Measurement of Free Ca\textsuperscript{2+} in Sarcoplasmic Reticulum in Perfused Rabbit Heart Loaded with 1,2-Bis(2-amino-5,6-difluorophenoxy)ethane-N,N,N',N'-tetraacetic Acid by \textsuperscript{19}F NMR\textsuperscript{*}

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Measurements of free calcium ion concentration in the sarcoplasmic reticulum ([Ca\textsuperscript{2+}]\textsubscript{SR}) and an evaluation of its relationship to changes in cytosolic free calcium and energy state of the cell, as well as heterogeneity of the SR calcium pool, were performed using \textsuperscript{19}F NMR in Langendorff perfused rabbit hearts loaded with acetoxymethyl ester of 1,2-bis(2-amino-5,6-difluorophenoxy)ethane-N,N,N',N'-tetraacetic acid. We report a base-line time-average [Ca\textsuperscript{2+}]\textsubscript{SR} value of 1.5 mM (n = 13) in the beating heart, similar to the value measured at diastole. We further report that [Ca\textsuperscript{2+}]\textsubscript{SR} decreases by ~30% at the start of systole and that there is no evidence of spacial heterogeneity in [Ca\textsuperscript{2+}]\textsubscript{SR} during the contraction cycle. However, there appears to be a heterogeneous response to SR calcium channel release activator (caffeine) and SR calcium-ATPase inhibitor (cyclopiazonic acid), consistent with studies suggesting that there are subpopulations of SR. Raising cytosolic free calcium by depolarizing the cell with 30 mM extracellular KCl, resulted in an increase in [Ca\textsuperscript{2+}]\textsubscript{SR}; however, the calcium gradient was unchanged. Lowering cell phosphorylation potential, which would reduce the free energy available for the SR Ca\textsuperscript{2+}-ATPase, leads to a decrease in the calcium gradient across the SR, but this reduced gradient was primarily due to an increase in cytosolic free calcium and not a net release of SR calcium.

The sarcoplasmic reticulum (SR)\textsuperscript{1} plays an important role in regulation of mammalian cardiac muscle contraction. It is generally accepted that contraction is activated by Ca\textsuperscript{2+} influx through the sarcolemmal L-type channel, which subsequently releases SR Ca\textsuperscript{2+} via the Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release mechanism (1). During relaxation, Ca\textsuperscript{2+} is resequestered into the SR by the SR Ca\textsuperscript{2+}-ATPase and extruded by the sarcolemmal Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger (2, 3). The SR Ca\textsuperscript{2+} content available for release is an important determinant of contractile state. In spite of the importance of SR Ca\textsuperscript{2+}, little is known about the levels of free SR Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{SR}) and how it is altered by physiologic or pathologic perturbations. Although previous studies (4, 5) using rapid cooling contractures have provided a valuable index of SR Ca\textsuperscript{2+} load in cultured myocytes, there are currently no direct measurements of [Ca\textsuperscript{2+}]\textsubscript{SR}.

It is generally agreed that the SR Ca\textsuperscript{2+}-ATPase maintains a calcium gradient between the SR matrix and the cytosol which is close to the theoretical limit based on the free energy available from ATP hydrolysis. However, the exact degree of efficiency is debated due to the lack of precise knowledge of [Ca\textsuperscript{2+}]\textsubscript{SR} (see Ref. 6). Values for free SR calcium have been estimated (based on binding to calsequestrin) to be in the range of 0.3–5 mM (6–8). Furthermore, if the SR Ca\textsuperscript{2+}-ATPase is operating near its theoretical limit, a fall in phosphorylation potential, for example under conditions of ischemia (9, 10), could affect the Ca\textsuperscript{2+} gradient across the SR.

The present study provides direct measurements of [Ca\textsuperscript{2+}]\textsubscript{SR} using \textsuperscript{19}F NMR in Langendorff perfused rabbit hearts loaded with acetoxymethyl ester of 1,2-bis(2-amino-5,6-difluorophenoxy)ethane-N,N,N',N'-tetraacetic acid (TF-BAPTA). Our laboratory has recently developed TF-BAPTA and applied it to measurements of cytosolic [Ca\textsuperscript{2+}] in perfused rat hearts (11, 12). TF-BAPTA has a high KD for Ca\textsuperscript{2+} (65 µM) and combines both a large shift sensitivity with fast-intermediate exchange kinetics at typical magnetic field strengths (11, 12). Such an indicator offers the potential for simultaneous determinations of Ca\textsuperscript{2+} concentrations in different cellular compartments, contingent on the degree of indicator loading into these compartments. For TF-BAPTA, the chemical shift rather than the ratio of resonance intensities provides information regarding [Ca\textsuperscript{2+}] (11, 12). The 6-fluorine resonance in TF-BAPTA is insensitive to calcium binding and serves as a shift reference, and the 5-fluorine resonance shifts downfield on Ca\textsuperscript{2+} complexation. Using this approach we directly measure an ionized calcium concentration in the SR of ~1.5 mM, a value in good agreement with estimates obtained using calcequestrin binding constants (7). In addition we calculate the free energy required for the Ca\textsuperscript{2+} gradient across the SR under control conditions, under conditions of cardiac arrest induced by high extracellular potassium concentration, and under conditions of reduced phosphorylation potential.

**EXPERIMENTAL PROCEDURES**

*Isolated Rabbit Heart Preparations*—Male New Zealand White rabbits (~1–1.5 kg) were used and received humane care in accordance with National Institutes of Health standards (35). Rabbits were anesthetized by intravenous injection of pentobarbital (~100 mg) into a marginal ear vein. The heart was excised rapidly, and the aorta was cannulated. Retrograde perfusion was begun under constant pressure...
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(100 cm H$_2$O). The nonrecirculating perfusate was a Krebs-Henseleit buffer containing (in mM): NaCl 120, KCl 4.7, MgSO$_4$ 1.2, KH$_2$PO$_4$ 1.2, CaCl$_2$ 1.25, NaHCO$_3$ 25, and glucose 11. The buffer was maintained at pH 7.4 by bubbling with a mixture of 95% O$_2$, 5% CO$_2$, and at a temperature of 37°C.

Hearts were placed in a standard 30-mm NMR tube. After 10 min of control perfusion, loading with 1000 ml of 5 μM acetylcholine ester of TF-BAPTA was started. With typical flow rates of 30–50 ml/min, loading took about 30 min. To monitor contractility, a latex balloon was inserted into the left ventricle. The balloon was inflated to give an end diastolic pressure of 5–10 cm H$_2$O. As observed in our previous study of perfused rat hearts (12), TF-BAPTA did not cause a significant reduction in contractility.

**NMR Measurements—** 19F NMR measurements were performed on a Varian 400 wide-bore NMR spectrometer at 376.27 MHz at 37°C. We shimmed on the proton signal from the unheated bath, and we routinely obtained a non-spinning line width at one-half height of ~0.25 ppm. Ungated spectra were acquired every 5 min using 0.26-s intervals between scans with a pulse angle of 40° (20 μs). The spectral width was ±7060 Hz, and 4000 data points were collected. The free-induction decay was multiplied by an exponential function corresponding to 100-Hz line broadening before Fourier transformation.

To examine whether [Ca$^{2+}$]$_{iSR}$ varies during systole and diastole, the NMR pulse was gated to the contraction cycle through a homemade gating system, which provides a precise, adjustable trigger and a delay relative to the standard pulse signal. The NMR measurement was initiated at the desired time during the cardiac cycle. At exactly the same time as the NMR pulse, a triggering signal (TS) was sent out. We recorded simultaneously left ventricular pressure (LVP), dP/dt, and TS to verify the timing of the NMR acquisition. Once the NMR pulse was generated, a free induction decay was acquired. Since the peak of the cytosolic Ca$^{2+}$ transient occurs near the beginning of the upstroke of LVP wave (13, 14), one NMR pulse was gated at the time shown as TS 1 to measure systolic [Ca$^{2+}$]$_{iSR}$, and another NMR pulse was delayed by 150 ms (TS 2) to measure diastolic [Ca$^{2+}$]$_{iSR}$. The time interval between the scans depended on the heart rate. For a typical heart rate of 150–200 beats/min, the interval was ~300–400 ms. 1000–2000 consecutive gated scans were acquired to achieve an acceptable signal-to-noise ratio. The other parameters were the same as those used in the ungated study.

For the gating data to be valid, the kinetics of calcium binding to the indicator should be fast and the relaxation time should be short on the NMR time scale. These conditions were met in these experiments. First, the apparent transverse relaxation time is ~1.2 ms, so the acquisition time for the [Ca$^{2+}$] measurements was only 2 ms. Thus the time resolution should be <3 ms, which is ~1% of the typical cardiac cycle.

Calculation of Free Energy for ATP hydrolysis (ΔG$_{ATP}$) for SR Ca$^{2+}$-ATPase—We calculated the ΔG required for the Ca$^{2+}$-ATPase using Equation 2 (15), where R and T are the gas constant and temperature and we assume no membrane potential (16).

\[
\Delta G = 2RT \ln([Ca^{2+}]_{iSR}/[Ca^{2+}])
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The free-induction decay was multiplied by an exponential function corresponding to 100 Hz line broadening before Fourier transformation. Immediately frozen by freeze-clamping at the temperature of liquid nitrogen and ATP, creatine phosphate, and creatine contents were measured enzymatically following perchloric acid extraction (17).

The following equation was used to calculate ΔG$_{ATP}$, with ΔG$_0$ = -30.5 kJ/mol.

\[
\Delta G_{ATP} = \Delta G_0 + RT \ln([ADP]_0/[P_i]/[ATP]_0)
\]

By thermodynamic convention values for ΔG$_0$ and ΔG$_{ATP}$ are negative for exergonic reactions, but after calculating ΔG$_{ATP}$ we refer to the values in the text as absolute values. The [ATP]/[ADP]$_0$ ratio was calculated by assuming that the creatine kinase reaction is at equilibrium. The apparent equilibrium constant (K$_a$) was calculated according to Equation 1 (18).

\[
K_a = [H^+] \cdot [K_a] = 10^{-6.87} \mu M^{-1} \cdot 1 \cdot 10^{-8.31}
\]

PCC and Cr contents were measured biochemically and converted to concentration by assuming that these metabolites are entirely cytosolic and that cytosolic volume equals 2.3 ml/g (dry weight). [P]$_i$ was obtained by comparing the NMR peak area to that of the basal PCr peak, after correction for NMR saturation, and assuming that the P$_r$ peak is entirely extracellular.

Materials—Cyclopiazonic acid (CPA; Sigma) was dissolved in dimethyl sulfoxide (16.8 mg/200 μl) and diluted in 1 liter of perfusate to a final concentration of 50 μM. Caffeine (1.94 g/liter) and BDM (1.01 g/liter) were dissolved directly in the perfusate to a final concentration of 10 mM each.

RESULTS

Ionized free calcium in compartments that contain different calcium concentrations can be measured from the 19F NMR spectra of TF-BAPTA loaded hearts from the shift difference between the Ca$^{2+}$-insensitive 6F resonance and the Ca$^{2+}$-sensitive 5F resonance of TF-BAPTA. In rat heart we observe one Ca$^{2+}$-sensitive peak (~5 ppm) corresponding to a time-average cytosolic free Ca$^{2+}$ concentration ([Ca$^{2+}$]$_c$) of ~600 nM (12). However, in rabbit heart, as shown in Fig. 1, the 19F spectrum shows two calcium-sensitive resonances: a cytosolic free calcium resonance at ~5 ppm, and an additional resonance peak at ~14 ppm that corresponds to a [Ca$^{2+}$]$_c$ of ~1.5 mM. To assess if the ~14 ppm peak represents SR Ca$^{2+}$, hearts were treated with a SR Ca$^{2+}$-ATPase inhibitor or a SR Ca$^{2+}$ release channel activator or were arrested with 30 mM [K]$_i$, to determine if these manipulations would alter the measured [Ca$^{2+}$]$_c$ as would be expected if the peak reflected indicator in the SR.

**FIG. 1.** 19F NMR spectrum (addition of spectra from four experiments), using 0.26-s intervals between scans with a pulse of 40° (20 μs). The spectral width was ±7060 Hz, and 4000 data points were collected. The free-induction decay was multiplied by an exponential function corresponding to 100 Hz-line broadening.
If the resonance at −14 ppm originates from the SR, then perfusion with 50 μM CPA, a specific SR Ca\(^{2+}\) -ATPase inhibitor (19), should decrease [Ca\(^{2+}\)]\textsubscript{SR}. Consistent with our expectation, after −10 min of perfusion with CPA, there was an upfield shift of the resonance near 14 ppm, indicating a decrease in [Ca\(^{2+}\)]\textsubscript{SR} (Fig. 2a). As shown in the inset, the shift and broadening observed with CPA addition is consistent with heterogeneity of the SR Ca\(^{2+}\) pool. The spectrum at 20–25 min of CPA perfusion could be modeled assuming that 66% of the SR pool had a [Ca\(^{2+}\)] of 100 μM and 33% of the SR had a [Ca\(^{2+}\)] of 260 μM. The change in contractility observed on addition of CPA is typical of SR Ca\(^{2+}\) -ATPase inhibition, i.e., a moderate decline in left ventricular developed pressure to 80 ± 14 mm Hg compared to the initial level of 106 ± 16 mm Hg, and a moderate increase in end diastolic pressure (see Fig. 2b).

We further tested whether SR Ca\(^{2+}\) could be depleted by the addition of caffeine, which activates the SR Ca\(^{2+}\)-dependent Ca\(^{2+}\) release channel (20). To minimize the caffeine-induced contracture so that we could maintain tissue perfusion in the presence of caffeine, 10 mM BDM was administered prior to caffeine (21). Subsequent combined perfusion with 10 mM caffeine + BDM resulted in a moderate increase in end diastolic pressure, while left ventricular developed pressure was reduced to 16.2 ± 3.1 mm Hg compared to the initial level of 101 ± 6 mm Hg. As shown in Fig. 3, addition of caffeine resulted in a loss of the resonance at 14.3 ppm, presumably due to lowering of [Ca\(^{2+}\)]\textsubscript{SR} and exchange broadening of the peak. Also shown in Fig. 3, when caffeine and BDM were washed out, the resonance at −14 ppm returned, as did contractility, consistent with an SR location for the calcium pool at −14 ppm. This broadening of the −14 ppm resonance during caffeine perfusion followed by the reappearance of a sharp resonance during caffeine washout indicates that the −14 ppm resonance is not due to extracellular indicator, and that the caffeine effect is not due to loss of indicator from the SR.

We were also interested in testing whether this resonance at −14 ppm could be shifted by perfusion with high [K\(^+\)], (30 mM), which depolarizes the myocytes, arrests the heart, and increases diastolic [Ca\(^{2+}\)] (22). If the peak at −14 ppm arises from SR Ca\(^{2+}\), one might expect a downfield shift on perfusion with high [K\(^+\)]. As demonstrated in Fig. 4, the putative SR resonance shifts from 14.4 ppm to 14.7 ppm, corresponding to an increase in [Ca\(^{2+}\)]\textsubscript{SR}. The shift to 14.7 ppm is very near saturation for the indicator, which makes it difficult to quantitate accurately the change in [Ca\(^{2+}\)]\textsubscript{SR}. The calculated value for [Ca\(^{2+}\)]\textsubscript{SR} increased from the control value of 1.5 mM to 5 mM during high [K\(^+\)] perfusion.

After confirming that the resonance at −14 ppm originates from SR, we investigated the magnitude of the change in [Ca\(^{2+}\)]\textsubscript{SR} during the cardiac cycle, which we thought might have a similar time course to the cytosolic Ca\(^{2+}\) transient. This is done by gating the NMR pulse and acquisition to the contraction cycle. As shown in Fig. 5a, [Ca\(^{2+}\)]\textsubscript{SR} was measured at the start of systole (TS 1) and after the heart had fully relaxed (TS 2). The corresponding spectra are shown in Fig. 5b. The chemical shift of the SR peak during diastole is 14.4 ± 0.04 (n = 3), corresponding to a [Ca\(^{2+}\)]\textsubscript{SR} of 1.5 mM, and the shift in early systole is 14.2 ± 0.03 (n = 3), corresponding to a [Ca\(^{2+}\)]\textsubscript{SR} of 1.0 mM. The difference (0.5 mM) represents a decrease in [Ca\(^{2+}\)]\textsubscript{SR} at the start of systole of ~30%. We also gated at other points during systole (for example at +10 ms compared to TS 1, near the middle of the upstroke). At this point there is already partial recovery of [Ca\(^{2+}\)]\textsubscript{SR} to a value of ~1.2 mM. Thus the fall in [Ca\(^{2+}\)]\textsubscript{SR} at the start of systole is very brief, which accounts for our finding that the time-average value of [Ca\(^{2+}\)]\textsubscript{SR} (1.49 ± 0.06 mM, n = 13) is essentially the same as the diastolic value.

The free energy required for the SR Ca\(^{2+}\)-ATPase can be
Free \( \text{Ca}^{2+} \) in SR in Perfused Rabbit Heart Loaded with TF-BAPTA

**TABLE I**

Biological determination of cellular contents of metabolites in control, high potassium arrested, and ischemic rabbit hearts

| Metabolite | Control (n = 3) | KCl (n = 3) | Ischemia (n = 4) |
|------------|---------------|------------|-----------------|
| ATP \( \mu \text{mol/g (dry weight)} \) | 22.7 ± 0.6 | 21.7 ± 1.0 | 9.4 ± 1.7 |
| PCr \( \mu \text{mol/g (dry weight)} \) | 34.2 ± 2.2 | 40.2 ± 2.3 | 22.2 ± 0.7 |
| Cr \( \mu \text{mol/g (dry weight)} \) | 54.6 ± 1.1 | 48.4 ± 4.2 | 85.7 ± 2.1 |
| P\(_i\) \( \mu \text{mol/g (dry weight)} \) | 2.18 ± 0.2 | 1.29 ± 0.4 | 60.2 ± 5.4 |

**TABLE II**

Calculation of free energy for the \( \text{Ca}^{2+} \)-ATPase and for ATP hydrolysis in control, high potassium arrested, and ischemic rabbit hearts

| \( [\text{Ca}^{2+}]_{\text{SR}} \) \( \mu \text{M} \) | \( [\text{Ca}^{2+}]_{o} \) \( \mu \text{M} \) | Gradient\(^a\) \( \times 10^3 \) | \( \Delta G_{\text{ATP}} \)\(^b\) \( \text{kJ/mol} \) | \( \Delta G_{\text{ATP}} \)\(^c\) \( \text{kJ/mol} \) |
|----------------|----------------|-----------------|-----------------|-----------------|
| Control | 1.5 | 0.1 | 150 | 49.5 | -59.3 |
| KCl | 5.0 | 0.35 | 14.3 | 49.3 | -61.7 |
| Ischemia | 1.5 | 3.0 | 0.5 | 32.0 | -47.7 |

\(^a\) Gradient = \( [\text{Ca}^{2+}]_{\text{SR}}/[\text{Ca}^{2+}]_{o} \).

\(^b\) \( \Delta G_{\text{ATP}} = 2RT \ln ([\text{Ca}^{2+}]_{\text{SR}}/([\text{Ca}^{2+}]_{o}) \).

\(^c\) \( \Delta G_{\text{ATP}} = -30.5 + RT \ln ([\text{ADP}]_{o} [\text{P}\(_i\)]/[\text{ATP}]_{o} \).

\(^d\) Values from Ref. 22.

is reduced during ischemia, the \( \Delta G_{\text{ATP}} \) is adequate to prevent net release of SR calcium during 30 min of total ischemia in the isolated rabbit heart.
DISCUSSION

We report a value for time-average \([\text{Ca}^{2+}]_{\text{SR}}\) of 1.5 mM measured in the beating perfused rabbit heart under basal conditions. When the \([\text{Ca}^{2+}]_{\text{SR}}\) measurement was gated to the contraction cycle, the diastolic value was essentially the same as the time-average value. This value agrees well with calculated values based on estimates of total calcium and calsequestrin binding sites (7). Calsequestrin, the major calcium-binding protein in the SR has a \(K_f\) for \([\text{Ca}^{2+}]\) in the range of 1 mM (24–26). Thus, the \([\text{Ca}^{2+}]_{\text{SR}}\) values measured in this study suggest that \([\text{Ca}^{2+}]_{\text{SR}}\) is being maintained near the \(K_f\) of calsequestrin. These data also allow calculation of the free energy requirement of the SR \(\text{Ca}^{2+}\)-ATPase. The data in this report suggest a calcium gradient across the SR of \(1.5 \times 10^4\) at diastole, and assuming no membrane potential, this would correspond to a \(\Delta G\) for the \(\text{Ca}^{2+}\)-ATPase of 49.5 kJ/mol, a value that agrees well with that estimated previously (6–8). We further show that high \(\text{K}^+\) perfusion, which we have shown previously (22) to increase diastolic \([\text{Ca}^{2+}]\), from ~100 nM to 350 nM, increases the \([\text{Ca}^{2+}]_{\text{SR}}\) to ~5 mM; this corresponds to a \(\Delta G\) for the \(\text{Ca}^{2+}\)-ATPase of 49.3 kJ/mol. Thus, perfusion with 30 mM potassium elevates both cytosolic and SR \([\text{Ca}^{2+}]\), but the \(\Delta G\) for the \(\text{Ca}^{2+}\)-ATPase is unchanged. We also show that during ischemia there is a parallel decline in the free energy of ATP hydrolysis and the energy requirement of the SR \(\text{Ca}^{2+}\)-ATPase. These data suggest that there is tight coupling between the SR calcium gradient and the free energy of ATP hydrolysis, and that the \(\text{Ca}^{2+}\)-ATPase works at near its theoretical limit.

SR calcium appears to be well buffered during a normal calcium transient. The difference in \([\text{Ca}^{2+}]_{\text{SR}}\) between the start of systole and diastole suggests that calcium release from the SR to trigger contraction causes a transient decrease in \([\text{Ca}^{2+}]_{\text{SR}}\) of ~30%. It has been reported that the fractional SR calcium release varies with SR calcium load and trigger calcium, but an estimate obtained under physiologic conditions suggests that ~35% of the SR calcium content may be released with each twitch (27). This is similar to an earlier report that only about half of SR \([\text{Ca}^{2+}]\) is released during a twitch (5). The relatively small and transient nature of the changes in \([\text{Ca}^{2+}]_{\text{SR}}\) during the contraction cycle is also indicated by the sharpness of the ~14 ppm resonance observed in the time-average measurement of \([\text{Ca}^{2+}]_{\text{SR}}\) in the beating heart, which is similar to that observed in hearts arrested with high \([\text{K}^+]\) (15). The sharp resonance also suggests that under basal conditions there is little spacial heterogeneity in \([\text{Ca}^{2+}]_{\text{SR}}\).

The modeling in the inset of Fig. 2 shows that the spectra observed after addition of CPA are consistent with a heterogeneous loss of calcium from the SR network. One possible explanation for this observation is that there are distinct types of SR, which may respond differently to SR calcium-ATPase inhibitors. Another possibility is that the signals were obtained from the whole heart, which displays a great deal of heterogeneity in cellular structure and function. In support of the first concept, Jorgensen et al. (28) showed that the total SR calcium measured by electron probe microanalysis (EPMA) varied within subpopulations of SR. They suggest that \([\text{Ca}^{2+}]\) is accumulated into the network SR, which immunoelectron microscopic studies show have \([\text{Ca}^{2+}]_{\text{SR}}\) during the contraction cycle is also indicated by the sharpness of the ~14 ppm resonance observed in the time-average measurement of \([\text{Ca}^{2+}]_{\text{SR}}\) in the beating heart, which is similar to that observed in hearts arrested with high \([\text{K}^+]\). The sharp resonance also suggests that under basal conditions there is little spacial heterogeneity in \([\text{Ca}^{2+}]_{\text{SR}}\).

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The total calcium content of the SR and the percentage released during an action potential, tetanus, or other interventions have been measured by EPMA and estimated by caffeine release and rapid cooling contractures, using fluorescent calcium indicators to measure the change in [Ca\(^{2+}\)]\(_{\text{SR}}\). The EPMA studies of frog skeletal muscle suggest that the resting content of total calcium in the terminal cisternae (TC) is \(\sim 120\) mmol/kg (dry weight) of TC; a 1.2-s tetanus releases \(\sim 70\) mmol/kg (dry weight) (32). Similarly, addition of caffeine reduces TC calcium to \(\sim 40\) mmol/kg (dry weight) (33). Thus, these studies show that greater than half the total TC calcium is released during tetanus or addition of caffeine in skeletal muscle. Using either caffeine or rapid cooling contractures, estimates of the percentage of SR calcium release have also been made in cardiac muscle. Baro et al. (34) report that caffeine released \(\sim 71\)% of the total calcium in cardiac muscle. The data presented here are generally in agreement with this estimate of caffeine-releasable calcium. We find that [Ca\(^{2+}\)]\(_{\text{SR}}\) decreases by more than 80% upon addition of CPA or caffeine.

The EPMA data also suggest that tetanus or caffeine addition results in a \(\sim 50\)% increase in total SR magnesium (16, 32, 33). The increase in free magnesium is likely to be much less than the increase in total magnesium, due to buffering. However, assuming that caffeine results in a 50% increase in free SR magnesium, this would increase our calculated [Ca\(^{2+}\)]\(_{\text{SR}}\) by only 0.01 mm.

In summary, several conclusions can be drawn from the data in this report. First, the time-average [Ca\(^{2+}\)]\(_{\text{SR}}\) in the beating rabbit heart is \(\sim 1.5\) mM, which is not significantly different than [Ca\(^{2+}\)]\(_{\text{SR}}\) measured at diastole. Second, [Ca\(^{2+}\)]\(_{\text{SR}}\) decreases by \(\sim 30\)% at the start of systole but this decrease is very brief, with \(\sim 50\)% recovery in 10 ms. Third, there is little spacial heterogeneity in [Ca\(^{2+}\)]\(_{\text{SR}}\) in the beating heart. Fourth, there may be a heterogeneous response to caffeine and CPA consistent with previous studies suggesting that there are subpopulations of SR with different calcium handling characteristics. Fifth, the data are consistent with tight coupling between the SR Ca\(^{2+}\) gradient and the free energy of ATP hydrolysis.

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