Minding the store of Ca\textsuperscript{2+} during ischaemia/reperfusion

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This editorial refers to ‘Transient Ca\textsuperscript{2+} depletion of the sarcoplasmic reticulum at the onset of reperfusion’ by C. A. Valverde et al., pp. 671–680, this issue.

Cardiac ischaemia puts a tremendous stress on cardiac myocytes, and abrupt reperfusion causes dramatic changes during the first seconds to minutes that profoundly affect cell survival vs. cell death.\textsuperscript{1–3} Myocyte ionic changes can contribute to these critical effects. During ischaemia, the concentration of intracellular Na\textsuperscript{+} ([Na\textsuperscript{+}]) rises because of a combination of increased Na\textsuperscript{+} entry via Na\textsuperscript{+}/H\textsuperscript{+} exchange and tetrodotoxin-sensitive Na\textsuperscript{+} channels and reduced extrusion via Na\textsuperscript{+}/K\textsuperscript{+}-ATPase (as ATP and phosphocreatine are gradually depleted and ΔG\textsubscript{ATP} declines). The rise in [Na\textsuperscript{+}], contributes to cellular Ca\textsuperscript{2+} gain via shifts in Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange, and thus elevation of diastolic [Ca\textsuperscript{2+}]. Contractile force and pressure development are abolished in the first minute or so of ischaemia because of rapidly developing acidosis. More gradually, phosphate rises and eventually rigor crossbridges form as [ATP] is dissipated. Reperfusion causes an abrupt normalization of extracellular pH, which drives a rapid rise in [Na\textsuperscript{+}] via Na\textsuperscript{+}/H\textsuperscript{+} exchange. This is thought to drive a rapid further rise in [Ca\textsuperscript{2+}] (via Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange), which along with pH normalization causes a rise in diastolic force. Spontaneous Ca\textsuperscript{2+} transients, oscillations, and arrhythmias are also observed upon reperfusion, and agents that inhibit Na\textsuperscript{+} and Ca\textsuperscript{2+} loading can improve the long-term recovery after reperfusion.\textsuperscript{4}

Despite intensive study of ischaemia/reperfusion, remarkably little is known about the dynamic changes in sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} handling at the critical moment of reperfusion. In this issue, Valverde et al.\textsuperscript{5} address this problem with innovative direct measurement of intra-SR free [Ca\textsuperscript{2+}] ([Ca\textsuperscript{2+}]\textsubscript{SR}) and [Ca\textsuperscript{2+}], in intact mouse hearts subjected to brief periods (12 min) of ischaemia and subsequent reperfusion.

SR and cytosolic Ca\textsuperscript{2+} were studied in these intact hearts with the novel and robust pulsed local field fluorescence (PLFF) technique,\textsuperscript{6} with SR-selective loading of the low-affinity fluorophore MagFura2 and cytosolic Rhod2, respectively. PLFF resolves a single cell’s fluorescence via photometry through a flexible light guide held in place with a suction pipette. This eliminates motion artifacts (verified with blebbistatin controls) without need for immobilization, which could alter electrophysiology and Ca\textsuperscript{2+} handling.\textsuperscript{7} Pulsed laser excitation (nsec duration) also limits photobleaching, and fast excitation-synchronized photodetection enhances the signal-to-noise ratio. One limitation, as the authors mention, is that PLFF detects fluorescence from not only the epicardial surface, whereas reperfusion-related arrhythmias may have complex origins related to transmural heterogeneity.\textsuperscript{8}

Cytosolic [Ca\textsuperscript{2+}], levels during ischaemia and reperfusion changed as expected. During ischaemia, diastolic [Ca\textsuperscript{2+}] increased more than systolic, within 30 s, and pacing-induced Ca\textsuperscript{2+} transients diminished to <20% of preischemic size within 4 min. Consistent with acidotic inhibition of SR Ca\textsuperscript{2+}-ATPase and inhibition of SR Ca\textsuperscript{2+} release, Ca\textsuperscript{2+} transient rise, and decay rates slowed dramatically. Upon reperfusion, a distinct ‘bump’ in the already-high diastolic [Ca\textsuperscript{2+}] signal occurred reliably in the first 30 s. Diastolic Ca\textsuperscript{2+} recovered gradually (4–5 min) whereas Ca\textsuperscript{2+} transient amplitude and decay rate recovered by 15 min. However, release rise rate never recovered, implying SR uptake function and loading were restored but release was not.

SR function was first inferred via cytosolic Ca\textsuperscript{2+} responses to caffeine. Although this approach is indirect and especially difficult to interpret quantitatively in intact hearts, the data suggested that SR Ca\textsuperscript{2+} content were reduced at 6 min of reperfusion vs. the initial preischemic level. In isolated cardiomyocytes, SR Ca\textsuperscript{2+} has been quantified directly with low-affinity Ca\textsuperscript{2+} indicators,\textsuperscript{9} dynamically during pacing-induced twitches and SR Ca\textsuperscript{2+} depletion induced by caffeine. SR Ca\textsuperscript{2+} buffering has also been measured.\textsuperscript{10}

Valverde et al.\textsuperscript{5} compared cytosolic and SR Ca\textsuperscript{2+} time courses in parallel experiments in intact hearts. Before ischaemia, pacing caused SR Ca\textsuperscript{2+} depletions of ~25% of the caffeine-releasable amount. Within 2 min of the start of ischaemia, SR content rose, although release (depletion transients) dwindled to essentially nothing, paralleling cytosolic [Ca\textsuperscript{2+}] increase, and loss of transiency. The dramatic [Ca\textsuperscript{2+}] increase and hypercontracture upon reperfusion has historically been attributed to influx.\textsuperscript{11} However, in the present study it was shown that within 30 s of the start of reperfusion, diastolic SR [Ca\textsuperscript{2+}] decreased suddenly and severely. Twitch SR depletions disappeared along with loss of SR Ca\textsuperscript{2+} (and/or electrical...
excitability). These events were closely parallel to accelerating hypercontracture and the cytosolic \([\text{Ca}^{2+}]_c\) bump, suggesting the bump was in large part due to unloading of SR at this time. As the authors point out, the cytosolic \(\text{Ca}^{2+}\) response to a massive SR release such as that which occurred here may be blunted by mitochondrial \(\text{Ca}^{2+}\) uptake.\(^{12}\)

By 5–6 min of reperfusion, SR depletion transients and caffeine-releasable \(\text{Ca}^{2+}\) had recovered only partially, but their proportional relationship (\(\sim 25\%\) fractional release) had largely returned. SR and cytosolic diastolic \([\text{Ca}^{2+}]_c\) recovered synchronously, with full recovery only upon 10 min or more of reperfusion.

So, why does \([\text{Ca}^{2+}]_{\text{SR}}\) rise during ischaemia (despite a decline in \(\Delta G_{\text{ATP}}\), which ought to limit maximal \([\text{Ca}^{2+}]_{\text{SR}}\) thermodynamically)? Presumably, this is because the combination of acidosis, reduced [ATP], and elevated [Mg\(^{2+}\)] inhibits RyR\(_2\) opening, allowing \([\text{Ca}^{2+}]_{\text{SR}}\) to rise toward the thermodynamically limiting \([\text{Ca}^{2+}]_{\text{SR}}/\ [\text{Ca}^{2+}]_c\) gradient. Even though \(\text{Ca}^{2+}\) current is likely to be occurring (based on the small \(\text{Ca}^{2+}\) transients), it does not seem to be able to trigger SR \(\text{Ca}^{2+}\) release.

The authors suggest that massive SR \(\text{Ca}^{2+}\) release upon reperfusion may be abetted as ryanodine receptor/Ca\(^{2+}\) release channels (RyR\(_2\)) recover from acidosis-induced inhibition during the preceding ischaemia.\(^{13}\) Not mentioned was a potentially stronger release trigger: burst generation of reactive oxygen species at the onset of reperfusion can hypersensitize RyR\(_2\) and synergistically inhibit SR \(\text{Ca}^{2+}\) uptake.\(^{14}\)

Although further study may be required to confirm which of the above mechanisms are responsible for the rise and fall of \([\text{Ca}^{2+}]_{\text{SR}}\) during critical stages of ischaemia/reperfusion, these observations provide a unique and novel window into SR \(\text{Ca}^{2+}\) handling.

A final issue is the electrical status of cells. During ischaemia the EKG inverted then dropped out (as did twitch \(\text{Ca}^{2+}\) transients), indicating a loss of excitability that could be in part due to ATP-sensitive K\(^{+}\) current activation.\(^{2}\) Action potentials that did occur shortened and became triangular (curtailment of phase 2), remained so during early reperfusion, and recovered only toward 30 min. Suppressing SR function without ischaemia similarly triangulizes phase 2. The SR was replete (overloaded) during ischaemia whereas depleted during reperfusion, but in both cases release was inoperative so that there was no drive for \(\text{Ca}^{2+}\) extrusion on Na\(^{+}\)/Ca\(^{2+}\) exchange, which in mouse largely shapes the late AP plateau phase. Triangular APs have often been associated with arrhythmias,\(^{15}\) which can also occur during reperfusion as SR function returns. A caveat is that AP determinants in mouse differ from those in larger species, including humans.

In summary, many aspects of SR \(\text{Ca}^{2+}\) control and ischaemia/reperfusion remain to be examined. By combining an intact heart with cellular-resolution \(\text{Ca}^{2+}\) signal detection, however, Valverde et al.\(^{8}\) have provided a reliable, relevant, scalable, and methodologically sound platform for further, ultimately quantitative study of ischaemia/reperfusion issues.

**Conflict of interest:** none declared.

**References**