Altered calcium sensitivity contributes to enhanced contractility of collateral-dependent coronary arteries

Cristine L. Heaps[1], **Janet L. Parker**[4], **Michael Sturek**[3], and **Douglas K. Bowles**[1,2]

[1]Department of Biomedical Sciences, University of Missouri, Columbia, Missouri 65211

[2]Dalton Cardiovascular Research Center, University of Missouri, Columbia, Missouri 65211

[3]Departments of Medical Pharmacology & Physiology and of Internal Medicine, Columbia, Missouri 65211

[4]Department of Medical Physiology, The Texas A&M University System Health Sciences Center, College Station, TX 77843

Abstract

Coronary arteries distal to chronic occlusion exhibit enhanced vasoconstriction and impaired relaxation when compared with nonoccluded arteries. In the present study, we tested the hypotheses that an increase in peak Ca^{2+} channel current density and/or increased Ca^{2+} sensitivity contributes to altered contractility in collateral-dependent coronary arteries. Ameroid occluders were surgically placed around the proximal left circumflex coronary artery (LCX) of female miniature swine. Segments of epicardial arteries (~1 mm luminal diameter) were isolated from the LCX and nonoccluded left anterior descending (LAD) arteries 24 wks following Ameroid placement. Contractile responses to depolarization (10-100 mM KCl) were significantly enhanced in LCX compared with size-matched LAD arterial rings (EC$_{50}$; LAD=41.7±2.3, LCX=34.3±2.7 mM). However, peak Ca^{2+} channel current was not altered in isolated smooth muscle cells from the LCX compared with LAD (–5.29±0.42 vs. –5.68±0.55 pA/pF, respectively). Furthermore, while half-maximal activation of Ca^{2+} channel current occurred at nearly the same membrane potential in LAD and LCX, half-maximal inactivation was shifted to a more positive membrane potential in LCX cells. Simultaneous measures of contractile tension and intracellular free Ca^{2+} (fura-2) levels in arterial rings revealed that significantly more tension was produced per unit change in fura-2 ratio in the LCX compared with the LAD in response to KCl-induced membrane depolarization, but not during receptor-agonist stimulation with endothelin-1. Taken together, our data indicate that coronary arteries distal to chronic occlusion display increased Ca^{2+} sensitivity in response to high KCl-induced membrane-depolarization, independent of changes in whole-cell peak Ca^{2+} channel current. Unaltered Ca^{2+} sensitivity in endothelin-stimulated arterial rings suggests more than one mechanism regulates Ca^{2+} sensitization in coronary smooth muscle.

Introduction

Collateral-dependent coronary arteries exhibit enhanced constriction and impaired relaxation responses when compared with control, nonoccluded arteries (7,8,13,20,22,28-32). These alterations in vasomotor responsiveness may have important implications in the regulation of blood flow to collateral-dependent myocardium (29). Indeed, episodes of myocardial ischemia in patients with chronic stable angina have been attributed to constriction of arteries distal to the site of stenosis (21). Previous studies have reported that the altered

Address correspondence to: Cristine L. Heaps, Ph.D., E102 Veterinary Biomedical Sciences, University of Missouri, Columbia, MO 65211, Telephone (573) 882-7121, FAX (573) 884-6890, heapsc@missouri.edu.

reactivity of coronary vasculature distal to chronic occlusion results from changes in both endothelial and smooth muscle function (7,8,13,20,22,28-32). Furthermore, alterations in vasomotor responsiveness of endothelium-denuded, collateral-dependent arterial rings are closely associated with altered smooth muscle intracellular free Ca^{2+} levels (13,22). The purpose of this study was to examine potential mechanisms related to smooth muscle function that may be responsible for the increased contractility observed in the collateral-dependent vasculature. We hypothesized that an increased peak Ca^{2+} channel current density and/or increased Ca^{2+} sensitivity in arterial smooth muscle distal to chronic occlusion would contribute to enhanced contractile activity of the collateral-dependent vasculature. We compared the Ca^{2+} sensitivity of collateral-dependent and nonoccluded coronary arteries by measuring simultaneous changes in contractile tension and intracellular free Ca^{2+} concentration (Ca_i) in response to high-KCl membrane depolarization and receptor-agonist stimulation with endothelin-1. Our results indicate that coronary arteries distal to chronic occlusion display increased Ca^{2+} sensitivity in response to high KCl-induced membrane-depolarization, independent of changes in whole-cell peak Ca^{2+} channel current. Unaltered Ca^{2+} sensitivity in endothelin-stimulated arterial rings suggests more than one mechanism regulates Ca^{2+} sensitization in coronary smooth muscle.

Methods

Experimental animals and surgical procedures

Animal protocols were approved by the University of Missouri Animal Care and Use Committee in accordance with the "Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training". Adult female Yucatan miniature swine (Charles River, Wilmington, MA). were surgically instrumented with Ameroid constrictors around the proximal left circumflex coronary (LCX) artery as described previously (7,13). Animals were preanesthetized with glycopyrrolate (0.004 mg·kg^{-1}, im) and midazolam (0.5 mg·kg^{-1}, im). Anesthesia was induced with ketamine (20 mg·kg^{-1}, im) and maintained with 3% isoflurane and 97% O_2 throughout aseptic surgery.

Preparation of coronary arteries

Twenty-four weeks following Ameroid placement, the animals were anesthetized with ketamine (30 mg·kg^{-1}) and pentobarbital sodium (35 mg·kg^{-1}). The hearts were removed and placed in ice cold Krebs bicarbonate buffer (0-4 °C) for isolation of the occluded LCX and the nonoccluded left anterior descending (LAD) coronary arteries (13). Visual inspection at the Ameroid occluder during dissection of the LCX artery indicated 100% occlusion in all animals used in this study.

With the aid of a dissection microscope, segments of the LCX and LAD arteries were trimmed of fat and connective tissue, cut into rings and measured with a calibrated Filar micrometer eyepiece (Hitschfel Instruments, St. Louis, MO) in a relaxed, nonpressurized state. Arterial rings (axial length 3.5-4.0 mm, luminal diameter ~1 mm) used for evaluation of concentration-response relationships were prepared in Krebs buffer. Additional arterial rings (axial length ~1.0 mm, luminal diameter ~1 mm) used for fura-2 experiments (fura-2/ acetoxymethyl ester; Molecular Probes, Eugene, OR) were denuded of endothelium by gently rubbing the luminal surface with a silk surgical suture. A segment of adventitia (~1.0 mm^2) was carefully removed from the arterial ring, creating a window that allowed direct excitation/emission of fura-2 in medial smooth muscle and concomitantly minimized autofluorescence of the adventitial connective tissue. Care was taken to ensure underlying smooth muscle was not damaged during removal of the adventitia. These arterial rings were incubated in fura-2/AM loading solution at 37°C for 2 h, followed by a 30-min rinse in sterile modified Eagle's minimal essential storage media.

KCl concentration-response relationships

Coronary rings (3.5-4.0 mm axial length) were mounted on two stainless steel wires passed through the vessel lumen. One wire was fixed to a force transducer (Grass FT03, Grass Instruments, Quincy, MA) and the other to a micrometer microdrive (Stoelting/Prior Microdrive, Wood Dale, IL) to allow precise changes in circumferential length of the vessel. The mounted arterial ring was lowered into a 20-ml tissue bath containing Krebs bicarbonate buffer at 37 ± 0.5 °C and aerated with 95% O_2, 5% CO_2. Coronary rings were progressively stretched to the maximum of the length-developed tension relationship (L_{max}) in increments equal to 10% of the initial vessel outer diameter. After each stretch, contraction was elicited with exposure to high KCl (80 mM) and L_{max} was defined as the circumferential length at which developed tension was <5% greater than the developed tension produced at the previous length. Arterial rings were allowed to equilibrate for 45-60 min at L_{max} prior to subsequent evaluation of pharmacological responsiveness. Following equilibration, concentration-response relationships were determined by cumulative additions of increasing concentrations of KCl (10-100 mM). Complete exchange of the tissue bath was achieved for each increase in KCl concentration to maintain the equimolar substitution of KCl for NaCl. KCl concentrations were increased when the response to the previous concentration had stabilized. Isometric contraction responses were measured on a Grass polygraph recorder (Grass Instruments, Quincy, MA). Percent constriction was defined as the percentage increase in developed tension.

Simultaneous measures of intracellular free Ca^{2+} (Ca_i) and contractile tension

In additional studies, Ca_i and contractile tension were measured simultaneously in arterial rings (13,22). Endothelium-denuded arterial rings were mounted on two stainless steel wires; one fixed to a force transducer (Kulite Semiconductor Products Inc., Leonia, NJ) and the other attached to a lever driven by a digital micrometer to permit precise changes in circumferential length of the vessel. The mounted arterial ring was lowered into a heated superfusion chamber of a microfluorometry system (Nikon Diaphot, Nikon, Garden City, NY) and stretched to L_{max} as determined by repeated exposure to high KCl (60 mM) at increasing vessel diameter. Excitation light from a 150 W xenon arc lamp was passed through a rotating filter wheel (50 ms intervals) containing alternating 340 and 380 nm interference filters. Fluorescence emission at 510 nm was synchronized with the appropriate excitation wavelength by a photodetector mounted on the filter wheel and was reflected to a photomultiplier tube with a dichroic mirror. Fluorescence was analyzed with an analog fluorescence signal processor and an analog-to-digital converter. The change in fluorescence ratio (F_{340}/F_{380}) was representative of relative Ca_i following off-line subtraction of vessel autofluorescence. Autofluorescence was determined at the end of each experiment by superfusion in physiological saline solution (PSS) containing 2 mM $MnCl_2$, followed by bathing the vessel in PSS containing 2 mM $MnCl_2$ plus ionomycin (10 μM) to quench intracellular fura-2. Fluorescence and contractile data were sampled every 5 s. Data acquisition and transformations were performed using AxoBASIC 1.0 software (Axon Instruments, Foster City, CA) customized for multichannel data acquisition.

Smooth muscle cell dissociation

All electrophysiology experiments were performed using freshly dispersed smooth muscle cells dissociated from vessels on the day following animal sacrifice. Previous comparison of first and second day voltage clamp experiments has demonstrated no detrimental effect of overnight storage on whole-cell Ca^{2+} channel currents. Segments of LCX and LAD coronary arteries were cut longitudinally and pinned lumen side up in low-Ca^{2+} (0.1 mM) physiological buffer containing 294 U/ml collagenase, 5 U/ml elastase, 2 mg/ml bovine serum albumin, 1 mg/ml soybean trypsin inhibitor, and 0.4 mg/ml DNase I. Cells were enzymatically dissociated by incubation in a 37 °C water bath for 1 h. The enzyme solution

was then replaced with enzyme-free low-Ca^{2+} solution and isolated single cells were obtained by repeatedly directing a stream of low-Ca^{2+} solution over the artery via fire-polished Pasteur pipette. Isolated cells were maintained in low-Ca^{2+} solution at 4 °C until use (0-4 h).

Whole-cell voltage clamp

Whole-cell Ca^{2+} channel currents were obtained from single cells using standard whole-cell voltage clamp techniques as used routinely (2,10). Cells were initially superfused with PSS containing (in mM): 138 NaCl, 5 KCl, 0.1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 20 HEPES, pH 7.4 during gigaseal formation. Following whole cell configuration, the superfusate was switched to PSS with tetraethylammonium chloride (TEACl) substituted for NaCl and 10 mM Ba^{2+} as the charge carrier. Heat-polished glass pipettes (2-5 MΩ) were filled with a solution containing (in mM): 120 CsCl, 10 TEACl, 1 $MgCl_2$, 20 HEPES, 5 Na_2ATP, 0.5 Tris-GTP, 10 EGTA, pH 7.1 with KOH. Ionic currents were amplified with an Axopatch 200B patch-clamp amplifier (Axon Instruments, Foster City, CA). Currents were low-pass filtered with a cutoff frequency of 1000 Hz, digitized at 2.5 kHz and stored on computer. Leak subtraction was not performed. Current densities (pA/pF) were obtained for each cell by normalization of whole cell current to cell capacitance to account for differences in cell membrane surface area. Capacity currents were measured for each cell during 10 ms pulses from a holding potential of −80 mV to a test potential of −75 mV. Data acquisition and analysis were accomplished using pClamp 8.0 software (Axon Instruments). Cells were continuously perfused under gravity flow at room temperature (22-25 °C).

Solutions

Krebs buffer contained (in mM): 131.5 NaCl, 5 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 11.2 glucose, 13.5 $NaHCO_3$ and 0.025 EDTA. Vessels used for fura-2 experiments were stored and rinsed in sterile modified Eagle's minimal essential storage media which contained (in mM): 135 NaCl, 5 KCl, 0.34 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 2.6 $NaHCO_3$, 0.44 KH_2PO_4 and 20 HEPES and (vol:vol) 0.02 amino acids, 0.01 vitamins, 0.002 phenol red, 0.01 penicillin/streptomycin and 2% horse serum, pH to 7.2 with NaOH at 23 °C and passed through a sterile filter to a final pH of 7.4. Fura-2/AM loading solution contained 10 μM fura-2/AM, 0.5% cremophor, and 5% bovine serum albumin. The loading solution was vortexed for 1 min and then sonicated for an additional 1 min to increase solubilization of fura-2/AM. Drugs were obtained from Sigma Chemical (St. Louis, MO) unless otherwise noted. Smooth muscle cell dispersion chemicals were obtained from Worthington Chemicals (Freehold, NJ), endothelin-1 from Peninsula Laboratories (Belmont, CA) and ionomycin from Calbiochem (La Jolla, CA).

Statistical Analysis

Dimensional characteristics of coronary arterial rings were compared using Student's *t*-tests. KCl concentration-response relationships and Ca^{2+} channel current-voltage (*I-V*) relationships were analyzed using repeated measures analysis of variance (RM ANOVA) and the Student Newman-Keuls correction for multiple comparisons when appropriate. The concentration of KCl causing 50% of the maximal contractile response was designated EC_{50} and was calculated by nonlinear regression analysis. The individual EC_{50} values were averaged for LAD and LCX and compared using Student's *t*-test. Ca^{2+} sensitivity data are presented as grams of tension as a function of fluorescence ratio and were fit by nonlinear regression analysis to a four parameter sigmoidal equation (SigmaPlot 8.0, SPSS) with tension produced at 60mM KCl set as maximal tension and EC_{50} values calculated. EC_{50} values for LAD and LCX were compared using Student's *t* test. Student's *t*-tests were used to compare half-maximal activation and inactivation values of whole-cell Ca^{2+} currents. For all analyses, a *P* value $\leq$ 0.05 was considered significant. Data are presented as mean ± SE, and

n values in parentheses reflect the number of animals for arterial ring studies and the number of animals and cells for voltage clamp studies. When more than one vascular ring from a single coronary artery was used in identical protocols, the responses from these rings were averaged before data analyses were conducted.

Results

Coronary vessel dimensions and characteristics

Dimensions of coronary arterial rings are divided into those used for KCl concentration-response relationships (Table 1) and those used for simultaneous Ca_i and contractile tension experiments (Table 2). No significant differences in dimensional characteristics were observed between nonoccluded LAD and collateral-dependent LCX arterial rings used in the concentration-response relationships (Table 1) or in LAD and LCX rings used for combined tension and fura-2 experiments (Table 2).

KCl concentration-response relationships

We compared steady-state contractile responses of the LAD and LCX to increasing concentrations of KCl (10-100 mM). Contraction to KCl was significantly enhanced in the collateral-dependent LCX compared with the nonoccluded LAD (Figure 1). Furthermore, as illustrated in Figure 1 *inset*, EC_{50} was attained at a significantly lower KCl concentration in LCX compared with LAD rings (34.3 ± 2.7 vs. 41.7 ± 2.3 mM, respectively) indicating that constriction to KCl was significantly enhanced in the collateral-dependent LCX compared with the nonoccluded LAD.

Ca^{2+} channel current density and kinetics

Ca^{2+} channel currents were elicited by 400-ms step-depolarizations to potentials ranging from -60 to $+60$ mV from a holding potential of -80 mV. Representative traces for currents from both LAD and LCX cells at selected test potentials are included in Figure 2A. *I-V* relationships for smooth muscle cells isolated from the collateral-dependent LCX and the nonoccluded LAD are presented in Figure 2B. Current is plotted as peak inward current and is normalized to cell membrane capacitance (pA/pF). Cell capacitance (17.5 ± 1.3 vs. 16.3 ± 1.5 pF) was not significantly different between smooth muscle cells isolated from LCX (n=4 animals, 21 cells) and LAD (n=4 animals, 19 cells), respectively. These data demonstrate that peak Ca^{2+} channel current was not altered by chronic coronary occlusion (-5.68 ± 0.55 vs. -5.29 ± 0.42 pA/pF, LAD vs. LCX, respectively). As shown in Figure 2C, the test potential at which inward current attained half-maximal activation was not significantly different between cells of the LAD and LCX (3.2 ± 0.4 and 3.5 ± 0.3 mV, respectively; *inset*). The slope of the activation curves, which provides an indication of the sensitivity of the current to voltage, was also not significantly different between cells from LAD and LCX (6.1 ± 0.4 and 6.5 ± 03 mV, respectively). Steady-state inactivation of inward current was measured using 5-sec conditioning pulses (-80 to $+50$ mV) in 10 mV increments from a holding potential of -80 mV, followed by a 600-ms test potential of $+20$ mV. As shown in Figure 2D, the test potential at which Ca^{2+} channel currents attained half-maximal inactivation was significantly shifted to a more positive membrane potential in cells from LCX when compared with LAD (-16.2 ± 0.6 vs. -19.3 ± 0.8 mV, respectively; *inset*). This rightward shift in Ca^{2+} channel current inactivation results in a 3% increase in channel availability in LCX cells at a membrane potential of -40 mV (Figure 2D). Slope values for inactivation curves were not different between LAD and LCX cells (-12.2 ± 0.7 and -12.3 ± 0.6 mV, respectively).

Simultaneous contractile tension and Ca$_i$

In additional experiments designed to further elucidate potential cellular mechanisms underlying alterations in KCl responsiveness resulting from chronic coronary occlusion, we simultaneously measured KCl-induced changes in contractile tension and Ca$_i$ in collateral-dependent LCX and nonoccluded LAD arteries. Figure 3A represents our experimental protocol in which endothelium-denuded arterial rings were constricted with increasing concentrations of KCl and both contractile response and Ca$_i$ data were obtained. Evaluation of grams of tension as a function of fura-2 ratio (Figure 3B) revealed that significantly more tension was produced per unit change in fura-2 ratio (EC$_{50}$, LAD=1.66±0.13, LCX=1.36±0.07 fluorescence ratio units) indicating that Ca^{2+} sensitivity was significantly enhanced in the collateral-dependent LCX compared with the nonoccluded LAD. Additional control experiments examining Ca^{2+} sensitivity in nonoccluded animals demonstrated no regional differences in Ca^{2+} sensitivity (Figure 3C; EC$_{50}$, LAD=1.54±0.17, LCX=1.70±0.05 fluorescence ratio units) and established that the nonoccluded LAD from chronically occluded hearts displayed Ca^{2+} sensitivity similar to that observed in vessels from nonoccluded hearts.

We also evaluated contractile tension and Ca$_i$ simultaneously in collateral-dependent LCX and nonoccluded LAD arteries in response to receptor-agonist stimulation with endothelin-1. Figure 4A represents our experimental protocol in which endothelium-denuded arterial rings were constricted with 10 and 100nM endothelin-1 and contractile responses and Ca$_i$ were measured. Evaluation of grams of tension as a function of fura-2 ratio (Figure 4B) revealed similar changes in tension per unit change in fura-2 ratio in both LAD and LCX in response to endothelin-1.

Discussion

These studies are the first to document that Ca^{2+} sensitivity is significantly enhanced in coronary arterial rings distal to chronic occlusion compared with control, nonoccluded arteries and provide a mechanism for the increased contractility commonly observed in collateral-dependent vasculature. Increased Ca^{2+} sensitivity was revealed in response to KCl-induced membrane depolarization but not receptor-agonist stimulation with endothelin-1. These data suggest that more than one mechanism for Ca^{2+} sensitization of the contractile apparatus in vascular smooth muscle as indicated previously (3,6,25,26,33,34). Contrary to our alternative hypothesis, we also provide evidence that whole-cell peak Ca^{2+} channel currents are not altered in isolated smooth muscle cells from the collateral-dependent LCX compared with the nonoccluded LAD. These data indicate that peak Ca^{2+} influx during a brief depolarization is not altered by chronic occlusion. Furthermore, although half-maximal activation of Ca^{2+} current occurred at nearly the same membrane potential in LAD and LCX smooth muscle cells, half- maximal inactivation was shifted to a more positive membrane potential in LCX cells indicating Ca^{2+} channels from these cells are less likely to inactivate at any given membrane potential and thus, potentially allow greater Ca^{2+} influx during sustained depolarization.

Our observation of an increased high KCl-induced Ca^{2+} sensitivity of the contractile apparatus in the endothelium-denuded collateral-dependent LCX artery may be due to numerous adaptations related to a reduced blood flow to the region distal to chronic occlusion including ischemia and/or changes in mechanical stimuli such as, decreases in distending pressure and altered pulsatile nature of the blood flow, as previously proposed (30). However, studies which have examined ischemia-associated perturbations in smooth muscle or reductions in arteriolar transmural pressure have typically documented a reduction in smooth muscle Ca^{2+} sensitivity (19,27,37). These findings provide evidence against ischemia or alterations in transmural pressure as direct contributors to the enhanced Ca^{2+}

sensitivity of the collateral-dependent LCX. However, the chronic nature of the reduced blood flow and associated stimuli in our model of occlusion may result in adaptations of Ca^{2+} sensitivity that differ from the acute adaptations observed in other studies.

Although we report that peak Ca^{2+} channel currents were not altered in smooth muscle cells distal to chronic occlusion, our finding that Ca^{2+} channels from these cells exhibit decreased voltage-dependent inactivation suggests that during sustained depolarizations, Ca^{2+} channels in LCX smooth muscle cells likely allow more Ca^{2+} influx than in cells from the nonoccluded LAD. Although small, the ~3 mV shift in voltage-dependent inactivation in LCX cells has great potential for impacting Ca^{2+} influx. For example, under physiological conditions, this shift results in a 3% increase in channel availability at a membrane potential of −40 mV. The resultant increase in Ca^{2+} influx through Ca^{2+} channels of the LCX cells would translate to a potential ~15 μM/s increase in Ca_i in the absence of Ca^{2+} buffering. This calculation is based on a unitary current amplitude of −0.18 pA or ~500,000 ions/s at −40 mV for Ca^{2+} channels (24), an estimated arterial smooth muscle cell volume of ~1 pL (1), and the estimation that one arterial smooth muscle cell contains ~5000 Ca^{2+} channels (18). As Ca_i increases in coronary smooth muscle are typically in the nM range, this represents a potentially large increase in Ca^{2+} influx into LCX smooth muscle and emphasizes the importance of Ca^{2+} regulatory processes (e.g., negative-feedback and buffering) in maintaining Ca^{2+} homeostasis. Despite this potential for greater Ca^{2+} channel availability, our data indicate that the global Ca^{2+} concentration (i.e., steady-state fura-2 signal; Figure 3B) in response to high KCl was not enhanced in the LCX compared with LAD suggesting that the increased Ca^{2+} influx must be compensated by another mechanism, such as Ca^{2+}-dependent inactivation of the Ca^{2+} channels or increased Ca^{2+} extrusion. We have previously concluded that Ca^{2+} extrusion by the Na/Ca exchanger and sarcolemmal Ca^{2+}-ATPase are not enhanced in smooth muscle from the LCX compared with LAD (12) suggesting other compensatory mechanisms. Furthermore, use of external Ba^{2+} as charge carrier in our voltage clamp experiments precludes comparison of Ca^{2+}-dependent inactivation in cells from LCX and LAD. On the other hand, we cannot disregard a potential increase in subsarcolemmal Ca^{2+} levels, undetectable by fura-2, in the LCX smooth muscle cells.

Previous studies have reported that altered subsarcolemmal Ca^{2+} concentrations, which are distinct from global Ca^{2+} regulation, influence cellular regulatory processes and may alter cellular contractile states (4,5,9,17,23,35). The presence of an increased Ca^{2+} concentration in a restricted subdomain of the plasma membrane might provide a link between the shift in inactivation and enhanced Ca^{2+} sensitivity observed in the collateral-dependent LCX. Previous studies have demonstrated that local increases in intracellular Ca^{2+} subsequent to KCl-induced membrane depolarization produce translocation and activation of PKC (14). Furthermore, KCl-induced Ca^{2+} influx also has been shown to stimulate the RhoA/Rho kinase signaling pathway (16,25,36). In turn, both PKC and Rho kinase have been reported to augment Ca^{2+} sensitivity of the contractile proteins through enhanced myosin light chain phosphorylation. Thus, the shift in Ca^{2+} channel current inactivation observed in the LCX cells and the potential resultant increased subsarcolemmal Ca^{2+} concentrations may produce Ca^{2+} sensitization via activation of a Ca^{2+}-dependent PKC, Rho kinase or other biochemical pathways recognized to increase Ca^{2+} sensitivity (34). Interestingly, increased activity of Rho kinase has been implicated in both increased contractility of vascular smooth muscle and coronary artery spasm in patients with vasospastic angina (15). Alternatively, our data using endothelin-1 (Figure 4) indicates that receptor-agonist stimulation-induced Ca^{2+} sensitivity was not altered by chronic occlusion. Previous studies have indicated that KCl-induced membrane depolarization and receptor-agonist stimulation may increase Ca^{2+} sensitivity via distinct pathways (25). Sakurada et al. (25) report that high KCl-mediated membrane depolarization induces Rho kinase-dependent Ca^{2+} sensitization, which is

completely Ca^{2+}-dependent. These investigators propose that in addition to Ca^{2+}-dependent Rho kinase activation, receptor agonists likely also activate non-Ca^{2+}-dependent G protein coupled pathways of Ca^{2+} sensitization, which likely are not stimulated by KCl. Based on these statements, the disparity in our Ca^{2+} sensitivity data with KCl and endothelin-1 suggests chronic occlusion may alter only the mechanism by which membrane depolarization increases Ca^{2+} sensitivity. Use of selective inhibitors of the RhoA/Rho kinase and other biochemical pathways recognized to increase Ca^{2+} sensitivity (e.g., PKC) in future experiments could further our understanding of the mechanisms contributing to enhanced Ca^{2+} sensitization in collateral-dependent coronary vasculature.

Our finding that the porcine collateral-dependent LCX demonstrated enhanced contractile responsiveness to KCl compared with the nonoccluded LAD is in contrast to a previous study in the chronically occluded dog heart, which documented that collateral-dependent and nonoccluded arterial rings responded similarly to increasing KCl concentrations (22). These contradictory findings support previous data documenting differences between species (pig vs. dog) in adaptive responses to vasoactive agents following chronic coronary occlusion (11). These species differences in the adaptive response of the coronary circulation to chronic occlusion may be a product of the inherent differences in the collateral circulations of the dog and pig (26).

In conclusion, this study provides the first evidence of enhanced myofilament Ca^{2+} sensitivity in the collateral-dependent artery of chronically occluded hearts. Enhanced Ca^{2+} sensitization was evident with KCl-induced membrane depolarization, however, receptor-agonist stimulation-induced Ca^{2+} sensitivity was not altered by chronic occlusion suggesting that chronic occlusion may alter only the mechanism by which membrane depolarization increases Ca^{2+} sensitivity. Moreover, although peak Ca^{2+} channel current density and half-maximal activation of Ca^{2+} channel current was not altered by chronic occlusion, the collateral-dependent artery did display a rightward shift in half-maximal inactivation of Ca^{2+} channel current, potentially allowing increased subsarcolemmal Ca^{2+} concentrations that may augment Ca^{2+} sensitivity through a Ca^{2+}-dependent mechanism. These data provide a mechanism for the enhanced vasoreactivity commonly observed in vasculature distal to chronic occlusion that may result in persistent ischemia of the collateral-dependent region.

Acknowledgments

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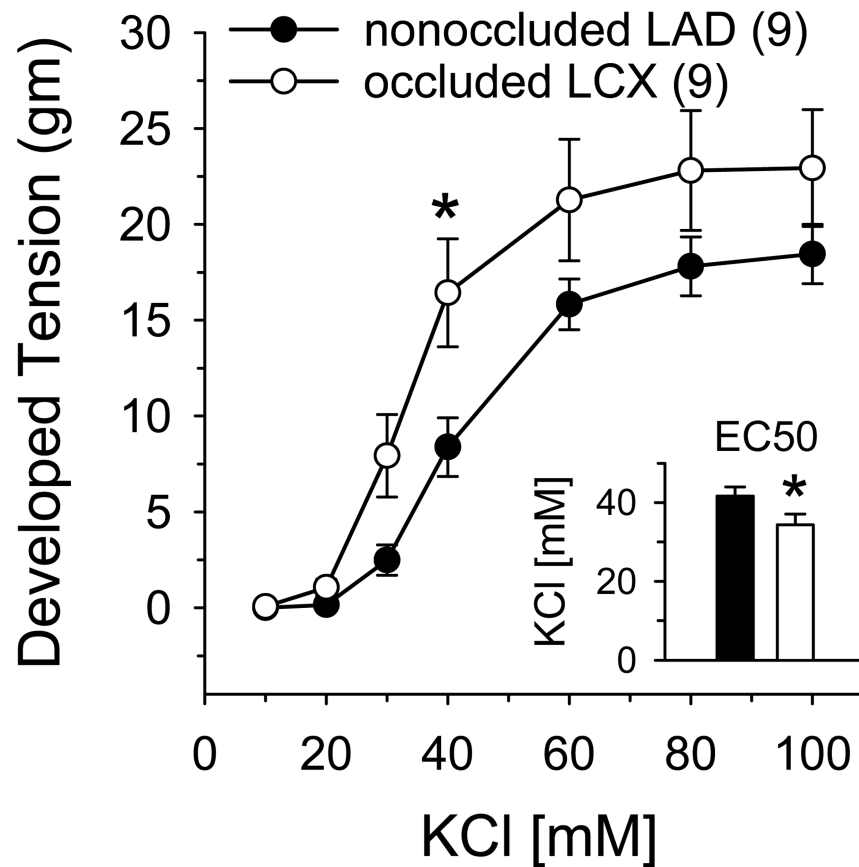
Figure 1.
Concentration-dependent contractile responses to KCl in collateral-dependent LCX and nonoccluded LAD arterial rings. KCl-induced constriction was significantly enhanced in LCX versus LAD arteries. The concentration of KCl yielding 50% maximum contraction (EC$_{50}$; *inset*) was significantly lower in LCX compared with LAD arterial rings. Values are means ± SE of the number of animals in parentheses; *P 0.05 versus LAD.

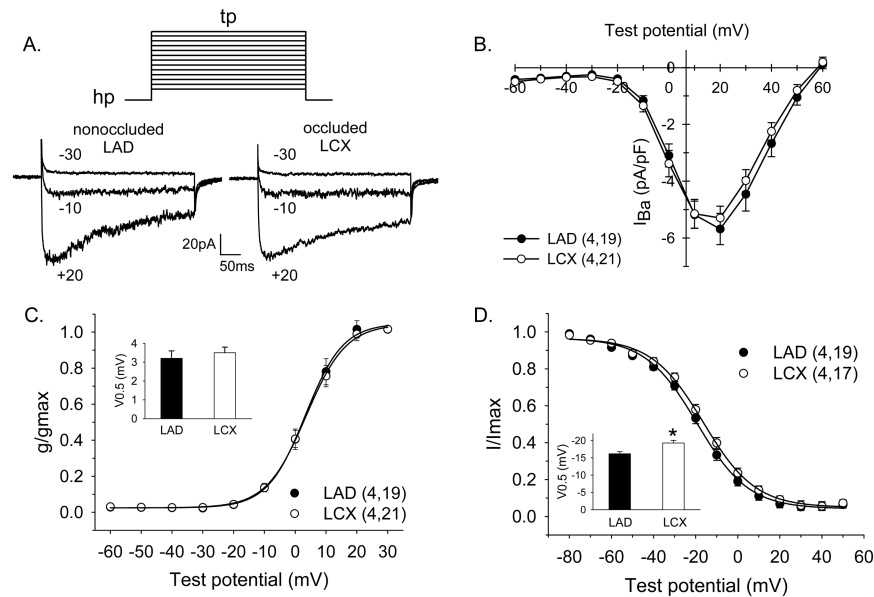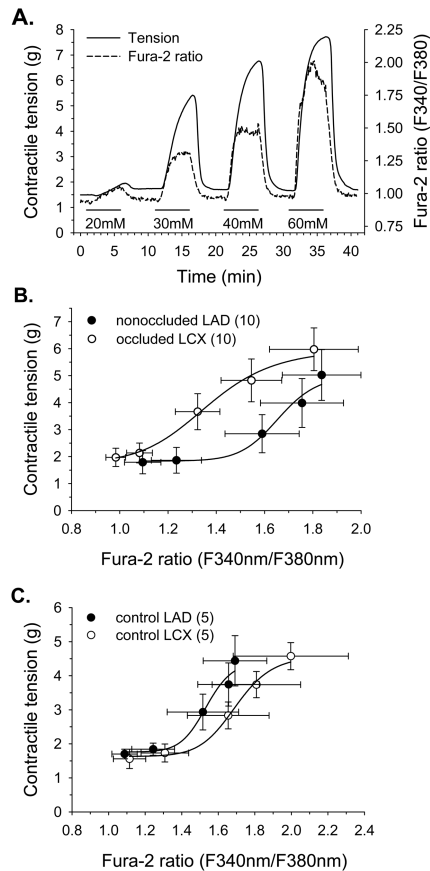**Figure 2.**
Effect of chronic coronary occlusion on whole-cell voltage-gated Ca^{2+} channel currents in isolated smooth muscle cells. A. Representative current traces of inward Ca^{2+} channel current in cells from LAD and LCX arteries at selected test potentials. Currents were elicited by 400-ms step depolarizations to potentials of −60 to +60 mV in 10 mV increments from a holding potential of −80 mV with 10 mM external Ba^{2+} as charge carrier. *B. I-V* relationships obtained by plotting peak inward current as a function of the indicated step potential. *C.* Voltage-dependent activation data were fit to a Boltzmann distribution equation. Half-maximal activation ($V_{0.5}$; *inset*) was not significantly different between cells of the LAD and LCX. *D.* Voltage-dependent inactivation of the inward current. Steady-state inactivation of inward current was measured using 5-sec conditioning pulses (−70 to +40 mV) in 10 mV increments from a holding potential of −80 mV, followed by a 600-ms test potential of +20 mV. Data were fit to a Boltzmann distribution equation. Currents attained half-maximal inactivation ($V_{0.5}$; *inset*) at a significantly more positive membrane potential in cells from collateral-dependent LCX when compared with nonoccluded LAD. Values in *B*, *C* and *D*, are means ± SE. Numbers in parentheses are number of animals, cells.

Figure 3.
Comparison of Ca^{2+}-sensitivity of collateral-dependent LCX and nonoccluded LAD arterial rings during KCl-induced membrane depolarization. *A.* Representative traces showing simultaneous measures of tension and Ca_i during exposure to noncumulative, increasing concentrations of KCl. Coronary arterial rings were superfused with KCl for 5 min, followed by 5-min recovery in normal PSS and subsequent exposure to a higher KCl concentration. *B.* Evaluation of tension as a function of Ca_i (fura-2 ratio) revealed that the collateral-dependent LCX developed significantly more tension per unit change in fura-2 ratio compared with the nonoccluded LAD (EC_{50}, LAD=1.66±0.13, LCX=1.36±0.07 fura-2 ratio units). *C.* Control experiments examining Ca^{2+} sensitivity in nonoccluded animals demonstrated no significant regional differences (LAD vs. LCX) in tension development as a function of fura-2 ratio in response to increasing KCl concentrations (EC_{50}, LAD=1.54±0.17, LCX=1.70±0.05 fura-2 ratio units). Values are means ± SE of the number of animals in parentheses.
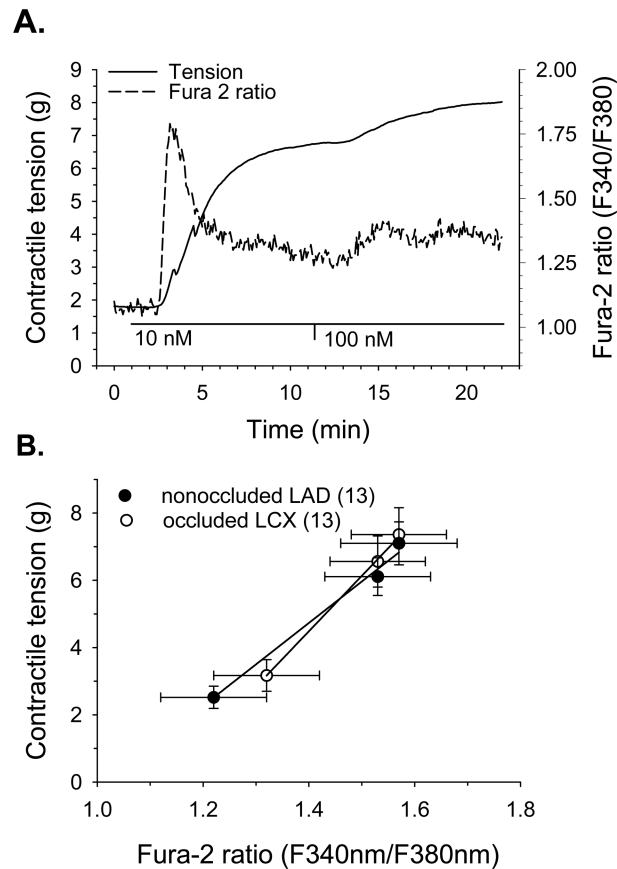
A.

B.

Figure 4.
Comparison of Ca^{2+}-sensitivity of collateral-dependent LCX and nonoccluded LAD arterial rings during receptor-agonist stimulation with endothelin-1. *A*. Representative traces showing simultaneous measures of tension and Ca_i during exposure to the cumulative addition of 10 and 100 nM endothelin-1. Coronary arterial rings were superfused with normal PSS for 1 min with subsequent exposure to each 10 and 100 nM endothelin-1 for 10 min. *B*. Evaluation of tension as a function of Ca_i (fura-2 ratio) revealed no difference in tension development per unit change in fura-2 ratio in the collateral-dependent LCX compared with the nonoccluded LAD.

Table
Dimensional characteristics of coronary artery rings

	Outer Diameter, mm	Lumen Diameter, mm	Wall Thickness, mm	Axial Length, mm
KCl concentration-response experiments				
LAD (n=9)	1.85 ± 0.05	1.25 ± 0.05	0.23 ± 0.02	3.90 ± 0.11
LCX (n=9)	1.70 ± 0.15	1.07 ± 0.12	0.22 ± 0.02	3.85 ± 0.10
Combined tension and fura-2 experiments				
LAD (n=10)	1.61 ± 0.08	0.98 ± 0.09	0.30 ± 0.03	
LCX (n=10)	1.70 ± 0.13	1.00 ± 0.10	0.29 ± 0.03	

Values are mean ± SE. LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery. Numbers in parentheses indicates number of arterial rings studied. No significant differences exist.