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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) and with the perinuclear cisterns of sole plate nuclei (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.

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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 a further 2 h.

Then, the portions were postfixed at 4 °C by one of the following procedures: (1) routine procedure using 1% osmium tetroxide for 3 h; (2) potassium ferrocyanide procedure using 1% osmium tetroxide with 0.08% potassium ferrocyanide for 3 h and (3) imidazole-osmium procedure using 1% osmium tetroxide in 0.1 M imidazole for 1 h. For all solutions a 0.1 M cacodylate buffer was used. Then, the samples were washed (3×5 min in cacodylate buffer), dehydrated in an ascending series of alcohol solutions and embedded in EPON.

The sections of muscle tissue fixed according procedures 1 and 2 were section stained with uranyl acetate and lead citrate (Reynolds, 1963); sections of muscle tissue fixed according procedure 3 were analysed in the unstained state.

All sections were analysed in a LEO912 Omega electron microscope

3. Results

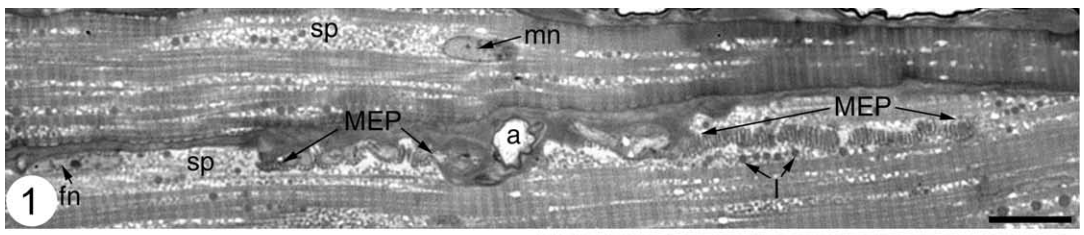
The present investigation of the sarcoplasm of MEPs of frogs verifies former findings about the shape, quantity and location of fundamental nuclei (Couteaux, 1981). These fundamental nuclei of the MEP cannot be located along the MEP effortlessly, because of their shape in frogs not to be distinguished 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 heavily stained, so that broad portions of sarcoplasm come out electron dense (Fig. 2). On the other hand, glycogen granules and ribosomes are poorly outlined by the imidazole-osmium postfixation (Figs. 3, 4a, 5, 6, 9a and b). 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 sarcomers (Figs. 3 and 6). Within the myofibril-free 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 subsynaptic folds the network forms cisterns in proximity to the folds (Figs. 5, 7, 8 and 10). The contact membranes between these cisterns and the subsynaptic folds display an enhanced contrast after imidazole-osmium postfixation. By means of the conventional postfixation with osmium or osmium-potassium ferrocyanide feet can be identified between the membrane of these cisterns and the sarcolemma of subsynaptic folds (Figs. 7 and 10), and electron dense granules can be shown in the lumen of the cisterns (Figs. 7, 8 and 10). These cisterns are therefore comparable to the cisternal SR of myofibrillar 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

Fig. 1. Semi-thin section through the MEP of a frog muscle fibre. A fundamental or sole plate nucleus (fn) is situated in a thin area of myofibril-free sarcoplasm (sp) in proximity to subsynaptic folds. These nuclei are oblong as the muscle fibre nuclei (mn) within the muscle fibre. The muscle fibre nuclei are surrounded by myofibril-free sarcoplasm too, which extend over several sarcomers in the longitudinal fibre axis; lipid droplets (l), axon (a). *Xenopus laevis*, imidazole-osmium tetroxide postfixation, unstained, bar = 20 μm. Fig. 2. Tangential longitudinal section through a MEP of a frog muscle fibre. The glycogen in the myofibril-free sarcoplasm (sp) around the subsynaptic folds (f) is stained electron dense by the potassium ferrocyanide osmium tetroxide postfixation; axon (a). *Xenopus laevis*, section staining, bar = 1 μm. Fig. 3. Section of a MEP in the area of a fundamental nucleus (fn). In the brightened myofibril-free sarcoplasm (sp) a smooth SR (sSR) is outlined, which can be traced from the cisternal SR cistern (cSR) of a myofibrillar triad to the lower third of a subsynaptic fold (f); axon (a), T-tubule (T). *Rana temporaria*, imidazole-osmium tetroxide postfixation, unstained, bar = 500 nm. Fig. 4a. Section through the sarcoplasm around a fundamental nucleus (fn) of a MEP. A branched tubular smooth SR (sSR) can be shown in the myofibril-free sole plate sarcoplasm. The rough SR (rSR) is characterized by a condensed sarcoplasm environment; Golgi apparatus (GA). *Rana temporaria*, imidazole-osmium tetroxide postfixation, unstained, bar = 2.5 μm. Fig. 4b. Section through the sarcoplasm around a fundamental nucleus (fn) of a MEP. The transition between the smooth SR (sSR) and rough SR (rSR) is characterized by a narrowing of its lumen. The membrane of the rough SR is more stained than the membrane of the smooth SR. *Rana temporaria*, imidazole-osmium tetroxide postfixation, unstained, bar = 500 nm. Fig. 5. Section of a MEP in the area of a fundamental nucleus. In the brightened myofibril-free sarcoplasm (sp) a smooth SR (sSR) expands from the myofibrils to the subsynaptic folds. Here, it forms a cisternal SR (cSR) and comes in close contact to the sarcolemma of the subsynaptic fold (f). *Rana temporaria*, imidazole-osmium tetroxide postfixation, unstained, bar = 500 nm. Fig. 6. Longitudinal section of a frog muscle fibre in the area of a muscle fibre nucleus (mn). The membrane of the central T-tubule (T) of a myofibrillar triad is stained more strongly. The cisternal SR (cSR) of the triad shows an empty lumen; myofibril-free sarcoplasm (sp); Golgi apparatus (GA). *Rana temporaria*, imidazole-osmium tetroxide postfixation, unstained, bar = 500 nm. Fig. 7. Section of a MEP in the contact area between subsynaptic folds (f) and myofibrils. The cisternal SR (cSR) forms a diad with the sarcolemma of a subsynaptic fold. Feet (∗) can be shown between the membranes which are facing each other. The cistern is filled with granules; axon (a). *Rana esculenta*, osmium tetroxide postfixation, section staining, bar = 250 nm. Fig. 8. Section of a MEP in the area of a fundamental nucleus (fn). A cisternal SR (cSR) forms a diad at the lower third of a subsynaptic fold (f). The cisternal SR is filled with granules and originates from the perinuclear cistern; axon (a). *Rana esculenta*, osmium tetroxide postfixation, section staining, bar = 250 nm. Fig. 9a. Longitudinal section through a frog muscle fibre in the area of a fundamental nucleus (fn). A closely linked smooth SR (sSR) can be shown in the brightened myofibril-free sarcoplasm. A structure that, lets think about a triad, is shown subsarcolemmal (∗); Golgi apparatus (GA). *Rana temporaria*, imidazole-osmium tetroxide postfixation, unstained, bar = 1 μm. Fig. 9b. The smooth SR expands to a cisternal SR (cSR). The membrane of the cisternal SR, facing the T-tubule (T), as well as the membrane of the T-tubule, facing the cisternal SR, are enhanced in contrast; fundamental nucleus (fn). *Rana temporaria*, imidazole-osmium tetroxide postfixation, unstained, bar = 500 nm. Fig. 10. Section of a MEP in the area of a fundamental nucleus (fn). A cisternal SR (cSR) forms a diad to the sarcolemma of a subsynaptic fold (f). Feet (∗) can be shown between the membranes which are facing each other. The cistern is filled with granules; axon (a). *Rana esculenta*, osmium tetroxide postfixation, section staining, bar = 250 nm.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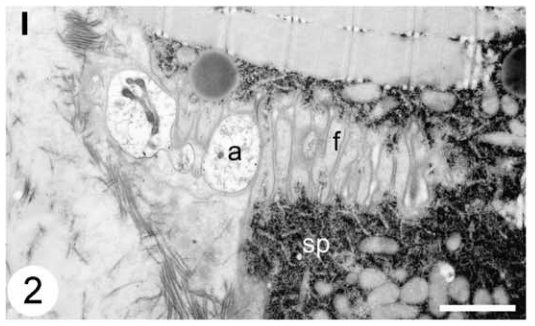
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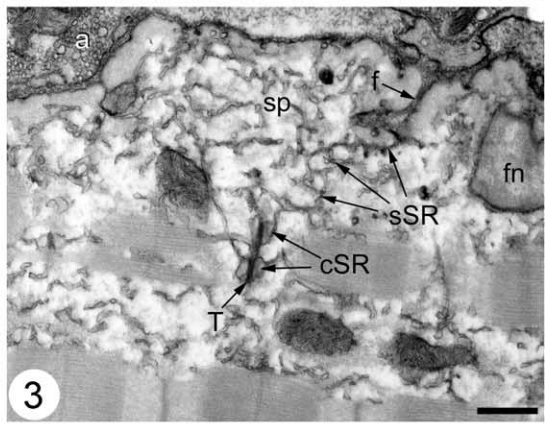
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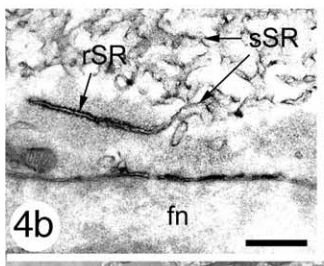
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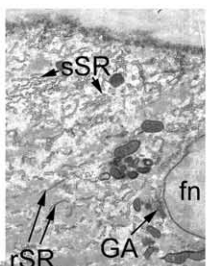
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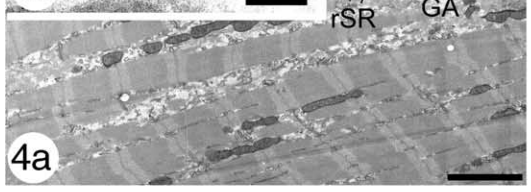
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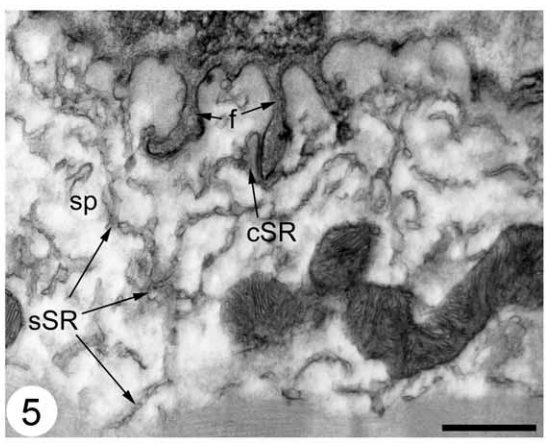
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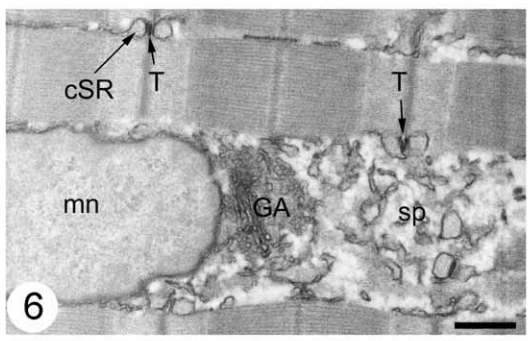
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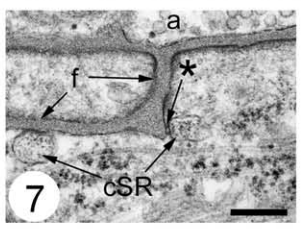
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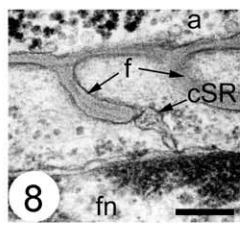
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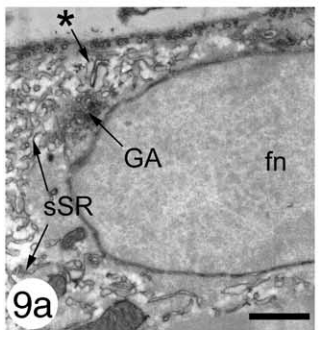
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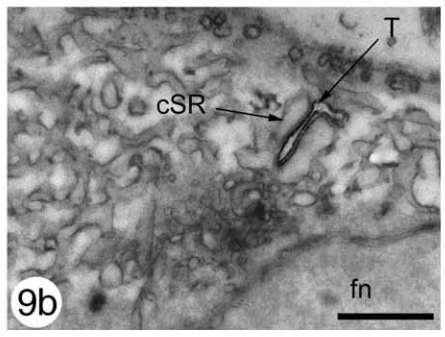
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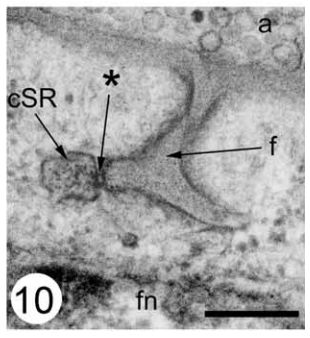
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Figs. 1–10.

determined as SR due to its contacts to the cisternal SR of myofibrillar 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 (Miledi et al., 1980). Additionally, an influx of Ca^{2+} by means of activated acetylcholine receptors (Takeuchi, 1963) or opened Na^{+}-channels of subsynaptic folds (Beam et al., 1985) could be the cause of a local increase of the Ca^{2+}-concentration.

But an increase of the Ca^{2+}-concentration must be buffered rapidly, on the one hand to counteract a desensitisation of acetylcholine receptors (Miledi, 1980), on the other hand to restrict the influence of the liberated Ca^{2+} to the metabolism (Decker and Dani, 1990; Zucker, 1985). In those MEP areas of frogs with proximity between subsynaptic folds and myofibrils a fast uptake of the influxed Ca^{2+} through the myofibrillar SR is to be expected because of short diffusion distances. However, as in mammals, the diffusion distances of the sarcoplasm around fundamental nuclei are larger so that the hazard of Ca^{2+}-accumulation could exist.

With regard to recent results that the local membrane activity controls the gene regulation in individual nuclei (Rossi et al., 2000), the morphology of the sole plate area must be considered. Since T-tubules meander through the sarcoplasm of the MEP (Voigt et al., 2000; Dauber et al., 2000), also the liberation of inositol-trisphosphate and an inositol-trisphosphate-mediated Ca^{2+} release (Carrasco et al., 1988; Volpe et al., 1986) through diads and triads of the MEP area must be thought of. Slow calcium waves, which do not have any influence on the contraction event, are stimulated by that (Jaimovich et al., 2000). These waves, however, cause an increase of the Ca^{2+}-concentration in the cell nuclei of the MEP and intervene in the gene regulation of the MEP (Powell et al., 2001, 2003).

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