

Measurement of absolute amount of calsequestrin 2 present in cardiac ventricular muscle

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Calsequestrin 2 (CSQ2) is generally regarded as the primary calcium buffering molecule present inside the sarcoplasmic reticulum (SR) in cardiac cells, though its role as a calcium buffer has been questioned recently (Knollmann, 2009). The aim of this study was to determine the absolute amount of CSQ2 present in cardiac ventricular cells, in order to gauge the likely influence of CSQ2 on the total and free calcium concentration within the SR. Whole hearts from freshly killed sheep were obtained from an abattoir. Ventricular tissue was homogenized (1:10) in Na-EGTA solution, and 5 to 10 µg samples loaded in their entirety and separated by 8% SDS-PAGE and CSQ2 detected by Western blotting, similar to our work with skeletal muscle (Murphy *et al.*, 2009). Intensities of the respective bands were compared to those obtained with various amounts (2.5 to 20 ng) of purified CSQ2 on the same blots. The fidelity of the quantification was verified by comparing signals from samples, purified CSQ2, and samples with added amounts of purified CSQ2. Ventricular tissue from n=8 sheep contained on average 23 ± 2 µmol CSQ2 per kg wet weight. Qualitative assessment of CSQ2 content by staining homogenate samples with Stains-All indicated that CSQ2 content of rat ventricular tissue was similar or even higher than that found in sheep heart. This amount of CSQ2 could bind a maximum of ~1 mmol calcium per kg of ventricular tissue, more than ample to account for current estimates of total SR calcium content of such tissue.

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