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Genetic ablation of caveolin-1 modifies Ca^{2+} spark coupling in murine arterial smooth muscle cells

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Abstract

L-type, voltage-dependent calcium (Ca^{2+}) channels, ryanodine-sensitive Ca^{2+} release (RyR) channels, and large-conductance Ca^{2+} -activated potassium (K_{Ca}) channels comprise a functional unit that regulates smooth muscle contractility. Here, we investigated whether genetic ablation of caveolin-1 (cav-1), a caveolae protein, alters Ca^{2+} spark to K_{Ca} channel coupling and Ca^{2+} spark regulation by voltage-dependent Ca^{2+} channels in murine cerebral artery smooth muscle cells. Caveolae were abundant in the sarcolemma of control (cav- $^{+/+}$) cells but were not observed in cav-1-deficient (cav-1 $^{-/-}$) cells. Ca^{2+} spark and transient K_{Ca} current frequency were approximately twofold higher in cav-1 $^{-/-}$ than in cav-1 $^{+/+}$ cells. Although voltage-dependent Ca^{2+} current density was similar in cav-1 $^{+/+}$ and cav-1 $^{-/-}$ cells, diltiazem and Cd^{2+} , voltage-dependent Ca^{2+} channel blockers, reduced transient K_{Ca} current frequency to ~55% of control in cav-1 $^{+/+}$ cells but did not alter transient K_{Ca} current frequency in cav-1 $^{-/-}$ cells. Furthermore, although K_{Ca} channel density was elevated in cav-1 $^{-/-}$ cells, transient K_{Ca} current amplitude was similar to that in cav-1 $^{+/+}$ cells. Higher Ca^{2+} spark frequency in cav-1 $^{-/-}$ cells was not due to elevated intracellular Ca^{2+} concentration, sarcoplasmic reticulum Ca^{2+} load, or nitric oxide synthase activity. Similarly, Ca^{2+} spark amplitude and spread, the percentage of Ca^{2+} sparks that activated a transient K_{Ca} current, the amplitude relationship between sparks and transient K_{Ca} currents, and K_{Ca} channel conductance and apparent Ca^{2+} sensitivity were similar in cav-1 $^{+/+}$ and cav-1 $^{-/-}$ cells. In summary, cav-1 ablation elevates Ca^{2+} spark and transient K_{Ca} current frequency, attenuates the coupling relationship between voltage-dependent Ca^{2+} channels and RyR channels that generate Ca^{2+} sparks, and elevates K_{Ca} channel density but does not alter transient K_{Ca} current activation by Ca^{2+} sparks. These findings indicate that cav-1 is required for physiological Ca^{2+} spark and transient K_{Ca} current regulation in cerebral artery smooth muscle cells.

Keywords

ryanodine-sensitive Ca^{2+} release channel; large-conductance Ca^{2+} -activated potassium channel; caveolae; voltage-dependent Ca^{2+} channel

Ca^{2+} is a signaling messenger that regulates a wide variety of cellular functions, including secretion, proliferation, and contraction (2). To regulate specific Ca^{2+} -dependent functions, cells can generate a variety of intracellular Ca^{2+} signals with distinct frequency, amplitude, and spatial-temporal characteristics.

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Smooth muscle cells generate several different modes of Ca^{2+} signaling (15,20). The global intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) arises because of extracellular Ca^{2+} influx and intracellular Ca^{2+} release. An elevation in global $[\text{Ca}^{2+}]_i$ stimulates contraction, whereas a reduction in global $[\text{Ca}^{2+}]_i$ results in relaxation. Localized $[\text{Ca}^{2+}]_i$ transients, termed “ Ca^{2+} sparks,” also occur in smooth muscle cells (20,27). Ca^{2+} sparks occur due to the opening of several ryanodine-sensitive Ca^{2+} release (RyR) channels on the sarcoplasmic reticulum (SR) (15,27). In smooth muscle cells, a Ca^{2+} spark does not directly elevate global Ca^{2+} but activates a number of nearby large-conductance Ca^{2+} -activated potassium (K_{Ca}) channels, resulting in a transient K_{Ca} current. Membrane hyperpolarization induced by asynchronous transient K_{Ca} currents reduces voltage-dependent Ca^{2+} channel activity, leading to a decrease in global $[\text{Ca}^{2+}]_i$ and relaxation. An elevation in $[\text{Ca}^{2+}]_i$ activates Ca^{2+} sparks and transient K_{Ca} currents, forming a negative-feedback loop that limits Ca^{2+} entry through voltage-dependent Ca^{2+} channels (15,27). Thus voltage-dependent Ca^{2+} channels, RyR channels, and K_{Ca} channels comprise a functional unit that regulates smooth muscle contractility (17). The differential regulation of contractility by local and global Ca^{2+} signaling is also effective because of differences in the frequency and amplitude of the Ca^{2+} signals and the Ca^{2+} sensitivities of the downstream targets for each signal mode (15). For local Ca^{2+} signaling mechanisms to operate, downstream targets must also be located in the proximity of the Ca^{2+} source. In smooth muscle cells, whether membrane proteins maintain spatial organization of voltage-dependent Ca^{2+} channels, K_{Ca} channels, and RyR channels to permit Ca^{2+} signaling between these proteins is unclear.

Compartmentalization of signaling molecules can occur in small, cholesterol-enriched, flask-shaped membrane invaginations termed “caveolae” (11,13,34, 39). Caveolins, of which three isoforms have been cloned (cav 1–3), are structural components required for caveolae formation (11,13,34,39). Although all three caveolins have been identified in vascular smooth muscle cells, caveolin-1 is the primary isoform (9,18, 28,34,39). Supporting a role for caveolae in the regulation of arterial smooth muscle local Ca^{2+} signaling is evidence that acute cholesterol depletion with dextrin inhibits Ca^{2+} sparks (23). Similarly, transient K_{Ca} current frequency is reduced in cerebral artery smooth muscle cells of cav-1-deficient (cav-1^{-/-}) mice, when compared with wild-type controls (10). In ureter smooth muscle cells, K_{Ca} channels are found in buoyant membrane fractions, and in cultured myometrial cells K_{Ca} channels are localized to caveolae (1,3). Thus cav-1 and caveolae may regulate Ca^{2+} sparks and K_{Ca} channels in smooth muscle.

Here, the communication between voltage-dependent Ca^{2+} channels and RyR channels that generate Ca^{2+} sparks and signaling between Ca^{2+} sparks and K_{Ca} channels was investigated in cerebral artery smooth muscle cells of cav-1^{+/+} and cav-1^{-/-} mice. Data indicate that cav-1 ablation abolishes caveolae, elevates Ca^{2+} spark frequency, and attenuates Ca^{2+} spark regulation by voltage-dependent Ca^{2+} channels. In contrast, transient K_{Ca} current activation by Ca^{2+} sparks is unaltered in cav-1^{-/-} cells, even though there is an increase in K_{Ca} channel density.

MATERIALS AND METHODS

Tissue preparation

Animal procedures used were reviewed and approved by the Animal Care and Use Committee policies at the University of Tennessee. Age-matched (4–6 wk) cav-1^{-/-} mice (Cav1^{tm1Mls}, stock no. 004585; see Ref. 33) or wild-type control (cav-1^{+/+}) mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were killed by peritoneal injection of a pentobarbital sodium overdose (130 mg/kg). The brain was removed and placed into ice-cold (4°C) physiological saline solution (PSS) containing (in mM) 112 NaCl, 4.8 KCl, 26 NaHCO_3 , 1.8 CaCl_2 , 1.2 MgSO_4 , 1.2 KH_2PO_4 , and 10 glucose and gassed with 74% N_2 –21%

O₂-5% CO₂ (pH 7.4). Posterior cerebral, cerebellar, and middle cerebral arteries (~150 µm in diameter) were removed, cleaned of connective tissue, and maintained in ice-cold Ca²⁺-free buffer (*solution A*) containing (in mM) 55 NaCl, 80 Na glutamate, 5.6 KCl, 2 MgCl₂, 10 HEPES, and 10 glucose (pH 7.3 with NaOH). For single cell isolation, smooth muscle cells were dissociated from cerebral arteries with the use of enzymes, as previously described (14).

Electron microscopy

The brain was fixed in PSS containing 2.5% glutaraldehyde for 1 h. Cerebral arteries were dissected from the fixed brain, cleaned of connective tissue, and postfixed in 1% osmium tetroxide in PSS for 4 h. Arteries were then rinsed briefly in deionized water and en bloc stained with 2% uranyl acetate in 0.85% sodium chloride overnight at 4°C. Arteries were dehydrated in graded solutions of ethanol, from 30% through 100% for 1 h each, infiltrated with 50% Spurr in 100% ethanol overnight at room temperature, 100% Spurr over an 8-h period involving at least three changes of Spurr, and then cured at 60°C for 2 days. Transverse sections (75 nm) were cut with a Reichert Ultracut E microtome and poststained with uranyl acetate and lead citrate. Cells were observed and photographed with a JEOL 2000EX TEM located in the Electron Microscope Facility at the University of Tennessee Health Science Center.

Patch-clamp electrophysiology

Potassium currents were measured by using the conventional whole cell, perforated-patch or inside-out patch-clamp configurations with an Axopatch 200B amplifier and Clampex 8.2 (Axon Instruments, Union City, CA). For transient K_{Ca} current measurement, the bath solution (*solution B*) contained (in mM) 134 NaCl, 6 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose (pH 7.4 with NaOH), and the pipette solution contained (in mM) 110 potassium aspartate, 30 KCl, 10 NaCl, 1 MgCl₂, 10 HEPES, and 0.05 EGTA (pH 7.2 with KOH). Amphotericin B (Sigma) was dissolved in DMSO and diluted into the pipette solution to give a final concentration of 200 µg/ml. For whole cell K⁺ current measurement, the bath solution was *solution B* and the pipette solution contained (in mM) 135 KCl, 5 EGTA, 1.0 BAPTA, 3.5 MgCl₂, 2.5 Na₂ATP, and 10 HEPES (pH 7.2 with KOH). For inside-out patch-clamp experiments to measure single K_{Ca} channel currents, the pipette and bath solution both contained (in mM) 140 KCl, 10 HEPES, 5 EGTA, and 1.6 1-*N*-(2-hydroxyethyl) ethylenediamine-*N,N',N'*-triacetic acid (pH 7.2 with KOH). MgCl₂ and CaCl₂ were supplemented to bath solution to provide 1 mM free Mg²⁺ and the required free Ca²⁺ concentration (1–100 µM). Free Ca²⁺ concentration in the pipette solution was 10 µM. Free Ca²⁺ concentrations were measured by using a Ca²⁺-sensitive and reference electrode (Corning). For voltage-dependent Ba²⁺ current measurements, the pipette solution contained (in mM) 125 CsCl, 20 tetraethylammonium chloride (TEACl), 1.0 MgCl₂, 0.5 EGTA, 10 HEPES, and 10 glucose (pH 7.2 with CsOH), and the bath solution contained (in mM) 60 NaCl, 60 TEACl, 1.0 MgCl₂, 20 BaCl₂, 10 glucose, and 10 HEPES (pH 7.4 with NaOH). Pipette resistance was measured by applying a 5-mV pulse using the seal test function of pClamp 8.2. Transient K_{Ca} currents were measured at a steady membrane potential of –40 mV. Voltage-dependent Ba²⁺ currents were activated from a holding potential of –80 mV by applying 300-ms voltage steps to voltages between –50 and +60 mV in increments of 10 mV. Whole cell K⁺ (*I_k*) currents were activated from a holding potential of –80 mV by applying 250-ms voltage steps to voltages between –70 and +80 mV in 10-mV increments. In inside-out patches, single K_{Ca} channel currents were measured at steady voltages of –40 or +40 mV. Transient K_{Ca} currents were filtered at 1 kHz and digitized at 5 kHz. Voltage-dependent Ba²⁺ currents were filtered at 1 kHz and digitized at 4 kHz. Other current measurements were filtered at 2 kHz and digitized at 10 kHz. Transient K_{Ca} current analysis was performed off-line using methodology described elsewhere (6). A transient K_{Ca} current was defined as the simultaneous opening of three or more K_{Ca} channels. Open probability (*P_o*) was calculated from the following equation: $P_o = (\sum t_i i) / nT$, where *t_i* is the time at each channel level *i*, *n* is the number

of channels in the patch, and T is the total time of analysis. The total number of K_{Ca} channels in an inside-out patch was determined at a voltage of +40 mV with 100 μ M free Ca^{2+} in the bath solution. In each patch under each condition, at least 5 min of continuous data were analyzed to calculate transient K_{Ca} current frequency and amplitude, and 2–5 min were analyzed to determine single K_{Ca} channel open probability.

Confocal Ca^{2+} imaging

Isolated smooth muscle cells were incubated in *solution A* containing fluo-4 AM (10 μ M) for 20 min at room temperature, followed by a 30-min wash to allow indicator deesterification. Smooth muscle cells were imaged with the use of a Noran Oz laser scanning confocal microscope (Noran Instruments, Middleton, WI) and a $\times 60$ water immersion objective (1.2 numerical aperture) attached to a Nikon TE300 microscope. Fluo-4 was excited by using the 488 nm line of a krypton-argon laser, and emitted light >500 nm was captured. Images ($56.3 \times 52.8 \mu$ m) were recorded every 8.3 ms (120 images/s). The laser intensity used did not alter transient K_{Ca} current frequency or amplitude. Current and fluorescence measurements were synchronized by using a light-emitting diode placed above the recording chamber that was triggered during acquisition. Each cell was imaged for 15 s. Ca^{2+} sparks in smooth muscle cells were analyzed with the use of software kindly provided by Dr. M. T. Nelson (University of Vermont). Detection of Ca^{2+} sparks was performed by dividing an area 1.54μ m (7 pixels) $\times 1.54 \mu$ m (7 pixels) (i.e., 2.37μ m²) in each image (F) by a baseline (F_0) that was determined by averaging 10 images without Ca^{2+} spark activity. The entire area of each image was analyzed to detect Ca^{2+} sparks. A Ca^{2+} spark was defined as a local increase in F/F_0 that was >1.2 .

Fura-2 imaging

Cerebral arteries were incubated with the ratio-metric fluorescent Ca^{2+} indicator fura-2 AM (5 μ M) and 0.05% pluronic F-127 for 20 min, followed by a 15-min wash. All experiments were performed using *solution B* (composition described in *Patch-clamp electrophysiology*). Fura-2 was alternately excited at 340 or 380 nm using a PC-driven hyperswitch (Ionoptix, Milton, MA). Background corrected ratios were collected every 1 s at 510 nm using a photomultiplier tube (Ionoptix). SR Ca^{2+} load ($[Ca^{2+}]_{SR}$) was estimated by rapidly applying a high concentration of caffeine (10 mM), an RyR channel activator, and measuring the amplitude of $[Ca^{2+}]_i$ transients (i.e., $\Delta[Ca^{2+}]_i$). Intracellular Ca^{2+} concentrations were calculated by using the following equation (12):

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

where R is the 340/380 nm ratio; R_{min} and R_{max} are the minimum and maximum 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). R_{min} , R_{max} , S_{f2} , and S_{b2} were determined at the end of experiments and in separate experiments by increasing the Ca^{2+} permeability of smooth muscle cells with ionomycin (10 μ M) and by perfusing cells with a high- Ca^{2+} (10 mM) or Ca^{2+} -free (no added Ca^{2+} , 5 mM EGTA) solution.

Measurement of cellular dimensions

Isolated smooth muscle cells were allowed to settle on a glass coverslip in a chamber with Ca^{2+} -free, HEPES-containing patch-clamp bath solution (composition described in *Patch-clamp electrophysiology*). Cell images were acquired with the use of a Zeiss LSM5 confocal microscope. Cell length and width were calculated using Pascal software (Zeiss).

Statistics

Values are expressed as means $\pm$ SE. Student's *t*-test and Student-Newman-Keuls test were used for comparing paired or unpaired data and multiple data sets, respectively. Simultaneous Ca^{2+} spark and transient K_{Ca} current amplitude data were fit with a linear regression function, and the slope $\pm$ SE of each fit was compared using Student's *t*-test and Graphpad Prism (San Diego, CA). $P < 0.05$ was considered significant.

RESULTS

Caveolae are absent in cerebral artery smooth muscle cells of $\text{cav-1}^{-/-}$ mice

Electron microscopy revealed that abundant caveolae were present in the sarcolemma of smooth muscle cells of small-diameter ($\sim 150 \mu\text{m}$) cerebral arteries of $\text{cav-1}^{+/+}$ mice (Fig. 1, A and B). In contrast, caveolae were not observed in cerebral artery smooth muscle cells of $\text{cav-1}^{-/-}$ mice (Fig. 1, C and D). These data indicate that cav-1 is necessary for caveolae formation in smooth muscle cells of small cerebral arteries.

Transient K_{Ca} current frequency is higher in $\text{cav-1}^{-/-}$ than in $\text{cav-1}^{+/+}$ cerebral artery smooth muscle cells

To investigate the effects of cav-1 ablation on Ca^{2+} spark-to- K_{Ca} channel signaling, transient K_{Ca} currents were measured in cerebral artery smooth muscle cells of $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ mice. Transient K_{Ca} currents were measured at -40 mV , which is the membrane potential of cerebral arteries pressurized to 60 mmHg (19). Mean transient K_{Ca} current frequency was $1.11 \pm 0.14 \text{ Hz}$ in $\text{cav-1}^{+/+}$ cells ($n = 35$) and $2.20 \pm 0.30 \text{ Hz}$ in $\text{cav-1}^{-/-}$ cells ($n = 31$), or approximately twofold higher (Fig. 2, A and B). In contrast, transient K_{Ca} current amplitude was similar in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cells (Fig. 2, A and C).

Cell capacitance of $\text{cav-1}^{-/-}$ cells ($\sim 8.4 \text{ pF}$) was smaller than for $\text{cav-1}^{+/+}$ cells ($\sim 12.1 \text{ pF}$; Fig. 2D). To investigate whether cell size underlies the difference in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cell capacitance, cellular dimensions were measured. Cells were observed in a Ca^{2+} -free bath solution to induce maximal relaxation. $\text{Cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cell length [in μm : $\text{cav-1}^{+/+}$, 37.1 ± 1.6 ($n = 32$); $\text{cav-1}^{-/-}$, 39.4 ± 2.6 ($n = 33$)] and width [in μm : $\text{cav-1}^{+/+}$, 9.6 ± 0.3 ($n = 32$); $\text{cav-1}^{-/-}$, 9.4 ± 0.3 ($n = 33$)] were not different ($P > 0.05$ for each). These data suggest that cav-1 ablation reduces the cell surface area but does not alter the dimensions of cerebral artery smooth muscle cells.

In arterial smooth muscle cells, transient K_{Ca} currents occur due to SR Ca^{2+} release (38). We sought to determine mechanisms that activate transient K_{Ca} currents in $\text{cav-1}^{-/-}$ cells. In $\text{cav-1}^{-/-}$ cells, thapsigargin (100 nM), an SR Ca^{2+} ATPase inhibitor, abolished transient K_{Ca} currents, indicating that these events occur due to SR Ca^{2+} release (Fig. 2E; $n = 4$).

Ca^{2+} spark frequency is elevated in $\text{cav-1}^{-/-}$ cells when compared with $\text{cav-1}^{+/+}$ cells, but the amplitude relationship between Ca^{2+} sparks and transient K_{Ca} currents is similar

Elevated transient K_{Ca} current frequency in $\text{cav-1}^{-/-}$ cells could occur because of an increase in Ca^{2+} spark frequency or an increase in the percentage of Ca^{2+} sparks that activate a transient K_{Ca} current (i.e., percent coupling). To investigate these possibilities and to compare Ca^{2+} spark properties in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cells, simultaneous measurements of Ca^{2+} sparks and evoked transient K_{Ca} currents were obtained by performing confocal Ca^{2+} imaging in combination with patch-clamp electrophysiology.

At -40 mV , Ca^{2+} spark frequency was ~ 1.8 -fold higher in $\text{cav-1}^{-/-}$ cells than in $\text{cav-1}^{+/+}$ cells (Fig. 3, A and B). In contrast, Ca^{2+} spark amplitude (Fig. 3C) and spatial spread [full width at half-maximal amplitude; $\text{cav-1}^{+/+}$, $1.82 \pm 0.13 \mu\text{m}$ ($n = 40$); $\text{cav-1}^{-/-}$, $1.94 \pm 0.11 \mu\text{m}$ ($n = 59$)]

were similar in *cav-1*^{+/+} and *cav-1*^{-/-} cells ($P > 0.05$ for each). The percentage of Ca^{2+} sparks that activated a transient K_{Ca} current (*cav-1*^{+/+}, $67 \pm 8\%$; *cav-1*^{-/-}, $71 \pm 4\%$) and the amplitude relationship between Ca^{2+} sparks and transient K_{Ca} currents were also similar (Fig. 3, A and D). Taken together, these data indicate that genetic ablation of *cav-1*^{-/-} elevates Ca^{2+} spark frequency, leading to an increase in transient K_{Ca} current frequency.

Cytosolic $[\text{Ca}^{2+}]_i$ and $[\text{Ca}^{2+}]_{\text{SR}}$ are similar in *cav-1*^{+/+} and *cav-1*^{-/-} cells

Cytosolic $[\text{Ca}^{2+}]_i$ and $[\text{Ca}^{2+}]_{\text{SR}}$ regulate Ca^{2+} sparks in arterial smooth muscle cells (6,38). However, $[\text{Ca}^{2+}]_i$ and $[\text{Ca}^{2+}]_{\text{SR}}$, as determined by caffeine (10 mM)-induced Ca^{2+} transients, were not different in *cav-1*^{+/+} and *cav-1*^{-/-} cerebral arteries (Fig. 4, A–C). Thus the higher Ca^{2+} spark frequency in *cav-1*^{-/-} cells is not because of elevated cytosolic or SR Ca^{2+} concentration.

***N*^ω-nitro-L-arginine does not inhibit transient K_{Ca} currents in *cav-1*^{+/+} or *cav-1*^{-/-} cells**

Cav-1 ablation leads to nitric oxide (NO) synthase (NOS) activation and an increase in NO generation (10,34). NO donors activate Ca^{2+} sparks in arterial smooth muscle cells (31). Therefore, we investigated whether Ca^{2+} spark frequency is higher in *cav-1*^{-/-} cells because of elevated NOS activity. *N*^ω-nitro-L-arginine (1 mM), a NOS blocker, did not alter transient K_{Ca} current frequency or amplitude in either *cav-1*^{+/+} [%control; frequency, 122 ± 16 ; amplitude, 107 ± 21 ($n = 6$)] or *cav-1*^{-/-} [frequency, 106 ± 13 ; amplitude, 90 ± 17 ($n = 5$)] cells ($P > 0.05$ for each). These data suggest that the Ca^{2+} spark frequency elevation in *cav-1*^{-/-} cells does not occur through NOS activation.

Transient K_{Ca} current regulation by voltage-dependent Ca^{2+} channels is abolished in *cav-1*^{-/-} cells

In smooth muscle cells, Ca^{2+} sparks are activated by Ca^{2+} entering through sarcolemma voltage-dependent Ca^{2+} channels (14,16,20). We investigated whether Ca^{2+} sparks are more frequent in *cav-1*^{-/-} cells because of enhanced coupling between voltage-dependent Ca^{2+} channels and RyR channels. In *cav-1*^{+/+} cells, Cd^{2+} (250 μM), a voltage-dependent Ca^{2+} channel blocker, or diltiazem (50 μM), an L-type Ca^{2+} channel blocker, reduced transient K_{Ca} current frequency to ~ 51 and $\sim 57\%$ of control, respectively, but did not alter transient K_{Ca} current amplitude (Fig. 5, A–C). In contrast, Cd^{2+} and diltiazem did not alter transient K_{Ca} current frequency or amplitude in *cav-1*^{-/-} cells (Fig. 5, A–C). Thus Ca^{2+} spark regulation by voltage-dependent Ca^{2+} channels is abolished in *cav-1*^{-/-} cells, indicating that higher Ca^{2+} spark frequency is not through enhanced coupling between voltage-dependent Ca^{2+} channels and RyR channels.

Voltage-dependent Ba^{2+} current density is similar in *cav-1*^{+/+} and *cav-1*^{-/-} cells

In *cav-1*^{-/-} cells, voltage-dependent Ca^{2+} channel density may be reduced or voltage-dependent Ca^{2+} channels may be insensitive to blockers. To test these hypotheses, voltage-dependent Ca^{2+} current density relationships were compared in *cav-1*^{+/+} and *cav-1*^{-/-} cells using Ba^{2+} as the charge carrier. Voltage-dependent Ba^{2+} current density was similar, and Cd^{2+} abolished voltage-dependent Ba^{2+} currents in both *cav-1*^{+/+} and *cav-1*^{-/-} cells (Fig. 6, A and B). Thus the absence of Ca^{2+} spark regulation by voltage-dependent Ca^{2+} entry in *cav-1*^{-/-} cells cannot be explained by alterations in sarcolemma voltage-dependent Ca^{2+} current density or Cd^{2+} sensitivity.

K_{Ca} and K_V current density is higher in *cav-1*^{-/-} than in *cav-1*^{+/+} cells

In cardiac myocytes, coupling between voltage-gated Ca^{2+} channels and RyR channels is tight, whereas in smooth muscle cells, this communication is loose (5,20,24,36). Conceivably, Ca^{2+} spark regulation by voltage-dependent Ca^{2+} channels may be abolished in *cav-1*^{-/-} cells

because of an increase in the distance between the sarcolemma and the SR. Therefore, we sought to study the distance between Ca^{2+} spark sites and a sarcolemmal Ca^{2+} spark target in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cells. To do this, K_{Ca} channel properties were measured, thereby allowing further investigation of Ca^{2+} spark to K_{Ca} channel coupling.

Transient K_{Ca} current amplitude is dependent on K_{Ca} channel conductance and Ca^{2+} sensitivity (4). In excised inside-out patches, single K_{Ca} channel slope conductance between -40 and $+40$ mV [$\text{cav-1}^{+/+}$, 268 ± 3 pS ($n = 9$); $\text{cav-1}^{-/-}$, 260 ± 3 pS ($n = 7$); $P > 0.05$] and K_{Ca} channel-apparent Ca^{2+} sensitivity were similar in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cells (Fig. 7, A and B). Transient K_{Ca} current amplitude also depends on the number of K_{Ca} channels activated by a Ca^{2+} spark. On average, inside-out patches pulled from $\text{cav-1}^{-/-}$ cells contained ~ 1.5 -fold more K_{Ca} channels than patches obtained from $\text{cav-1}^{+/+}$ cells (Fig. 7C). This finding was not due to differences in the size of patch pipettes used for these experiments [$\text{cav-1}^{+/+}$, 28.6 ± 1.2 M Ω ($n = 17$); $\text{cav-1}^{-/-}$, 27.1 ± 0.7 M Ω ($n = 22$); $P > 0.05$]. These data suggest that sarcolemma K_{Ca} channel density is higher in $\text{cav-1}^{-/-}$ cells than in $\text{cav-1}^{+/+}$ cells.

To further examine K_{Ca} channel properties, whole cell K^{+} current density was measured in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cells. K^{+} currents were measured by using a strongly buffered Ca^{2+} -free pipette solution to abolish Ca^{2+} -dependent K_{Ca} channel activation. Whole cell K^{+} current density was larger in $\text{cav-1}^{-/-}$ cells than in $\text{cav-1}^{+/+}$ cells (Fig. 8, A and D). Paxilline (300 nM), a selective K_{Ca} channel blocker (21,35), partially inhibited K^{+} currents in both $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cells (Fig. 8B). Paxilline-insensitive current density, which arises because of K_{V} channel activation, was larger in $\text{cav-1}^{-/-}$ cells than in $\text{cav-1}^{+/+}$ cells (Fig. 8, B and E). Similarly, paxilline-sensitive current density, which occurs through K_{Ca} channel activation, was larger in $\text{cav-1}^{-/-}$ cells than in $\text{cav-1}^{+/+}$ cells (Fig. 8, C and F). These data suggest that cav-1 ablation elevates the sarcolemmal current density of both K_{Ca} and K_{V} channels.

DISCUSSION

The impact of cav-1 ablation on the regulation of Ca^{2+} sparks by voltage-dependent Ca^{2+} channels and the communication between Ca^{2+} sparks and K_{Ca} channels were investigated in arterial smooth muscle cells. Caveolae were not observed in $\text{cav-1}^{-/-}$ cerebral artery smooth muscle cells, consistent with a role for this protein in caveolae formation (10,34,39). Cav-1 ablation attenuated coupling between voltage-dependent Ca^{2+} channels and RyR channels that generate Ca^{2+} sparks. Cav-1 ablation also elevated Ca^{2+} spark frequency, although this was not due to an increase in voltage-dependent Ca^{2+} current density, cytosolic or SR Ca^{2+} concentration, or NOS activity. Although there was an increase in K_{Ca} channel density in $\text{cav-1}^{-/-}$ cells, transient K_{Ca} current amplitude was similar to that in $\text{cav-1}^{+/+}$ cells. Taken together, the data suggest that cav-1 abolishment leads to uncoupling between voltage-dependent Ca^{2+} channels and RyR channels that generate Ca^{2+} sparks but does not alter transient K_{Ca} current activation by Ca^{2+} sparks.

In smooth muscle cells, coupling between voltage-dependent Ca^{2+} channels and RyR channels is “loos” (20). Although structural and molecular mechanisms that establish this coupling process are unclear, loss of voltage-dependent Ca^{2+} channel to RyR channel communication in $\text{cav-1}^{-/-}$ cells likely occurs because of spatial separation of these proteins. Supporting this hypothesis is evidence that although voltage-dependent Ca^{2+} current density was similar in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cells, regulation of transient K_{Ca} currents by voltage-dependent Ca^{2+} channel blockers was abolished in $\text{cav-1}^{-/-}$ cells. In addition, although K_{Ca} channel density was elevated in $\text{cav-1}^{-/-}$ cells, mean transient K_{Ca} current amplitude was similar to that in $\text{cav-1}^{+/+}$ cells. K_{Ca} channel conductance, Ca^{2+} spark amplitude and spatial spread, the effective coupling of Ca^{2+} sparks to K_{Ca} channels, and K_{Ca} channel-apparent Ca^{2+} sensitivity were similar in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cells. Caveolae are structural membrane invaginations that

may reduce the sarcolemma to SR distance and physically localize RyR channels nearby voltage-dependent Ca^{2+} channels and K_{Ca} channels in $\text{cav-1}^{+/+}$ cells. A flattening of the sarcolemma in $\text{cav-1}^{-/-}$ cells may explain the proposed increase in the signaling distance between RyR channels and voltage-dependent Ca^{2+} channels and K_{Ca} channels. In $\text{cav-1}^{-/-}$ cells, a distant spark would impact a smaller membrane area and induce a lower subsarcolemmal $[\text{Ca}^{2+}]_i$ elevation. Consistent with our observations, higher K_{Ca} channel density would be required for distant Ca^{2+} sparks to activate transient K_{Ca} currents of similar amplitude to those in $\text{cav-1}^{+/+}$ cells.

Another explanation for the proposed increase in signaling distance is that cav-1 abolishment leads to delocalization of voltage-dependent Ca^{2+} channels and K_{Ca} channels from within the vicinity of the spark site. Several lines of evidence support such a proposal. In smooth muscle cells, caveolae compartmentalize voltage-dependent Ca^{2+} channels and K_{Ca} channels (3,8). In arterial smooth muscle cells, K_{Ca} channels are proposed not to cluster above Ca^{2+} sparks sites, although in *Bufo marinus* stomach smooth muscle cells, such clustering may occur (30,42). In guinea pig bladder smooth muscle, peripheral SR and caveolae are in close proximity, and L-type Ca^{2+} channels located in caveolae strips may be closely opposed to SR RyR channels (25). It is not clear what causes the elevation in K_{Ca} channel density in $\text{cav-1}^{-/-}$ cells, but in cultured human myometrial smooth muscle cells, K_{Ca} channels associate with caveolins 1 and 2, and cholesterol depletion with cyclodextrin leads to an increase in whole cell K_{Ca} current density (3), consistent with the results here. Furthermore, in bovine endothelial cells, cav-1 physically interacts with and inhibits K_{Ca} channels and this effect can be removed by cholesterol depletion, leading to channel activation (37).

Investigating the effects of chronic caveolae deficiency on smooth muscle Ca^{2+} signaling can provide insights into physiological regulation by these membrane organelles in $\text{cav-1}^{+/+}$ cells and potential changes that occur during pathophysiology and ontogeny (11,29,32,34,39). In contrast to the observations here, acute cholesterol depletion with dextrin inhibited arterial smooth muscle Ca^{2+} sparks (23). Acute cholesterol depletion and chronic cav-1 ablation may differentially regulate Ca^{2+} spark frequency through opposing effects on signaling pathways that regulate these events, and in the case of the genetic model, through developmental changes that occur in the sustained absence of cav-1 (10,39). However, dextrin treatment also induces a change in membrane fluidity that may inhibit Ca^{2+} sparks (26). In agreement, both dextrin and cav-1 ablation abolished coupling between voltage-dependent Ca^{2+} channels and RyR channels that generate sparks (present data and Ref. 23). Whereas dextrin treatment disrupts both caveolar and noncaveolar lipid rafts, in the absence of cav-1 , noncaveolar lipid rafts would remain (see Ref. 40). Thus our data suggest that caveolae-localized voltage-dependent Ca^{2+} channels regulate Ca^{2+} sparks in arterial smooth muscle cells. In another previous study, transient K_{Ca} current frequency was reduced in arterial smooth muscle cells of $\text{cav-1}^{-/-}$ mice (10). It is unclear why data here are contradictory to those observations, but in the previous study, effects of cav-1 deficiency on Ca^{2+} sparks were not measured (10).

Through its amino-terminal scaffolding domain, cav-1 inhibits the activity of many binding partners, including NOS, protein kinase C, and adenylyl cyclase (28,34). Cav-1 is found in the cytosol (22) and, conceivably, may interact with and inhibit RyR channels. Cav-1 is also found in mitochondria, which regulate Ca^{2+} sparks in cerebral artery smooth muscle cells (7,41). Cav-1 abolishment may also activate RyR channels indirectly by modulating the activity of signaling pathways that regulate Ca^{2+} sparks (e.g., see Refs. 15 and 41). Data here indicate that cav-1 ablation does not activate Ca^{2+} sparks through NOS activation (31,33). Because cav-1 ablation may activate RyR channels directly and indirectly, future studies will be required to determine the specific underlying mechanisms. The physiological impact of the transient K_{Ca} current frequency elevation in $\text{cav-1}^{-/-}$ cells would be a membrane hyperpolarization that reduces voltage-dependent Ca^{2+} channel activity, leading to a decrease in global $[\text{Ca}^{2+}]_i$ and

vasodilation (15). Cav-1^{-/-} aortas contract less in response to vasoconstrictors and relax more in response to acetylcholine, a vasodilator, when compared with cav-1^{+/+} arteries (10,33), supporting upregulation of a vasodilatory pathway.

In summary, data show that genetic ablation of cav-1 leads to loss of caveolae, an increase in Ca²⁺ spark frequency, abolishment of Ca²⁺ spark regulation by voltage-dependent Ca²⁺ channels, and an increase in K_{Ca} channel density in cerebral artery smooth muscle cells. In contrast, cav-1 ablation does not attenuate transient K_{Ca} current activation by Ca²⁺ sparks.

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REFERENCES

1. Babiychuk EB, Smith RD, Burdiga T, Babiychuk VS, Wray S, Draeger A. Membrane cholesterol regulates smooth muscle phasic contraction. *J Membr Biol* 2004;198:95–101. [PubMed: 15138749]
2. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol* 2000;1:11–21. [PubMed: 11413485]
3. Brainard AM, Miller AJ, Martens JR, England SK. Maxi-K channels localize to caveolae in human myometrium: a role for an actin-channel-caveolin complex in the regulation of myometrial smooth muscle K⁺ current. *Am J Physiol Cell Physiol* 2005;289:C49–C57. [PubMed: 15703204]
4. Brenner R, Perez GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, Patterson AJ, Nelson MT, Aldrich RW. Vasoregulation by the β 1 subunit of the calcium-activated potassium channel. *Nature* 2000;407:870–876. [PubMed: 11057658]
5. Cannell MB, Cheng H, Lederer WJ. The control of calcium release in heart muscle. *Science* 1995;268:1045–1049. [PubMed: 7754384]
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. Mitochondrial modulation of Ca²⁺ sparks and transient K_{Ca} currents in smooth muscle cells of rat cerebral arteries. *J Physiol* 2004;556:755–771. [PubMed: 14766935]
8. Darby PJ, Kwan CY, Daniel EE. Caveolae from canine airway smooth muscle contain the necessary components for a role in Ca²⁺ handling. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L1226–L1235. [PubMed: 11076813]
9. Doyle DD, Upshaw-Earley J, Bell E, Palfrey HC. Expression of caveolin-3 in rat aortic vascular smooth muscle cells is determined by developmental state. *Biochem Biophys Res Commun* 2003;304:22–25. [PubMed: 12705878]
10. Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, Menne J, Lindschau C, Mende F, Luft FC, Schedl A, Haller H, Kurzchalia TV. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science* 2001;293:2449–2452. [PubMed: 11498544]
11. Gratton JP, Bernatchez P, Sessa WC. Caveolae and caveolins in the cardiovascular system. *Circ Res* 2004;94:1408–1417. [PubMed: 15192036]
12. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J Biol Chem* 1985;260:3440–3450. [PubMed: 3838314]
13. Isshiki M, Anderson RG. Function of caveolae in Ca²⁺ entry and Ca²⁺-dependent signal transduction. *Traffic* 2003;4:717–723. [PubMed: 14617355]
14. Jaggar JH. Intravascular pressure regulates local and global Ca²⁺ signaling in cerebral artery smooth muscle cells. *Am J Physiol Cell Physiol* 2001;281:C439–C448. [PubMed: 11443043]
15. Jaggar JH, Porter VA, Lederer WJ, Nelson MT. Calcium sparks in smooth muscle. *Am J Physiol Cell Physiol* 2000;278:C235–C256. [PubMed: 10666018]
16. Jaggar JH, Stevenson AS, Nelson MT. Voltage dependence of Ca²⁺ sparks in intact cerebral arteries. *Am J Physiol Cell Physiol* 1998;274:C1755–C1761.

17. Jaggar JH, Wellman GC, Heppner TJ, Porter VA, Perez GJ, Gollasch M, Kleppisch T, Rubart M, Stevenson AS, Lederer WJ, Knot HJ, Bonev AD, Nelson MT. Ca^{2+} channels, ryanodine receptors and Ca^{2+} -activated K^{+} channels: a functional unit for regulating arterial tone. *Acta Physiol Scand* 1998;164:577–587. [PubMed: 9887980]
18. Je HD, Gallant C, Leavis PC, Morgan KG. Caveolin-1 regulates contractility in differentiated vascular smooth muscle. *Am J Physiol Heart Circ Physiol* 2004;286:H91–H98. [PubMed: 12969891]
19. Knot HJ, Nelson MT. Regulation of arterial diameter and wall $[\text{Ca}^{2+}]$ in cerebral arteries of rat by membrane potential and intravascular pressure. *J Physiol* 1998;508:199–209. [PubMed: 9490839]
20. Kotlikoff ML. Calcium-induced calcium release in smooth muscle: the case for loose coupling. *Prog Biophys Mol Biol* 2003;83:171–191. [PubMed: 12887979]
21. Li G, Cheung DW. Effects of paxilline on K^{+} channels in rat mesenteric arterial cells. *Eur J Pharmacol* 1999;372:103–107. [PubMed: 10374720]
22. Li WP, Liu P, Pilcher BK, Anderson RG. Cell-specific targeting of caveolin-1 to caveolae, secretory vesicles, cytoplasm or mitochondria. *J Cell Sci* 2001;114:1397–1408. [PubMed: 11257005]
23. Lohn M, Furstenau M, Sagach V, Elger M, Schulze W, Luft FC, Haller H, Gollasch M. Ignition of calcium sparks in arterial and cardiac muscle through caveolae. *Circ Res* 2000;87:1034–1039. [PubMed: 11090549]
24. Lopez-Lopez JR, Shacklock PS, Balke CW, Wier WG. Local calcium transients triggered by single L-type calcium channel currents in cardiac cells. *Science* 1995;268:1042–1045. [PubMed: 7754383]
25. Moore ED, Voigt T, Kobayashi YM, Isenberg G, Fay FS, Gallitelli MF, Franzini-Armstrong C. Organization of Ca^{2+} release units in excitable smooth muscle of the guinea-pig urinary bladder. *Biophys J* 2004;87:1836–1847. [PubMed: 15345562]
26. Munro S. Lipid rafts: elusive or illusive? *Cell* 2003;115:377–388. [PubMed: 14622593]
27. 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]
28. Ostrom RS, Liu X, Head BP, Gregorian C, Seasholtz TM, Insel PA. Localization of adenylyl cyclase isoforms and G protein-coupled receptors in vascular smooth muscle cells: expression in caveolin-rich and noncaveolin domains. *Mol Pharmacol* 2002;62:983–992. [PubMed: 12391260]
29. Park DS, Cohen AW, Frank PG, Razani B, Lee H, Williams TM, Chandra M, Shirani J, De Souza AP, Tang B, Jelicks LA, Factor SM, Weiss LM, Tanowitz HB, Lisanti MP. Caveolin-1 null (–/–) mice show dramatic reductions in life span. *Biochemistry* 2003;42:15124–15131. [PubMed: 14690422]
30. 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]
31. Porter VA, Bonev AD, Knot HJ, Heppner TJ, Stevenson AS, Kleppisch T, Lederer WJ, Nelson MT. Frequency modulation of Ca^{2+} sparks is involved in regulation of arterial diameter by cyclic nucleotides. *Am J Physiol Cell Physiol* 1998;274:C1346–C1355.
32. Ratajczak P, Damy T, Heymes C, Oliviero P, Marotte F, Robidel E, Sercombe R, Boczkowski J, Rappaport L, Samuel JL. Caveolin-1 and -3 dissociations from caveolae to cytosol in the heart during aging and after myocardial infarction in rat. *Cardiovasc Res* 2003;57:358–369. [PubMed: 12566108]
33. Razani B, Engelman JA, Wang XB, Schubert W, Zhang XL, Marks CB, Macaluso F, Russell RG, Li M, Pestell RG, Di VD, Hou H Jr, Kneitz B, Lagaud G, Christ GJ, Edelmann W, Lisanti MP. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. *J Biol Chem* 2001;276:38121–38138. [PubMed: 11457855]
34. Razani B, Woodman SE, Lisanti MP. Caveolae: from cell biology to animal physiology. *Pharmacol Rev* 2002;54:431–467. [PubMed: 12223531]
35. Sanchez M, McManus OB. Paxilline inhibition of the alpha-subunit of the high-conductance calcium-activated potassium channel. *Neuropharmacology* 1996;35:963–968. [PubMed: 8938726]
36. Wang SQ, Song LS, Lakatta EG, Cheng H. Ca^{2+} signaling between single L-type Ca^{2+} channels and ryanodine receptors in heart cells. *Nature* 2001;410:592–596. [PubMed: 11279498]
37. Wang XL, Ye D, Peterson TE, Cao S, Shah VH, Katusic ZS, Sieck GC, Lee HC. Caveolae targeting and regulation of large conductance Ca^{2+} -activated K^{+} channels in vascular endothelial cells. *J Biol Chem* 2005;280:11656–11664. [PubMed: 15665381]

38. Wellman GC, Santana LF, Bonev AD, Nelson MT. Role of phospholamban in the modulation of arterial Ca^{2+} sparks and Ca^{2+} -activated K^{+} channels by cAMP. *Am J Physiol Cell Physiol* 2001;281:C1029–C1037. [PubMed: 11502581]
39. Williams TM, Lisanti MP. The caveolin genes: from cell biology to medicine. *Ann Med* 2004;36:584–595. [PubMed: 15768830]
40. Woodman SE, Park DS, Cohen AW, Cheung MW, Chandra M, Shirani J, Tang B, Jelicks LA, Kitsis RN, Christ GJ, Factor SM, Tanowitz HB, Lisanti MP. Caveolin-3 knock-out mice develop a progressive cardiomyopathy and show hyperactivation of the p42/44 MAPK cascade. *J Biol Chem* 2002;277:38988–38997. [PubMed: 12138167]
41. 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]
42. 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. Genetic ablation of caveolin-1 (*cav-1*) abolishes caveolae in cerebral artery smooth muscle cells. *A*: electron micrograph illustrates sarcolemma of smooth muscle cell in cerebral artery from *cav-1*^{+/+} mouse. Abundant caveolae are present within sarcolemma. *B*: magnification of area illustrated by black box in *A* showing caveolae. *C*: caveolae were absent in sarcolemma of *cav-1*^{-/-} cerebral artery smooth muscle cells. *D*: magnification of area highlighted by black box in *C*.

Fig. 2.

Cav-1 ablation elevates transient large-conductance Ca^{2+} -activated potassium (K_{Ca}) current frequency in cerebral artery smooth muscle cells. **A:** original traces illustrate transient K_{Ca} currents in a $cav-1^{+/+}$ (top) and $cav-1^{-/-}$ (bottom) cell at -40 mV. **B:** mean transient K_{Ca} current frequency was higher in $cav-1^{-/-}$ ($n = 31$) when compared with $cav-1^{+/+}$ ($n = 35$) cells. **C:** mean transient K_{Ca} current amplitude was similar in $cav-1^{+/+}$ ($n = 35$) and $cav-1^{-/-}$ ($n = 31$) cells. **D:** mean cell capacitance of $cav-1^{-/-}$ cells ($n = 49$) was smaller than for $cav-1^{+/+}$ cells ($n = 55$). **E:** thapsigargin (100 nM) abolishes transient K_{Ca} currents in a $cav-1^{-/-}$ cell voltage-clamped at -40 mV. Similar results were obtained in 4 cells. * $P < 0.05$ when compared with $cav-1^{+/+}$ cells.

Fig. 3.

Ca²⁺ spark frequency is higher in cav-1^{-/-} cells than in cav-1^{+/+} cells. **A:** simultaneous measurements of Ca²⁺ sparks (bottom colored traces) and transient K_{Ca} currents (top black traces) at -40 mV in a cav-1^{+/+} cell (left) and cav-1^{-/-} cell (right). *Inset:* peak of Ca²⁺ spark, labeled by asterisk in **A**, with yellow box in image indicating region from where F/F₀ trace was obtained. **B:** mean Ca²⁺ spark frequency in cav-1^{+/+} ($n = 9$) and cav-1^{-/-} ($n = 7$) cells. **C:** mean Ca²⁺ spark amplitude in cav-1^{+/+} ($n = 88$) and cav-1^{-/-} ($n = 124$) cells. **D:** amplitude correlation of Ca²⁺ sparks and evoked transient K_{Ca} currents in cav-1^{+/+} (black squares) and cav-1^{-/-} cells (red circles) with linear fit and 95% confidence bands to each data set [slope $\pm$ SE: cav-1^{+/+}, 14.2 ± 1.4 ($r = 0.25$, $n = 62$, $P < 0.0001$); cav-1^{-/-}, 16.0 ± 1.1 ($r = 0.32$, $n = 91$, $P = 0.01$)]. Amplitude relationship was not significantly different in cav-1^{+/+} and cav-1^{-/-} cells ($P > 0.05$). * $P < 0.05$ when compared with cav-1^{+/+} cells.

Fig. 4. Intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and sarcoplasmic reticulum Ca^{2+} load are similar in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cerebral arteries. *A:* $[\text{Ca}^{2+}]_i$ and caffeine (10 mM)-induced Ca^{2+} transients were measured in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cerebral arteries using fura-2. *B:* $[\text{Ca}^{2+}]_i$ was similar in $\text{cav-1}^{+/+}$ ($n = 7$) and $\text{cav-1}^{-/-}$ ($n = 9$) cerebral arteries. *C:* caffeine-induced Ca^{2+} transients were similar in $\text{cav-1}^{+/+}$ ($n = 7$) and $\text{cav-1}^{-/-}$ ($n = 9$).

Fig. 5.

Voltage-dependent Ca^{2+} channel blockers reduce transient K_{Ca} current frequency in $\text{cav-1}^{+/+}$ cells but not in $\text{cav-1}^{-/-}$ cells. *A*: original traces illustrating effect of CdCl_2 (250 μM) on transient K_{Ca} currents in a $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cell at -40 mV. *B*: mean effects of CdCl_2 on transient K_{Ca} current frequency and amplitude in $\text{cav-1}^{+/+}$ ($n = 5$) and $\text{cav-1}^{-/-}$ ($n = 6$) cells. *C*: mean effect of diltiazem (50 μM) on transient K_{Ca} currents in $\text{cav-1}^{+/+}$ ($n = 5$) and $\text{cav-1}^{-/-}$ ($n = 4$) cells. $*P < 0.05$ when compared with control obtained in same cells before Cd^{2+} or diltiazem application; $\#P < 0.05$ when compared with $\text{cav-1}^{+/+}$ cells.

Fig. 6.

Voltage-dependent Ca^{2+} current density is similar in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cells. *A*: original traces illustrating voltage-dependent Ba^{2+} currents (I_{Ba}) elicited by a voltage step from a holding potential of -80 to $+20$ mV in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cerebral artery smooth muscle cells. CdCl_2 ($250 \mu\text{M}$) abolished (I_{Ba}) in $\text{cav-1}^{+/+}$ and $\text{cav-1}^{-/-}$ cells. *B*: current density-voltage relationship of CdCl_2 ($250 \mu\text{M}$)-sensitive currents in $\text{cav-1}^{+/+}$ ($n = 9$) and $\text{cav-1}^{-/-}$ ($n = 9$) cells. $P > 0.05$ for each voltage when compared with $\text{cav-1}^{+/+}$.

Fig. 7.

Single K_{Ca} channel properties in $cav-1^{+/+}$ and $cav-1^{-/-}$ cells. *A*: original records illustrate single K_{Ca} channel openings in $10\ \mu M\ Ca^{2+}$ at -40 and $+40$ mV in inside-out patches from a $cav-1^{+/+}$ and $cav-1^{-/-}$ cell. *B*: at -40 and $+40$ mV, apparent Ca^{2+} sensitivity of K_{Ca} channels was similar for $cav-1^{+/+}$ ($n = 8-9$ for each $[Ca^{2+}]$) and $cav-1^{-/-}$ ($n = 7$ for each $[Ca^{2+}]$) cells ($P > 0.05$ for each). P_o , open probability. *C*: inside-out patches from $cav-1^{-/-}$ cells ($n = 22$) contained more K_{Ca} channels than patches from $cav-1^{+/+}$ cells ($n = 17$). C, closed; O, open. $*P < 0.05$.

Fig. 8.

Whole cell K^+ , K_{Ca} , and K_V current density is elevated in $cav-1^{-/-}$ cells. *A*: original recordings of whole cell K^+ currents activated by depolarizing voltage steps in a $cav-1^{+/+}$ and $cav-1^{-/-}$ cell. *B*: paxilline (300 nM) inhibits K^+ currents in the same cells illustrated in *A*. *C*: paxilline-sensitive K^+ currents. *D*: mean whole cell K^+ current density (pA/pF) in $cav-1^{+/+}$ ($n = 11$) and $cav-1^{-/-}$ ($n = 9$) cells. *E*: paxilline-insensitive K^+ current density. *F*: paxilline-sensitive K^+ current density. $*P < 0.05$ when compared with $cav-1^{+/+}$ cells.