Smooth muscle sparklet Ca\textsubscript{v} channels defined: 1.2 is the number

Jonathan H. Jaggar
Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee

SYSTEMIC BLOOD PRESSURE and tissue blood flow are regulated by the contractile status of smooth muscle cells located within the wall of small arteries and arterioles. One important signal that regulates arterial smooth muscle cell contractility is the intracellular calcium ion (Ca\textsuperscript{2+}) concentration. Although Ca\textsuperscript{2+} was once considered to act only as a global intracellular signaling element, research performed over the past decade has revealed Ca\textsuperscript{2+} to act in a far more dynamic and versatile manner than previously thought possible.

The ability of Ca\textsuperscript{2+} to regulate a wide variety of cellular functions stems primarily from the diversity of local and global Ca\textsuperscript{2+} signals that can be generated. In arterial smooth muscle cells, local and global Ca\textsuperscript{2+} signals differ with respect to their spatial, temporal, and amplitude properties. In addition, whether proteins detect local or global Ca\textsuperscript{2+} signals depends on several factors, including their Ca\textsuperscript{2+} sensitivity and proximity to the source of a Ca\textsuperscript{2+} signal. In arterial smooth muscle cells, the global intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) occurs because of Ca\textsuperscript{2+} influx through the plasma membrane and Ca\textsuperscript{2+} release from intracellular stores (8). A nanomolar elevation in global [Ca\textsuperscript{2+}]\textsubscript{i}, activates Ca\textsuperscript{2+}/calmodulin-dependent myosin light chain kinase, leading to contraction. In contrast, a reduction in global [Ca\textsuperscript{2+}]\textsubscript{i}, results in relaxation.

Localized [Ca\textsuperscript{2+}]\textsubscript{i} transients, termed “Ca\textsuperscript{2+} sparks,” occur because of the opening of multiple ryanodine-sensitive Ca\textsuperscript{2+} channels on the sarcoplasmic reticulum (SR) (8, 14). When imaged by using fluorescent Ca\textsuperscript{2+} indicators, Ca\textsuperscript{2+} sparks are transient events that last for only ~100 ms. Within the vicinity of RyR channels, Ca\textsuperscript{2+} sparks produce a localized micromolar [Ca\textsuperscript{2+}]\textsubscript{i}, elevation that activates several nearby plasma membrane large-conductance Ca\textsuperscript{2+}-activated potassium (K\textsubscript{Ca}) channels, resulting in a transient K\textsubscript{Ca} current. Consistent with Ca\textsuperscript{2+} sparks acting as local signals, large-conductance K\textsubscript{Ca} channels are sensitive to micromolar Ca\textsuperscript{2+} concentrations and are relatively insensitive to nanomolar Ca\textsuperscript{2+} concentrations found globally. Asynchronous transient K\textsubscript{Ca} currents cause an arterial wall membrane hyperpolarization that reduces voltage-dependent Ca\textsuperscript{2+} channel activity, leading to a decrease in global [Ca\textsuperscript{2+}]\textsubscript{i}, and relaxation. Thus Ca\textsuperscript{2+} sparks induce vasodilation by reducing global [Ca\textsuperscript{2+}]\textsubscript{i}. In smooth muscle cells, Ca\textsuperscript{2+} sparks occur at ~1 event/s and do not directly elevate global Ca\textsuperscript{2+} because of their transient and localized temporal and spatial properties. Although global [Ca\textsuperscript{2+}]\textsubscript{i} and Ca\textsuperscript{2+} sparks are discrete Ca\textsuperscript{2+} signals with distinct targets, these events are functionally coupled. Ca\textsuperscript{2+} sparks regulate global Ca\textsuperscript{2+}, and global Ca\textsuperscript{2+} feeds back to regulate Ca\textsuperscript{2+} sparks by modulating the activity of RyR channels, which are Ca\textsuperscript{2+} sensitive.

Another primary Ca\textsuperscript{2+} signal that occurs in arterial smooth muscle cells is a “Ca\textsuperscript{2+} wave” (6). These propagating Ca\textsuperscript{2+} transients occur due the activation of SR inositol trisphosphate (IP\textsubscript{3})-gated Ca\textsuperscript{2+}-release channels and RyR channels. Ca\textsuperscript{2+} waves have been proposed to stimulate contraction, but Ca\textsuperscript{2+} waves and oscillations produced in response to alkaline pH or vasoconstrictors have also been described that do not induce contraction (3–5, 7, 10, 15) Thus the physiological functions of Ca\textsuperscript{2+} waves are less clear.

Junctional Ca\textsuperscript{2+} transients (jCaTs) are localized [Ca\textsuperscript{2+}]\textsubscript{i} elevations that occur in arterial smooth muscle cells in response to ATP release from nerve fibers (9). jCaTs are caused by Ca\textsuperscript{2+} influx through purinergic P2X receptors and stimulate mesenteric artery contraction. Additional Ca\textsuperscript{2+} signals have also been described in arterial smooth muscle cells, including “Ca\textsuperscript{2+} ripples,” which occur because of IP\textsubscript{3}-induced Ca\textsuperscript{2+} release, and “Ca\textsuperscript{2+} flashes,” which occur in a synchronous manner in small groups of cells at a very low rate (2). Thus the inventory of Ca\textsuperscript{2+} signals generated in arterial smooth muscle cells is diverse, and novel Ca\textsuperscript{2+} signals are still being discovered.

A series of elegant studies (1, 11, 12) by the Santana group at the University of Washington has recently described another intracellular Ca\textsuperscript{2+} signal produced in arterial smooth muscle cells. Termed “Ca\textsuperscript{2+} sparklets,” these subsarcolemmal transient Ca\textsuperscript{2+} sparks are generated by Ca\textsuperscript{2+} influx through one or several tightly clustered voltage-dependent Ca\textsuperscript{2+} channels. Previously, arterial smooth muscle cell voltage-dependent Ca\textsuperscript{2+} channels were studied by using patch-clamp electrophysiology. Electro-physiological techniques generated high-resolution temporal information about currents generated by Ca\textsuperscript{2+} channels but could not provide spatial data concerning channel location and the impact of channel opening on subjacent Ca\textsuperscript{2+} concentrations. A significant advantage to imaging Ca\textsuperscript{2+} sparks is that important spatial information can be obtained regarding the location of active voltage-dependent Ca\textsuperscript{2+} channels in the membrane and the profiles of the local Ca\textsuperscript{2+} transients that are produced by these channels. Combining imaging and electrophysiological techniques provides an even more powerful approach, because both high-resolution temporal and spatial information about intracellular Ca\textsuperscript{2+} changes that occur in response to voltage-dependent Ca\textsuperscript{2+} channel activation can be obtained.

With the use of these techniques, it has been found that arterial smooth muscle cell Ca\textsuperscript{2+} sparklets occur in two distinct functional modes: low and high activity. Low-activity Ca\textsuperscript{2+} sparklets occur due to the opening of a single voltage-dependent Ca\textsuperscript{2+} channel, at low frequency, and in an apparent stochastic manner throughout the plasma membrane. In contrast, high-activity sparklets occur at recurring sites through the opening of small clusters of voltage-dependent Ca\textsuperscript{2+} channels that exhibit such a high open probability that they produce almost constant Ca\textsuperscript{2+} influx. An important distinguishing feature of these apparently different Ca\textsuperscript{2+} signals is that the high-activity mode results from protein kinase C (PKC)-\textalpha\textsubscript{Ca}\textsuperscript{2+}-induced Ca\textsuperscript{2+} channel activation. Protein phosphatases 2A and 2B counter PKC-mediated Ca\textsuperscript{2+} sparklets because phosphatase 2A

Address for reprint requests and other correspondence: J. H. Jaggar, Dept. of Physiology, Univ. of Tennessee Health Science Center, Memphis, TN 38163 (e-mail: jaggar@physiol.utmem.edu).
inhibition stimulates previously silent high-activity-mode sparklet sites. One major discovery is that Ca\textsuperscript{2+} sparklets not only cause a local [Ca\textsuperscript{2+}], elevation within the vicinity of the Ca\textsuperscript{2+} channel, but these events also increase global [Ca\textsuperscript{2+}]. A striking finding is that at physiological voltages and external Ca\textsuperscript{2+} concentrations (2 mM), \sim50\% of voltage-dependent Ca\textsuperscript{2+} influx in arterial smooth muscle cells occurs because of the PKC-\alpha-induced high-activity Ca\textsuperscript{2+} sparklet mode.

In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, Navedo et al. (13) investigated the molecular identity of voltage-dependent Ca\textsuperscript{2+} channels that generate Ca\textsuperscript{2+} sparklets in murine arterial smooth muscle cells. Previous studies using pharmacological approaches had suggested that sparklets occur due to the opening of Cav\textsubscript{1.2} L-type voltage-dependent Ca\textsuperscript{2+} channels (1, 11, 12). However, because Ca\textsuperscript{2+} sparklets are observed at hyperpolarized voltages where Cav\textsubscript{1.2} channel activity would be extremely low, additional Ca\textsuperscript{2+} channels with more negative voltage sensitivities than Cav\textsubscript{1.2} may also generate sparklets. Because Cav\textsubscript{1.3} channels have been detected in basilar artery and because the voltage sensitivity of Cav\textsubscript{1.3} is more negative than for Cav\textsubscript{1.2}, the authors tested the hypothesis that Cav\textsubscript{1.3} channels may also produce Ca\textsuperscript{2+} sparklets in cerebral artery smooth muscle cells. The authors demonstrate that Cav\textsubscript{1.3} channels expressed in tsA201 cells have a similar conductance to Cav\textsubscript{1.2} channels. Similarly, when coexpressed with PKC-\alpha, Cav\textsubscript{1.3} channels generate Ca\textsuperscript{2+} sparklets that function in low- and high-activity modes. Cav\textsubscript{1.3} sparklets were also similar in amplitude and dihydropyridine sensitivity to those generated by Cav\textsubscript{1.2} channels. However, several important findings support the conclusion that Ca\textsuperscript{2+} sparklets in cerebral artery smooth muscle cells arise due to the opening of Cav\textsubscript{1.2} channels. These include the observations that in arterial smooth muscle cells, Ca\textsuperscript{2+}-current voltage dependence and steady-state inactivation were similar to Cav\textsubscript{1.2} but not Cav\textsubscript{1.3} currents and that RT-PCR and immunofluorescence detected only Cav\textsubscript{1.2} in isolated myocytes. In addition, the authors found that nifedipine did not alter Ca\textsuperscript{2+} sparklets in arterial smooth muscle cells isolated from mice that express dihydropyridine-insensitive Cav\textsubscript{1.2} channels. If Ca\textsuperscript{2+} sparklets also occurred due to the activation of dihyropyridine-sensitive Ca\textsuperscript{2+} channels other than Cav\textsubscript{1.2}, nifedipine would have reduced Ca\textsuperscript{2+} sparklet frequency in these cells. Together, these data indicate that Cav channels other than Cav\textsubscript{1.2} can generate Ca\textsuperscript{2+} sparklets but that Cav\textsubscript{1.2} channels produce sparklets in cerebral artery smooth muscle cells.

Although the present study and earlier studies provide some answers as to the source, regulation, and physiological functions of Ca\textsuperscript{2+} sparklets in arterial smooth muscle cells, many questions remain. A major question is what, if any, are local targets for Ca\textsuperscript{2+} sparklets? Many different Ca\textsuperscript{2+}-sensitive proteins could be located in close proximity to voltage-dependent Ca\textsuperscript{2+} channels and may be exposed to Ca\textsuperscript{2+} sparklets, including kinases, ion channels, and transporters. Whether Ca\textsuperscript{2+} sparklets regulate local Ca\textsuperscript{2+}-sensitive proteins will depend on several factors, including the Ca\textsuperscript{2+} sensitivity of the protein and how close the protein is to the Ca\textsuperscript{2+}-channel pore, which would determine the Ca\textsuperscript{2+} concentration to which it is exposed. Any Ca\textsuperscript{2+}-permeant channel should generate Ca\textsuperscript{2+} sparklets, with the spatial and temporal properties of the Ca\textsuperscript{2+} transients produced being contingent on the properties of the channel, such as conductance. Arterial smooth muscle cells located in vascular beds other than those in the brain may express voltage-dependent Ca\textsuperscript{2+} channels other than Cav\textsubscript{1.2}. As such, the properties and physiological functions of individual Ca\textsuperscript{2+} sparklets would be defined by those of the Ca\textsuperscript{2+} channel isoforms that generate the signals.

A more specific question that arises from the current study is what is different molecularly between voltage-dependent Ca\textsuperscript{2+} channels that generate low- and high-activity-mode Ca\textsuperscript{2+} sparklets. Although the importance of PKC has been established, it is unclear what predisposes only some Cav\textsubscript{1.2} channels to PKC activation and thus the high-activity mode. Several possibilities exist, including that PKC is located within the vicinity of only some channels or within certain channel complexes, that Cav\textsubscript{1.2} splice variants with differing sensitivities to PKC are present, or that protein phosphatases which limit channel phosphorylation are associated with only some Cav\textsubscript{1.2} channels.

In summary, the excellent study by Navedo et al. (13) demonstrates that although Cav\textsubscript{1.2} and Cav\textsubscript{1.3} channels generate quantitatively similar Ca\textsuperscript{2+} sparklets, Cav\textsubscript{1.2} channels generate sparklets in cerebral artery smooth muscle cells. Data also indicate that Cav\textsubscript{1.2} channels are responsible for voltage-dependent Ca\textsuperscript{2+} influx in cerebral artery smooth muscle cells. When considering the importance of voltage-dependent Ca\textsuperscript{2+} channels in arterial smooth muscle physiology, future studies are likely to uncover additional functions of Ca\textsuperscript{2+} sparklets in these cells.

REFERENCES

