Calcium sparks in smooth muscle

JONATHAN H. JAGGAR,1 VALERIE A. PORTER,1 W. JONATHAN LEDERER,2 AND MARK T. NELSON1
1Department of Pharmacology, College of Medicine, The University of Vermont, Burlington, Vermont 05405; and 2Medical Biotechnology Center, University of Maryland Biotechnology Institute, and Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

Jaggar, Jonathan H., Valerie A. Porter, W. Jonathan Lederer, and Mark T. Nelson. Calcium sparks in smooth muscle. Am. J. Physiol. Cell Physiol. 278: C235–C256, 2000.—Local intracellular Ca2+ transients, termed Ca2+ sparks, are caused by the coordinated opening of a cluster of ryanodine-sensitive Ca2+ release channels in the sarcoplasmic reticulum of smooth muscle cells. Ca2+ sparks are activated by Ca2+ entry through dihydropyridine-sensitive voltage-dependent Ca2+ channels, although the precise mechanisms of communication of Ca2+ entry to Ca2+ spark activation are not clear in smooth muscle. Ca2+ sparks act as a positive-feedback element to increase smooth muscle contractility, directly by contributing to the global cytoplasmic Ca2+ concentration ([Ca2+]i) and indirectly by increasing Ca2+ entry through membrane potential depolarization, caused by activation of Ca2+ spark-activated Cl− channels. Ca2+ sparks also have a profound negative-feedback effect on contractility by decreasing Ca2+ entry through membrane potential hyperpolarization, caused by activation of large-conductance, Ca2+-sensitive K+ channels. In this review, the roles of Ca2+ sparks in positive- and negative-feedback regulation of smooth muscle function are explored. We also propose that frequency and amplitude modulation of Ca2+ sparks by contractile and relaxant agents is an important mechanism to regulate smooth muscle function.

ryanodine-sensitive calcium-release channel; voltage-dependent calcium channel; calcium-sensitive potassium channel; calcium-activated chloride channel; sarcoplasmic reticulum

CALCIUM IONS DIRECTLY REGULATE the contraction of smooth muscle through the activation of Ca2+/calmodulin-dependent myosin light-chain kinase (MLCK). For this to occur, an elevation of Ca2+ must be broadly or globally distributed throughout the cytoplasm. The view that a spatially homogeneous elevation in cytoplasmic Ca2+ is necessary for effective Ca2+ signaling has been radically altered by the discovery of local Ca2+ transients (“Ca2+ sparks”) in cardiac (37), skeletal (99,193), and smooth (140) muscle cells. Ca2+ sparks are caused by the opening of ryanodine-sensitive Ca2+-release (RyR) channels in the sarcoplasmic reticulum (SR). A single Ca2+ spark is capable of producing a very high (10–100 µM) local (~1% of the cell volume) increase in [Ca2+], while increasing the global intracellular concentration of Ca2+ ([Ca2+]i) by <2 nM (85, 140). A Ca2+ spark, by virtue of its high [Ca2+]i elevation, has the potential to modulate Ca2+-dependent processes that are not responsive to global increases in [Ca2+]i.

Early evidence for local Ca2+ release in cells came from measurements of spontaneous transient currents through Ca2+-sensitive K+ channels in frog sympathetic ganglia neurons (26, 125, 126). Subsequently, Benham & Bolton (13) measured transient outward currents through large-conductance Ca2+-sensitive K+ (BKCa) channels (referred to as “spontaneous transient outward currents” or STOCs) in isolated single cells of longitudinal smooth muscle from rabbit jejunum and ear artery (13). STOCs, which are caused by Ca2+ sparks (140), have since been described in a wide variety of types of smooth muscle (13, 19, 43, 75, 79, 82, 89, 95, 137, 140, 143, 145, 157, 163, 167, 217–219). The existence of STOCs suggested the possibility that subsarcolemmal [Ca2+]i is different from global cytoplasmic [Ca2+]i and that, under certain circumstances, subsarcolemmal [Ca2+]i could be significantly higher than global [Ca2+]i. Further support for this idea has come from studies that demonstrated a dissociation between glo-
RYANODINE RECEPTORS IN SMOOTH MUSCLE

Smooth muscle SR elements are very close (within 20 nm) to the plasma membrane (Fig. 3A) (45). RyR channels have been localized to both the peripheral (Fig. 3A) and central SR (45, 61, 118). Dihydropyridine receptors (voltage-dependent Ca\(^{2+}\) channels) and RyR channels colocalize in smooth muscle (33, 61), suggesting an intimate communication between these proteins in the plasma membrane and the SR. A strong spatial association has also been shown for the Na\(^+\)/K\(^+\) pump and the Na\(^+\)/Ca\(^{2+}\) exchanger, and the Na\(^+\)/Ca\(^{2+}\) exchanger and Ca\(^{2+}\) released from the SR (18, 136). This close spatial localization between RyR channels and the plasma membrane suggests a special communication between the SR and sarcoplasmic reticulum (SR) and ion transport systems.

RyR channels are found in all muscle types and many other cell types, including those from mammals, birds, amphibians, reptiles, fish, insects, crustaceans, and molluscs (for a review see Ref. 183). RyR channels were first identified in insect and invertebrate striated muscles, due to the action of the plant alkaloid, ryanodine, a potent channel antagonist (see Ref. 183). Presently, three molecularly distinct subtypes of RyR channels (RYR1, RYR2, RYR3) have been identified and cloned, with each thought to exist as a homotetramer in the SR membrane. RyR1 is found primarily in skeletal muscle, whereas RyR2 and RyR3 are predominantly located in smooth muscle.
found in cardiac tissue and brain, respectively. mRNA transcripts for RyR1, RyR2, and RyR3 have been identified in smooth muscle preparations (see Refs. 67, 90, 117, 142). However, these results should be interpreted with some caution, since the detection of RyR1–3 transcripts may reflect contamination from cells other than smooth muscle cells (e.g., endothelium, neurons) and does not necessarily reflect the level of functional protein.

A major breakthrough in the investigation of the functional properties of RyR channels involved the incorporation of RyR channels from cardiac and skeletal muscle SR microsomes into planar lipid bilayers (176). One difficulty with isolating RyR channels from smooth muscle is that smooth muscle SR membranes cannot be readily separated from other membranes. As an alternative approach, RyR channels have been partially purified from aortic smooth muscle (71) (Table 1) and from toad stomach smooth muscle (214) and incorporated into microsomes and then into planar lipid bilayers. The molecular and functional properties of the three cloned RyR channel subtypes and the smooth muscle RyR channel are summarized in Table 1. RyR channels from smooth muscle are activated by micromolar cytoplasmic [Ca\textsuperscript{2+}] (Fig. 4) and by caffeine and are inhibited by Mg\textsuperscript{2+} and ruthenium red (71), similar to RyR2 and RyR3. The single-channel conductance of the smooth muscle RyR channels is also similar to the cardiac (RyR2) and skeletal muscle (RyR1) channels. Ca\textsuperscript{2+} release through RyR channels could also contribute to global [Ca\textsuperscript{2+}] (for contraction i.e., Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR); positive feedback element). Contribution of Ca\textsuperscript{2+} sparks to global [Ca\textsuperscript{2+}] may be significant in phasic smooth muscle and less important in tonic smooth muscle. Ca\textsuperscript{2+} sparks activate BK\textsubscript{Ca} channels in virtually all types of smooth muscle to cause a very substantial membrane potential (\(V_m\)) hyperpolarization (negative-feedback element). Sparks activate Cl\textsubscript{Ca} channels in some types of smooth muscle to cause membrane depolarization (positive-feedback element). Ca\textsuperscript{2+} release through RyR channels could also activate SK\textsubscript{Ca} channels to cause membrane potential hyperpolarization in some types of smooth muscle (effect not illustrated). The final outcome of these various signaling elements depends on a number of factors, including proximity of the various elements, Ca\textsuperscript{2+} sensitivity, and phosphorylation state. MLCK, myosin light-chain kinase.

Fig. 2. Possible relationships among voltage-dependent Ca\textsuperscript{2+} channels, ryanodine-sensitive Ca\textsuperscript{2+}-release (RyR) channels, large-conductance Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} (BK\textsubscript{Ca}) channels, and Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} (Cl\textsubscript{Ca}) channels to regulate smooth muscle contractility. Voltage-dependent Ca\textsuperscript{2+} channels (VDCC) are the primary Ca\textsuperscript{2+} entry pathway in most types of smooth muscle. For tonic smooth muscle, such as resistance arteries, steady Ca\textsuperscript{2+} entry through VDCC determines intracellular [Ca\textsuperscript{2+}] ([Ca\textsuperscript{2+}]\textsubscript{i}); indicated by thick arrow). Ca\textsuperscript{2+} entry through VDCCs also stimulates RyR channels as Ca\textsuperscript{2+} spark events or as non-Ca\textsuperscript{2+} spark events. The type of Ca\textsuperscript{2+} communication from VDCCs to RyR channels (e.g., local Ca\textsuperscript{2+} control) in smooth muscle is not known. RyR channels can serve as negative- and positive-feedback transducers to VDCCs. Ca\textsuperscript{2+} release from RyR channels could inactivate VDCCs (negative-feedback element); this is not known for smooth muscle. Ca\textsuperscript{2+} release from RyR channels may also contribute to global [Ca\textsuperscript{2+}] for contraction (i.e., Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR); positive feedback element). Contribution of Ca\textsuperscript{2+} sparks to global [Ca\textsuperscript{2+}] may be significant in phasic smooth muscle and less important in tonic smooth muscle. Ca\textsuperscript{2+} sparks activate BK\textsubscript{Ca} channels in virtually all types of smooth muscle to cause a very substantial membrane potential (\(V_m\)) hyperpolarization (negative-feedback element). Sparks activate Cl\textsubscript{Ca} channels in some types of smooth muscle to cause membrane depolarization (positive-feedback element). Ca\textsuperscript{2+} release through RyR channels could also activate SK\textsubscript{Ca} channels to cause membrane potential hyperpolarization in some types of smooth muscle (effect not illustrated). The final outcome of these various signaling elements depends on a number of factors, including proximity of the various elements, Ca\textsuperscript{2+} sensitivity, and phosphorylation state. MLCK, myosin light-chain kinase.
in cardiac cells and smooth muscle cells have similar biophysical and pharmacological characteristics (e.g., see Refs. 37 and 140; Table 2). Furthermore, the ryanodine-binding receptors purified from toad stomach, and from cerebral artery smooth muscle cells, cross-react with monoclonal antibodies for RyR2 (Fig. 3B) (61, 214). Therefore, the RyR channel responsible for Ca\(^{2+}\) release from the SR in smooth muscle appears to be similar to the RyR2 subtype, although an involvement of other RyR channel subtypes is also possible.

**Table 1. Characteristics of ryanodine receptor subtypes**

<table>
<thead>
<tr>
<th></th>
<th>Mammalian Skeletal Muscle (ryr1)</th>
<th>Mammalian Cardiac Muscle (ryr2)</th>
<th>Mammalian Brain (ryr3)</th>
<th>Mammalian Smooth Muscle (?) (71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human chromosome</td>
<td>19q13.1 (124, 129)</td>
<td>1a (148, 149)</td>
<td>15q14-q15 (180)</td>
<td></td>
</tr>
<tr>
<td>Mouse chromosome</td>
<td>7A2-7A3 (34, 127)</td>
<td>13A1-13A2 (34, 127)</td>
<td>2E5-2F3 (34, 127)</td>
<td>500 kDa (130)</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>564 kDa (220), 565 kDa (188)</td>
<td>565 kDa (138; 149)</td>
<td>15q14-q15 (180)</td>
<td>500 kDa (130)</td>
</tr>
<tr>
<td>Number of residues</td>
<td>5,032 (220), 5,037 (188)</td>
<td>4,968 or 4,976 (138), 4,969 (149)</td>
<td>4,968 (138), 4,969 (149)</td>
<td>4,872 (67)</td>
</tr>
<tr>
<td>Morphology Conductance (50–100 mM Ca(^{2+}))</td>
<td>Quatrefoil (111)</td>
<td>Quatrefoil (5)</td>
<td>Quatrefoil (90, 110)</td>
<td>140 pS (110)</td>
</tr>
<tr>
<td>Mechanism of activation</td>
<td>Direct coupling, CICR</td>
<td>CICR</td>
<td>Ca(^{2+})</td>
<td>Ca(^{2+})</td>
</tr>
<tr>
<td>Activation by (\mu)M Ca(^{2+})</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (90)</td>
<td>Yes (in the presence of (\mu)M Ca(^{2+}))</td>
</tr>
<tr>
<td>ATP</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (90)</td>
<td>Yes</td>
</tr>
<tr>
<td>Inhibition by mM Mg(^{2+}) (b)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (90)</td>
<td>Yes</td>
</tr>
<tr>
<td>Ruthenium red</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (90)</td>
<td>Yes</td>
</tr>
<tr>
<td>Modulation by Protein kinase (A)</td>
<td>Activation (59, 66, 73) (c)</td>
<td>Activation (195) (d)</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Ryanodine binding (K_d)</td>
<td>540–650 pmol/mg protein (4–8) nM(^{2+}) (111, 113)</td>
<td>450 pmol/mg protein (2) nM(^{2+}) (5)</td>
<td>460 pmol/mg protein (3–12) nM(^{2+}) (90, 110, 130)</td>
<td>1–3 pmol/mg protein</td>
</tr>
</tbody>
</table>

\(c\) CICR, Ca\(^{2+}\)-induced Ca\(^{2+}\) release. \(a\) Located at the interval between 1q42.1 and 1q43. \(b\) Mg\(^{2+}\) has been suggested to competitively displace Ca\(^{2+}\) from its activation site and may also interact with the low-affinity Ca\(^{2+}\) inhibitory site (42). \(c\) Phosphorylation by PKA reduced Mg\(^{2+}\) inhibition. \(d\) Phosphorylation by protein kinase A increased the responsiveness of the channel to Ca\(^{2+}\). \(e\) One high-affinity and 3 low-affinity binding sites. Nos. in parentheses are reference nos.
**Ca^{2+}** SPARK PROPERTIES IN SMOOTH MUSCLE

**Basic Properties**

Ca^{2+} sparks have been observed in cardiac (37), skeletal (99, 193), and smooth (140) muscle cells. These measurements have been made using laser scanning confocal microscopy and the fluorescent Ca^{2+} indicator, fluo 3. A number of lines of evidence indicate that Ca^{2+} sparks are caused by the opening of RyR channels in the SR, including modulation by ryanodine, voltage, Ca^{2+}, and caffeine, and location of events (30, 37, 99, 121, 140, 193). Table 2 summarizes the properties of Ca^{2+} sparks in cardiac, skeletal, and smooth muscle cells. Ca^{2+} sparks recorded in skeletal muscle are approximately one-third the size of those observed in cardiac and smooth muscle (Table 2), although the single-channel currents through skeletal and cardiac and smooth muscle RyR channels incorporated into bilayers are about the same (Table 1).

Table 2. Ca^{2+} sparks in cardiac, skeletal, and smooth muscle cells

<table>
<thead>
<tr>
<th>Muscle Type</th>
<th>Cardiac</th>
<th>Skeletal</th>
<th>Smooth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Ca^{2+} *</td>
<td>114–180 nM (37, 62, 123)</td>
<td>20–60 nM (99, 193)</td>
<td>50–200 nM (85, 133, 140, 172)</td>
</tr>
<tr>
<td>Rise time</td>
<td>10 ms (37)</td>
<td>4.7 ms (109)</td>
<td>20–95 ms (133, 140, 153, 172)</td>
</tr>
<tr>
<td>Decay</td>
<td>t_{1/2} = 20–37 ms (30, 37, 123)</td>
<td>t_{1/2} = 8.5 ms (109)</td>
<td>t_{1/2} = 27–60 ms (19, 63, 85, 133, 140, 153)</td>
</tr>
<tr>
<td>Spread (FWHM)</td>
<td>1.7–2 µm (30, 35, 37) (62, 123)</td>
<td>2.4 µm (99)</td>
<td>1.4–1.5 µm (133, 140), 14 µm² (153)</td>
</tr>
<tr>
<td>Unstimulated frequency</td>
<td>100 s⁻¹-cell⁻¹ (37)</td>
<td>0.176 triad⁻¹-s⁻¹ (99)</td>
<td>0.5–1 s⁻¹-cell⁻¹ (19, 85, 140, 157, 172)</td>
</tr>
<tr>
<td>Intracellular location</td>
<td>t-Tubule (ventricular) (37, 169); subplasmalemmal and central (atrial) (15, 80)</td>
<td>t-Tubule (99)</td>
<td>Subplasmalemmal (140)</td>
</tr>
<tr>
<td>Stimulation</td>
<td>Local Ca^{2+} (30, 37, 121)</td>
<td>Coupled to DHPR/Ca^{2+} (99)</td>
<td>Ca^{2+} (local?)</td>
</tr>
<tr>
<td>Proposed effect on muscle</td>
<td>Contraction (37)</td>
<td>Contraction (99)</td>
<td>Relaxation (140) and contraction</td>
</tr>
<tr>
<td>Pharmacology</td>
<td>≤2 µM ryanodine</td>
<td>Activation (37, 166)</td>
<td>Activation (133, 172)</td>
</tr>
<tr>
<td>10–30 µM ryanodine</td>
<td>Inhibition (37)</td>
<td>Inhibition (99)</td>
<td>Inhibition (9, 19, 63, 85, 133, 140, 157)</td>
</tr>
<tr>
<td>Effect of caffeine</td>
<td>Activation (500 µM) (166)</td>
<td>Activation (41, 99)</td>
<td>Activation (50 µM–1 mM) (61, 133, 172)</td>
</tr>
</tbody>
</table>

FWHM, full width of the Ca^{2+} spark spread at the half-maximal amplitude. *Ca^{2+} sparks were measured in unstimulated cells using Ca^{2+}-sensitive fluorescent dyes such as fluo 3. †Assessed using rapid 2-dimensional (2D) planar imaging.
is due to the number of RyR channels contributing to the Ca$^{2+}$ spark or to different RyR channel kinetics (99, 131).

Ca$^{2+}$ sparks in smooth muscle cells were first described in myocytes from rat cerebral arteries [Figs. 5 and 6 (140), see also Refs. 19, 61, 85, 86, 153, 157]. Subsequently, Ca$^{2+}$ sparks have been measured in smooth muscle cells from coronary arteries (86, 157), mesenteric artery (132), rat portal vein (9), guinea pig ileum (63), guinea pig urinary bladder (83), guinea pig vas deferens (83), and toad stomach (219). The properties of Ca$^{2+}$ sparks appear to be similar in these different smooth muscle preparations (Table 3). Figure 5 illustrates recordings of Ca$^{2+}$ sparks in the smooth muscle cells of an intact pressurized (60 mmHg) cerebral artery. In arterial smooth muscle, Ca$^{2+}$ sparks have a rise time of $\sim$20 ms, a half time of decay of 50–60 ms (Figs. 5 and 6), and a spatial spread (full width at the half-maximum amplitude) of 2.4 $\mu$m (19, 85, 140, 153). These unitary events occur most frequently close to the cell membrane (140), although events away from the cell membrane have been reported in smooth muscle cells from portal vein (9) and ileum (63). Ca$^{2+}$ sparks occur in intact, nonpressurized (61, 85) and pressurized cerebral (Fig. 5) and mesenteric arteries (132), with an apparent frequency of $\sim$1·s$^{-1}$.cell$^{-1}$ at physiological membrane potentials (−60 to −40 mV). These results support the idea that [Ca$^{2+}$]$\text{c}$ is dynamically and heterogeneously distributed in intact tonic smooth muscle.

A Ca$^{2+}$ Spark is due to the Simultaneous Activation of a Cluster of RyR Channels

Ca$^{2+}$ sparks appear to represent the near-simultaneous activation of a cluster, or plaque, of RyR channels. The flux of Ca$^{2+}$ from a spark site is substantial and corresponds to a Ca$^{2+}$ current of 4 pA for $\sim$10 ms (37). With physiological levels of SR luminal [Ca$^{2+}$], the unitary current of a single cardiac RyR channel is <0.6 pA (131). Therefore, a Ca$^{2+}$ spark would correspond to the coordinated opening of at least 10 RyR channels (131). In cardiac muscle, the elementary physiological event appears to be Ca$^{2+}$ sparks, since the global Ca$^{2+}$ transient is composed of many Ca$^{2+}$ sparks (29). In a similar fashion, the miniature end plate potential represents the release of one presynaptic vesicle (the elementary physiological event), and not the release of one neurotransmitter molecule.

Fate of Ca$^{2+}$ From a Ca$^{2+}$ Spark

The decay of the Ca$^{2+}$ spark may be determined by diffusion, uptake into the SR by the thapsigargin-sensitive Ca$^{2+}$-ATPase, and extrusion from the cell through the plasmalemmal Ca$^{2+}$-ATPase and Na$^{+}$/Ca$^{2+}$ exchanger. In cardiac muscle, Ca$^{2+}$ spark decay reflects diffusion and SR Ca$^{2+}$ uptake, with a small contribution from Ca$^{2+}$ extrusion (165). As measured with fluorescent dyes, the decay of Ca$^{2+}$ sparks in arterial smooth muscle is biexponential, with both rate constants ($t_1 = 32$ ms and $t_2 = 275$ ms) contributing equally to the decay (49 and 51%, respectively) (153). It is unclear why Ca$^{2+}$ sparks demonstrate a biexponential decay pattern, but this may reflect limited space diffusion, fluorescent dye kinetics, or extrusion/uptake of Ca$^{2+}$ by SR or sarcolemmal Ca$^{2+}$ pumps. The faster of the two time constants for decay is similar to that which has been measured for Ca$^{2+}$ sparks in heart muscle (62, 165), consistent with diffusion of Ca$^{2+}$ in the cytoplasm. The contribution of Ca$^{2+}$ extrusion/sequestration mechanisms to the decay of Ca$^{2+}$ sparks in smooth muscle remains to be addressed. However, the decay of a whole cell Ca$^{2+}$ transient, which reflects Ca$^{2+}$ extrusion and SR Ca$^{2+}$ uptake, is much slower [decay constant ($\tau$) = 2.0–8.6 s (49, 207); see also Refs. 53–55, 57, 93, 94] than the decay of a Ca$^{2+}$ spark. This observation suggests that Ca$^{2+}$ spark decay is largely determined by diffusion (see Ref. 62).

The smooth muscle cell Ca$^{2+}$ spark represents the spatially localized, unitary release of a significant amount of Ca$^{2+}$ very close to the plasma membrane, with essentially no effect on global [Ca$^{2+}$]$\text{c}$, or on contraction (140). The localized release of Ca$^{2+}$ into a subsarcolemmal domain can thus represent a measurable element of the "superficial buffer barrier" (SBB) hypothesis.

![Image](http://ajpcell.physiology.org/)

Fig. 5. Ca$^{2+}$ sparks in a pressurized cerebral artery. Arteries were loaded with 10 $\mu$M fluo 3 and cannulated at each end as previously described (25). A steady luminal pressure of 60 mmHg was applied and the artery imaged using a Noran laser scanning confocal microscope. For similar experimental procedure see Ref. 85. Areas (56 × 52 $\mu$m) were acquired at a rate of 60 images/s for 10 s. Ca$^{2+}$ sparks were observed in the smooth muscle cells of the artery (top; average of 100 images) as transient localized changes in fluorescence. Bottom: fractional increase in fluorescence (F/F$_{0}$) vs. time for analysis boxes a–d. Gray lines illustrate the smooth muscle cells (3 cells are clearly identifiable) (G. C. Wellman and M. T. Nelson, unpublished observations).
This hypothesis suggests that Ca\(^{2+}\) entering a smooth muscle cell is continuously buffered by the SR and is then discharged "vectorially" toward the plasma membrane without any effect on global \([\text{Ca}^{2+}]_i\). Subsequently, this Ca\(^{2+}\) is extruded from the cell via the sarcolemmal Na\(^+/\)Ca\(^{2+}\) exchanger and Ca\(^{2+}\)-ATPase. The anatomic requirements for a Ca\(^{2+}\) spark to activate BK\(_{\text{Ca}}\) channels are similar to those proposed for the SBB hypothesis. Additional studies on the decay of Ca\(^{2+}\) sparks should provide new insights into local Ca\(^{2+}\) extrusion and uptake mechanisms as well as the SBB hypothesis.

**CA\(^{2+}\) SPARKS ARE INVOLVED IN BOTH POSITIVE AND NEGATIVE FEEDBACK REGULATION OF GLOBAL INTRACELLULAR \([\text{CA}^{2+}]\) IN SMOOTH MUSCLE**

The role of local Ca\(^{2+}\) signaling is cell type dependent and should be viewed in the context of cell function. For example, cardiac muscle must contract forcefully and...
relax rapidly at high rates (1–7 Hz). In these cells, activation of voltage-dependent Ca\textsuperscript{2+} channels, which is synchronized by the action potential, leads to an immediate rise in Ca\textsuperscript{2+} influx, which activates, in a coordinated and rapid fashion, large numbers of Ca\textsuperscript{2+} sparks, which summate to cause the global [Ca\textsuperscript{2+}]\textsubscript{i} transient. Thus, Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+}-release (CICR) through RyR channels acts as an amplifier for Ca\textsuperscript{2+} influx, contributing 90% of the cellular Ca\textsuperscript{2+} needed for contraction of heart muscle (Fig. 1). Although cardiac and smooth muscle share many of the same key components for excitation-contraction coupling (e.g., L-type Ca\textsuperscript{2+} channels and RyR channels), these elements are arranged to regulate function in very different ways. Importantly, cardiac muscle lacks BK\textsubscript{Ca} channels. In smooth muscle, the triumvirate of dihydropyridine-sensitive voltage-dependent Ca\textsuperscript{2+} channels, RyR channels, and BK\textsubscript{Ca} channels represent a functional unit to control the levels of [Ca\textsuperscript{2+}]\textsubscript{i} (86).

Most types of smooth muscle are involved in maintaining tonic contraction. One important element in graded, tonically contracted smooth muscle is the negative-feedback control of membrane potential, and hence Ca\textsuperscript{2+} entry, through activation of BK\textsubscript{Ca} channels (Figs. 1, 2, and 6; cf. Refs. 25 and 140). Some types of smooth muscle (e.g., urinary bladder) exhibit phasic contractions, suggesting that Ca\textsuperscript{2+} release through RyR channels may contribute directly to contraction, like cardiac muscle, and indirectly to relaxation through activation of BK\textsubscript{Ca} channels (Fig. 6) (69). Furthermore, as discussed later, some types of smooth muscle have SK\textsubscript{Ca} channels and Cl\textsubscript{Ca} channels (see Fig. 2 and Table 4), which will also contribute to the regulation of smooth muscle excitability.

### Communication Between Voltage-Dependent Ca\textsuperscript{2+} Channels and RyR Channels Is a Critical Element in the Control of Global Intracellular [Ca\textsuperscript{2+}]

The first step in the flow of information following electrical excitation in heart muscle is an increase in Ca\textsuperscript{2+} influx through voltage-dependent Ca\textsuperscript{2+} channels. The communication between voltage-dependent Ca\textsuperscript{2+} channels and RyR channels is intimate, rapid, and

### Table 3. Ca\textsuperscript{2+} sparks in smooth muscle cells from arteries, veins, and nonvascular tissues

<table>
<thead>
<tr>
<th>Smooth Muscle Source</th>
<th>Arteries</th>
<th>Veins</th>
<th>Non-vascular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Ca\textsuperscript{2+}</td>
<td>110–200 nM (85, 140)</td>
<td>10–200 nM† (133)</td>
<td>50–120 nM (172)</td>
</tr>
<tr>
<td>Rise time</td>
<td>20 ms (140)</td>
<td>22 ms (133)</td>
<td>18–180 ms (63, 172)</td>
</tr>
<tr>
<td>Decay (t\textsubscript{1/2})</td>
<td>48–56 ms (19, 85, 140, 153)</td>
<td>27 ms (133)</td>
<td>30–60 ms (63)</td>
</tr>
<tr>
<td>Spread (FWMH)</td>
<td>2.4 µm (140), 14 µm\textsuperscript{2} (153)§</td>
<td>1.5 µm (133)</td>
<td>0.9–2 µm (63)</td>
</tr>
<tr>
<td>Frequency</td>
<td>Unstimulated 0.24 Hz§ (85)</td>
<td>Ca\textsuperscript{2+} (local?)</td>
<td>Ca\textsuperscript{2+} (local?)</td>
</tr>
<tr>
<td>Location</td>
<td>Subplasmalemmal</td>
<td>Ca\textsuperscript{2+} (local?)</td>
<td>Ca\textsuperscript{2+} (local?)</td>
</tr>
<tr>
<td>Stimulation</td>
<td>Ca\textsuperscript{2+} (local?)</td>
<td>Ca\textsuperscript{2+} (local?)</td>
<td>Ca\textsuperscript{2+} (local?)</td>
</tr>
<tr>
<td>Effect on muscle</td>
<td>Relaxation (140; 157)</td>
<td>Contraction (9)</td>
<td>Relaxation and contraction</td>
</tr>
<tr>
<td>Effect of caffeine</td>
<td>Activation (0.3 mM) (61)</td>
<td>Activation (0.5–1 mM) (133)</td>
<td>Activation (50 µM) (172)</td>
</tr>
</tbody>
</table>

All measurements are obtained using Ca\textsuperscript{2+}-sensitive fluorescent dyes such as fluo 3. *Determined in unstimulated cells. †Peak of a Gaussian distribution (133). ‡Assessed using rapid 2D planar imaging of isolated, voltage-clamped (−40 mV) cerebral artery myocytes under conditions where approximately one-half of the cell volume is captured. §Rapid 2D confocal imaging of myocytes in an intact cerebral artery. Frequency measurements were obtained from 56 µm × 53 µm areas, or −8 smooth muscle cells.

### Table 4. Cellular targets for Ca\textsuperscript{2+} sparks

<table>
<thead>
<tr>
<th>Mechanism of activation by Ca\textsuperscript{2+}</th>
<th>BK\textsubscript{Ca} Channels</th>
<th>Cl\textsuperscript{−} Channels</th>
<th>SK\textsubscript{Ca} Channels</th>
<th>MLCK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct channel activation</td>
<td>Unknown (calmodulin?)</td>
<td>Calmodulin (213)</td>
<td>Calmodulin</td>
<td>K\textsubscript{d} &lt; 1 µM (see Ref. 24)</td>
</tr>
<tr>
<td>K\textsubscript{d} = 1.8 µM at −11 mV and 10 µM at −83 mV (31)</td>
<td>K\textsubscript{d} = 0.37 µM (150)</td>
<td>K\textsubscript{d} = 0.74 µM at −80 mV (74)</td>
<td>0.5–10 Channels/µm\textsuperscript{2} (see Ref. 116)</td>
<td>Described in murine colon (104) and murine ileum (200)</td>
</tr>
<tr>
<td>6 × 10\textsuperscript{−4} % (at −40 mV)</td>
<td>1.5%</td>
<td>3.2%</td>
<td>Unknown</td>
<td>All</td>
</tr>
<tr>
<td>80 pS</td>
<td>2.6 pS (100)</td>
<td>10 pS</td>
<td>2–3%</td>
<td></td>
</tr>
<tr>
<td>1–4 Channels/µm\textsuperscript{2} (13, 173, 190)</td>
<td>All</td>
<td>0.5–10 Channels/µm\textsuperscript{2} (see Ref. 116)</td>
<td>Described in murine colon (104) and murine ileum (200)</td>
<td>All</td>
</tr>
<tr>
<td>Voltage dependence</td>
<td>eFold per 12- to 14-mV depolarization (14, 115)</td>
<td>None</td>
<td>None</td>
<td>Activation of &lt;1% of MLCK</td>
</tr>
<tr>
<td>Functional impact of one Ca\textsuperscript{2+} spark</td>
<td>Up to 20-mV hyperpolarization (52)</td>
<td>5-mV depolarization (198)</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

BK\textsubscript{Ca}, large-conductance Ca\textsuperscript{2+}-sensitive; SK\textsubscript{Ca}, small-conductance Ca\textsuperscript{2+}-sensitive; MLCK, myosin light-chain kinase.
bidirectional. RyR channels are positioned in junctional SR elements within short distances (~20 nm) of voltage-dependent Ca\(^{2+}\) channels in the t-tubules of ventricular heart cells (for review see Ref. 51). RyR channels are activated by local Ca\(^{2+}\) shortly after they enter the cell through voltage-dependent Ca\(^{2+}\) channels ("local control") (30, 36, 121). Local Ca\(^{2+}\) in the subsarcolemmal junctional space is likely to reach relatively high concentrations (>10 μM), corresponding to levels required for significant RyR channel activation. Thus local Ca\(^{2+}\) control of Ca\(^{2+}\) release can explain the graded and stable increases in Ca\(^{2+}\) release through RyR channels in response to Ca\(^{2+}\) influx (see Fig. 1) (30, 121, 178). The communication is bidirectional, since Ca\(^{2+}\) sparks can also induce inactivation of voltage-dependent Ca\(^{2+}\) channels (1, 65, 170).

The communication between Ca\(^{2+}\) channels and RyR channels/Ca\(^{2+}\) sparks is less clear in smooth muscle. In smooth muscle, Ca\(^{2+}\) sparks are also regulated by Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels, since Ca\(^{2+}\) spark frequency and amplitude increase with activation of voltage-dependent Ca\(^{2+}\) channels in the smooth muscle cells of intact arteries (85). Membrane potential depolarization of intact cerebral arteries from about −60 to −40 mV, a membrane potential that would occur in pressurized arteries with tone, increases Ca\(^{2+}\) spark frequency about fivefold (85). The voltage dependence is similar to that observed in isolated cardiac myocytes over a similar voltage range (30, 164). This increase in Ca\(^{2+}\) spark frequency and amplitude depends on Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels, since it is reversed by diltiazem, a selective blocker of L-type Ca\(^{2+}\) channels (85). Additionally, in voltage-clamped rat portal vein myocytes, Ca\(^{2+}\) sparks have been observed to occur immediately following a depolarizing voltage step that elicits a Ca\(^{2+}\) current (9).

Although these studies indicate that Ca\(^{2+}\) spark frequency is modulated by Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels, it is still unresolved whether local Ca\(^{2+}\) regulates Ca\(^{2+}\) spark probability in smooth muscle.

Steady membrane depolarization of arterial smooth muscle increases voltage-dependent Ca\(^{2+}\) channel open probability (e.g., see Ref. 162), global Ca\(^{2+}\), and presumably SR Ca\(^{2+}\). An elevation in local [Ca\(^{2+}\)] from Ca\(^{2+}\) channels, global Ca\(^{2+}\), or SR Ca\(^{2+}\) could each contribute to the observed depolarization-induced increase in Ca\(^{2+}\) spark frequency and amplitude (Figs. 1 and 7). Indeed, recent evidence indicates that Ca\(^{2+}\) spark and STOC frequency in smooth muscle cells depends steeply on the SR Ca\(^{2+}\) load (219). Figure 7 illustrates modulation of the Ca\(^{2+}\) dependence of Ca\(^{2+}\) spark frequency by SR Ca\(^{2+}\) load and other factors (see FM AND AM OF CA\(^{2+}\) SPARKS: PHYSIOLOGICAL IMPLICATIONS).

Ca\(^{2+}\) release from RyR channels can inactivate voltage-dependent Ca\(^{2+}\) channels in cardiac myocytes (23, 134, 170), which would serve as another negative-feedback mechanism to regulate Ca\(^{2+}\) entry. The possibility that a similar mechanism plays a role in smooth muscle has not been extensively examined (although see Ref. 97). Indirect evidence from measurements of arterial wall [Ca\(^{2+}\)] and diameter in pressurized arteries suggests that this pathway is not significant (103), at least in the absence of receptor agonists.

Direct Contribution of Ca\(^{2+}\) Sparks to Cytoplasmic Ca\(^{2+}\)

In cardiac muscle, Ca\(^{2+}\) release through RyR channels (CICR) contributes most (~90%) of the Ca\(^{2+}\) for contraction. In tonic smooth muscle (e.g., arteries), in the absence of smooth muscle constrictors, global Ca\(^{2+}\), is largely determined by steady-state Ca\(^{2+}\) entry through voltage-dependent Ca\(^{2+}\) channels, and Ca\(^{2+}\) release likely contributes little to global Ca\(^{2+}\) (102, 103, 140). Ca\(^{2+}\) spark frequency is low in intact arteries (~1 Ca\(^{2+}\) spark · s\(^{-1}\) · cell\(^{-1}\)) (19, 61, 85, 140, 153, 157), and blocking Ca\(^{2+}\) sparks or RyR channels (in the presence of blockers of BKCa channels) has little direct effect on global Ca\(^{2+}\) and arterial tone (102, 103, 140). However, it is conceivable that, under conditions of elevated arterial tone (e.g., in hypertension), Ca\(^{2+}\) sparks might have a significant impact on global Ca\(^{2+}\).

In smooth muscle types that exhibit phasic contractions (e.g., urinary bladder), Ca\(^{2+}\) release through RyR channels may contribute Ca\(^{2+}\) directly for contraction. Unlike tonic smooth muscle, phasic smooth muscle exhibits action potentials, which activate voltage-dependent Ca\(^{2+}\) channels to trigger Ca\(^{2+}\) release. Ca\(^{2+}\) release through RyR channels can contribute as much as 70% of the increment in global Ca\(^{2+}\) (30% to Ca\(^{2+}\) influx) in isolated voltage-clamped urinary bladder myocytes (54). The excitability and duration of action potentials in phasic smooth muscle are also regulated in a negative-feedback manner by BKCa and SKCa channels (69, 70). Therefore, blocking RyR channels affects both positive- and negative-feedback regulation of Ca\(^{2+}\) in these types of smooth muscle. The precise contribution of Ca\(^{2+}\) release through RyR channels to global Ca\(^{2+}\) in intact phasic smooth muscle remains to be determined.

The ability of voltage-dependent Ca\(^{2+}\) influx to stimulate Ca\(^{2+}\) release through RyR channels appears to
vary considerably among different types of smooth muscle. All types of smooth muscle are capable of releasing significant amounts of Ca\(^{2+}\) through RyR channels, since caffeine, an activator of RyR channels (Table 1), can induce global ryanodine-sensitive Ca\(^{2+}\) transients in virtually all smooth muscle types (cf. Refs. 12, 39, 49, 54, 61). Yet, influx through voltage-dependent Ca\(^{2+}\) channels can induce ryanodine-sensitive elevations in global [Ca\(^{2+}\)] in some (54, 94), but not all (49, 128), types of smooth muscle. The lack of effective CICR through RyR channels suggests that, in smooth muscle, many of the RyR channels are spatially distant from the high local [Ca\(^{2+}\)] caused by the opening of voltage-dependent Ca\(^{2+}\) channels. However, even in cell types (e.g., urinary bladder) that exhibit significant ryanodine-sensitive global Ca\(^{2+}\) transients, the situation is very different from cardiac muscle. CICR, when it does occur in smooth muscle, is very sluggish, developing slowly over hundreds of milliseconds (93, 94). In contrast, the close proximity of cardiac Ca\(^{2+}\) channels and RyR channels enables rapid and effective communication, i.e., Ca\(^{2+}\) influx stimulates Ca\(^{2+}\) release (Ca\(^{2+}\) sparks) within milliseconds (30, 121). Therefore, the failure of voltage-dependent Ca\(^{2+}\) influx to induce substantial Ca\(^{2+}\) transients does not seem to reflect a lack of releasable Ca\(^{2+}\) through RyR channels, but may reflect a physical separation of voltage-dependent Ca\(^{2+}\) channels from a majority of the RyR channels. Communication between Ca\(^{2+}\) channels and RyR channels in smooth muscle may be analogous to that in atrial muscle where the t-tubular network is virtually absent. Atrial muscle has been found to contain “foot-like” structures similar in appearance to those in smooth muscle (45) that occur close to the plasmalemma (50). Electrical stimulation of atrial myocytes has been demonstrated to elicit Ca\(^{2+}\) sparks immediately beneath the plasmalemma, which then trigger Ca\(^{2+}\) sparks in more central cellular regions to elicit a global Ca\(^{2+}\) transient (15, 80).

In heart, the Ca\(^{2+}\) transient is composed of the simultaneous activation of many Ca\(^{2+}\) sparks. However, in smooth muscle, the contribution of Ca\(^{2+}\) sparks to ryanodine-sensitive global Ca\(^{2+}\) transients is not known. It is also conceivable that, in smooth muscle, Ca\(^{2+}\) spark sites communicate only with local targets (e.g., BK\(_{\text{Ca}}\) channels), and ryanodine-sensitive global Ca\(^{2+}\) transients are caused by Ca\(^{2+}\) release through RyR channels, which are not in Ca\(^{2+}\) spark sites (these channels may be located in the central SR rather than at peripheral subsarcolemmal locations). The precise regulation of Ca\(^{2+}\) sparks and RyR channels by local Ca\(^{2+}\) influx and the direct contribution of Ca\(^{2+}\) release to contraction remain to be elucidated.

**Negative Feedback Regulation of Global [Ca\(^{2+}\)] Through Ca\(^{2+}\) Spark Activation of BK\(_{\text{Ca}}\) Channels in Smooth Muscle**

BK\(_{\text{Ca}}\) channels have an estimated single-channel conductance of \(-80\) pS at \(-40\) mV with a physiological K\(^+\) gradient and occur at a density of \(-1\)–4 channels/\(\mu\)m\(^2\) (Table 4) (13, 173, 190). Nelson and colleagues (140) proposed that Ca\(^{2+}\) sparks activate a number of nearby BK\(_{\text{Ca}}\) channels to cause a macroscopic BK\(_{\text{Ca}}\) channel current, which had been previously described as a STOC (140). Simultaneous optical and electrical measurements in isolated smooth muscle cells support the idea that Ca\(^{2+}\) sparks activate STOCs (133, 153, 218). Quantitative analysis of such simultaneous measurements indicates that virtually all Ca\(^{2+}\) sparks are associated with STOCs in arterial myocytes (Fig. 6, A and B) (153). These measurements also indicate that Ca\(^{2+}\) sparks increase the open probability of BK\(_{\text{Ca}}\) channels \(-10^6\)-fold, which is consistent with subsarcolemmal activator [Ca\(^{2+}\)] in the order of 10–100 \(\mu\)M (153). STOCs rise and decay quite rapidly (time to peak, 10–20 ms; half time of decay, 9 ms (140, 153)), and are consistent with the simultaneous activation of at least 15 BK\(_{\text{Ca}}\) channels in a membrane surface area of \(-15\) \(\mu\)m\(^2\) (\(-1\)% of the surface area of an arterial myocyte). In contrast, the Ca\(^{2+}\) spark, as measured with the fluorescent indicator fluo 3, rises from baseline to peak in \(-20\) ms but decays more slowly (half time of decay 50–60 ms (85, 140, 153)) (Tables 2 and 3). These results indicate that the RyR channels that cause Ca\(^{2+}\) sparks are located very close to the sarcolemma BK\(_{\text{Ca}}\) channels. Disparities in the peak [Ca\(^{2+}\)] of a Ca\(^{2+}\) spark measured with fluo 3 (up to 0.5 \(\mu\)M) and the Ca\(^{2+}\) requirement for an associated increase in BK\(_{\text{Ca}}\) channel activity (local Ca\(^{2+}\) may actually reach levels of 10–100 \(\mu\)M), as well as kinetic differences between Ca\(^{2+}\) sparks and STOCs, suggest that the fluorescent Ca\(^{2+}\) indicator is unable to accurately track Ca\(^{2+}\) in the subsarcolemmal space. This is consistent with the known chemical properties of fluo 3.

Ca\(^{2+}\) sparks cause up to 20 mV hyperpolarizations of isolated arterial myocytes through activation of BK\(_{\text{Ca}}\) channels (Fig. 8, A and B, and Table 4) (52). Figure 8A illustrates a simultaneous measurement of Ca\(^{2+}\) sparks and associated membrane potential hyperpolarizations in an isolated cerebral artery myocyte. Smooth muscle cells are electrically coupled in the intact tissue, such that asynchronized hyperpolarizations that are caused by Ca\(^{2+}\) sparks should summate and lead to a graded level of hyperpolarization (see Fig. 8, B and C) (48). Ca\(^{2+}\) spark-induced membrane potential hyperpolarizations contribute significantly to the membrane potential of isolated smooth muscle cells. For example, in Fig. 8B, Ca\(^{2+}\) spark-induced hyperpolarizations caused an average membrane potential change of \(-15\) mV. Ryanodine caused a 15-mV depolarization by inhibiting the transient hyperpolarizations (Fig. 8B). In support of this mechanism, inhibition of Ca\(^{2+}\) sparks or BK\(_{\text{Ca}}\) channels causes a membrane potential depolarization of \(-10\) mV in pressurized cerebral arteries (Fig. 8C) (103), which leads to an elevation in global [Ca\(^{2+}\)] and vasoconstriction (61, 103, 140, 157). The effects of blocking Ca\(^{2+}\) sparks and BK\(_{\text{Ca}}\) channels on membrane potential, arterial wall [Ca\(^{2+}\)], and diameter are not additive, supporting the idea that Ca\(^{2+}\) sparks regulate BK\(_{\text{Ca}}\) channel activity (61, 103, 140, 157). These results support the concept that Ca\(^{2+}\) sparks, in part, regulate vascular tone, in a negative feedback manner, through activation of BK\(_{\text{Ca}}\) channels (19, 48, 61, 85, 86, 103, 140, 153, 157).
Positive Feedback Regulation of Global [Ca$^{2+}$]_i Through Ca$^{2+}$ Spark Activation of Cl$\text{Ca}$ Channels in Smooth Muscle

Some types of smooth muscle, but not all, express Cl$\text{Ca}$ channels. To date, Cl$\text{Ca}$ currents have been described in smooth muscle cells from rat (206) and rabbit (68, 205) pulmonary artery, rabbit ear artery (3, 4), rabbit (114) and porcine (101) coronary artery, rat renal artery (64), human mesenteric artery (100, 101), rat (A7r5) (156, 199) and pig (46) aorta, rabbit (28, 203, 204) and rat portal vein (151), guinea pig mesenteric vein (197), rat small intestine (84, 144), rabbit colon (182), canine (87, 89) and guinea pig (87–89) trachea, rabbit esophagus (2), guinea pig uterus (40), rat myometrium (8), and rat anococcygeus (27). Cl$\text{Ca}$ channels are activated by increases in [Ca$^{2+}$]_i with a half-maximal activation at 365 nM and full activation around 600 nM (150) and have a single-channel conductance of 2.6 pS (Table 4) (100). The simultaneous activation of a number of Cl$\text{Ca}$ channels underlies the spontaneous transient inward currents (“STICs”) first described in tracheal (87) and portal vein smooth muscle cells (203) (for a review see Ref. 116). STICs are activated by Ca$^{2+}$ sparks (218) in a manner similar to that of STOCs. Interestingly, the same Ca$^{2+}$ sparks that activate STICs also activate STOCs in these cells, indicating that Cl$\text{Ca}$ channels and BK$\text{Ca}$ channels are colocalized in this preparation (tracheal smooth muscle) (218). Figure 9 reproduced from ZhuGe et al. (218) illustrates the biphasic nature of the current activated by a Ca$^{2+}$ spark, which initially evokes an outward current due to the activation of BK$\text{Ca}$ channels, followed by a long-lasting inward current due to the activation of Cl$\text{Ca}$ channels (218).

There are some notable differences in the properties of STOCs and STICs. First, the apparent number of

---

**Fig. 8.** Ca$^{2+}$ sparks hyperpolarize the membrane potential of arterial smooth muscle. A: Ca$^{2+}$ sparks elicit transient membrane hyperpolarization. Simultaneous recording of Ca$^{2+}$ sparks (top) and membrane potential (bottom) in an isolated cerebral artery smooth muscle cell. Changes in subcellular Ca$^{2+}$ fluorescence were recorded using a laser scanning confocal microscope and the Ca$^{2+}$-sensitive dye fluo 3. Top: fluorescence ratio recording of 2 Ca$^{2+}$ sparks that occur at a single Ca$^{2+}$-spark site. Bottom: simultaneous patch-clamp recording from same cell under current clamp. Both Ca$^{2+}$ sparks elicit spontaneous transient membrane potential hyperpolarizations (STHs) due to activation of BK$\text{Ca}$ channels (L. F. Santana and M. T. Nelson, unpublished observations). B: STHs contribute to membrane potential hyperpolarization in arterial smooth muscle cells and are inhibited by application of 10 µM ryanodine. STHs were observed in an isolated cerebral artery smooth muscle cell, under current clamp, using perforated-patch configuration of patch-clamp technique. Data were acquired using an Axopatch 200 amplifier (Axon Instruments) at 2 kHz and subsequently filtered at 500 Hz with a bessel filter. Mean contribution of STHs to membrane potential (red line), obtained by averaging 5,000 adjacent data points, was –15 mV. Application of 10 µM ryanodine inhibited STHs and reduced mean STH contribution to membrane potential. Green and blue dotted lines illustrate the basal membrane potential and peak Ca$^{2+}$-spark-induced transient membrane potentials, respectively. Ryanodine (10 µM) induced membrane potential depolarization due to a reduction of STHs and the impact of these events on mean membrane potential (red line). Depolarization was not due to a steady-state depolarization of basal membrane potential (green dotted line) (J. H. Jaggar and M. T. Nelson, unpublished observations). C: ryanodine depolarizes membrane potential of smooth muscle cells in pressurized (60 mmHg) cerebral arteries. Inhibition of Ca$^{2+}$ sparks by application of 10 µM ryanodine depolarizes membrane potential of a pressurized (60 mmHg) cerebral artery by 9 mV (103). [Modified from Knot et al. (103).] STHs are not observed in this cell, due presumably to cell-cell contact between smooth muscle cells in the artery. i.e., hyperpolarization due to individual Ca$^{2+}$ sparks is averaged throughout the artery.
channels involved in STOCs and STICs may be very different. The amplitudes of STICs and STOCs are similar (with similar driving forces for Cl\(^-\) and K\(^+\); e.g., Fig. 9), even though the unitary conductance of the BK\(_{Ca}\) channel (80 pS) is ~30-fold greater than the Cl\(_{Ca}\) channel (2.6 pS (100); for reviews see Refs. 31, 116, 141). Therefore, a STIC appears to represent the activation of at least 600 Cl\(_{Ca}\) channels by a Ca\(^{2+}\) spark. Cl\(_{Ca}\) channels may be clustered above Ca\(^{2+}\) spark sites, since tracheal myocytes have <10,000 Cl\(_{Ca}\) channels/cell (116), and a STIC represents the activation of at least 5% of the Cl\(_{Ca}\) channels in 0.25% of the membrane surface area in this preparation. Second, the decay of STICs is much slower than the decay of STOCs and mirrors the decay of the fluorescence transient (see Figs. 6 and 9) (218).

The precise effect of the simultaneous activation of Cl\(_{Ca}\) and BK\(_{Ca}\) channels by a Ca\(^{2+}\) spark on membrane

Fig. 9. A single Ca\(^{2+}\) spark activates biphasic currents (STOICs) in isolated voltage-clamped tracheal myocytes. (Modified from ZhuGe et al. (218).) For experimental protocol see Ref. 218. A: time course of a single Ca\(^{2+}\) spark acquired at times designated in B (top). Images in bottom row show entire cell. Contour plots in top row show spark at higher spatial resolution. Bar = 5 \(\mu\)m. B: top: time course of the change in [Ca\(^{2+}\)] in the one pixel (333 nm \(\times\) 333 nm) where peak [Ca\(^{2+}\)] is reached during the course of the Ca\(^{2+}\) spark. Bottom: current simultaneously recorded at a holding potential (HP) of \(-30\) mV. Transient outward current is due to Ca\(^{2+}\) spark activation of BK\(_{Ca}\) channels, whereas longer inward current represents Cl\(_{Ca}\) channel activation.
potential would depend on a number of factors, including the intrinsic voltage dependence of ClCa (none) and BKCa channels [open probability changes about e-fold for 12-14 mV (14, 115)] and the voltage and Ca2+ dependence of other conductances in the cell membrane (Table 4). It is curious that ClCa channels and STICs have only been reported in a handful of smooth muscle preparations [rabbit portal vein (77, 78, 203), rabbit pulmonary artery (76), and canine and guinea pig tracheal myocytes (87, 218, and see Ref. 116)], suggesting that these channels subserve a functional role unique to these tissues. The coexistence of ClCa and BKCa channels in the sarcolemmal membrane adjacent to Ca2+ spark sites suggests another level of fine control of membrane excitability through Ca2+ sparks (see Figs. 2 and 9). Indeed, differential modulation of ClCa and BKCa channels by vasoactive substances would provide a means to alter the control of membrane potential by Ca2+ sparks. For example, vasoconstrictor-induced activation of ClCa channels and inhibition of BKCa channels (see Decrease in Ca2+ Spark Frequency by PKC) could be a mechanism to enhance membrane excitability.

The decay of STICs [t1/2 = 65-182 ms (89, 218)] is much slower than the decay of STOCs [t1/2 = 9-43 ms (153, 218)] and is similar to the decay of Ca2+ sparks as measured with the fluorescent indicator fluo 3 (see Figs. 6 and 9) (218). This observation is consistent with the idea that the BKCa channels (STOCs) are activated by high (10–100 µM) local Ca2+ (Fig. 6). The rapid decay of the STOC is determined by dissipation of this local Ca2+ following closure of the RyR channels. In contrast, ClCa channels are saturated with Ca2+ above 1 µM, and these currents do not decay until [Ca2+]i falls below 1 µM, which are concentrations measured accurately by fluo 3. Interestingly, whole cell ClCa currents decay much faster than whole cell Ca2+ transients, as the decay of the current is determined by CAM kinase-induced inactivation of ClCa channels (207). Therefore, the decay of a STIC appears to be determined by the decline in [Ca2+]i, whereas the decay of the whole cell ClCa currents is regulated by [Ca2+]i and CAM kinase.

FM and AM of Ca2+ Sparks: Physiological Implications

A number of factors regulate the apparent cytoplasmic Ca2+ sensitivity of RyR channels, including phosphorylation state, pharmacological agents, and the SR Ca2+ load (Fig. 7). Modulation of RyR channels is manifest as a change in Ca2+ spark frequency and/or amplitude. Ca2+ spark amplitude should depend on the open time of the RyR channels and the Ca2+ load of the SR, i.e., the driving force for Ca2+ (165). Increasing SR Ca2+ load (219) or elevating cAMP or cGMP (157) leads to an increase in Ca2+ spark frequency in smooth muscle, without elevating global [Ca2+]i. Activators of protein kinase C (PKC) have the opposite effect (19).

FM and AM of Ca2+ sparks by SR Ca2+ load

Increases in the cytoplasmic [Ca2+]i increase the open probability of smooth muscle RyR channels (Fig. 4 and 7) through a direct effect on cytosolic Ca2+-activating sites on the RyR channel protein (71). SR luminal [Ca2+] also modulates RyR channel activity, producing profound effects on both the frequency and the amplitude of Ca2+ sparks in smooth muscle (219). Skeletal and cardiac muscle RyR channels, incorporated into planar lipid bilayers, are activated by increases in luminal [Ca2+]i (for reviews see Refs. 42, 51, 184). It has been proposed that skeletal and cardiac muscle RyR channels have luminal Ca2+ binding sites that interact with regulatory sites located in the cytosol, since luminal Ca2+ will only activate RyR channels when cytosolic ATP is present (174, 175). Recent evidence indicates that luminal Ca2+ that passes through the RyR channel may activate by interaction with cytosolic binding sites (72, 192). Regardless of the mechanism, increasing SR luminal Ca2+ elevates RyR channel open probability in bilayers (42) and Ca2+ spark frequency in intact myocytes (219).

In stomach smooth muscle cells isolated from the toad Bufo marinus, simultaneous measurements of SR [Ca2+], cytoplasmic [Ca2+]i, and Ca2+ sparks (or STOCs) have provided new insights into the relationship between SR Ca2+ load and Ca2+ spark properties (219). After emptying the SR with a bolus of caffeine, the frequency of Ca2+ sparks and STOCs increased steeply with the SR [Ca2+]i (219), as has been reported in heart (30, 36, 164). Therefore, SR Ca2+ load appears to be an important regulator of Ca2+ spark frequency and amplitude in smooth muscle. However, the mechanism by which SR Ca2+ regulates Ca2+ spark properties in smooth muscle remains to be elucidated.

Increase in Ca2+ Spark Frequency by cGMP and cAMP

Nitric oxide, a potent vasodilator produced by both the vascular endothelium and by clinically used nitrovasodilators, activates guanylyl cyclase, leading to increased production of cGMP and stimulation of cGMP-dependent protein kinase (PKG) (119). Other endogenous smooth muscle relaxants coupled to Gs proteins (e.g., calcitonin gene-related peptide, adenosine, β-adrenergic receptor agonists) activate adenyl cyclase and increase cAMP, resulting in the stimulation of cAMP-dependent protein kinase (PKA). Several mechanisms of action have been proposed for PKG and PKA, including increased Ca2+ uptake into the SR and activation of BKCa channels (158, 161, 186, 212). Indeed, cGMP- and cAMP-mediated relaxations in many types of smooth muscle are, in part, through activation of BKCa channels, causing membrane potential hyperpolarization, which closes voltage-dependent Ca2+ channels and leads to smooth muscle relaxation (see Table 5) (6, 7, 17, 32, 91, 92, 96, 107, 135, 146, 152, 157–159, 168, 186, 189, 201, 212).

Smooth muscle relaxants that increase cGMP and cAMP have been shown to activate BKCa channels through direct phosphorylation effects on the channel protein (108, 161, 191, 208) and through elevation of Ca2+ spark frequency (86, 157). Agents that elevate cGMP or cAMP increase Ca2+ spark frequency, and therefore BKCa channel activity, threefold (86, 157). The
Table 5. Inhibitors of BK<sub>c</sub> channels attenuate the relaxation to agents that increase cAMP or cGMP

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Relaxant</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral artery</td>
<td>Forskolin, SIN-1, SNP, 8-BrcGMP, zaprinast, Adenosine, DBcAMP, Forskolin, DBaOMP</td>
<td>IbTX-sensitive relaxation (157) ChTX-sensitive relaxation (146)</td>
</tr>
<tr>
<td>Coronary artery</td>
<td>Adenosine, isoproterenol, forskolin, SNP, ACh, ANP, 8-BrcGMP, SNP</td>
<td>IbTX- and TEA&lt;sup&gt;+&lt;/sup&gt; (1 mM)-sensitive relaxation (152) IbTX- and ChTX-sensitive relaxation (186) IbTX- and TEA&lt;sup&gt;+&lt;/sup&gt; (1 mM)-sensitive relaxation (158) IbTX- and TEA&lt;sup&gt;+&lt;/sup&gt; (1 mM)-sensitive relaxation (159) IbTX-sensitive relaxation and hyperpolarization (212)</td>
</tr>
<tr>
<td>Mesenteric artery</td>
<td>SNP, SNAP, ANP, 8-BrcGMP, Nitroglycerine, NO, ACh</td>
<td>TEA&lt;sup&gt;+&lt;/sup&gt; (1 mM)-sensitive relaxation (32) IbTX-sensitive relaxation (189) IbTX- and ChTX-sensitive relaxation (96)</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>NO, 8-BrcGMP, zaprinast</td>
<td>ChTX- and TEA&lt;sup&gt;+&lt;/sup&gt; (10 mM)-sensitive relaxation (7) IbTX- and ChTX-sensitive relaxation (17)</td>
</tr>
<tr>
<td>Aorta</td>
<td>SIN-1, SNAP, TEA&lt;sup&gt;+&lt;/sup&gt;</td>
<td>IbTX- and ChTX-sensitive relaxation (146) IbTX-sensitive relaxation (92)</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>SIN-1, SNAP, TEA&lt;sup&gt;+&lt;/sup&gt;</td>
<td>IbTX- and ChTX-sensitive relaxation (17) ChTX-sensitive relaxation (91) IbTX-sensitive relaxation (92)</td>
</tr>
<tr>
<td>Tail artery</td>
<td>Iloprost, Sp-dBIMPS</td>
<td>IbTX- and ChTX-sensitive relaxation (17) ChTX-sensitive relaxation (135)</td>
</tr>
<tr>
<td>Trachea</td>
<td>Isoproterenol, salbutamol, aminophylline, AcMP, SNP</td>
<td>IbTX- and ChTX-sensitive relaxation (17) ChTX-sensitive relaxation (135)</td>
</tr>
<tr>
<td>Human airway</td>
<td>Isoproterenol</td>
<td>ChTX-sensitive relaxation (201)</td>
</tr>
<tr>
<td>Mesenteric lymphatic vessel</td>
<td>Isoproterenol, forskolin</td>
<td>ChTX-sensitive relaxation (201)</td>
</tr>
</tbody>
</table>

SiN, 1,3-morpholinosydnonimine; SNP, sodium nitroprusside; 8-BrcGMP, 8-bromoguanosine 3',5'-cyclic monophosphate; DBcAMP, dibutyryl cAMP; TEA<sup>+</sup>, tetracyethylammonium; ANP, atrial natriuretic peptide; SNMP, S-nitroso-N-acetylpenicillamine; Sp-dBIMPS, (Sp)-5,6-dichloro-1-ß-p-ribofuranosylbenzimidazole-3',5'-cyclic monophosphorothioate; NO, authentic nitric oxide; AcMP, N-formyl-2'-O-adenosine 3',5'-cyclic monophosphate; IbTX, iberiotoxin; ChTX, charybdotoxin.

direct effect of PKG and PKA on BK<sub>c</sub> channel activity in intact cells appears to be much smaller (1.3-fold) than the activation caused by increased Ca<sup>2+</sup> spark frequency. These results suggest that cyclic nucleotide-mediated smooth muscle relaxation may occur partially through an increase in the frequency of Ca<sup>2+</sup> sparks and a subsequent increase in the activity of BK<sub>c</sub> channels.

An increase in Ca<sup>2+</sup> spark frequency following elevation of cGMP and cAMP may be due to phosphorylation of RyR channels, phospholamban, or an unknown regulatory protein (Figs. 7 and 10). Phospholamban, when phosphorylated by PKG or PKA, dissociates from the components of RyR channels, phospholamban, or an unknown regulatory protein (Figs. 7 and 10). Phospholamban, when phosphorylated by PKG or PKA, dissociates from the components of RyR channels, phospholamban, or an unknown regulatory protein (Figs. 7 and 10). Phospholamban, when phosphorylated by PKG or PKA, dissociates from the components of RyR channels, phospholamban, or an unknown regulatory protein (Figs. 7 and 10).

PKC may also contribute to the action of vasoconstrictors on smooth muscle through a novel mechanism, by decreasing the frequency of Ca<sup>2+</sup> sparks, which would lead to membrane depolarization and activation of voltage-dependent Ca<sup>2+</sup> channels (Fig. 10) (19). Activators of PKC, phorbol 12-myristate 13-acetate and sn-1,2-dioctanoyl-glycerol, decreased Ca<sup>2+</sup> spark and STOC frequency by ~60% in isolated cells from rat cerebral arteries (19). Activators of PKC also decreased STOC amplitude by ~20%, which could be explained by a direct inhibition of BK<sub>c</sub> channels (Fig. 10) (19). The inhibitory effect of PKC activators on Ca<sup>2+</sup> spark frequency does not involve a reduction of SR Ca<sup>2+</sup> load, suggesting that PKC directly inhibits RyR channels (Fig. 10) (19). The precise molecular mechanisms of PKC inhibition of Ca<sup>2+</sup> sparks remains to be determined.

IP<sub>3</sub> induced Ca<sup>2+</sup> release has been described in terms of a hierarchical nomenclature based on the spatiotemporal characteristics of the observed events. Fundamental Ca<sup>2+</sup> release through a single IP<sub>3</sub> receptor has been termed a blip (amplitude, 20–30 nM; spread, 1–3 µm; decay (t<sub>1/2</sub>) = 45 ms (22)), whereas intermediate release...

Downloaded from http://ajpcell.physiology.org/ by 10.220.33.3 on August 3, 2017.
due the recruitment of blips are termed Ca$^{2+}$ puffs (22, 216). Propagating Ca$^{2+}$ waves occur due to the summation of these smaller events. Although these elementary and intermediate events have not been described in smooth muscle cells, application of an agonist that elevates cytoplasmic levels of IP$_3$ should activate fundamental Ca$^{2+}$ release through IP$_3$ receptors.

Vasoconstrictors have been shown to increase Ca$^{2+}$ wave frequency and amplitude in the smooth muscle cells of rat tail artery, an effect that has been attributed to the activation of IP$_3$ receptors by IP$_3$, and subsequent activation of RyR channels by Ca$^{2+}$ released through IP$_3$ receptors (81). IP$_3$-induced Ca$^{2+}$ release would increase the cytoplasmic [Ca$^{2+}$] and would tend to increase Ca$^{2+}$ spark frequency, particularly if RyR channels are situated close to IP$_3$ receptors and sense the high local [Ca$^{2+}$]. However, the reduction in SR Ca$^{2+}$ load would tend to decrease Ca$^{2+}$ spark frequency (219). The extent to which IP$_3$-induced Ca$^{2+}$ release increases or decreases Ca$^{2+}$ spark activity (16, 52, 98, 106) would depend on a balance between an increase in Ca$^{2+}$ near the cytoplasmic face of the RyR channels and the reduction in stored Ca$^{2+}$ that would follow. It remains to be resolved how PKC and IP$_3$ affect the cytoplasmic and SR luminal communication between RyR channels and IP$_3$ receptors.

CONCLUSIONS

Ca$^{2+}$ sparks occur in a wide variety of smooth muscle types and are caused by the activation of a cluster of RyR channels. In smooth muscle, the majority of Ca$^{2+}$ sparks occur very close to the cell membrane, consistent with a role in signaling ion channels in the plasma membrane. The close proximity of Ca$^{2+}$ spark sites to the plasma membrane support earlier studies indicating that, under certain circumstances, subsarcolemmal [Ca$^{2+}$] could be significantly higher than global [Ca$^{2+}$], (215). Further support for this idea has come from a demonstrated dissociation between global [Ca$^{2+}$], and BK$_{Ca}$ channel currents, which should be a measure of subsarcolemmal [Ca$^{2+}$] (56, 153, 181).

Voltage-dependent Ca$^{2+}$ channels, Ca$^{2+}$ sparks (RyR channels), and BK$_{Ca}$ channels act as a functional unit to regulate smooth muscle function (Figs. 1 and 2). In some cases Cl$_{Ca}$ channels may be regulated by Ca$^{2+}$ sparks, adding another level of control to smooth muscle membrane potential (Fig. 2). Apamin-sensitive SK$_{Ca}$ channels are also expressed in some types of smooth muscle (Table 4), and the effect of Ca$^{2+}$ sparks on SK$_{Ca}$ channels remains to be established.

Ca$^{2+}$ entry through dihydropyridine-sensitive, voltage-dependent Ca$^{2+}$ channels regulates Ca$^{2+}$ spark
properties. The speed and efficacy of communication of voltage-dependent Ca\(^{2+}\) channels to activate Ca\(^{2+}\) sparks depends on proximity, and this issue is not resolved for smooth muscle. In contrast, it is clear that Ca\(^{2+}\) sparks in cardiac muscle are activated by local Ca\(^{2+}\) entry through voltage-dependent Ca\(^{2+}\) channels (see Fig. 1). Ca\(^{2+}\) spark communication to BK\(_{\text{Ca}}\) channels is local, and the activator [Ca\(^{2+}\)] for BK\(_{\text{Ca}}\) channels likely reaches 10–100 µM (153) within the 20-nm gap between the SR and plasma membrane. Activation of BK\(_{\text{Ca}}\) channels by Ca\(^{2+}\) sparks causes membrane potential hyperpolarization (e.g., Fig. 8), which doses voltage-dependent Ca\(^{2+}\) channels, leading to a decrease in global intracellular [Ca\(^{2+}\)] and smooth muscle relaxation (Figs. 1 and 2) (102, 103, 140, 157). This negative-feedback pathway may be of particular importance for tonic smooth muscle.

The ability of Ca\(^{2+}\) entry through voltage-dependent Ca\(^{2+}\) channels to stimulate Ca\(^{2+}\) release through RyR channels and contribute to a global Ca\(^{2+}\) transient seems to be quite variable and is not consistent with local Ca\(^{2+}\) controlling Ca\(^{2+}\) release. The precise mechanisms and significance of Ca\(^{2+}\)-induced Ca\(^{2+}\) release to global Ca\(^{2+}\) transients in smooth muscle are still unclear. It is likely that CICR does contribute Ca\(^{2+}\) for contraction in some types of phasic smooth muscle. Nonetheless, blocking Ca\(^{2+}\) release with ryanodine contracts most types of smooth muscle (10, 11, 38, 39, 44, 103, 105, 122, 140, 155, 209–211), suggesting that RyR channel-mediated negative-feedback control of [Ca\(^{2+}\)] dominates in smooth muscle (Fig. 2). It is possible that local Ca\(^{2+}\) release through RyR channels (Ca\(^{2+}\) sparks) provides a means for Ca\(^{2+}\) extrusion, thus decreasing cytoplasmic Ca\(^{2+}\) (the SBB hypothesis). RyR channels could exert negative-feedback control of [Ca\(^{2+}\)], through membrane potential hyperpolarization caused by activation of BK\(_{\text{Ca}}\) channels (Fig. 2) and SK\(_{\text{Ca}}\) channels and through inactivation of voltage-dependent Ca\(^{2+}\) channels (Fig. 2).

Modulation of Ca\(^{2+}\) spark frequency and amplitude by smooth muscle relaxants and contractors appears to regulate smooth muscle membrane potential and hence contractility. PKA, PKG, and PKC have especially prominent roles in frequency and amplitude modulation of Ca\(^{2+}\) sparks. These kinases appear to exert coordinated and concerted actions on the key elements of Ca\(^{2+}\) signaling, including voltage-dependent Ca\(^{2+}\) channels, RyR channels, phospholamban, and BK\(_{\text{Ca}}\) channels (see Fig. 10). The exact molecular targets that determine the frequency and amplitude modulation of Ca\(^{2+}\) sparks by different kinases are still unresolved. However, it is clear that modulation of Ca\(^{2+}\) sparks provides a powerful means to regulate smooth muscle function.

We thank Adrian D. Bonev, Joseph Brayden, Laura Cartin, Del-Rae Eckman, Thomas J. Heppner, Gerald Herrera, David Hill-Eubanks, and George C. Wellman for helpful comments on the manuscript. We also thank the following persons who have contributed to research on smooth muscle Ca\(^{2+}\) sparks at the University of Vermont: Adrian D. Bonev, Heping Cheng, Maik Gallasch, Thomas J. Heppner, Gerald Herrera, Thomas Kleppisch, Harn J. Knot, J. B. Patlait, Guillermo J. Perez, Michael Rubart, L. Fernando Santana, Andrá S. Stevenson, and George C. Wellman. Unpublished observations have been kindly provided by Drs. George C. Wellman and L. Fernando Santana.

This review was supported by National Institutes of Health Grants HL-44455, HL-51728, and DK-53832 (to M. T. Nelson) and HL-25675, HL-36974, and HL-61602 (to W. J. Lederer), by National Science Foundation Grants IBN-9631416 and BIR-9601682 (to M. T. Nelson), and by fellowships from the American Heart Association, New England (to H. J.) and Vermont (to V.A. Porter) affiliates. Present address of V. A. Porter: Dept. of Pediatrics, Univ. of Minnesota, 420 Delaware St. SE, Minneapolis, MN 55455.

Address for reprint requests and other correspondence: M. T. Nelson, Dept. of Pharmacology, College of Medicine, The Univ. of Vermont, Burlington, VT 05405 (E-mail: nelson@salus.med.uvm.edu).

REFERENCES


93. Khan, S. A., W. R. Mathews, and K. D. Meisner. Role of calcium-activated K⁺ channels in vasodilation induced by nitro-


149. Piacaud, P., G. Loirand, G. Gregoire, C. Mironneau, and J. Mironneau. Calcium-depending of the calcium-activated chlo-

150. Piacaud, P., G. Loirand, G. Gregoire, C. Mironneau, and J. Mironneau. Calcium-depending of the calcium-activated chlo-

151. Piacaud, P., G. Loirand, G. Gregoire, C. Mironneau, and J. Mironneau. Calcium-depending of the calcium-activated chlo-

152. Piacaud, P., G. Loirand, G. Gregoire, C. Mironneau, and J. Mironneau. Calcium-depending of the calcium-activated chlo-


Downloaded from http://ajpcell.physiology.org/ on 10/22/2013 by 3.33.10.220 on August 3, 2017


