Myogenic contraction in rat skeletal muscle arterioles: smooth muscle membrane potential and Ca$^{2+}$ signaling

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Kotecha, Neela, and Michael A. Hill. Myogenic contraction in rat skeletal muscle arterioles: smooth muscle membrane potential and Ca$^{2+}$ signaling. Am J Physiol Heart Circ Physiol 289: H1326–H1334, 2005.—The present studies examined relationships between intraluminal pressure, membrane potential ($E_m$), and myogenic tone in skeletal muscle arterioles. Using pharmacological interventions targeting Ca$^{2+}$ entry/release mechanisms, these studies also determined the role of Ca$^{2+}$ entry and release being necessary for myogenic contraction (5). However, although Ca$^{2+}$ influx across the plasma membrane is essential for the myogenic response, there are disparate reports regarding whether voltage-gated Ca$^{2+}$ entry is the prime determinant of myogenic tone (8, 10, 13, 14, 21). Supporting the involvement of alternate mechanisms, studies of single smooth muscle cells undergoing controlled stretch, as a model of myogenic activation, have shown that this mode of mechanical stimulation leads to Ca$^{2+}$ entry, which is only partially blocked by nifedipine (6). Furthermore, evidence has been presented for the involvement of mechanisms utilizing signaling pathways involving protein kinase C and Rho kinase (21). A further complicating factor is that heterogeneity in the mechanisms underlying myogenic reactivity may exist between tissues. Thus the contribution of non-voltage-dependent mechanisms to myogenic contraction remains uncertain (12).

In addition to Ca$^{2+}$ entry, intracellular Ca$^{2+}$ dynamics play a pivotal role in the electromechanical coupling in vascular smooth muscle cells of the arterial wall (16, 17, 20, 23, 28). Thus Ca$^{2+}$ release from the sarcoplasmic reticulum (SR) and localized events including Ca$^{2+}$ sparks, which may affect the activity of plasma membrane large-conductance Ca$^{2+}$-activated K$^+$ (BK$_{Ca}$) channels, have been implicated in the expression of myogenic reactivity (16, 17, 20). Although this is an attractive hypothesis, the exact links between these events and mechanically induced events such as changes in membrane potential ($E_m$) and ion channel activation remain unresolved.

To date, much of our knowledge of the contribution of changes in $E_m$ to myogenic contraction has been derived from studies of cerebral vessels (7, 17, 20, 28). The general applicability of these observations has not been established, and given observed heterogeneity with respect to the ability of various tissues to demonstrate autoregulatory responses (19), it may be reasonable to expect variation in the relationships between $E_m$ and levels of myogenic tone. Additionally, the degree of negative feedback by mechanisms such as BK$_{Ca}$ channels may vary between tissues. Thus such channels may act more to limit myogenic vasoconstriction in cerebral vessels compared with those in resting skeletal muscle (15).

The present studies therefore aimed to define the 1) relationships between intraluminal pressure, $E_m$, and extent of myogenic tone for skeletal muscle arterioles and 2) implications of these relationships on physiological function. Using pharmacological interventions targeting Ca$^{2+}$ entry/release mechanisms, these studies also sought to determine the role of these Ca$^{2+}$ pathways and $E_m$ in determining steady-state myogenic constriction.
MEMBRANE POTENTIAL AND Ca^{2+} PATHWAYS IN MYOGENIC TONE

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats (200–300 g body wt; n = 76) were used. Animals were housed in a facility equipped with 12:12-h light-dark cycle and free access to rat chow and drinking water. The Animal Ethics and Experimentation Committee of RMIT University approved all procedures.

**Isolated Arteriole Preparation**

Rats were anesthetized with thiopental sodium (100 mg/kg) given intraperitoneally. Cremaster muscles were removed, and segments of first-order arteriole were isolated as previously described (25). Arteriole segments were cannulated onto glass micropipettes, secured using 10-0 monofilament suture, and mounted in a 3-ml tissue chamber. The vessel preparation was positioned on the stage of an inverted microscope and continually superfused (2–4 ml/min) with physiological salt solution (PSS; in mM: 111 NaCl, 25.7 NaHCO₃, 4.9 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 11.5 glucose, and 10 HEPES). Arterioles were gradually pressurized, under zero flow, to 70 mmHg by connecting the inflow pipette to a height-adjustable fluid reservoir and warmed to 34°C during a 60-min equilibration period and allowed to develop spontaneous tone. Vessel length was adjusted before development of tone by increasing segment length such that pressure steps to 120 mmHg did not cause lateral bowing of the vessel. This procedure has been verified to result in optimal myogenic and agonist responsiveness. Lumen diameter (as an index of mechanical response) was determined using video microscopy in conjunction with an image-based tracking program (Diamtrak, Adelaide, Australia) (27), and the data were stored using a MacLab analog-to-digital system.

**E_m Measurements**

Intracellular recordings of E_m using glass microelectrodes filled with 2 M KCl (tip resistances of 100–200 MΩ) and an Axoclamp 2B amplifier (Axon Instruments), were made in pressurized arteriole preparations. Impalements were made using a Leitz precision micromanipulator (Leica Microsystems, Victoria, Australia) within the field of microscope view and in a region of the vessel demonstrating typical vasoreactivity.

**Drugs and Chemicals**

Nifedipine (Sigma) was prepared daily as a stock solution (10 mM) in DMSO. Cyclopiazonic acid (CPA; Sigma) was dissolved (10 mM) in DMSO and stored at −20°C. 2-Aminoethoxydiphenylborate (2-APB; Aldrich) was prepared as a 100-mM stock solution in ethanol. Iberiotoxin (100 nM, Sigma) was dissolved in 0.95 saline and stored at −20°C. Phenylephrine (10 mM), ACh (10 mM), and adenosine (100 mM) were dissolved in water. All subsequent dilutions were made in PSS as required, and 75 mM KCl (75KPSS) was made by raising the extracellular [K⁺] of the PSS to 75 mM with direct replacement of NaCl with KCl.

**Protocols**

**Effect of intraluminal pressure and vasoactive agents on myogenic tone and E_m.** After initial equilibration at 70 mmHg, intraluminal pressure was varied between 0 and 150 mmHg. At each chosen pressure, the arteriole was allowed to equilibrate for 5–10 min to reach steady-state diameter before determining the corresponding E_m. Additionally, the steady-state effect of a hyperpolarizing stimulus, i.e., ACh, was determined at 50 and 120 mmHg.

KCl, an agent that primarily relies on Ca^{2+} entry through voltage-operated Ca^{2+} channels (VOCC) to cause constriction, was used to assess whether a limit on VOCC-mediated vascular contractility occurs at higher intraluminal pressures. 75KPPS-induced constriction and depolarization were compared at two pressures (50 and 120 mmHg). Additional studies were performed to determine the contribution of BKCa channels to the level of pressure-induced membrane depolarization and myogenic tone. After taking baseline measurements, vessels were treated with iberiotoxin (100 nM, 15 min), and measurements were subsequently repeated.

**Effect of nifedipine, CPA, and 2-APB on vascular tone and E_m at 70 mmHg.** The endothelial layer was removed from all vessels by intraluminal passage of air bubbles followed by PSS to remove cellular debris. Endothelial removal was demonstrated functionally by a lack of vasodilatation to ACh (10 μM), while dilator responses to adenosine (0.3 mM) were maintained. When steady-state diameter had been attained, smooth muscle cells were impaled to determine E_m.

To determine the contribution of selected Ca^{2+} release/entry pathways, arterioles were exposed to either CPA (50 μM, an inhibitor of SR Ca-ATPase), 2-APB (10 μM, inhibitor of inositol 1,4,5-triphosphate (IP₃)-mediated Ca^{2+} influx and release), or nifedipine (1 μM, an inhibitor of Ca^{2+} influx through VOCC) for at least 20 min before repeating measurements of diameter and E_m. Additional experiments were performed to assess the effects of a combination of nifedipine and 2-APB. Furthermore, the effects of inhibiting a single Ca^{2+} entry pathway in combination with the Ca^{2+} release pathway were studied (nifedipine + CPA or 2-APB + CPA). In all experiments, E_m and diameter measurements were performed when steady-state diameter was reached.

Control experiments were conducted to verify the efficacy of nifedipine and CPA. In one series, arterioles were exposed to 75KPSS to obtain a control KCl-induced constriction, which was then compared with that after 20-min exposure to 1 μM nifedipine. In separate experiments, arterioles were exposed to 0 mM Ca^{2+} PSS containing 2 mM EGTA for 1 min before measuring a control constriction (an indicator of intracellular Ca^{2+} release) to phenylephrine (10 μM). The arteriole was then superfused for 30 min in Ca^{2+}-containing PSS and a further 10 min in PSS containing 30 μM CPA to deplete the intracellular Ca^{2+} stores. The arteriole was then exposed to 0 mM Ca^{2+} PSS, as above, for 1 min before repeating the response to phenylephrine.

**Comparative effect of nifedipine on the development and maintenance of myogenic tone.** As differences have been suggested in the events underlying the development and maintenance of myogenic tone (29), the effectiveness of nifedipine was compared when applied at 0 or 70 mmHg. Studies were conducted using paired vessel segments, and responses were determined as changes in vessel diameter. After basal myogenic tone was established at 70 mmHg, intraluminal pressure was decreased to 0 mmHg for 60 min to negate all pressure-induced signals. One group of vessels was treated with nifedipine (1 μM) for 20 min before increasing the intraluminal pressure to 70 mmHg. The second set of vessels was exposed to vehicle for 20 min before being returned to 70 mmHg, and nifedipine (1 μM) was added subsequently.

**Measurement of passive arteriolar diameter.** At the completion of each protocol, arterioles were made passive by superfusion (10 min) with 0 mM Ca^{2+} PSS containing 2 mM EGTA. Passive diameters were then measured at appropriate intraluminal pressures.

**Analysis and Statistics**

Data are expressed as means ± SE. Simple comparisons of means were performed using Student’s t-test. Multiple comparisons were determined using ANOVA with the paired least-squares difference post hoc test. Values of P < 0.05 were considered significant. Sugimoto curves were generated using Graphpad Prism software.

Myogenic tone refers to active vessel diameter expressed as percentage of passive diameter at 70 mmHg unless stated otherwise. The extent of myogenic contraction (active myogenic tone) occurring in response to changes in intraluminal pressure was obtained by subtracting the diameter of the active vessel at a particular pressure from its passive diameter in 0 mM Ca^{2+} PSS and expressed in micrometers.
RESULTS

Increasing intraluminal pressures over the range 0 to 150 mmHg were associated with progressive membrane depolarization from $-55.3 \pm 4.1$ to $-29.4 \pm 0.7$ mV (Fig. 1A). Plotting active myogenic tone against $E_m$ yielded a steep sigmoidal relationship (Fig. 1B).

Implications of $E_m$-Myogenic Tone Relationship

From the data in Fig. 1B it can be seen that a 10-mV change in $E_m$ from $-30$ to $-40$ mV is associated with arteriolar relaxation of $\approx 20 \mu m$, whereas the same magnitude of membrane hyperpolarization (from $-40$ mV to $-50$ mV) leads to a more pronounced relaxation of $\approx 70 \mu m$. This was experimentally supported by examining arteriole responsiveness to ACh at intraluminal pressures of 50 and 120 mmHg. The amplitude of hyperpolarization and the corresponding changes in diameter in response to ACh at these intraluminal pressures are shown in Fig. 2A. While there is no significant difference in the amplitude of hyperpolarization at the two pressures, there is a significant difference in the resulting change in diameter. This supports the proposition that the functional effect of a change in $E_m$, at the two different pressures, is governed by the sigmoidal relationship between $E_m$ and myogenic tone.

To assess whether there was a limit on VOCC-mediated contractility at higher intraluminal pressures, 75KPSS-induced constriction was compared at pressures of 50 and 120 mmHg and therefore different levels of myogenic tone. Figure 2, B and C, shows that while there were significant differences between the amplitude ($\Delta$) of KCl-induced depolarization (29.2 $\pm$ 1.3 mV at 50 mmHg compared with 21.5 $\pm$ 1.5 mV at 120 mmHg; $P = 0.002$; $n = 6$) and constriction (47.8 $\pm$ 4.5 vs. 31.4 $\pm$ 3.8 $\mu m$; $P = 0.02$) at the two pressures, there were no differences in either the final (or absolute) lumen diameter (19.6 $\pm$ 1.1 vs.
MEMBRANE POTENTIAL AND CA\(^{2+}\) PATHWAYS IN MYOGENIC TONE

Effect of Nifedipine, CPA, and 2-APB on \(E_m\) and Myogenic Tone at 70 mmHg

All experiments examining \(Ca^{2+}\) sources were conducted in deendothelialized arterioles (70 mmHg) with myogenic tone of 50.3 ± 1.4% (n = 58) and \(E_m\) of −39.04 ± 1.02 mV (n = 25). Inhibition of L-type \(Ca^{2+}\) channels with nifedipine (1 \(\mu\)M) resulted in a significant but incomplete loss of myogenic tone (68 ± 2.1% vs. control of 51.9 ± 2.2%; n = 11; Fig. 5). In contrast, there was no significant change in the associated \(E_m\) (−37.2 ± 1.6 vs. control of −37.0 ± 0.8 mV; n = 10). Nifedipine (1 \(\mu\)M) was shown to be effective by its ability to inhibit 75KPSS-induced constriction (10.9 ± 2.0 \(\mu\)m vs. control of 73.5 ± 7.7 \(\mu\)m, \(P = 0.006\); n = 4), suggesting that this concentration was functionally effective in blocking voltage-gated \(Ca^{2+}\) entry.

The SR \(Ca^{2+}\) ATPase inhibitor CPA (30 \(\mu\)M) had a biphasic effect on arteriole diameter at 70 mmHg (Fig. 5). After an initial constriction, there was a significant subsequent loss of tone (66.7 ± 3.3%) relative to pre-CPA treatment diameter (50.0 ± 3.2%; n = 6). After 20 min exposure to CPA, \(E_m\) remained unchanged (−40.8 ± 2.4 vs. control of −40.6 ± 2.1 mV; n = 6) despite loss of tone. The efficacy of CPA to deplete the stores was verified by its ability to attenuate the contraction to phenylephrine (10 \(\mu\)M) in 0 mM \(Ca^{2+}\) superfusate. Phenylephrine-induced contractions (54.2 ± 13.7 \(\mu\)m) were significantly attenuated in the presence of 30 \(\mu\)M CPA (1.8 ± 1.8 \(\mu\)m; \(P = 0.02\); n = 5).

Unlike nifedipine and CPA, 2-APB (50 \(\mu\)M) did not have any significant effect on either steady-state diameter (43.1 ± 4% vs. control of 44.9 ± 4.2%; n = 9) (Fig. 5) or \(E_m\) (−35.3 ± 1.7 mV vs. control of −36.3 ± 1.9 mV; n = 9). Combining any of the two pharmacological interventions of \(Ca^{2+}\) entry/release pathways resulted in almost complete loss of myogenic tone (Fig. 6) again without a significant change in \(E_m\). These observations were not affected by the order in which the various inhibitors were added. Addition of CPA to arterioles

22.8 ± 1.6 \(\mu\)m) or \(E_m\) (−9.8 ± 0.3 vs. −10.7 ± 0.3 mV). Hence, the difference in amplitude of constriction would appear to reflect differences in the level of myogenic tone as, importantly, arterioles remained responsive to KCl at the higher pressure.

The relationship between \(E_m\) and mechanical response was observed in arterioles that displayed spontaneous vasomotion and by application of ACh, a hyperpolarizing stimulus. Vasomotion was observed in ~5% of the isolated, pressurized arterioles that were studied. Simultaneous continuous measurements of diameter and \(E_m\) were recorded in five such experiments. Peak spike depolarization preceded peak arteriole constriction by 2.05 ± 0.51 s (n = 5; for each n value, an average was determined for 5–10 spikes). An example tracing is shown in Fig. 3A with an expanded timescale tracing in Fig. 3C. In response to ACh, hyperpolarization preceded relaxation; an example tracing is shown in Fig. 3B together with an expanded timescale in Fig. 3D.

Contribution of BK\(_{Ca}\) Channels to Arteriolar Smooth Muscle \(E_m\)

To determine the contribution of BK\(_{Ca}\) channels, pressure-\(E_m\) relationships were compared in the absence and presence of iberiotoxin (100 nM). At pressures of 30, 50, 70, and 120 mmHg, iberiotoxin caused significant vasoconstriction and depolarization. For example, at 70 mmHg, iberiotoxin caused a depolarization of 6.9 mV with a corresponding vasoconstriction of 15% (Fig. 4).

Fig. 3. A: simultaneous recording of \(E_m\) and diameter in an arteriole displaying vasomotion (at 0.11 Hz) at 70 mmHg. In this example, peak spike depolarization preceded peak constriction by 2.04 ± 0.26 s, calculated over 7 spikes. B: simultaneous recording of \(E_m\) and diameter during ACh exposure for an arteriole at 50 mmHg. Hyperpolarization in response to 1 \(\mu\)M ACh preceded relaxation response by 0.75 s. C and D: sections of the above tracings using an expanded scale to highlight that \(E_m\) changes precede diameter changes.

Fig. 4. Effect of large-conductance \(Ca^{2+}\)-activated K\(^+\) channel (BK\(_{Ca}\)) inhibition on arteriolar diameter (A) and \(E_m\) (B). At intraluminal pressures of 30, 50, 70, and 120 mmHg, iberiotoxin (IBTx; 100 nM) caused vasoconstriction (A) and depolarization (B). Results are shown as means ± SE; n = 6–9. *P < 0.05.
were not significantly different from control values (refer to text).

arterioles, without endothelium, at an intraluminal pressure of 70 mmHg.

DISCUSSION

Comparative Effect of Nifedipine on the Development and Maintenance of Myogenic Tone

Figure 7 illustrates the comparative effect of nifedipine on the development and maintenance of myogenic tone. When added to arterioles in which myogenic tone had been abolished by lowering intraluminal pressure to 0 mmHg, nifedipine totally prevented the development of tone on returning arterioles to 70 mmHg (Fig. 7A). In contrast, in arterioles where tone was allowed to redevelop (by returning to 70 mmHg), subsequent addition of nifedipine only partially dilated vessels toward passive (Fig. 7B). The differential effect of the same concentration of nifedipine suggests a difference in the relative contribution of dihydropyridine-sensitive VOCCs to the developmental and maintenance phases of myogenic constriction.

that had previously been exposed to 2-APB and nifedipine had no further effect on myogenic tone (data not shown).

Although the underlying reason for the plateau in the myogenic constriction-\(E_m\) relationship (Fig. 1B) is uncertain, it has important implications in that hyperpolarizing stimuli may have limited capacity to elicit dilation at higher intraluminal pressures. Support for a limitation to endothelium-dependent
dilation at higher pressures was demonstrated in the present study by comparing the effectiveness of ACh at pressures of 50 and 120 mmHg. Although extensive studies were not performed to dissect the various components of the ACh-mediated dilation, we have previously shown that there is significant involvement of an endothelium-dependent hyperpolarizing component (26). Nevertheless, as the nonhyperpolarizing components of the ACh response were not inhibited in the present studies, the effect of pressure-induced changes in $E_m$ on the hyperpolarizing effect of ACh may be underestimated. As a result of these effects, the relationship between myogenic tone and $E_m$ should be taken into account when considering the effectiveness of vasoactive stimuli that exert their actions through modulation of arteriolar smooth muscle $E_m$.

The results of the present studies, while supporting previous data from Knot et al. (20) obtained from cerebral arterioles, also suggest that differences may exist between vascular beds. Comparing the current data to that of Knot et al. (20) shows an upward shift (toward more depolarized values for a given intraluminal pressure) in the pressure-$E_m$ relationship for the skeletal muscle arterioles (Fig. 8A). Interestingly, the largest differences between the data sets are evident at pressures below $\approx 80$ mmHg, which correspond to the region of most rapid change in the $E_m$-myogenic tone relationship (Fig. 8). Given evidence for heterogeneity in the distribution of ion channels in vascular tissues, a possible explanation could relate to underlying differences in reliance on a hyperpolarizing influence such as has been suggested for the BKCa channel. Studies using the BKCa inhibitor iberiotoxin did demonstrate a contribution of these channels to both $E_m$ and vessel diameters over the pressure range 30–120 mmHg. Over this pressure range, intracellular Ca$^{2+}$ is known to increase (37) and progressive depolarization occurs favoring activation of BKCa channels. However, compared with the work of Brayden and Nelson (3), it appears that the contribution of the BKCa channel is greater in cerebral vessels compared with cremaster muscle arterioles. Thus, in the study of Brayden and Nelson (3), inhibition of BKCa channels caused a vasoconstriction of 41 ± 9% (at 60 mmHg), whereas in the present study, a vasoconstriction of only 15 ± 3% was observed (at 70 mmHg). However, in terms of change in $E_m$, there was a similar change of 7 ± 1 mV in cerebral vessels (3) and 6.9 ± 1.3 mV in the present study. It is worthwhile noting that a 15% constriction is predictably associated with a change of 7 mV (at 70 mmHg) in the skeletal muscle arterioles (Fig. 1B).

In contrast to the cerebral vessel studies referred to above, Jackson and Blair (15) have suggested that in small myogenically active skeletal muscle arterioles, BKCa channels are “silent” under resting in vivo conditions but can be recruited...
during agonist-induced vasoconstriction. This may reflect heterogeneity in KCa channel subtypes with a possible underlying difference in Ca\(^{2+}\) set point (15). Alternatively, while estimates of global cytoplasmic Ca\(^{2+}\) appear similar in the two tissues, interactions between the cell membrane and the superficial SR controlling focal Ca\(^{2+}\) release may differ for the vessel types. If such heterogeneity exists, it remains uncertain as to whether this reflects differences in tissue distribution (i.e., skeletal muscle vs. cerebral) or vessel size. At a minimum, comparison at the molecular level is required to establish whether regional differences exist with respect to BKCa subtype distribution. At a physiological level, such differences may importantly reflect adaptive or protective mechanisms such that relative to resting skeletal muscle arterioles, cerebral vessels are able to maintain a more dilated state (Fig. 8B).

It is also conceivable that the apparent difference in the relationship between diameter and smooth muscle membrane potential for cerebral and skeletal muscle arterioles is unrelated to differences in the activity, and/or expression, of BKCa channels. Preliminary analysis of the iberiotoxin experiments of the present study suggests that the data points approximate a similar relationship to that for control conditions as shown in Fig. 8B. Further studies are, however, required to obtain data in the presence of BKCa inhibition over the entire range of pressures presented in Figs. 1 and 8. Regardless of this, it appears that resting E\(_m\), per se, cannot be used to predict myogenic tone when comparing between vessels from different vascular beds.

While this study did not find an exclusive role for voltage-gated Ca\(^{2+}\) entry (see below) in myogenic constriction, evidence was provided for a temporal relationship between E\(_m\) and mechanical activity. In particular, this was shown for hyperpolarizing stimuli and in continuous records of vessels exhibiting spontaneous vasomotion (Fig. 3). With regard to the latter, spikes in E\(_m\) were observed, on average, to occur 2 s before peak vasoconstriction. In separate experiments, it was found that peak changes in intracellular [Ca\(^{2+}\)] precde constriction by \(\approx 1.5\) s (data not shown). Collectively, these data suggest a sequence of events whereby in skeletal muscle arterioles, vasomotion is triggered by rhythmic changes in E\(_m\) that promote Ca\(^{2+}\) entry and subsequent constriction (1, 9).

Interestingly, in earlier in vivo studies of small arterioles (diameter \(\approx 20\) \(\mu\)m), it was shown that while inhibition of voltage-dependent Ca\(^{2+}\) entry did not abolish myogenic tone, it effectively blocked vasomotion (10). These results are consistent with the notion that voltage-gated Ca\(^{2+}\) entry may not be the only source of activator Ca\(^{2+}\) contributing to the myogenic response.

**Sources of Ca\(^{2+}\) Underlying Myogenic Constriction**

In the presence of VOCC blockade by nifedipine, there was a significant, but incomplete, loss of myogenic tone. Thus while a significant influx of Ca\(^{2+}\) appeared to be mediated by nifedipine-sensitive channels, the data differ from studies suggesting that depolarization-linked Ca\(^{2+}\) entry is solely responsible for the generation of active myogenic tone (5). In contrast, data consistent with the present results are provided by studies demonstrating that \(~50\%\) of Ca\(^{2+}\) influx into isolated smooth muscle cells subjected to controlled stretch was not prevented by nifedipine (6). In addition, as mentioned above, we have previously shown (10) that nifedipine did not abolish myogenic tone in third-order arterioles (diameter \(\approx 20\) \(\mu\)m) although rhythmic vasomotion was markedly inhibited. Collectively, it therefore appears that Ca\(^{2+}\) required for myogenic tone can be supplied via both VOCC- and non-VOCC-dependent sources.

Exposure of arterioles to CPA to deplete SR Ca\(^{2+}\) stores resulted in a significant loss of myogenic tone, suggesting a role for Ca\(^{2+}\) stores in the generation and/or maintenance of myogenic constriction. These data are consistent with several studies examining the effect of SR Ca\(^{2+}\) ATPase inhibitors on pressure- and stretch-induced vascular tone (20, 35). The present study further suggests that both Ca\(^{2+}\) entry and SR Ca\(^{2+}\) release are required as the combination of CPA and nifedipine effected an almost complete loss of tone. The combination of inhibitors did not, however, prevent pressure-induced depolarization, consistent with both of these Ca\(^{2+}\) mobilizing events being distal to membrane depolarization in the overall signaling pathway.

As an additional approach for studying the role of Ca\(^{2+}\) entry and Ca\(^{2+}\) release, studies were performed using the putative IP\(_3\) receptor blocker 2-APB. This agent has been previously reported to inhibit store depletion-mediated Ca\(^{2+}\) entry (22, 34), and such pathways are active in pressurized arterioles (30). Caution must, however, be taken in interpretation of such data as 2-APB has also been suggested to inhibit store depletion-mediated Ca\(^{2+}\) entry by more direct actions (31); nevertheless, the agent inhibits this axis of Ca\(^{2+}\) entry and was considered suitable for the goals of this study.

2-APB alone showed little effect on myogenic tone under steady-state conditions (70 mmHg), indicating that Ca\(^{2+}\) entry mechanisms inhibited by this compound were not essential. Similarly to the combination of CPA and nifedipine described above, 2-APB and nifedipine resulted in a near total loss of myogenic tone. Furthermore, treatment of arterioles with 2-APB and CPA led to a nearly complete loss of myogenic tone. While the presence of 2-APB by itself did not compromise myogenic tone, there was complete loss of myogenic tone in the presence of 2-APB and CPA. It is not clear why all tone was lost under these conditions, as Ca\(^{2+}\) influx through VOCCs would be expected to be maintained as these agents do not alter pressure-induced changes in E\(_m\). Furthermore, 2-APB does not inhibit depolarization-mediated constriction in the vessels studied (30). With regard to this, Wesselman et al. (36) have demonstrated that in rat mesenteric resistance vessels, sensitization to VOCC-mediated Ca\(^{2+}\) influx is necessary to affect myogenic constriction. While it is plausible that 2-APB and CPA somehow interfere with Ca\(^{2+}\) sensitization mechanisms, this was beyond the scope of the current study. Collectively these data are, however, consistent with a dynamic relationship between Ca\(^{2+}\) entry and SR Ca\(^{2+}\) release being necessary for the maintenance of myogenic constriction, as has been proposed by Nelson and colleagues (17, 20, 28).

Disparate results regarding the role of voltage-dependent Ca\(^{2+}\) entry in myogenic phenomena may arise from several factors. The strength of the myogenic response varies between arterioles of similar size from different vascular beds and also between vessels within a given microcirculatory bed (32). Among the possible reasons for this apparent functional het-
erogeneity is the variation in the extent and involvement of intracellular stores (33). In addition to differences relating to regional variation in vascular smooth muscle cells, differing results may be a function of protocols employed. For example, protocols involving long time periods of exposure of arterioles to 0 mM Ca²⁺, or decreases in intraluminal pressure, may compromise pressure-evoked \( E_m \) signals and hence the development of tone when VOCCs are blocked. Whether the endothelium has been removed from a given preparation may also contribute to discrepancies. While the endothelium does not directly contribute to arteriolar myogenic tone under no-flow conditions, pharmacological interventions adopted to assess the role of various sources of Ca²⁺ would also have effects on the endothelial Ca²⁺ levels, thus modulating the release of endothelial vasoactive stimuli that in turn modulate smooth muscle Ca²⁺ levels and hence the level of tone. In addition, consideration must be given to drug specificity. For example, the endothelial Ca²⁺ of Australia.

ences of neural and endothelial origin can be predicted. Furthermore, the variation in the extent and involvement of previously appreciated and is suggestive of heterogeneity between vascular beds. This relationship has significant physiological implications for responsiveness to stimuli that target \( E_m \) and provides a base against which the hyperpolarizing influences of neural and endothelial origin can be predicted. Furthermore, the data may be relevant to understanding the action of hyperpolarizing vasodilators in pathophysiological states such as hypertension.

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