Alterations in PKC signaling underlie enhanced myogenic tone in exercise-trained porcine coronary resistance arteries

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Submitted 3 October 2003; accepted in final form 8 December 2003

Korzick, D. H., M. H. Laughlin, and D. K. Bowles. Alterations in PKC signaling underlie enhanced myogenic tone in exercise-trained porcine coronary resistance arteries. J Appl Physiol 96: 1425–1432, 2004. First published December 12, 2003; 10.1152/japplphysiol.01077.2003.—The intracellular mechanisms underlying enhanced myogenic contraction (MC) in coronary resistance arteries (CRAs) from exercise-trained (EX) pigs have not been established. The purpose of this study was to test the hypothesis that exercise-induced alterations in protein kinase C (PKC) signaling underlie enhanced MC. Furthermore, we sought to determine whether modulation of intracellular Ca2+ signaling by PKC underlies enhanced MC in EX animals. Male Yucatan miniature swine were treadmill trained (n = 7) at ~75% of maximal O2 uptake for 16 wk (6 miles/h, 60 min) or remained sedentary (SED, n = 6). Diameter measurements in response to intraluminal pressure (60, 75, and 90 cmH2O) or 60 mM KCl were determined in single, cannulated CRAs (~100 μm ID) with and without the PKC inhibitor chelerythrine (CE, 1 μM). Confocal imaging of Ca2+ signaling [myogenic Ca2+ (Ca m)] was also performed in CRAs of similar internal diameter after abluminal loading of the Ca2+ indicator dye fluo 4 (1 μM, 37°C, 30 min). We observed significantly greater MC in CRAs isolated from EX than from SED animals at 90 cmH2O, as well as greater reductions in MC after CE at all pressures studied. At intraluminal pressures of 75 and 90 cmH2O, CE produced greater decreases in Ca m from CRAs isolated from EX than from SED animals (64% vs. 25%, P < 0.05). Inhibition of KCl constriction by CE, bisindolymaleimide, and staurosporine in isolated smooth muscle cells from CRAs, providing evidence for a mechanistic link between VGCCs and PKC in our experimental paradigm. These results suggest that enhanced MC in CRAs from EX animals involves PKC-dependent modulation of intracellular Ca2+, including regulation of VGCCs.

MYOGENIC RESPONSIVENESS is an inherent property of vascular smooth muscle and an important determinant of local blood flow autoregulatory responses in animals and humans (for review see Refs. 10, 17, and 31). The contribution of myogenic constriction to basal levels of vascular tone is also well established (36), and this mechanism has been implicated in the coordinated vascular response to a variety of vasoactive stimuli (8, 37). Interestingly, the myogenic response is amenable to adaptation by chronic exercise training in the coronary circu-

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modulation of intracellular Ca\textsuperscript{2+}-signaling mechanisms by PKC in CRAs is affected by exercise training.

**METHODS**

**Animals.** Male Yucatan miniature swine (Charles River) weighing 25–40 kg were randomized to an exercise-training (EX) group (n = 7) or remained sedentary (SED, n = 6). The animal protocols associated with this study were approved by the University of Missouri Animal Care and Use Committee and carried out in accordance with the Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (US Government).

**Exercise training program.** Animals in the EX group were treadmill trained at ~75% of maximal O\textsubscript{2} uptake for 16 wk, as described previously (3, 28, 32). Briefly, progressive exercise sessions (85 min) were conducted as follows: 1) 5-min warm-up run at 2.5 miles/h (mph), 2) 15-min sprint at 5–8 mph, 3) 60-min endurance run at 4–5 mph, and 4) 5-min cool-down run at 2 mph. To document the presence of an endurance-trained state, citrate synthase activity assays were previously demonstrated the use of CE as a potent inhibitor of PKC inhibitor CE (1 \textmuM). At the conclusion of each experiment, passive vessel diameter was assessed after administration of 100 \textmuM SNP (60 \textmuM H\textsubscript{2}O\textsubscript{2}). Diameter measurements were expressed relative to passive diameter.

**Measurement of Ca\textsuperscript{2+} signaling by confocal microscopy.** In a separate series of experiments, CRAs (~100 \textmuM) were isolated from EX (6 vessels from 6 pigs) and SED (6 vessels from 6 pigs) animals, cannulated, and studied as described above. Before experimental manipulation and after equilibration at 37°C for 1 h, vessels were incubated with the Ca\textsuperscript{2+} indicator dye fluo 4 (1 \textmuM) and 0.01% Pluronic for 30 min and washed three times with physiological saline solution. Coronary resistance vessels were visualized with a Nikon Diaphot inverted microscope and a \times40 VWD objective after confocal argon laser excitation at 488 nm (Noran Instruments). All acquisition parameters (e.g., exposure time and excitation intensity) were kept constant in all experiments.

**Western blotting.** CRAs (~100 \textmuM ID) were isolated as described above, snap frozen on dry ice, and stored at ~70°C until they were processed. Three to four vessels from each heart (n = 1 pig) were placed in 200–300 \textmul of extraction buffer containing 20 mM Tris-HCI, 0.1 mM EDTA, 0.1 mM EGTA, 0.1% β-mercaptoethanol, 1 mM PMSF, 2 \textmuM leupeptin, 1 \textmuM pepstatin A, and 20 mM 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate and sonicated. Samples were centrifuged at 100,000 g for 30 min at 4°C. Equal amounts of sample (~15 \mu g protein) were loaded per lane, electrophoresed on 7.5% SDS-polyacrylamide gels, transferred to polyvinylidene difluoride membranes, and incubated with primary anti-rabbit antibodies for PKC-α or PKC-ε (1:400; Santa Cruz Biochemicals). Phosphospecific antibodies for PKC-α (Ser\textsuperscript{65}) were also purchased from Santa Cruz Biochemicals (1:1,000). Antibody binding was detected by enhanced chemiluminescence (Amersham). Rat brain (2–μg protein) was used as positive control. Densitometric analysis of immunoblot films (Hyperfilm, Amersham) was performed utilizing NIH Scion Image Analysis software. Care was taken to prevent saturation of the densitometric signal on film development.

**Coronary myocyte isolation.** CRAs were incubated in 200 \textmul of enzyme solution consisting of low-Ca\textsuperscript{2+} (0.1 mM) physiological saline solution plus 294 U/ml collagenase (CLS II, Worthington), 6.5 U/ml elastase (Worthington), 2 mg/ml BSA (fraction V, Sigma), 1 mg/ml soybean trypsin inhibitor (type I-S, Sigma), and 0.4 mg/ml DNase I (type IV, Sigma), as previously described (3). CRAs were incubated for 45–60 min in a water bath at 37°C and then incubated for 5 min at room temperature with enzyme-free low-Ca\textsuperscript{2+} solution. Isolated single cells were obtained by gentle trituration with a fire-polished Pasteur pipette.

**Whole cell VGCC characteristics in isolated coronary myocytes.** Macroscopic VGCC current (I\textsubscript{Ca}) was determined by using dialyzing, whole cell voltage clamp, as previously described (3). Briefly, current-voltage relations were determined by 400-ms step depolarizations from a holding potential of ~80 mV to test potentials from ~60 to +60 mV. Voltage clamp and current amplification were performed with an Axopatch 200B patch-clamp amplifier. Current records were filtered at 1 kHz (~3 dB) and digitized at 5 kHz. Control of voltage protocols and analysis were performed by Clampex 7.0 software (Axon Instruments, Foster City, CA). All experiments were conducted at room temperature (22–25°C).

**Statistics.** All variables of interest are reported as means ± SE. Data were analyzed with the Statistical Analysis System (SAS). Specifically, group comparisons of vasoreactivity after changes in intraluminal pressure were analyzed with the SAS general linear models procedure for a two-way ANOVA with repeated measures on one factor (group × pressure). Higher order terms were employed to reveal significant interaction effects with respect to varying pressure.
on vasoreactivity between groups. For immunoblot data, a one-way ANOVA was employed. The Tukey-Kramer method was used for all post hoc comparisons. An alpha level of $P < 0.05$ was considered statistically significant.

RESULTS

Efficacy of the exercise training program. As we previously demonstrated (3, 28, 32), the exercise paradigm described here resulted in significant group differences in several markers associated with an endurance-trained state (Table 1). Specifically, chronic exercise was associated with increases of 17.5% and 13% in heart weight and heart weight-to-body weight ratio, respectively, whereas no group differences were observed for body weight. Furthermore, deltid muscle citrate synthase activity was significantly greater in EX than in SED animals (Table 1), as was exercise run time to exhaustion ($P < 0.05$).

Taken together, the physiological and biochemical data indicate exercise-induced enhancements in aerobic metabolic capacity as a result of chronic treadmill running.

Baseline vessel characteristics. Mean maximal intraluminal diameter of isolated coronary resistance vessels measured at 60 cmH2O after administration of 100 μM SNP was identical between groups: 100 ± 4 μm in EX ($n = 20$ vessels) and SED ($n = 15$ vessels). After vessel equilibration for 1 h at 37°C, mean spontaneous tone development across all series of experiments was also similar between experimental groups with normalized diameter: 0.78 ± 0.04 vs. 0.73 ± 0.04 in SED and EX groups, respectively ($P > 0.05$; Fig. 1, see Fig. 3).

Myogenic responses and dependence on PKC. Myogenic responsiveness after step increases in intraluminal pressure in the absence and presence of the PKC inhibitor CE is presented in Fig. 1. Consistent with our previous findings in coronary resistance vessels isolated from exercise-trained female pigs (32), myogenic constriction was significantly greater in CRAs isolated from EX than from SED male pigs at 90 cmH2O ($P < 0.05$). After PKC inhibition with CE, myogenic tone was significantly attenuated at all intraluminal pressures studied in EX and SED animals; however, CE elicited greater reductions in myogenic constriction in EX than in SED animals ($P < 0.05$). Specifically, ANOVA revealed a significant interaction effect between group and drug, whereby EX increased the CE-sensitive component of myogenic tone by ~50% compared with vessels isolated from SED animals (28 ± 5% vs. 19 ± 6%) at 90 cmH2O (Fig. 1). In a separate series of experiments, confocal imaging of Ca2+ signaling [myogenic Ca2+ (Ca_m)] was performed on resistance arteries isolated from SED and EX animals using the Ca2+ indicator dye fluo 4 in response to changes in intraluminal pressure before and after PKC inhibition with 1 μM CE. It is clear from Fig. 2 that PKC inhibition resulted in the attenuation of Ca_m levels during myogenic constriction. At intraluminal pressures of 75 and 90 cmH2O, CE produced greater decreases in Ca_m in arterioles from EX than from SED animals (64 ± 8% vs. 25 ± 3%, $P < 0.05$).

Depolarization-induced constriction and dependence on PKC. To provide insight into the relation between depolarization, PKC, and VGCCs, vasoreactivity was assessed after abolumal administration of 60 mM KCl in the absence and presence of 1 μM CE (60 cmH2O). It is clear from Figs. 3 and 4 that constrictor responses induced by KCl were significantly attenuated in EX vs. SED animals ($P < 0.05$). However, inhibitory responses of CE on KCl-mediated constrictor responses were significantly greater in EX than in SED animals (Fig. 3). KCl constriction was completely blocked by CE in EX animals but only attenuated by CE in SED animals. Furthermore, the CE inhibition of vasoconstriction was accompanied by reductions in Ca_m (Fig. 4). To confirm a possible association between PKC and VGCCs, smooth muscle cells were isolated from CRAs, and whole cell VGCC current (I(Ca)) was assessed before and after PKC inhibition with three different PKC inhibitors: CE (1 or 10 μM), bisindolylmaleimide (10 μM), and staurosporine (10 μM). Approximately 60% of I(Ca) was effectively blocked by CE, bisindolylmaleimide, and staurosporine (Fig. 5), a finding similar to that in A7r5 cells, where PKC inhibition reduced I(Ca) by 70–75% (34). Inclusion of a phosphatase inhibitor, okadaic acid, in the pipette prevented

![Fig. 1. Dependence of myogenic contraction on PKC in sedentary (SED) and exercise-trained (EX) pigs. Myogenic responses to increasing intraluminal pressure in coronary microvessels isolated from SED (n = 9 vessels, ~100 μm ID) and EX (n = 9 vessels, ~98 μm ID) pigs are shown in the presence and absence of the PKC blocker chelerythrine (CE, 1 μM). Values are means ± SE. ANOVA revealed greater reductions in relative diameter in EX than in SED animals in response to PKC inhibition: *$P < 0.05$ vs. EX; ‡$P < 0.05$ vs. EX + CE.)](image)

Table 1. Efficacy of the exercise training program

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart Wt, g</th>
<th>Body Wt, kg</th>
<th>Heart Wt/Body Wt, g/kg</th>
<th>CS Activity, μmol/min·g−1</th>
<th>Exercise Time, min</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TLth</td>
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<tr>
<td>EX</td>
<td>184.86±7.54*</td>
<td>33.83±1.31</td>
<td>5.47±0.15*</td>
<td>18.60±1.50*</td>
<td>30.05±0.72*</td>
</tr>
<tr>
<td>SED</td>
<td>152.60±6.22</td>
<td>32.26±1.37</td>
<td>4.75±0.21</td>
<td>16.82±0.97</td>
<td>23.38±1.40</td>
</tr>
</tbody>
</table>

Values are means ± SE. EX, exercise trained; SED, sedentary; TLth, triceps, lateral head; CS, citrate synthase. *$P < 0.05$ vs. SED.
the CE effect, consistent with a PKC-dependent phosphorylation and activation of the VGCC. Together, these data support an important interaction between PKC and VGCCs in coronary vascular smooth muscle.

**PKC-α and PKC-ε immunoreactivity in CRAs.** Figures 6 and 7 present results of immunoblot analysis of PKC-α (Ca\(^{2+}\)-dependent PKC) and PKC-ε (Ca\(^{2+}\)-independent PKC) levels in isolated CRAs. We observed no significant group differences for PKC-ε (Fig. 6). However, PKC-α levels were elevated in CRAs from EX pigs and were significantly increased by 45% (P < 0.01) compared with CRAs isolated from SED pigs. Interestingly, increases in phosphorylated PKC-α levels were also observed in EX compared with SED animals (Fig. 6). These results are consistent with previous studies implicating a role for PKC-α in myogenic responses (11, 30).

**DISCUSSION**

A major focus of the present study was that alterations in PKC signal transduction mechanisms underlie, in part, adaptations in CRAs isolated from EX pigs. Accordingly, we investigated the role of PKC as an important determinant of myogenic contraction in coronary arterioles isolated from porcine myocardium. Novel findings include the following: 1) myogenic constriction and inhibitory responses after PKC blockade were greater in CRAs isolated from EX than from SED pigs, 2) KCl constriction was significantly less in resistance arteries isolated from EX hearts and blocked to a greater extent by PKC inhibition, 3) PKC-α (Ca\(^{2+}\)-dependent PKC) but not PKC-ε (Ca\(^{2+}\)-independent PKC) levels were significantly greater in CRAs isolated from EX animals, and 4) \(I_{\text{Ca}}\) was effectively blocked by PKC inhibition, as was the Ca\(^{2+}\) signal induced by myogenic constriction and KCl. These results suggest, for the first time, a potential mechanism for enhanced myogenic constriction known to occur in CRAs isolated from trained myocardium. Our data also provide an important circumstantial link between VGCC and PKC-α as a basis, at least in part, for training-induced adaptations in coronary myogenic responses.

An important role for PKC signal transduction pathways in the modulation of vascular smooth muscle myogenic responses is well established (for review see Refs. 10, 17, 18, and 24). Recent experimental evidence has identified multiple roles for PKC activation in the genesis of myogenic responses, including depolarization-induced VGCC channel activation, Ca\(^{2+}\) sensitization, and/or myosin light chain phosphatase inhibition (5, 33, 40, 47). Activation of a G protein-coupled receptor-dependent mechanism may also lead to activation of PKC under myogenic conditions, whereby activation of G protein-coupled receptors results in Ca\(^{2+}\) release from inositol trisphosphate-sensitive stores and activation of PKC (18). In this regard, recent experimental evidence suggests a key role for the phospholipase C-diacylglycerol-PKC signal transduction axis as a basis for cation channel activation in the generation of myogenic tone (43). Therefore, mechanisms involving pressure-induced increases in intracellular Ca\(^{2+}\) and ion channel phosphorylation seem likely, and a final common pathway...
for the modulation of myogenic contraction via receptor-dependent and receptor-independent mechanisms occurs at the level of PKC.

In the present study, we observed significant increases in myogenic tone at 90 cmH\textsubscript{2}O in coronary resistance arterioles isolated from male EX pigs relative to SED control animals, extending our previous findings in female pigs (32). In both studies, a significant training effect became apparent only at intraluminal pressures \(>60 \text{ mmHg} \ (\approx 80 \text{ cmH}_2\text{O})\). The myogenic mechanism that underlies the “threshold” for the training response is yet to be determined; however, this intraluminal pressure range coincides with the transition to a phase where depolarization, intracellular Ca\textsuperscript{2+} increases, and VGCC activation occur (36). Thus this range of pressures would be especially sensitive to training-induced changes in Ca\textsuperscript{2+}-dependent PKC (e.g., PKC-\(\alpha\)) and VGCC activity. The enhanced myogenic response may be a compensatory mechanism to the increased flow-mediated dilation that occurs with exercise training (26). An increased myogenic response would be necessary to allow training-induced increases in coronary flow reserve in the presence of increased flow-mediated dilation (8).

In response to PKC blockade, we observed significant attenuation of myogenic tone at all intraluminal pressures studied in SED and EX pigs, consistent with previous studies suggesting an important role for PKC in the genesis of myogenic responses in a variety of vascular beds, including cerebral (2, 22), mesenteric (45, 46), and skeletal (16, 30) circulations. Of notable interest was the finding that the degree of inhibition of myogenic contraction after CE was significantly greater in CRAs isolated from EX than from SED animals. Confocal microscopy revealed that inhibitory effects of PKC blockade on myogenic tone were associated with reductions in Ca\textsubscript{m} (Fig. 2). Our data are consistent with previous studies (9, 22, 36); however, they are at odds with experimental evidence attri-
uating PKC effects on myogenic contraction to Ca\textsuperscript{2+} sensitization (11, 33, 40, 47).

To investigate the possibility of key regulatory interactions between PKC and Ca\textsuperscript{2+}/H\textsuperscript{11001} in flux via VGCCs, depolarization-induced vasoreactivity was assessed after abluminal administration of KCl in the absence and presence of PKC inhibition. It is clear from Fig. 3 that vasoconstrictor responses induced by KCl depolarization were diminished by PKC inhibition, supporting a key role for PKC in modulating Ca\textsuperscript{2+}/H\textsuperscript{11001} flux via depolarization-induced activation of VGCCs. Confirmation of this association was observed by confocal imaging of Ca\textsubscript{m}, whereby KCl-induced increases in Ca\textsubscript{m} were significantly reduced by PKC inhibition (Fig. 4). Although KCl constriction was significantly less in resistance arteries isolated from EX than from SED animals, effects of PKC inhibition were greater in EX than in SED animals, indicating an increased reliance on a PKC-mediated mechanism for constriction. Although it is impossible to rule out training-induced alterations in PKC on myofilament Ca\textsuperscript{2+} sensitization in our experimental paradigm, one interpretation of the present results is that, because the PKC-dependent portion of depolarization-induced contraction was greater in EX animals, regulation of VGCC activation by PKC assumes a greater role in mediating CRA contractile responses after chronic exercise training. Indeed, confocal data presented in Fig. 6 provide evidence for this supposition, inasmuch as Ca\textsubscript{m} increases were blocked to a greater extent by PKC inhibition in EX than in SED animals after KCl depolarization. It is also important to note that three different, general PKC inhibitors inhibited whole cell I\textsubscript{Ca} (Fig. 5), providing additional circumstantial evidence for important regulatory interactions between PKC and VGCCs in porcine CRAs.

Taken together, our data support the hypothesis that PKC plays an important role in determining VGCC activity and vasoconstrictor tone in CRAs and that training-induced adaptations in PKC contribute to alterations in myogenic regulation.

Fig. 5. Dependence of basal voltage-gated Ca\textsuperscript{2+} channel current (I\textsubscript{Ca}) on PKC. Inhibition of I\textsubscript{Ca} (10 nM external Ba\textsuperscript{2+}) by the specific PKC inhibitors CE (10 \textmu M), bisindolylmaleimide (Bis, 10 \textmu M), and staurosporine (St, 10 \textmu M) is shown. Superfusion with CE, Bis, or St produced inhibition of I\textsubscript{Ca}, compared with time control (C). Inset: inclusion of the phosphatase inhibitor okadaic acid [+OA (○), 1 \textmu M] in the pipette inhibited the effect of CE (●, CE added at 4 min). *P < 0.05 vs. C; n = 3 cells (SED) per condition.

Fig. 6. PKC-α immunoreactivity in coronary resistance arteries (CRAs). Equal amounts of protein were loaded per lane (15 \textmu g). Lanes 1–4, microvessels isolated from SED pigs (n = 4); lanes 5–8, CRAs isolated from EX pigs (n = 4). Each lane represents 3–4 CRAs per pig (~100 \textmu M ID, see METHODS); isolated rat brain (RB) was utilized as a positive control. Values are means ± SE. *P < 0.01. PKC-α (Ca\textsuperscript{2+}-dependent PKC) levels were significantly greater in CRAs isolated from EX animals (A). Phosphorylated PKC-α levels (pSer\textsuperscript{657}) were similarly increased in EX and SED animals (B).

Fig. 7. PKC-α and phosphorylated PKC-α immunoreactivity in CRAs. Equal amounts of protein were loaded per lane (15 \textmu g). Lanes 1–4, microvessels isolated from SED pigs (n = 4); lanes 5–8, CRAs isolated from EX pigs (n = 4). Each lane represents 3–4 CRAs per pig (~100 \textmu M ID, see METHODS); isolated RB was utilized as a positive control. Values are means ± SE. *P < 0.01. PKC-α (Ca\textsuperscript{2+}-dependent PKC) levels were significantly greater in CRAs isolated from EX animals (A). Phosphorylated PKC-α levels (pSer\textsuperscript{657}) were similarly increased in EX and SED animals (B).
reactivity remains poorly understood. In cultured smooth muscle cells isolated from human aorta, PKC-α, βI, βIII, -δ, and -ε, but not PKC-γ and -ζ, are detected (13). In the porcine coronary arterial microcirculation, we have documented the presence of PKC-α, βI, βIII, -δ, and -ε (unpublished observations). In the present investigation, we observed significant increases in the Ca\textsuperscript{2+}-dependent PKC-α, whereas group differences in the Ca\textsuperscript{2+}-independent PKC-ε were not observed. These findings are consistent with experimental observations from the laboratory of Dessy and colleagues (11) as well as Massett et al. (30), whereby PKC-α activation was identified as a key transducer of myogenic responses in the ferret coronary and rodent skeletal muscle microcirculations, respectively. Additionally, we provide evidence, for the first time that PKC-α phosphorylation is enhanced as a result of chronic endurance exercise training in porcine CRAs, possibly identifying an important modulator of PKC-α-dependent signaling in coronary vascular smooth muscle after exercise training. PKC-ε involvement in myogenic reactivity has been shown to be linked to MAPKs (30, 38) and/or RhoA/Rho kinase (7, 40). That group differences in PKC-ε were not observed suggests that MAPKs and/or RhoA/Rho kinase are less likely to be a target of exercise-induced adaptation in the porcine coronary circulation under conditions of myogenic contraction. Future studies are indicated to resolve this issue. Collectively, our data suggest that the amount and phosphorylation of PKC-α protein levels are enhanced by chronic exercise. One important limitation of the present study is use of the general PKC inhibitor CE in our experimental paradigm. Additional studies are necessary to more precisely define the role of PKC-α in mediating exercise-induced adaptations in myogenic responses. In this regard, it will be necessary to utilize isoform-specific PKC inhibitors to directly address the involvement of PKC-α in exercise-induced alterations in myogenic reactivity.

Chronic endurance exercise has been implicated in a myriad of beneficial effects on the coronary circulation, and, of particular clinical interest, are adaptive responses known to occur in vascular smooth muscle regulatory mechanisms (3, 4, 27, 28). One logical issue to be addressed is the apparent dichotomy between pathological increases in myogenic contraction, as observed in the hypertensive heart (12, 19, 41), vs. physiological adaptations in myogenic responses, as described here. It is likely that these adaptations represent only one component of an integrated, balanced process that, when expressed in a coordinated fashion, contributes to a beneficial adaptation, e.g., increased coronary reserve. Disruption or inhibition of one or more of these components in disease may prevent the beneficial adaptation and predispose to pathology. Alternatively, increased myogenic responses in pathological and physiological conditions may represent identical compensatory responses of varying magnitude, analogous to compensated vs. uncompensated cardiac hypertrophy. Further study to determine the precise mechanism of the exercise-induced response will allow identification of critical components that separate the physiological and the pathological response.

In conclusion, this study provides novel evidence that endurance exercise training increases the myogenic response in the coronary microcirculation through a PKC-dependent mechanism. Increased expression and phosphorylation of PKC-α by exercise training suggests that this PKC isoform may mediate this training adaptation. The increased reliance on PKC in mediating C\textsubscript{a0} responses further suggests a training-induced increase in VGCC regulation by PKC. Together, these results suggest that enhanced myogenic responses in coronary arterioles by exercise training involve PKC-dependent modulation of intracellular Ca\textsuperscript{2+}, including regulation of VGCCs.

ACKNOWLEDGMENTS

The authors thank Pam Thorne for expert technical assistance with the cannulated vascular studies.

GRANTS

This study was funded by National Institutes of Health Grants PO1 HL-52490 (to M. H. Laughlin, D. K. Bowles, and D. H. Kozick) and K01 AG-08075 (to D. H. Kozick).

REFERENCES


42. Shistik E, Kerov-Raifman T, Idelson GH, Blumenstein Y, Dascal N, and Ivanina T. The N terminus of the cardiac L-type Ca\(^{2+}\) channel \(\alpha_{1C}\)-subunit. The initial segment is ubiquitous and crucial for protein kinase C modulation, but is not directly phosphorylated. J Biol Chem 274: 31145–31149, 1999.


