Opposing roles of smooth muscle BK channels and ryanodine receptors in the regulation of nerve-evoked constriction of mesenteric resistance arteries

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Krishnamoorthy G, Sonkusare SK, Heppner TJ, Nelson MT. Opposing roles of smooth muscle BK channels and ryanodine receptors in the regulation of nerve-evoked constriction of mesenteric resistance arteries. Am J Physiol Heart Circ Physiol 306: H981–H988, 2014. First published February 7, 2014; doi:10.1152/ajpheart.00866.2013.—In depolarized smooth muscle cells of pressurized cerebral arteries, ryanodine receptors (RyRs) generate “Ca2+ sparks” that activate large-conductance, Ca2+- and voltage-sensitive potassium (BK) channels to oppose pressure-induced (myogenic) constriction. Here, we show that BK channels and RyRs have opposing roles in the regulation of arterial tone in response to sympathetic nerve activation by electrical field stimulation. Inhibition of BK channels with paxilline increased both myogenic and nerve-induced constrictions of pressurized, resistance-sized mesenteric arteries from mice. Inhibition of RyRs with ryanodine increased myogenic constriction, but it decreased nerve-evoked constriction along with a reduction in the amplitude of nerve-evoked increases in global intracellular Ca2+. In the presence of L-type voltage-dependent Ca2+ channel (VDCC) antagonists, nerve stimulation failed to evoke a change in arterial diameter, and BK channel and RyR inhibitors were without effect, suggesting that nerve-induced constriction is dependent on activation of VDCCs. Collectively, these results indicate that BK channels and RyRs have different roles in the regulation of myogenic versus neurogenic tone: whereas BK channels and RyRs act in concert to oppose myogenic vasoconstriction, BK channels oppose neurogenic vasoconstriction and RyRs augment it. A scheme for neurogenic vasoconstriction is proposed in which RyRs act in conjunction with VDCCs to regulate nerve-evoked constriction in mesenteric resistance arteries.

BK channels; ryanodine receptors; myogenic tone; nerve-evoked constriction; resistance arteries

Constriction to pressure, myogenic tone, is an inherent property of smooth muscle cells from resistance arteries, playing an important role in local blood flow autoregulation, setting of basal peripheral vascular resistance, and regulation of capillary hydrostatic pressure (5). In the mesenteric circulation, proximal artery diameter is regulated by sympathetic innervation, whereas the diameters of small arteries and arterioles are regulated by both myogenic tone and neurohumoral factors. An important negative feedback element that opposes pressure-induced constriction and regulates the myogenic tone of cerebral arteries is the activation of large-conductance, Ca2+- and voltage-sensitive potassium (BK) channels by local Ca2+-release events (Ca2+ sparks) mediated by ryanodine receptors (RyRs) in the sarcoplasmic reticulum of smooth muscle (2, 3, 17, 27). Whether the same mechanism is involved in regulating the tone of myogenic mesenteric arteries is not clear.

Sympathetic nervous system activity regulates total peripheral resistance and systemic blood pressure and is a key regulator of tone in mesenteric arteries. Sympathetic nervous system hyperactivity is a characteristic feature of all manifestations of human hypertension and heart failure (23) and forms the basis for the physiological response to stress. The roles of BK channels and RyRs in the regulation of sympathetic tone are not clearly understood, although studies have shown that inhibition of RyRs decreases adrenergic contractions in swine renal arteries (6), whereas inhibition of BK channels increases the late phase of adrenoceptor-mediated contractions in guinea pig vas deferens (30). In rat mesenteric arteries, constriction evoked by electrical stimulation of perivascular nerves is significantly reduced by ryanodine (9).

Upon stimulation, sympathetic nerves release ATP and norepinephrine (NE) to constrict mesenteric arteries (12). ATP activates smooth muscle purinergic P2X1 receptor (P2X1R) channels, causing an influx of Na+ and Ca2+ ions that excites the smooth muscle and creates an excitatory junction potential (7, 37). Neurally released ATP can be monitored optically as a local elevation of Ca2+ in smooth muscle, referred to as a junctional Ca2+ transient (jCaT) (21). NE acts via α1-adrenergic receptors to promote constriction through multiple mechanisms, including the induction of inositol 1,4,5-trisphosphate receptor-mediated Ca2+ waves, membrane depolarization, and increased Ca2+-sensitivity (19, 25, 33, 34).

Resistance-sized mesenteric arteries constrict to both pressure and sympathetic nerve stimulation and play a key role in blood pressure regulation (8, 10, 18, 24). Here, we investigated the roles of BK channels and RyRs in regulating the myogenic and neurogenic tone of resistance-sized mesenteric arteries from mice. Our results support the concept that RyR-activated BK channels oppose myogenic tone, as has been shown for cerebral arteries. However, RyRs appear to have a dual role in nerve-evoked constriction: Ca2+ release through RyRs activates BK channels to oppose constriction but also contribute the majority of Ca2+ for neurogenic constriction.

MATERIALS AND METHODS

Tissue preparation. Adult male mice (C57BL/6, 2–4 mo old) were euthanized with an intraperitoneal injection of pentobarbital sodium (150 mg/kg), followed by decapitation in accordance with protocols approved by the Institute of Animal Care and Use Committee of the University of Vermont. The mesenteric tissue was extracted and placed in ice-cold HEPES-buffered physiological saline solution (PSS), consisting of (in mM) 134 NaCl, 6 KCl, 1 MgCl2, 2 CaCl2, 10 HEPES, and 10 glucose (pH 7.4).

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Diameter measurements. Third-order branches of mesenteric arteries were dissected, cannulated, and pressurized at either 60 or 80 mmHg. Arteries were equilibrated for 30 min, followed by two treatments with 60 mM KCl solution to test the viability of the vessel and the repeatability of observations. All experiments were conducted at 37°C in bicarbonate-buffered PSS, consisting of (in mM) 118.5 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 1.2 MgCl₂, 2.5 CaCl₂, 22 NaHCO₃, 7 glucose, and 0.025 EDTA, bubbled with 20% O₂–5% CO₂ to obtain a pH of 7.4. Internal diameter changes in response to electrical field stimulation (EFS) were recorded using a charged-coupled device camera and an edge-detection system (IonOptix, Milton, MA). Control solutions contained the transient receptor potential vanilloid-1 channel-desensitizing agent capsaicin (1 μM), β-adrenergic receptor blocker propranolol (10 μM), and muscarinic receptor antagonist atropine (10 μM) to inhibit the actions of sensory nerve stimulation and muscarinic and other adrenergic nerve-evoked responses. After steady, repeatable EFS-induced responses under control conditions were obtained, paxilline, ryanodine (Enzo Life Sciences, PA), diltiazem, or nifedipine, alone or in combination, was added to this cocktail. The passive diameter of an artery was always measured at the end of the experiment in Ca²⁺-free solution containing 5 mM EGTA. Papaverine hydrochloride (100 μM; Fluka Biochemicals, St. Louis, MO), a phosphodiesterase inhibitor that increases cAMP levels in cells, was added to Ca²⁺-free solutions to maximally dilate the arteries. Where Dia is diameter, myogenic tone was calculated as \[ \frac{\text{Dia}_{\text{Control}} - \text{Dia}_{\text{Ca-free}}}{\text{Dia}_{\text{Control}}} \times 100 \]. Percent changes in tone were calculated as \[ \frac{\text{Dia}_{\text{Control}} - \text{Dia}_{\text{Drug}}}{\text{Dia}_{\text{Control}}} \times 100 \]. Percent changes in amplitudes and area under the curve (AUC) of nerve-evoked constriction were calculated similarly. Stock solutions of drugs were prepared in DMSO (or ethanol in the case of capsaicin) and working stocks, diluted fresh for every experiment, and were prepared so as to ensure that final DMSO concentration in solutions was <0.05% of solution volume.

Measurement of resting and nerve-evoked smooth muscle Ca²⁺ signals and global Ca²⁺. Ca²⁺ signals were measured in smooth muscle cells of pressurized arteries with a laser-scanning confocal microscope (OZ; Noran Instruments, Middleton, WI), attached to a Nikon Diaphot microscope. Tissues were visualized using a ×60 water immersion objective (Nikon, numerical aperture, 1.2). Images were acquired using software (Prairie Technologies, Middleton, WI), controlled by an OZ–PC workstation. Arteries were preincubated in HEPES PSS containing 10 μM Fluo-4 AM (Invitrogen, Eugene, OR) and pluronic acid (2.5 μg/mL; Invitrogen) in the dark for 60 min at 37°C. Tissues were excited at 488 nm with a krypton-argon laser, and the emitted light was captured at wavelengths >500 nm. Images were acquired at a rate of ~58 images/s (approximately every 17 ms) over a field size of 126 × 126 μm (256 × 256 pixels). Collected images were analyzed using in-house, custom-written analysis software (SparkAn) developed by Dr. Adrian D. Boney (University of Vermont, Burlington, VT). In all instances, 10 images without the signal of interest were averaged as baseline fluorescence (F₀). Nerve-evoked global Ca²⁺ traces were obtained from a region of interest (ROI) positioned over the entire field of recording (256 × 256 pixels). Resting global Ca²⁺ intensity was measured in an ROI drawn around all the smooth muscle cells visible in the recording frame. Ca²⁺ sparks/jCaTs were detected by measuring increases in the fractional fluorescence of Ca²⁺ over a threshold value of F/F₀ > 1.2 above the background noise. Events detected automatically were cross-checked manually. Experiments for jCaT recordings were done at a pressure of 60 mmHg. To aid visualization of jCaTs, 20 μM ryanodine (Enzo LifeSciences) and 200 nM prazosin were added to the incubating solution. jCaTs (Fig. 5) and spark (Fig. 2) traces were generated from 2.6 × 2.6 μm (5 × 5 pixels) and 4.9 × 4.9 μm ROIs positioned over the active sites, respectively. Unless specified otherwise, all drugs and salts were purchased from Sigma-Aldrich (St. Louis, MO).

Electrical field stimulation. Sympathetic nerves on the arteries were stimulated with a pair of platinum electrodes placed on either side of pressurized arteries. For diameter experiments and global Ca²⁺ measurements, stimulation pulses (40–120V, 10 Hz, 0.25 ms) were delivered to arteries in 5-s bursts with 5 min between bursts. For jCaT measurements, stimulation pulses (0.25 ms, 0.5 Hz) were delivered for 15 s following a 10-s rest period recording. Pulse amplitude was adjusted to a value that successfully evoked jCaTs upon stimulation.

Statistical analysis. Averages of the specified number of data points was calculated from data collected on different days from at least three animals. Comparisons between groups were made using paired, two-tailed t-tests. Values of \( P < 0.05 \) were considered statistically significant. Data are reported as means ± SE.

RESULTS

Inhibition of BK channels or RyRs constricts pressurized mesenteric arteries. BK channels and RyRs have been previously shown to oppose myogenic constriction of cerebral arteries (2, 17, 27). In rat and mouse cerebral arteries with myogenic tone, application of BK channel or RyR blockers depolarizes smooth muscle cells and causes vasoconstriction, effects that are nonadditive (2, 3, 17, 27). This suggests that elevation of pressure activates RyR-mediated Ca²⁺ sparks and BK channels to provide a negative feedback mechanism that opposes myogenic constriction (13, 27).

We found that elevation of intravascular pressure to 80 mmHg constricted resistance-sized mesenteric arteries (~100–200 μm passive diameter at 80 mmHg) by 23.1 ± 1.1% (\( n = 25 \) arteries), which is similar to values of myogenic tone previously reported for mesenteric arteries of similar size (18, 26, 31). Paxilline (5 μM) and iberiotoxin (100 nM), selective blockers of BK channels, constricted mesenteric resistance arteries by 7 ± 1 and 7 ± 2%, respectively (Fig. 1, A and C), consistent with the effects of BK channel inhibition on cerebral arteries. Addition of ryanodine (10 μM), an inhibitor of RyRs, constricted arteries by 13 ± 1% (Fig. 1, B and C). Addition of paxilline or iberiotoxin in the presence of ryanodine did not significantly affect arterial diameter (Fig. 1, B and C). The effects of BK channel and RyR inhibition on myogenic tone indicate that BK channels and RyRs in smooth muscle cells of mesenteric resistance arteries act to oppose pressure-induced constriction and that the action of BK channels requires functional RyRs.

Inhibition of RyRs eliminates Ca²⁺ sparks and elevates global Ca²⁺ in smooth muscle cells of pressurized mesenteric arteries. Smooth muscle cells of pressurized mesenteric arteries exhibited Ca²⁺ sparks (Fig. 2A), reflecting localized Ca²⁺ release through RyRs. The average increase in fluorescence during a spark was 1.3 ± 0.1 F/F₀ (\( n = 39 \)). Using a frame rate of 58 frames/s, we found that the average decay time to 50% of maximum amplitude (t₀₂) was 34 ± 1 ms (\( n = 39 \)). The average frequency of sparks per recording field (126 × 126 μm) containing ~6–8 smooth muscle cells was 4.6 ± 1.4 Hz (\( n = 4 \) fields from 3 arteries) corresponding to a Ca²⁺ spark frequency of 0.7 Hz/cell. As expected, ryanodine abolished all sparks in smooth muscle cells (Fig. 2A). Although ryanodine eliminated these local Ca²⁺ signals (sparks), it caused a global elevation of Ca²⁺ (Fig. 2B), as evidenced by the difference between global Ca²⁺ in the presence of ryanodine + paxilline (1.4 ± 0.11 F/F₀) and that in the presence of paxilline alone (1.20 ± 0.03 F/F₀).
BK channel inhibition increases nerve-evoked constriction, whereas RyR inhibition decreases nerve-evoked constriction and suppresses the elevation of vascular smooth muscle cell Ca\(^{2+}\) in pressurized mesenteric arteries. Stimulation of perivascular sympathetic nerves evokes release of ATP and NE, which act through P2X\(_1\)Rs and \(\beta_2\)-adrenergic receptors, respectively, to constrict mesenteric arteries (36). ATP acts rapidly through P2X\(_1\)R channels and NE acts through slower G\(_{q}\)PCR mechanisms (19, 20). EFS (10 Hz for 5 s) of perivascular nerves led to a constriction of pressurized mesenteric arteries (29) that is largely prevented by \(\beta_2\)-methylene ATP (\(\beta_2\)-meATP), an activator and desensitizer of P2X\(_1\)Rs (26). Thus nerve-evoked constriction of mesenteric arteries under these experimental conditions is largely through activation of vascular smooth muscle P2X\(_1\)Rs by ATP, as previously reported (29).

Activation of vascular smooth muscle cell P2X\(_4\)Rs by EFS-evoked ATP release elevates smooth muscle Ca\(^{2+}\) directly via Ca\(^{2+}\) influx through P2X\(_4\)R channels and indirectly through membrane potential depolarization and subsequent activation of L-type voltage-dependent Ca\(^{2+}\) channels (VDCCs), leading to vasoconstriction. Therefore, inhibition of BK channels and Ca\(^{2+}\) sparks, which normally act to oppose these influences, should enhance EFS-induced constrictions. Consistent with this prediction, inhibition of BK channels with paxilline increased nerve-evoked constrictions in pressurized arteries (Fig. 3). The experiments were performed at two physiologically relevant pressures (60 and 80 mmHg) to rule out the pressure dependence of the regulation of nerve stimulation-induced constriction by RyRs. At 60 mmHg, the amplitude of the initial rapid constriction increased by 29.6 ± 8.9%, whereas the total constriction measured as AUC increased by 63.6 ± 17.6% (n = 16) relative to controls (Fig. 3E). At 80 mmHg, nerve-evoked constrictions in the presence of paxilline showed a 48.6 ± 7.8% increase in amplitude and an 89.7 ± 17.2% increase in AUC (n = 6) relative to controls. This effect was dependent on functional RyRs, as evidenced by the negligible effect of BK channel inhibition on constriction (0.5 ± 5.1% amplitude, and 12.1 ± 9.2% AUC; n = 5) in the presence of ryanodine (Fig. 3E). These results indicate that inhibition of BK channels removes an inhibitory influence on nerve-evoked constriction, suggesting that these channels normally oppose nerve-induced constriction. As was the case for the regulation of myogenic tone, inhibition of RyRs rendered BK channels ineffective, indicating that the regulatory action of BK channels depends on this Ca\(^{2+}\) release mechanism. Thus BK channel inhibition has the same effect on nerve-induced (neu-
rogenic) vasoconstriction as it has on pressure-induced (myogenic) vasoconstriction.

Myogenic constriction and resting Ca\(^{2+}\) in pressurized mesenteric arteries was increased by inhibition of RyRs (see Figs. 1 and 2). In striking contrast, inhibition of RyRs exerted the opposite effect on nerve-evoked constrictions (Fig. 3, C, D, and F). At 60 mmHg, ryanodine decreased constrictions by 62.8 \(\pm\) 5.7\% in amplitude and 72.6 \(\pm\) 10.4\% in AUC \((n = 6)\) compared with controls. At 80 mmHg, ryanodine induced a decrease of 52.3 \(\pm\) 8.1\% in amplitude and 55.6 \(\pm\) 4.7\% in AUC \((n = 5)\) compared with controls (Fig. 3F). Prior inhibition of BK channels with paxilline had no significant effect on the ryanodine-induced decrease in the amplitude \((37.6 \pm 11.6\%; \; n = 6)\) or AUC \((66.1 \pm 15.4\%; \; n = 6)\) of nerve-evoked constrictions compared with that observed with ryanodine (Fig. 3F). These results suggest that RyR-mediated Ca\(^{2+}\) release serves a dual purpose in neurogenic constrictions: 1) it activates BK channels to oppose constriction, and 2) it contributes Ca\(^{2+}\) for constriction.

To further elucidate the contribution of RyR-mediated Ca\(^{2+}\) release to nerve-evoked smooth-muscle Ca\(^{2+}\), we measured EFS-induced global Ca\(^{2+}\) transients in the absence and presence of inhibitors of BK channels and RyRs (Fig. 4). At rest, smooth muscle cells exhibited Ca\(^{2+}\) sparks, Ca\(^{2+}\) waves, and, rarely, spontaneous jCaTs, with nerve-stimulation evoking a number of jCaTs. This was followed by a rapid elevation of smooth muscle global Ca\(^{2+}\) accompanied by a robust constriction. Diameter and Ca\(^{2+}\) transients gradually returned to pre-stimulus states upon termination of the stimulus (Figs. 3 and 4).

Global Ca\(^{2+}\) changes induced by stimulation were determined by placing a ROI over the entire recording field. The peak of the nerve-evoked Ca\(^{2+}\) transient under control conditions.
Inhibition of BK channels does not alter elementary purinergic Ca\(^{2+}\) signals. It is conceivable that inhibition of BK channels or RyRs could indirectly affect nerve-evoked constrictions by affecting neurotransmitter release (9, 11). Garcha and Hughes (9, 11) showed that ryanodine does not affect NE release from perivascular nerves of mesenteric arteries. To address potential effects of BK channel blockers on neurotransmission, we measured jCaTs in vascular smooth muscle cells of pressurized mesenteric arteries; these signals can be readily distinguished from Ca\(^{2+}\) sparks by virtue of their larger amplitudes and longer decay times (21). The frequency of evoked jCaTs, which reflect postsynaptic Ca\(^{2+}\) entry through P2X1R channels, correlates with the probability of neurotransmitter release at individual synaptic varicosities (1, 11, 37). If BK channels directly regulate neurotransmitter release, inhibition of BK channels with paxilline would be predicted to increase neurotransmission, which should register as an increase in the frequency of jCaTs. jCaTs were measured in pressurized resistance arteries using the Ca\(^{2+}\)-sensitive dye Fluo-4 AM (Fig. 5) and in the presence of ryanodine (10 μM) to block Ca\(^{2+}\) sparks. To avoid movement artifacts during measurements and thus facilitate observation of jCaTs in isolation, we used EFS parameters that were below the threshold for constriction (0.5 Hz for 15 s). The α\(_{1}\)-adrenergic agonist, prazosin (200 nM), was also added to the bath solution to eliminate possible interference from Ca\(^{2+}\) signals resulting from nerve-induced α\(_{1}\)-adrenergic signaling. Previous studies have shown that ryanodine causes a very slight (13%) reduction in the amplitude of jCaTs and does not affect their frequency (20, 21). These conditions enabled us to record jCaTs in isolation and to estimate the effect of BK channel inhibition on jCaT frequency and, hence, neurotransmitter release. Under these experimental conditions, no spontaneous jCaTs were detected. Upon nerve stimulation, jCaTs were induced (images in Fig. 5, A and B). These events were abolished by desensitizing the P2X1R with 10 μM α,β-meATP, (7.7 ± 0.9 jCaTs in 15 s in control vs. none in 10 μM α,β-meATP; n = 3), confirming that the recorded signals were indeed jCaTs. Over a 15-s recording period of stimulation (0.5 Hz), 11.1 ± 1.1 jCaTs were observed in controls, a number that did not change significantly with the addition of paxilline (12.4 ± 1.3; Fig. 5C), indicating that BK channel inhibition did not stimulate neurotransmission of ATP.

Inhibition of VCDCs eliminates myogenic and nerve-evoked constriction. In vascular smooth muscle cells, membrane depolarization opens VCDCs, and the ensuing Ca\(^{2+}\) influx drives the increase in global Ca\(^{2+}\) that causes vasoconstriction (15, 16, 28). To test the role of VCDCs in myogenic and nerve-induced constriction, we stimulated pressurized mesenteric arteries (80 mmHg) in the absence and presence of selective VCDC blockers. Inhibition of VCDCs with diltiazem (50 μM) dilated arteries by 93.4 ± 2.0%, and further addition of the...
VDCC blocker nifedipine (1 μM) had no significant effect (98.2 ± 1.1%; n = 4). In the presence of diltiazem or nifedipine, addition of ryanodine (10 μM) or paxilline (1 and 5 μM) failed to constrict the arteries. EFS-induced nerve-evoked constrictions were also almost completely abolished in the presence of diltiazem (50 μM; Fig. 6A), which reduced constrictions to 8.2 ± 3.1% of those observed under control conditions (Fig. 6B). These results show that both ryanodine-induced myogenic constriction and RyR-dependent nerve-evoked constriction require functional VDCCs.

**DISCUSSION**

In cerebral arteries, intravascular pressure induces membrane potential depolarization of vascular smooth muscle, and the ensuing Ca²⁺ influx via VDCCs stimulates the local release of sarcoplasmic reticulum Ca²⁺ through RyRs in the form of Ca²⁺ sparks. Ca²⁺ sparks, in turn, activate closely coupled BK channels to oppose the depolarizing and vasoconstricting influence of pressure (2, 14, 17, 22). In the absence of functional BK channels, Ca²⁺ sparks contribute little to global Ca²⁺ and constriction in this context (3, 17, 27). Here, we provide the first evidence that RyR-dependent-BK channel activation opposes pressure-induced and nerve-evoked constrictions in small mesenteric arteries. Unexpectedly, we found that RyR-mediated Ca²⁺ release serves to augment nerve-induced vasoconstriction, an effect that is dependent on Ca²⁺ influx via VDCCs. This is the first instance where BK channels and RyRs have been demonstrated to play different roles in modulating vascular tone depending on the nature of the vasoconstriction stimulus—myogenic versus neurogenic.

The Ca²⁺ spark/BK channel pathway modulates the tone of mesenteric arteries. Inhibition of BK channels or RyRs increased intracellular Ca²⁺ and constricted pressurized (80 mmHg) resistance-sized mesenteric arteries (Figs. 1 and 2). Interestingly, the constriction induced by the inhibition of RyRs was twice that caused by inhibition of BK channels alone. This is unlike observations in cerebral arteries, where the increase in global Ca²⁺ and the constriction induced by inhibition of BK channels and RyRs are equal and nonadditive. The greater effect of ryanodine suggests the presence of additional dilatory mechanisms that are dependent on RyR-mediated Ca²⁺ release. The absence of an effect of ryanodine in the presence of VDCC inhibitors suggests that these dilatory processes regulate VDCC activity. It is plausible that Ca²⁺ release from RyRs plays a role in Ca²⁺-dependent Ca²⁺ release. Opposing effects of BK channels and RyRs on neurogenic constrictions. Electrical stimulation of sympathetic nerves induces the release of ATP and NE. Brief nerve stimulation such as used in this study constricts pressurized mesenteric arteries largely through ATP activation of smooth muscle purinergic receptors (P2X1R) (29). Inhibition of BK channels enhanced nerve-evoked constriction, but this effect was absent when RyRs were already inhibited (Fig. 3). This is consistent with
the idea that activation of BK channels by RyR-mediated Ca\(^{2+}\) release provides a braking effect on increases in nerve-evoked Ca\(^{2+}\) elevation and vasoconstriction. BK channels have been shown to play a similar role in urinary bladder smooth muscle, where deletion of the BK channel \(\alpha\)-subunit gene enhances nerve-mediated contractility (32, 35).

The more complex role of RyRs in regulating neurogenic tone provides an interesting contrast with the uniformly vasodilatory role of BK channels. Our results indicate that Ca\(^{2+}\) release through RyRs opposes nerve-evoked constriction by a direct supply of Ca\(^{2+}\). A number of observations indicate that Ca\(^{2+}\) release via RyRs is necessary for nerve-evoked constriction. First, inhibition of RyRs decreased, rather than increased, nerve-evoked contractions (Fig. 3). Second, under control conditions, nerve stimulation triggered a rapid global elevation of Ca\(^{2+}\); however, in the presence of ryanodine, nerve-induced elevation of global Ca\(^{2+}\) was modest (Fig. 4). Although our study does not exclude a direct effect of ryanodine on the nerves, Lamont and Wier (21) have previously shown that jCaTs are largely unaffected by ryanodine in the rat small mesenteric arteries, indicating that ryanodine acts downstream of jCaTs to augment nerve-induced constriction (Fig. 7).

Previous studies have implicated the involvement of RyR-mediated Ca\(^{2+}\) release in rapid, nerve-induced vascular responses. Notably, in rat small mesenteric arteries, ryanodine has been shown to abolish Ca\(^{2+}\) increases and associated contractions in response to short single-pulse stimulations (0.3 ms), without affecting maximal force or Ca\(^{2+}\) increases during long-term stimulations or exogenous application of norepinephrine (9). Though this latter study used a single pulse whereas we used a train of short pulses (10 Hz, 0.25-ms pulses for 5s), these results collectively support the idea that RyR-mediated Ca\(^{2+}\) release may mediate rapid responses during intermittent stimulations of short duration.

**Inhibition of VDCCs abolishes neurogenic vasoconstriction.** The myogenic response of arteries is mediated by Ca\(^{2+}\) influx via VDCCs and subsequent smooth muscle contraction. Our data indicate that this is true for both myogenic and nerve-induced constriction of mesenteric resistance arteries. In the presence of VDCC blockers, RyR and BK channel inhibition had no effect on diameter and stimulation-induced constriction was virtually abolished (Fig. 6). Lamont et al. (19) have earlier shown that inhibition of VDCCs with nifedipine did not affect jCaT amplitude or the constriction produced by jCaTs. These results are consistent with the concept that neurally released ATP causes membrane depolarization (excitatory junction potential), which rapidly increases Ca\(^{2+}\) influx through VDCCs, and this stimulates a synchronized activation of RyRs to cause a global Ca\(^{2+}\) increase through RyR-mediated Ca\(^{2+}\) release, and thereby vasoconstriction (Fig. 7).

In summary, the regulation of tone in mesenteric resistance arteries by BK channels and RyRs depends on the nature of the stimulus for constriction. When the constricting stimulus is pressure, Ca\(^{2+}\) release from RyRs in the form of Ca\(^{2+}\) sparks activates BK channels to hyperpolarize the smooth muscle cells, which results in deactivation of VDCCs, thereby opposing the increases in intracellular Ca\(^{2+}\). On the other hand, in response to stimulation of sympathetic nerves, Ca\(^{2+}\) release via RyRs together with Ca\(^{2+}\) influx via VDCCs, leads to a global elevation of Ca\(^{2+}\) and aids in the rapid amplification of the vasoconstricting stimulus. BK channels oppose and regulate this nerve-induced tone (Fig. 7), an action that is dependent on functional RyRs. The stimulus-dependent, vasodilatory-vs.-vasoconstricting influence of the RyR-mediated Ca\(^{2+}\) release emphasizes the complex nature of the regulation of vascular tone and smooth muscle Ca\(^{2+}\) by intracellular signals in mesenteric resistance arteries.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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