PERIPHERAL COUPLINGS IN ADULT VERTEBRATE SKELETAL MUSCLE

Anatomical Observations and Functional Implications

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INTRODUCTION

The sarcoplasmic reticulum (SR) of striated muscle (1) is differentiated into certain discrete morphologic specializations, the couplings, which are presumed to be prominently involved in the process of excitation-contraction coupling (2, 3). The coupling is composed of sarcolemma and junctional sarcoplasmic reticulum (JSR), from which periodic junctional processes (4, 5, 6), or SR feet (7), project towards the sarcolemmal membrane. Couplings occurring at the surface sarcolemma have been called peripheral couplings and those occurring at transverse tubules, interior couplings (5). Peripheral couplings have until now not been reported in adult skeletal muscle of the higher vertebrates (8). The present report shows the existence of peripheral couplings in several such adult vertebrate skeletal muscles, and discusses the anatomical and functional significance of these findings.

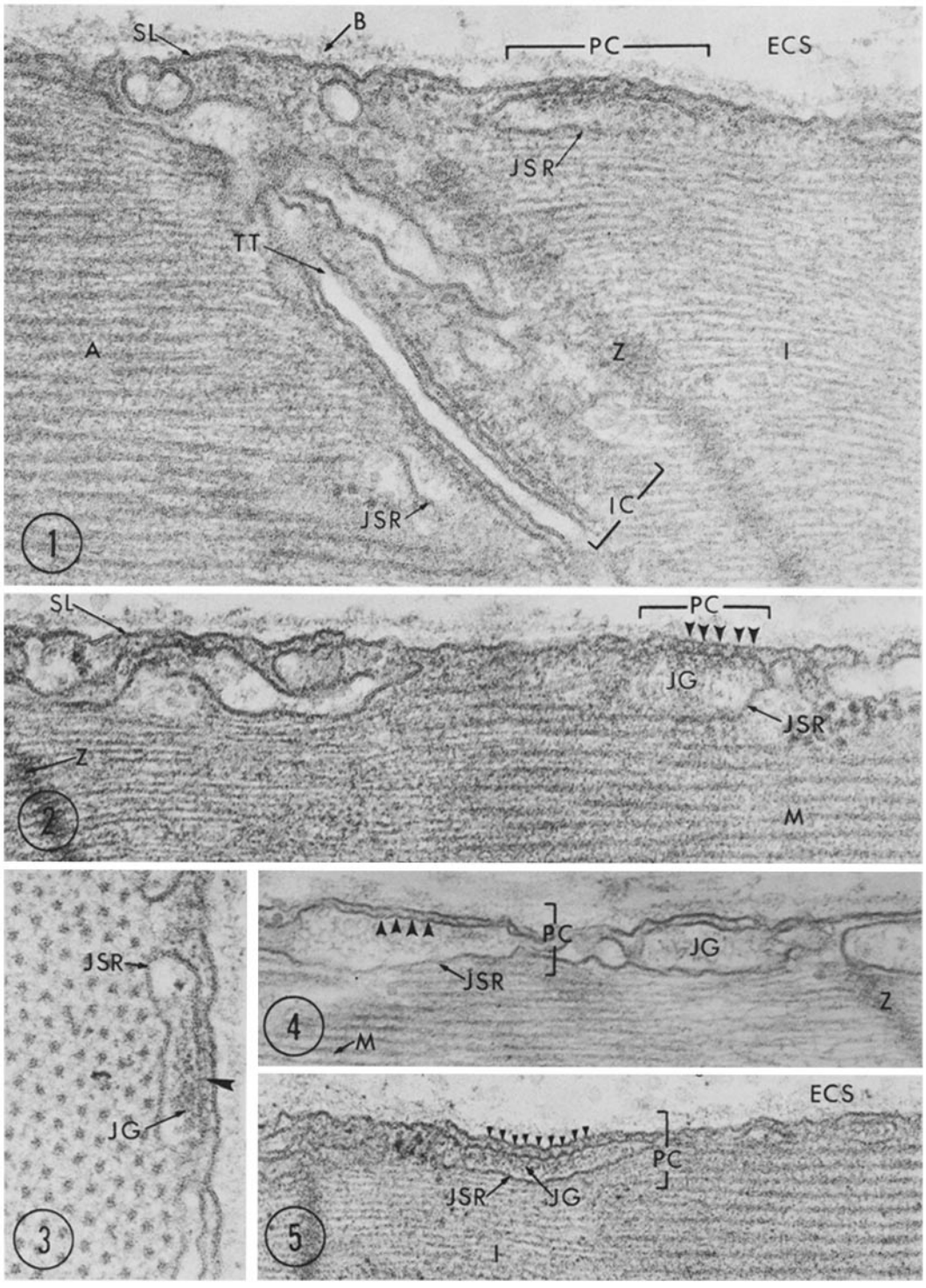
MATERIALS AND METHODS

Animals

Species studied included the zebra finch *Taeniopygia castanotis*, the strawberry finch *Amandava amandava*, the parakeet *Melopsittacus undulatus*, the mouse C3H strain, and the frog *Rana pipiens*. Anesthesia was accomplished by intraperitoneal injection of Nembutal except for the frogs, which were pithed. For the bird experiments, the feathers were removed and the pectoralis muscle with adherent fascia was incised adjacent to the sternum and stripped laterally. These muscle strips (about 1.0 × 0.5 × 0.1 cm) were clamped under slight tension and excised. Bird leg muscles (biceps femoris or strap mucles) were delineated by blunt dissection, clamped under slight tension, and excised. For specimens from the mouse, a strip of pectoralis muscle and a hindlimb adductor were clamped before excision. The frog sartorius muscles were clamped at rest length or greater and then removed. All clamped muscles were rinsed briefly in cold (4°C), oxygenated Krebs-Henseleit solution (9) (for the frog specimens this solution was diluted to 220 mosmol), and then placed in cold (4°C) fixative. After 0.5–1 h in the fixative, the specimens were cut into smaller pieces suitable for embedding and left overnight in the same fixative at 4°C.

Fixatives

For the specimens from both the birds and the mouse, 3% glutaraldehyde (diluted from 50% biological grade from the Fisher Scientific Co., Pittsburgh, Pa.) in either 0.2 M cacodylate buffer or 0.1 M phosphate buffer, pH 7.2, was used. After fixation overnight at 4°C, the specimens were rinsed briefly (30 min) in several changes of cold buffer and then postfixed for 90–120 min in 2% OsO_4 in phosphate or cacodylate buffer. All fixative solutions were prepared so that the osmolarity of the buffer was about 300 mosmol for the birds and the mouse, and 220 mosmol for the frog. After postfixation, specimens were either rinsed briefly in distilled water and then blockstained in the dark for 1.5–2 h with 1% aqueous uranyl acetate (at room temperature or at 4°C), or rinsed in 50% ethanol for 20 min and then stained overnight in the dark at 4°C in a solution of 70 parts ethanol and 30 parts 1% aqueous uranyl acetate (10). The tissues were then rinsed again in distilled water, dehydrated through graded ethanols, cleared in propylene oxide, and embedded in Epon 812.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Preparation for Examination

Gray-silver sections were cut with diamond knives, suspended on colloidion-coated and carbon-stabilized grids, and stained with saturated uranyl acetate (30 min) and lead citrate (10–15 min) (11). Sections were examined with a JEM 100B electron microscope operating at 60 kV.

RESULTS AND DISCUSSION

In the finch and parakeet leg muscles, transverse tubules (TT) were located at the A-I-junctions (Fig. 1). Sacs of JSR were attached along the surface sarcolemma forming peripheral couplings (Figs. 1–3). The JSR which formed the peripheral couplings was continuous with the free SR in some areas. Junctional granules sometimes formed a cribriform matrix inside the JSR sac. As described by Franzini-Armstrong (7, 12), junctional processes displayed several patterns depending on the plane of section (compare Fig. 1–3). The peripheral couplings were numerous in the finch leg muscle, and, as in cardiac muscle, were located predominantly along the I band but also occasionally along the M line (Fig. 2). Similar peripheral couplings were found in the pectoral muscles of both the finches and the parakeet, and, like those of the leg muscle, were generally located along the I band with an occasional appearance at the M line.[1] Thus, the location of the transverse tubules per se does not determine the location of the peripheral couplings, suggesting that additional factors are operative, as if to assure that most couplings are kept in the vicinity of the Z-I region. In their location and morphology, the peripheral couplings were thus similar in all avian fibers studied. In the avian leg muscles and both skeletal muscles from the mouse, numerous fenestrations were observed in the SR retes over both the M line and Z line in accordance with earlier observations on the distribution of SR fenestrations in rat soleus muscle (14), in certain other avian muscle (15), and in the mouse diaphragm (16).

Although frog sartorius muscle has been studied carefully in the past (7, 17), peripheral couplings have not been reported in it. In our preparations of frog sartorius, the large sacs of the JSR (terminal cisternae, 3, 17) were frequently seen just beneath the surface sarcolemma (Fig. 4). The peripheral couplings in frog sartorius muscle, as in other muscles, were located occasionally at an M line in addition to the more common location along the I band (Fig. 4). Although dilated sacs of SR filled

[1] In contrast to their location in the leg muscle and analogous to their distribution in chicken pectoral muscle (13), the transverse tubules in finch pectoral muscle were present at the Z lines.

FIGURE 1 Zebra finch leg muscle. A peripheral coupling (*PC*) is shown at the surface sarcolemma (*SL*) along the I-band region (*I*) of the sarcomere. Note the junctional SR (*JSR*) and the junctional processes which attach the JSR to the sarcolemma. A transverse tubule (*TT*) is shown at the *A-I* junction. Interior couplings (*IC*), which are morphologically identical to the *PC*, are located at the *TT*. Basement membrane (*B*), Z line (*Z*), A band (*A*), I band (*I*), extracellular space (*ECS*). × 90,000.

FIGURE 2 Zebra finch leg muscle. Note the peripheral coupling (*PC*) located at the M line (*M*). The PC is distinguished by the junctional SR (*JSR*) which contains junctional granules (*JG*), and by the junctional processes (arrowheads). Sarcolemma (*SL*), Z line (*Z*). × 76,000.

FIGURE 3 Parakeet leg muscle. This cross section through a peripheral coupling shows the junctional granules (*JG*) which characterize the junctional SR (*JSR*). In this projection, the junctional processes appear to form a leaflet (arrowhead) between the JSR membrane and the sarcolema. × 114,500.

FIGURE 4 Frog sartorius muscle. A peripheral coupling (*PC*) composed of junctional SR (*JSR*), sarcolemma, and intervening junctional processes (arrowhead) is located at the M line (*M*). Note the appearance of the junctional processes in this projection. Other areas of SR containing junctional granules (*JG*) do not show distinct junctional processes and therefore cannot clearly be identified as part of a peripheral coupling. Z line (*Z*). × 84,500.

FIGURE 5 Mouse adductor muscle. In the I region (*I*) of the myofiber, the sarcoplasmic reticulum forms a peripheral coupling (*PC*) as defined by junctional SR (*JSR*) with contained junctional granules (*JG*) in close apposition to the sarcolemma with intervening junctional processes (arrowheads). Extracellular space (*ECS*). × 72,000.

with a granular material were frequently noted in a subsarcolemmal location in the frog's sartorius (Fig. 4), junctional processes were visualized infrequently. Therefore, by applying rigorous criteria for defining the peripheral coupling, only rarely could such a structure be identified unequivocally.

Recognition of peripheral couplings in the mouse pectoral and leg muscles (Fig. 5) was complicated because the JSR in these muscles was not as distinct as in the other muscles studied, and because it seemed to extend for long distances just beneath the sarcolemma while passing in and out of the plane of the section, making it difficult at times to distinguish the JSR from other subsarcolemmal vesicles. Junctional processes, though present, were visualized infrequently.

Anatomically interior couplings were originally described in vertebrate and invertebrate skeletal muscle as dyads and triads (3). Peripheral couplings have been reported in skeletal muscle from a primitive chordate, the amphioxus (18), but the myofibers in this animal have a peculiar geometry in that they are arranged in lamellar sheets approximately equal in thickness to one myofibril. Peripheral couplings have also been described in smooth muscle (19). Both of these latter muscles lack a transverse tubular system. Peripheral couplings have also been seen in cardiac muscle of all vertebrates so far examined, albeit they are rudimentary in some. Neonatal studies have shown that peripheral couplings in skeletal muscle of young rats become interior couplings as the transverse tubules develop and that this process is essentially complete by an early neonatal stage (8, 20). Our present investigations indicate, however, that, at least in those skeletal muscles examined, peripheral couplings persist into the adult state.

Functionally, the significance of the present findings relates particularly to the experiments of Howell and Jenden (21), Howell (22), Gage and Eisenberg (23, 24), and Eisenberg and Eisenberg (25). These authors among other things were able to dissociate excitation from contraction presumably by disrupting the transverse tubules in frog skeletal muscle. Since it was shown that the experimental procedure did not damage the contractile machinery itself, one must conclude that transverse tubules are critically involved in the process of excitation-contracting coupling. This function may reside either within the transverse tubules themselves, or within the interior couplings that they bear. The fact that the above authors found no muscular contraction after disruption of the transverse tubules in spite of the fact that they used muscle fibers in which peripheral couplings occur, does suggest that couplings have nothing to do with the process of electromechanical coupling per se. However, at the moment such a conclusion is premature because the possibility cannot be ruled out that the experimental manipulation which severs the transverse tubules may coincidentally also damage the couplings functionally, if not morphologically. Moreover and alternatively, the number of peripheral couplings, e.g., in the frog, may be quantitatively insufficient to effect electromechanical coupling except, perhaps, in the most superficial fibrils, in a fiber of large diameter after the more numerous interior couplings have been isolated from the surface sarcolemma.

The occurrence of peripheral couplings in all the skeletal muscles so far examined stresses the remarkably uniform topographic distribution of these structures in different kinds of muscle from different species, and suggests that they are part of an evolutionary continuum ranging from lower invertebrates to the mammals and, in addition, emphasizes the anatomically homologous nature of the couplings in skeletal and cardiac muscle. Furthermore, the morphologic persistence of large numbers of peripheral couplings in some adult skeletal muscles implies persistence of function; their structural homology to interior couplings implies a similar and thus nonatavistic function.

Contemplating that function, it is perhaps worth pointing out in passing that in cardiac muscle of some birds, the extended junctional SR, occurring as it does at almost every Z-I region deep in the interior of the muscle fibers, has no direct contact with sarcolemma (6).

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