THE ORGANIZATION OF THE SARCOPLASMIC RETICULUM AND T SYSTEM IN THE FEMORAL MUSCLE OF THE HOUSEFLY, *MUSCA DOMESTICA*

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INTRODUCTION

The role of the T system in the conduction of the stimulus from the sarcolemma inward to the contractile material has been increasingly apparent since the original electron microscopic works by Bennett and Porter (2) and Andersson-Cedergren (1) and the outstanding experiments by Huxley and coworkers (7) on the effects of local stimulation. At present, it is well established that in all muscles so far studied the T system originates from invaginations of the sarcolemmal membrane, thus providing a continuous structural basis for the transmission of the stimulus to the contractile material (4, 8, 10, 16, 17).

In higher animals (amphibians, birds, and mammals), the T system makes close contact, within the muscle cell, with two cisternal enlargements of the sarcoplasmic reticulum (SR): the resulting structure (triad unit) is thought to be the basis for the electrochemical coupling phenomena (8, 10).

Triadic structures have been shown to be present also in the muscle fibers of lower animals (12, 14); however, either more complex (pentadic) or apparently simpler (dyadic) structural relations between the T system and the SR membranes have also been reported (3–6, 11, 12, 16). In the case of the dyads, which are by far the alternative to the triads most frequently observed, the sarcoplasmic reticulum forms a continuous sheet onto the surface of the myofibril, and the T system runs alongside the cisternae of the sarcoplasmic reticulum (18); hence the dyadic appearance of the whole structure. However, little attention has been paid to the problem of the very precise tridimensional organization of the dyads, and still poorly understood are the spatial relationships between the membranes of the two systems (3, 15).

In this paper, a tridimensional reconstruction of the structural relationships of the T system and the SR, as they appear in the femoral muscle of the housefly (*Musca domestica*), is presented, and the suggestion is advanced that the so called dyad is, in fact, in this insect muscle, a mere variation of the typical triadic structure, since the sarcoplasmic reticulum is segmentally arranged and interrupted by the presence of the T system.

MATERIAL AND METHODS

The femoral muscles from the housefly (*Musca domestica*) were fixed for 3 hr in an ice-cold solution of 6% glutaraldehyde buffered with 0.13 M Na-K-phosphate, pH 7.4. The muscles were kept at their original resting length. They were then rinsed in the same phosphate buffer, to which 0.2 M sucrose was added, and postfixed in a 0.1% OsO_4 solution buffered at pH 7.4 with 0.13 M phosphate. After short rinsing, the specimens were cut into small pieces, dehydrated through a series of graded acetones, and stained with 0.3% uranyl acetate in absolute acetone overnight. Fixation, rinsing, and dehydration up to 90% acetone were performed at 0°C; all subsequent operations were done at room temperature. The specimens were then embedded in Araldite. Sectioning was performed on a Porter-Blum ultramicrotome or an LKB Ultrotome ultramicrotome. Unstained sections and sections stained with lead (13) were viewed in a Siemens Elmiskop IA with double condenser lens system operating at 80 kv and with a 50-μ-objective lens aperture. The primary magnifications varied from 1,500 to 20,000.

RESULTS

The femoral muscle of the housefly shows the typical ultrastructural organization of the syn-

chronous muscles of insects (9). The central nucleus is surrounded by layers of laminated myofibrils which are separated from each other by a well developed sarcoplasmic reticulum and by mitochondria. The ratio between primary and secondary filaments is 1 to 6, and no M lines are detectable in the central region of the sarcomere.

When seen in longitudinal section, the T system appears located at the level of the regions in which primary and secondary filaments overlap (Fig. 1). As can be seen in the same figure and in Fig. 2, considerable enlargements of the T tubules are rather regularly spaced with respect to each other. These saccular dilatations have a diameter of up to 0.2–0.3 μ, and appear to protrude from both sides of the T tubule and to project toward either the Z band or the A band. The location and structure of these formations are made particularly clear by the presence, enclosed within the membranes, of an electron-opaque matrix. This matrix appears to be made up of granular, structureless material, which is found also, although less tightly packed, in the tubular regions of the T system (Figs. 1–2). In the same figures, it is also apparent that at the level of these enlargements the membranes of the T system are adjacent to the membranes of the SR. The SR membranes are organized as a complex system of tubules which are oriented parallel to the long axis of the myofilaments at the A-band level (Figs. 1–2), forming almost continuous septa between two adjacent myofibrils. The longitudinal tubules of the SR tend to anastomose in the regions in which contact occurs with the T system; however, real terminal cisternae of the SR were never observed (Figs. 1 and 2). Direct continuity between the two membrane systems was never seen; on the contrary, a constant distance of 110–115 A between them was observed. It was also apparent, from inspection of a number of specimens, that the SR never completely overlaps the T system, and that it is not continuous with the membranes of the SR

FIGURE 1 Electron micrograph illustrating a longitudinal section of femoral muscle. The tubules of the T system are located at the level of the regions in which overlapping of the primary and secondary filaments occurs. The rather regularly spaced enlargments of the T tubule are clearly visible. In between two subsequent T tubules, the SR appears as a complex system of membranes that makes contact with the membranes of the T system. × 24,000.

of the adjacent portion of the sarcomere. On the contrary, a tubule of SR located on the side of the Z band may be continuous with that located on the homologous side of the adjacent sarcomere (Fig. 2, arrow). Thus, portions of the SR enclosed between two adjacent T tubules are structurally isolated and independent of each other.

When seen in cross-sections, the T system appears as a tubule of a rather constant diameter (1,500–2,000 A) arising from the sarcolemma. Each T tubule is confluent with the T tubules of the adjacent myofibrils and thus a completely anastomotic system is formed by which the individual myofibrils are surrounded (Fig. 3). As seen in the same figure, depending on the plane of sectioning, no lateral contacts are seen between the SR and the T system in certain regions in which the myofibrils appear isolated from each other by the T tubule only. This observation confirms the conclusion, drawn from inspection of longitudinal sections, that the membranes of the SR do not overlap the T tubule completely and that no continuity exists between the membranes of the SR adjacent to the same T system from opposite directions.

DISCUSSION

As reported in the Results, when seen in longitudinal section, the T system, which arises from the sarcolemma as a tubular invagination, shows saccular enlargements in the regions in which the system comes into contact with the membranes of the SR. The enlargements project from both sides of the T tubule, with respect to the long axis of

FIGURE 2 This figure illustrates in finer detail the structure of the T system and its spatial relationship with the membranes of the SR. It is apparent that both the tubular and the enlarged portions of the T system contain an electron-opaque substance. The T system is in close contact with the membranes of the SR; however, direct continuity of the two membrane systems is not visible. It is also apparent that the tubules which form the SR do not completely overlap the T system. The SR appears as a very complex and anastomotic system of cavities and tubules. These have a predominantly longitudinal orientation at the A-band level, and tend to be confluent in the regions in which the contact with the T system occurs. However, no real terminal cisternae of the SR are formed. Note the structural continuity of the membrane of the SR over the Z band (arrow). × 42,000.

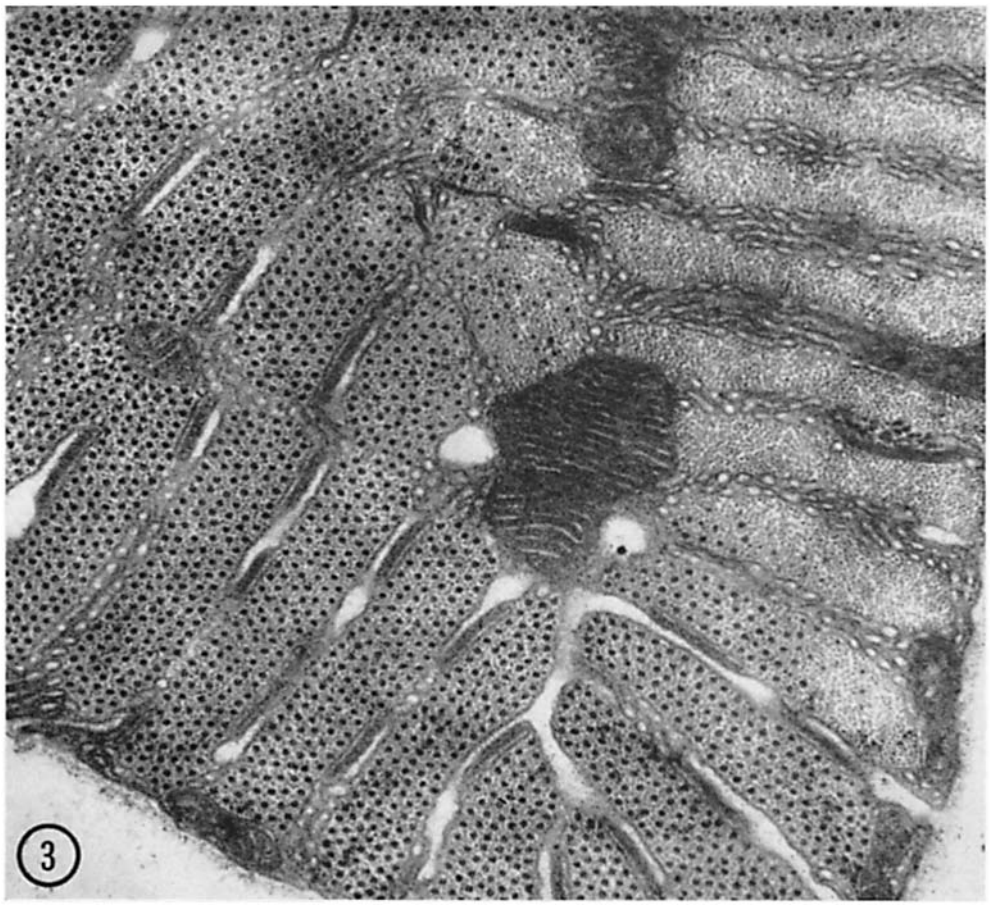

FIGURE 3 This electron micrograph illustrates the anastomosis of the T tubules to form a continuous and complex system surrounding the contractile material. It is apparent that the membranes of the SR also contribute to the separation of the individual myofibrils, but at a different level of the sarcomere. Note that the systems come into contact with each other only at the level of the enlargements of the T system. × 48,000.

the myofilaments. However, any single sacculus protrudes in only one direction. These dilatations of the T system come into large contact with membranes of the SR. The contact between the membranes of the two systems occurs at the level of the apical regions of the saccular enlargements of the T tubules and, to a less extent, by lateral apposition. It is noteworthy that, at variance with previous reports on similar material (18), the membranes of the SR do not overlap completely the T system but are segmentally interrupted by the presence of the T system. Thus, no continuity exists between the SR membranes that are located on adjacent sides of the same T tubule. It is also noteworthy that a material, similar in appearance to that which seems to cement the vesicles of the triads in the skeletal muscle fibers of the higher animals, is also seen in the case of the triadic organization described in the muscle examined in the present study.

From a functional point of view, this means that a stimulus coming from the sarcolemma may be transmitted toward the contractile material on both sides of the T system, but that no transmission occurs from a portion of the SR to the adjacent portion of SR located on the opposite side of the same T system tubule. Thus, from both structural and functional viewpoints, the organization of the T system and its relationship with the SR do not differ, except in minor details, from the

organization and relationship characteristic of a typical triad. However, the triadic organization in this insect muscle differs in significant details from that observed in mammalian skeletal muscles. In fact, in the muscles of these higher animals, the T system appears as a tubular structure of rather constant diameter (about 200 A) (1), which makes contact with cisternal enlargements of the SR. In the femoral muscle of *Musca domestica*, however, the T system is widely expanded (up to 1,500 A) in the regions in which contact with the SR occurs, but cisternal enlargements of the SR were never observed.

It seems reasonable to suggest that this structural diversity of the T system may be related to some very precise functional requirements for the contractile cycle in this insect muscle. However, the alternative possibility should also be considered that the characteristic organization observed in this insect muscle might be referred to a particular stage in the evolution of the T system that is related to the evolution of the whole contractile system in the various animal species.

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