KCa channel insensitivity to Ca\(^{2+}\) sparks underlies fractional uncoupling in newborn cerebral artery smooth muscle cells

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**Li, Anlong, Adebowale Adebiyi, Charles W. Leffler, and Jonathan H. Jaggar.** KCa channel insensitivity to Ca\(^{2+}\) sparks underlies fractional uncoupling in newborn cerebral artery smooth muscle cells. *Am J Physiol Heart Circ Physiol* 291: H1118–H1125, 2006. First published April 7, 2006; doi:10.1152/ajpheart.01308.2005.—In smooth muscle cells, localized intracellular Ca\(^{2+}\) transients, termed “Ca\(^{2+}\) sparks,” activate several large-conductance Ca\(^{2+}\)-activated K\(^+\) (KCa) channels, resulting in a transient KCa current. In some smooth muscle cell types, a significant proportion of Ca\(^{2+}\) sparks do not activate KCa channels. The goal of this study was to explore mechanisms that underlie fractional Ca\(^{2+}\) spark-KCa channel coupling. We investigated whether membrane depolarization or ryanodine-sensitive Ca\(^{2+}\) release (RyR) channel activation modulates coupling in newborn (1-to 3-day-old) porcine cerebral artery myocytes. At steady membrane potentials of −40, 0, and +40 mV, mean transient KCa current frequency was ~0.18, 0.43, and 0.26 Hz and KCa channel activity [number of KCa channels activated by Ca\(^{2+}\) sparks × open probability of KCa channels at peak of Ca\(^{2+}\) sparks (NP\(_{Ca}\))] at the transient KCa current peak was ~4, 12, and 24, respectively. Depolarization between −40 and +40 mV increased KCa channel sensitivity to Ca\(^{2+}\) sparks and elevated the percentage of Ca\(^{2+}\) sparks that activated a transient KCa current from 59 to 86%. In a Ca\(^{2+}\)-free bath solution or in diltiazem, a voltage-dependent Ca\(^{2+}\) channel blocker, steady membrane depolarization between −40 and +40 mV increased transient KCa current frequency up to ~1.6-fold. In contrast, caffeine (10 μM), an RyR channel activator, increased mean transient KCa current frequency but did not alter Ca\(^{2+}\) spark-KCa channel coupling. These data indicate that coupling is increased by mechanisms that elevate KCa channel sensitivity to Ca\(^{2+}\) sparks, but not by RyR channel activation. Overall, KCa channel insensitivity to Ca\(^{2+}\) sparks is a prominent factor underlying fractional Ca\(^{2+}\) spark uncoupling in newborn cerebral artery myocytes.

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The brain was removed and maintained in ice-cold HEPES-buffered saline solution (PSS) containing (in mM) 134 NaCl, 6 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose, with pH adjusted to 7.4 with NaOH. Isolated arteries (50–200 μm) were dissected from the brain and cleaned to remove basolateral connective tissue. Individual smooth muscle cells were dissociated from cerebral arteries by a procedure described previously (13).

Confocal Ca²⁺ imaging. Arterial smooth muscle cells were placed in HEPES-buffered PSS containing 10 μM fluo 4-AM for 20 min at room temperature. The cells were then washed with HEPES-buffered PSS for 30 min to allow indicator deesterification. Fluorescence imaging was performed using a laser scanning confocal microscope (Oz, Noran Instruments, Middleton, WI) and a ×60 water immersion objective (1.2 NA) attached to a microscope (model TE300, Nikon). Fluo 4 was illuminated at 488 nm with use of a krypton-argon laser, and emitted light >500 nm was captured. Images (56.3 × 52.8 μm) were recorded every 8.3 ms (i.e., 120 images per second). When a slit width of 100 μm was used, the z resolution (full width at half-maximal amplitude) of the imaging system was 7 μm, as determined by subresolution (100-nm-diameter) fluorescent beads. Electrophysiological and fluorescence measurements were synchronized using a light-emitting diode placed above the recording chamber that was triggered during acquisition. Each isolated smooth muscle cell was imaged for 10 s under each condition. Custom analysis software (kindly provided by Dr. M. T. Nelson, University of Vermont) was used to detect Ca²⁺ sparks in smooth muscle cells. For detection of Ca²⁺ sparks, an area 1.54 × 1.54 μm (7 × 7 pixels, i.e., 2.37 μm²) in each image (F) was divided by a baseline (F₀) that was determined by averaging 10 images without Ca²⁺ spark activity. The entire image area was analyzed to detect Ca²⁺ sparks. A Ca²⁺ spark was identified as a local increase in F/F₀ that was >1.2. Mean Ca²⁺ spark frequency and standard error of the mean under each condition were calculated by averaging individual cellular frequencies. Spatial spread of the Ca²⁺ spark was calculated at half-maximal amplitude. Changes in local or global [Ca²⁺]i were calculated using the pseudoratio method (5):

\[
[Ca^{2+}] = \frac{KR}{K_f[Ca^{2+}]_{rest} + 1 - R}
\]

where K is the apparent affinity of fluo 4 for Ca²⁺ [770 nM (28)], R is the fractional fluorescence increase (F/F₀), and [Ca²⁺]rest is [Ca²⁺]i at F₀. Global Ca²⁺ fluorescence was calculated from the same images used for Ca²⁺ spark analysis and was the mean pixel value of 100 different images acquired over 10 s. Global [Ca²⁺]i at 0 and +40 mV were calculated from the cellular change in F/F₀ from −40 mV (determined with fura 2; see Intracellular Ca²⁺ measurements using fura 2).

Patch-clamp electrophysiology. Isolated cells were allowed to adhere to a glass coverslip in the bottom of a chamber for 10 min before experimentation. K⁺ currents were measured using the perforated-patch configuration of the patch-clamp technique with an Axopatch 200B amplifier (Axon Instruments, Union City, CA). The bath solution was HEPES-buffered PSS. Where appropriate, Ca²⁺-free bath solution was prepared by substitution of equimolar CaCl₂ with NaCl and addition of 1 mM EGTA. The pipette solution contained (in mM) 110 potassium aspartate, 30 KCl, 10 NaCl, 1 MgCl₂, 10 HEPES, and 0.05 EGTA, with pH adjusted to 7.2 with KOH. Membrane currents were filtered at 1 kHz and digitized at 4 kHz. In each patch under each condition, transient Kᵦ current frequency and amplitude were calculated from ≥5 min of continuous gap-free data. At −40, 0, and +40 mV, in the presence of thapsigargin (500 nM), an SR Ca²⁺-ATPase blocker that inhibits Ca²⁺ sparks (16), a maximum of two, three, and six Kᵦ channel openings, respectively, were observed (n = 5 cells). Therefore, at −40, 0, and +40 mV in control, a transient Kᵦ current was defined as the simultaneous opening of three, four, or seven Kᵦ current, respectively. Single Kᵦ channel current amplitude at each voltage was calculated using amplitude histograms.

Intracellular Ca²⁺ measurements using fura 2. Cerebral arteries were incubated in HEPES-buffered PSS containing 5 μM fura 2-AM and 0.05% Pluronic F-127 for 45 min at room temperature. After they were washed, the arteries were allowed 15 min for indicator deesterification. Fura 2 was alternately excited with 340- or 380-nm light with use of a xenon arc lamp and a personal computer-driven hyperswitch (Ionoptix, Milton, MA). Background corrected ratios were collected every 1 s at 510 nm with use of a photomultiplier tube (Ionoptix). For calibration of confocal Ca²⁺ imaging data, the extracellular K⁺ concentration was elevated from 6 to 30 mM by substitution of equimolar K⁺ for Na⁺; 30 mM K⁺ depolarizes arterial smooth muscle cells to −40 mV (10), a voltage applied in transient Kᵦ current measurements. [Ca²⁺]i values were calculated from fura 2 fluorescence measurements using the following equation (9):

\[
[Ca^{2+}] = \frac{R - R_{min}}{R_{max} - R} (S_2 - S_0)
\]

where R is the ratio of fluorescence at 340 nm to fluorescence at 380 nm, R_min and R_max are the minimum and maximum fluorescence ratios determined in Ca²⁺-free and saturating Ca²⁺ solutions, respectively. S₂/S₀ is the ratio of Ca²⁺-free to Ca²⁺-replete emissions at 380-nm excitation, and Kᵦ is the dissociation constant for fura 2 (282 nM (19)). For determination of R_min, R_max, S₂, and S₀ at the end of the experiments and in separate experiments, the Ca²⁺ permeability of smooth muscle cells was increased with 10 μM ionomycin and the cells were perfused with a high-Ca²⁺ (10 mM) or Ca²⁺-free (no added Ca²⁺, 5 mM EGTA) solution. Elevation of extracellular K⁺ from 6 to 30 mM or from approximately −60 to −40 mV increased arterial wall Ca²⁺ from 104 ± 17 to 244 ± 29 nM (n = 7 arteries, P < 0.05).

Statistical analysis. Values are means ± SE; n refers to the number of events analyzed, unless otherwise specified. Student’s t-tests were used for comparison of paired or unpaired data and Student-Newman-Keuls test for comparison of multiple data sets. When data sets were not normally distributed, the Kruskal-Wallis test with Dunn’s multiple comparisons test was used for statistical comparison. Linear regression was used to calculate statistical correlation between the amplitude of Ca²⁺ sparks and evoked transient Kᵦ currents (Origin, OriginLab, Northampton, MA). Analysis of covariance of linear regression was used to compare amplitude correlation data sets (Graphpad Prism, San Diego, CA). P < 0.05 was considered significant.

Chemicals. Unless otherwise stated, all chemicals were obtained from Sigma Chemical (St. Louis, MO). Papain was purchased from Worthington Biochemical (Lakewood, NJ) and fluo 4-AM from Molecular Probes (Eugene, OR).

RESULTS

Membrane depolarization elevates transient Kᵦ current frequency and activity in newborn cerebral artery smooth muscle cells. Steady membrane depolarization between −40 and 0 mV increased mean transient Kᵦ current frequency from −0.18 to 0.43 Hz (Fig. 1, A and B). Further depolarization to +40 mV reduced transient Kᵦ current frequency to −0.26 Hz (Fig. 1, A and B). In contrast, depolarization between −40 and +40 mV continually increased mean transient Kᵦ current amplitude (Fig. 1, A and C). Transient Kᵦ current amplitude (I) is dependent on the number of Kᵦ channels activated by a Ca²⁺ spark (N), the open probability of Kᵦ channels at the Ca²⁺ spark peak (P₀), and single Kᵦ channel amplitude (i), giving iNPᵦ. Membrane depolarization increases the driving force for K⁺ and, thus, i. Therefore, transient Kᵦ current...
amplitude data were normalized for voltage-dependent changes in driving force as follows: \( NP_o = I/I_o \). In the same patches used for transient KCa current analysis, single KCa channel amplitudes at \(-40\), 0, and \(+40\) mV were \(2.8 \pm 0.1\), \(4.8 \pm 0.1\), and \(9.0 \pm 0.1\) pA, respectively \((n = 13)\). Over the voltage range of \(-40\) to \(+40\) mV, transient KCa channel activity \((i.e., NP_o)\) increased from 4 to 23 (Fig. 1D). These data indicate that membrane depolarization elevates transient KCa current frequency and Ca\({}^{2+}\) spark-induced KCa channel activity in newborn porcine cerebral artery smooth muscle cells.

Membrane depolarization activates Ca\({}^{2+}\) sparks and augments Ca\({}^{2+}\) spark-induced KCa channel activation. To examine the mechanisms by which membrane depolarization elevates transient KCa current frequency and activity in newborn arterial smooth muscle cells, simultaneous measurements of Ca\({}^{2+}\) sparks and transient KCa currents were acquired using confocal Ca\({}^{2+}\) imaging in combination with patch-clamp electrophysiology.

At \(-40\) mV, \(-59\%\) of Ca\({}^{2+}\) sparks activated a transient KCa current (Fig. 2, Table 1). Steady membrane depolarization from \(-40\) to 0 mV elevated global F/F\(_0\) 1.33-fold, which translates to an increase in global [Ca\({}^{2+}\)], from \(224 \pm 29\) nM (see MATERIALS AND METHODS) to 363 nM. Depolarization from \(-40\) to 0 mV elevated the amplitude of coupled and uncoupled Ca\({}^{2+}\) sparks, with the mean amplitude of all Ca\({}^{2+}\) sparks increasing from \(-874\) to 1,424 nM. In contrast, mean Ca\({}^{2+}\) spark spread was smaller and decay was faster at 0 mV than at \(-40\) mV. Depolarization from \(-40\) to 0 mV increased the percentage of Ca\({}^{2+}\) sparks that activated a transient KCa current to \(-77\%\). Further depolarization from 0 to \(+40\) mV reduced global [Ca\({}^{2+}\)], to 271 nM, which is expected because of a reduction in the driving force for Ca\({}^{2+}\) influx, decreased mean Ca\({}^{2+}\) spark amplitude to \(-1.121\) nM and reduced coupled and uncoupled Ca\({}^{2+}\) spark amplitude. However, depolarization to \(+40\) mV increased the percentage of Ca\({}^{2+}\) sparks that activated a transient KCa current to \(-86\%\). Taken together, membrane depolarization between \(-40\) and \(+40\) mV is estimated to increase KCa channel sensitivity to Ca\({}^{2+}\) sparks from \(-0.015\) to 0.026 NP\(_o\) nM Ca\({}^{2+}\) when the [Ca\({}^{2+}\)], detected by fluo 4 is taken as an indicator of Ca\({}^{2+}\) spark amplitude.

Diltiazem or removal of extracellular Ca\({}^{2+}\) blocks depolarization-induced elevations in transient KCa current frequency, but not activity. To investigate the contribution of Ca\({}^{2+}\) influx to the depolarization-induced increase in Ca\({}^{2+}\) spark-KCa channel coupling, voltage-dependent transient KCa current regulation was measured in a Ca\({}^{2+}\)-free bath solution or in the presence of diltiazem (50 \(\mu\)M), a voltage-dependent Ca\({}^{2+}\) channel blocker.

At \(-40\) mV, removal of extracellular Ca\({}^{2+}\) reduced transient KCa current frequency to \(0.41 \pm 0.10\) of control \((P < 0.05)\) but did not change transient KCa current amplitude \((0.99 \pm 0.05\) of control, \(P > 0.05, n = 5\) cells). At \(-40, 0,\) and \(+40\) mV, 50 \(\mu\)M diltiazem reduced transient KCa current frequency to \(0.43 \pm 0.06, 0.24 \pm 0.03,\) and \(0.46 \pm 0.03\) of control, respectively \((P < 0.05\) for each), but did not alter transient KCa current amplitude \((1.00 \pm 0.10, 1.17 \pm 0.10,\) and \(1.07 \pm 0.05\) respectively).
of control, respectively, $P > 0.05$ for each, $n = 6$ cells). More importantly, in the absence of extracellular Ca$^{2+}$ or in the continued presence of diltiazem, steady membrane depolarization between $-40$ and $+40$ mV increased mean transient $K_{\text{Ca}}$ current frequency up to 1.6-fold (Fig. 3A). Over the same voltage range, transient $K_{\text{Ca}}$ current activity ($NP_o$) increased up to approximately fourfold (Fig. 3B). These data indicate that steady membrane depolarization elevates transient $K_{\text{Ca}}$ current frequency and activity in the absence of extracellular Ca$^{2+}$ entry or voltage-dependent Ca$^{2+}$ channel activation.

Caffeine activates Ca$^{2+}$ sparks and transient $K_{\text{Ca}}$ currents. To determine whether RyR channel activation alters Ca$^{2+}$ spark-$K_{\text{Ca}}$ channel coupling in arterial smooth muscle cells, we studied transient $K_{\text{Ca}}$ current regulation by caffeine, an RyR channel activator.

![Diagram](image)

**Fig. 2. Membrane depolarization elevates $K_{\text{Ca}}$ channel sensitivity to Ca$^{2+}$ sparks.** A: simultaneous recordings of whole cell current (top traces) and Ca$^{2+}$ sparks (bottom traces) at $-40$, $0$, and $+40$ mV. Fluo 4 fluorescence changes ($F/F_0$) were measured in 2 different $1.54 \times 10^{-3}$ areas of the cell in which Ca$^{2+}$ sparks occurred. At $-40$ mV, 1 Ca$^{2+}$ spark occurred at 1 location; at $0$ mV, 4 Ca$^{2+}$ sparks were observed at 2 locations; at $+40$ mV, 3 Ca$^{2+}$ sparks occurred at 1 location. *Ca$^{2+}$ spark at its peak in the pseudocolored inset image. B: voltage dependence of relation between peak Ca$^{2+}$ spark amplitude and activity ($NP_o$) of evoked transient $K_{\text{Ca}}$ currents ($NP_o$ 14, 13, and 10 cells for $-40$, $0$, and $+40$ mV, respectively). Linear regression with 95% confidence bands is illustrated with slopes of 0.005, 0.010, and 0.016 for $-40$, $0$, and $+40$ mV, respectively. Membrane depolarization elevated linear correlation coefficient as follows: 0.04 for $-40$ mV, 0.28 for $0$ mV, and 0.51 for $+40$ mV. Amplitudes of Ca$^{2+}$ sparks and evoked transient $K_{\text{Ca}}$ currents were significantly correlated at each voltage ($P < 0.05$ for each).

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<th>Table 1. Regulation of Ca$^{2+}$ spark and transient $K_{\text{Ca}}$ current properties by membrane potential</th>
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Values are means±SE of number of events in parentheses. $t_{1/2}$, half time; $K_{\text{Ca}}$, Ca$^{2+}$-activated K$^+$; $NP_o$, number of $K_{\text{Ca}}$ channels activated by Ca$^{2+}$ spark × open probability of $K_{\text{Ca}}$ channels at peak of Ca$^{2+}$ spark. *$P < 0.05$ vs. $-40$ mV.
At −40, 0, and +40 mV, 10 μM caffeine increased transient KCa current frequency ~1.5-, 1.6-, and 1.5-fold, respectively (Fig. 4A). In contrast, over the same voltage range, caffeine did not alter transient KCa channel activity (NP_o, Fig. 4B). To investigate the effects of caffeine on Ca^{2+} spark properties and Ca^{2+} spark-KCa channel coupling, we used simultaneous patch-clamp electrophysiology and confocal Ca^{2+} imaging. Experiments were performed at 0 mV, because caffeine was most effective at activating transient KCa currents at this voltage. Caffeine increased mean global Ca^{2+} from ~363 to 419 nM but reduced mean peak Ca^{2+} spark amplitude from ~1,956 to ~1,375 nM (Table 2). Caffeine also increased mean Ca^{2+} spark spatial spread from ~2.9 to 3.6 μm². Caffeine did not alter Ca^{2+} spark decay, the percentage of Ca^{2+} sparks that activated a transient KCa current, the amplitude relation between sparks and transient KCa currents, or transient KCa channel activity (NP_o; Table 2, Fig. 5). These data indicate that RyR channel activation decreases Ca^{2+} spark amplitude (i.e., the local subsarcolemmal [Ca^{2+}], activating KCa channels) and elevates spatial spread of Ca^{2+} sparks, which would increase the number of KCa channels impacted by the spark. The combination of these changes in Ca^{2+} spark properties results in no net change in Ca^{2+} spark-KCa channel coupling.

**DISCUSSION**

The regulation of Ca^{2+} spark-KCa channel coupling by mechanisms that activate KCa and RyR channels was studied in newborn cerebral artery smooth muscle cells, in which a significant proportion of Ca^{2+} sparks do not activate a transient KCa current. Membrane depolarization between −40 and +40 mV increased 1) transient KCa current frequency and activity (NP_o), 2) the percentage of Ca^{2+} sparks that activated a transient KCa current from 59 to 86%, and 3) the sensitivity of KCa channels to Ca^{2+} sparks. Ca^{2+} influx or voltage-dependent Ca^{2+} channel activation was not obligatory for membrane depolarization to elevate transient KCa current frequency and activity. In contrast, RyR channel activation elevated transient KCa current frequency solely by causing an increase in Ca^{2+} spark frequency. RyR channel activation did not change Ca^{2+} spark-KCa channel coupling or transient KCa current activity. These data indicate that KCa channel Ca^{2+} sensitivity, rather than RyR channel activity, is a principal factor that underlies

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<td>KCa transient activity (NP_o)</td>
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Values are means ± SE of number of events in parentheses. *P < 0.05 vs. control.
fractional Ca\textsuperscript{2+} sparks in newborn cerebral artery smooth muscle cells.

Membrane depolarization between -40 and 0 mV increased global [Ca\textsuperscript{2+}], Ca\textsuperscript{2+} spark amplitude, K\textsubscript{Ca} channel sensitivity to Ca\textsuperscript{2+} sparks, and the percentage of Ca\textsuperscript{2+} sparks that activated a transient K\textsubscript{Ca} current. Further depolarization to +40 mV decreased Ca\textsuperscript{2+} spark amplitude and reduced global [Ca\textsuperscript{2+}], which was expected because of a reduction in driving force for Ca\textsuperscript{2+} influx. However, depolarization from 0 to +40 mV further increased the percentage of Ca\textsuperscript{2+} sparks that activated a transient K\textsubscript{Ca} current and elevated K\textsubscript{Ca} channel sensitivity to Ca\textsuperscript{2+} sparks. These data suggest that, in newborn arterial smooth muscle cells, effective coupling and percent coupling of Ca\textsuperscript{2+} sparks to K\textsubscript{Ca} channels are modulated primarily by K\textsubscript{Ca} channel sensitivity to Ca\textsuperscript{2+} sparks, rather than by RyR channel activity. An explanation for these findings is that membrane depolarization increases K\textsubscript{Ca} channel apparent Ca\textsuperscript{2+} sensitivity, which would increase the impact of sparks on K\textsubscript{Ca} channel P\textsubscript{0} (4, 12, 20). The depolarization-induced elevation in transient K\textsubscript{Ca} current frequency most likely occurs through an increase in the percentage of Ca\textsuperscript{2+} sparks that activate K\textsubscript{Ca} channels. In support of this conclusion, in the presence of diltiazem or in the absence of extracellular Ca\textsuperscript{2+}, both of which would block depolarization-induced Ca\textsuperscript{2+} spark activation (13, 17), depolarization elevated transient K\textsubscript{Ca} current frequency and activity. In murine colonic myocytes, a reduction in extracellular Ca\textsuperscript{2+} reduced local intracellular Ca\textsuperscript{2+} transients but elevated transient K\textsubscript{Ca} current frequency and amplitude by removing protein kinase C-mediated K\textsubscript{Ca} channel inhibition (2). In contrast, in the present study, removal of extracellular Ca\textsuperscript{2+} or diltiazem reduced transient K\textsubscript{Ca} current frequency but did not alter amplitude. These data suggest Ca\textsuperscript{2+} sparks are activated by Ca\textsuperscript{2+} influx through voltage-dependent Ca\textsuperscript{2+} channels, as previously reported (13, 17), and illustrate differences in the mechanisms by which Ca\textsuperscript{2+} spark-K\textsubscript{Ca} channel coupling is modulated by Ca\textsuperscript{2+} influx pathways in colonic and arterial smooth muscle cells.

K\textsubscript{Ca} channel “Ca\textsuperscript{2+} sensitivity” has previously been used to describe 1) the Ca\textsuperscript{2+} concentration that induces half-maximal activation at a given voltage, 2) the slope of the Ca\textsuperscript{2+}-activity relation at a defined voltage, and 3) a shift in half-maximal potential for a given Ca\textsuperscript{2+} concentration change (4). Depolarization shifts the Ca\textsuperscript{2+} concentration-K\textsubscript{Ca} channel activity relation leftward (4) and increases the percentage of Ca\textsuperscript{2+} sparks that activate K\textsubscript{Ca} channels. The present data dispute the possibility that uncoupling occurs because K\textsubscript{Ca} channels within the vicinity of Ca\textsuperscript{2+} spark sites are absent or incapable of activation. The K\textsubscript{d} for Ca\textsuperscript{2+} of newborn porcine arteriole smooth muscle cell K\textsubscript{Ca} channels is 31 \mu M at 0 mV, which is high compared with that of K\textsubscript{Ca} channels in other smooth muscle cell preparations, including human coronary artery and rat cerebral artery (22, 26, 30). Conceivably, uncoupling may occur because K\textsubscript{Ca} channel Ca\textsuperscript{2+} sensitivity is lower in uncoupled than in strongly coupled cell types. Other likely explanations are that uncoupled Ca\textsuperscript{2+} sparks are of lower amplitude (present study and Ref. 14) and/or the distance between uncoupled spark release sites and the sarcolemma is greater, both of which would result in lower spark-induced subsarcolemmal Ca\textsuperscript{2+} elevations. In B. marinus stomach smooth muscle cells, some Ca\textsuperscript{2+} spark sites generate sparks that reliably activate transient K\textsubscript{Ca} currents, whereas other locations consistently generate uncoupled sparks (31). In the amphibian preparation, sites that generate uncoupled Ca\textsuperscript{2+} sparks may be located near sarcolemma that is devoid of K\textsubscript{Ca} channels or populated by inactivatable K\textsubscript{Ca} channels (31). However, in newborn cerebral artery smooth muscle cells, Ca\textsuperscript{2+} spark-K\textsubscript{Ca} channel coupling is increased by membrane depolarization and carbon monoxide, which elevate K\textsubscript{Ca} channel apparent Ca\textsuperscript{2+} sensitivity (14, 15, 30). Similarly, in guinea pig bladder smooth muscle cells, membrane depolarization between -50 and -20 mV elevated Ca\textsuperscript{2+} spark coupling (11). Thus K\textsubscript{Ca} channel localization near Ca\textsuperscript{2+} spark sites and regulation by Ca\textsuperscript{2+} sparks appear to differ in mammalian and amphibian smooth muscle cells.

Regardless of voltage, caffeine, which elevates RyR channel Ca\textsuperscript{2+} sensitivity (24), induced a similar relative increase in transient K\textsubscript{Ca} current frequency but did not change transient K\textsubscript{Ca} channel activity. These data suggest that caffeine activates transient K\textsubscript{Ca} currents by elevating Ca\textsuperscript{2+} spark frequency. Caffeine also elevated global [Ca\textsuperscript{2+}], and reduced Ca\textsuperscript{2+} spark amplitude, presumably by causing SR Ca\textsuperscript{2+} leak and a reduction in SR Ca\textsuperscript{2+} load, respectively (6). Caffeine also increased Ca\textsuperscript{2+} spark spread, presumably by elevating the number of RyR channels that contribute to sparks through localized Ca\textsuperscript{2+} induced Ca\textsuperscript{2+} release. In pulmonary artery smooth muscle cells, 500 \mu M caffeine did not change Ca\textsuperscript{2+} spark amplitude (calculated as F/F\textsubscript{0}) but elevated Ca\textsuperscript{2+} spark frequency, duration, and spread (25). In B. marinus stomach smooth muscle cells, caffeine increased the number of spark sites from ~42 to 400 (31). Conceivably, caffeine may have also generated Ca\textsuperscript{2+} sparks at additional sites in newborn cerebral artery smooth muscle cells. However, the low Ca\textsuperscript{2+} spark frequency in newborn arterial smooth muscle cells and the 10-s time limit required for imaging to avoid laser-induced cell damage precluded systematic examination of this possibility. Nevertheless, the net effect of Ca\textsuperscript{2+} spark spatial and temporal changes was no net change in the mean percentage or effective Ca\textsuperscript{2+} spark-K\textsubscript{Ca} channel coupling. Thus, in newborn porcine cerebral artery smooth muscle cells, RyR channel activation elevates...
transient $K_{Ca}$ current frequency by elevating $Ca^{2+}$ spark frequency, and not by altering $Ca^{2+}$ spark-$K_{Ca}$ channel coupling.

Caffeine, at low micromolar concentrations, induces a $K_{Ca}$ channel-sensitive vasodilation in pressurized newborn cerebral arteries (1). Carbon monoxide increases $K_{Ca}$ channel apparent $Ca^{2+}$ sensitivity and $Ca^{2+}$ spark-$K_{Ca}$ channel coupling in smooth muscle cells and dilates newborn porcine cerebral arteries (14, 15, 30). These findings show that an elevation in $Ca^{2+}$ spark frequency alone or an increase in $Ca^{2+}$ spark-$K_{Ca}$ channel coupling induces vasodilation through $K_{Ca}$ channel activation. In the present study, membrane depolarization within the physiological range [i.e., ca. $-60$ to $-20$ mV (19)] would increase $Ca^{2+}$ spark-$Ca^{2+}$ channel coupling by only $\sim 10\%$. The increase in coupling alone would be predicted to have only a small effect on membrane potential. However, the combination of an increase in coupling and depolarization-induced transient $K_{Ca}$ current frequency and amplitude elevation would increase $K^{+}$ current through $K_{Ca}$ channels, produce membrane hyperpolarization, and oppose pressure-induced constriction (16). Within the physiological range of voltages, $Ca^{2+}$ spark-$K_{Ca}$ channel coupling in newborn myocytes does not reach $100\%$, allowing additional mechanisms that enhance $K_{Ca}$ channel $Ca^{2+}$ sensitivity to augment coupling and further enhance $K_{Ca}$ channel activity [e.g., carbon monoxide (14)]. As such, signalings elements that increase $K_{Ca}$ channel $Ca^{2+}$ sensitivity will be more effective vasodilators in myocytes that exhibit fractional coupling than in cells with $100\%$ coupling. Furthermore, messengers that elevate $Ca^{2+}$ spark frequency and coupling to $K_{Ca}$ channels, including reactive oxygen species (7, 29) and carbon monoxide (14, 15), should produce the most significant $K_{Ca}$ channel-dependent vasodilation.

In summary, the present data indicate that, in newborn porcine cerebral artery smooth muscle cells, fractional $Ca^{2+}$ spark coupling occurs through $K_{Ca}$ channel insensitivity to $Ca^{2+}$ sparks. Uncoupled $Ca^{2+}$ sparks can be coupled by mechanisms that elevate $K_{Ca}$ channel $Ca^{2+}$ sensitivity. In contrast, RyR channel activation alone reduces $Ca^{2+}$ spark amplitude and increases $Ca^{2+}$ spark spread, resulting in no net change in $Ca^{2+}$ spark coupling.

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