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2-Aminoethoxydiphenyl borate inhibits KCl-induced vascular smooth muscle contraction

Paul H. Ratz *, Krystina M. Berg

Departments of Biochemistry and Pediatrics, Virginia Commonwealth University, School of Medicine, 1101 East Marshall Street, PO Box 980614, Richmond, VA 23298-0614, USA

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Abstract

K^+-depolarization (KCl)-activated Ca^{2+} entry permitting sustained force-maintenance in tonic vascular smooth muscle has long been attributed solely to activation of L-type voltage-operated Ca^{2+} channels (VOCs). We used the transient receptor potential channel (TRP) blocker, 2-aminoethoxydiphenyl borate (2-APB), to test the hypothesis that KCl activates additional Ca^{2+} entry pathways. 2-APB alone caused a transient weak *increase* in force, a sustained weak *increase* in basal $[Ca^{2+}]_i$ and myosin light chain phosphorylation, and inhibition of KCl-induced force, $[Ca^{2+}]_i$ and myosin light chain phosphorylation. 2-APB did not appear to block VOCs, because 2-APB did not inhibit 30 nM Bay k 8644-induced increases in $[Ca^{2+}]_i$. Moreover, although 1 μM nifedipine abolished the increase in $[Ca^{2+}]_i$ produced by α-adrenergic receptor activation, 2-APB produced an additional reduction in $[Ca^{2+}]_i$ below the basal level. These data support the conclusion that membrane depolarization activates 2-APB-sensitive TRPs in addition to VOCs to permit strong force-maintenance in tonic vascular smooth muscle.

Keywords: 2-APB; Signal transduction; Cell signaling; Calcium channel blocker; Fura-2; Myosin light chain phosphorylation; Contraction

1. Introduction

K^+-depolarization (KCl) causes smooth muscle contraction by activation of L-type voltage-operated Ca^{2+} channels (VOCs) leading to increases in $[Ca^{2+}]_i$ ((Ganitkevich and Isenberg, 1991; Imaizumi et al., 1989; Morgan and Morgan, 1982) and reviewed by (Bolton, 1979; Van Breemen et al., 1978)), activation of Ca^{2+}-calmodulin-dependent myosin light chain kinase (reviewed by (Kamm and Stull, 1985)) and elevations in the degree of myosin light chain phosphorylation ((Himpens and Somlyo, 1988; Ratz and Murphy, 1987) and reviewed by (Murphy, 1988)). Acceptance of this model is due in large measure to the ability of highly selective organic Ca^{2+} channel blockers, such as dihydropyridines, to abolish KCl-induced tonic increases in $[Ca^{2+}]_i$, myosin light chain phosphorylation and force ((Meisheri et al., 1981; Moreland and Moreland, 1987; Peiper et al., 1971; Takuwa et al., 1987) and reviewed by (Godfraind et al., 1986; Karaki et al., 1997)). Stimuli that activate G protein-coupled receptors often cause increases in Ca^{2+} sensitivity of contractile proteins through activation of rhoA kinase and protein kinase C that inhibit myosin light chain phosphatase activity. Until recently, Ca^{2+} sensitization was thought to be activated only by G protein-coupled receptor agonists, and not KCl. However, studies from several laboratories show that KCl can also cause increases in Ca^{2+} sensitization (reviewed by (Ratz et al., 2005)) by stimulation of ROK activity that is inhibitable by nifedipine (Urban et al., 2003).

There is strong evidence that KCl can also cause release of intracellular Ca^{2+} by direct depolarization of the sarcoplasmic reticulum or by causing Ca^{2+}-induced Ca^{2+} release (Kobayashi et al., 1985, 1986). Depletion of sarcoplasmic reticulum Ca^{2+} either by activation of inositol 1,4,5-trisphosphate receptors or ryanodine receptors can cause activation of store-operated cation channels (SOCs) (Ay et al., 2004; Fellner and Arendshorst, 2000; Usachev and Thayer, 1999; Wayman et al., 1998), but whether SOCs contribute to KCl-induced maintenance of tonic increases in $[Ca^{2+}]_i$, myosin light chain phosphorylation and force in smooth muscle, remains to be determined. This question may not yet have been addressed because dihydropyridine Ca^{2+} channel

* Corresponding author.
E-mail address: phratz@vcu.edu (P.H. Ratz).

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blockers and related agents that inhibit KCl-induced Ca^{2+} entry and force in smooth muscle have long been considered highly selective L-type VOC blockers (Bean et al., 1986; Bolger et al., 1982). However, recent data suggests that dihydropyridine Ca^{2+} channel blockers may be highly potent inhibitors of certain SOCs (Curtis and Scholfield, 2001; Krutetskaia et al., 1997; Willmott et al., 1996). Moreover, TRP channels are responsible for store-operated Ca^{2+} entry ((Zagranichnaya et al., 2005) and reviewed by (Albert and Large, 2003)), and because there is evidence for sequence similarity between TRP channels and the α_1 subunit of L-type VOCs (Phillips et al., 1992), it is conceivable that any stimulus that causes membrane depolarization leading to sarcoplasmic reticulum Ca^{2+} store depletion may also cause sustained smooth muscle contraction, in part, by activation of nifedipine-sensitive TRPs. Thus, a fundamental question that remains to be addressed is whether KCl can cause maintenance of arterial smooth muscle contractions, in part, by activation of TRPs.

In this study, we examined the effects of 2-APB, a TRP channel blocker, on KCl-induced increases in $[Ca^{2+}]_i$, myosin light chain phosphorylation and force in isolated rings of rabbit femoral artery. 2-APB was originally reported in 1997 to be a selective inositol 1,4,5-trisphosphate receptor inhibitor (Maruyama et al., 1997), although subsequent data suggest that this effect is somewhat variable (reviewed by (Bootman et al., 2002)). Recent studies provide compelling evidence that a primary action of 2-APB is to inhibit influx of Ca^{2+} through SOCs independently of the function of inositol 1,4,5-trisphosphate receptors ((Broad et al., 2001; Dobrydneva and Blackmore, 2001; Gregory et al., 2001; Iwasaki et al., 2001) and reviewed by (Bootman et al., 2002)). Recently, 2-APB has also been shown to directly inhibit TRP channels (Ma et al., 2000; Poburko et al., 2004; Trebak et al., 2002; van Rossum et al., 2000; Xu et al., 2005), however the role of TRP channels in KCl-induced contractions has not been examined. KCl has long been used as a stimulus that bypasses G protein-coupled receptors. As such, KCl provides a unique tool to examine regulation of certain cell signaling systems downstream of G protein-coupled receptor activation directly related to mobilization of cellular Ca^{2+}. By measuring the inhibitory activity of 2-APB on force, $[Ca^{2+}]_i$ and myosin light chain phosphorylation, we tested the hypothesis that KCl-induced tonic force-maintenance is due, in part, to activation of TRPs.

2. Materials and methods

2.1. Tissue preparation

All experiments involving animals were conducted within the appropriate animal welfare regulations and guidelines and were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee. Tissues were prepared as described previously (Ratz, 1993). Femoral and renal arteries from adult female New Zealand White rabbits were cleaned of adhering tissue and stored in cold (0–4 °C) physiological saline solution: PSS, composition in mM: NaCl, 140; KCl, 4.7; $MgSO_4$, 1.2; $CaCl_2$, 1.6; Na_2HPO_4, 1.2; morpholino-propanesulfonic acid, 2.0 (adjusted to pH 7.4 at either 0 °C or 37 °C, as appropriate); Na_2ethylenediamine tetraacetic acid (EDTA; to chelate trace heavy metals), 0.02; and D-glucose, 5.6. High purity (greater than 17 MΩ distilled and deionized) water was used throughout. The endothelium of each artery was removed by gently rubbing the intimal surface with a metal rod, and tissues were cut into artery rings ~3–4 mm wide. Each muscle ring was secured between a micrometer for length adjustments and isometric force transducer (model 52, Harvard Apparatus, South Natick, MA) in a water-jacketed muscle chamber (Radnoti Glass Technology, Inc., Monrovia, CA). In one set of force experiments, strips of rabbit urinary bladder (detrusor) free from underlying urothelium were isolated as described previously (Shenfeld et al., 1998), cleaned of overlying fat and serosal connective tissues, and secured between tissue clips for force measurements.

Isometric contractions were measured as described previously (Ratz, 1993). Voltage signals from force transducers were digitized (CIO-DAS16F, ComputerBoards, Middleboro, MA), visualized on a computer screen as force (g), and stored by software command to a hard disk for later analyses. All data analyses were accomplished using DasyLab (DasyTec, Amherst, NH) and an electronic spread sheet.

2.2. Isometric force

Contractile force (F) was measured as described previously (Ratz, 1993). Tissues were allowed to equilibrate at 37 °C for 1 h, and the muscle length at which active force was maximum (L_o) was then determined for each tissue with K^+ as agonist (110 mM KCl substituted isosmotically for NaCl) using an abbreviated length-tension curve (Herlihy and Murphy, 1973; Ratz, 1993). Once tissues were stretched to L_o, no further length changes were imposed. For each pre-loaded tissue, the degree of steady-state F produced at L_o by incubation for 5–10 min in 110 mM KCl was equal to the optimal force for muscle contraction (F_o), and subsequent contractions were calculated as F/F_o. Arteries contracted with KCl were incubated with 1 μM phentolamine to block potential α-adrenergic receptor activation caused by release of norepinephrine from peri-arterial nerves. Detrusor strips contracted with KCl were incubated with 1 μM atropine to block potential muscarinic receptor activation by release of acetylcholine. In experiments designed to activate α-adrenergic receptors, 10 μM phenylephrine was used because this concentration produces a strong, sustained contraction in rabbit arteries (Ratz, 1993).

2.3. Myosin light chain phosphorylation

The degree of myosin light chain phosphorylation was measured as described previously (Ratz, 1993; Ratz and Murphy, 1987). Arteries were quick-frozen in an acetone-dry ice slurry, slowly warmed to room temperature, dried, weighed and homogenized on ice in 8 M urea, 2% Triton X-100 and 20 mM dithiothreitol. Isoelectric variants of the 20 kDa myosin light chains were separated by 2-D (isoelectric focusing/sodium dodecyl-sulfate) polyacrylamide gel electrophoresis followed by Western Blot, visualized by colloidal gold stain, and the relative amounts of phosphorylated and non-phosphorylated myosin light chains were quantified by digital image analysis.

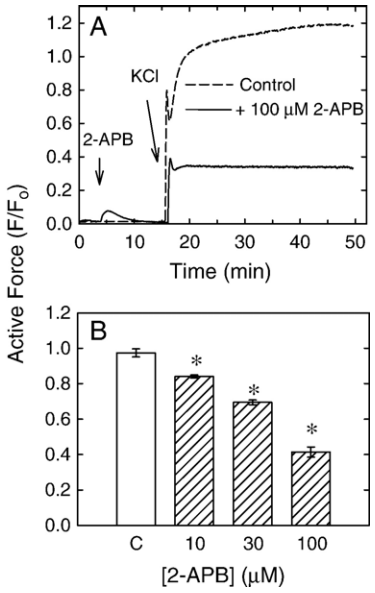

Fig. 1. Typical force tracings (A) of KCl-induced contractions in the absence ("Control") and presence ("+2-APB") of 100 μM 2-APB. Notice that addition of 100 μM 2-APB produced a weak, transient contraction. Summary of tonic data (B) for control ("C" in panel B) and 10, 30 and 100 μM 2-APB. Data in panel B are mean±S.E.M.; $n=4$; *$P<0.05$ compared to control.

2.4. Intracellular free calcium ($[Ca^{2+}]_i$) measurement

$[Ca^{2+}]_i$ was measured as described previously with minor modifications (Ratz, 1993). Arterial rings were secured in a temperature-controlled myograph suitable for simultaneous measurement of force and fluorescence (Danish Myo Technology, Denmark) which was positioned on the stage of an Olympus IX71 inverted microscope with attachments to a Deltaram V fluorometer and photomultiplier tube (Photon Technology International, Lawrenceville, NJ). Tissues were maintained at 37 °C and loaded for 2.5 h with the 7.5 μM of the Ca^{2+} indicator, Fura-2(PE3)/AM (Teflabs, Austin, TX) with 0.01% Pluronic F-127 added to enhance solubility. Tissues were washed for 30 min prior to the start of the experiment to remove extracellular Fura-2(PE3)/AM. Fluorescence emissions at 510 nm were collected by a photomultiplier tube in response to alternate excitation at 340 and 380 nm with a 75-W xenon lamp (Photon Technologies International). At the end of the experiment, background tissue fluorescence was recorded by adding 4 mM $MnCl_2$ plus 10 μM ionomycin to quench the fura-2 signal. $[Ca^{2+}]_i$ was presented as the fluorescence ratio (F_{340}/F_{380}) corrected for background fluorescence. To reduce tissue-to-tissue variability for statistical comparisons, $[Ca^{2+}]_i$ for each tissue was reported as the fraction of that produced at 2 min of a KCl stimulus and the minimum value produced during exposure of tissues to a Ca^{2+}-free solution containing 1 mM ethylene glycol bis-(2-aminoethyl ether)-*N*,*N*,*N*′*N*′-tetraacetic acid (EGTA) plus 10 μM ionomycin.

2.5. Drugs

2-APB was from Aldrich Chemical Co., Inc, and all other compounds were from Sigma Corporation. 2-APB, nifedipine and (−)-1,4-dihydro-2,6-dimethyl-5-nitro-4-[2′-(trifluoromethyl)phenyl]-3-pyridinecarboxylic acid methyl ester (Bay k 8644) were dissolved in ethanol, which was added at final concentration no greater than 0.1% that had no effect on stimulus-induced responses. Tissues were first exposed to 5 mM KCl prior to addition of Bay k 8644 to produce slight membrane depolarization and enhance the rate at which Bay k 8644, which increases the open state probability of L-type VOCs, causes contraction. Addition of 5 mM KCl alone did not produce an increase in $[Ca^{2+}]_i$ or force.

2.6. Statistics

The null hypothesis was examined using Student's *t*-test (when 2 groups were compared) or using a one-way Analysis of Variance (ANOVA). To determine differences between groups following ANOVA, the Student–Neuman–Keuls post-hoc test was used. In all cases, the null hypothesis was rejected at $P<0.05$. For each study described, the *n* value was equal to the number of rabbits from which arteries were obtained.

3. Results

3.1. Effect of 2-APB on peak and tonic KCl-induced force

KCl produced a biphasic contraction consisting of a rapid increase, then decrease in force yielding an early peak response that was immediately followed by a slower increase in force producing a sustained, tonic response (Fig. 1A, Control). 2-APB (100 μM) produced a strong inhibition of both peak and

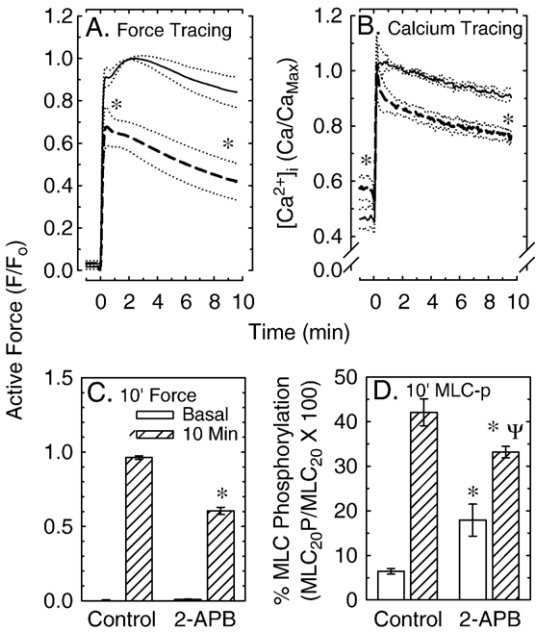

Fig. 2. Mean (solid and dashed lines)±S.E.M. (dotted lines) of force (A) and $[Ca^{2+}]_i$ (B) tracings, and mean±S.E.M. of force (C) and myosin light chain phosphorylation (D) produced by tissues stimulated by KCl in the absence (solid lines in A and B and "Control" in C and D) and presence (dashed lines in A and B and "2-APB" in C and D) of 30 μM 2-APB; $n=7$; *$P<0.05$ compared to control, $^{\Psi}P<0.05$ compared to basal 2-APB.

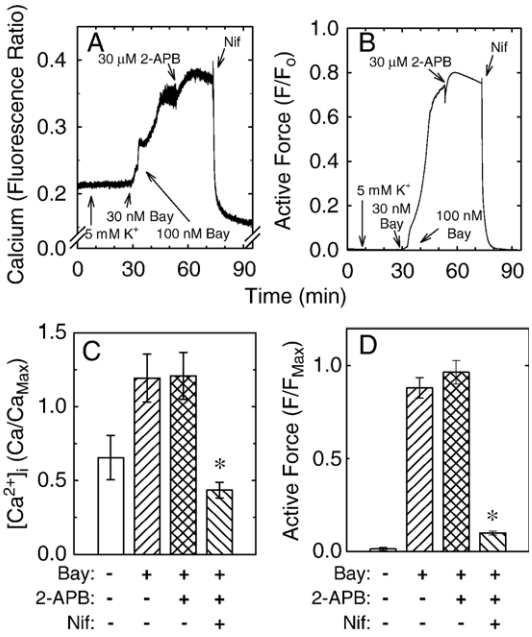

Fig. 3. Typical $[Ca^{2+}]_i$ (A) and force (B) tracings, and summary $[Ca^{2+}]_i$ (C) and force (D) data for tissues contracted with the L-type VOC activator, Bay k 8644 ("Bay") followed by exposure to 30 μM 2-APB and 1 μM nifedipine ("Nif"). Data in panels C and D are mean±S.E.M. taken at ~ 30 min of stimulation with Bay k 8644, 15 min of stimulation with 2-APB, and 15 min of stimulation with nifedipine; $n=3$; $^*P<0.05$ compared to effect of Bay k 8644.

tonic force (Fig. 1A). Moreover, inhibition of force by 2-APB was concentration-dependent (Fig. 1B for tonic force). Inhibition of peak force as a percentage of the control response was similar to inhibition of tonic force (peak F/F_o values were 0.71±0.03, 0.63±0.02, 0.58±0.02 and 0.38±0.01 for, respectively, control, and 10 μM, 30 μM and 100 μM 2-APB). Thus, 2-APB at a concentration as low as 10 μM significantly reduced peak and tonic KCl-induced force by 11–14% in rabbit artery. Interestingly, when added at 100 μM, 2-APB alone produced a weak, transient contraction peaking at ~10% of the force produced by KCl, and lasting ~5 min before declining back to the basal level (see force tracing immediately after addition of 2-APB in Fig. 1A). This weak contraction to 2-APB was absent when tissues were incubated in a Ca^{2+}-free solution. Inhibition of KCl-induced force was not tissue-specific because 30 μM 2-APB also inhibited both peak and tonic contractions of rabbit detrusor, a phasic smooth muscle (data not shown).

3.2. Effect of 2-APB on KCl-induced increases in $[Ca^{2+}]_i$ and myosin light chain phosphorylation

To determine whether inhibition of KCl-induced contraction by 2-APB was due to a reduction in $[Ca^{2+}]_i$, fura-2-loaded tissues were incubated for 15 min with 30 μM 2-APB and contracted with KCl for 10 min. Interestingly, 30 μM 2-APB alone caused a small but significant *increase* in basal $[Ca^{2+}]_i$ (Fig. 2B, ∗ before time zero) without significantly increasing force (Fig. 2A). 2-APB caused a reduction in KCl-induced tonic increases in force (Fig. 2A, ∗ at 9 min) and $[Ca^{2+}]_i$ (Fig. 2B, ∗ at 9 min). 2-APB also inhibited the early peak increase in force (Fig. 2A, ∗ at 1 min) but not the early peak $[Ca^{2+}]_i$ (Fig. 2B) produced by KCl.

The primary mechanism responsible for contraction directly downstream from increases in $[Ca^{2+}]_i$ is myosin light chain phosphorylation. 2-APB (30 μM) significantly inhibited KCl-induced tonic (10 min) increases in force (Fig. 2C) and myosin light chain phosphorylation (Fig. 2D). These data together suggest that 2-APB reduced KCl-induced tonic contraction by inhibiting KCl-induced increases in $[Ca^{2+}]_i$ and myosin light chain phosphorylation. However, 2-APB alone caused an increase in basal $[Ca^{2+}]_i$ (Fig. 2B) and myosin light chain phosphorylation (Fig. 2D) without a concomitant sustained increase in basal force (Fig. 2A and C). These data together suggest that 2-APB can partially uncouple myosin light chain phosphorylation from force.

3.3. Effect of 2-APB and nifedipine on $[Ca^{2+}]_i$ and force produced by the dihydropyridine Ca^{2+} channel agonist, Bay k 8644

To determine whether 2-APB could inhibit increases in $[Ca^{2+}]_i$ and force produced by a sub-maximum concentration of the selective L-type VOC activator, Bay k 8644, tissues were contracted with 100 nM Bay k 8644 and then exposed to 30 μM 2-APB followed by 1 μM nifedipine. Rather than causing inhibition of $[Ca^{2+}]_i$ and relaxation, 2-APB produced a small

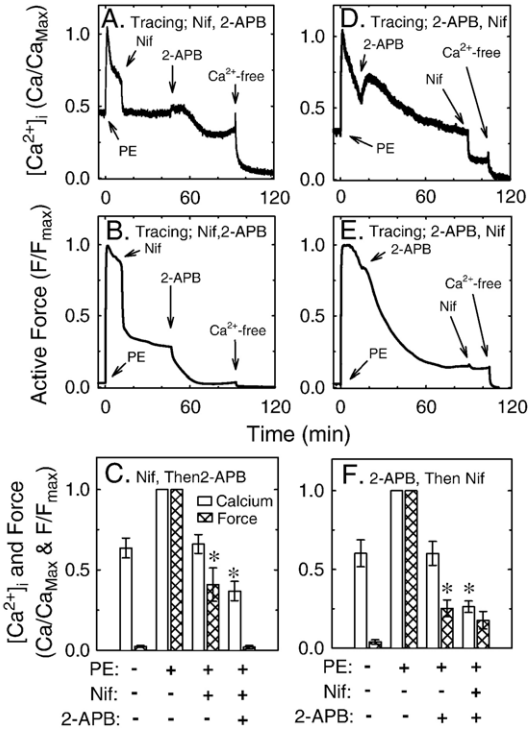

Fig. 4. Typical $[Ca^{2+}]_i$ (A and D) and force (B and E) tracings with summary bar graphs (C and F) for tissues contracted with 10 μM phenylephrine ("PE") followed by inhibition with 1 μM nifedipine ("Nif") and 30 μM 2-APB to test for additivity of inhibition. Panels A–C show responses when nifedipine was added first followed by addition of 2-APB, while panels D–F show responses when 2-APB was added before nifedipine. At the end of the experiment, tissues were exposed to a Ca^{2+}-free solution containing 1 mM EGTA and 10–20 μM ionomycin, which further reduced $[Ca^{2+}]$ to a minimum level. Data in panel C and F are mean±S.E.M. of steady-state data normalized to the peak phenylephrine-induced responses, $n=3$; * $P<0.05$ compared to basal values.

and transient increase in $[Ca^{2+}]_i$ (Fig. 3A) and force (Fig. 3B) when added to tissues contracted by 100 nM Bay k 8644, but within 15 min, $[Ca^{2+}]_i$ and force were not different than control (Fig. 3C and D). Nifedipine successfully competed with Bay k 8644 to reduce $[Ca^{2+}]_i$ to a level significantly below the basal level (Fig. 3A and C) and cause tissue relaxation (Fig. 3B and D). These data support the hypothesis that 2-APB does not inhibit L-type VOCs.

3.4. Effect of 2-APB and nifedipine on $[Ca^{2+}]_i$ and force produced by the G protein-coupled receptor agonist, phenylephrine

To determine whether 2-APB could inhibit phenylephrine-induced increases in $[Ca^{2+}]_i$ and force independently of inhibition of VOCs, tissues were contracted with phenylephrine, and at steady-state (~10 min), relaxed by addition of nifedipine at a concentration known to maximally inhibit L-type VOCs (1 μM), followed by addition of 2-APB, and by addition of 2-APB followed by addition of nifedipine. Nifedipine alone abolished the increase in $[Ca^{2+}]_i$ induced by phenylephrine, but 2-APB additionally reduced $[Ca^{2+}]_i$ to a level below the basal level. Likewise, nifedipine reduced $[Ca^{2+}]_i$ below the basal level after 2-APB abolished the phenylephrine-induced increase. Thus, these data support the contention that 2-APB does not inhibit VOCs, because the VOC-blocker, nifedipine, acted synergistically with 2-APB to reduce $[Ca^{2+}]_i$, suggesting that 2-APB and nifedipine inhibited different Ca^{2+} channels produced during the sustained-phase of a phenylephrine-induced contraction (Fig. 4).

4. Discussion

Ca^{2+} causes smooth muscle contraction by activation of myosin light chain kinase resulting in increases in myosin light chain phosphorylation, the "switch" responsible for "turning on" crossbridge cycling leading to increases in force (reviewed by (Kamm and Stull, 1985)). In the present study, concomitant with reductions in stimulus-induced increases in $[Ca^{2+}]_i$, 2-APB, a TRP channel blocker (Xu et al., 2005), partially inhibited KCl-induced increases in myosin light chain phosphorylation and force. Thus, most of the relaxant effect of 2-APB on KCl-induced contraction can be attributed to inhibition of stimulus-induced increases in $[Ca^{2+}]_i$. However, a portion of the relaxant effect of 2-APB may also have been mediated by an action downstream from myosin light chain phosphorylation. With the addition of 2-APB, force became temporally dissociated from Ca^{2+} and myosin light chain phosphorylation as demonstrated by the weak transient increase in force in the face of sustained increases in $[Ca^{2+}]_i$ and myosin light chain phosphorylation. This finding is consistent with results showing that 2-APB inhibits myogenic contractions in rat cremaster arteriole independently of reductions in $[Ca^{2+}]_i$ (Potocnik and Hill, 2001), but the specific cell system downstream from myosin light chain phosphorylation affected by 2-APB was not identified in this study. This finding is also consistent with recent data showing that 2-APB can *activate* Ca^{2+} entry through certain TRPV isoforms (Hu et al., 2004), and therefore support the hypothesis that TRPVs are functionally responsive in vascular smooth muscle (Jia et al., 2004; Rousseau et al., 2005).

The present study was designed to test the hypothesis that KCl may cause contraction through activation of 2-APB-sensitive TRPs. This hypothesis was based on the fact that KCl can cause sarcoplasmic reticulum Ca^{2+} depletion (Kobayashi et al., 1985, 1986), and sarcoplasmic reticulum Ca^{2+} depletion should theoretically lead to Ca^{2+} entry through 2-APB-sensitive SOCs (Ay et al., 2004; Fellner and Arendshorst, 2000; Usachev and Thayer, 1999; Wayman et al., 1998). 2-APB was initially thought to be selective for inhibition of inositol 1,4,5-trisphosphate receptors, and thus, for Ca^{2+} release from intracellular stores (Maruyama et al., 1997). However, the current and generally accepted view (reviewed by (Bootman et al., 2002)) is that 2-APB more potently inhibits Ca^{2+} entry through SOCs, and does not inhibit L-type VOCs (Luo et al., 2001; Poteser et al., 2003) ryanodine receptors (Maruyama et al., 1997; Wu et al., 2000), arachidonic acid-activated cation channels (Luo et al., 2001; Mignen et al., 2003), *S*-nitrosylation-activated channels (van Rossum et al., 2000) and Ca^{2+}-activated Cl^- entry channels (Wu et al., 2000). Considerable evidence now supports the concept that TRP-dependent cation channels are the molecular entities responsible for SOC and receptor-operated Ca^{2+} channels in smooth muscle (Beech et al., 2004; Thebault et al., 2005; Zagranichnaya et al., 2005). Thus, 2-APB may be considered a TRP inhibitor. Membrane depolarization by KCl has long been known to activate Ca^{2+} entry through L-type VOCs to cause smooth muscle contraction (Briggs, 1962; Nelson et al., 1988). Because 2-APB can inhibit KCl-induced increases in $[Ca^{2+}]_i$, 2-APB is either an effective inhibitor of L-type VOCs, or, in addition to activating 2-APB-insensitive L-type VOCs, KCl also activates another 2-APB-sensitive Ca^{2+} entry pathway. Our data support the latter possibility.

KCl-induced increases in $[Ca^{2+}]_i$ and force can be abolished by nifedipine and related Ca^{2+} channel blockers, and these data provide strong support for the hypothesis that L-type VOCs are wholly responsible for maintenance of KCl-induced Ca^{2+} entry and contraction. However, recent data suggest that certain SOCs may be as sensitive to inhibition by nifedipine and related compounds as are L-type VOCs (Curtis and Scholfield, 2001; Krutetskaia et al., 1997; Willmott et al., 1996). Our data shows that an elevation in $[Ca^{2+}]_i$ and force produced by the selective activation of L-type VOCs by 100 nM Bay k 8644 was not inhibited by 30 μM 2-APB, a concentration that strongly inhibited KCl-induced increases in $[Ca^{2+}]_i$ and force. These data indicate that part of a KCl-induced contraction was most likely produced by Ca^{2+} entry through a channel type distinct from L-type VOCs.

Contractions of vascular smooth muscle are mediated by Ca^{2+} entry through receptor-operated Ca^{2+} channels as well as through VOCs and SOCs, and evidence is emerging that ROCs and SOCs are related and are likely constructs of TRP isomers ((Thebault et al., 2005; Zagranichnaya et al., 2005) and reviewed by (McFadzean and Gibson, 2002)). Data from the present study indicate that 2-APB inhibited increases in $[Ca^{2+}]_i$ induced by G protein-coupled receptor activation in the presence of sufficient nifedipine to abolish VOC activity, suggesting that 2-APB inhibited TRP channels characterized phenomenologically as ROCs

and/or SOCs. But perhaps more importantly, our data showed that, in G protein-coupled receptor-activated tissues, nifedipine could still reduce $[Ca^{2+}]_i$ in the presence of 2-APB, which support our hypothesis that 2-APB did not substantially inhibit VOCs in rabbit tonic vascular smooth muscle.

In conclusion, our data support the hypothesis that KCl-induced contraction is due, in part, to Ca^{2+} entry through one or more of the TRP cation channel isoforms. We propose that nifedipine inhibited both channel types activated by KCl, resulting in complete inhibition of KCl-induced contraction, while 2-APB inhibited only TRPs, resulting in partial inhibition of contraction.

Acknowledgments

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