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

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Abstract

In smooth muscle cells, localized intracellular Ca^{2+} transients, termed "Ca^{2+} sparks," activate several large-conductance Ca^{2+}-activated K^+ (K_{Ca}) channels, resulting in a transient K_{Ca} current. In some smooth muscle cell types, a significant proportion of Ca^{2+} sparks do not activate K_{Ca} channels. The goal of this study was to explore mechanisms that underlie fractional Ca^{2+} spark-K_{Ca} 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 K_{Ca} current frequency was ~0.18, 0.43, and 0.26 Hz and K_{Ca} channel activity [number of K_{Ca} channels activated by Ca^{2+} sparks × open probability of K_{Ca} channels at peak of Ca^{2+} sparks (NP_o)] at the transient K_{Ca} current peak was ~4, 12, and 24, respectively. Depolarization between −40 and +40 mV increased K_{Ca} channel sensitivity to Ca^{2+} sparks and elevated the percentage of Ca^{2+} sparks that activated a transient K_{Ca} 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 K_{Ca} current frequency up to ~1.6-fold. In contrast, caffeine (10 μM), an RyR channel activator, increased mean transient K_{Ca} current frequency but did not alter Ca^{2+} spark-K_{Ca} channel coupling. These data indicate that coupling is increased by mechanisms that elevate K_{Ca} channel sensitivity to Ca^{2+} sparks, but not by RyR channel activation. Overall, K_{Ca} channel insensitivity to Ca^{2+} sparks is a prominent factor underlying fractional Ca^{2+} spark uncoupling in newborn cerebral artery myocytes.

Keywords

ryanodine-sensitive calcium release channel; calcium-activated potassium channel; membrane potential

Arterial smooth muscle cell contractility is differentially regulated by local and global elevations in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (16). Global nanomolar $[Ca^{2+}]_i$ elevations, caused by Ca^{2+} influx from the extracellular space and release from intracellular stores, stimulate contraction via the activation of Ca^{2+}/calmodulin-dependent myosin light chain kinase (8,16). In contrast, localized micromolar $[Ca^{2+}]_i$ transients, termed "Ca^{2+} sparks," oppose contraction (16,21).

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Ca^{2+} sparks are induced by activation of several ryanodine-sensitive Ca^{2+} release (RyR) channels on the sarcoplasmic reticulum (SR) (5,16). In smooth muscle cells, a Ca^{2+} spark can activate multiple large-conductance Ca^{2+}-activated K^+ (K_{Ca}) channels, resulting in a transient K_{Ca} current (3,16,21). In the arterial wall, transient K_{Ca} currents induce membrane hyperpolarization, which reduces voltage-dependent Ca^{2+} channel activity and, thus, global $[Ca^{2+}]_i$ and contractility (21). The differential regulation of arterial smooth muscle contractility by local and global Ca^{2+} signals exemplifies how a single signaling element can control opposing cellular functions in the same cell.

Several important features facilitate differential regulation of arterial smooth muscle contractility by local and global $[Ca^{2+}]_i$ elevations, including the spatial and temporal nature of the Ca^{2+} signals and the proximity and Ca^{2+} sensitivity of downstream target proteins (16). One important feature of Ca^{2+} sparks that allows specificity of signaling is that these events do not contribute significantly to global $[Ca^{2+}]_i$ because of their rapid and localized properties (16,21). Another important aspect is that K_{Ca} channels are sensitive to micromolar $[Ca^{2+}]_i$ elevations, such as those generated by Ca^{2+} sparks (22,33). As such, K_{Ca} channels are relatively insensitive to global nano-molar $[Ca^{2+}]_i$ elevations that signal contraction (22,33).

In adult rat cerebral artery smooth muscle cells, the effective coupling of Ca^{2+} sparks to K_{Ca} channels is strong, and at a physiological membrane potential of −40 mV, essentially all Ca^{2+} sparks activate a transient K_{Ca} current (6,23). However, in adult human and newborn porcine cerebral arterial, adult feline esophageal, and *Bufo marinus* stomach smooth muscle cells, the effective coupling of Ca^{2+} sparks to K_{Ca} channels is considerably weaker (14,18, 27,32). In these smooth cell types, a significant proportion of Ca^{2+} sparks do not activate a transient K_{Ca} current (~20–40%), and the amplitude correlation between these events is less robust than in rat cerebral artery smooth muscle cells (14,18,27,32). However, underlying causes of weak coupling and mechanisms that enhance coupling in these smooth muscle cell types are unclear.

The goal of this study was to investigate mechanisms that underlie fractional Ca^{2+} spark-K_{Ca} channel coupling in smooth muscle cells. Ca^{2+} spark-K_{Ca} channel coupling was studied in newborn porcine cerebral artery smooth muscle cells, which exhibit a weak coupling phenotype similar to that observed in other smooth muscle cell types, including human cerebral artery smooth muscle cells (27). We investigated whether an increase in K_{Ca} channel Ca^{2+} sensitivity or RyR channel activation enhances coupling. Data suggest that coupling is determined primarily by K_{Ca} channel sensitivity to Ca^{2+} sparks and indicate that RyR channel activation alone does not influence coupling.

MATERIALS AND METHODS

Tissue preparation

All procedures used were approved by the University of Tennessee Animal Care and Use Committee. Newborn pigs (1–3 days old, 1–2.5 kg body wt) were anesthetized with ketamine hydrochloride (33 mg/kg im) and acepromazine (3.3 mg/kg im). The brain was removed and maintained in ice-cold HEPES-buffered physiological saline solution (PSS) containing (in mM) 134 NaCl, 6 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 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 Ca^{2+} 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. Fluo 4 was imaged 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 Ca^{2+} sparks in smooth muscle cells. For detection of Ca^{2+} sparks, an area 1.54 × 1.54 μm (7 × 7 pixels, i.e., 2.37 μm^2) in each image (F) was divided by a baseline (F_0) that was determined by averaging 10 images without Ca^{2+} spark activity. The entire image area was analyzed to detect Ca^{2+} sparks. A Ca^{2+} spark was identified as a local increase in F/F_0 that was >1.2. Mean Ca^{2+} spark frequency and standard error of the mean under each condition were calculated by averaging individual cellular frequencies. Spatial spread of the Ca^{2+} spark was calculated at half-maximal amplitude. Changes in local or global $[Ca^{2+}]_i$ were calculated using the pseudoratio method (5)

$$\left[Ca^{2+}\right] = \frac{K\mathrm{R}}{K / \left[Ca^{2+}\right]_{\mathrm{rest}} + 1 - \mathrm{R}}$$

where *K* is the apparent affinity of fluo 4 for Ca^{2+} [770 nM (28)], R is the fractional fluorescence increase (F/F_0), and $[Ca^{2+}]_{rest}$ is $[Ca^{2+}]_i$ at F_0. Global Ca^{2+} fluorescence was calculated from the same images used for Ca^{2+} spark analysis and was the mean pixel value of 100 different images acquired over 10 s. Global $[Ca^{2+}]_i$ at 0 and +40 mV were calculated from the cellular change in F/F_0 from −40 mV (determined with fura 2; see *Intracellular Ca^{2+} 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 Axo-patch 200B amplifier (Axon Instruments, Union City, CA). The bath solution was HEPES-buffered PSS. Where appropriate, Ca^{2+}-free bath solution was prepared by substitution of equimolar $CaCl_2$ with NaCl and addition of 1 mM EGTA. The pipette solution contained (in mM) 110 potassium aspartate, 30 KCl, 10 NaCl, 1 $MgCl_2$, 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 K_{Ca} 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 Ca^{2+}-ATPase blocker that inhibits Ca^{2+} sparks (16), a maximum of two, three, and six K_{Ca} channel openings, respectively, were observed (n = 5 cells). Therefore, at −40, 0, and +40 mV in control, a transient K_{Ca} current was defined as the simultaneous opening of three, four, or seven K_{Ca} channels, respectively. Single K_{Ca} channel current amplitude at each voltage was calculated using amplitude histograms.

Intracellular Ca^{2+} 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 Ca^{2+} 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 K_{Ca} current measurements. $[Ca^{2+}]_i$ values were calculated from fura 2 fluorescence measurements using the following equation (9)

$$\left[Ca^{2+}\right] = K_d \frac{\left(R - R_{min}\right)}{\left(R_{max} - R\right)} \frac{\left(S_{f2}\right)}{\left(S_{b2}\right)}$$

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 Ca^{2+}-free and saturating Ca^{2+} solutions, respectively, S_{f2}/S_{b2} is the ratio of Ca^{2+}-free to Ca^{2+}-replete emissions at 380-nm excitation, and K_d is the dissociation constant for fura 2 [282 nM (19)]. For determination of R_{min}, R_{max}, S_{f2}, and S_{b2} at the end of the experiments and in separate experiments, the Ca^{2+} permeability of smooth muscle cells was increased with 10 μM ionomycin and the cells were perfused with a high-Ca^{2+} (10 mM) or Ca^{2+}-free (no added Ca^{2+}, 5 mM EGTA) solution. Elevation of extracellular K^+ from 6 to 30 mM or from approximately −60 to −40 mV increased arterial wall Ca^{2+} 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 Ca^{2+} sparks and evoked transient K_{Ca} 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 K_{Ca} current frequency and activity in newborn cerebral artery smooth muscle cells

Steady membrane depolarization between −40 and 0 mV increased mean transient K_{Ca} current frequency from ~0.18 to 0.43 Hz (Fig. 1, *A* and *B*). Further depolarization to +40 mV reduced transient K_{Ca} current frequency to ~0.26 Hz (Fig. 1, *A* and *B*). In contrast, depolarization between −40 and +40 mV continually increased mean transient K_{Ca} current amplitude (Fig. 1, *A* and *C*). Transient K_{Ca} current amplitude (*I*) is dependent on the number of K_{Ca} channels activated by a Ca^{2+} spark (*N*), the open probability of K_{Ca} channels at the Ca^{2+} spark peak (P_o), and single K_{Ca} channel amplitude (*i*), giving iNP_o. Membrane depolarization increases the driving force for K^+ and, thus, *i*. Therefore, transient K_{Ca} current amplitude data were

normalized for voltage-dependent changes in driving force as follows: $NP_o = I/i$. In the same patches used for transient K_{Ca} current analysis, single K_{Ca} channel amplitudes at −40, 0, and +40 mV were 2.8 ± 0.1, 4.8 ± 0.1, and 9.0 ± 0.1 pA, respectively (n = 13). Over the voltage range of −40 to +40 mV, transient K_{Ca} channel activity (i.e., NP_o) increased from 4 to 23 (Fig. 1D). These data indicate that membrane depolarization elevates transient K_{Ca} current frequency and Ca^{2+} spark-induced K_{Ca} channel activity in newborn porcine cerebral artery smooth muscle cells.

Membrane depolarization activates Ca^{2+} sparks and augments Ca^{2+} spark-induced K_{Ca} channel activation

To examine the mechanisms by which membrane depolarization elevates transient K_{Ca} current frequency and activity in newborn arterial smooth muscle cells, simultaneous measurements of Ca^{2+} sparks and transient K_{Ca} 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 K_{Ca} 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 $[Ca^{2+}]_i$ from 224 ± 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 that at −40 mV. Depolarization from −40 to 0 mV increased the percentage of Ca^{2+} sparks that activated a transient K_{Ca} current to ~77%. Further depolarization from 0 to +40 mV reduced global $[Ca^{2+}]_i$ 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 K_{Ca} current to ~86%. Taken together, membrane depolarization between −40 and +40 mV is estimated to increase K_{Ca} channel sensitivity to Ca^{2+} sparks from ~0.015 to 0.026 NP_o/nM Ca^{2+} when the $[Ca^{2+}]_i$ 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 K_{Ca} current frequency, but not activity

To investigate the contribution of Ca^{2+} influx to the depolarization-induced increase in Ca^{2+} spark-K_{Ca} channel coupling, voltage-dependent transient K_{Ca} current regulation was measured in a Ca^{2+}-free bath solution or in the presence of diltiazem (50 μM), a voltage-dependent Ca^{2+} channel blocker.

At −40 mV, removal of extracellular Ca^{2+} reduced transient K_{Ca} current frequency to 0.41 ± 0.10 of control ($P < 0.05$) but did not change transient K_{Ca} current amplitude (0.99 ± 0.05 of control, $P > 0.05$, n = 5 cells). At −40, 0, and +40 mV, 50 μM diltiazem reduced transient K_{Ca} current frequency to 0.43 ± 0.06, 0.24 ± 0.03, and 0.46 ± 0.03 of control, respectively ($P < 0.05$ for each), but did not alter transient K_{Ca} current amplitude (1.00 ± 0.10, 1.17 ± 0.10, and 1.07 ± 0.05 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_{Ca} current frequency up to 1.6-fold (Fig. 3A). Over the same voltage range, transient K_{Ca} current activity (NP_o) increased up to approximately fourfold (Fig. 3B). These data indicate that steady membrane depolarization elevates transient K_{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_{Ca} currents

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

At −40, 0, and +40 mV, 10 μM caffeine increased transient K_{Ca} 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 K_{Ca} channel activity (NP_o; Fig. 4B). To investigate the effects of caffeine on Ca^{2+} spark properties and Ca^{2+} spark-K_{Ca} 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 K_{Ca} 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 μm^2. Caffeine did not alter Ca^{2+} spark decay, the percentage of Ca^{2+} sparks that activated a transient K_{Ca} current, the amplitude relation between sparks and transient K_{Ca} currents, or transient K_{Ca} 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 $[Ca^{2+}]_i$ activating K_{Ca} channels) and elevates spatial spread of Ca^{2+} sparks, which would increase the number of K_{Ca} channels impacted by the spark. The combination of these changes in Ca^{2+} spark properties results in no net change in Ca^{2+} spark-K_{Ca} channel coupling.

DISCUSSION

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

Membrane depolarization between −40 and 0 mV increased global $[Ca^{2+}]_i$, Ca^{2+} spark amplitude, K_{Ca} channel sensitivity to Ca^{2+} sparks, and the percentage of Ca^{2+} sparks that activated a transient K_{Ca} current. Further depolarization to +40 mV decreased Ca^{2+} spark amplitude and reduced global $[Ca^{2+}]_i$, which was expected because of a reduction in driving force for Ca^{2+} influx. However, depolarization from 0 to +40 mV further increased the percentage of Ca^{2+} sparks that activated a transient K_{Ca} current and elevated K_{Ca} channel sensitivity to Ca^{2+} sparks. These data suggest that, in newborn arterial smooth muscle cells, effective coupling and percent coupling of Ca^{2+} sparks to K_{Ca} channels are modulated primarily by K_{Ca} channel sensitivity to Ca^{2+} sparks, rather than by RyR channel activity. An explanation for these findings is that membrane depolarization increases K_{Ca} channel apparent Ca^{2+} sensitivity, which would increase the impact of sparks on K_{Ca} channel P_o (4,12,20). The depolarization-induced elevation in transient K_{Ca} current frequency most likely occurs through an increase in the percentage of Ca^{2+} sparks that activate K_{Ca} channels. In support of this conclusion, in the presence of diltiazem or in the absence of extracellular Ca^{2+}, both of which would block depolarization-induced Ca^{2+} spark activation (13,17), depolarization elevated transient K_{Ca} current frequency and activity. In murine colonic myocytes, a reduction in extracellular Ca^{2+} reduced local intracellular Ca^{2+} transients but elevated transient K_{Ca} current

frequency and amplitude by removing protein kinase C-mediated K_{Ca} channel inhibition (2). In contrast, in the present study, removal of extracellular Ca^{2+} or diltiazem reduced transient K_{Ca} current frequency but did not alter amplitude. These data suggest Ca^{2+} sparks are activated by Ca^{2+} influx through voltage-dependent Ca^{2+} channels, as previously reported (13,17), and illustrate differences in the mechanisms by which Ca^{2+} spark-K_{Ca} channel coupling is modulated by Ca^{2+} influx pathways in colonic and arterial smooth muscle cells.

K_{Ca} channel "Ca^{2+} sensitivity" has previously been used to describe *1*) the Ca^{2+} concentration that induces half-maximal activation at a given voltage, *2*) the slope of the Ca^{2+}-activity relation at a defined voltage, and *3*) a shift in half-maximal potential for a given Ca^{2+} concentration change (4). Depolarization shifts the Ca^{2+} concentration-K_{Ca} channel activity relation leftward (4) and increases the percentage of Ca^{2+} sparks that activate K_{Ca} channels. The present data dispute the possibility that uncoupling occurs because K_{Ca} channels within the vicinity of Ca^{2+} spark sites are absent or incapable of activation. The K_d for Ca^{2+} of newborn porcine arteriole smooth muscle cell K_{Ca} channels is 31 μM at 0 mV, which is high compared with that of K_{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_{Ca} channel Ca^{2+} sensitivity is lower in uncoupled than in strongly coupled cell types. Other likely explanations are that uncoupled Ca^{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^{2+} elevations. In *B. marinus* stomach smooth muscle cells, some Ca^{2+} spark sites generate sparks that reliably activate transient K_{Ca} currents, whereas other locations consistently generate uncoupled sparks (31). In the amphibian preparation, sites that generate uncoupled Ca^{2+} sparks may be located near sarcolemma that is devoid of K_{Ca} channels or populated by inactivatable K_{Ca} channels (31). However, in newborn cerebral artery smooth muscle cells, Ca^{2+} spark-K_{Ca} channel coupling is increased by membrane depolarization and carbon monoxide, which elevate K_{Ca} channel apparent Ca^{2+} sensitivity (14,15,30). Similarly, in guinea pig bladder smooth muscle cells, membrane depolarization between −50 and −20 mV elevated Ca^{2+} spark coupling (11). Thus K_{Ca} channel localization near Ca^{2+} spark sites and regulation by Ca^{2+} sparks appear to differ in mammalian and amphibian smooth muscle cells.

Regardless of voltage, caffeine, which elevates RyR channel Ca^{2+} sensitivity (24), induced a similar relative increase in transient K_{Ca} current frequency but did not change transient K_{Ca} channel activity. These data suggest that caffeine activates transient K_{Ca} currents by elevating Ca^{2+} spark frequency. Caffeine also elevated global $[Ca^{2+}]_i$ and reduced Ca^{2+} spark amplitude, presumably by causing SR Ca^{2+} leak and a reduction in SR Ca^{2+} load, respectively (6). Caffeine also increased Ca^{2+} spark spread, presumably by elevating the number of RyR channels that contribute to sparks through localized Ca^{2+}-induced Ca^{2+} release. In pulmonary artery smooth muscle cells, 500 μM caffeine did not change Ca^{2+} spark amplitude (calculated as F/F_0) but elevated Ca^{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^{2+} sparks at additional sites in newborn cerebral artery smooth muscle cells. However, the low Ca^{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^{2+} spark spatial and temporal changes was no net change in the mean percentage or effective Ca^{2+} spark-K_{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 ~10–15%. 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, signaling 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.

REFERENCES

1. Ahmed A, Waters CM, Leffler CW, Jaggar JH. Ionic mechanisms mediating the myogenic response in newborn porcine cerebral arteries. Am J Physiol Heart Circ Physiol 2004;287:H2061–H2069. [PubMed: 15284060]
2. Bayguinov O, Hagen B, Kenyon JL, Sanders KM. Coupling strength between localized Ca^{2+} transients and K^+ channels is regulated by protein kinase C. Am J Physiol Cell Physiol 2001;281:C1512–C1523. [PubMed: 11600414]
3. Benham CD, Bolton TB. Spontaneous transient outward currents in single visceral and vascular smooth muscle cells of the rabbit. J Physiol 1986;381:385–406. [PubMed: 2442353]
4. Carl A, Lee HK, Sanders KM. Regulation of ion channels in smooth muscles by calcium. Am J Physiol Cell Physiol 1996;271:C9–C34.
5. Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. Science 1993;262:740–744. [PubMed: 8235594]
6. Cheranov SY, Jaggar JH. Sarcoplasmic reticulum calcium load regulates rat arterial smooth muscle calcium sparks and transient K_{Ca} currents. J Physiol 2002;544:71–84. [PubMed: 12356881]
7. Cheranov SY, Jaggar JH. TNF-α dilates cerebral arteries via NAD(P)H oxidase-dependent Ca^{2+} spark activation. Am J Physiol Cell Physiol 2006;290:C964–C971. [PubMed: 16267103]
8. Davis MJ, Hill MA. Signaling mechanisms underlying the vascular myogenic response. Physiol Rev 1999;79:387–423. [PubMed: 10221985]
9. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985;260:3440–3450. [PubMed: 3838314]
10. Harder DR. Comparison of electrical properties of middle cerebral and mesenteric artery in cat. Am J Physiol Cell Physiol 1980;239:C23–C26.
11. Herrera GM, Heppner TJ, Nelson MT. Voltage dependence of the coupling of Ca^{2+} sparks to BK_{Ca} channels in urinary bladder smooth muscle. Am J Physiol Cell Physiol 2001;280:C481–C490. [PubMed: 11171567]

12. Jackson WF, Blair KL. Characterization and function of Ca^{2+}-activated K^+ channels in arteriolar muscle cells. Am J Physiol Heart Circ Physiol 1998;274:H27–H34.
13. Jaggar JH. Intravascular pressure regulates local and global Ca^{2+} signaling in cerebral artery smooth muscle cells. Am J Physiol Cell Physiol 2001;281:C439–C448. [PubMed: 11443043]
14. Jaggar JH, Leffler CW, Cheranov SY, Tcheranova D,ES, Cheng X. Carbon monoxide dilates cerebral arterioles by enhancing the coupling of Ca^{2+} sparks to Ca^{2+}-activated K^+ channels. Circ Res 2002;91:610–617. [PubMed: 12364389]
15. Jaggar JH, Li A, Parfenova H, Liu J, Umstot ES, Dopico AM, Leffler CW. Heme is a carbon monoxide receptor for large-conductance Ca^{2+}-activated K^+ channels. Circ Res 2005;97:805–812. [PubMed: 16166559]
16. Jaggar JH, Porter VA, Lederer WJ, Nelson MT. Calcium sparks in smooth muscle. Am J Physiol Cell Physiol 2000;278:C235–C256. [PubMed: 10666018]
17. Jaggar JH, Stevenson AS, Nelson MT. Voltage dependence of Ca^{2+} sparks in intact cerebral arteries. Am J Physiol Cell Physiol 1998;274:C1755–C1761.
18. Kirber MT, Etter EF, Bellve KA, Lifshitz LM, Tuft RA, Fay FS, Walsh JV, Fogarty KE. Relationship of Ca^{2+} sparks to STOCs studied with 2D and 3D imaging in feline oesophageal smooth muscle cells. J Physiol 2001;531:315–327. [PubMed: 11230506]
19. Knot HJ, Nelson MT. Regulation of arterial diameter and wall [Ca^{2+}] in cerebral arteries of rat by membrane potential and intravascular pressure. J Physiol 1998;508:199–209. [PubMed: 9490839]
20. Magleby KL. Gating mechanism of BK (Slo1) channels: so near, yet so far. J Gen Physiol 2003;121:81–96. [PubMed: 12566537]
21. Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, Lederer WJ. Relaxation of arterial smooth muscle by calcium sparks. Science 1995;270:633–637. [PubMed: 7570021]
22. Perez GJ, Bonev AD, Nelson MT. Micromolar Ca^{2+} from sparks activates Ca^{2+}-sensitive K^+ channels in rat cerebral artery smooth muscle. Am J Physiol Cell Physiol 2001;281:C1769–C1775. [PubMed: 11698234]
23. Perez GJ, Bonev AD, Patlak JB, Nelson MT. Functional coupling of ryanodine receptors to K_{Ca} channels in smooth muscle cells from rat cerebral arteries. J Gen Physiol 1999;113:229–238. [PubMed: 9925821]
24. Pessah IN, Stambuk RA, Casida JE. Ca^{2+}-activated ryanodine binding: mechanisms of sensitivity and intensity modulation by Mg^{2+}, caffeine, and adenine nucleotides. Mol Pharmacol 1987;31:232–238. [PubMed: 2436032]
25. Remillard CV, Zhang WM, Shimoda LA, Sham JS. Physiological properties and functions of Ca^{2+} sparks in rat intrapulmonary arterial smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2002;283:L433–L444. [PubMed: 12114206]
26. Tanaka Y, Meera P, Song M, Knaus HG, Toro L. Molecular constituents of maxi K_{Ca} channels in human coronary smooth muscle: predominant α + β subunit complexes. J Physiol 1997;502:545–557. [PubMed: 9279807]
27. Wellman GC, Nathan DJ, Saundry CM, Perez G, Bonev AD, Penar PL, Tranmer BI, Nelson MT. Ca^{2+} sparks and their function in human cerebral arteries. Stroke 2002;33:802–808. [PubMed: 11872907]
28. Woodruff ML, Sampath AP, Matthews HR, Krasnoperova NV, Lem J, Fain GL. Measurement of cytoplasmic calcium concentration in the rods of wild-type and transducin knock-out mice. J Physiol 2002;542:843–854. [PubMed: 12154183]
29. Xi Q, Cheranov SY, Jaggar JH. Mitochondria-derived reactive oxygen species dilate cerebral arteries by activating Ca^{2+} sparks. Circ Res 2005;97:354–362. [PubMed: 16020754]
30. Xi Q, Tcheranova D, Parfenova H, Horowitz B, Leffler CW, Jaggar JH. Carbon monoxide activates K_{Ca} channels in newborn cerebral arteriole smooth muscle cells by increasing the apparent Ca^{2+} sensitivity of α-subunits. Am J Physiol Heart Circ Physiol 2004;286:H610–H618. [PubMed: 14563665]
31. ZhuGe R, Fogarty KE, Baker SP, McCarron JG, Tuft RA, Lifshitz LM, Walsh JV Jr. Ca^{2+} spark sites in smooth muscle cells are numerous and differ in number of ryanodine receptors, large-conductance K+ channels, and coupling ratio between them. Am J Physiol Cell Physiol 2004;287:C1577–C1588. [PubMed: 15306542]

32. ZhuGe R, Fogarty KE, Tuft RA, Lifshitz LM, Sayar K, Walsh JV Jr. Dynamics of signaling between Ca^{2+} sparks and Ca^{2+}-activated K^+ channels studied with a novel image-based method for direct intracellular measurement of ryanodine receptor Ca^{2+} current. J Gen Physiol 2000;116:845–864. [PubMed: 11099351]

33. ZhuGe R, Fogarty KE, Tuft RA, Walsh JV Jr. Spontaneous transient outward currents arise from microdomains where BK channels are exposed to a mean Ca^{2+} concentration on the order of 10 μM during a Ca^{2+} spark. J Gen Physiol 2002;120:15–27. [PubMed: 12084772]

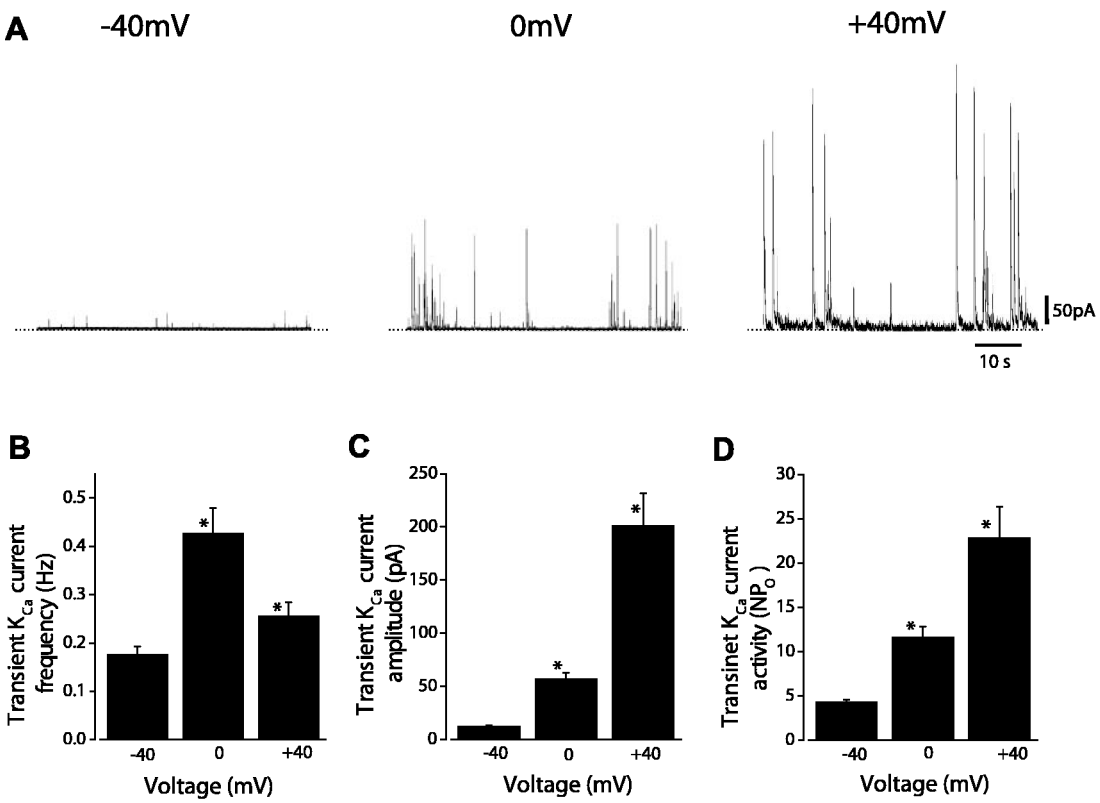

Fig. 1.

Voltage dependence of transient Ca^{2+}-activated K^+ (KCa) current frequency, amplitude, and activity. *A*: original recordings obtained in the same newborn arterial smooth muscle cell illustrating effect of steady membrane potentials between −40 and +40 mV on transient K_{Ca} current frequency and amplitude. *B*: voltage dependence of transient K_{Ca} current frequency (n = 13 cells). *C*: depolarization-induced elevation in transient K_{Ca} current amplitude (n = 13 cells). *D*: depolarization-induced elevation in transient K_{Ca} current activity (n = 13 cells). NP_o, number of K_{Ca} channels activated by Ca^{2+} sparks × open probability of K_{Ca} channels at peak of Ca^{2+} sparks. *P < 0.05 vs. −40 mV.

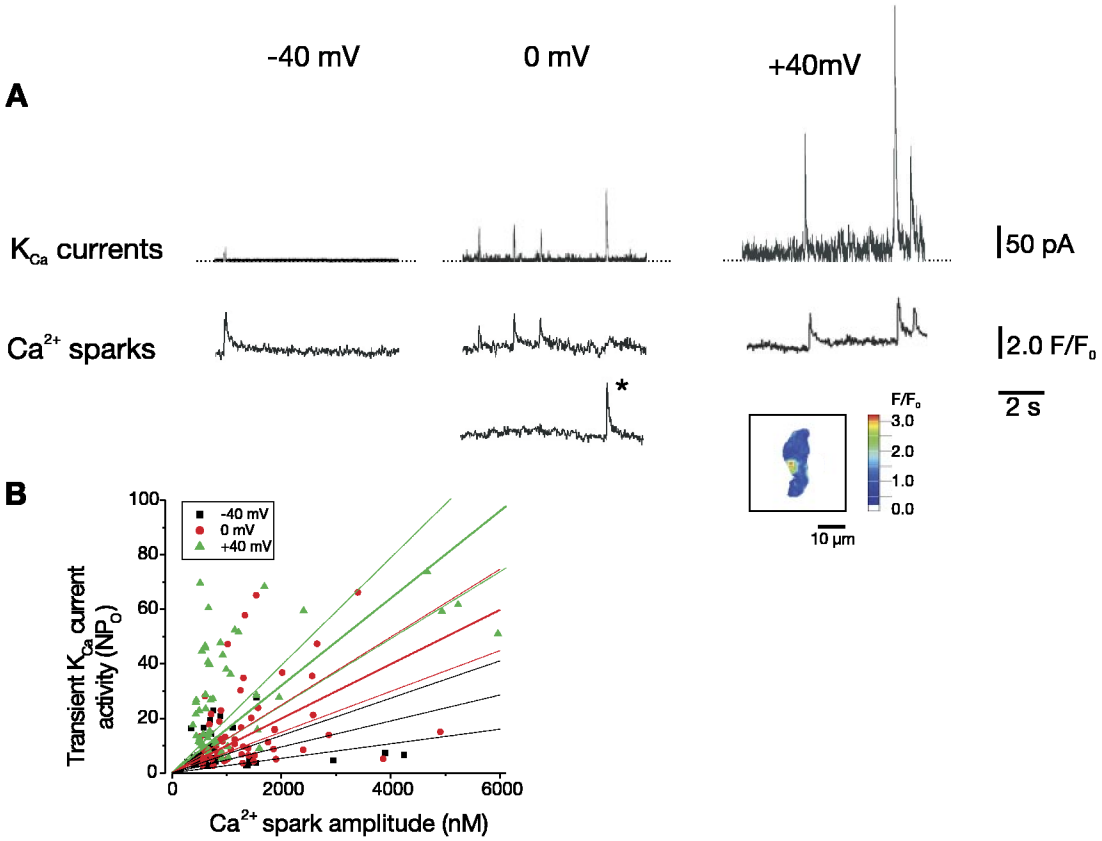

Fig. 2.

Membrane depolarization elevates K_{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 × 1.54 µm (i.e., 2.37 μm^2) 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_{CA} currents (n = 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_{CA} currents were significantly correlated at each voltage ($P < 0.05$ for each).

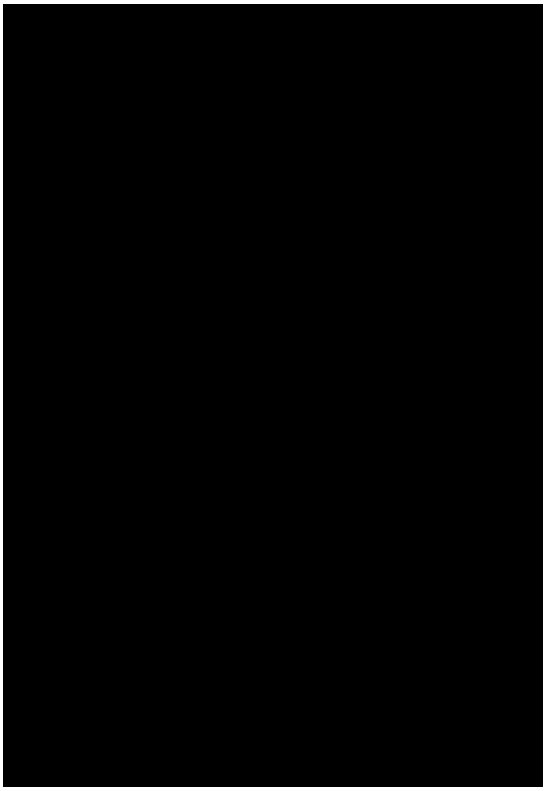

Fig. 3.

In the absence of Ca^{2+} influx, membrane depolarization elevates transient K_{CA} current frequency and amplitude. In a Ca^{2+}-free bath solution ($n = 5$ cells) or in the continued presence of 50 μM diltiazem ($n = 6$ cells), steady membrane depolarization elevates transient K_{CA} current frequency (*A*) and activity (*B*). Dotted lines indicate control level. **P* < 0.05 vs. −40 mV. #*P* < 0.05 vs. 0 mV.

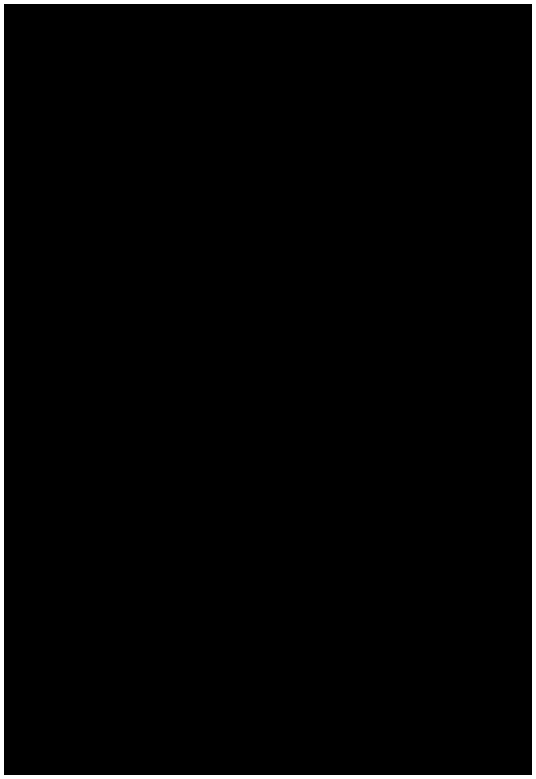

Fig. 4.

Caffeine elevates transient K_{CA} current frequency, but not activity. *A*: 10 μM caffeine increased transient K_{CA} current frequency at steady membrane potentials of −40, 0, and +40 mV ($n = 6$ cells). *B*: caffeine did not alter transient K_{CA} current activity at −40, 0, and +40 mV ($n = 6$ cells). *$P < 0.05$ vs. control at the same voltage.

Fig. 5.

Caffeine does not alter Ca^{2+} spark-K_{CA} channel coupling. Caffeine did not alter relation between peak Ca^{2+} spark amplitude and transient K_{CA} current activity (NP_O). Control and caffeine data were obtained from the same 7 cells. Linear regression with 95% confidence bands are illustrated with slopes of 0.009 and 0.011 for control and caffeine, respectively. Correlation coefficients were 0.11 and 0.53 for control and caffeine data, respectively. Amplitudes of Ca^{2+} sparks and evoked transient K_{CA} currents were significantly correlated for control and caffeine ($P < 0.0001$ for each).

Table 1

Regulation of Ca^{2+} spark and transient K_{Ca} current properties by membrane potential

	−40 mV	0 mV	+40 mV
Ca^{2+} sparks			
Amplitude, All nM	874±89 (65)	1,424±100(74)*	1,121±147 (62)
Coupled	927±123 (45)	1,377±114(58)*	1,187±180 (50)
Uncoupled	755±77 (20)	1283±97(16)*	849±99 (12)
Spread, μm^2	3.56±0.35 (17)	3.08±0.39(17)*	3.58±0.30 (17)
Decay ($t_{1/2}$), ms	61.3±5.3 (17)	52.6±3.0(17)*	59.2±3.6 (17)
Coupling, %	58.6±6.4	76.5±4.2*	86.2±5.1*
Global Ca^{2+}, nM	224±29	363	271
K_{Ca} transients			
Amplitude, pA	34.7±5.9 (39)	88.4±11.6(52)*	251.5±24.3(41)*
NP_o	13.9±2.4 (39)	22.1±2.9(52)*	31.4±3.0(41)*

Values are means±SE of number of events in parentheses. $t_{1/2}$, half time; K_{Ca}, Ca^{2+}-activated K^+; NP_o, number of K_{Ca} channels activated by Ca^{2+} spark × open probability of K_{Ca} channels at peak of Ca^{2+} spark.

* $P < 0.05$ vs. −40 mV.

Table 2

Regulation of Ca^{2+} spark and transient K_{Ca} current properties by caffeine

	Control	Caffeine (10 μM)
Ca^{2+} sparks		
Amplitude: all, nM	1,915±213 (33)	1,375±81(42)*
Spread, μm^2	2.92±0.29 (20)	3.61±0.31(20)*
Decay ($t_{½}$), ms	54.5±3.9 (16)	50.7±5.7 (16)
Coupling, %	75.4±4.9	75.8±5.1
Global Ca^{2+}, nM	363	419±9*
K_{Ca} transient activity (NP_o)	15.5±1.4 (25)	15.7±1.0 (32)

Values are means ± SE of number of events in parentheses.

*$P < 0.05$ vs. control.