Electromechanical Coupling III. Estimation of the Ca Storage Capacity of the SR by Analysing the Time Course of Caffeine-Induced Tension Transients of Skinned Muscle Fibres

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The Ca uptake of the SR of a skinned fibre, which was studied with an indirect method, shows a biphasic time course. A total Ca concentration between 8 and 23 mM was accumulated in the SR by an initial fast process ($\tau \approx 4.7$ s). This Ca-uptake activity of the SR is high enough to bring about relaxation of twitch contraction *in vivo*. The magnitude of a subsequent slow process ($\tau \approx 10^3$ s) indicates that the total Ca storage capacity of the SR of skeletal muscle is in the order of 60 – 75 mM.

The sarcoplasmic reticulum (SR) from mechanically skinned skeletal muscle fibres has been widely used to study Ca-uptake and -release processes of this compartment under more physiological conditions than those occurring in vesicle preparations of SR (see [1]). In the present experiments the SR of skinned fibres was loaded with Ca and the stored Ca was subsequently released by the application of a high concentration of caffeine. Information about the amount of Ca accumulated by the SR during the loading period was obtained from an analysis of the associated force transients.

Frog muscle fibres (M. iliofibularis, *Rana esculenta*) were skinned after Natori [2]. The experimental procedure corresponded to that of Moisescu [3] and the bathing solutions used were prepared as described by Moisescu and Thieleczek [4]. Diffusion problems which normally occur in experiments with myofibrillar preparations can be minimized by using this type of solutions. The SR of the skinned fibre was loaded with Ca to different levels by immersing the preparation into a loading solution (total EGTA 50 mM, pCa 6.1) for different periods of time, after having been equilibrated in a relaxing solution with a low EGTA concentration (0.08 mM EGTA, 49.92 mM HDTA, pCa > 8). With this loading procedure a rapid (within ≈350 ms) and uniform equilibration of the free Ca concentration of the loading solution around the surface of the SR could be established. The loading with Ca was terminated by introducing the preparation into a high-EGTA relaxing solution (50 mM EGTA, pCa > 10) for 30 s in order to lower suddenly the concentration of ionized Ca within the myofibrillar space to $< 10^{-8}$ M. Thereafter the preparation was incubated for 60 s into an intermediary low-EGTA relaxing solution containing either 0.41 mM EGTA and 49.59 mM HDTA (pCa > 8) or 2.0 mM EGTA and 48 mM HDTA (pCa > 8). In order to release the stored Ca, the fibre was subsequently transferred to a corresponding "releasing" solution, $R_{0.41}$ or $R_{2.0}$, identical to the previous solution but containing in addition 25 mM caffeine. This high caffeine concentration is assumed to completely release the mobilizable Ca stored in the SR of the skinned fibre [5]. All solutions contained 60 mM TES, 8 mM ATP, 10 mM Creatine phosphate, 15 U/ml Creatine kinase, 1 mM Mg^{2+}, 133 mM K^+, 36 mM Na^+, 20 mM Cl^- at pH 7.10; 11 °C. With these solutions up to 20 cycles of Ca- loading and -release could be carried out on the same preparation without reducing the maximum induceable tension by more than 20%.

The peak of the caffeine-induced tension transients was reached within ≈ 2 s in the releasing solution $R_{0.41}$ and within ≈ 3 s in $R_{2.0}$. Peak tension response corresponded to maximum isometric force in these solutions if the loading intervals were > 33 s and ≳ 792 s, respectively. For longer loading times the transients showed a plateau phase from which the tension declined relatively slowly with half times of about 6 s. This tension decay was too slow to be due to radial diffusion of Ca^{2+} and CaEGTA out of the fibre. The time course of the plateau phase and tension decline could be described in a quantitative manner using the following assumptions:

(1) Diffusion limited processes within the preparation are much faster than the time course of Ca^{2+}-efflux from the SR.

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(2) The caffeine-induced Ca^{2+}-efflux from the SR can be approximated with an exponential process having a rate constant β [6].

(3) Local free Ca^{2+} within the myofibrils is determined by the ratio CaEGTA/$EGTA_{free}$ and local force could be regarded as being in equilibrium with the local Ca^{2+}-concentration during the relatively slow force transient [4].

(4) The average total EGTA concentration inside the myofibrils could be as low as 1/5 of that present in the releasing solution, due to the negatively charged filaments [7].

(5) in order to calculate the Ca^{2+}-distribution within the fibre for conditions approximating the experimental situation (preparation incubated in an unstirred solution) the following additional group of assumptions had to be made. The effective radius of the myofibrillar preparation is extended to include an "unstirred" layer of solution of thickness b around the surface of the cylinder which represents the myofibrillar bundle (radius r). The Ca concentration at the outer surface of this layer is zero at all times and the SR is assumed to be uniformly distributed within the extended cylinder. The diffusion coefficients of Ca^{2+} and all other Ca complexes (mainly CaEGTA) are considered to be similar to that of CaEGTA, $D_{CaEGTA} \simeq 4.6 \cdot 10^{-6}$ cm^2 s^{-1} [4].

The time course of an individual tension transient could be accurately predicted when $\beta = 0.08$ s^{-1} and $b = 20$ µm. Using these constants all further tension transients obtained from the same preparation ($r = 40$ µm, length 2.5 mm) could be described by only varying the total Ca concentration released in the caffeine solutions after preceding loading times of 3 – 800 sec. The value for β is very similar to the rate constant of the caffeine-induced mean Ca efflux from the SR, directly measured on skinned fibres [6]. The total concentrations of Ca released during caffeine treatment, plotted as a function of loading time, could be explained by the sum of two exponential functions.

The first component can be described by a relatively fast process with a time constant of 4.7 s based on the results obtained in solution $R_{0.41}$. The magnitude of releasable Ca during this fast process can be estimated to lie between 0.8 mM (obtained in solution $R_{0.41}$) and 2.3 mM (obtained in solution $R_{2.0}$). The lower value is probably an underestimate, because in the weaker Ca-buffered solution $R_{0.41}$ the local Ca concentration within the preparation could significantly exceed that of EGTA. This would imply a higher apparent diffusion coefficient for Ca_{total} ($D_{Ca} > D_{Ca\,EGTA}$) than that assumed above. A higher apparent diffusion coefficient would in turn lead to higher estimates of releasable Ca. The higher value obtained in solution $R_{2.0}$ is more likely an overestimate, since the local pH value could significantly decrease following a Ca release in the presence of higher EGTA concentrations rather than in the presence of lower total EGTA concentrations. This acidification would, in turn, significantly decrease the apparent Ca affinity to EGTA and thereby indirectly increase the local free Ca^{2+} concentration [4]. Or, in other words, one would assume a higher magnitude of releasable Ca than is actually present.

The second, slow component of total releasable Ca can be more accurately characterized in both types of solutions $R_{0.41}$ and $R_{0.2}$. It is remarkable that both independent sets of results suggest essentially the same time constant (10^3 s) and magnitude (5 mM) for this process.

Taking a fractional fibre volume of 10% for the SR [8] the results would suggest a total Ca storage capacity of the SR of skeletal muslce fibres of about 58 – 73 mM. These values lie in the range of data obtained from SR vesicles, provided that all stored Ca can be transformed into the ionized form [9]. If the rapid exponential phase of the released Ca-loading time relationship reflects the Ca-uptake activity of the SR during the twitch of a muscle fibre, it could terminate twitch tension within 250 – 300 ms using the values obtained with releasing solution $R_{2.0}$. This time would be within the range of observed twitch durations of single fibres at this temperature. Furthermore, the rapid process would be fast enough to account for the observed decline in free Ca concentration during a twitch, as deduced from aequorin responses [10] provided that a substantial amount of Ca is still bound to the myofilaments when the light signal has returned to its resting level [11], see also [12].

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