Letter to the Editor

Ca^{2+} Spark Termination: Inactivation and Adaptation May Be Manifestations of the Same Mechanism

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Several perspectives on Ca^{2+} sparks were recently published. A common theme was the importance of defining the mechanisms that terminate local SR Ca^{2+} release. We propose that the time- and Ca^{2+}-dependent modal gating behavior of single ryanodine receptor (RyR) channels is the negative control mechanism that terminates local Ca^{2+} release. Specifically, the observed "inactivation" and "adaptation" phenomena are two manifestations of the same general mechanism (i.e., modal RyR gating). We hope this new unified view of RyR negative control mechanisms may lead to new insights into local intracellular Ca^{2+} signaling in heart.

The local Ca^{2+} spark is thought to represent the elementary intracellular Ca^{2+} release unit in adult mammalian cardiac muscle (Cheng et al., 1993, 1996). Intuitively, the kinetics and geometry of the Ca^{2+} removal and Ca^{2+} release machinery in the cell govern the spatiotemporal nature of the Ca^{2+} spark. Ryanodine receptor (RyR) channels in the sarcoplasmic reticulum (SR) mediate local intracellular Ca^{2+} release in cardiac muscle (Cheng et al., 1993). The Ca^{2+} spark may arise from the opening of a single ryanodine receptor (RyR) Ca^{2+} release channel (Cheng et al., 1993) or from the concerted opening of several RyR channels (Lipp and Niggli, 1994; Blatter et al., 1997). In any event, it is clear that the mechanism(s) that terminate SR Ca^{2+} release are fundamental to local intracellular Ca^{2+} signaling.

In 1993, we argued that conventional wisdom was insufficient to explain why repeated fast Ca^{2+} stimuli triggered transiently and repeatedly activated single RyR channels in planar bilayers (Györke and Fill, 1993). In response to a fast Ca^{2+} stimulus, single RyR channel activity peaked and then spontaneously decayed. The spontaneous decay was not mediated by a conventional Ca^{2+}-dependent inactivation mechanism because the apparently "inactivated" channels (i.e., refractory channels) could be reactivated by a second Ca^{2+} stimulus. We proposed that the spontaneous decay was mediated by a different mechanism, which we called adaptation (Györke and Fill, 1993). This original hypothesis "sparked" sometimes heated debate (Györke and Fill, 1994; Lamb and Stephenson, 1995) and several studies of Ca^{2+}-dependent RyR gating kinetics (Schiefer et al., 1995; Sitsapesan et al., 1995; Valdivia et al., 1995; Laver and Curtis, 1996; Laver and Lamb, 1998). The overall result has been a better understanding of how Ca^{2+} may regulate single RyR channels.

Adaptation: Phenomenon Not Mechanism

6 yr and volumes of new data have provided further insight into RyR adaptation. We believe RyR adaptation should be viewed as a physiologically important phenomenon and not as a molecular mechanism. There is now substantial evidence that the adaptation phenomenon is due to a transient, Ca^{2+}-dependent shift in the modal gating behavior of the RyR channel (Zahradníková and Zahradník, 1995, 1996; Armisén et al., 1996; Zahradníková et al., 1999). Fast trigger Ca^{2+} stimuli drive the channel into a high open probability (P_o) mode. If the trigger Ca^{2+} stimulus is sustained (even at a lower level), RyR activity spontaneously decreases as a new steady state between high and low P_o modes is reached. A second rapid elevation of $[Ca^{2+}]$ disrupts the equilibrium again, causing another transient increase in activity. Repeated activations can occur only within a certain range of $[Ca^{2+}]$ because the Ca^{2+} binding sites that govern the equilibrium between the high and low P_o modes can saturate. It is reasonable to assume that Ca^{2+} binding to the same sites that govern the RyR's steady state Ca^{2+} dependence may be responsible for this phenomenon (Zahradníková and Zahradník, 1995, 1996; Armisén et al., 1996). Thus, the activity of single RyR channels may represent a dynamic Ca^{2+}-dependent balance between the time spent in high, low, and zero activity modes. This balance would be governed by multiple Ca^{2+} binding sites with different affinities and kinetics (Cheng et al., 1995).

The RyR adaptation phenomenon is observed when the channel is activated by a free Ca^{2+} waveform generated by laser flash photolysis of DM-nitrophen (Györke

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and Fill, 1993; Velez et al., 1997). The Ca^{2+} waveform has a complex time course composed of a fast Ca^{2+} step (0.1 to 1.0 μM), with a very fast (∼150 μs), large (∼100 μM) Ca^{2+} overshoot at its leading edge (Velez et al., 1997). The impact of the fast Ca^{2+} spike on data interpretation has been debated (Györke and Fill, 1994; Lamb and Stephenson, 1995). Direct measurement of RyR response to fast Ca^{2+} spikes alone (albeit smaller and briefer; Zahradníková and Györke, 1997.) showed that channel deactivation after these brief Ca^{2+} changes was ∼1,000× faster than the observed adaptation phenomenon (Zahradníková et al., 1999; Györke and Fill, 1993). Fast Ca^{2+} spikes alone trigger only a single open event, while adaptation is characterized by a prolonged transient burst of channel activity. Additionally, repetitive transient bursts of channel activity can be induced only over a relatively narrow Ca^{2+} concentration range, and this Ca^{2+} concentration range is defined by the sustained Ca^{2+} step, not the properties of the fast Ca^{2+} spike (Györke and Fill, 1993; Györke et al., 1994; Valdivia et al., 1995). Therefore, it is highly unlikely that the adaptation phenomenon is due to simple deactivation after the fast Ca^{2+} spike, or that it artifactually induced the fast Ca^{2+} spike. Instead, the impact of the Ca^{2+} spike appears to be limited to "super charging" the trigger Ca^{2+} signal in that it may accelerate the transition into the high P_o mode.

When true step Ca^{2+} stimuli (without fast Ca^{2+} spikes) are composed of single RyR channels in planar bilayers, these step-like Ca^{2+} stimuli trigger bursts of RyR channel activity that spontaneously decay over time (τ_{decay}, ∼1–2 s range; Schiefer et al., 1995; Sitsapesan et al., 1995; Laver and Curtis, 1996; Laver and Lamb, 1998). In some studies, the spontaneous decay in channel activity was not always observed (Sitsapesan et al., 1995). In other studies, the spontaneous decay occurred only if the channel was initially in a high activity state (Laver and Lamb, 1998). This decay has been interpreted as a conventional "inactivation" mechanism. An alternative interpretation is that smaller, slower Ca^{2+} stimuli are simply less efficient at triggering the initial high activity burst. In this latter view, the spontaneous decay is due to a time- and Ca^{2+}-dependent shift in the channel's modal gating behavior. Thus, the apparent "inactivation" here and the apparent "adaptation" described above are actually two manifestations of the same underlying mechanism (i.e., modal gating).

RyR Negative Control: The Cellular Level

Fabiato (1985) proposed that Ca^{2+}-dependent inactivation is the negative control mechanism that regulates the SR Ca^{2+} release process in heart. However, early patch clamp studies of intact ventricular myocytes found no evidence of inactivation (i.e., refractory behavior) of cell-averaged SR Ca^{2+} release in experiments using conventional two-pulse protocols (Cleeman and Morad, 1991). Subsequent studies have shown that SR Ca^{2+} release does indeed "turn-off" when activated by a sustained trigger Ca^{2+} stimulus (Yasui et al., 1994). Paradoxically, the apparently inactivated Ca^{2+} release process could be reactivated by the suddenly increased trigger Ca^{2+} stimulus carried by the tail current upon repolarization. This ability of incremental macroscopic trigger Ca^{2+} stimuli to trigger multiple transient SR Ca^{2+} releases qualitatively resembles the adaptation phenomenon observed at the single RyR channel level described above. Thus, it was proposed that this reactivation of SR Ca^{2+} release is a whole cell manifestation of the RyR adaptation phenomenon (Yasui et al., 1994). A recent study using confocal Ca^{2+} imaging, however, suggests the situation is more complicated and may involve both complex single channel behavior and multichannel interactions (Sham et al., 1998).

Defining the mechanisms that terminate elementary SR Ca^{2+} release events (i.e., Ca^{2+} sparks) is an important step towards understanding how release is regulated. The candidate negative control mechanisms include: (a) Ca^{2+}-dependent inactivation, (b) adaptation, and (c) use-dependent inactivation (Lukyanenko et al., 1998; Sham et al., 1998). These mechanisms have been viewed as potentially independent and mutually exclusive RyR regulatory entities. This view, however, may not be accurate. Modal RyR gating behavior may provide a framework in which these apparently different mechanisms can be integrated.

The hallmark of the adaptation phenomenon is thought to be the ability of apparently "refractory" RyR channels to reactivate in response to a larger Ca^{2+} stimulus. This, however, is not likely to be relevant to regulation of CICR in situ, as even small trigger Ca^{2+} stimuli in situ may elevate the local free Ca^{2+} concentration in the diadic cleft to very high levels representing maximal activating stimuli for the local RyRs. These high Ca^{2+} levels should result in maximal occupation of Ca^{2+} binding sites that govern the equilibrium between the high and low P_o modes, and thus the reactivation by even larger Ca^{2+} stimuli would not occur. Perhaps the more physiologically relevant feature of adaptation is the underlying modal gating shift. In the presence of a sustained trigger Ca^{2+} signal, a time- and Ca^{2+}-dependent shift to the low- and zero-P_o mode would cause a decline in channel activity. The implication is that the decreasing RyR channel activity would always appear as a consequence of earlier channel activation. Thus (provided it is sufficiently fast) the shift in gating modes could account for apparent use-dependent properties of Ca^{2+} release inactivation in situ (Pizarro et al., 1997; Sham et al., 1998).

Many vesicle Ca^{2+} flux studies (Chamberlain et al.,

1984; Zimanyi and Pessah, 1991; Chu et al., 1993) and single RyR channel studies (Laver et al., 1995; Copello et al., 1997; Györke and Györke, 1998; Marengo et al., 1998) demonstrate an inhibition of RyR activity at high (>50 μM) steady [Ca^{2+}]. Traditionally, inactivation by Ca^{2+} is thought to be mediated by a Ca^{2+}-dependent transition to an absorbing inactivated state (Fabiato, 1985). The modal nature of RyR channel behavior suggests an alternative Ca^{2+}-dependent mechanism in which channel activity is decreased by stabilizing low- or zero-P_o modes. Binding of Ca^{2+} to the low affinity inhibition sites could accelerate the rate of shift in gating modes, bringing it to a more physiologically relevant range. Intuitively, this may be analogous to the modal mechanism of Ca^{2+}-dependent inactivation proposed for the L-type Ca^{2+} channels (Imredy and Yue, 1994). In this sense, adaptation and Ca^{2+}-dependent inactivation may represent the different aspects of a common underlying mechanism; i.e., time- and Ca-dependent shifts in modal gating.

Thus, we propose that the modal RyR gating behavior may represent a common factor that underlies the apparently different mechanisms of Ca^{2+}-dependent inactivation, use-dependent inactivation, and adaptation. The implication is that the negative control mechanisms that counter the inherent positive feedback of CICR may be a time- and Ca^{2+}-dependent shift in the modal gating behavior of the RyR channel. The intent of our proposition is to simply stimulate discussion. It is clear that additional experimentation and a far more detailed theoretical framework is required to understand termination of the SR Ca^{2+} release process in heart. Nevertheless, even a relatively speculative exchange of scientific ideas can generate new and interesting ideas and future directions.

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