PKC, Ca^{2+}, and Myogenic Constriction

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Journal Review of:

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

by:

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Journal of Applied Physiology, 2004

Abstract

- Myogenic responses originate within the smooth muscle cells of vascular structures in response to a change in transmural pressure.
- Exercise enhances the myogenic response, but the mechanism remains unknown.
- This study investigated the role of changes in protein kinase C (PKC) as the cause of exercise-enhanced myogenic contraction and the role of PKC-dependant intracellular Ca^{2+} signaling.
- Pigs were subjected to an exercise regimen and the internal diameter of coronary resistance arteries measured in response to intraluminal pressure, with or without the PKC inhibitor chelerythrine.
- Microperfusion, immunoblotting, patch clamp techniques and confocal imagery were used to assess the vessel response, measure PKC levels, assess voltage gated calcium current, and visualize myogenic Ca^{2+} release.
- Exercise training served to: increase the myogenic response, decrease the myogenic response after chelerythrine treatment, and increase the level of PKC-α and degree of phosphorylation.

Background

Protein kinase C (PKC) is an enzyme with several isoforms such as α, β_I, β_{II}, γ, and □. Some isoenzymes such as PKC-α requires Ca^{2+} for activation, while others such as PKC-□ do not. PKC's actions vary between somatic cells. In vascular smooth muscle, the PKC-α isoform has been shown to be involved in the myogenic response transduction process.

The relationship between PKC and the L-type voltage-gated calcium channel is thought to play an important role in the development of myogenic tone in response to changing transmural pressure.

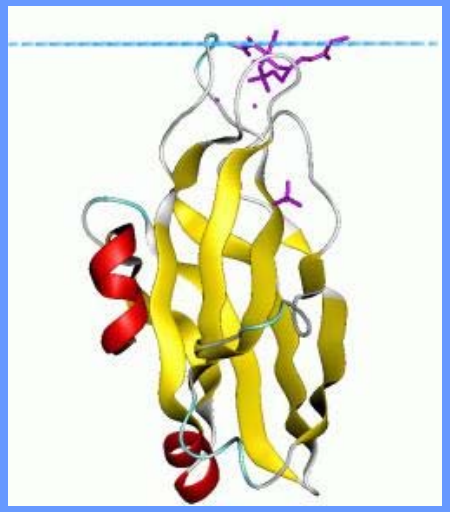
Specifically, this study sought to elucidate the connection between PKC modulation and exercise enhanced myogenic contraction. Additionally, the study investigated the relationship between exercise and PKC interaction with Ca^{2+} signaling mechanisms.

In the present study, Yucatan Miniature swine were subjected to exercise training

Phorbol esters can directly activate PKC

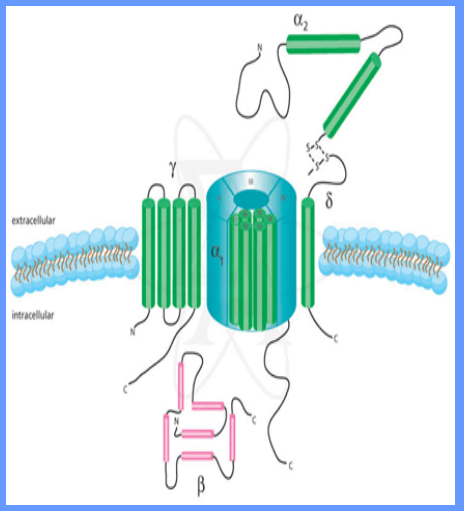
Hypothesis

- The exercise-induced enhancement of myogenic contraction in pig coronary arteries is a result of protein kinase C signaling modulation
- Exercise training affects intracellular Ca^{2+} signaling through PKC modifications



Protein Kinase C-α

Chelidonium majus, source of cheleythrine



L-type voltage gated calcium ion channel

Objectives

- Determine efficacy of exercise program:
  - Compare heart/ body weight and measure skeletal muscle citrate synthase activity
- Determine the dependence of myogenic response on PKC:

  - increase intraluminal pressure with or without chelerythrine in exercised or sedentary pigs and assess vasoreactivity of coronary microvessels (microperfusion)
- Determine the role of PKC and voltage-gated Ca^{2+} channels in depolarization-induced constriction:
  - Dose vessels from either exercised or sedentary pigs with 60 mM KCl, with or without chelerythrine (microperfusion/confocal and voltage clamp)
- Determine the levels of PKC-α and PKC-□ in coronary resistance arteries:
  - Compare immunoblot results from exercised and sedentary pigs

Results

- Exercise Training:
  - exercise trained pigs had increased heart weight and heart/body weight ratio
  - exercise trained pigs demonstrated increased citrate synthase activity in skeletal muscle
  - exercise trained pigs had increased physical endurance and stamina

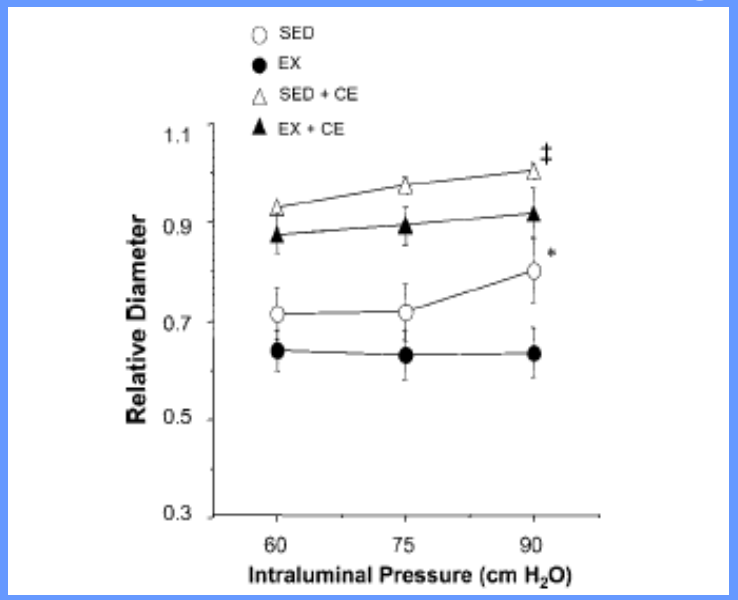
Table 1. *Efficacy of the exercise training program*

	Heart Wt, g	Body Wt, kg	Heart Wt/Body Wt, g/kg	CS Activity, μmol·min^{-1}·g^{-1}		Exercise Time, min
				TLth	Deltoid	
EX	184.86±7.54*	33.83±1.31	5.47±0.15*	18.60±1.50*	21.23±1.97*	30.05±0.72*
SED	152.60±6.22	32.26±1.37	4.75±0.21	16.82±0.97	17.11±1.03	23.38±1.40

Values are means ± SE. EX, exercise trained; SED, sedentary; TLth, triceps, lateral head; CS, citrate synthase. *$P < 0.05$ vs. SED.

Results

- Dependence of myogenic response to changing transmural pressure on PKC:
  - Inhibition of PKC with chelelythrine decreased myogenic tone in both sedentary and active pigs, but CE had an increased reduction in myogenic response
  - Exercise increased the sensitivity of the myogenic constriction to chelelythrine attenuation
  - Confocal imaging revealed decreased myogenic Ca^{2+} with cheleythrine treatment but more significant decreases in exercised animals



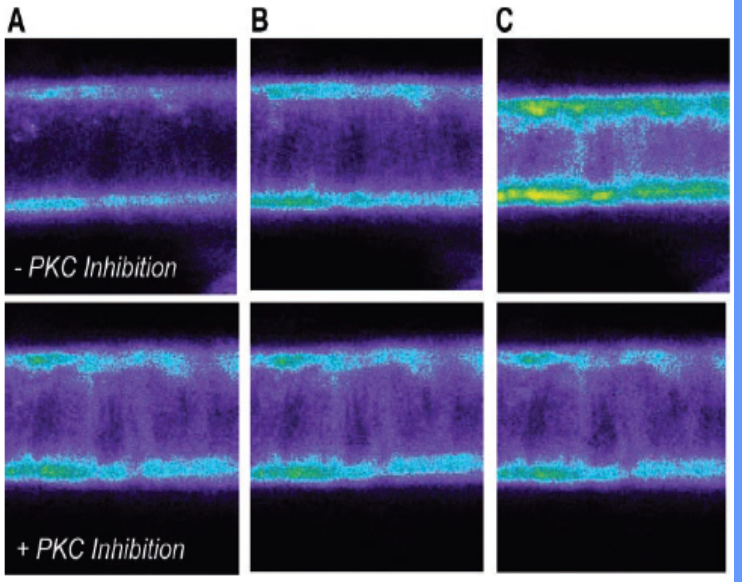
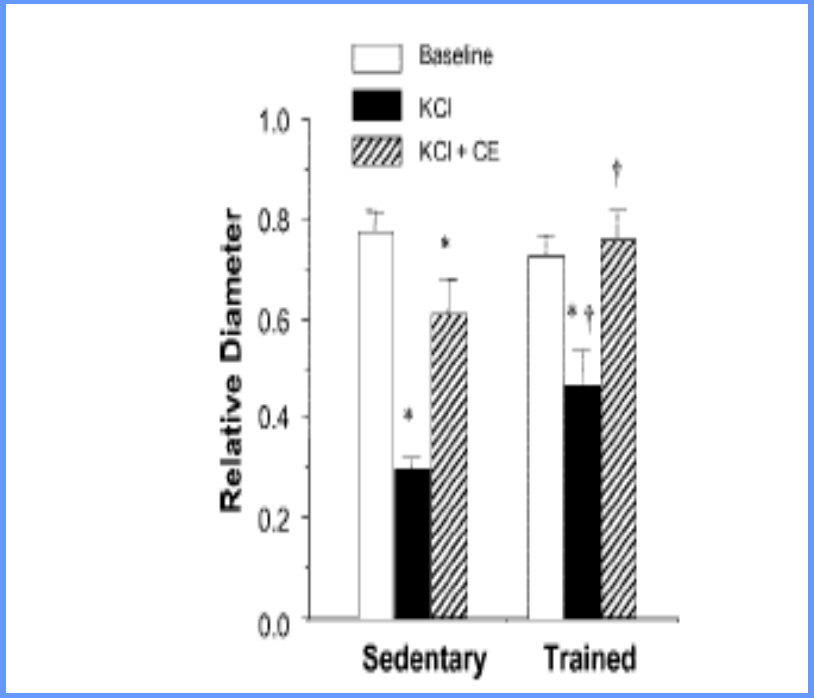


Fig. 2. Dependence of myogenic contraction on Ca^{2+} and PKC. Confocal imaging of myoplasmic Ca^{2+} (Ca_m) using the Ca^{2+} indicator dye fluo 4 in response to changes in intraluminal pressure at 60 cmH_2O (*A*) and during transition (*B*) to 90 cmH_2O (*C*) is shown in a representative coronary arteriole (~100 μm ID) isolated from EX animals in the absence (*top*) and presence (*bottom*) of PKC inhibition with 1 μM CE.

Results

- Dependence of depolarization-induced constriction on PKC:
  - 60 mM KCl induced a weaker vasoconstriction in exercised vs. sedentary pigs
  - cheleythrine completely inhibited KCl-induced constriction in exercised pigs but merely decreased constriction in sedentary animals



Results

•Dependence of depolarization-induced constriction on PKC:

PKC inhibition resulted in decreased myogenic Ca^{2+}, but exercised pigs exhibited a greater decrease versus sedentary animals

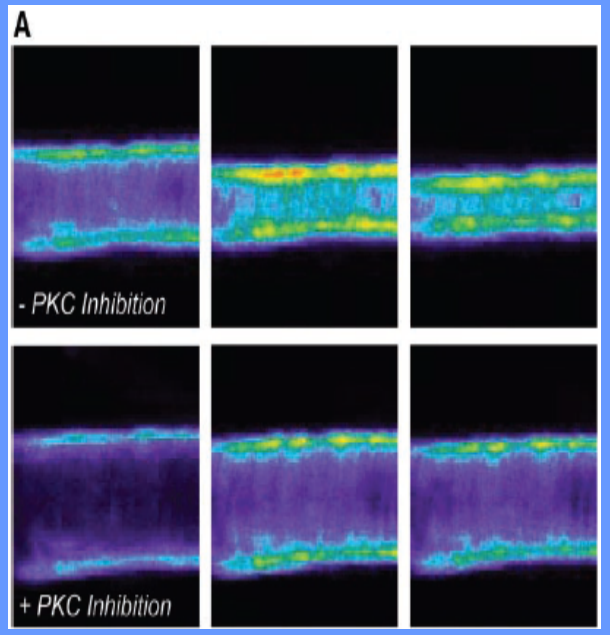
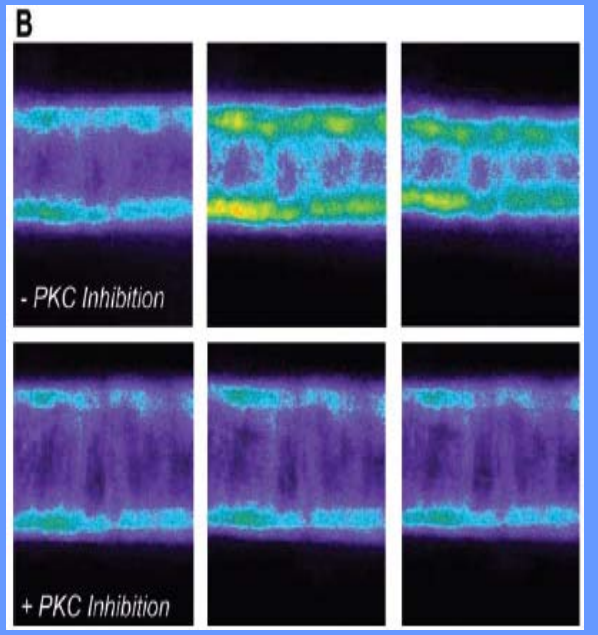


Fig. 4. Regulation of KCl contraction by Ca^{2+} and PKC in resistance arteries from SED and EX pigs. Confocal imaging of Ca_m using the Ca^{2+} indicator dye fluo 4 is shown in response to 60 mM KCl in the absence and presence of 1 μM CE in coronary resistance arteries (~100 μm ID) isolated from SED (*A*) and EX (*B*) male swine.



Results

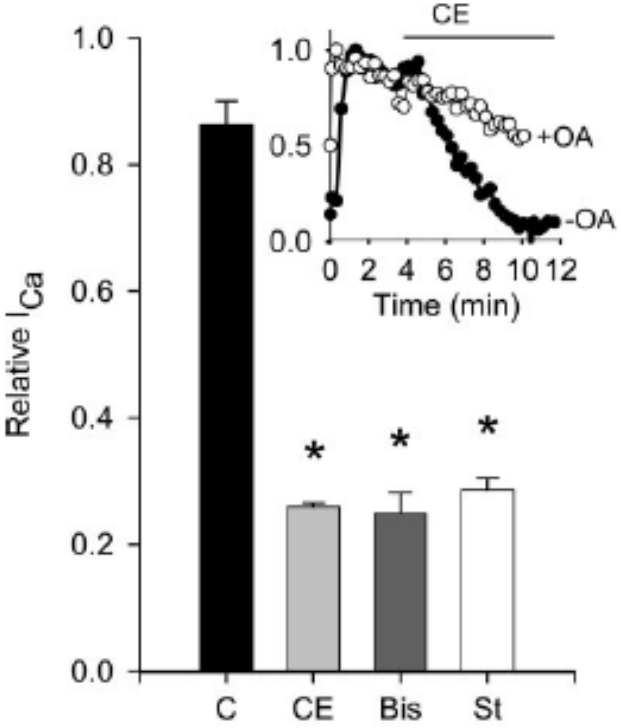


Fig. 5. Dependence of basal voltage-gated Ca^{2+} channel current (I_{Ca}) on PKC. Inhibition of I_{Ca} (10 mM external Ba^{2+}) by the specific PKC inhibitors CE (10 μM), bisindolylmaleimide (Bis, 10 μM), and staurosporine (St, 10 μM) is shown. Superfusion with CE, Bis, or St produced inhibition of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

- Dependence of depolarization-induced constriction on PKC:
  - Voltage gated calcium channel current in isolated smooth muscle cells was decreased with specific PKC inhibitors (chelelythrine, bisindolylmalemide, staurosporine)
  - The phosphatase inhibitor okadaic acid negated the chelethryrine effect, implying PKC-dependent phosphorylation of the voltage gated Ca^{2+} channel

Results

- PKC-α and PKC-□ levels in coronary resistance arteries:
  - Immunoblotting demonstrated equivalent PKC-□ levels (Ca^{2+} independent)
  - However, in exercised pigs, PKC- α levels were increased as well as levels of phosphorylated PKC- α

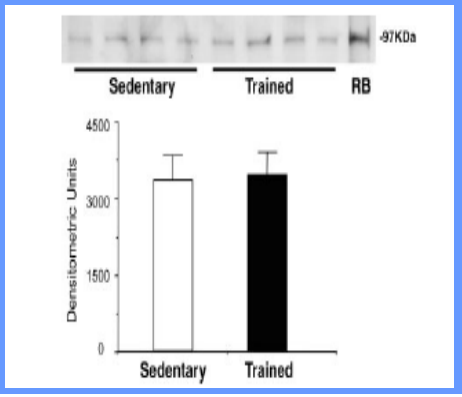
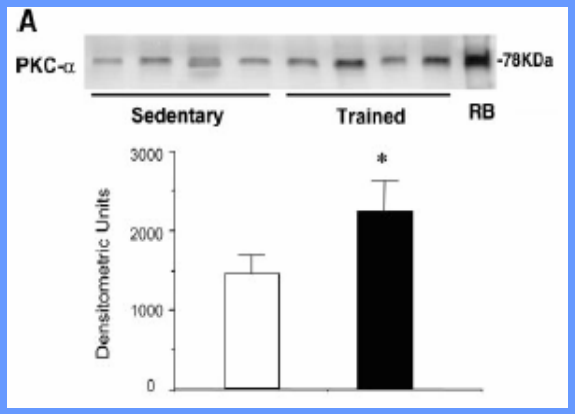


Fig. 6. PKC-ε immunoreactivity in coronary resistance arteries (CRAs). Equal amounts of protein were loaded per lane (15 μg). *Lanes 1–4*, CRAs isolated from SED (n = 4) pigs; *lanes 5–8*, CRAs isolated from EX (trained) pigs (n = 4). Each lane represents 3–4 CRAs per pig (~100 μM ID, see METHODS); isolated rat brain (RB) was utilized as a positive control. Values are means ± SE. PKC-ε (Ca^{2+}-independent PKC) levels were similar in CRAs isolated from EX and SED animals.



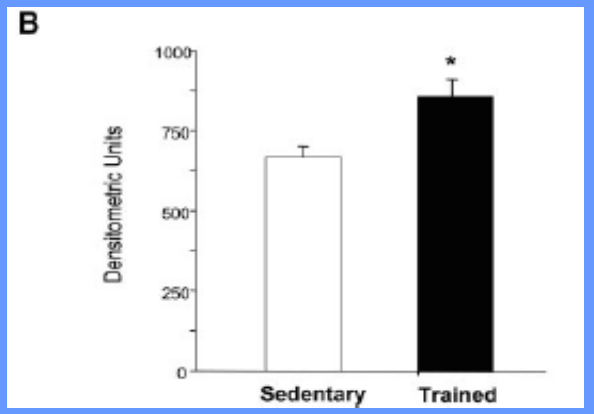


Fig. 7. PKC-α and phosphorylated PKC-α immunoreactivity in CRAs. Equal amounts of protein were loaded per lane (15 μg). *Lanes 1–4*, microvessels isolated from SED pigs (n = 4); *lanes 5–8*, CRAs isolated from EX pigs (n = 4). Each lane represents 3–4 CRAs per pig (~100 μM ID, see METHODS); isolated RB was utilized as a positive control. Values are means ± SE. $^*P <$ 0.01. PKC-α (Ca^{2+}-dependent PKC) levels were significantly greater in CRAs isolated from EX animals (*A*). Phosphorylated PKC-α levels ($pSer^{657}$) were similarly increased in EX and SED animals (*B*).

Discussion

- This study centered on the connection between myogenic tone and modifications of PKC signal transduction through exercise in coronary resistance arteries from pigs. The previously unreported discoveries are:
  - Exercise induces a greater myogenic constriction and sensitivity to PKC inhibition
  - KCl-induced constriction was reduced and inhibited to a greater degree by chelethyrine in exercised pigs vs. sedentary pigs
  - Ca^{2+}-dependent PKC-α levels were increased in excercised pigs but Ca^{2+}-independent PKC-□ levels were consistent between sedentary and active pigs
  - Intracellular calcium signals and voltage gated Ca^{2+} current were blocked by PKC-specific inhibitors

Discussion

- Exercise appears to increase PKC levels, enhancing myogenic tone and increasing resistance to changes in intraluminal pressure. PKC-α levels were increased in exercised pigs, which were also more affected by cheleyrthrine inhibition.
- The activation of PKC due to increased intraluminal pressure appears to be related to the phosphorylation of Ca^{2+} channels and subsequent ion influx. The exercised pigs showed decreased sensitivity to depolarization-induced constriction, implying a PKC-mediated nature of contraction.
- The increased levels of Ca^{2+}-dependant PKC-α isoform and degree of phosphorylation in exercised pigs suggest this as a transduction pathway in coronary smooth muscle after exercise training. This was further supported by the inhibitory effects of cheleythrine.
- The addition of general PKC inhibitors decreased Ca^{2+} current, suggesting that it is a regulatory interaction between PKC and voltage gated calcium channels that is involved in training-induced myogenic enhancement.

Conclusion

- The PKC- α isoform is involved in the development of exercise-training induced myogenic enhancement.
- Exercise induces changes in PKC- α, which affects the myogenic Ca^{2+} levels, suggesting an interaction between PKC and voltage gated calcium channels.

Questions and Future Directions

-Conflicting information on myogenic contraction, PKC -α , and Ca^{2+} sensitivity:

-does PKC -α induce a Ca^{2+} sensitive myogenic reaction or does increased Ca^{2+} alter the activity of PKC -α ?

-Myogenic involvement of PKC- and the role of MAPK and/or RhoA-Rho kinase:

-these enzymes have been shown to be active in response to mechanotransduction in relation to altered wall tension

-Use of cheleythrine as a PKC inhibitor:

-cheleythrine is non-specific to PKC isoenzymes

-Relationship between pathological and exercise-induced increases in myogenic activity:

-hypertension vs. exercise