Short communication

Disintegration of Junctional Feet in Crayfish Muscle Fibres Kept *in vitro*

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The significance of junctional feet (Franzini-Armstrong 1971) for excitation-contraction (E-C) coupling is suggested by their location in the gap separating the T-system from the sarcoplasmic reticulum (SR).

Until now, however, no specific treatment for selectively disrupting junctional feet has been known and the direct evidence relating the presence of feet to the transfer of signals across the T-SR gap is thus still lacking.

The importance of morphological integrity of tubulo-reticular (T-SR) junction for E-C coupling was invoked by Oota and Nagai (1973) and Frank and Treffers (1977) who showed widening of the junctional gap following urea-removal treatment and by Eisenberg and Eisenberg (1982) reporting an increased number of pillars spanning the T-SR gap in fibres activated by potassium-rich solution. On the other hand, E-C decoupling in crayfish after glycerol-removal (Zacharová and Uhrík 1978) or urea-removal (Krolenko et al. 1980) treatments could be satisfactorily explained by the detachment of T-tubules from the sarcolemma.

An interesting type of E-C decoupling was described by Rýdlová et al. (1984) on crayfish (*Astacus fluviatilis*) muscle fibres kept *in vitro*. When the fibres were maintained in a culture medium consisting of a mixture (2 : 1) of crayfish saline with calf serum supplemented with penicillin and streptomycin (Daig and Spindler 1979; Rýdlová et al. 1983) the dissociation of the E-C link was developing gradually. A significant decrease in the mechanical response upon depolarisation (induced either by potassium or electrical stimulation) could be recorded on the 4th day after the isolation of fibres. Prolonged maintenance of fibres in the culture medium (for 8 days or more) mostly resulted in nearly complete E-C decoupling. At the same time the membrane potential, action potentials in strontium solution or inward calcium currents under voltage clamp were not significantly different from controls. Caffeine contractures were normal or higher and ultrastructurally intact T-system connected to sarcolemma and SR with normal appearance could be seen.

The aim of the present study was to examine the ultrastructure of T-SR junctions of crayfish muscle fibres isolated and kept *in vitro* for different periods of time.

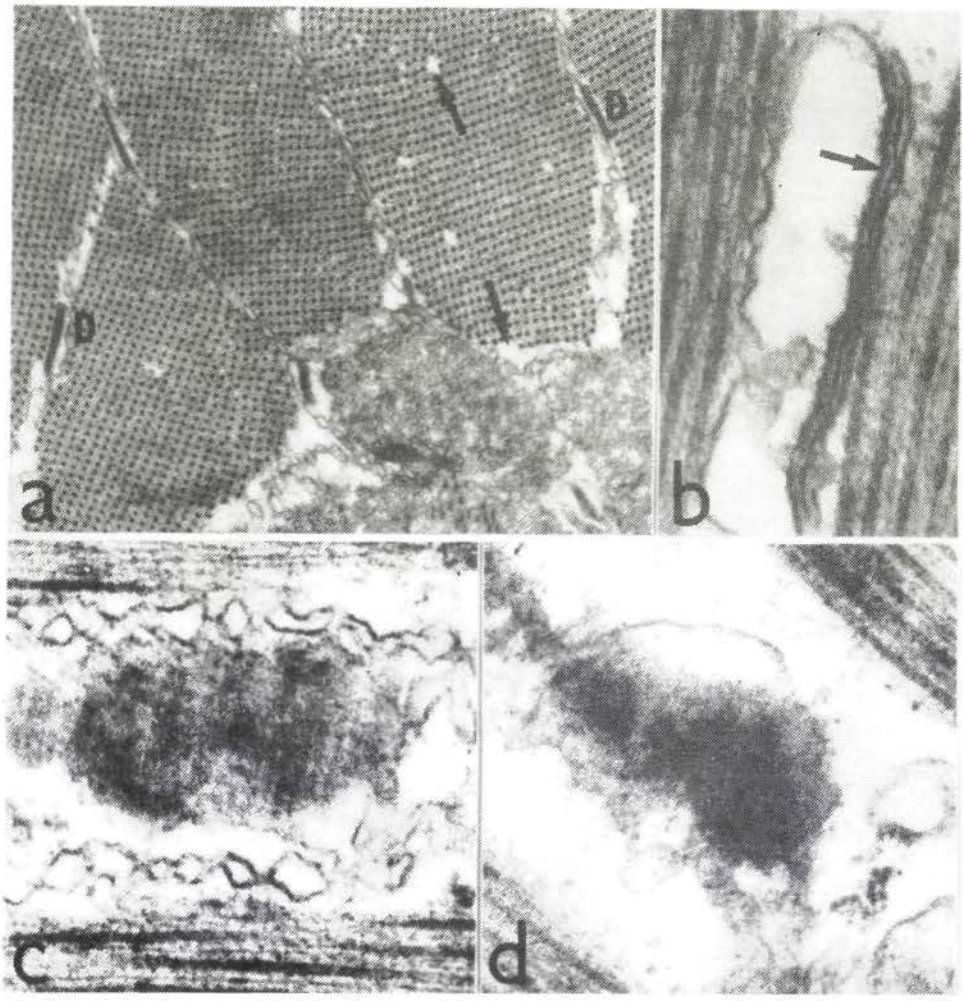

Fig. 1. (*a*) Transverse section of the crayfish muscle fibre kept 11 days *in vitro*. D — diads (× 22,000). (*b*) A diad sectioned transversally. Arrow points to T-SR gap. 10 days *in vitro* (× 90,000). (*c*) Normal appearance of the grazing section of the junctional gap (× 80,000). (*d*) Grazing section of the junctional gap of the fibre kept 11 days *in vitro* (× 80,000).

Diads, the site of T-SR junctions in crayfish, were conspicuous by the presence of a dense material in the junctional gaps (Fig. 1*a*; 11 days *in vitro*). Inspection of gaps at higher magnification revealed a partial disorganisation of feet: they were shallow, extended laterally or fused to form a homogenous, dense material (Fig. 1*b*; 10 days *in vitro*). Since the appearance of feet in diads sectioned transversally (Fig. 1*b*) depends on the plane of the section and may not be representative, grazing sections of junctional gaps were searched for. Normally,

grazing sections of junctional gaps in crayfish are characterised by the presence of tetragonally arranged dots corresponing to feet bridging the gap (Fig. 1*c*; Uhrík et al. 1984). A different image was seen in fibres kept *in vitro* (Fig. 1*d*; 11 days *in vitro*): the gap material had a diffuse, homogenous appearance, the outline of feet was not distinct enough to form recognisable dots.

E-C decoupling in fibres from isolated frog muscles maintained in Ringer solution for 24 h was described by Kiku-Iri (1962) yet not further analysed. The loss of force to a half the initial value during twitch or tetanic tension in the sartorius muscle of a toad kept in organ culture for 15 days (McDonagh 1984) was tentatively explained as a consequence of a failure of action potential propagation in the sarcolemma or in the T-tubules. In rat skeletal muscles cultured for 4 days a changed kinetics of the decay phase of charge movement was described (Hollingworth et al. 1984). This observation may be relevant to our results since junctional feet were assumed (Schneider and Chandler 1973) to be the site where displacement currents may be generated.

On the other hand in a study by Harris and Miledi (1972) both twitch and slow frog muscle fibres were able to contract in response to acetylcholine or potassium-rich solution after having been kept in a culture medium (of a different composition) for about 4 weeks.

The reasons for the disruption of feet under experimental conditions used in our study are not evident at present.

Insufficiencies of the culture medium may have resulted in a decrease in the turnover of the feet material in the protein-synthesising machinery of the cell and in feet decay.

Another mechanism was suggested by the occurrence of enlarged interfibrillar spaces and erosion areas at the level of A bands (Fig. 1*a*; arrows). Morphologically similar erosions were described in crustaceans during molting and explained as a consequence of an increased protein degradation by Ca^{2+}-dependent proteinases (for a review see Mykles and Skinner 1982).

Proteinases released from lysosomes as a result of some adverse influences of *in vitro* conditions could in addition to contractile proteins also attack feet material, the feet being freely accessible from the sarcoplasm as demonstrated for ferritin particles (Franzini-Armstrong 1971).

Since muscle fibres kept *in vitro* are devoid of nerve supply the multiplication of lysosomes (Schiaffino and Hanzlíková 1972) and an increase in proteinases activity (Pluskal and Pennington 1973) both shown after muscle denervation add further support to this explanation.

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