Calcium loading properties of sarcoplasmic reticulum from rat ventricular myocardium

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There is a very large range of estimates for SR Ca^{2+} capacity of cardiac muscle in the literature, as reviewed by Bers (2001). For example estimates for ventricular myocardium SR Ca^{2+} capacity may vary by a factor of up to 6 times. In part this range may be attributed to the differences in the functional state of the SR. In this study we tried to emulate the conditions prevalent in "active" and "resting" cardiac muscle by exposing preparations to ATP containing solutions at different concentrations of EGTA and pCa during the skinning procedure. Such conditions are known to either facilitate a prolonged activation (0.1 mmol L^{-1} $EGTA_{Total,}$ pCa 7.1) or prevent any activation (10.0 mmol L^{-1} $EGTA_{Total}$, pCa 9). Kabbara & Stephenson (1997) showed that when Ca^{2+} entry was facilitated during the skinning procedure the SR was loaded with more Ca^{2+} under the same loading conditions compared with skinning when Ca^{2+} entry was prevented. They regarded these responses as being reminiscent of those from "active" cardiac muscle compared with more quiescent or "resting" cardiac muscle.

Right ventricular muscle bundles were homogenised in either highly (10.0 mmol L^{-1} $EGTA_{Total}$, pCa 9) or weakly (0.1 mmol L^{-1} $EGTA_{Total}$, pCa 7.1) calcium buffered skinning solutions to mechanically render the sarcolemma "leaky". The preparations were then subjected to a simple protocol developed to estimate the SR Ca^{2+}content. Briefly, the preparation was immersed into a solution of known $[EGTA]_{Total}$ at a desired pCa for up to 10.0 minutes to allow the SR to equilibrate with calcium. Then the preparation was moved into an identical solution, which also contained 30 mmol L^{-1} caffeine where a caffeine-induced force transient was recorded. This procedure was then repeated without reloading the SR with Ca^{2+}. On second immersion into the caffeine containing solution, there was no SR Ca^{2+} release, and force only increased due to the higher sensitivity of the contractile apparatus for Ca^{2+} in the presence of caffeine. By overlaying the force responses we could subtract the myofibrillar force response component due to caffeine, which allowed measurement of the SR Ca^{2+} released which could then be converted to the amount of Ca^{2+} released by the fibre volume at the peak of the caffeine-induced force response.

The SR Ca^{2+} content estimated from the caffeine-induced force responses for preparations loaded at pCa 7.0, over a range of $[EGTA]_{Total}$ (0.02 to 0.2 mmol L^{-1}) were on average 197 ± 35 (mean ± SEM, n=3) mmol Ca^{2+} L^{-1} cytosol for preparations skinned under weakly buffered conditions for calcium, emulating "active" myocardium. When preparations were skinned in 10 mmol l^{-1} $EGTA_{Total}$ (pCa 9) however, this was reduced to 142 ± 2 (mean ± SEM, n=3) mmol Ca^{2+} L^{-1} cytosol where the preparations were expected to behave more like "resting" muscle. Hence there appeared to be a shift towards higher calcium loading and release by the SR when the preparations have been skinned under conditions analogous to "active" cardiac muscle compared with conditions more like those of the "resting" cardiac muscle.

Bers, D.M. (2001) *E-C Coupling and Cardiac Contractile Force, 2nd Ed.* Kluwer Academic Publishers, Dordrecht, p 179.

Kabbara, A.A. & Stephenson, D.G. (1997) *American Journal of Physiology,*. 273, H1347-H1357.