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TRPML1ng on sparks

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Abstract

In this issue of *Science Signaling*, Thakore *et al.* report that the Ca^{2+} permeable channel TRPML1 closely associates with ryanodine receptors to induce Ca^{2+} sparks in native arterial myocytes. Functional studies revealed a key role for TRPML1 channels in regulation of arterial myocyte contractility and blood pressure.

Arterial myocytes encircling resistance arteries provide moment-to-moment control of vessel diameter, thus influencing blood flow and blood pressure regulation (1). Arterial myocyte contractility is modulated by a dynamic interplay of ion channels to control membrane potential and the magnitude and shape of intracellular Ca^{2+} signals (1). Among them, the discovery of Ca^{2+} sparks in arterial myocytes 25-years ago was a major breakthrough (2). Ca^{2+} sparks are produced by Ca^{2+} release mediated by ryanodine receptors (RyRs) located in the junctional sarcoplasmic reticulum (SR) (2). Arterial myocytes Ca^{2+} sparks are confined to 1–2 sites per cell and their frequency is low compared to that in ventricular myocytes (2, 3). Thus, arterial myocyte Ca^{2+} sparks are rare enough not to cause cell-wide increases in intracellular Ca^{2+} and instead cause local intracellular Ca^{2+} elevations that activate closely apposed large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels at the plasma membrane (1, 2). This leads to K^+ efflux as spontaneous transient outward currents (STOCs) that result in membrane hyperpolarization, arterial myocyte relaxation, and vasodilation (1, 2). Activation of this RyR/BK_{Ca} axis provides negative feedback control of arterial myocyte contraction (1). The association of Ca^{2+} sparks in arterial myocytes with relaxation starkly contrasts with their role in cardiomyocytes where Ca^{2+} sparks are the elementary Ca^{2+} release events that trigger contraction (2, 3). These results highlight distinct physiological roles for similar Ca^{2+} signals in different excitable cells.

Ca^{2+} sparks can be initiated by a localized increase in Ca^{2+} that activates RyRs through Ca^{2+}-induced Ca^{2+} release mechanisms (4). Indeed, cardiac Ca^{2+} sparks can be triggered by Ca^{2+} influx through L-type Ca^{2+} channels at the plasma membrane that stimulates RyR activity (5). Although both indirect (such as L-type Ca^{2+} channels) and direct (such as TRPV4 and $Ca_V3.2$ channels) coupling of various Ca^{2+} permeable channels have been implicated in Ca^{2+}-mediated RyR activation and Ca^{2+} sparks in arterial myocytes (1), the

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underlying mechanistic details remains unresolved. In this issue of *Science Signaling*, Thakore *et al. (6)* provide unexpected and exciting data supporting an unorthodox role for TRPML1 (transient receptor potential mucolipin 1) channels in contractile arterial myocytes (6). Using a multifacted approach, the authors offer convincing evidence suggesting that TRPML1 channels in late endosome and lysosomes (LELs) are localized close to RyR clusters where they ignite Ca^{2+} sparks and hence become key regulators of vascular contractility and blood pressure (Figure 1).

The first set of key experiments found exclusive mRNA and protein expression for TRPML1 in isolated cerebral and mesenteric arterial myocytes and arterial lysates, respectively (6). Super-resolution microscopy subsequently revealed nanometer separation of most TRPML1 clusters with the LEL marker Lamp-1 in native arterial myocytes (6). These results suggest prominent subcellular localization of TRPML1 in LEL structures where they could modulate LEL trafficking, which is highly dynamic in many cells (7). Unexpectedly, LELs showed minimal displacement in contractile arterial myocytes in isolation and in intact pressurized arteries, a feature that was further exacerbated in TRPML1-deficient cells (6).

These results raised a fundamental question: What was the functional relevance of immobile TRPML1-containing LELs in contractile arterial myocytes? Knowing of the steep Ca^{2+} gradient between the LEL lumen and cytosol, Thakore *et al.* cleverly hypothesized that TRPML1 in immobile LELs could form complexes with other intracellular proteins, such as RyRs, to modulate their function. Accordingly, super-resolution imaging showed close association (between 0–40 nm) and non-coincidental overlap of subpopulations of clustered TRPML1 and RyRs (6). Moreover, a substantial number of RyR clusters resided ≤40 nm away from Lamp-1 positive structures, presumably LELs (6). These results suggest that TRPML1 in immobile LELs could associate with RyRs in arterial myocytes and could regulate RyR-mediated Ca^{2+} signals and vascular contractility.

A testable prediction of this hypothesis was that Ca^{2+} sparks and underlying STOC activity, as well as vascular function, should be impaired in TRPML1-deficient cells. Remarkably, Thakore *et al.* found that spontaneous Ca^{2+} sparks were nearly completely suppressed in contractile arterial myocytes from TRPML1-deficient mice (6), which correlated with a decrease in the frequency and amplitude of STOCs. Intriguingly, Ca^{2+} sparks/STOC coupling could still be stimulated in TRPML1-deficient cells by engaging the independent TRPV4/RyR/BK_{Ca} pathway. These results suggest that the RyR/BK_{Ca} structural organization was maintained in TRPML1-deficient cells and that TRPML1 was necessary for spontaneous activation of Ca^{2+} sparks in arterial myocytes. As expected, the reduction in Ca^{2+} sparks/STOC activity in TRPML1-deficient cells led to an increase in myogenic tone and hyperreactivity to vasoconstricting agonists in both TRPML1-deficient cerebral and mesenteric arteries and increased blood pressure in TRPML1-deficient mice. Together, these results suggest a key role for TRPML1 in the regulation of arterial myocyte contractile state, which may contribute to modulate blood pressure.

The work by Thakore *et al.* provides insight into the signaling module formed by TRPML1, RyR and BK_{Ca} for the initiation of Ca^{2+} sparks and regulation of arterial myocyte contractility and blood pressure (Figure 1). This study raises many intriguing questions. For

example, can TRPML1 activation produce an optically detectable Ca^{2+} signal? If yes, what are the biophysical properties of this TRPML1-generated Ca^{2+} signal and its relationship with Ca^{2+} sparks? If there are so many TRPML1/RyR/BK_{Ca} units, why are Ca^{2+} spark sites limited to 1–2 per cell? Do vasodilators require TRPML1 activation to increase Ca^{2+} spark frequency? Why do LELs not move in contractile arterial myocytes? What targets and anchors LELs near RyRs? How does this new TRPML1/RyR/BK_{Ca} signaling axis integrate with other mechanisms that have been proposed to initiate Ca^{2+} sparks and regulate arterial myocyte contractility? What are the physiological activators of TRPML1 in arterial myocytes and how does TRPML1 activity change during pathologies such as hypertension? The answers to these questions may require the integration of new and innovative experimental tools (such as live Ca^{2+} and super-resolution imaging similar to those used for L-type Ca^{2+} channels (8)) and powerful analytical approaches (such as computational modeling) to further uncover the functional importance of TRPML1 in arterial myocytes.

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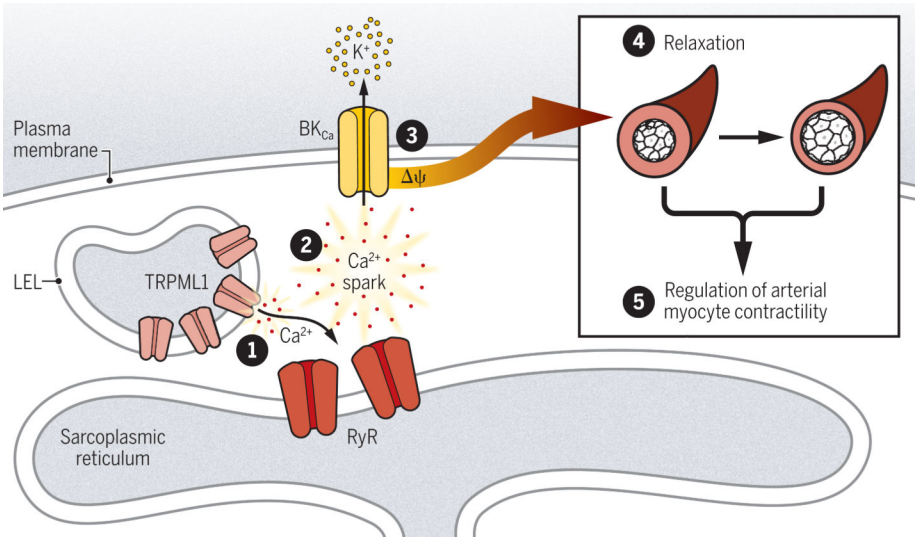

Figure 1. TRPML1 regulation of Ca^{2+} sparks and vascular contractility.

Data support a model in which TRPML1 are located in immobile LELs near RyRs (1). This close proximity is necessary for TRPML1 to evoke spontaneous RyR-mediated Ca^{2+} sparks (2). The ensuing sparks activate BK_{Ca} channels to produce K^+ efflux as STOCs (3). STOCs hyperpolarize the membrane potential (indicated by $\Delta\psi$), which may lead to arterial myocyte relaxation (4). This pathway appears important for regulation of arterial myocyte contractility (5). Accordingly, genetic ablation of TRPML1 suppresses Ca^{2+} sparks and STOCs and leads to enhanced myogenic tone and hyperreactivity of arterial myocyte to vasoconstricting agonists.