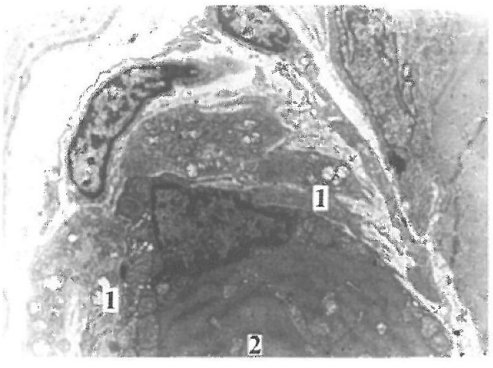

Figure 3. Transversal ultrathin section through the gamma motor axon terminal (1) being located on the chain 1 intrafusal fiber (2). Mag´nification x 7,500.

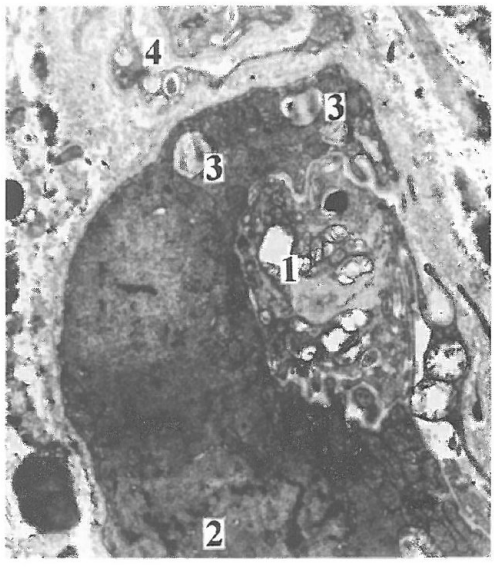

Figure 4. Beta motor axon ending (1) on the bag1 (2) intrafusal fiber in extracapsulated region. Lipid inclusions (3), myelinized motor nerve (4). Magnification x 9,000.

zone in comparison with motor endings of juxtaequatorial region. In the postjunctional sarcoplasm a muscle nucleus, lots of mitochondria, polyribosomes, profiles of rough-surfaced endoplasmic reticulum, elements of the Golgi complex, small secondary lysosomes can be observed. The surface of the bag1 intrafusal fibers is devoid of typical postsynaptic folds, but always forms some finger-like projections in the motor axon terminals (Figure 1ab). The length of the sixth motor ending is 38 ìm.

The bag2 intrafusal fiber in Q.FEMOR is innervated at fewer sites than is the bag1 in the same spindle. Three motor endings are observed on the bag2 fiber.The first of them is at the distance of about 100 ìm from the first motor ending on the bag1 fiber. It has two terminals (long and short) and lies on the smooth surface of an intrafusal fiber. To some extent the bag2 motor endings look similar to those being located in juxtaequatorial zone on the bag1 fiber, but they have a larger size of terminal and postsynaptic area. The length of the third ending is about 40 ìm, its postsynaptic membrane forms small protrusions and single finger-like projections (Figure 2ab).

Only one pole of the chain intrafusal fiber is innervated in intracapsulated region of Q.FEMOR muscle spindle. At the transversal sections of muscle spindle, motor axon terminal surrounds about a half perimeter of the chain intrafusal fiber. Postsynaptic membrane of the chain fiber does not form protrusions and projections, its surface is smooth. Large mitochondria, polyribosomes, muscle nuclei, secondary lysosomes and other organelles are concentrated in synaptic sarcoplasm (Figure 3). The second chain intrafusal fiber is devoid of motor innervation. This muscle spindle pole is thinner and shorter than the innervated pattern. It should be pointed out that in the same fixation conditions all mithochondria in this noninnervated chain fiber are swollen.

In extracapsulated region of muscle spindle in rat's

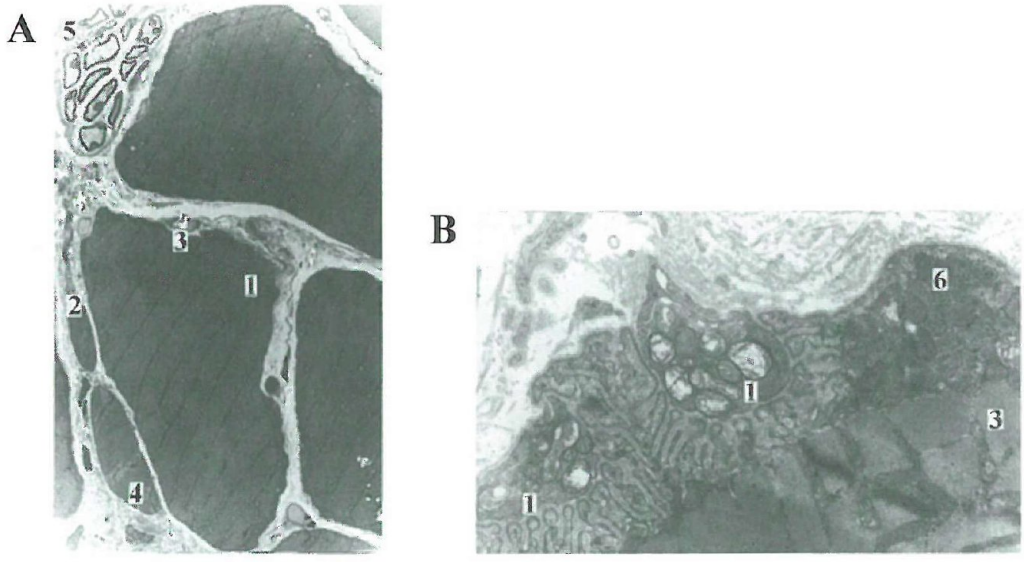

Figure 5.

A. Ultrathin section of the beta motor end-plate (1) in the vicinity of extrafusal muscle fiber (2), adjacent to the bag1 intrafusal fiber (3). The bag2 (4) intrafusal fiber and intramuscular nerve bundle (5). Magnification x 2,200.

B. Two beta axonal terminals (1). Mitochondria (6) in postsynaptic region of extrafusal muscle fiber (3). Magnification x 15,000.

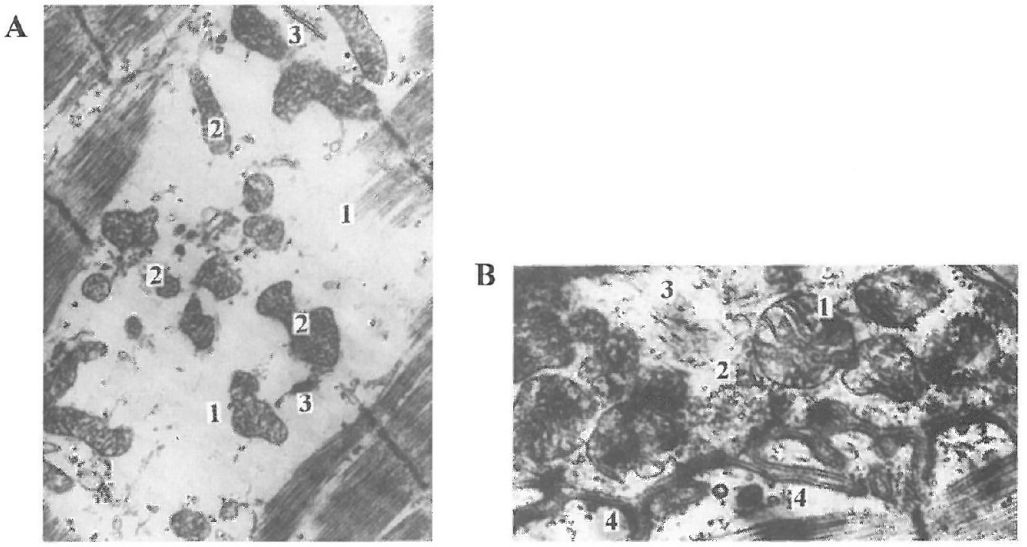

Figure 6.
A. Destruction of myofibrils (1) in the central part of intrafusal fiber of soleus muscle, isolated mitochondria (2) and single T-tubules (3) can be seen in sarcoplasm. Magnification x 20,000.
B. Motor ending on the chain intrafusal fiber of soleus muscle. Mitochondrion (1), synaptic vesicles (2), neurofilaments (3) and postsynaptic folds (4). Magnification x 27,000.

Q.FEMOR only one motor ending can be observed. It is located on the bag1 intrafusal fiber at the distance of 100 ìm from the outer capsule of spindle and is indented into its surface. The length of this ending is about 21 ìm. Axon terminal has short nonmyelinized segment. Postsynaptic membrane forms finger-like projections in the proximal part of motor endings and short wide junctional folds along the side surfaces of motor axon terminal. At the bottom of axon terminal, postsynaptic membrane of the bag1 fiber is parallel to presynaptic membrane and does not form junctional folds. Muscle nucleus, large mitochondria, polyribosomes, vesicles, lysosome-like bodies, partly resorbed light lipid inclusions occur in synaptic sarcoplasm (Figure 4). Extrafusal motor endplate also has short nonmyelinized preterminal segment. Motor endplates are found on 6 extrafusal muscle fibers neighbouring the bag1 intrafusal muscle fiber bundles. Motor endplates of extrafusal muscle fibers have (1-5) small oval axon terminals, indented into muscle fibers (Figure 5). Postsynaptic membrane of extrafusal fibers form a lot of regular, deep, narrow junctional folds which are separated from myofibrils by some muscle nuclei, polyribosomes, elements of granular reticulum, aggregations of mitochondria. Extracapsulated region of the bag2 and the chain intrafusal fibers of this spindle pole has no motor innervation.

In the polar region of muscle spindles of exercised and control rats' satellite cells occur under the basal membrane of intrafusal fibers in both types of muscles (Q.FEMOR and SOL). 12 satellite cells (5 - in the bag2, 4 - in the bag1, 3 - in the chain intrafusal fibers) are being located in the Q.FEMOR spindle pole within the distance of 240-300 ìm. Large secondary lysosome-like bodies were observed in the sarcoplasm of the chain fibers and in cytoplasm of some satellite cells.

After muscle activity there are no conspicuous destructive changes in the myofibrils of Q.FEMOR intrafusal fibers. However, lysosome-like bodies are always prersent in perinuclear and synaptic sarcoplasm. At the same time the destruction of myofibrils in the peripheral, central and perinuclear parts of three types of intrafusal fibers occurs in SOL.

Intermyofibrillar space increases simultaneously. Lyosome-like bodies and big vacuole-like structures are formed in the sarcoplasm of SOL intrafusal fibers (Figure 6a). In case of muscular activity no prominent lesions in SOL axon terminals can be found, but postsynaptic membrane of chain fiber forms short postjunctional folds with unfused basal membranes in synaptic clefts (Figure 6b).

Discussion

In order to study changes in the biggest muscle of the rat's hindlimb Q.FEMOR, the ultrastructure of intrafusal muscle fibers and their motor endings under increased muscular activity, it is essential to find out the peculiarities of these structures in the usual cage conditions.

Studies of serial ultrathin sections of Q.FEMOR muscle spindle pole have revealed 10 motor endings in intracapsulated region on all intrafusal fibers. It is known that the factors which affect diversity of motor endings may be the following: neuron type, function of motor axon (dynamic or static), afferent innervation of intrafusal fibers (*Walro & Kucera* 1985a). The bag1 intrafusal fiber has most of the sites of motor innervation (in our case - 6 endings). It is supposed that the small size and short length of axon terminals being located on the bag1 fiber in juxtaequatorial region is explained by suppressive action of muscle spindle sensory region (*Walro & Kucera* 1985a). Ultrastructure of motor endings becoming more complicated with the icrease of the distance from equator of muscle spindle confirms this supposition. Undulating contour and finger-like projections, formed by the bag1 postsynaptic membrane may probably be used for identification of the bag1 fiber in rat's Q.FEMOR. Postsynaptic membrane of the cat's bag1 fiber has the same property (*Boyd & Gladden* 1985).

On the bag2 intrafusal fiber there are three motor endings being located in the intracapsulated region. The postsynaptic membrane of the bag2 fiber has smoother contour than the bag1 fiber.

The motor ending being located on the chain fiber has the largest size. One chain intrafusal fiber lacking motor innervation on this pole is thinner and shorter than the innervated chain fiber. Some investigators have observed noninnervated intrafusal fibers in other small muscles of rat's hindlimb (*Walro & Kucera* 1985 ab, *Kucera et al* 1991). They believe that motor innervation has influence upon intrafusal fiber size, oxidative capacity and development of sarcotubular system (*Walro & Kucera* 1985a). It is also necessary to note that under the same fixation conditions in noninnervated chain fiber, mitochondria are swollen. It may be suggested that chain fibers are in different metabolic state or differ in their biochemical properties (*Walro & Kucera* 1988, *Kucera & Walro* 1987), but it may be possible as well, that this chain fiber pole lacks motor innervation.

All motor endings, being located in intracapsulated area of Q.FEMOR muscle spindle on three types of intrafusal fibers occupy superficial position in relation to fiber surface. They are not indented into intrafusal fibers and have a small postsynaptic area. Taking this factor into consideration we suggest that motor endings being located in intracapsulated region of Q.FEMOR muscle spindle are formed by gamma motor axons.

In addition it is essential to compare the ultrastructure of muscle spindles and nerve innervation of fast-twitch and slow-twitch extrafusal muscle fibres.

The satellite cells being located under the basal membrane of intrafusal fibers may be the source of the formation of new fibers after neonatal deefferentation of rat's hindlimb (*Zelena & Soukup* 1993) and under autografting all the small skeletal muscles (*Soukup et al* 1990, *Umnova et al* 1994). Extracapsulated region of the bag1 intrafusal fiber and neighbouring extrafusal muscle fibers are coinnervated by beta axon. Motor ending, located on the bag1 in extracapsulated region of the spindle pole, is more indented into the intrafusal fiber in comparison with the superficial synapses formed by the gamma axons in intracapsulated region on the same fiber (*Umnova & Seene* 1995). Nonmyelinized preterminal segments of motor axon, forming endings on the bag1 fiber in extracapsulated region and on the neighbouring extrafusal muscle fibers, are short. This feature is also characteristic of the beta axon endings (*Kucera* 1985). The length of the motor ending may be considered as a sign of skeletofusimotor innervation of fibers. Length of the motor ending, located in extracapsulated region on the bag1 fiber is 2 times shorter than the length of gamma motor endings on the same fiber in intracapsula

ted region of this pole. It is similar to average length of beta axon terminals in muscles of the other mammalian species (*Boyd & Gladden* 1985, *Kucera* 1985). As the bag1 fiber is innervated by the gamma and beta axons, has well developed myofibrillar apparatus (at least in extracapsulated region) and lipid drops, which are subjected to resorption, we suggest that the bag1 fiber may be able to contract. The diversity of motor endings located on the bag1 intrafusal fiber reflects its polyneural innervation. This fact probably causes the morphological and histochemical heterogenity of the bag1 muscle fiber in its different regions (*Walro & Kucera* 1985a, *Kucera & Walro* 1988, *Khan & Soukup* 1988). It is suggested that the location of beta-innervated muscle spindles in motor region of skeletal muscle, where muscle contractions originate, probably helps to receive immediate afferent response.

In the studied region of Q.FEMOR we registered at least 6 red oxidative type extrafusal muscle fibers coinnervated together with bag1 intrafusal fiber and forming one motor unit. In usual conditions of skeletal muscle function the intrafusal muscle fibers very weakly participate in the process of contraction (*Kucera et al.* 1991). Considering some sings of destruction in myofibrils and other organelles of intrafusal fibers we may suggest that SOL is probably subjected to the majority of workload under prolonged muscular activity as predominantly slow-twitch skeletal muscle. Summing up, it can be concluded that intrafusal muscle fibers being located in the region of slow oxidative muscle fibers of SOL adapt themselves to increased activity probably by using a mechanism of response reaction similar to that of extrafusal fibers, although the changes in intrafusal muscle fibers are considerably smaller than in extrafusal fibers.

Acknowledgments

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Summary

The ultrastructures of intrafusal muscle fibers and motor endings in the rat's Quadriceps femoris (Q.FEMOR) have some peculiarities. They are expressed by a deficit of static component in motor innervation, as one static nuclear chain intrafusal fiber is devoid of motor innervation, and polyneuronal innervation of the dynamic bag1 intrafusal fiber with gamma and beta motor axons of appropriate motoneurons. Reduced motor static component and polyneuronal innervation strengthen the influences of the dynamic intrafusal fiber in muscle spindle of Q. FEMOR muscle. Ability of the bag1 plasma membrane to form finger-like projections in the vicinity of motor axonal terminals establishes more close synaptic contact and quick transmission of information concerning changes of muscle length of Q.FEMOR muscle to the CNS. The bag1 intrafusal fiber is coinnervated by the beta axon together with oxidative type of extrafusal muscle fiber.

Comparison of the effect of prolonged muscular activity on the ultrastructure of the muscle spindles in Q.FEMOR and SOL shows that in SOL intrafusal muscle fibres indicate some signs of destruction of myofibrils and other cell organelles. Intrafusal muscle fibers being located in the SOL muscle adapt themselves to increased activity with a mechanism of response reaction similar to that of extrafusal fibers, although the changes in intrafusal muscle fibers are considerably smaller than those in extrafusal fibers.

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