Z AND T TUBULES IN STOMACH MUSCLES OF THE SPINY LOBSTER

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INTRODUCTION

Two systems of intracellular tubules which open to the extracellular environment have been described in striated muscle fibers of crustaceans: the T or A tubules, which form diadic contacts with the sarcoplasmic reticulum, usually near the ends of the A band; and the Z tubules, which have been reported to penetrate the fiber along the Z line (12, 13).

Local stimulation experiments on crab fibers with an external current-passing microelectrode implicated the T tubules in excitation-contraction coupling. Local contractions were obtained only when the electrode was positioned along a sarcomere near the end of an A band, where the T tubules invaginate. No contraction was produced by local stimulation at the middle of a sarcomere or at the Z line (6, 12). Thus, the Z tubules apparently do not mediate excitation-contraction coupling, and their function is unknown.

Since Huxley's and Peachey's original reports (6, 13), the existence of Z tubules in crustacean muscle fibers has been supported by some workers (5, 14), but not by others (3). No mention of Z tubules in crayfish muscle fibers is made by Brandt et al. (3), although structures similar to Peachey's Z tubules can be seen in their pictures. They describe T tubules, which sometimes pass through the Z line from one sarcomere to another, and have an electron-opaque wall at the Z line. They also describe "radial tubules" and sarcolemmal invaginations, neither of which structure shows the distribution and properties of Peachey's Z tubules. Thus, there has been controversy over the existence of the Z tubules, and more observations are desirable.

The stomach muscles of the spiny lobster (10, 11) have proven to be especially favorable for the study of the Z and T tubules. As the present report will show, the Z system is distinct and unmistakable in these muscles. In addition, the T system has a distribution not seen previously in skeletal muscles of crustaceans.

MATERIALS AND METHODS

Stomach muscles of *Panulirus argus*, collected at the Bermuda Biological Station, were used in this study.

The muscles described here are those of the pyloric region of the stomach. Approximately 10 discrete muscles, with different innervation (11), occur in the pyloric region; of these, the five more anterior ones were examined, and proved to be similar in structure. After an animal was killed, the stomach was removed intact, split open by a mid-ventral incision, washed in marine animal solution (1), and pinned out in a wax dish. Some of the connective tissue and fat overlying the muscles was dissected away before fixatives were applied.

Fixation, embedding, sectioning, and staining procedures followed those of Jahromi and Atwood (8). Sections were examined in a Philips EM 200 electron microscope.

RESULTS

Longitudinal sections through fibers of the pyloric muscles of the stomach showed sarcomeres of 3–5 μm with A bands of 2–2.5 μm , in which an H zone could readily be distinguished (Fig. 1). At the H zone, in the very center of the sarcomere, structures similar to the diads of other crustacean muscles (3, 5) were usually present between adjacent myofibrils. These structures appeared to consist of a T tubule in close contact with one or two vesicles of the sarcoplasmic reticulum (SR). The diads or "triads" occurred only at the H zone and were not seen anywhere else.

The presence of diads at the H zone, but not elsewhere, was also demonstrated in transverse sections. In the region of overlap of the thick and thin filaments, only SR was seen around the myofibrils (Fig. 3). When an H zone was transected, numerous diads or triads were seen around each myofibril (Fig. 4).

The presence of T tubules and diads at mid-sarcomere level marks a departure from the usual crustacean pattern, in which they occur near the ends of the A band. However, a central T system has been described previously in crustacean heart muscle (7, 9).

The myofibrils of the pyloric muscles were rather small by crustacean standards (averaging less than 1 μm in diameter). They were well separated by single or double layers of SR throughout their length, except at the Z line (Figs. 1–3).

In the Z line itself, a series of tubular structures

FIGURE 1 Longitudinal section through a pyloric muscle fiber, showing the general features of the sarcomeres. Diads (circled) occur at mid-sarcomere level in the H zone (H). Z tubules (ZT) occur between myofibrils in the Z line. When a Z tubule is absent at this location (large arrowhead), the sarcoplasmic reticulum is continuous across the Z line. Scale mark, 1 μ m.

FIGURE 2 Enlarged longitudinal section to show Z tubules (ZT), T tubules (T), and sarcoplasmic reticulum (SR). The sarcoplasmic reticulum is interrupted at the Z line by the Z tubules. Although the two membranous elements approach each other closely, there is no specialized contact (such as a diad) between them. The lumens of the Z tubules contain an amorphous material. Scale mark, 1 μ m.

was present between adjacent myofibrils (Figs. 1 and 2). These Z tubules were variable in size, but usually larger than the T tubules. Except for those of very small diameter, they contained a material which looked like a part of the basement mem-

brane (Figs. 2, 5, 6). The SR approached these tubules closely, but no structure resembling a diad was ever seen in this location (Fig. 2). The Z tubules interrupted the passage of the SR from one sarcomere to the next. When a Z tubule was

missing (Fig. 1, large arrowhead), the SR was continuous across the Z line.

The Z tubules were not observed to give off any longitudinally oriented tubules between the myofibrils, as suggested for another crustacean muscle (14). However, the possibility that such extensions occasionally occur is not excluded.

In transverse sections, the Z tubules could be seen to invaginate from the surface of the fiber and to surround most of the myofibrils (Figs. 5 and 6). Basement membrane material was clearly present in the lumens of these tubules, except at very fine terminations.

The arrangement of thick and thin myofilaments in the A band was similar to that previously described for various crustacean "fast" muscles (8, 15). Each thick filament had an orbit of six thin filaments, giving an over-all thin:thick ratio of 3:1.

DISCUSSION

In the pyloric muscles of the lobster stomach, the Z and T systems are very regularly arranged and are quite distinct from each other. The Z tubules are distinguished from T tubules by their location, their possession of a basement membrane-like material, their lack of associated diads, and their larger size. They appear to be a specialized type of sarcolemmal invagination.

The lack of consensus about the Z tubules in previous accounts of crustacean leg muscle fibers (3, 12) could have been occasioned by the much less orderly structure of the leg fibers in comparison with those of the stomach muscles. The less regular structure of leg fibers would make definition of a distinct Z system more difficult. Possibly, a difference in terminology is also involved, as Brandt et al. (3) may have used the term "sarcolemmal invagination" to include parts of Peachey's Z system.

The structure and location of the Z system do not provide much of a clue to its function. As Peachey has suggested (12), it probably is not involved in excitation-contraction coupling. Also, the presence of basement membrane in the Z tubules would perhaps lessen their efficiency as organs for transfer of metabolites into or out of the fiber. They may have a supportive function (12), for when they were absent between adjacent myofibrils, separation often occurred during fixation (Fig. 1, large arrowhead).

The Z tubules may contribute substantially to the membrane capacitance of the muscle fiber and would have to be taken into account in explaining the results of electrical measurements of muscle fiber membrane properties.

In crustacean heart muscle, a similar system of Z tubules may occur, although in previous accounts, this has been labeled (or mislabeled) as T system (9). The pyloric muscles of the stomach, and the heart muscle, have in common the generation of regular rhythmic contractile activity. Their similarity of structure may reflect a functional requirement. The pyloric muscles also contain many mitochondria and have an abundance of associated connective tissues as well as numerous blood vessels—features which undoubtedly adapt them for sustained vigorous activity.

In contrast with crustacean leg muscles (2, 4), the pyloric muscles appeared rather uniform in fiber composition, as judged from their structure. The structural features suggest a fast or fast-intermediate intrinsic contraction speed. Other muscles from the more anterior cardiac portion of the stomach showed fibers with a completely different structure, rather similar to that of "tonic" fibers of crustacean leg and abdominal muscles—long sarcomeres, diads near the ends of the A band, a high ratio of thin to thick myofilaments, etc.

FIGURE 3 Transverse section through the A band in the region of overlap between thick and thin filaments, to show myofibrils delimited by sarcoplasmic reticulum, and absence of diads. Scale mark, 1 μ m.

FIGURE 4 Transverse section through the H zone (note absence of thin filaments in central myofibril), showing the numerous diads surrounding the myofibrils. T tubule (T) and sarcoplasmic reticulum (SR) are indicated. Scale mark, 1 μ m.

FIGURE 5 Transverse section through the Z line, to show invagination of Z tubules (arrowheads), from the surface of the fiber. Basement membrane material (B) fills the lumens of the tubules. Scale mark, 1 μ m.

FIGURE 6 Transverse section through the Z line, to show the branching of the Z tubules between the myofibrils. Scale mark, 1 μ m.

Further work is necessary to clarify the relationships between structure and function in the various stomach muscles.

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