Intermittent hypoxia in rats reduces activation of Ca\(^{2+}\) sparks in mesenteric arteries

Olan Jackson-Weaver, Jessica M. Osmond, Jay S. Naik, Laura V. Gonzalez Bosc, Benjimen R. Walker, and Nancy L. Kanagy

Vascular Physiology Group, Department of Cell Biology and Physiology, School of Medicine, University of New Mexico, Albuquerque, New Mexico

Submitted 11 March 2015; accepted in final form 17 September 2015

Intermittent hypoxia in rats reduces activation of Ca\(^{2+}\) sparks in mesenteric arteries. Am J Physiol Heart Circ Physiol 309: H1915–H1922, 2015. First published September 25, 2015; doi:10.1152/ajpheart.00179.2015.—Ca\(^{2+}\) sparks are vascular smooth muscle cell (VSMC) Ca\(^{2+}\)-release events that are mediated by ryanodine receptors (RyR) and promote vasodilation by activating large-conductance Ca\(^{2+}\)-activated potassium channels and inhibiting myogenic tone. We have previously reported that exposing rats to intermittent hypoxia (IH) to simulate the hypoxic episodes of sleep apnea augments myogenic tone in mesenteric arteries through loss of hydrogen sulfide (H\(_2\)S)-induced dilation. Because we also observed that H\(_2\)S can increase Ca\(^{2+}\) spark activity, we hypothesized that loss of H\(_2\)S after IH exposure reduces Ca\(^{2+}\) spark activity and that blocking Ca\(^{2+}\) spark generation reduces H\(_2\)S-induced dilation. Ca\(^{2+}\) spark activity was lower in VSMC of arteries from IH compared with sham-exposed rats. Furthermore, depolarizing VSMC by increasing luminal pressure (from 20 to 100 mmHg) or by elevating extracellular [K\(^+\)] increased spark activity in VSMC of arteries from sham but had no effect in arteries from IH rats. Inhibiting endogenous H\(_2\)S production in sham arteries prevented these increases. NaHS or phosphodiesterase inhibition increased spark activity to the same extent in sham and IH arteries. Depolarization-induced increases in Ca\(^{2+}\) spark activity were due to increased sparks per site, whereas H\(_2\)S increases in spark activity were due to increased spark sites per cell. Finally, inhibiting Ca\(^{2+}\) spark activity with ryanodine (10 \(\mu\)M) enhanced myogenic tone in arteries from sham but not IH rats and blocked dilation to exogenous H\(_2\)S in arteries from both sham and IH rats. Our results suggest that H\(_2\)S regulates RyR activation and that H\(_2\)S-induced dilation requires Ca\(^{2+}\) spark activation. IH exposure decreases endogenous H\(_2\)S-dependent Ca\(^{2+}\) spark activation to cause membrane depolarization and enhance myogenic tone in mesenteric arteries.

myogenic tone; ryanodine receptors; sleep apnea; vascular smooth muscle; endothelium

NEW & NOTEWORTHY

This study provides the first evidence that hydrogen sulfide (H\(_2\)S) increases Ca\(^{2+}\) spark activity through an endothelium-dependent effect to contribute to H\(_2\)S-induced dilation. Additionally, it appears that endothelial H\(_2\)S is required for depolarization-induced activation of Ca\(^{2+}\) sparks in intact arteries.

EPIDEMIOLOGICAL STUDIES have established that sleep apnea is an independent risk factor for cardiovascular disease, in particular hypertension (31). Potential mechanisms to explain this association include increased sympathetic activity (5), endothelial dysfunction (22), systemic inflammation (36), and increased endothelin-1-dependent vasoconstriction (10). We previously reported that arteries from rats made hypertensive by exposure to intermittent hypoxia (IH) to simulate the hypoxic episodes of sleep apnea have enhanced myogenic tone and depolarized vascular smooth muscle cell (VSMC) membrane potential (\(E_m\)) through an apparent loss of the vasodilator hydrogen sulfide (H\(_2\)S) (14, 19, 35, 37). Because previous studies have linked H\(_2\)S-mediated vasodilation with Ca\(^{2+}\) spark activation and VSMC hyperpolarization (12, 25), this study examined the effect of IH exposure on that pathway.

Ca\(^{2+}\) sparks are spatially and temporally limited Ca\(^{2+}\)-release events from ryanodine-sensitive Ca\(^{2+}\) channels (RyR) in the sarcoplasmic reticulum (SR) of muscle cells. In cerebral artery VSMC, Ca\(^{2+}\) sparks activate large-conductance Ca\(^{2+}\)-activated K\(^+\) (BK\(_{Ca}\)) channels and cause \(E_m\) hyperpolarization, followed by decreased activity of L-type voltage-gated calcium channels (VGCC) and decreased cytosolic [Ca\(^{2+}\)] (26). Ca\(^{2+}\) spark activity is increased in response to \(E_m\) depolarization downstream of increased intraluminal pressure and subsequent stretch of VSMC. This increase in Ca\(^{2+}\) spark activity has been hypothesized to act as an intrinsic negative feedback mechanism to regulate myogenic tone (11, 18). Ca\(^{2+}\) sparks are also regulated by cyclic nucleotides after activation of G protein-coupled receptors. Both cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG) have been shown to increase spark activity (29). The gasotransmitter H\(_2\)S has recently been shown to be another activator of Ca\(^{2+}\) sparks in cerebral arteries (25) and in mesenteric arteries (12).

Our previous observation that IH exposure in rats enhances myogenic tone in mesenteric arteries through the loss of H\(_2\)S production led us to hypothesize that IH reduces H\(_2\)S-dependent Ca\(^{2+}\) spark activity, leading to depolarization and enhanced myogenic tone in mesenteric arteries. Studies were designed to determine how H\(_2\)S regulates Ca\(^{2+}\) sparks in VSMC.

METHODS

Animals. Male Sprague-Dawley rats (275–325 g) were used for all studies and were exposed to IH as described previously (20). Briefly, animals were housed in Plexiglas boxes with free access to food and water and exposed to either IH (nadir 5% O\(_2\); 5% CO\(_2\)) to peak 21% O\(_2\); 0% CO\(_2\)) or air only cycling at a rate of 20 cycles/h for 7 h/day for 14 days (20). This IH protocol reduces P\(_{O2}\) to ~35 mmHg, maintains P\(_{CO2}\) at ~30 mmHg (32), and increases arterial blood pressure about 15 mmHg (2, 8, 35). On the day of the experiment, animals were anesthetized with pentobarbital sodium (200 mg/kg, ip) and mesenteric arteries were collected for Ca\(^{2+}\) imaging, dilation studies, \(E_m\) recordings, and myogenic tone studies. All animal protocols were reviewed and approved by the Institutional Animal Care and Use

Address for reprint requests and other correspondence: N. Kanagy, Dept. of Cell Biology and Physiology, MSC 08-4750, Univ. of New Mexico, Albuquerque, NM 87131 (e-mail: nkanagy@salud.unm.edu).

http://www.ajpheart.org 0363-6135/15 Copyright © 2015 the American Physiological Society H1915
Committee of the University of New Mexico School of Medicine and conform to National Institutes of Health guidelines for animal use.

*Isolated vessel preparation.* The intestinal artery was removed and placed in a Silastic-coated Petri dish containing chilled physiological saline solution (PSS; in mmol/l: 129.80 NaCl, 5.40 KCl, 0.83 MgSO4, 0.43 NaH2PO4, 19.00 NaHCO3, 1.80 CaCl2, and 5.50 glucose). Fifth- to seventh-order artery segments (80–120-µm inner diameter) were dissected from the mesenteric vascular arcade and placed in fresh PSS. Arteries were transferred to a vessel chamber (Living Systems Instrumentation), cannulated onto glass micropipettes, and secured with silk ligatures. The arteries were pressurized to 60 mmHg with PSS using a servo-controlled peristaltic pump (Living Systems Instrumentation) and superfused with warmed, oxygenated PSS at a rate of 5 ml/min.

**Vessel reactivity studies.** Luminal pressure was increased in 40-mmHg steps from 20 to 180 mmHg using a servo-controlled peristaltic pump. Myogenic tone was allowed to develop for a minimum of 5 min at each pressure step. Pressure was reduced, and arteries were subsequently incubated with Ca2+-free PSS for 60 min. The pressure curve was repeated to determine passive diameter at each pressure. Myogenic tone was calculated as [(Ca2+-free diameter) – (Ca2+-containing diameter)]/(Ca2+-free diameter)*100.

In separate arteries, tone was induced to 50% resting diameter using phenylephrine, and dilation responses to the H2S donor, NaHS (10 µM), were recorded. Dilation was calculated as [(NaHS diameter – PE diameter)/(Ca2+-free diameter)] – (PE diameter*100) and represents reversal of active tone.

**Ca2+ spark imaging.** Arteries used for Ca2+ spark imaging studies were incubated in a fluo-4 AM (10 µmol/l, Invitrogen) solution containing 0.25% pluronic acid in HEPES buffer (in mmol/l: 134.000 NaCl, 6.0000 KCl, 1.0000 MgCl2, 2.0000 CaCl2, 10.0000 HEPES, 0.026 EDTA, 10.0000 glucose, pH 7.4) for 60 min at 28°C before cannulation. After being loaded with fluo-4, arteries were transferred to a vessel chamber, cannulated as described above, and pressurized to 60 mmHg. After 5-min equilibration in oxygenated PSS at 32°C, fluo-4-loaded arteries were excited at 488 nm by a solid-state laser, and emitted light was collected using an Olympus IX71 microscope with a ×60 water-immersion lens and a spinning-disk confocal scanning unit (Andor Technology). A 75 × 50 µm area was imaged at 50–60 Hz using a laser power of 15%.

**Spark analysis.** Spark movies were analyzed using SparkAn software, developed by A. D. Bonev and M. T. Nelson (University of Vermont). Ten images without spark activity were averaged to determine background fluorescence levels (F0). Regions of interest of 10 pixels2 (4 µm2) were used to detect sparks with a minimum F/F0 of 1.2. The average F/F0 was 1.27 ± 0.20 with a duration of 0.057 ± 0.003 s, and neither parameter varied between conditions. Each image contained 10–20 cells, and spark frequency was averaged for all cells visible. The number of active sites was counted for each field of view and divided by number of cells to obtain spark sites/cell. To determine the effect of inhibitors and activators on spark activity, a baseline level of sparks was recorded, and then arteries were equilibrated for 15 min before a second recording was made. Where possible, the order of conditions was randomized. For irreversible inhibitors, vehicle time controls were performed. In some arteries, the endothelium was disrupted by gentle abrasion of the lumen with a strand of moose mane as previously described (14). To evaluate the effect of modest depolarization on Ca2+ spark activity, extracellular [KCl] was increased from 5 to 15 mM, the minimum concentration to cause VSMC depolarization (Table 2) without a significant vasoconstriction.

**VSMC Em measurement.** Small mesenteric arteries (80–100-µm inner diameter) were cannulated and pressurized to 20 mmHg. After 60-min equilibration, VSMC were pierced from the exterior using sharp electrodes (filling solution 3M KCl in dH2O, R = 40–60 MΩ). Em was recorded for at least 1 min from 3–8 sites and averaged to obtain n = 1 for each artery. Then intraluminal pressure was increased to 100 mmHg, and Em was recorded from another 3–8 sites and averaged. In another set of arteries pressurized to 60 mmHg, Em was recorded in normal PSS (5 mM K+) and high-K-PSS (15 mM K+ with KCl substituted equimolar for NaCl).

**Statistical analysis.** Myogenic tone, dilation, and spark frequency were analyzed using two-way ANOVA with Student-Newman-Keuls post hoc analysis for differences between groups. Em differences were evaluated using a paired t-test. For all studies, P < 0.05 was considered statistically significant.

**RESULTS**

**IH reduces RyR-mediated regulation of myogenic tone.** To determine whether Ca2+ spark activation regulates myogenic tone in mesenteric arteries, tone was assessed with and without blockade of RyR using ryanodine (10 µM). Vehicle-treated arteries from IH rats developed significant tone, whereas arteries from sham rats did not (Fig. 1), consistent with previous work (14). Furthermore, ryanodine treatment enhanced myogenic tone in arteries from sham rats but not in arteries from IH rats (Fig. 1). These results are consistent with Ca2+ sparks acting to inhibit arterial myogenic tone under normal conditions. This inhibition is impaired following IH exposure, con-

---

**Fig. 1.** Effect of ryanodine (10 µM) on myogenic tone in mesenteric arteries from sham (A) and intermittent hypoxia (IH) (B) rats; n = 6–7 animals/group. *P < 0.05 vs. vehicle.
consistent with loss of H$_2$S-dependent activation of RyR through loss of Ca$^{2+}$ spark activity.

IH impairs basal Ca$^{2+}$ spark activity. Ca$^{2+}$ spark activity was measured in VSMC in sham and IH arteries pressurized to 60 mmHg using software identification of Ca$^{2+}$ sparks with F/F$_O$ $\geq$ 1.2 of fluorescence (Fig. 2A). Spark activity was significantly lower in endothelium-intact arteries from IH rats compared with arteries from sham rats (Fig. 2B).

IH impairs activation of Ca$^{2+}$ spark activity. NaHS (10 $\mu$M) administration increased Ca$^{2+}$ spark activity in VSMC in arteries from sham rats (Fig. 3A). Conversely, inhibiting endogenous synthesis of H$_2$S via cystathionine $\gamma$-lyase (CSE) with the CSE inhibitor $\beta$-cyanoalanine (BCA; 100 $\mu$M) decreased sparks in arteries from sham rats. However, CSE inhibition did not affect spark activity in arteries from IH rats. Similarly, depolarizing VSMC with elevated [KCl] (15 mM) or by increasing luminal pressure from 20 to 100 mmHg also increased Ca$^{2+}$ spark activity in arteries from sham but not IH rats, suggesting that IH exposure impairs VSMC Ca$^{2+}$ spark activation by certain stimuli. To determine whether cyclic nucleotide activation of Ca$^{2+}$ sparks was also impaired in arteries from IH rats, phosphodiesterase was inhibited with 3-isobutyl-1-methylxanthine (IBMX, 40 $\mu$M) to elevate cyclic nucleotide activation of RyR (29). IBMX increased spark activity in arteries from both groups, showing that RyR are present and can generate Ca$^{2+}$ sparks (Fig. 3A). Raw traces of Ca$^{2+}$ sparks in sham arteries show increased activity at basally active sites as well as recruitment of previously inactive sites (Fig. 3B).

Endothelium is required for H$_2$S- and depolarization-induced Ca$^{2+}$ spark activation. The endothelium was disrupted in arteries from sham rats to determine whether the site of action for H$_2$S regulation of Ca$^{2+}$ spark activity is the endothelium. Endothelium removal suppressed changes in spark activity to all perturbations except for phosphodiesterase inhibition (Fig. 4). Thus H$_2$S- and depolarization-dependent spark activation both require intact endothelium, but activation of sparks by cyclic nucleotide elevation is endothelium independent.

Depolarization-induced increase in Ca$^{2+}$ spark activity is H$_2$S dependent. VSMC $E_m$ was recorded to determine whether the diminished spark response to KCl and increased luminal pressure in arteries from the IH rats was caused by a smaller change in VSMC $E_m$. We observed that either increased luminal pressure or increased [KCl] caused similar depolarization in arteries from sham and IH rats (Fig. 5, insets). In arteries from sham rats, inhibiting CSE to decrease H$_2$S production (BCA, 100 $\mu$M) decreased basal Ca$^{2+}$ spark activity and prevented increases in spark activity after exposure to KCl (Fig. 5A). BCA treatment also prevented increased Ca$^{2+}$ spark activity after a pressure step (Fig. 5B). Therefore, Ca$^{2+}$ spark activation in response to small depolarizations appears to require endogenous H$_2$S. In contrast with VSMC in arteries from sham rats, VSMC in arteries from IH rats have fewer spark sites/cell (Fig. 6A) but a similar activity at individual sites (Fig. 6B). KCl depolarization increased the total number of sparks/site per second (Fig. 6B) without increasing the number of spark sites/cell.

RyR are required for NaHS-induced dilation. Previous work from our laboratory demonstrated that endogenous H$_2$S production limits generation of myogenic tone in arteries from sham rats, but arteries from both sham and IH rats dilate in response to an exogenous H$_2$S donor, NaHS. Because NaHS-induced Ca$^{2+}$ spark activity is similar in VSMC from sham and IH rats (Fig. 3A), dilation to NaHS was assessed in small mesenteric arteries from sham and IH rats in the absence and presence of ryanodine (10 $\mu$M). In arteries pressurized to 100 mmHg and constricted to $\sim$50% resting diameter with phenylephrine, arteries from both sham and IH rats demonstrated a robust dilation to NaHS, which was almost fully blocked by pretreatment with ryanodine (Fig. 7). Therefore, Ca$^{2+}$ sparks appear to contribute to NaHS-induced dilation in arteries from both sham and IH rats.

DISCUSSION

This study establishes that ryanodine channel activity inhibits myogenic tone, but this effect is lost after IH exposure (Fig. 1). The loss of a response to ryanodine is accompanied by decreased spark activity in arteries from IH rats (Fig. 2). Exogenous H$_2$S increases Ca$^{2+}$ spark activity above baseline, whereas inhibiting H$_2$S synthesis decreases Ca$^{2+}$ spark activity in arteries from sham but not IH rats, consistent with endogenous H$_2$S regulating Ca$^{2+}$ spark activity (Fig. 3) and validating that H$_2$S activates Ca$^{2+}$ sparks in these arteries. In arteries from sham rats, depolarizing VSMC increases Ca$^{2+}$ spark activity as expected, but this is impaired in arteries from IH rats and is prevented by inhibiting H$_2$S synthesis (Fig. 3). Thus
endogenous H$_2$S appears to be required for depolarization-induced increases in Ca$^{2+}$ spark activity. Disrupting the endothelium eliminated exogenous H$_2$S regulation of Ca$^{2+}$ spark activity and abrogated the effect of CSE inhibition (Fig. 4). Inhibiting CSE in arteries with intact endothelium also prevented KCl and pressure-induced increases in Ca$^{2+}$ spark activity (Fig. 5). Together, these data show that H$_2$S facilitates basal and depolarization-induced Ca$^{2+}$ spark activity and suggest that this effect is mediated through actions on the endothelium. Furthermore, NaHS-induced dilation was prevented.
by ryanodine blockade of spark activity (Fig. 7), suggesting that Ca\(^{2+}\) spark activity contributes to H\(_2\)S-induced dilation. Finally, CSE inhibition and endothelium disruption did not affect Ca\(^{2+}\) spark activity in arteries from IH rats, consistent with our previous observation that IH leads to decreased endothelial expression of CSE (10). These data support the conclusion that H\(_2\)S regulates Ca\(^{2+}\) spark-mediated dilation as illustrated in the diagram in Fig. 8. Loss of this pathway after exposure to IH for 14 days may contribute to increased vasoconstriction and elevated blood pressure in this model of sleep apnea-induced hypertension (6, 9, 20).

Myogenic tone regulation of appropriate organ perfusion is gaining recognition as a potential therapeutic target for preventing end-organ damage in diabetes, hypertension, and aging (30). Thus the H\(_2\)S pathway is a potential therapeutic target to modulate myogenic tone and alleviate vascular disease in patients with sleep apnea and other populations. These findings provide a theoretical basis for exploring the upregulation of endogenous H\(_2\)S signaling as a prophylactic and therapeutic target in peripheral vascular disease.

Ca\(^{2+}\) sparks have been shown to regulate myogenic tone and vascular function through activation of VSMC BK\(_{Ca}\) channels (26), hyperpolarizing VSMC \(E_m\) (7). The activation of a cluster of BK\(_{Ca}\) channels by RyR-dependent Ca\(^{2+}\) sparks causes a spontaneous transient outward current (STOC) (28). These STOCs sum to cause a steady-state \(E_m\) hyperpolarization of 10 mV or greater (23) so that depolarization-induced increases in Ca\(^{2+}\) spark activity act as a feedback regulator of membrane potential and vascular tone (17). Our data suggest that this

Fig. 4. Changes in Ca\(^{2+}\) spark activity from baseline in arteries from sham rats with endothelium intact (endothelial cell, EC intact) or disrupted (EC disrupted) in response to increased H\(_2\)S (NaHS, 10 \(\mu\)M), inhibition of H\(_2\)S production (BCA, 100 \(\mu\)M), depolarization (KCl, 15 mM), stretch-induced depolarization (increased luminal pressure from 20 to 100 mmHg), or phosphodiesterase inhibition (IBMX, 40 \(\mu\)M); \(n = 5–6\) animals/group. *\(P < 0.05\) vs. EC intact within treatment.

Fig. 5. A: changes in Ca\(^{2+}\) spark activity in response to increased KCl in arteries with intact endothelium from sham rats treated with vehicle or BCA (100 \(\mu\)M). Inset: membrane potential (\(E_m\)) in VSMC in pressurized arteries (60 mmHg) from sham or IH rats in PSS with normal (5.4 mM) or elevated (15.0 mM) KCl. *Different from vehicle for \(P < 0.05\); \#different from 5 mM KCl for \(P < 0.05\); $different from sham for \(P < 0.05\). B: changes in Ca\(^{2+}\) spark activity in response to increased luminal pressure (from 20 to 100 mmHg) in arteries from sham rats with endothelium intact treated with vehicle or BCA (100 \(\mu\)M). Membrane potential in VSMC in arteries from sham or IH rats pressurized to low (20 mmHg) or high (100 mmHg) pressures. *Different from vehicle; \#different from 20 mmHg; or $different from Sham for \(P < 0.05\); \(n = 5–9\) animals/group.
Feedback regulation is impaired after acute inhibition of H2S generation in arteries from healthy rats or after loss of H2S synthesis in arteries from rats chronically exposed to IH. Hypoxia has been previously shown to inhibit Ca\textsuperscript{2+} spark-dependent dilation. Acute hypoxia uncouples Ca\textsuperscript{2+} sparks from BK\textsubscript{Ca} channel activation, reducing their hyperpolarizing effect (40). This effect is mediated by reduced Ca\textsuperscript{2+} sensitivity of BK\textsubscript{Ca} channels, with no effect on Ca\textsuperscript{2+} spark activity. In contrast, our data show that 2 wk of IH decreases both basal and depolarization-induced Ca\textsuperscript{2+} spark activity over the same time course that myogenic (14) and agonist-induced vasoconstriction increase (1). Furthermore, the IH-induced decrease in Ca\textsuperscript{2+} spark activity is persistent under the normoxic conditions in which experiments were conducted, whereas hypoxic uncoupling of Ca\textsuperscript{2+} sparks and BK\textsubscript{Ca} channels is seen only during acute hypoxic exposure. Thus the effects of acute, sustained hypoxia vs. chronic intermittent exposure to hypoxia appear to be distinct. Our previous observations that IH exposure decreases endothelial expression of CSE (14) and the current data showing that IH abolishes the effect of CSE inhibition to decrease Ca\textsuperscript{2+} spark activity point to H2S as a primary regulator of Ca\textsuperscript{2+} spark activity and basal vasoreactivity.

In arteries from sham rats, either inhibiting CSE or abrading the endothelium similarly decreased basal Ca\textsuperscript{2+} spark activity and prevented depolarization-induced increases in Ca\textsuperscript{2+} spark activity (Figs. 3 and 4). Because CSE is primarily expressed in the endothelium in small mesenteric arteries (12), endogenous endothelium-derived H2S appears requisite for depolarization-induced Ca\textsuperscript{2+} spark activation. Potential mechanisms for H2S facilitation of Ca\textsuperscript{2+} spark generation include direct effects on VSMC RyR or VGCC, the source and trigger, respectively, of Ca\textsuperscript{2+} sparks (17). However, exogenous H2S does not increase Ca\textsuperscript{2+} spark activity in endothelium-denuded arteries, suggesting that neither RyR nor VGCC are direct targets of H2S. Rather, H2S appears to release an endothelial factor that increases coupling of VGCC to RyR. Future studies to identify this factor should help to elucidate this novel pathway for vasodilation.

Fig. 6. Ca\textsuperscript{2+} spark activity in arteries from sham or IH rats. The average number of sites/cell (A) or average number of sparks/site per second (B) observed in arteries from sham (Open bars) and IH (shaded bars) rats exposed to different KCl concentrations after treatment with vehicle (open bars) or BCA (100 μM, hatched bars). *Different from 5.4 mM KCl; $different from vehicle; or Sdifferent from sham for P < 0.05; n = 7 (sham) or 6 (IH) animals.
A requirement for the endothelium in depolarization-induced Ca$^{2+}$ spark activation contrasts with previous reports of depolarization regulation of Ca$^{2+}$ sparks and STOCs in isolated VSMCs. For example, short depolarizing pulses increased Ca$^{2+}$ spark activity in isolated vein myocytes (3), and depolarization from −50 to −10 mV increased STOCs in VSMC from Sprague Dawley rat cerebral arteries in a nifedipine-dependent manner (15). Thus large depolarizations in isolated VSMC increase Ca$^{2+}$ influx through VGCC. This depolarization only exposed the EC. If indeed there is hyperpolarization of the EC, that depolarization does not always increase Ca$^{2+}$ sparks and can instead lead to decreased spark activity.

In the present study, the low concentration of KCl used might increase the number of sparks/site, consistent with increased Ca$^{2+}$ influx. In contrast, we observed that a modest depolarization of the VSMC from cerebral but not cremaster arteries exhibited Ca$^{2+}$ sparks even though both had similar $E_m$ and that spark activity could be increased by caffeine but not by changes in resting $E_m$. Thus several laboratories have observed dissociations between $E_m$ and spark activation in isolated myocytes. In studies showing depolarization-mediated activation of Ca$^{2+}$ spark activity, the degree of change in $E_m$ is larger than that used in the present study (16). In the present study, the low concentration of KCl used might cause a modest hyperpolarization of the endothelial cell (EC) attributable to activation of Kir. We did not directly measure this because it is not possible to cut open these small arteries to expose the EC. If indeed there is hyperpolarization of the EC, this should increase Ca$^{2+}$, which could be a stimulus for H$_2$S release. With the stretch, there are also reports of increases in EC [Ca$^{2+}$] so that this might be a common pathway to implicate Ca$^{2+}$ in the final VSMC response. Thus VSMC depolarization increases spark activity, especially in cerebral artery myocytes, but this does not exclude an additional modulatory role of endothelial H$_2$S on Ca$^{2+}$ spark activation in response to a modest depolarization.

The mechanism of this modulation is not clear, but H$_2$S appears to stimulate the release of an endothelium-derived factor (12) rather than exerting a direct effect on the VSMC. Because inhibition of CSE appears to decrease the number of active sites/cell, it appears that the H$_2$S pathway increases the sensitivity of RyR to increased Ca$^{2+}$ influx. In contrast, we observed that KCl-induced depolarization of the VSMC $E_m$ increases the number of sparks/site, consistent with increase activator Ca$^{2+}$ delivery through VGCC. This suggests that, at any given $E_m$, H$_2$S enhances the coupling between RyR and VGCC Ca$^{2+}$ influx, consistent with our observation that increasing KCl from 5.4 to 15.0 mM similarly depolarized VSMC from sham and IH rats, but this depolarization only increased Ca$^{2+}$ spark activity in arteries with a functional H$_2$S system. Future investigations are needed to directly test this hypothesis.

In summary, this study provides the first evidence that H$_2$S increases Ca$^{2+}$ spark activity through an endothelium-dependent effect to contribute to H$_2$S-induced dilation. Additionally, it appears that endothelial H$_2$S is required for depolarization-induced activation of Ca$^{2+}$ sparks in intact arteries. Finally, both H$_2$S activity and Ca$^{2+}$ spark activity are impaired by IH exposure, leading to enhanced myogenic tone, and may contribute to therapy resistant hypertension common in the sleep apnea population (27).

**REFERENCES**


**AUTHOR CONTRIBUTIONS**


**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**REFERENCES**


Kanagy NL, Walker BR, Nelin LD.  

Kanagy NL.  

Knot HJ, Standen NB, Nelson MT.  


Ji G, Barsotti RJ, Feldman ME, Kotlikoff MI.  

Jaggar JH, Stevenson AS, Nelson MT.  


