Calcium buffering in coronary smooth muscle after chronic occlusion and exercise training

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Abstract

Objective: Exercise promotes “sarcoplasmic reticulum (SR) Ca2+ unloading” in porcine coronary smooth muscle, resulting in decreased agonist-induced Ca2+ release. We studied Ca2+ handling in healthy, non-occluded right coronary artery cells from hearts chronically occluded at the circumflex artery.

Methods: Myoplasmic free Ca2+ (Ca2+) was assessed with fura-2 in cells from sedentary (n=8) and aerobically exercise-trained (n=6) female Yucatan pigs after 6-month circumflex artery ameroid occlusion (OCC) and in cells from non-occluded, sedentary pigs (SED, n=5). First, Ca influx was induced by 80 mM KCl depolarization (priming step) followed by 5 mM caffeine to elicit maximal Ca release and depletion. The SR was Ca-loaded again by depolarization and then exposed to caffeine after 2- or 11-min recovery to compare SR Ca unloading.

Results: Baseline Ca2+, caffeine-induced peak Ca2+ and depolarization-induced maximum Ca2+ were decreased, and depolarization-induced time-to-half-maximum was increased in OCC vs. SED pigs, suggesting a tonic Ca2+ buffering (lowering) effect of occlusion. Exercise did not alter these effects. SR Ca2+ unloading occurred only in SED, as evidenced by decreased caffeine-induced Ca2+ release after 11 min of recovery, and was inhibited by low extracellular Na+. 

Conclusions: SR Ca2+ unloading can be demonstrated in coronary smooth muscle from sedentary pigs using a novel SR Ca2+ unloading protocol, and Ca2+ unloading partly depends on Na+/Ca2+ exchange activity. Furthermore, SR Ca2+ unloading in cells from non-occluded right coronary arteries of chronically circumflex-occluded pig hearts was not altered by exercise, perhaps due to enhanced tonic Ca2+ extrusion versus cells from normal, sedentary animals.

Keywords: Calcium (cellular); Collateral circulation; Coronary circulation; Coronary disease; Na/Ca-exchanger; SR (function)

1. Introduction

Moderate levels of exercise training are associated with delayed progression of coronary artery disease. Although mechanisms that underlie the effects of exercise on the coronary vasculature are not clear, several recent studies report training-induced adaptations within the coronary circulation [1–5], with beneficial effects of exercise being attributed to an overall pattern of increased coronary vasodilation and decreased agonist-induced vasoconstriction [2,5]. In addition, various groups have reported the existence of functional alterations throughout the coronary arterial tree in coronary artery disease, mostly in regions distal to the diseased vessel [6,7]. Accordingly, knowledge of exercise-induced cellular adaptations in non-occluded arteries from diseased hearts is essential for understanding interactions of exercise and coronary artery disease, especially since blood flow through these healthy, non-occluded arteries is increased in the presence of a coronary occlusion, thus providing collateral flow to distal myocardial regions [8].

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Calcium handling by vascular myocytes is fundamental in regulation of blood pressure [9], blood flow [10], and growth/atherogenesis [11]. The sarcoplasmic reticulum (SR) is widely known to play a crucial role in Ca\(^{2+}\) homeostasis through its regulation of both agonist-induced Ca\(^{2+}\) release and Ca\(^{2+}\) sequestration after removal of vasoactive agonists [12,13]. Increasing evidence indicates that the SR attenuates increases in averaged myoplasmic free Ca\(^{2+}\) (Ca\(_m\)) by sequestering a portion of the Ca\(^{2+}\) that enters the cell across the sarcolemma [12]. The SR also provides a “superficial buffer barrier” for Ca\(_m\) in quiescent cells by slowly extruding Ca\(^{2+}\) into the extracellular space [12,14–16]. This process, termed “SR Ca\(^{2+}\) unloading”, is time-dependent [14,15] and involves the release of Ca\(^{2+}\) from the superficial SR into a restricted junctional domain near the sarcolemma for extrusion by Na\(^+\)-Ca\(^{2+}\) exchange [17] and/or the sarcolemmal Ca\(^{2+}\) pump. Consequently, the unloading process results in attenuated agonist-induced SR Ca\(^{2+}\) release [18] and decreased steady-state Ca\(_m\) [19].

Previous data from our laboratory demonstrate a reduced endothelin-mediated Ca\(_m\) response in exercise-trained swine [18,20], consistent with tonically lower Ca\(^{2+}\) stores. Stehno-Bittel et al. have shown that SR Ca\(^{2+}\) unloading occurs in quiescent bovine, but not porcine, coronary artery smooth muscle cells [16], and that coronary smooth muscle cells of exercised trained, but not sedentary, pigs exhibit SR Ca\(^{2+}\) unloading that is dependent on ryanodine-sensitive Ca\(^{2+}\) release channels [15]. Finally, a major role has been ascribed to Na\(^+\)-Ca\(^{2+}\) exchange in SR Ca\(^{2+}\) handling in rat-tail artery [21] and rabbit vena cava [22]. It is not known whether Na\(^+\)-Ca\(^{2+}\) exchange is necessary for SR Ca\(^{2+}\) unloading in porcine coronary arteries.

Using a new protocol for assessment of SR Ca\(^{2+}\) unloading, the present study demonstrates that cells from coronary arteries of sedentary pigs can exhibit time-dependent SR Ca\(^{2+}\) unloading seen previously only after adaptation to exercise [14,15]. We tested the hypothesis that after chronic occlusion of the circumflex coronary artery, SR Ca\(^{2+}\) unloading in cells from the healthy, non-occluded right coronary artery would be enhanced by exercise and would be affected by Na\(^+\)-Ca\(^{2+}\) exchange activity.

### 2. Materials and methods

#### 2.1. Animals

All animal procedures were approved by the Animal Care and Use Committee at the University of Missouri and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Adult female Yucatan miniature swine from multiple litters, ranging in age from 8 to 10 months (Charles River, 25–40 kg), were housed in pens at the College of Veterinary Medicine. This pig model, which is well characterized for the study of coronary physiology, coronary collateral circulation and exercise physiology [23], has many physiological characteristics similar to humans, including: (1) maximal coronary blood flow [23] and O\(_2\) consumption during exercise [24]; (2) redistribution of cardiac output from visceral tissue to skeletal muscle during exercise [24]; (3) sparse coronary collaterals and collateral flow [23]; (4) heart/body weight ratios [23]; and (5) a right dominant coronary anatomy [25].

#### 2.2. Coronary occlusion

Ameroid occluders (Research Instruments and MFG, Corvallis, OR) were surgically placed around the proximal region of the circumflex coronary artery (Fig. 1) as described previously [26–28]. This occlusion method stimulates the development of the collateral circulation supplying the myocardium-at-risk distal to occlusion. A period of 2 months after surgery, when occlusion and collateral development are complete [28], occluded-heart animals were divided randomly into either an exercise group (OCC+EX) that participated in a progressive treadmill training program for 16 weeks (described below), or a sedentary group (OCC+SED) cage-confined for 16 weeks. Matched non-occluded (control, not sham-operated) pigs (SED) were also cage-confined for 16 weeks. We have previously documented that smooth muscle responses of coronary arteries isolated from sham-occluded animals are essentially identical to responses from coronary arteries of animals not exposed to surgery [29]. In addition, a non-occluded exercise trained group was not included in this...
study because it represented only a minor variation of our previous studies on SR Ca\(^{2+}\) unloading in exercise [18,20].

2.3. Exercise training

The training paradigm consisted of a previously described 16-week treadmill-running program used extensively by Laughlin and colleagues [1,4,14,15,18,27,30]. During the first week, pigs ran at 3 miles per hour (mph), 0% grade, for 20–25 min (endurance) and at 5 mph for 5–10 min (sprint). Speed and duration of running were increased progressively at a rate dependent on the tolerance of each pig such that during the last week of training, a typical training session consisted of: (1) 5 min warm-up run at 2.5 mph; (2) 5–15 min sprint at 6 mph; (3) 60 min endurance run at 4–5.5 mph; and (4) 5 min warm-down run at 2 mph. Post-exercise feeding was used as positive reinforcement.

2.4. Tissue removal

At least 24 h after completion of the 16-week training or inactivity period, pigs were anesthetized with ketamine (30 mg/kg, IM) and pentobarbital sodium (35 mg/kg, IV), heparinized (1000 U/kg, IV), and the hearts rapidly removed and placed in iced (4°C) Krebs bicarbonate solution. The right coronary artery was isolated and carefully cleaned of fat and adventitia in a sterile low Ca\(^{2+}\) solution (0.5 mM Ca, modified Eagle’s Minimal Essential storage media containing 20 mM HEPES) on the same day of sacrifice.

2.5. Smooth muscle cell dispersion

All experiments were performed on freshly dispersed myocytes and completed within 36 h of tissue dissection. Cells were isolated as previously described [1,14–16,18,30–32]. Briefly, a segment of the right coronary artery was cut longitudinally and pinned lumen side up in a shaking water bath (90 excursions per min) for 1.5 h at 37°C, cells were loaded with 2.5 μM of the membrane-permeant acetoxyxethyl ester of fura-2 (fura-2/AM; Molecular Probes) for 20 min at 37°C. Cells were then washed for 20 min at 37°C in serum-containing media to promote fura-2 ester cleavage and resuspended in normal Ca\(^{2+}\) (2 mM) physiological saline solution (PSS) containing BSA (0.2%) at 4°C until use.

2.6. Myoplasmic Ca\(^{2+}\) determination

Fura-2 measurements of bulk Ca\(_m\) in isolated cells were obtained at room temperature (22–23°C) using the InCa\(^{2+}\) Calcium Imaging System and Double-Wavelength Ion Measurement Software (Version 1.5, Intracellular Imaging Inc., Cincinnati, OH) as described by Wahl et al. [33], using methodology similar to previously published methods from our laboratory [1,14–16,18,30–32]. Briefly, a drop of fura-2-loaded cellular suspension was placed in a superfusion chamber mounted on the stage of an inverted epifluorescence microscope, and cells were allowed to settle and adhere to the glass coverslip. Fura-2 was excited sequentially by ultraviolet light from 340 and 380 nm filters (10 nm bandwidth) and the fluorescence emission (510 nm) corresponding to each excitation wavelength was collected by a monochrome CCD Camera (COHU, Inc., San Diego, CA) attached to a 100 MHz Pentium data acquisition computer at a sampling rate of 0.5 Hz. Whole cell fluorescence data are expressed as the ratio of intensity of the 340–380 excitation wavelengths. Previous data from our laboratory have demonstrated comparable fura-2 loading between experiments carried out on vascular myocytes isolated from sedentary, exercise-trained, and coronary-occluded pigs [18,27].

2.7. Solutions

Cells were superfused at a rate of 2 ml/min with a PSS solution consisting of (in mM): 2 CaCl\(_2\), 10 glucose, 10 HEPES, 5 KCl, 1 MgCl\(_2\), and 138 NaCl (pH 7.4 with NaOH). Cell depolarization by equimolar replacement of NaCl with 80 mM KCl resulted in Ca\(^{2+}\) influx via voltage-dependent Ca\(^{2+}\) channels and subsequent loading of the SR Ca\(^{2+}\) stores. Caffeine (1,3,7-trimethylxanthine, Sigma Chemical), dissolved in PSS at a concentration of 5 mM, is routinely used in our laboratory [14–16,31,32] and others [13,17,19,21,22] to induce maximal SR Ca\(^{2+}\) release. A PSS solution containing 5 mM Na\(^+\) was used to assess the effects of low extracellular Na\(^+\), with Li\(^+\) as an equimolar Na\(^+\) substitute. Caffeine and KCl were applied to the cells via bath exchange.

2.8. “Priming” and the SR Ca\(^{2+}\) unloading protocol

SR Ca\(^{2+}\) unloading is accompanied by minimal increases in Ca\(_m\) [14–16], thereby making the Ca\(^{2+}\) unloading process difficult to measure directly with traditional whole-cell Ca\(_m\) measurement protocols. However, by varying the recovery time following depolarization, we have previously demonstrated SR Ca\(^{2+}\) unloading by comparing SR Ca\(^{2+}\) content before (2-min post-depolarization) and after (11–14 min post-depolarization) unloading occurs [14–16] using fura-2. Cells demonstrating unloading exhibit lower caffeine-induced Ca\(_m\) transients
with increasing recovery time periods following depolarization, consistent with loss of $\text{Ca}^{2+}$ from the SR and subsequent $\text{Ca}^{2+}$ extrusion [34]. The current study’s modified protocol (Fig. 5A and B) consisted of a depolarization “priming” step (80 mM KCl for 7 min, “1st 80 K”) followed by depletion of SR $\text{Ca}^{2+}$ with 5 mM caffeine (“CAF1”) for 2 min, in order to maximally fill and then empty the caffeine-sensitive $\text{Ca}^{2+}$ stores for “normalizing” the stores to the same pre-experimental filling state. The experimental portion of the protocol, which was identical to our original SR $\text{Ca}^{2+}$ unloading protocol, consisted of a second 7-min depolarization with 80 mM KCl (“2nd 80 K”), then a 2- or 11-min recovery (SR $\text{Ca}^{2+}$ unloading) period in normal PSS, followed by another 5 mM caffeine exposure (“CAF2’) to elicit maximum $\text{Ca}^{2+}$ release for group comparisons of SR $\text{Ca}^{2+}$ content. In some experiments, cells were superfused with low sodium (5 mM Na) PSS to block Na–Ca exchange during the 2 or 11 min recovery and the second caffeine exposure. In these experiments CAF2 exposure was extended from 2 to 4 min to assure full release of the caffeine-sensitive $\text{Ca}^{2+}$ store and to prevent re-uptake of $\text{Ca}^{2+}$ by the SR.

2.9. Relative $\text{Ca}_m$ response to caffeine — an index of SR $\text{Ca}^{2+}$ unloading

A maximal caffeine-induced $\text{Ca}_m$ response is proportional to the functional size of the SR $\text{Ca}^{2+}$ store [17,31,35]. In the current study, we define SR $\text{Ca}^{2+}$ content as proportional to the maximal caffeine-induced $\text{Ca}_m$ response minus the baseline $\text{Ca}_m$ value just before the onset of the caffeine response, or “$\Delta \text{CAF}$”. This measure was used since 5 mM Na$^+$ significantly increased basal $\text{Ca}_m$ levels in our cells (Fig. 7A) due to inhibition of Na$^+-\text{Ca}^{2+}$ exchange and a resultant net accumulation of $\text{Ca}^{2+}$ in the cytosol. Otherwise, an absolute measure of the $\text{Ca}_m$ response to caffeine may overestimate $\text{Ca}^{2+}$ release. The ratio of the second $\text{Ca}_m$ response to caffeine ($\Delta \text{CAF2}$) to the first $\text{Ca}_m$ response to caffeine ($\Delta \text{CAF1}$) was taken as the index of the extent of SR $\text{Ca}^{2+}$ unloading; a smaller $\Delta \text{CAF2}/\Delta \text{CAF1}$ ratio in cells from the 11-min recovery protocol vs. cells from the 2-min recovery protocol would indicate the presence of $\text{Ca}^{2+}$ unloading, consistent with extrusion of $\text{Ca}^{2+}$ from the cells between 2 and 11 min after depolarization. In control animals, $\Delta \text{CAF2}/\Delta \text{CAF1}$ values were not different when comparing the use of Li$^+$ (0.97±0.05, n=56 cells) or choline (0.98±0.07, n=17 cells) as a Na$^+$ substitute.

2.10. Time-to-half maximum and maximum determinations of $\text{Ca}_m$ responses to high K

Time-to-half maximum $\text{Ca}_m$ response ($t_{1/2,\text{max}}$) was calculated for the first and second 80 mM K$^+$ exposures, with the initial time point for $t_{1/2,\text{max}}$ determination when solution exchange occurred. Maximum $\text{Ca}_m$ responses to the 1st 80 K and 2nd 80 K exposures were calculated as the averaged 80 K maximum response, minus the pre-80 K-exposure $\text{Ca}_m$ level.

2.11. Statistical analysis

All group data are presented as mean±S.E.M. and were based on cell number. Statistical analyses were performed using SigmaStat software (Jandel Scientific Software). Single-factor group comparisons (i.e., SED vs. OCC+SED vs. OCC+EX) were made with one-way analysis of variance (ANOVA), while multi-factorial group comparisons (i.e., 2-min vs. 11-min unloading in SED, OCC+SED, and OCC+EX) were assessed using two-way ANOVA. Post-hoc differences were determined using the Student–Newman–Keuls Method. A value of $P<0.05$ was considered significant.

3. Results

3.1. Effects of occlusion and exercise on resting $\text{Ca}_m$, SR $\text{Ca}^{2+}$ content, and SR $\text{Ca}^{2+}$ sequestering ability

Fig. 2A shows two representative tracings from the first 20 min of the SR $\text{Ca}^{2+}$ unloading protocol, the thin tracing from the cell of a normal SED pig, and the thick tracing from an OCC+SED pig. In Fig. 2B, group baseline $\text{Ca}_m$ and $\Delta \text{CAF1}$ were significantly larger in SED than in OCC+SED and OCC+EX. Fig. 3 directly compares the 1st 80 K and 2nd 80 K tracings from a single SED cell. Within groups, a greater $t_{1/2,\text{max}}$ and a depressed maximum $\text{Ca}_m$ response during the 2nd 80 K exposure compared to the 1st 80 K exposure occurred (Fig. 4, open vs. cross-hatched bars). When comparing the 1st and 2nd 80 K $\text{Ca}_m$ responses between groups, $t_{1/2,\text{max}}$ values were less in cells from SED animals than in cells from the other two groups (Fig. 4A), indicating more rapid rates of response. The maximum $\text{Ca}_m$ response to the 1st or 2nd 80 K (Fig. 4B) was greater in the SED animals compared to OCC+SED and OCC+EX, while no differences existed between OCC+SED and OCC+EX in the measures of $t_{1/2,\text{max}}$ and maximum $\text{Ca}_m$ for either 80 K exposure.

3.2. Effect of “priming” on SR $\text{Ca}^{2+}$ unloading

Fig. 5 shows representative $\text{Ca}_m$ traces in isolated cells from normal SED pigs using either the 2-min (Fig. 5A) or 11-min (Fig. 5B) SR $\text{Ca}^{2+}$ unloading protocol. Minimal SR $\text{Ca}^{2+}$ unloading ($\Delta \text{CAF2}/\Delta \text{CAF1}=0.99$) occurred during the 2 min recovery period. In contrast, SR $\text{Ca}^{2+}$
Fig. 3. Increased Ca\textsuperscript{2+} sequestration by an acutely Ca-depleted SR store. This figure illustrates two overlaid responses to 80 mM K\textsuperscript{+} (1st 80 K and 2nd 80 K) from the same cell of a sedentary pig. Circle and square symbols denote \( t_{1/2} \) values for the Ca responses to the 1st 80 K and the 2nd 80 K, respectively. Arrowheads emerging from symbol drop lines stop at the ordinate value corresponding to the start time of the 80 K solution change (time 0), and the abscissa value associated with their respective Ca\textsuperscript{2+} level at the time point of the solution change (also time 0).

3.3. Effect of coronary occlusion and exercise on SR Ca\textsuperscript{2+} unloading

Fig. 6 also shows group data for SR Ca\textsuperscript{2+} unloading in cells of the right coronary artery from occluded pigs (OCC\textsuperscript{+}SED) and exercise-trained occluded pigs (OCC\textsuperscript{+}EX). In contrast to the results from cells of SED pigs, SR Ca\textsuperscript{2+} unloading appears to be absent in cells from OCC\textsuperscript{+}SED and OCC\textsuperscript{+}EX pigs as indicated by no significant differences between the 2- and 11-min recovery results.

3.4. Effect of a low sodium-containing bath solution during the recovery period in the SR Ca\textsuperscript{2+} unloading protocol

In Fig. 7A, a thick-lined tracing taken from a cell exposed to the 11-min unloading protocol is directly overlaid on a tracing from another cell exposed to the low-sodium variation of the same protocol (thin line), demonstrating the increase in the Ca\textsubscript{m} signal that typically occurred in response to changing to low Na\textsuperscript{+}. Group \( \Delta \text{CAF2}/\Delta \text{CAF1} \) results (Fig. 7B) illustrate that although a trend existed towards lower values in OCC when low sodium (5 mM Na) was used to block Na\textsuperscript{+}–Ca\textsuperscript{2+} exchange activity during the recovery portion of the SR Ca\textsuperscript{2+} unloading protocol, statistical analysis revealed that unloading did not occur in any of the three treatment groups.

4. Discussion

Little is known about Ca\textsuperscript{2+} handling mechanisms in non-occluded vessels from occluded hearts, but other
Fig. 4. Effects of coronary occlusion and exercise on $t_{1/2,\text{max}}$ and maximum Ca in response to 80 mM K. Group cell data for the $t_{1/2,\text{max}}$ (Panel A) and maximum Fura-2 ratio (Panel B) of the 1st 80 K (open bars) and 2nd 80 K (cross-hatched bars) Ca responses are presented (SED n=242/5, OCC+SED n=346/8, OCC+EX n=309/6 cells/animal). Asterisks refer to a statistically significant difference from the 80 K-matched SED control value, while plus signs indicate significant differences between the 1st and 2nd 80 K Ca response within groups, $P<0.05$.

Fig. 5. Using the “SR priming” protocol on coronary smooth muscle cells from sedentary pigs. Cells from normal (non-occluded) SED animals were subjected to a priming step (min 0–12), followed by the experimental step (min 12–34) of the SR Ca$^{2+}$ unloading protocol (the two phases of the protocol are delineated by a vertical dashed line in Panels A and B). Representative tracings were taken from separate single cells isolated from the same control vessel.

effects of occlusion on surrounding healthy vessels have been reported. For instance, during coronary ischemia, the relative contribution of larger epicardial coronary arteries to flow regulation is increased to restore myocardial function [28,36,37]. Also, non-occluded conduit vessels: (1) contribute to the production of collateral vessel growth into the region at risk to restore blood flow distal to occlusion [28]; and (2) dilate to allow more blood flow to undamaged regions of the heart [8]. Since Ca$^{2+}$ is involved in multiple cellular processes, it is conceivable that the pathophysiological manifestations of coronary artery occlusion may extend into non-ischemic “donor” arteres that supply collaterals. Ca-handling processes altered by occlusion may include sarcolemmal voltage gated calcium channels (VGCC), SR Ca-ATPase (SERCA), plasmalemmal Ca-ATPase, the plasmalemmal Na$^+-$Ca$^{2+}$ exchanger, and mitochondrial Ca$^{2+}$ uptake [34]. Although we did not study all of these processes specifically, our results shed some light on possible mechanisms for the effects of occlusion on Ca$^{2+}$ handling in donor vessels. The present study’s results reveal three major findings about the occluded porcine coronary vasculature: (1) resting Ca$^{2+}$ levels are decreased and Ca$_{\text{in}}$ responses to depolarization are attenuated in smooth muscle from a non-occluded artery; (2) SR Ca$^{2+}$ unloading in vascular myocytes from hearts of sedentary, but not occluded, pigs is demonstrated for the first time using a novel priming protocol [34], and this process is linked to Na$^+$–Ca$^{2+}$ exchange activity; (3) exercise does not alter Ca$^{2+}$ homeostasis in donor arteries.

We have previously demonstrated time-dependent de-
Fig. 6. SR priming-induced depletion of SR Ca\(^{2+}\) in porcine coronary smooth muscle cells: effects of coronary artery occlusion and exercise. The ratio of the second to the first caffeine-induced rise in the Fura-2 ratio \(\Delta(F_{340}/F_{380})\) has been plotted vs. the recovery (unloading) time and pig group. The number of cells in each group is indicated inside each bar. The number of animals per group was 5, 8 and 6 for SED, OCC+SED, and OCC+EX, respectively. According to two-way ANOVA, only cells from sedentary pigs exhibited differences between the 2- and 11-min protocols, as indicated by an asterisk, \(P<0.05\).

Fig. 7. Effects of low sodium on SR Ca\(^{2+}\) unloading. Panel A shows overlaid tracings of two cells from SED pigs, one exposed to the 11-min priming protocol, and the other exposed to the same protocol but with low sodium. Panel B illustrates the effects of the low sodium protocol on SR Ca\(^{2+}\) unloading (2- vs. 11-min \(\Delta(F_{340}/F_{380})\)) in SED, OCC+SED and OCC+EX groups. The number of cells in each group is indicated inside their respective bars. The number of animals per group was 5, 8 and 6 for SED, OCC+SED, and OCC+EX, respectively. No significant differences were noted between any groups according to two-way ANOVA.

polarization-induced SR Ca\(^{2+}\) unloading in exercised-trained, but not sedentary, pigs [14,15]. Fig. 5 illustrates that SR Ca\(^{2+}\) unloading can now be elicited in cells from the same porcine model (SED miniature pigs) using a novel “priming” protocol that normalizes cellular pre-experimental Ca\(_{m}\) levels [34], a finding that was essential for our comparison of SR Ca\(^{2+}\) unloading in cells from sedentary normal vs. coronary occluded pigs. The fact that SR Ca\(^{2+}\) unloading did not occur in right coronary artery cells from the OCC+SED pigs (Fig. 6) was interesting. However, caffeine-induced Ca\(^{2+}\) release is initially reduced in occluded pigs (CAF1, Fig. 2B, cross-hatched bars), consistent with decreased maximal net content of the SR Ca\(^{2+}\) store. This observation, along with the significantly lower baseline Ca\(_{m}\) in the OCC+SED and OCC+EX groups versus the control SED group (Fig. 2B), supports an occlusion-related balance of Ca handling mechanisms that tonically favors a net loss of Ca\(^{2+}\) from the SR and the cell. Indeed, if cells from non-occluded SED were depolarized with lower K concentrations or shorter exposure to 80 K to elicit less Ca influx via voltage-gated Ca channels, the initial SR Ca load would be similar to the lower levels in occluded pigs. Accordingly, one would predict that SR Ca unloading would not occur in the non-occluded SED pigs.

When comparing 80 K-induced Ca\(_{m}\) responses in all three groups (Fig. 4A), the relative onset rate of the 2nd 80 K Ca\(_{m}\) response is less rapid (longer \(t_{1/2}\) max) than the 1st 80 K Ca\(_{m}\) response, a phenomenon which has been shown previously by our laboratory and others [12,31,35]. One explanation is that the recently emptied superficial SR, which is intimately associated with the sarcolemma, is more efficient at sequestering Ca\(^{2+}\) that enters the cell via voltage-gated Ca\(^{2+}\) channels during a second depolarization, resulting in a lower whole-cell fura-2 signal [35]. Importantly, this interpretation does not exclude the possibility that differences in plasmalemmal Ca\(^{2+}\) influx and other Ca-handling mechanisms may be involved. We can, however, exclude the possibility of Ca-induced Ca\(^{2+}\) release (CICR) in the first Ca\(^{2+}\) response to 80 K, because we have previously shown in coronary smooth muscle that the CICR inhibitor, ryanodine, did not decrease the Ca\(^{2+}\) response during exposure to 80 K [31]. Thus, CICR has little effect on the bulk Ca\(^{2+}\) response to depolarization, in marked contrast to the well-known contribution of CICR to
depolarization-induced Ca\(^{2+}\) transients in cardiac muscle [38]. This does not exclude localized CICR involved in Ca\(^{2+}\) sparks [39].

When comparing both high K\(^+\) responses between the treatment groups, the increased \(t_{1/2}\) \(\text{Max}\) (Fig. 4A) and decreased maximal \(C_{m}\) (Fig. 4B) in cells from occluded vs. sedentary hearts suggest more effective Ca\(^{2+}\) buffering during depolarization. The steady-state (maximum) \(C_{m}\) response to 80 K almost certainly must require increased Ca\(^{2+}\) extrusion coupled to increased SR Ca\(^{2+}\) uptake, otherwise the SR Ca\(^{2+}\) store would saturate (maximally fill) within just minutes and cytoplasmic Ca\(^{2+}\) levels could subsequently approach control cells’ levels. Experiments in our laboratory on coronary vascular tissue rings from control pigs have shown that during mild depolarization (25 mM extracellular K), the presence of ryanodine, a blocker of the caffeine-sensitive Ca\(^{2+}\) release channel, significantly elevates sustained tension (\(P=0.01\)), consistent with elevated \(C_{m}\). This observation supports a balance of SR Ca\(^{2+}\) release with Ca\(^{2+}\) extrusion during depolarization that is partly dependent on SR Ca\(^{2+}\) release. Therefore, SR Ca\(^{2+}\) release and subsequent extrusion (or redistribution to other subcellular Ca\(^{2+}\) stores, i.e. mitochondria) can occur to a significant degree even during depolarization and could be primarily responsible for differences in the steady-state response to sustained depolarization in response to occlusion.

Numerous beneficial adaptations of cellular mechanisms within the coronary circulation in normal hearts [1–5] and in hearts affected by pathophysiological states (gradual occlusive coronary artery disease) [26,27,40–44] in response to exercise training have been recently reported. An unexpected result of the present study was that exercise did not alter the effects of occlusion on the Ca\(^{2+}\) homeostasis, since we have previously shown that exercise promotes SR Ca\(^{2+}\) unloading in coronary vascular myocytes in swine [14,15,18,20]. Considering that Ca\(^{2+}\) influx through voltage-gated Ca\(^{2+}\) channels is increased after exercise training in pigs [30,34], clearly Ca\(^{2+}\) buffering via SR Ca\(^{2+}\) unloading should be tremendously increased after exercise training of occluded pigs. Otherwise, the SR Ca\(^{2+}\) store would be larger after exercise because increased Ca\(^{2+}\) influx in the absence of SR Ca\(^{2+}\) unloading loads the SR Ca\(^{2+}\) store [31]. Indeed, previous data from our lab indicate that Ca\(^{2+}\) buffering mechanisms are increased to compensate for increased Ca\(^{2+}\) current density resulting from exercise training in normal pigs [34]. We suggest that vascular myocytes from healthy vessels from occluded hearts are already in a tonically compensated or “protected” state with no need for additional Ca\(^{2+}\) buffering offered by exercise training.

Recently, Lankford et al. [45] proposed that myocardial Ca\(^{2+}\) efflux was augmented by exercise training and that Na\(^{+}\)–Ca\(^{2+}\) exchange is the primary cardiac Ca\(^{2+}\) efflux pathway. Data from other laboratories also support the influence of Na\(^{+}\)–Ca\(^{2+}\) exchange on SR Ca\(^{2+}\) unloading in vascular myocytes [21,22]. The decrease of SR Ca\(^{2+}\) unloading in low sodium (Fig. 7B) vs. normal sodium (Fig. 6) condition in cells from sedentary animals supports the involvement of Na\(^{+}\)–Ca\(^{2+}\) exchange in SR Ca\(^{2+}\) unloading. However, whether Na\(^{+}\)–Ca\(^{2+}\) exchange was the key mediator of Ca\(^{2+}\) efflux pathway during SR Ca\(^{2+}\) unloading in coronary cells from sedentary animals is not entirely clear from our study.

We suggest that cells from a non-occluded donor vessel (right coronary artery) of a coronary-occluded heart may have adapted in such a way that they are already in a “protected” state at rest, as suggested by decreases in baseline \(C_{m}\), depolarization \(t_{1/2}\) max, depolarization maximal \(C_{m}\), and post-depolarization SR Ca\(^{2+}\) content. At least two advantages could be gained from these effects: (1) less agonist-induced Ca\(^{2+}\) release in response to endogenous vasoconstrictor agonists, such as endothelin [18,20] and, therefore, less tone generation due to decreased SR Ca\(^{2+}\) content; and (2) due to increased Ca\(^{2+}\) buffering, less Ca\(^{2+}\) would reach the cytoplasmic contractile mechanisms of the cell in response to Ca\(^{2+}\) influx, leading to a smaller vasoconstrictor response. In addition, exercise training may not add to these potentially beneficial effects of occlusion, since resting SR Ca\(^{2+}\) content would already be tonically optimized (lowered) for Ca\(^{2+}\) handling in those cells. Therefore, adaptation in cellular Ca\(^{2+}\) homeostasis may act in concert with structural/angiogenic adaptations to beneficially optimize blood flow to myocardium distal to a chronic coronary occlusion.

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