About the morphological relationships of the sarcoplasmic reticulum in the sole plate area of the frog

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Abstract

In the present investigation the sole plate area of motor end plates of the frog is ultrastructurally examined with different postfixation methods. We concentrated in this case on the proof of the smooth and rough sarcoplasmic reticulum of the sole plate. The relations of the smooth and rough sarcoplasmic reticulum to subsynaptic folds and the local T-system and its connections to diads and triads in the sole plate area are represented. The morphological differences between mammal and frog are pointed out. The possible functions of the sarcoplasmic reticulum in the myofibril-free sarcoplasm are discussed.

Keywords: Sarcoplasmic reticulum; Myonuclei; Sole plate; Motor end plate; Frog; Imidazole

1. Introduction

Previous investigations of motor end plates (MEPs) of mammalian skeletal muscle fibres demonstrated that there are two separate tubular networks in the sole plate sarcoplasm. One of these tubular networks, i.e. the T-system, is in contact with subsynaptic folds in mammals (Dauber et al., 2000) and frogs (Voigt et al., 2000; Couteaux and Pecot Dechavassine, 1968). The other tubular network consists of the smooth and rough sarcoplasmic reticulum (SR). In mammals, the smooth SR of sole plate is in contact with the smooth SR between the myofibrils (Segretain, 1995; Voigt et al., 2003). In mammals, this SR of sole plate builds triads with the T-system of this region (Dauber et al., 1999) and diads with subsynaptic folds (Dauber and Meister, 1986). In frogs, comparable sarcoplasm areas, with a tubular network surrounding the sole plate nuclei, better known as fundamental nuclei, were also demonstrated when more scarce (Couteaux, 1981). But the relation of this network to the T-system of sole plate (Voigt et al., 2000) or the smooth SR between the myofibrils was not investigated. The aim of the present investigation is to portray this tubular network structurally and its possible relations to other cell structures.

2. Materials and methods

For the investigation frogs (Xenopus laevis (n = 1), Rana esculenta (n = 1) and Rana temporaria (n = 3)) were anaesthetised with 0.1% MS222 in water (Sandoz Co.) (Couteaux, 1981) and decapitated. In a Petri dish filled with 2.5% glutaraldehyde in a 0.1 M cacodylate buffer, the legs were exarticulated at the pelvic joint, the skin of the leg was removed, the superficial sartorius muscle was exposed, and its fascia was completely removed. Then, the entire leg was immersion fixed in the same fixative for 1 h. Subsequently, the muscle was divided into small portions and allowed to remain in the fixative agent for 1 h. For all solutions a 0.1 M cacodylate buffer was used. Then, the portions were washed (3 × 5 min in cacodylate buffer), dehydrated in an ascending series of alcohol solutions and embedded in Epon.
All sections were analysed in a LE0912 Omega electron microscope.

3. Results

The present investigation of the sarcoplasm of MEPs of frogs verifies former findings about the shape, quantity and localization of fundamental nuclei (Conteaux, 1981). These fundamental nuclei of the MEP cannot be located along the MEP effortlessly, because of their shape in frogs not to be distinguished from muscle fibre nuclei, and their sporadic appearance (Fig. 1).

Different aspects of sole plate are shown with various postfixation methods. After conventional postfixation with osmium or osmium-potassium ferrocyanide, ribosomes and glycogen granules are poorly distinguished from muscle fibre nuclei, and their sporadic appearance (Fig. 1). With this postfixation a tubular network can be shown in the sarcoplasm of the MEP, which is in contact with the myofibrillar smooth SR at all levels of the sarcromers (Figs. 3 and 6). Within the myofibrillar sarcoplasm of the MEP the smooth SR is in contact with the rough SR (Fig. 4b). In spite of the poor staining of the ribosomes after the imidazole-osmium postfixation, the rough SR can be differentiated due to its enhanced contrast of the membrane, its smaller diameter, its straight unbranched course and the more electron dense sarcoplasm surrounding it (Fig. 4b). At the lower third of the sarcoplasmic triads. Due to these criteria, a triad can be identified in the sole plate of the frog in proximity to a fundamental nucleus after imidazole-osmium postfixation (Fig. 9b).

4. Discussion

The investigation has shown that, as in mammals, a tubular network can be shown in the subsynaptic sarcoplasm of the MEP of frogs. As in mammals (Voigt et al., 2002), the use of the imidazole-osmium postfixation proves to be suitable for a sharp membrane outlining at frogs. Furthermore, the blackening of the sarcoplasm of sole plate, mainly caused by the glycogen, is suppressed. Because of these facts and the fact that there are less mitochondria obscuring the view within the sole plate at frogs, this network can be shown in greater expansion. As in mammals, this network can be
determined as SR due to its contacts to the cisternal SR of myobrillar triads and to the rough SR (Voigt et al., 2003). As in mammals, the tubules expand cistern-like in proximity to the lower third of the subsynaptic folds (Segretain, 1995) and form diads with them (Dauber and Meister, 1986). And, as in mammals (Dauber et al., 1999), it forms triads with the T-system of the sole plate sarcoplasm (Voigt et al., 2000). Different to mammals, this SR is more cross-linked and the lumen width of individual tubules is raised, but we could not constitute a subdivision into a wide and fine-meshed part. So, there are no similarities of parts of this network to the fenestrated collar around myofibrils (Voigt et al., 2003).

The functions of these structures, i.e. the diads and triads in the sole plate, are unknown. According to the generally accepted ideas about their function in the event of the electrochemical coupling, it is to assume that also in frogs, Ca\(^{2+}\) is released, recovered and stored by these structures in the sole plate sarcoplasm. Myofibrils, lying in proximity to the sole plate area of the frog, could be activated. So, a local shortening of the MEP was attributed to a local increase of the Ca\(^{2+}\)-concentration (Battaglia et al., 1988; Volpe et al., 1986) through diads and triads of the MEP (Powell et al., 2001, 2003).


References


