Voltage dependence of the coupling of Ca\textsuperscript{2+} sparks to BK\textsubscript{Ca} channels in urinary bladder smooth muscle

Gerald M. Herrera, Thomas J. Heppner, and Mark T. Nelson

Departments of Molecular Physiology and Biophysics and Pharmacology, University of Vermont College of Medicine, Burlington, Vermont 05405

Received 11 August 2000; accepted in final form 2 October 2000

Herrera, Gerald M., Thomas J. Heppner, and Mark T. Nelson. Voltage dependence of the coupling of Ca\textsuperscript{2+} sparks to BK\textsubscript{Ca} channels in urinary bladder smooth muscle. Am J Physiol Cell Physiol 280: C481–C490, 2001.—Large-conductance Ca\textsuperscript{2+}-dependent K\textsuperscript{+} (BK\textsubscript{Ca}) channels play a critical role in regulating urinary bladder smooth muscle (UBSM) excitability and contractility. Measurements of BK\textsubscript{Ca} currents and intracellular Ca\textsuperscript{2+} revealed that BK\textsubscript{Ca} currents are activated by Ca\textsuperscript{2+} release events (Ca\textsuperscript{2+} sparks) from ryanodine receptors (RyRs) in the sarcoplasmic reticulum. The goals of this project were to characterize Ca\textsuperscript{2+} sparks and BK\textsubscript{Ca} currents and to determine the voltage dependence of the coupling of Ca\textsuperscript{2+} sparks to BK\textsubscript{Ca} channels in UBSM. Ca\textsuperscript{2+} sparks in UBSM had properties similar to those described in arterial smooth muscle. Most Ca\textsuperscript{2+} sparks caused BK\textsubscript{Ca} currents at all voltages tested, consistent with the BK\textsubscript{Ca} channels sensing ~10 \textmu M Ca\textsuperscript{2+}. Membrane potential depolarization from -50 to -20 mV increased Ca\textsuperscript{2+} spark and BK\textsubscript{Ca} current frequency threefold. However, membrane depolarization over this range had a differential effect on spark and current amplitude, with Ca\textsuperscript{2+} spark amplitude increasing by only 30% and BK\textsubscript{Ca} current amplitude increasing 16-fold. A major component of the amplitude modulation of spark-activated BK\textsubscript{Ca} current was quantitatively explained by the known voltage dependence of the Ca\textsuperscript{2+} sensitivity of BK\textsubscript{Ca} channels. We, therefore, propose that membrane potential, or any other agent that modulates the Ca\textsuperscript{2+} sensitivity of BK\textsubscript{Ca} channels, profoundly alters the coupling strength of Ca\textsuperscript{2+} sparks to BK\textsubscript{Ca} channels.

Guinea pig; sarcoplasmic reticulum; ryanodine receptor; ibotenate; thapsigargin; large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels

Large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (BK\textsubscript{Ca}) channels play a key role in regulating the excitability and contractility of urinary bladder smooth muscle (UBSM). Blocking BK\textsubscript{Ca} channels with iberiotoxin dramatically increases the amplitude and duration of action potentials (11) and phasic contractions (12) in UBSM.

BK\textsubscript{Ca} channel activity in UBSM depends primarily on two factors: voltage and Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) (21). Elevation of [Ca\textsuperscript{2+}] and depolarization increase the open probability of BK\textsubscript{Ca} channels from UBSM cells. The UBSM action potential is a potent activator of BK\textsubscript{Ca} channels through membrane depolarization and elevation of Ca\textsuperscript{2+} entry through dihydropyridine-sensitive, voltage-dependent Ca\textsuperscript{2+} channels (VDCCs) (11). BK\textsubscript{Ca} channels are also activated by Ca\textsuperscript{2+} release, in the form of Ca\textsuperscript{2+} sparks. Ca\textsuperscript{2+} sparks are local Ca\textsuperscript{2+} transients caused by the opening of ryanodine-sensitive Ca\textsuperscript{2+} release channels [referred to as ryanodine receptors (RyRs)] in the sarcoplasmic reticulum (SR) membrane (22). In smooth muscle cells from rat cerebral arteries, every Ca\textsuperscript{2+} spark activates nearby BK\textsubscript{Ca} channels to cause a transient K\textsuperscript{+} current (24). In this preparation, each Ca\textsuperscript{2+} spark increases the open probability of BK\textsubscript{Ca} channels 10\textsuperscript{-4} to 10\textsuperscript{-6}-fold, indicating that the Ca\textsuperscript{2+} spark delivers 10–100 \textmu M Ca\textsuperscript{2+} to the nearby BK\textsubscript{Ca} channels (1, 24).

Another potentially important effect of membrane potential depolarization is to increase the apparent Ca\textsuperscript{2+} sensitivity of the BK\textsubscript{Ca} channel (5, 6). This effect could theoretically increase the impact of Ca\textsuperscript{2+} sparks on BK\textsubscript{Ca} channel activity, such that a given-size Ca\textsuperscript{2+} spark causes a larger BK\textsubscript{Ca} current transient at depolarized potentials.

Ca\textsuperscript{2+} sparks have recently been described in UBSM. Collier and co-workers (4) observed a process whereby Ca\textsuperscript{2+} sparks in UBSM are evoked by Ca\textsuperscript{2+} entry through VDCCs. However, unlike classical Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release in cardiac muscle, which occurs on the millisecond time scale (3), this process in UBSM was relatively slow and could be attributed to the accumulation of Ca\textsuperscript{2+} through activation of VDCCs, rather than local control by Ca\textsuperscript{2+} entry through a single VDCC (4).

Although membrane potential is thought to regulate the coupling of Ca\textsuperscript{2+} sparks to BK\textsubscript{Ca} channels in smooth muscle, little is known about this important relationship. Membrane potential depolarization could act on the BK\textsubscript{Ca} channels by increasing the driving force for K\textsuperscript{+}, increasing channel open probability, and by increasing channel Ca\textsuperscript{2+} sensitivity. Membrane potential could indirectly affect BK\textsubscript{Ca} channel activity by altering Ca\textsuperscript{2+} spark frequency and amplitude.

The primary goal of the present study is to understand the effect of voltage on the coupling of Ca\textsuperscript{2+} sparks to BK\textsubscript{Ca} channels. To accomplish this, we characterize Ca\textsuperscript{2+} sparks in UBSM and provide the first characterization of the effect of membrane potential on
the coupling of Ca\textsuperscript{2+} sparks to BK\textsubscript{Ca} channels by simultaneously measuring Ca\textsuperscript{2+} sparks and K\textsuperscript{+} currents in isolated UBSM cells. We provide the first evidence that voltage modulates the strength of coupling of Ca\textsuperscript{2+} sparks to BK\textsubscript{Ca} channels, such that Ca\textsuperscript{2+} sparks cause larger BK\textsubscript{Ca} currents as the membrane potential is depolarized. The results can be explained by the voltage dependence of the Ca\textsuperscript{2+} sensitivity of BK\textsubscript{Ca} channels and provide a mechanism for enhanced negative-feedback regulation of UBSM membrane potential by BK\textsubscript{Ca} channels.

MATERIALS AND METHODS

Cell isolation. All procedures were reviewed and approved by the Office of Animal Care Management at the University of Vermont. Guinea pigs (250–350 g) were euthanized by halothane overdose and then exsanguinated. The urinary bladder was removed and placed in a bath solution to remove extraneous fluo 3 from the extracellular fluid. This wash progressed for 20–45 min, allowing the bath solution to remove extraneous fluo 3 from the extracellular fluid. The urinary bladder was cut into small strips (0.5 mm wide, 1 mm long) and transferred to a water-jacketed vial (2 ml volume, 37°C) containing DS supplemented with 1 mg/ml BSA, 1 mg/ml papain (Worthington), and 1 mg/ml dithioerythritol for 35 min. The papain-containing solution was then replaced with fresh DS containing 1 mg/ml BSA, 1 mg/ml collagenase (Fluka), and 100 μM CaCl\textsubscript{2}. The tissue was left in this solution for 5 min (37°C), and then the solution was replaced with cold BSA-containing DS and kept on ice. After two subsequent washes with cold BSA-containing DS, the tissue pieces were passed through the tip of a fire-polished glass Pasteur pipette to free individual cells. Cells were kept on ice until use, generally within 6 h. All experiments were performed at room temperature (22°C).

Confocal fluorescence microscopy. Isolated myocytes were loaded with the Ca\textsuperscript{2+}-sensitive fluorophore fluo 3-AM (Molecular Probes) by mixing 250 μl of cell suspension with 250 μl of bath solution containing (in mM) 134 NaCl, 6 KCl, 1 MgCl\textsubscript{2}, 2 CaCl\textsubscript{2}, 10 glucose, 10 HEPES, and 0.05 EGTA, with pH adjusted to 7.4 with NaOH. This solution was passed through the tip of a fire-polished glass Pasteur pipette to free individual cells. Cells were kept on ice until use, generally within 6 h. All experiments were performed at room temperature (22°C).

Fluorescence measurements. Fluorescence measurements were performed using a single image. Scan durations were 10–20 s. Pharmacological agents were applied to the recording chamber via a gravity-fed reservoir at a flow rate of ~2.5 ml/min. For the cells used in the present study, resting membrane potential was ~36 ± 2 mV, cell capacitance was 35 ± 1 pF, and series resistance was 38 ± 3 MΩ (n = 44 cells). Currents were recorded using an Axopatch 200A amplifier (Axon Instruments) filtered at 0.5–1 kHz and digitized at 1–4 kHz. For pharmacological characterization of transient outward currents in UBSM cells, experiments were conducted at a holding potential of −20 mV. These currents were characterized pharmacologically in a subset of 24 cells for a 5-min period. For control data, the 5-min period immediately before drug application was used for analysis, and to determine the effect of a given drug, a 5-min period was used during which activity was at a steady state. Transient outward currents were analyzed using Mini Analysis (Synaptosoft) with an amplitude threshold of three times the unitary BK\textsubscript{Ca} channel current for guinea pig UBSM cells at the given holding potential (11, 22).

Simultaneous current and Ca\textsuperscript{2+} measurements. To examine the temporal relationship between Ca\textsuperscript{2+} sparks and BK\textsubscript{Ca} channel activation, Ca\textsuperscript{2+} sparks and whole cell currents at holding potentials of −50 to −20 mV were measured simultaneously using the methods described above. All simultaneous current and fluorescence recordings were conducted in a standing bath. A trigger source output on the confocal imaging system allow the majority of the cell to be scanned in a single image. Scan durations were 10–20 s. Pharmacological agents were applied to the test chamber at stated concentrations via a gravity-fed reservoir at a flow rate of ~2.5 ml/min.

Imaging experiments were analyzed with custom software written by Dr. Adrian Bonev using IDL 5.0.2 (Research Systems). Ca\textsuperscript{2+} sparks were defined as local increases in fluorescence of 1.2 F/F\textsubscript{0} (where F is the instantaneous fluorescence at a given time point and F\textsubscript{0} is the baseline fluorescence) that persisted for at least two images. F\textsubscript{0} was obtained by averaging 10 images containing no discernible Ca\textsuperscript{2+} transients. For quantitation of Ca\textsuperscript{2+} sparks, a square-box region (2.2 × 2.2 μm or 10 × 10 pixels) was placed over an area of the cell in which Ca\textsuperscript{2+} sparks were observed, and F/F\textsubscript{0} traces were generated for this region of the cell. Event frequency, amplitude, and kinetics were determined from entire experimental recordings lasting for 10–20 s.

Electrophysiology. A single drop of cell suspension was placed on a glass coverslip in the bottom of a recording chamber (~1 ml volume). Cells were allowed up to 20 min to adhere to the coverslip, and then fresh bath solution (see above for composition) was applied. Whole cell currents were measured using the perforated-patch technique (14). The pipette solution contained (in mM) 110 potassium aspartate, 30 KCl, 10 NaCl, 1 MgCl\textsubscript{2}, 10 HEPES, and 0.05 EGTA, with pH adjusted to 7.2 with NaOH. Amphotericin B (200 μg/ml) was also added to the pipette solution. Pharmacological agents were applied to the recording chamber via a gravity-fed reservoir at a flow rate of ~2.5 ml/min. For the cells used in the present study, resting membrane potential was ~36 ± 2 mV, cell capacitance was 35 ± 1 pF, and series resistance was 38 ± 3 MΩ (n = 44 cells). Currents were recorded using an Axopatch 200A amplifier (Axon Instruments) filtered at 0.5–1 kHz and digitized at 1–4 kHz. For pharmacological characterization of transient outward currents in UBSM cells, experiments were conducted at a holding potential of −20 mV. These currents were characterized pharmacologically in a subset of 24 cells for a 5-min period. For control data, the 5-min period immediately before drug application was used for analysis, and to determine the effect of a given drug, a 5-min period was used during which activity was at a steady state. Transient outward currents were analyzed using Mini Analysis (Synaptosoft) with an amplitude threshold of three times the unitary BK\textsubscript{Ca} channel current for guinea pig UBSM cells at the given holding potential (11, 22).

Chemicals. Unless otherwise stated, all chemicals used in this study were purchased from Sigma Chemical. Rynadine was obtained from L. C. Laboratories.

Calculations and statistics. Values are means ± SE. Data were compared using one-way ANOVA or one- or two-tailed t-test, where appropriate. Student-Newman-Keuls method was used for all pairwise multiple comparisons. P < 0.05 was considered statistically significant.

In experiments where Ca\textsuperscript{2+} sparks were recorded in cells voltage clamped to different membrane potentials, the [Ca\textsuperscript{2+}]\textsubscript{c} associated with a fluo 3 transient (Ca\textsuperscript{2+} spark) was estimated...
using the following equation to normalize for changes in background fluorescence, $F_0$ (global $[\text{Ca}^{2+}]$)

$$[\text{Ca}^{2+}] = \frac{KR}{(K/[\text{Ca}^{2+}]_{\text{rest}} + 1) - R}$$

where $K$ is the apparent affinity of fluo 3 for Ca$^{2+}$ (~400 nM; see 3), $R$ is the fractional fluorescence increase ($F/F_0$), and $[\text{Ca}^{2+}]_{\text{rest}}$ is the cytosolic $[\text{Ca}^{2+}]$ at $F_0$.

RESULTS

Pharmacological characterization of transient outward currents in UBSM cells. Transient outward currents have been previously observed in UBSM cells (9, 15). These currents were proposed to be conducted by BK$_{Ca}$ channels, since tetraethylammonium (1 mM) or iberiotoxin (30 nM) attenuated these currents by 80% (15). Furthermore, transient outward currents in UBSM are thought to be activated by Ca$^{2+}$ released from the SR, since the RyR agonist caffeine has an initial stimulatory effect on these currents (15). However, a detailed analysis of transient outward currents in UBSM has not been performed, so our first objective was to characterize transient outward currents in this tissue and determine the source of Ca$^{2+}$ initiating these electrical events.

Single UBSM cells were held at a potential of $-20$ mV using the perforated-patch configuration of whole cell voltage clamp (see MATERIALS AND METHODS). The frequency of transient BK$_{Ca}$ currents was $1.23 \pm 0.24$ Hz ($n = 24$), and the average amplitude of these currents was $43.2 \pm 9.9$ pA. Iberiotoxin (100 nM), a selective blocker of BK$_{Ca}$ channels (8, 23), inhibited the...
the possibility that the transient currents remaining in the presence of iberiotoxin were conducted by K⁺ channels other than BKCa channels, the potent and selective small-conductance Ca²⁺-dependent K⁺ (SKCa) channel blocker apamin (100 nM) was applied in the presence of 100 nM iberiotoxin. Apamin did not affect these currents (n = 3), suggesting that they are not conducted by SKCa channels (average amplitude of transient currents in the presence of iberiotoxin = 15.6 ± 2.3 pA, average amplitude in the presence of iberiotoxin and apamin = 14.2 ± 1.5 pA, P > 0.05). Furthermore, increasing the concentration of iberiotoxin to 600 nM completely abolished all transient outward current activity in UBSM cells, with an apparent EC₅₀ of 27 nM at −20 mV (n = 3). Thus the transient currents in UBSM can be attributed to activation of BKCa channels.

Transient BKCa currents in other preparations are caused by local Ca²⁺ release (Ca²⁺ sparks) through RyRs in the SR (17, 22). To determine the importance of SR Ca²⁺ release in initiating BKCa currents in UBSM, UBSM cells were treated with thapsigargin (100 nM), which blocks the SR Ca²⁺-ATPase and thereby leads to depletion of SR Ca²⁺ stores (n = 5). Figure 1C shows an original record of BKCa currents measured from a cell before and after thapsigargin. Thapsigargin reduced the BKCa current frequency by 96% (from 1.05 ± 0.26 to 0.04 ± 0.04 Hz, P < 0.05, n = 5; Fig. 1D). This observation suggests that BKCa channels are activated by SR Ca²⁺ release. Next, the role of RyRs in causing transient BKCa currents was examined by treating UBSM cells (n = 9) with ryanodine (10 μM) at a concentration that inhibits RyRs (18, 22, 26). Figure 1E shows an original recording of BKCa currents in a single UBSM cell before and after ryanodine (10 μM) treatment. Within 15 min of application, ryanodine caused a marked reduction in transient BKCa current activity. At steady state, ryanodine reduced BKCa current frequency by 94%, from 1.32 ± 0.57 to 0.09 ± 0.03 Hz (P < 0.05, n = 9 cells; Fig. 1F). These observations suggest that BKCa currents are caused by brief Ca²⁺ release events through RyRs in the SR.

Identification of local Ca²⁺ transients (Ca²⁺ sparks) in UBSM cells. To determine whether localized transient increases in intracellular [Ca²⁺] (l[Ca²⁺]), could be observed that may serve as a stimulus for BKCa channel activity in UBSM cells, myocytes were loaded with the Ca²⁺-sensitive indicator fluo 3 and scanned with a laser-scanning confocal microscope (Fig. 2). Localized transient increases in fluorescence were observed in UBSM cells. The spatial and temporal characteristics of these events suggest that they correspond to Ca²⁺ sparks, which have been described in other types of smooth muscle (17, 22), as well as skeletal (19) and cardiac (3) muscle. An average of 2.9 ± 0.3 spark sites per cell were observed (n = 18). Spark frequency was 0.62 ± 0.08 Hz. The fractional fluorescence increase (F/F₀, see MATERIALS AND METHODS) associated with Ca²⁺ sparks in UBSM cells was 2.12 ± 0.03 (n = 344 sparks from 18 cells). Figure 2A is a pseudocolor image of a single UBSM cell loaded with the fluorescent Ca²⁺ indicator fluo 3. In this cell, transient increases in [Ca²⁺], corresponding to Ca²⁺ sparks, were found in three distinct regions (red, orange, and green boxes).

Pharmacological characterization of Ca²⁺ sparks in UBSM cells. In other preparations, Ca²⁺ sparks are attributed to Ca²⁺ release via RyRs in the SR (3, 17, 19, 22, 27). To examine the nature of the Ca²⁺ sparks in UBSM, cells were treated with thapsigargin (100 nM) to inhibit the SR Ca²⁺-ATPase (n = 6). Figure 3A shows an image of a single fluo 3-loaded UBSM cell before (control) and after application of thapsigargin. Under basal conditions, Ca²⁺ sparks were observed at five sites in this cell (regions a–e, Fig. 3A, left). Fluo-
**Simultaneous measurements of Ca$^{2+}$ sparks and BK$_{Ca}$ currents in UBSM cells.** To examine directly the hypothesis that SR Ca$^{2+}$ release from RyRs activates BK$_{Ca}$ channels in the cell surface, Ca$^{2+}$ spark activity and whole cell membrane currents were recorded simultaneously. Figure 4A shows the simultaneous electrical and fluorescence recordings from a 20-s scan in a UBSM cell held at −40 mV. Each Ca$^{2+}$ spark is associated with a nearly synchronous transient BK$_{Ca}$ current. Larger Ca$^{2+}$ sparks are associated with larger BK$_{Ca}$ currents (see below). The peak of the current preceded the peak of the Ca$^{2+}$ spark by 21.5 ± 4.4 ms (n = 27). The onset time for BK$_{Ca}$ currents and sparks can be determined using the rise times (~16 ms for BK$_{Ca}$ currents, ~35 ms for Ca$^{2+}$ sparks; Table 1), and it is evident that these events start within the imaging resolution (8.33 ms/image). The close temporal relationship (Fig. 4B) between Ca$^{2+}$ sparks and BK$_{Ca}$ currents suggests that SR Ca$^{2+}$ release from RyRs (detected as a change in fluorescence) activates BK$_{Ca}$ channels.

Membrane potential depolarization increases frequency and amplitude of Ca$^{2+}$ sparks and transient BK$_{Ca}$ currents. The relationship between Ca$^{2+}$ sparks and BK$_{Ca}$ currents was assessed at membrane potentials ranging from −50 to −20 mV. Figure 5A shows Ca$^{2+}$ sparks from two sites and the associated membrane currents in a cell held at −50 mV. At this membrane potential, some Ca$^{2+}$ sparks (11 of 55) were observed that were not associated with resolvable BK$_{Ca}$ currents. Figure 5B shows Ca$^{2+}$ sparks from the same cell shown in Fig. 5A and membrane currents at a holding potential of −20 mV. At −20 mV, all Ca$^{2+}$ sparks (n = 127) were associated with BK$_{Ca}$ currents, but many currents (61 of 188) were observed with no detectable fluorescence transients. This result is not unexpected, since only about one-third of the cell volume is scanned with the confocal microscope, whereas current is recorded across the entire cell membrane (24). Thus, although we can detect electrical events associated with nearly every Ca$^{2+}$ spark (94%), we only detect Ca$^{2+}$ sparks that occur in the volume of the cell that is scanned.

Figure 6A illustrates the relationship between holding potential and Ca$^{2+}$ spark and associated BK$_{Ca}$ current frequency. Fewer sparks than transient currents were detected at all potentials positive to −50 mV. A summary of kinetic data for Ca$^{2+}$ sparks and BK$_{Ca}$ currents recorded at various membrane potentials is given in Table 1.

Global [Ca$^{2+}$], increased with depolarization. Baseline whole cell fluorescence at −50 mV was taken as F$_{0}$, and when expressed relative to −50 mV, whole cell
fluorescence (F/F₀) increased with depolarization 1.04 ± 0.17-fold at −40 mV (n = 8), 1.12 ± 0.19-fold at −30 mV (n = 8), and 1.76 ± 0.49-fold at −20 mV (n = 5); these values are consistent with previous measurements of steady-state [Ca²⁺]ᵢ in UBSM (9). Ca²⁺ spark amplitude also increased with depolarization. The fractional increase in fluorescence, F/F₀, of the Ca²⁺ sparks was used to calculate [Ca²⁺]ᵢ during a spark (see Eq. 1).

Values for [Ca²⁺]ᵢ were taken from measurements of [Ca²⁺]ᵢ in voltage-clamped UBSM cells and were 115 nM at −50 mV, 150 nM at −40 mV, 180 nM at −30 mV, and 250 nM at −20 mV (9). Figure 6B shows the increase in Ca²⁺ spark amplitude at depolarized potentials. The increase in spark amplitude and increased global [Ca²⁺]ᵢ suggest that SR Ca²⁺ content increases at depolarized potentials.

Membrane potential depolarization increases the coupling strength of RyRs to BKCa channels. A striking observation is that BKCa current amplitude increases dramatically at depolarized potentials (cf. Fig. 5, A and B). BKCa current amplitude increased from 8.6 ± 0.8 pA at −50 mV to 144.1 ± 14.3 pA at −20 mV (−16-fold). To account for the effects of the Kᵢ driving force on measured BKCa currents, the peak transient BKCa current (I_BK) were divided by the unitary BKCa current (i) (11) at each potential (Fig. 6C). BKCa channel activity, measured as I_BK/i, increased sixfold over the range of −50 to −20 mV (Fig. 6C). The elevation in

---

**Table 1. Summary of BKCa current and Ca²⁺ spark kinetics from simultaneous measurements**

<table>
<thead>
<tr>
<th>Membrane Potential, mV</th>
<th>BKCa Currents</th>
<th>Ca²⁺ Sparks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rise Time, ms</td>
<td>Duration, ms</td>
</tr>
<tr>
<td>−20</td>
<td>18.9 ± 1.8</td>
<td>30.8 ± 2.3</td>
</tr>
<tr>
<td>−30</td>
<td>15.7 ± 1.2</td>
<td>28.5 ± 1.3</td>
</tr>
<tr>
<td>−40</td>
<td>15.9 ± 1.3</td>
<td>27.1 ± 1.9</td>
</tr>
<tr>
<td>−50</td>
<td>15.7 ± 2.1</td>
<td>16.1 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± SE from n individual events measured in 5–8 cells at each membrane potential. BKCa current, large-conductance Ca²⁺-activated K⁺ current; rise time, time from baseline to peak; duration, full width at half-maximum amplitude; t½, half-time for decay determined by a single exponential fit. *P < 0.05 vs. −50 mV.
BKCa current at depolarized potentials could reflect an increase in the Ca\(^{2+}\) released during a Ca\(^{2+}\) spark (Fig. 6B) or an increase in the sensitivity of BKCa channels to Ca\(^{2+}\) sparks, such that a Ca\(^{2+}\) spark of a given amplitude causes a larger transient BKCa channel current at more depolarized voltages.

To explore the issue of the elevation of transient BKCa currents with membrane depolarization, the relationship between peak BKCa channel activity (\(I_{BK}/i\)) and peak [Ca\(^{2+}\)] during a spark was determined at each membrane potential. Peak transient BKCa channel activity, corrected for driving force (\(I_{BK}/i\)), increased with peak [Ca\(^{2+}\)] during a spark at all voltages (Fig. 7). Membrane potential depolarization increased the steepness of the relationship between peak [Ca\(^{2+}\)] during a spark and \(I_{BK}/i\), indicating that a given-size Ca\(^{2+}\) spark is more effective at activating BKCa channels at more positive voltages. Thus the coupling strength of a Ca\(^{2+}\) spark to BKCa channels increased with depolarization.

Fig. 6. Ca\(^{2+}\) spark and BKCa current amplitude and frequency increase with depolarization. A: voltage dependence of Ca\(^{2+}\) spark and BKCa current frequency. \(E_m\), membrane equilibrium potential. B: voltage dependence of Ca\(^{2+}\) spark amplitude (peak spark Ca\(^{2+}\) concentration ([Ca\(^{2+}\)])) minus resting intracellular [Ca\(^{2+}\)]. C: BKCa channel activity (\(I_{BK}/i\), where \(I_{BK}\) is BKCa current and \(i\) is unitary BKCa current) increases with membrane potential depolarization from -50 to -20 mV (\(n=5–8\) cells at each membrane potential).

Fig. 7. Membrane potential depolarization increases the coupling strength of RyRs to BKCa channels. BKCa channel activity (\(I_{BK}/i\)) is plotted as a function of Ca\(^{2+}\) spark amplitude (peak spark [Ca\(^{2+}\)]) at a given membrane potential, fit by least-squares regression (\(n=5–8\) cells at each potential). Peak Ca\(^{2+}\) spark amplitudes were obtained using Eq. 1. Correlation coefficients were 0.75, 0.80, 0.81, and 0.83 at -20, -30, -40, and -50 mV, respectively. Dashed lines, 95% confidence intervals for each fit.
It is possible that the increase in Ca$^{2+}$ spark-induced BK$_{Ca}$ channel activity with depolarization reflects a greater spatial spread of Ca$^{2+}$ sparks, such that a spark would recruit more BK$_{Ca}$ channels on a greater surface of the cell at more positive voltages. Despite the slight increase in Ca$^{2+}$ spark amplitude with depolarization (Fig. 6B), Ca$^{2+}$ spark spatial spread (area of spark at half-maximum amplitude) did not change with membrane potential depolarization (Fig. 8). This observation is in contrast to reports in striated muscle, where larger-amplitude sparks also have a larger spatial profile (10), and suggests that the increased BK$_{Ca}$ channel activity ($I_{BK}/i$) at depolarized potentials cannot be explained by an increased spread of the Ca$^{2+}$ sparks but, instead, is due to a higher activity of BK$_{Ca}$ channels activated by a given-size Ca$^{2+}$ spark.

**BK$_{Ca}$ channel activity in the absence of Ca$^{2+}$ sparks.** To estimate the increase in BK$_{Ca}$ channel activity caused by a Ca$^{2+}$ spark, it is necessary to determine BK$_{Ca}$ channel activity in the absence of sparks. To do this, whole cell membrane currents were recorded (perforated patch) in UBSM cells that were pretreated with thapsigargin (100 nM) for 10 min to eliminate Ca$^{2+}$ sparks (see Ref. 25 for a similar procedure in vascular smooth muscle). UBSM cells were held at 0 mV, and single BK$_{Ca}$ channel activity was recorded. BK$_{Ca}$ channel activity was $0.073 \pm 0.039$ (n = 4; not shown).

**DISCUSSION**

**BK$_{Ca}$ channels as targets for Ca$^{2+}$ sparks in UBSM.** BK$_{Ca}$ channels are key elements in the regulation of UBSM function. BK$_{Ca}$ channels are involved in the repolarization of the action potential (11, 20). The specific BK$_{Ca}$ channel inhibitor iberiotoxin causes a membrane potential depolarization and increases the action potential amplitude, duration, and frequency (11), and this causes a dramatic increase in the amplitude and duration of phasic UBSM contractions (12).

Step depolarization of UBSM cells leads to transient SR Ca$^{2+}$ release, which is associated with activation of Ca$^{2+}$-dependent K$^+$ channels (16). These Ca$^{2+}$ transients (referred to as “hot spots”) coalesced into a global Ca$^{2+}$ transient over the course of ~100 ms and were rarely observed in cells at rest (16). This situation is different from the present study, where Ca$^{2+}$ sparks occurred in unstimulated cells, and had very little effect on global [Ca$^{2+}$]$_i$. However, the observation that SR Ca$^{2+}$ release can activate BK$_{Ca}$ channels provides a possible mechanism to account for the involvement of BK$_{Ca}$ channels in the regulation of the membrane potential.

**Ca$^{2+}$ sparks and SK$_{Ca}$ channels.** Apamin-sensitive SK$_{Ca}$ channels play an important role in regulating UBSM contractility (12). Because these channels are activated by Ca$^{2+}$ in the submicromolar range (13), Ca$^{2+}$ sparks should activate these channels. We found that transient outward currents in UBSM were not sensitive to the SK$_{Ca}$ channel blocker apamin. This finding suggests that apamin-sensitive SK$_{Ca}$ channels are not present in sufficient density near a spark site to generate measurable currents.

**Ca$^{2+}$ sparks dramatically elevate BK$_{Ca}$ channel activity.** The results of the present study have significant implications for communication between RyRs and BK$_{Ca}$ channels. On the basis of our measurements, the increase in BK$_{Ca}$ channel activity during a Ca$^{2+}$ spark can be estimated (24). Dividing the transient BK$_{Ca}$ current by the unitary current gives an estimate for the minimum number of BK$_{Ca}$ channels activated by a Ca$^{2+}$ spark. At ~20 mV, a Ca$^{2+}$ spark activates ~35 BK$_{Ca}$ channels in ~0.3% of the cell membrane (Figs. 6C and 8). The whole cell activity (open channel probability) of BK$_{Ca}$ channels in the absence of sparks is ~10$^{-7}$ at ~20 mV on the basis of our single BK$_{Ca}$ channel measurements in whole cells. Therefore, a Ca$^{2+}$ spark increases the mean activity of BK$_{Ca}$ channels above the spark site 10$^6$-fold at ~20 mV. For such a substantial increase in BK$_{Ca}$ channel activity to occur, [Ca$^{2+}$]$_i$ in the vicinity of BK$_{Ca}$ channels would have to increase ~30-fold, with the assumption that channel activity increases with the fourth power of [Ca$^{2+}$]$_i$ (2). This means that a Ca$^{2+}$ spark increases [Ca$^{2+}$]$_i$ in its local domain from ~250 nM at rest to ~7.5 \mu$M at ~20 mV (see Ref. 9 for resting [Ca$^{2+}$]$_i$ in UBSM). Therefore, the [Ca$^{2+}$]$_i$ sensed by the BK$_{Ca}$ channel is far greater than that reported by the fluorescent indicator fluo 3 (24). To communicate high [Ca$^{2+}$]$_i$ (~10 \mu$M) to the BK$_{Ca}$ channel, the RyRs in a spark site must be close to the BK$_{Ca}$ channels in the surface membrane (7, 17).

**Voltage dependence of coupling strength of RyRs to BK$_{Ca}$ channels.** In the present study, we characterized the relationship between Ca$^{2+}$ sparks and their associated BK$_{Ca}$ currents over a range of membrane potentials (~50 to ~20 mV). Ca$^{2+}$ spark frequency and amplitude increased with depolarization (Fig. 6, A and B). Membrane depolarization from ~50 to ~20 mV elevated global [Ca$^{2+}$] from 115 to 260 nM on the basis of an increase in whole cell fractional fluorescence (resting [Ca$^{2+}$]$_i$, at ~50 mV from Ref. 9). This would lead to an elevation of SR Ca$^{2+}$ content. The elevation of cytoplasmic and SR Ca$^{2+}$ should elevate Ca$^{2+}$ spark frequency (28). An elevation of SR Ca$^{2+}$ would also increase spark amplitude (28).
Membrane potential dramatically impacted the amplitude of the $\text{BK}_{\text{Ca}}$ channel currents activated by a spark (Fig. 6C). Because the $\text{Ca}^{2+}$ sensitivity of the $\text{BK}_{\text{Ca}}$ channel increases with depolarization (5, 6, 21), we propose that changes in the apparent $\text{Ca}^{2+}$ sensitivity of the $\text{BK}_{\text{Ca}}$ channel could underlie the depolarization-induced increase in transient $\text{BK}_{\text{Ca}}$ current amplitude activated by a given-size $\text{Ca}^{2+}$ spark. At each membrane potential, $\text{BK}_{\text{Ca}}$ current amplitude correlated with spark $[\text{Ca}^{2+}]_i$ (Fig. 7). Depolarization caused a steepening of the relationship between $\text{BK}_{\text{Ca}}$ currents and peak $[\text{Ca}^{2+}]_i$ during a spark, such that a given $\text{Ca}^{2+}$ spark caused a larger increase in $\text{BK}_{\text{Ca}}$ channel activity. In fact, the voltage dependence of $\text{BK}_{\text{Ca}}$ channel activity ($I_{\text{BKCa}}$) for a given-size $\text{Ca}^{2+}$ spark mirrors the voltage dependence of the $\text{Ca}^{2+}$ sensitivity of the $\text{BK}_{\text{Ca}}$ channel (6-fold increase in $\text{BK}_{\text{Ca}}$ channel activity at 10 $\mu\text{M} \text{Ca}^{2+}$ from −50 to −20 mV). Cui and co-workers (6) found that the apparent dissociation constant of the $\text{BK}_{\text{Ca}}$ channel decreased approximately sixfold over the voltage range −50 to −20 mV, and their results suggest that the coupling strength of $\text{Ca}^{2+}$ sparks to $\text{BK}_{\text{Ca}}$ channels should increase approximately $e$-fold per 20 mV of depolarization, until $\text{BK}_{\text{Ca}}$ channels saturate with $[\text{Ca}^{2+}]_i$.

In addition to the enhanced $\text{Ca}^{2+}$ sensitivity, two other factors contribute to the elevation of transient $\text{BK}_{\text{Ca}}$ current with membrane potential depolarization. The amplitude of $\text{Ca}^{2+}$ sparks increased by 30% with membrane depolarization from −50 to −20 mV (Fig. 6B). The $K^+$ driving force also increased with depolarization. These three factors lead to a 16-fold increase in $\text{BK}_{\text{Ca}}$ current amplitude over the range of −50 to −20 mV. In contrast, the frequency of $\text{Ca}^{2+}$ sparks and transient $\text{BK}_{\text{Ca}}$ currents increased nearly fivefold over this same range of membrane potentials (Fig. 6A). Therefore, amplitude modulation of $\text{BK}_{\text{Ca}}$ currents at depolarized potentials reflects three main factors: 1) an increase in the driving force for $K^+$, 2) an increase in the amount of $\text{Ca}^{2+}$ released during a spark, and 3) augmented coupling strength of RyRs to $\text{BK}_{\text{Ca}}$ channels. The coupling strength increased about sixfold for 30 mV (Fig. 6C) and, as such, was the most significant contributor to the amplitude modulation of the transient $\text{BK}_{\text{Ca}}$ currents by voltage.

In conclusion, the present study supports the idea of local communication between RyRs in the SR and $\text{BK}_{\text{Ca}}$ channels in the sarcolemma of UBSM cells. This communication involves $\text{Ca}^{2+}$ release events ($\text{Ca}^{2+}$ sparks), which serve as the stimulus to activate $\text{BK}_{\text{Ca}}$ channels, thus comprising a negative-feedback system to regulate $\text{Ca}^{2+}$ entry via voltage-dependent $\text{Ca}^{2+}$ channels (22). Surprisingly, the coupling strength between $\text{Ca}^{2+}$ sparks and $\text{BK}_{\text{Ca}}$ channels increases with membrane potential depolarization, resulting in much larger $\text{BK}_{\text{Ca}}$ currents for a given-size $\text{Ca}^{2+}$ spark at depolarized potentials. On the basis of previous studies (24), we expected that $\text{BK}_{\text{Ca}}$ channels would be saturated with $\text{Ca}^{2+}$ during a spark at all membrane potentials, and the coupling strength would not increase with membrane potential depolarization but would simply reflect the electrochemical gradient for $K^+$. Our data suggest that $\text{BK}_{\text{Ca}}$ channels are not saturated by $\text{Ca}^{2+}$ during a spark and that the $\text{BK}_{\text{Ca}}$ channel $\text{Ca}^{2+}$ sensitivity is under dynamic regulation, depending on the membrane potential. Thus the gain on this feedback system is effectively increased at depolarized potentials, owing to the voltage-dependent nature of the $\text{BK}_{\text{Ca}}$ channel $\text{Ca}^{2+}$ sensitivity (5, 6). It is likely that this local signaling scheme plays an important role in controlling UBSM contractility by affecting membrane excitability, especially at depolarized membrane potentials when the coupling of RyRs to $\text{BK}_{\text{Ca}}$ channels is enhanced. These results suggest that other factors (e.g., the $\beta$-subunit or protein kinase A or G) that regulate the $\text{Ca}^{2+}$ sensitivity of $\text{BK}_{\text{Ca}}$ channels should also modulate the coupling of $\text{Ca}^{2+}$ sparks to $\text{BK}_{\text{Ca}}$ channel activation.

The authors thank Drs. Adrian Bonev, David Hill-Eubanks, and Guillermo Pérez for comments and discussions regarding the manuscript. This work was supported by National Institutes of Health Grants DK-53832 and HL-44455 (to M. T. Nelson). G. M. Herrera is a National Science Foundation Graduate Research Fellow.

REFERENCES


