



# Ryanodine receptors: waking up from refractoriness

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**This editorial refers to ‘Recovery of cardiac calcium release is controlled by sarcoplasmic reticulum refilling and ryanodine receptor sensitivity’ by H.R. Ramay et al., pp. 598–605, this issue.**

Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR) from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyRs) ultimately governs cardiac muscle force and can be considered as an amplification system for cardiac Ca<sup>2+</sup> signals. CICR is initiated by Ca<sup>2+</sup> influx via voltage-dependent L-type Ca<sup>2+</sup> channels.<sup>1</sup> Unlike skeletal muscle fibres, each cardiac myocyte can produce an amount of force (and a Ca<sup>2+</sup> transient) that is graded by the amplitude of the Ca<sup>2+</sup> current. How this works had remained a mystery for years because CICR is a positive feedback mechanism and one would expect, and mathematically predict, an all-or-none behaviour for these Ca<sup>2+</sup> signals.<sup>2</sup> As it turns out, regulation of the amplitude of the cardiac Ca<sup>2+</sup> transients is implemented by virtue of a local control process, recruiting fewer or more Ca<sup>2+</sup> sparks as needed to generate a small or large Ca<sup>2+</sup> transient. Ca<sup>2+</sup> sparks are subcellular elementary SR Ca<sup>2+</sup> release signals, each of which is an all-or-none CICR event by itself, consistent with the predictions for a positive feedback system.

Since the discovery of Ca<sup>2+</sup> sparks, the combined efforts of many research groups have particularized features of their activation. However, much less is known about the mechanism(s) restraining CICR and leading to Ca<sup>2+</sup> spark termination. In an elegant study published in the present issue of *Cardiovascular Research*, Ramay et al. combined an ingenious experimental approach with mathematical modelling of RyR gating to derive further insight into these mechanisms.<sup>3</sup> It is obvious that SR Ca<sup>2+</sup> release has to stop after each heartbeat in order to allow efficient Ca<sup>2+</sup> removal from the cytosol, SR refilling, and relaxation of the myocytes. So far, three fundamentally different mechanisms for Ca<sup>2+</sup> spark termination have been considered: (i) Ca<sup>2+</sup>-induced inactivation of the RyRs, whereby Ca<sup>2+</sup> released from the SR via each RyR would exert a negative feedback on the RyRs themselves and eventually shut them down.<sup>1</sup> While this mechanism was evident in early experiments with permeabilized cardiac myocytes, it has been difficult to reproduce in intact cell experiments and more recent studies seem to suggest that it may play a less important role,<sup>4</sup> similar to the somewhat related RyR adaptation.<sup>5</sup> (ii) An alternative mechanism for Ca<sup>2+</sup> spark termination was proposed on theoretical grounds, termed ‘stochastic attrition’.<sup>2</sup> Since the Ca<sup>2+</sup> concentration

within the dyadic cleft is expected to rise and drop very rapidly after opening and closing of a RyR channel (i.e. within less than a millisecond), there is a certain probability that the mutual activation by CICR within a cluster of RyRs would be interrupted, by chance. This would occur as soon as all channels within that cluster are closed simultaneously. However, the probability for this to occur drops dramatically, as the number of channels in a spark-forming couplon increases. With the reported number of RyRs present in each couplon, stochastic attrition becomes a less likely mechanism unless coupled gating of several RyRs reduces the number of independently gating channels.<sup>6</sup> (iii) The third mechanism proposes the presence of an SR luminal Ca<sup>2+</sup> sensor, either on the RyR itself or implemented as a backwards signal from calsequestrin to the RyR, via junctin and triadin.<sup>7</sup> The presence of such a backwards signal would imply that the SR Ca<sup>2+</sup> release does not terminate by Ca<sup>2+</sup> exhaustion of the SR, but rather by a mechanism desensitizing the RyRs for cytosolic Ca<sup>2+</sup>, leading to their deactivation once a certain threshold of low intra-SR Ca<sup>2+</sup> has been reached.<sup>8</sup>

While it has been previously reported that the CICR availability, or the recovery of the RyRs from refractoriness, was to some extent governed by the rate of SR refilling, the presence of additional time-consuming steps has been suspected.<sup>9</sup> In the present study, Ramay et al. applied a very low concentration of ryanodine to resting rat cardiomyocytes. As expected, ryanodine binding to only very few RyRs led to ‘eager’ Ca<sup>2+</sup> spark sites, where a single ryanodine-modified RyR entrained Ca<sup>2+</sup> sparks repeatedly at the same site (i.e. couplon), but at random intervals. Using confocal imaging of a large number of these sparks, the researchers followed and analysed two parameters: (i) the recovery of the Ca<sup>2+</sup> spark amplitude, a parameter that is believed to follow SR refilling, (ii) the evolution of the Ca<sup>2+</sup> spark trigger probability, as an indicator of RyR availability. First of all, the amplitude was found to recuperate faster than the recovery of the trigger probability, suggesting a combined effect of amplitude and trigger sensitivity, or additional time-consuming steps between SR refilling and the moment when RyRs become available. In experiments where the Ca<sup>2+</sup> sensitivity of the RyRs was either enhanced (with caffeine) or reduced (with tetracaine), the rate of recovery for the spark trigger probability increased or decreased, respectively. However, the Ca<sup>2+</sup> spark amplitude recovered at an unchanged rate. Interestingly,  $\beta$ -adrenergic stimulation accelerated both recovery of spark amplitude and triggering probability.

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For a detailed analysis of these findings, the authors modified their mathematical 'sticky cluster' model of RyR gating and  $\text{Ca}^{2+}$  diffusion in the junctional SR (jSR) and dyadic cleft.<sup>10</sup> Based on this analysis they were able to derive a number of important conclusions from their findings. First of all, it appears that RyR  $\text{Ca}^{2+}$  sensitivity determines the recovery of spark trigger probability but does not affect the  $\text{Ca}^{2+}$  spark amplitude. Furthermore, SR refilling via SERCA seems to significantly contribute to the rate of local jSR refilling after a spark-induced  $\text{Ca}^{2+}$  depletion (i.e. after a  $\text{Ca}^{2+}$  'blink'),<sup>11</sup> in addition to the  $\text{Ca}^{2+}$  diffusion inside the SR. Interestingly,  $\beta$ -adrenergic stimulation seems not only to speed up SR refilling but also sensitizes the RyRs, presumably by PKA- and/or CaMKII-dependent RyR phosphorylation. Finally, the data are consistent with the hypothesis that  $\text{Ca}^{2+}$  release termination depends predominantly on changes of the  $\text{Ca}^{2+}$  concentration inside the SR and not on  $\text{Ca}^{2+}$ -induced inactivation of RyRs.

In the future, the experimental approach adopted in this study should reveal more detailed information about the kinetics and inner workings of the molecular mechanisms regulating CICR. Understanding the mechanism of  $\text{Ca}^{2+}$  release termination is very important, because it is responsible for the refractoriness of  $\text{Ca}^{2+}$  release during diastole. Alterations of the refractory state due to SR  $\text{Ca}^{2+}$  overload, RyR mutations, and other causes of RyR sensitization (e.g. RyR phosphorylation, redox modifications) may underlie the generation of arrhythmias such as delayed afterdepolarizations and catecholaminergic polymorphic ventricular tachycardias.<sup>12,13</sup>

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