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Arrangement and Density of Junctional Feet in Crayfish Muscle Fibres

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Abstract. Junctional feet in tubulo-reticular junctions of crayfish muscle fibres are arranged tetragonally with a centre-to-centre spacing of 30—34 nm. The resulting density of 860—1110 feet per 1 μm^2 of the junctional membrane is similar to that reported for other animal species. Using data of a previous stereological study, there are 150—190 feet per 1 μm^2 of the total T-tubule surface and 6000—7800 feet per 100 μm^3 of the fibre volume.

Key words: Tubulo-reticular junction — Junctional feet — Crayfish muscle — Excitation-contraction coupling

Introduction

The role of T-tubules in excitation-contraction coupling is well established. Junctional feet between tubular membranes and sarcoplasmic reticulum (SR) cisternae are assumed to form a structural basis for the transfer of the tubular signal to calcium releasing SR vesicles (for a review see Ebashi 1976).

Molecular mechanisms underlying this signal transfer and operating at the feet are largely unknown. To test hypotheses on the mechanism of the feet function in E—C coupling, e.g. the presence of hydrophilic pores in feet centres (Birks 1965; Franzini—Armstrong 1971; Mathias et al. 1980) or potential-dependent movements of long signalling molecules located in the feet (Schneider and Chandler 1973), quantitative data on the feet density and distribution are necessary.

Recently, two types of asymmetry currents in crayfish muscle fibres were described differing in amplitude and time parameters (Zachar et al. 1981; Henček et al. 1982). The slow asymmetry currents were tentatively related to the activation of contraction. The feet in tubulo-reticular (T—SR) junctions could represent a site where these displacement currents are generated.

In the present work the arrangement of junctional feet in crayfish muscle fibres was studied on longitudinally oriented grazing sections of T—SR junctions. Quantitative data on the density of feet related to the total T-tubule area or the

fibre volume were estimated from the results of a previous stereological study (Uhrik et al. 1980).

Materials and Methods

Long-sarcomere fibres from m. extensor carpopoditi of crayfish *(Astacus fluviatilis)* were fixed in situ with 2% glutaraldehyde in 0.15 mol/l Na-cacodylate (pH 7.4) for 40 min. Individual fibres or small bundles of fibres were then dissected, washed in the same buffer for 20 min and postfixed with 1% OsO_4 (dissolved in the same buffer) for 30 min. The specimens were left overnight in 2% uranyl acetate in H_2O, dehydrated in ethanol series and embedded in Durcupan.

Longitudinal ultrathin sections (of grey interference colour) were cut with a diamond knife mounted in a Porter-Blum MT2 ultramicrotome. The sections were picked up on copper grids, stained with lead citrate, carbon coated in a HV B 30.1 coating-unit and studied in a Tesla BS 613 electron microscope at a direct magnification of about 22,000 x. The final magnification on micrographs (of about 110,000 x) was determined with the use of a diffraction grating replica (1220 lines per 1 mm).

Goniometer stage was operated so as to ascertain that the distances between feet in particular junctions to be exposed were maximal.

For quantitative evaluation 14 junctions with distinct feet were selected.

Results and Discussion

Grazing sections of areas containing junctional feet were identified as dense patches with dots arranged periodically in rows (Fig. 1). To eliminate the effect of

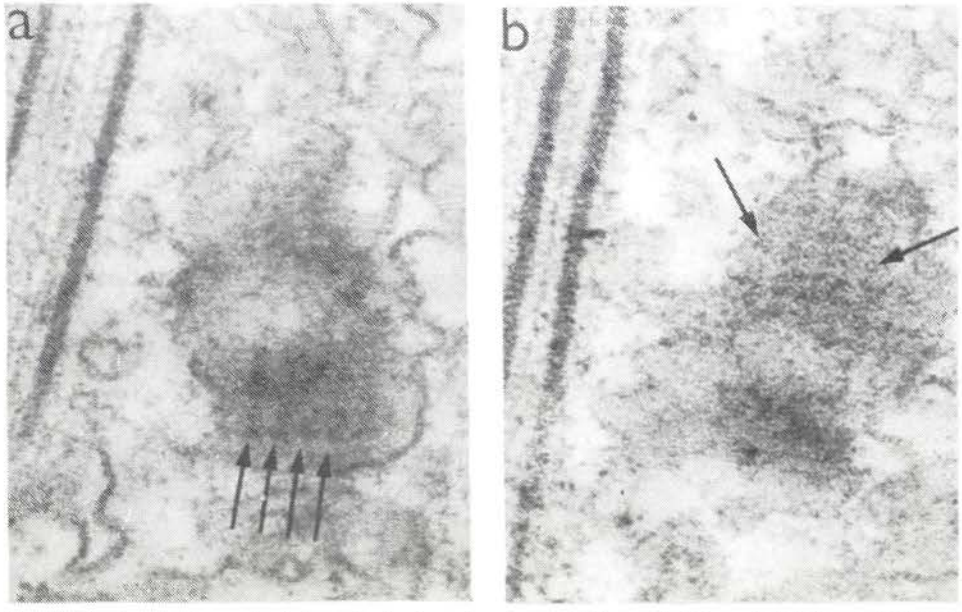

Fig. 1. Grazing sections of tubulo-reticular junctions in crayfish muscle fibres. (a) Distances between arrows are perpendicular to the direction of sectioning. (b) Arrows point to feet with electron lucent cores (×110,000).

plastic deformation during sectioning (compression artifact) on feet spacing only distances perpendicular to the direction of sectioning were measured (Fig. 1a).

Fig. 1a shows junctional feet arranged in an oblong lattice. If compression artifact is considered (myofilaments indicate the direction of sectioning), an originally square lattice may be assumed.

Markham rotation of micrographs for accentuating arrangement with a radial symmetry (Markham et al. 1963) could not generally be used due to the presence of compression artifacts.

The feet in Fig. 1b are conspicuous by their electron lucent cores suggesting the presence of pores; the overall feet arrangement is similar to that seen in Fig. 1a.

Table 1. Quantitative data on feet spacing and density in crayfish T—SR junctions.

Distance between feet (nm)	30—34
Number of feet per 1 μm of the junctional membrane	860—1110
Number of feet per 1 μm^2 of the total T-system area	150—190
Number of feet per 100 μm^3 of the fibre volume	6000—7800

Table 2. Feet spacing and densities in different animal species

Animal species	Distance between feet (nm)	Number of feet per 1 μm^2 of the junctional membrane	Number of feet per 1 μm^3 of the total T-system area
(1) Amphioxus	34—38	690—860	
(2) Opsanus tau	30	1110	435
(3) Rana pipiens	25—30	1110—1580	790
(4) Astacus fluviatilis	30—34	860—1110	150—190
(5) Eurypelma californicum	30	1110	

(1) Grocki (1982); (2) Franzini—Armstrong and Nunzi (1983);
(3) Franzini—Armstrong (1975); (4) the present study;
(5) Franzini—Armstrong (1974)

Quantitative data obtained by morphometric analysis are summarized in Table 1. An arrangement of the feet in a square lattice was assumed; the number of feet per 1 μm^2 of the total T-system area and the number per 100 μm^3 of the fibre volume were calculated from data of a previous stereological study (Uhrík et al. 1980).

Table 2 summarizes data on the feet spacing and density in different animal species. The differences in the feet spacing and their density on the junctional membranes may be considered insignificant due to inaccuracy in determining magnification on electron micrographs. With respect to ultrastructural organization the T—SR junction seems to be phylogenetically stable. The feet densities calculated per total area of T-tubules vary with the variations in the surface density of T-system membranes.

In the same volume of frog muscle fibres, about twice as many feet are contained as in crayfish muscle fibres as calculated from data of the stereological study on frog skeletal muscle (Mobley and Eisenberg 1975).

Despite a lower density of junctional feet in crayfish than in frog a much higher charge is displaced by slow asymmetry currents in crayfish (Henček et al. 1984). As pointed out by Henček et al. (1984), this discrepancy suggests that structures other than junctional feet may be the site of origin of slow asymmetry currents in crayfish.

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