Ultrastructural localisation of Ca^{2+} release channels in the nuclear envelope of human cardiac myocytes

S. Jäger[1], J. Kockskämper[2], and G. Leitinger[1,3]

1. Core Facility Ultrastructure Analysis, Center for Medical Rerearch (ZMF), Medical Unversity of Graz, Austria, 2. Division of Cardiology, 3. Institute of Cell Biology, Histology and Embryology, Center for Molecular Medicine, Medical University of Graz, Austria

Silke.Jäger@chello.at

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In cardiac myocytes, large rhythmic changes in cytoplasmic Ca^{2+} concentration [Ca^{2+}] underlie contraction and relaxation. Recent evidence indicates that nucleoplasmic [Ca^{2+}] can be regulated independently from cytoplasmic [Ca^{2+}] and that localized increases in nucleoplasmic [Ca^{2+}], via increased transcription, may be involved in the development of hypertrophy and heart failure. The structural basis for this compartmentalized regulation of [Ca^{2+}] in cardiac myocytes, however, remains poorly understood. Therefore, we studied the characteristics of the nuclear envelope, i.e. the perinuclear Ca^{2+} storage compartment, and the subcellular distribution of the two major intracellular Ca^{2+} release channels, the ryanodine receptor (RyR) and the inositol 1,4,5-trisphosphate receptor (IP3R), in human atrial myocardium.

To detect the subcellular localisation of these release channels, we used a post-embedding immunogold technique. We made thin (70nm) sections of human atrial material that had been embedded in LR White resin and applied both the Calbiochem anti-IP3R antibody (cat. Nr. NR09) and a polyclonal antibody against RyR (gift of Dr Tony Lai, Cardiff) as primary antibodies. British Biocell goat-anti-rabbit immunoglobulins coupled to 10nm gold particles were used as secondary antibodies.

We were thus able to locate both RyR and IP3R to the nuclear envelope (Figs 1 and 2), and a statistical evaluation of the gold granule localisation showed that most gold granules could be attributed to the inner nuclear membrane rather than the outer nuclear membrane. The density of gold granules at the inner nuclear membrane was statistically significantly higher than background for both RyR and IP3R localisation (t-test, $p<0.05$). A high density of gold granules was also visible in the periphery of the myofibrils, where sarcoplasmic reticulum is to be expected.

These results indicate that the two Ca^{2+} release channels are localised to the inner nuclear membrane and could be responsible for localised Ca^{2+} release into the nucleoplasm.

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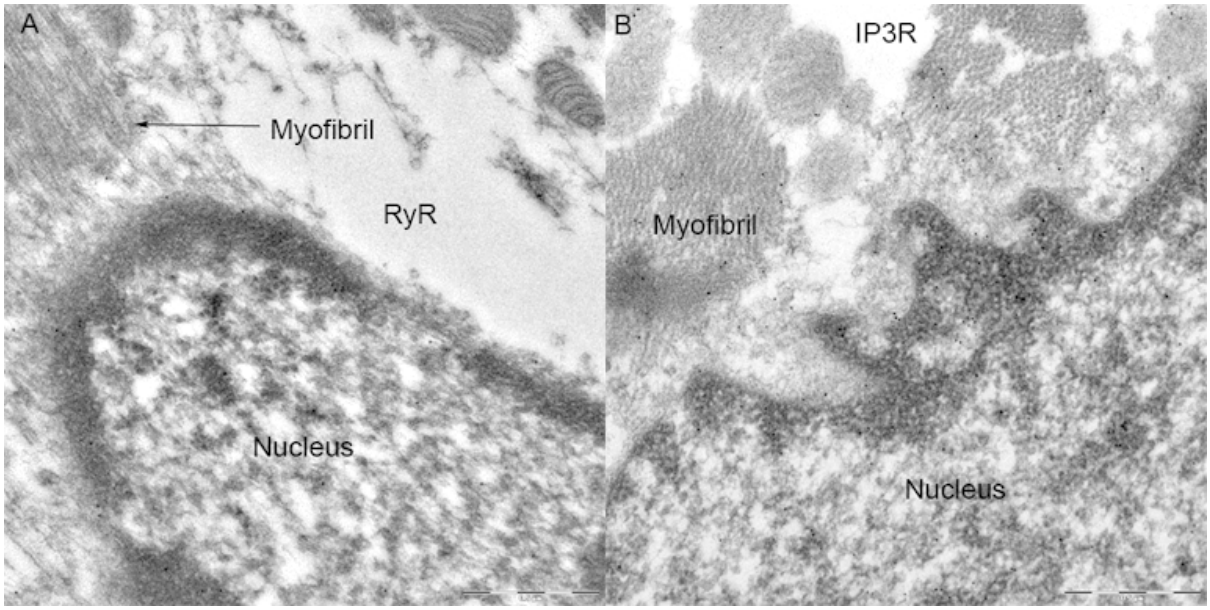

Figure 1. A RyR immunocytochemistry, **B** IP3R immunocytochemistry in human atrial cardiac myocytes. Most gold granules are visible at the nuclear envelope as well as surrounding the myofibrils, where sarcoplasmic reticulum is to be expected. Scale bars 0.5µm.

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