Role of Ca\(^{2+}\)- and swelling-activated Cl\(^{-}\) channels in \(\alpha_1\)-adrenoceptor-mediated tone in pressurized rabbit mesenteric arterioles

Carmelle V. Remillard, Marie-Andrée Lupien, Valérie Crépeau, Normand Leblanc*

Department of Physiology, University of Montréal and Montréal Heart Institute Research Centre, 5000 East Bélanger St., Montreal, Quebec, Canada H1T 1C8

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Abstract

**Background:** Ca\(^{2+}\)-activated \((I_{\text{Cl(Ca)}})\) and swelling-induced \((I_{\text{Cl(swell)}})\) Cl\(^{-}\) channels have, respectively, been postulated to participate in the membrane depolarization and contraction mediated by activation of \(\alpha_1\)-adrenoceptors and vascular wall distension during pressurization. Their respective function in generating active force in pressurized arterioles during \(\alpha_1\)-adrenoceptor stimulation remains unsettled. **Objectives:** Experimental protocols were designed to: (1) assess the relative contribution of \(I_{\text{Cl(Ca)}}\) to the pressure-dependence of lumen diameter of mesenteric arterioles at different states of activation of the \(\alpha_1\)-adrenoceptor, and (2) investigate the potential role of \(I_{\text{Cl(swell)}}\) in spontaneous and agonist-mediated myogenic reactivity. **Methods:** Segments of endothelium-denuded rabbit mesenteric arterioles with a lumen diameter of \(\sim 70\) \(\mu\)m were cannulated at both ends and studied under isobaric conditions at 36°C. Steady-state lumen diameter at each pressure step investigated (0–100 mmHg, in 20-mmHg increments) was measured by a video-microscopy edge-detection technique. **Results:** Under control conditions, 23% of the arterioles developed nifedipine-sensitive spontaneous myogenic tone. In the presence of 1 mM tetraethylammonium chloride (TEA) to inhibit Ca-dependent K channels, the \(\alpha_1\)-agonist phenylephrine (PE) contracted the vessels in a concentration-dependent manner (0.1–10 \(\mu\)M) and potentiated myogenic reactivity. The contraction mediated by 1 \(\mu\)M PE / TEA was abolished by 1 \(\mu\)M nifedipine, indicating that Ca entry through voltage-gated Ca channels was a necessary step in the cascade leading to contraction. Niflumic acid (NfA, 100 \(\mu\)M), a relatively selective inhibitor of \(I_{\text{Cl(Ca)}}\), had no effect on myogenic tone but reversed the PE-induced contraction, varying with the concentration of PE and transmural pressure. For PE concentrations between 0.1 and 1 \(\mu\)M, but not for 10 \(\mu\)M PE, the relaxing efficacy of NfA decreased as applied pressure was raised from 0 to 100 mmHg. At all pressure steps, the NfA-induced relaxation was inversely related to the concentration of PE and transmural pressure. For PE concentrations between 0.1 and 1 \(\mu\)M, another Cl channel blocker, inhibited spontaneous myogenic tone, and partially suppressed a component of contraction at elevated transmural pressures in arterioles incubated in 1 \(\mu\)M PE / 1 mM TEA / 100 \(\mu\)M NfA. **Conclusions:** Our data indicate that under low to moderate stimulation of the \(\alpha_1\)-adrenoceptor signaling pathway, \(I_{\text{Cl(Ca)}}\) channels play an important role in the sustained contraction produced. Their declining contribution to contraction with increasing transmural pressure may be explained, at least in part, by a progressive enhancement of stretch-induced ionic conductances, possibly volume-sensitive Cl\(^{-}\) channels.

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1. Introduction

Noradrenaline (NA), a putative neurotransmitter released in blood vessel varicosities, induces a vasoconstriction that is usually accompanied in most vascular beds by a sustained membrane depolarization [1]. Among several postulated ionic mechanisms, it has been hypothesized that activation of a Ca\(^{2+}\)-activated Cl\(^{-}\) current \((I_{\text{Cl(Ca)}})\) by NA and other neurotransmitters and hormones plays a prime role in the associated depolarization observed in many vascular networks [2]. Consistent with single cell studies from which a putative physiological role was originally proposed, niflumic acid (NfA), a relatively specific blocker of \(I_{\text{Cl(Ca)}}\) channels [2], and other fenamate compounds,
partially or fully reversed the contraction mediated by activation of $\alpha_1$-adrenoceptors [3–7]. These investigations have led support to the idea that activation of $I_{\text{Cl(Ca)}}$ plays a critical function in the sustained depolarization and contraction mediated by contractile agonists.

Many resistance arteries contract in response to stretch or an increase in transmural pressure. This endothelium-independent state of contraction, so-called myogenic tone or response, is thought to play a prime role in the autoregulation of blood flow in several vascular beds [8]. Besides a few exceptions (for a review, consult Davis and Hill [8]), myogenic tone generally occurs as a consequence of membrane depolarization [9] that leads to graded enhanced $Ca^{2+}$ entry and modest elevations of intracellular $Ca^{2+}$ concentration of $\sim 100–200 \text{ nM}$ from a resting level of $\sim 100 \text{ nM}$ [10,11]. Several ionic mechanisms may participate actively in the depolarization and activation of myogenic tone. These include: (1) stretch-activated non-selective cation channels that are permeable to $Ca^{2+}$ [12], (2) direct modulation of voltage-dependent $Ca^{2+}$ channels by stretch [13], and (3) block of charybotoxin- or TEA-sensitive $Ca^{2+}$-dependent $K^+$ channels [14]. Recently, Nelson et al. [15] proposed that in cerebral arteries, myogenic tone may be the consequence of the activation of $Cl^-$ channels that are distinct from $I_{\text{Cl(Ca)}}$ since: (1) niflumic acid was ineffective at reversing the depolarization and contraction evoked by increased transmural pressures, (2) DIDS and IAA-94, two commonly used chloride channel inhibitors, were shown to hyperpolarize cerebral arterioles and contribute to the myogenic contraction, and (3) lowering the extracellular $Cl^-$ concentration potentiated the myogenic response. A $Cl^-$ channel belonging to the CIC-3 subfamily of voltage-gated $Cl^-$ channels was recently cloned from mammalian heart [16] and expressed in canine vascular smooth muscle cells [17]. This channel is regulated by changes in cell volume, is inhibited by DIDS, and is postulated to participate in the depolarization associated with the development of myogenic tone [17–19].

We hypothesized that the relative contribution of $I_{\text{Cl(Ca)}}$ in vasoactive tone induced by a constricting agonist changes when transmural pressure is altered in a manner consistent with a variable participation of these channels to overall membrane conductance. This hypothesis was tested by using niflumic acid as a pharmacological tool to assess the contribution of $Ca^{2+}$-dependent $Cl^-$ channels to changes in active diameter of pressurized rabbit mesenteric arterioles stimulated with the $\alpha_1$-adrenergic agonist phenylephrine. Two specific aims were sought: (1) to determine the effects of NfA on the pressure-diameter relationships obtained at different levels of activation of the $\alpha_1$-adrenergic receptor; (2) to investigate the potential role of volume-sensitive $Cl^-$ channels in spontaneous and agonist-mediated myogenic reactivity. Our results suggest that the contribution of $I_{\text{Cl(Ca)}}$ to membrane depolarization declines when transmural pressure is increased and this may be due, at least in part, to an enhanced contribution of volume-sensitive $Cl^-$ channels recruited in response to the increase in wall stress. Preliminary data have been published previously in abstract form [20].

2. Methods

2.1. Tissue preparation

This study conforms with the Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care, and was approved by the Animal Care Ethics Committee of the Montréal Heart Institute. New Zealand White rabbits were sacrificed by cervical dislocation. Mesenteric arterioles 2–3 mm in length (mean lumen diameter 71±2 $\mu$m, $n=106$) were carefully dissected from the vascular bed, mounted on borosilicate canulae in a perfusion chamber, and attached using silk suture threads. Criteria for viability of the arterioles included contraction to a bolus of 1 M KCl and/or 10 mM PE, and no pressure leaks between the proximal and distal ends of the vessel. In all experiments, the endothelium was removed by passage of a large air bubble through the vessel lumen; effective removal was assessed by the failure of acetylcholine (1 $\mu$M) to relax vessels pre-contracted with phenylephrine (1 $\mu$M). Suitable arterioles were then allowed to equilibrate at 40 mmHg for 30–60 min at $36±0.5^\circ$C before initiating experimental protocols.

2.2. Diameter measurements

An image of the arteriole was projected onto a video monitor via a microscope-mounted CCD camera (KP-113, Hitachi) at a final magnification of 10X. Lumen diameter of the vessel was monitored continuously by edge-detection (Living Systems Instrumentation Inc., Burlington, VT). Transmural pressures ranging from 0 to 100 mmHg were applied for a minimum of 2 min at 20-mmHg increments, maintained by a pressure-servo control system, and monitored with a PM-4 pressure monitor (Living Systems Instrumentation Inc., Burlington, VT). Pressure transducers were attached at both the proximal and distal ends of the artery. Pressures reported throughout the study were registered from the proximal transducer.

Video (diameter) and pressure were recorded simultaneously on a conventional video tape (A.R. Vetter Co., Rebersburg, PA) and on a 486-IBM-PC using Axotape (version 2.0) or Axoscope software (version 7, Axon Instruments Inc., Foster City, CA) at a sampling rate of 25 or 33.3 Hz. Final analysis was performed using either Axotape or Axoscope, and Origin (version 4.1, Microcal Software Inc., Northampton, MA) softwares.

2.3. Solutions

The dissecting solution contained (mM): 120 NaCl, 25 NaHCO$_3$, 4.2 KCl, 0.6 KH$_2$PO$_4$, 1.2 MgCl$_2$, 11 glucose,
1.8 CaCl₂. The normal bathing (control) solution was identical to the dissecting solution. Passive diameter changes to pressure were obtained by exposing the arteriole to a Ca²⁺-free solution of similar composition to the dissecting solution, except for the omission of CaCl₂ and addition of 100 μM EGTA. The high K⁺ (45 mM) solution was obtained by equiosmolar replacement of NaCl by KCl in the control solution. Test solutions were prepared by adding agents to the final concentrations into the control solution. Phenylephrine was prepared as a 10-mM stock in H₂O. Niflumic acid and nifedipine stock solutions were prepared in dimethyl sulfoxide (DMSO) at concentrations of 100 and 10 mM, respectively; the final concentration of DMSO never exceeded 0.1%. Tetraethylammonium chloride and DIDS were added in powder form to the final desired concentration. All drugs were analyzed. The concentration of phenylephrine, in the presence of slightly but significantly dilated vessel measured in Ca²⁺-free medium. The relative contraction elicited by a given PE concentration and pressure (% Contraction(P=x)) was estimated using the following equation:

\[
\% \text{Contraction}_{(P=x)} = \left[ \frac{\text{Diam}_{(PE-x)} - \text{Diam}_{(PE-10)}}{\text{Diam}_{(0Ca)}} \right] \times 100
\]

where Diam_{(PE-x)} and Diam_{(PE-10)} are diameters measured at a given PE concentration and 10 μM PE, respectively, and Diam_{(0Ca)} corresponds to the diameter obtained 0 Ca²⁺–100 μM EGTA.

Percent relaxation at a given pressure step (% Relaxation_{(P=x)}) due to niflumic acid was calculated using Eq. (2):

\[
\% \text{Relaxation}_{(P=x)} = \left[ \frac{\text{Diam}_{(NFA)} - \text{Diam}_{(PE/TEA)}}{\text{Diam}_{(Ctrl)}} \right] \times 100
\]

where Diam_{(NFA)}, Diam_{(PE/TEA)} and Diam_{(Ctrl)} are the diameters at the applied pressure in the presence of 100 μM NFA/PE/TEA, [x] PE/1 mM TEA and control solutions, respectively.

Data are expressed as means±SE.M. with n referring to the number of arterioles. Using the software Statistica for Windows 99 (version 5.5), statistical significance between individual means of steady-state lumen diameter (measured at the end of the pressure step) was assessed using a paired Student’s t-test when two groups were compared, or one-way ANOVA test with a Duncan’s post-hoc multiple range test for repeated measure when more than two groups were analyzed. P<0.05 was considered to be statistically significant.

3. Results

3.1. Passive properties of rabbit mesenteric vessels

When pressurized to 40 mmHg during equilibration, 23% (24/106) of the arterioles developed spontaneous myogenic tone, sometimes accompanied by slow cycles of constriction that usually disappeared after 30 min of pressurization. Fig. 1A reports the passive characteristics of 30 of these vessels. Exposure to Ca²⁺-free medium slightly but significantly dilated the arterioles at all pressures above 0 mmHg. The relatively poor reactivity of these vessels in the absence of a contractile agent is consistent with other studies performed on the mesenteric vasculature [21,22].

3.2. The α₁-adrenoceptor-induced contraction is nifedipine-sensitive

Many chloride channel inhibitors, including members of the fenamate family such as niflumic acid, also stimulate large conductance Ca²⁺-dependent K⁺ (KCa) channels in vascular smooth muscle [23]. We first evaluated the effects of tetracylammonium chloride (TEA) at a concentration (1 mM) that would be considered as relatively selective and potent for blocking KCa in vascular myocytes [24]. TEA significantly reduced lumen diameter relative to control at pressures between 20 and 60 mmHg (Fig. 1B). These results suggest that KCa inhibition enhanced myogenic reactivity in our preparations.

We next tested the effects of TEA on pressure-diameter relationships in the presence of 0.1 or 1 μM phenylephrine (PE), a selective α₁-agonist. At 0.1 μM, PE had no effect on lumen diameter in the absence of pressure, but induced contraction with increasing pressure. Whereas TEA further contracted the arterioles in the presence of 0.1 μM PE (Fig. 1C), it failed to potentiate the contraction caused by 1 μM PE (Fig. 1D). These results suggest that KCa may partly regulate the resting membrane potential and tone at low levels of α₁-adrenoceptor stimulation; however, this K⁺ channel does not appear to play an important role at higher levels of receptor stimulation. In the presence of TEA, the EC₅₀ for the PE-induced contraction was 352
Fig. 1. Passive properties of pressurized vessels and contribution of TEA-sensitive K⁺ channels. (A) Pressure steps were applied with 1.8 mM Ca²⁺ (control, ■) or 0 Ca²⁺/100 μM EGTA (□) in the bathing medium. A graph reporting the steady-state diameter±S.E.M. (n=30) vs. applied pressure is shown. (B) Arterioles were subjected to a similar pressure protocol with 1 mM TEA (n=6) in the bathing solution to block K⁺ channels. Vessels contracted slightly to TEA (□) relative to control (■). Panels (C) and (D) depict graphs showing the effects of 1 mM TEA (●) on the contraction elicited by 0.1 (n=6) or 1 (n=6) μM PE (○), respectively. As for panels A and B, the two graphs show mean steady-state diameter vs. pressure relationships. (E) Dihydropyridine inhibition of PE-induced tone. Mean diameter±pressure relationships from a total of five arterioles are displayed. The arterioles were subjected to pressure steps in control (■), 1 mM PE/1 mM TEA (●) and 1 mM nifedipine/PE/TEA (○) conditions. For all panels: * Significantly different from control (P<0.05); panel C: † Significantly different from 0.1 μM PE (P<0.05); panel E: ‡ Significantly different from 1 μM PE + 1 mM TEA (P<0.05).

(n=45), 177 (n=75) and 75 (n=38) at 0, 40 and 80 mmHg, respectively. These observations are consistent with the reported facilitation of myogenic reactivity following α₁-adrenoceptor stimulation [25].

Fig. 1E shows that the contraction elicited by 1 μM PE in the presence of 1 mM TEA was totally reversed at all pressure steps by 1 μM nifedipine, a specific L-type Ca²⁺ channel blocker [26]. Our results are compatible with those obtained by others in rat small mesenteric arteries [21] and suggest that Ca²⁺ entry through voltage-dependent Ca²⁺ channels is mainly responsible for the sustained contraction induced by PE.

3.3. Role of Ca²⁺-dependent Cl⁻ channels in α₁-adrenoceptor-induced tone

Fig. 2 shows the results of two typical experiments in which we tested the effects of the Iisci(Ca) blocker niflumic...
Fig. 2. Representative traces showing relaxation by NfA of PE-induced vasoconstriction. Data from sample experiments with two PE concentrations are shown: 0.5 (A) and 10 (B) μM. In panel A, PE/1 mM TEA (top right trace) contracted the arteries at all pressures (P) and promoted myogenic reactivity. NfA (100 μM) reversed the contraction induced by PE/TEA at pressures between 0 and 60 mmHg, and between 0 and 100 mmHg for 0.5 and 10 μM PE, respectively (bottom left panels). At the lower right hand side of each panel is shown a graph illustrating the diameter–pressure relationships derived from each particular experiment.

acid (NfA) on vascular reactivity mediated by an intermediate (0.5 μM) and a saturating concentration (10 μM) of PE, in the presence of TEA. NfA produced differential pressure-dependent effects that varied with the concentration of PE. For the experiment using 0.5 μM PE (panel A), the arteriole passively dilated in the absence of drug (control) in response to incremental pressure steps. Exposure to 0.5 μM PE/1 mM TEA contracted the arteriole from 84 to 18 μm in the absence of pressure and promoted myogenic reactivity when pressure was applied, consistent with studies in skeletal muscle arterioles [25]. In the continued presence of PE/TEA, 100 μM NfA dilated the
arteriole in a pressure-dependent manner, but did not decrease myogenic reactivity. The graph displayed at the lower right of Fig. 2A summarizes the diameter-pressure relationships derived from this experiment. In the presence of NfA, the arteriole strongly autoregulated, displaying a fully relaxed state at 0 mmHg, and a nearly fully contracted state at 80 and 100 mmHg, relative to that measured in the absence of NfA. In the experiment using 10 µM PE (Fig. 2B) myogenic reactivity was apparent for pressure steps above 40 mmHg in control conditions. Incubation of the arteriole with 10 µM PE/1 mM TEA led to a potent contraction at all pressures. Exposure to 100 µM NfA partially reversed the constriction induced by PE/TEA (lower right graph). Fig. 3A shows pooled data in which we tested four concentrations of PE (0.1, 0.5, 1 and 10 µM) in separate series of experiments. At all PE concentrations except 10 µM, NfA was more effective at increasing lumen diameter at low transmural pressures. Its efficacy generally declined as pressure increased; this behavior was most striking with 0.5 µM, where no significant effect of NfA was apparent at 80 and 100 mmHg. These differential effects of NfA are reflected better in panels A–D of Fig. 4 where the percentage of relaxation produced by NfA is plotted as a function of pressure for each concentration of PE studied. Separate series of experiments showed that the contraction remaining following exposure to NfA (in the presence of 1 µM PE/1 mM TEA) could be abolished by 1 µM nifedipine (n = 6; data not shown). Panel E also shows that NfA was progressively less potent at vasodilating the arterioles as the concentration of PE was increased for three selected pressure levels. In the absence of TEA (n = 5; data not shown), NfA completely reversed the contraction induced by PE, resulting in a 'nifedipine-like' response (see Fig. 1E). These results are consistent with the idea that NfA stimulated K, an action that would hyperpolarize the resting membrane potential of the smooth muscle cells to an extent that activation of I, by PE and/or I, is prevented.

3.4. Effects of niflumic acid and DIDS on myogenic and α-adrenoceptor-mediated tone

The inverse pressure-dependent response to NfA with
concentrations of PE in the range of 0.1–1 μM (Fig. 4) may be explained by the declining contribution of $I_{\text{Cl(Ca)}}$, as swelling-induced Cl$^-$ channels ($I_{\text{Cl(swell)}}$) and/or other depolarizing ionic conductances are recruited by pressure-induced stretching of the myocytes. As reported by others in rat small mesenteric arteries [21], nifedipine (1 μM) abolished myogenic tone ($n = 3$; data not shown), consistent with a prerequisite function of $I_{\text{Cl(Ca)}}$ channels in the activation of this process. Since 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), a compound known to inhibit $I_{\text{Cl(swell)}}$ in vascular myocytes [17,19], has been recently shown to inhibit myogenic tone and hyperpolarize cerebral resistance arteries [15], we therefore tested the effects of NfA and DIDS on mesenteric arteries that exhibited spontaneous myogenic tone. In these experiments, 1 mM TEA was also included to counteract the NfA-induced stimulation of $K_{Ca}$. Whereas NfA produced no effect on pressure-induced tone (Fig. 5A), 200 μM DIDS significantly alleviated the constriction apparent at pressures above 20 mmHg (Fig. 5B), an observation.

Fig. 4. Relaxation by NfA is pressure- and [PE]-dependent. The relative relaxation produced by 100 μM NfA (expressed as mean % relaxation relative to the control level) was calculated from the steady-state diameters measured in individual vessels for the series of experiments described in Fig. 3 and plotted as a function of transmural pressure for the four experimental PE concentrations tested in the presence of 1 mM TEA: 0.1 μM (A), 0.5 μM (B), 1 μM (C), and 10 μM (D). Percent relaxation was calculated using Eq. (2) (see Methods). (E) Graph reporting mean % Relaxation by NfA as a function of PE concentration on a logarithmic scale for three selected pressure steps (P: 0, 40 and 80 mmHg) as indicated. For panels A and C, the solid lines are least-squares mono-exponential fits to the mean data points with the error bars as weighing factors. For panels B, D and E, the solid lines are linear regressions to the data points. Except for panel D, all regressions yielded slopes which were significantly different from 0 ($P < 0.01$).
consistent with that made by Nelson et al. [15] in cerebral vessels. Separate experiments revealed that 100 μM NFA or 200 μM DIDS did not affect the contraction induced by 45 mM KCl (with TEA) at 0 or 40 mmHg (n = 6 for both), suggesting that these compounds likely exert little, if any, effect on $I_{Ca(L)}$ channels.

The effects of NFA and DIDS in the same preparation after pre-contraction with 1 μM PE/1 mM TEA were then investigated. Fig. 6A reveals that combined PE/TEA contracted the arterioles at all pressures to a similar extent to experiments described in Figs. 1 and 3. Relative to the passive diameter of these vessels obtained after incubation in Ca$^{2+}$-free medium, 100 μM NFA relaxed the pre-contracted arterioles at all pressures, but with a more
prominent effect at lower pressures. A subsequent exposure to 200 μM DIDS in the presence of PE/TEA/NfA caused a vasorelaxation at all pressures but with greater efficacy for pressures >20 mmHg. Fig. 6B reports the relative relaxation (expressed as percent relaxation) produced by NfA, with and without DIDS. In the absence of DIDS, the percent relaxation mediated by NfA was still pressure-dependent exhibiting a progressive decline as pressure increased. Although the preparations significantly dilated at 0 mmHg in response to DIDS, this compound’s major effect was to eliminate the pressure-dependence of relaxation in the range of 20–100 mmHg. The vasodilation mediated by DIDS was not due to receptor desensitization since the constriction caused by 1 μM PE/1 mM TEA was maintained for over 90 min in separate experiments (n = 3).

4. Discussion

4.1. Major findings

The major goal of this study was to investigate the role of Ca2+-dependent Cl− channels at α1-adrenoceptor-mediated tone in pressurized rabbit mesenteric resistance-sized arteries. To our knowledge, our study is the first to demonstrate that niflumic acid (100 μM), a relatively selective blocker of 12channel in vascular smooth muscle [2], reversed the contraction produced by the α1-agonist phenylephrine (PE) in a manner that depended both on the concentration of the agonist and transmural pressure. For PE concentrations between 0.1 and 1 μM, the potency of NfA at causing relaxation decreased as applied pressure was raised from 0 to 100 mmHg. In contrast, the relaxation exerted by NfA was relatively independent of pressure when the arterioles were exposed to 10 μM PE, a concentration that elicited maximal contraction in our preparation. The NfA-induced relaxation was inversely related to the concentration of PE. While NfA did not influence spontaneous myogenic tone, DIDS (200 μM), another Cl− channel blocker, inhibited myogenic tone in the absence of PE, and partially suppressed a component of contraction at elevated transmural pressures in arterioles incubated in the presence of 1 μM PE/1 mM TEA/100 μM NfA. Our data indicate that at low to moderate stimulation of the α1-adrenergic receptor signaling pathway, 12channel play an important role in the sustained depolarization and contraction induced by these receptors. Their declining relative contribution to membrane potential and tone as transmural pressure or PE concentration is elevated may be explained by a progressive enhancement of stretch-induced ionic conductances, possibly volume-sensitive Cl− channels as recently proposed by one group [17], or by other ionic or contractile mechanisms that are downstream of the α1-adrenoceptor signaling pathway.

4.2. α1-Adrenoceptor-induced contraction and role of Ca2+ channels

It is well known that noradrenaline (NA), an endogenous neurotransmitter released from nerve terminals of most blood vessels, induces vasoconstriction and membrane depolarization by predominantly interacting with α1-adrenergic receptors. Phenylephrine, a full α1-agonist, was used to assess the possible contribution of 12channel on the diameter–pressure relationships induced by variable levels of stimulation of this pathway. We cannot rule out the possibility that shifts in the sensitivity to [Ca2+] may play a role in the response to various concentrations of PE [1,27,28]. However, inhibition by a dihydropyridine of PE-induced tone (Fig. 1) clearly indicates that Ca2+ entry through L-type Ca2+ channels is a prerequisite for the sustained contraction induced by α1-adrenoceptors, a finding consistent with observations made by Wesselman et al. [21] in rat mesenteric small arteries. Similar to another study in rat small arteries [29], the complete suppression of tone by nifedipine also argues against a direct contribution from Ca2+ entry through non-selective cation channels.

4.3. 12Channels participate in α1-adrenoceptor-mediated tone

Phenylephrine may elicit tone by an indirect activation of voltage-dependent Ca2+ channels resulting from the triggering of one or several depolarizing conductances. Among several postulated mechanisms, a possible role for Ca2+-dependent Cl− channels agonist-induced membrane depolarization has recently received support in many vascular beds [2]. Niflumic acid was chosen rather than other Cl− channel blockers because it is considered the most potent and relatively selective inhibitor of 12channel [2]. In single cell studies, 100 μM NfA has been reported to nearly completely block 12channel [23], have no effect on Ca2+ channels [30,31], and cause ~50% block of swelling-induced Cl− channels [19]. This concentration was chosen to achieve near complete block of 12channel [32] and took into account its reduced efficacy at blocking 12channel in the physiological range of membrane potentials [33]. One setback of using NfA is that it also enhances the activity of Kca channels [23]. To alleviate the contribution of Kca channel activation to the NfA-induced relaxation, we relied on TEA to effectively block these channels, an approach also used by Yuan in pulmonary arteries [7]. In vascular smooth muscle, TEA causes a relatively high affinity block of Kca channels with a KIC of 200 μM. At 1 mM, TEA would result in over 95% block of Kca channel activity [24,34]. This agent is known to increase the sensitivity of NA-induced contraction [35]. Consistent with this, TEA produced modest effects on the diameter–pressure curve in the absence of agonist (Fig. 1B) and potentiated the myogenic response in the presence of 100 nM PE (Fig.
We cannot rule out the possibility that NfA might have other non-specific effects on tension, other ionic channels or signal transduction mechanisms associated with α₁-adrenergic receptors. Although additional experiments at the single cell level will be required to fully address these issues, our results suggest that the main action of NfA was probably to exert its vasorelaxing effect by inhibiting I_{Cl(Ca)}: (1) NfA-sensitive I_{Cl(Ca)} channels have been clearly identified in mesenteric arterial smooth muscle cells and suggested to play a major role in the depolarization mediated by hormone receptors coupled to G_{q} and phospholipase C [36,37]; (2) the NfA-induced vasoconstriction is not consistent with a direct inhibitory action on L-typeCa^{2+} channels or contractile mechanisms since NfA had no effect on KCl-induced contractions; (3) the latter observation also lends support to the hypothesis that α₁-adrenoceptor-induced activation of I_{Cl(Ca)} depolarizes the myocytes since 45 mM KCl would be expected to shift membrane potential (−26 mV [38]) near the predicted equilibrium potential for Cl− in smooth muscle (−25 to −35 mV [39]); (4) NfA had no effect on spontaneous myogenic tone which suggests that it did not influence stretch-activated non-selective cation channels or I_{Cl(swell)}, both speculated to participate in the depolarization and contraction associated with this process [8]; (5) the level of tone produced by 0.5 μM PE and 1 mM TEA was not significantly altered at 80 and 100 mmHg; (6) the stimulation of K_{Ca} by norepinephrine is not influenced by NfA [33] suggesting that this agent does not interfere with the α₁-adrenoceptor signaling pathway.

Similar to our study, 10 μM NfA has been shown to block the increase in perfusion pressure to 1 nM NA by 34% in the isolated rat mesenteric vascular bed [6] and the contraction to 1 μM NA by 38% in isolated rat aorta [3]. In pulmonary arteries, 10 and 50 μM NfA decreased the PE (0.5 μM)/TEA (1 mM)-induced contraction by approximately 50 and 86%, respectively [7]. By comparison, we measured an average relaxation of 61, 44, 48, and 38% KCl-induced contraction at 0 or 40 mmHg indicating that approximately 50 and 86%, respectively [7]. By comparison, previously observed, DIDS did not significantly affect myogenic tone which suggests that it did not influence myogenic tone (Fig. 5A), or any depolarizing conductance [12], would tend to diminish the relative contribution of I_{Cl(Ca)} to membrane potential and vascular tone. As similarly reported in cerebral arterioles [15], 200 μM DIDS/1 mM TEA potently inhibited but did not abolish myogenic tone at a concentration that would block I_{Cl(swell)} by ≥80% at −50 mV [19], an effect not shared by NfA (Fig. 5). In rabbit portal vein myocytes, DIDS has been reported to also inhibit I_{Cl(Ca)} albeit with less potency than NfA (IC_{50} > 200 μM [41]). However, this was unlikely to have an impact in our experiments since DIDS was always added after NfA in the experiments with PE (Fig. 6). As previously observed, DIDS did not significantly affect KCl-induced contraction at 0 or 40 mmHg indicating that its dilatory action does not involve a direct inhibition of L-typeCa^{2+} channels. These results are consistent with the reported lack of effect of DIDS on KCl-induced contractions [4,15] and whole-cell Ca^{2+} current [30,31]. However, since a systematic study examining the possible effects of DIDS on stretch-activated non-selective cation channels is presently lacking, interpretation regarding the possible involvement of I_{Cl(swell)} channels must be undertaken with caution.

In arteries pre-contracted with 1 μM PE/1 mM TEA, the application of DIDS in the presence of NfA mainly inhibited the remaining contraction at pressures above 20 mmHg (Fig. 6A) resulting in flattening of the percent relaxation–pressure relationship (Fig. 6B). The DIDS-induced removal of the pressure-dependence of relaxation exerted by NfA, combined with the fact that the latter had no influence myogenic tone (Fig. 5A), or the constriction elicited by 0.5 μM PE/1 mM TEA at 80 and 100 mmHg
(Fig. 3B), support the following hypotheses: (1) swelling-induced Cl\(^-\) channels (and/or possibly a DIDS-sensitive stretch-activated non-selective cation channel) play an important role in the depolarization that triggers the myogenic response in mesenteric arterioles; (2) this \(I_{\text{Cl(swell)}}\)-induced depolarization persists during stimulation of \(\alpha_1\)-adrenoceptors and accounts, at least in part, for the reduced participation of \(I_{\text{Cl(Ca)}}\) to overall membrane conductance as transmural pressure increases.

Taken together, our data strengthen further the concept that activation of distinct types of chloride channels may represent a key determinant of the electromechanical properties of rabbit mesenteric arterioles submitted to physiological transmural pressures during sympathetic stimulation. However, other mechanisms likely participate in driving or modulating tone during stimulation of this pathway. Consistent with this was the observation that the efficacy of NFA to induce relaxation decreased as the concentration of phenylephrine increased from 0.1 to 10 \(\mu\text{M}\). Moreover, with 10 \(\mu\text{M}\) PE, the relative relaxation produced by NFA did not vary with pressure, measuring between 27 and 37\% in the range of 0 to 100 mmHg (Fig. 5D). Whether the lack of pressure-dependence and reduced contribution of \(I_{\text{Cl(Ca)}}\) to \(\alpha_1\)-adrenoceptor-induced contraction at moderate to saturating agonist concentrations (via stimulation of \(G_q, PLC\) and/or PKC) involves: (1) a shift in the sensitivity of the contractile apparatus to intracellular \(\text{Ca}^{2+}\) concentrations [1,27,28], (2) a direct stimulation [40] or stretch-induced modulation of \(\text{Ca}^{2+}\) channels [10,13], (3) inhibition of \(I_{\text{Cl(swell)}}\) [42], (4) activation of non-selective cation channels [12,43], or (5) block of other \(K^+\) channels [44,45], will necessitate further experiments.

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[14] Wesselman JPM, Schubert R, VanBavel E, Nilsson H, Mulvany M. Moreover, with \(10^{-5}\) \(\mu\text{M}\) PE, the relative relaxation produced by NFA did not vary with pressure, measuring between 27 and 37\% in the range of 0 to 100 mmHg (Fig. 5D). Whether the lack of pressure-dependence and reduced contribution of \(I_{\text{Cl(Ca)}}\) to \(\alpha_1\)-adrenoceptor-induced contraction at moderate to saturating agonist concentrations (via stimulation of \(G_q, PLC\) and/or PKC) involves: (1) a shift in the sensitivity of the contractile apparatus to intracellular \(\text{Ca}^{2+}\) concentrations [1,27,28], (2) a direct stimulation [40] or stretch-induced modulation of \(\text{Ca}^{2+}\) channels [10,13], (3) inhibition of \(I_{\text{Cl(swell)}}\) [42], (4) activation of non-selective cation channels [12,43], or (5) block of other \(K^+\) channels [44,45], will necessitate further experiments.


