

# On the Loose: Uncaging Ca<sup>2+</sup>-induced Ca<sup>2+</sup> Release in Smooth Muscle

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Movement of Ca<sup>2+</sup> from intracellular stores into the cytosol is an essential component of excitation–contraction coupling in muscle. In cardiac and smooth muscle, Ca<sup>2+</sup> is released from the sarcoplasmic reticulum (SR) in response to Ca<sup>2+</sup> influx by a process of Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR). First described by Lederer and co-workers in cardiac myocytes (Cheng et al., 1993), highly localized cytosolic Ca<sup>2+</sup> transients, called Ca<sup>2+</sup> sparks, originating from ryanodine-sensitive Ca<sup>2+</sup>-release channels (ryanodine receptors [RyRs]) in the SR membrane, are thought to be the elementary intracellular Ca<sup>2+</sup> release events in excitation–contraction coupling, not only in cardiac muscle but also in skeletal (Klein et al., 1996) and smooth muscle (Nelson et al., 1995). RyRs are formed by a family of intracellular Ca<sup>2+</sup> channel subunits (RyR1–3), which span the SR membrane of all muscle cells. RyR1 is limited primarily to skeletal muscle, and cardiac cells express chiefly RyR2 (Fill and Copello, 2002). Although RyR2 and RyR3 may be functional there is sufficient evidence to suggest that RyR2 predominates with respect to Ca<sup>2+</sup> sparks in smooth muscle (Ji et al., 2004).

Smooth muscle Ca<sup>2+</sup> sparks, which result from a concerted opening of clusters of RyRs (Blatter et al., 1997; Mejia-Alvarez et al., 1999), generate microdomains of elevated (>10 μM) Ca<sup>2+</sup> without changing global intracellular Ca<sup>2+</sup> concentrations (Nelson et al., 1995; Soeller and Cannell, 1997; Perez et al., 2001). Because the surface membrane and peripheral SR of smooth muscle cells often are closely apposed (≈10 nm) and form periodic “surface couplings” (Devine et al., 1972), Ca<sup>2+</sup>-sensitive plasmalemmal proteins, such as ion channels, are exposed to intense pulses of elevated Ca<sup>2+</sup>. Consistent with this scenario, Ca<sup>2+</sup> sparks in smooth muscle are important regulators of membrane potential and hence smooth muscle excitability. In these cells, the Ca<sup>2+</sup> signal produced by a spark is tightly coupled to large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> (BK) channels. Stimulation of BK channel activity by Ca<sup>2+</sup> sparks produces spontaneous transient outward currents (STOCs) resulting in membrane hyperpolarization, which causes relaxation (Nelson et al., 1995). In contrast, Ca<sup>2+</sup> spark activation of Ca<sup>2+</sup>-activated Cl<sup>−</sup> channels in portal vein

produces analogous inward currents (STICs) and membrane depolarization (Wang et al., 1992). Thus, the functional role(s) of Ca<sup>2+</sup> sparks depend on the molecular identity and relative location of nearby Ca<sup>2+</sup>-sensitive proteins.

The close apposition between plasma and SR membranes has functional consequences on CICR as well. In ventricular myocytes, brief openings of voltage-gated, dihydropyridine-sensitive L-type Ca<sup>2+</sup> channels allows a small amount of Ca<sup>2+</sup> to enter the small cytosolic volume separating this junctional space. This local increase in [Ca<sup>2+</sup>]<sub>i</sub> activates nearby RyRs (to cause the Ca<sup>2+</sup> sparks) by the mechanism of CICR. Thus, in ventricular myocytes, the function of sarcolemmal L-type Ca<sup>2+</sup> channels and SR RyRs is tightly coupled. Ca<sup>2+</sup> influx during the action potential results in a coordinated, synchronous activation of multiple Ca<sup>2+</sup> sparks that culminates in a global [Ca<sup>2+</sup>]<sub>i</sub> transient that activates the contractile apparatus. Such a relationship is essential for efficient, spatially uniform excitation–contraction coupling in the heart.

Because of the physical proximity of plasma and SR membranes in smooth muscle, one would expect the same relationship to hold also in smooth muscle. Experimental evidence suggests otherwise. In contrast to cardiac myocytes, the functional coupling between L-type Ca<sup>2+</sup> channels and RyRs is not obvious, and minimal at best. As proposed by Kotlikoff and coworkers, CICR in smooth muscle, when it does occur, results from a “loose coupling” between L-type Ca<sup>2+</sup> channels and RyRs (Collier et al., 2000). The basis for loose coupling is unclear at present. Perhaps related to the loose coupling between L-type Ca<sup>2+</sup> channels and RyRs is the observation that the spatial distribution of Ca<sup>2+</sup> sparks in smooth muscle, spontaneous and evoked, is not stochastic but rather is limited to discrete subcellular sites called “frequent discharge sites” (Gordienko et al., 1998). These sites of Ca<sup>2+</sup> spark activity generally correspond to a region of perinuclear SR just underneath the plasma membrane (Pucovsky and Bolton, 2006). Interestingly, RyRs are broadly expressed throughout smooth muscle cells (Devine et al., 1972; Gordienko

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Abbreviations used in this paper: BK, large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup>; CICR, Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release, RyR, ryanodine receptor; SR, sarcoplasmic reticulum.

et al., 1998). These previous studies raise two important questions. Are RyRs outside of frequent discharge sites functional? And, if so, how are they activated? Addressing these two questions is critical to understanding the function and mechanisms underlying spatially heterogeneous SR Ca<sup>2+</sup> release activity in smooth muscle.

In this issue of the *Journal of General Physiology*, Ji et al. (p. 225) describe an elegant series of experiments designed to examine the mechanisms of Ca<sup>2+</sup> release from the SR of urinary bladder smooth muscle. Ji et al. (2006) used two-photon and confocal microscopy to image [Ca<sup>2+</sup>]<sub>i</sub> and locally uncage Ca<sup>2+</sup> via flash photolysis in urinary bladder smooth muscle, respectively. Using these approaches, the authors make three novel observations regarding the mechanisms of SR Ca<sup>2+</sup> release in these cells. First, they found that local photorelease of Ca<sup>2+</sup> evoked SR Ca<sup>2+</sup> release in the form of Ca<sup>2+</sup> sparks and Ca<sup>2+</sup> waves throughout the cell. Second, loss of FK506-binding protein 12.6 expression lowers the threshold and increases the rate of activation of RyRs by local increases in Ca<sup>2+</sup> in smooth muscle. Third, SR Ca<sup>2+</sup> release in response to local photorelease of Ca<sup>2+</sup> required activation of RyRs and IP<sub>3</sub> receptors.

These findings provide the first direct and compelling demonstration that smooth muscle expresses functional RyRs throughout the cell and that they can be activated by CICR. Related to that, the findings by Ji et al. (2006) suggest that RyRs outside frequent discharge sites could, if the appropriate signal for activation is provided, produce Ca<sup>2+</sup> sparks and, together with nearby IP<sub>3</sub> receptors, be involved in the propagation of Ca<sup>2+</sup> waves in smooth muscle. The latter observation provides strong support for the view that IP<sub>3</sub> receptors can be activated by CICR and that there may be bidirectional communication between RyRs and IP<sub>3</sub> receptors.

The findings from Ji et al. (2006) imply that during membrane depolarization, activation of Ca<sup>2+</sup> influx throughout the cell may provide a Ca<sup>2+</sup> signal to trigger SR Ca<sup>2+</sup> by the mechanism of CICR. As the authors convincingly demonstrate, given the appropriate signal, Ca<sup>2+</sup> release from the SR can be evoked throughout the cell. Because BK channels are broadly distributed throughout the sarcolemma of smooth muscle cells (Amberg and Santana, 2003), these findings indicate that RyR–BK channel communication is not necessarily limited to the frequent discharge sites but could occur in multiple locations within the cell.

However, as with any good study, the work by Ji et al. (2006) not only answers several important questions, it also raises a new set of issues. For example, why are spontaneous Ca<sup>2+</sup> sparks limited to specific locations within a cell (i.e., frequent discharge sites) even though Ca<sup>2+</sup> sparks can be evoked throughout the cell? Recent studies, together with the study by Ji et al. (2006), may hint a potential answer to this difficult, yet important, question.

In smooth muscle, Ca<sup>2+</sup> influx, like Ca<sup>2+</sup> sparks, seems to occur through specialized regions of the cell in which small clusters of Ca<sup>2+</sup> channels operate in high open probability mode that create sites of nearly continual Ca<sup>2+</sup> influx (“persistent Ca<sup>2+</sup> sparklets”) (Navedo et al., 2005). Persistent Ca<sup>2+</sup> sparklets could increase local [Ca<sup>2+</sup>]<sub>i</sub> to a similar extent as Ca<sup>2+</sup> sparks or the artificial Ca<sup>2+</sup> sparks evoked by Ji et al. (2006) using flash photolysis. Although multiple aspects of Ca<sup>2+</sup> sparklet activity need to be elucidated (e.g., molecular identity of the channels involved, mechanisms of activation), maybe persistent Ca<sup>2+</sup> sparklets allow for spatially restricted modulation of SR Ca<sup>2+</sup> release by providing a localized source of Ca<sup>2+</sup> that could increase, presumably via loose coupling mechanisms (Collier et al., 2000), Ca<sup>2+</sup> spark activity. Future studies should examine the relationship between Ca<sup>2+</sup> sparklet and frequent Ca<sup>2+</sup> spark sites in smooth muscle.

To conclude, the work described here by Ji et al. (2006) highlights the exquisite, localized control of SR Ca<sup>2+</sup> release in smooth muscle and the role of local Ca<sup>2+</sup> signals in activating SR Ca<sup>2+</sup> release. Future studies will undoubtedly expand on these findings and reveal the mechanisms underlying recurrent, spontaneous Ca<sup>2+</sup> spark activity in smooth muscle.

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