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Myosin phosphatase isoforms and related transcripts in the pig coronary circulation and effects of exercise and chronic occlusion

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Abstract

Myosin phosphatase (MP) is a key target of signaling pathways that regulate smooth muscle tone and blood flow. Alternative splicing of MP targeting subunit (MYPT1) exon 24 (E24) generates isoforms with variable presence of a C-terminal leucine zipper (LZ) required for activation of MP by NO/cGMP. Here we examined the expression of MP and associated genes in a disease model in the coronary circulation. Female Yucatan miniature swine remained sedentary or were exercise-trained beginning eight weeks after placement of an ameroid constrictor around the left circumflex (LCX) artery. Fourteen weeks later epicardial arteries (~1 mm) and resistance arterioles (~125 μ m) were harvested and assayed for gene expression. MYPT1 isoforms were distinct in the epicardial arteries (E24–/LZ+) and resistance arterioles (E24+/LZ–) and unchanged by exercise training or coronary occlusion. MYPT1, CPI-17 and PDE5 mRNA levels were not different between arteries and arterioles while Kir2.1 and eNOS were 6.6-fold and 3.9-fold higher in the arterioles. There were no significant changes in transcript abundance in epicardial arteries of the collateralized (LCX) vs. non-occluded left anterior descending (LAD) territories, or in exercise-trained vs. sedentary pigs. There was a significant 1.2 fold increase in CPI-17 in collateral-dependent arterioles, independent of exercise, and a significant 1.7 fold increase in PDE5 in arterioles from exercise-trained pigs, independent of occlusion. We conclude that differences in MYPT1 E24 (LZ) isoforms, eNOS, and Kir2.1 distinguish epicardial arteries and resistance coronary arterioles. Up-regulation of coronary arteriolar PDE5 by exercise and CPI-17 by chronic occlusion could contribute to altered vasomotor responses and requires further study.

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Keywords

coronary artery disease; gene expression; gene conservation; splice variants; leucine zipper; smooth muscle; myosin phosphatase targeting subunit; exercise; coronary occlusion; coronary arteries and arterioles

Introduction

De-phosphorylation of myosin by the myosin phosphatase (MP) is the primary mechanism for vascular smooth muscle relaxation. Vasoconstrictor and vasodilator signals regulate vascular smooth muscle tone in part through inhibition or activation, respectively, of MP (reviewed in (Grassie et al., 2011; Hartshorne et al., 2004)). Based on the pattern of expression of MP subunits and their isoforms in different vascular and smooth muscle tissues, it has been proposed that their regulated expression may determine vessel-specific responses to these signals (reviewed in (Fisher, 2010)). The myosin phosphatase targeting (regulatory) subunit (MYPT1) Exon 24 (E24) is alternatively spliced, generating isoforms that contain or lack a C-terminal leucine zipper motif required for NO/cGMP-mediated activation of MP (Huang et al., 2004; Khatri et al., 2001; Surks et al., 1999). Protein Kinase C (and Rho Kinase)-potentiated myosin phosphatase inhibitor of 17 kDa (CPI-17) mediates contractile agonist-triggered inhibition of MP (reviewed in (Eto, 2009; Eto et al., 1997)). Variable levels of expression of CPI-17 correlate with sensitivity to agonist-mediated inhibition of MP (Kitazawa and Kitazawa, 2012; Su et al., 2013; Woodsome et al., 2001).

The contractile function and gene programs of the resistance arterioles that regulate blood flow are distinct from that of larger conduit arteries (reviewed in (Chilian, 1997; Tanko and Matrougui, 2002)). In the splanchnic circulation the expression of myosin phosphatase subunits is vessel-specific, developmentally regulated and modulates in disease (Payne et al., 2006; Payne et al., 2004; Zhang and Fisher, 2007) (reviewed in (Fisher, 2010)). In the coronary circulation functional differences between conduit and resistance vessels have been described (reviewed in (Camici and Crea, 2007; Duncker and Bache, 2008)), yet there is little understanding of the molecular basis of these functional differences particularly with regards to smooth muscle contractile function. In disease models of reduced coronary blood flow, such as chronic coronary occlusion induced by placement of an ameroid constrictor, a number of changes in the contractile function of the collateral-dependent arterioles have been described in sedentary or exercise-trained pigs (reviewed in (Heaps and Parker, 2011)). The molecular bases of these functional changes are mostly unknown. Given the critical role of MP in setting calcium sensitivity and vascular smooth muscle responses to constrictor and dilator signals, we hypothesized that the increased basal tone and increased responses to vasoconstrictors and vasodilators in this model is due to altered expression of MP subunits and related gene products that determine vascular function. In addition to the MP subunits, we measured eNOS and PDE5 given their critical role in determining vasodilator function, and Kir2.1 as a mediator of Endothelium-Derived Hyperpolarizing Factor (EDHF)-triggered vasorelaxation predominant in the smaller arteries (Quayle et al., 1996). These were measured in pig epicardial arteries and resistance arterioles from the chronically occluded

collateral-dependent left circumflex territory and reference non-occluded left anterior descending artery in sedentary and exercise-trained pigs.

Material and methods

Animals

Coronary arteries and arterioles were obtained from a well-established porcine model of chronic coronary artery occlusion and collateral-dependent perfusion (Heaps et al., 2000). Female Yucatan miniature pigs (Sinclair Research Center, Auxvasse, MO) were used according to the National Institutes of Health (NIH) Guide of “U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training” and approved by the Institutional Animal Care and Use Committee at Texas A&M University. Pigs were subjected to proximal left circumflex coronary (LCX) artery ameroid occlusion and allowed to recover for eight weeks. Animals were randomly assigned to sedentary (n=4) or exercise-training (n=4) groups, in which pigs underwent a progressive treadmill exercise training program for 14 weeks, 5 days/week or remained pen-confined, as described previously (Heaps et al., 2000; Xie et al., 2012; Xie et al., 2013). With the aid of a dissection microscope, epicardial arteries (~1 mm) and subepicardial arterioles (~125 µm) were dissected from the non-occluded (LAD) and collateral-dependent (LCX) myocardial regions, respectively. Vessels were cleaned of myocardium and trimmed of fat and connective tissue.

Immunoblots

Coronary epicardial arteries (~1 mm internal diameter; 1 cm length) and arterioles (~100–150 µm diameter; ~15–20 mm total length) were isolated from pig hearts obtained from a local abattoir, quick-frozen in liquid N₂ and stored at –80 °C for later immunoblot analysis as described previously (Fogarty et al., 2009). Arterial and arteriole lysates (20 µg of total protein) were subjected to SDS-polyacrylamide gel electrophoresis (4–20 % gradient gel), transferred to polyvinylidene difluoride (PVDF) membrane, and probed overnight with primary antibody. Primary antibodies included rabbit polyclonal antibodies that specifically recognize the MYPT1 LZ- isoform (1:1000; rabbit polyclonal IgG (Bhetwal et al., 2011)), β-actin (1:1,000; Novus Biologicals NB600-501), and GAPDH (1:1000; Advanced Immunochemical RGM2). Membranes were incubated with HRP conjugated secondary antibodies followed by SuperSignal West Dura Substrate (Thermo Scientific Pierce) detection. Scanning densitometry was used to quantify signal intensities which were normalized against the on-blot signals for β-actin or GAPDH.

Conventional PCR and Real-Time PCR

Total RNA from pig vessels was isolated using the RNeasy mini or micro kits (QIAGEN) as per instructions. The yield of total RNA was ~50 ng and ~500 ng from the arterioles and arteries, respectively. Pig bladder RNA was purchased from ZYAGEN (PR902). Total RNA was reverse transcribed by standard methods and splice variants of MYPT1 amplified by conventional PCR: sense primer 5'-gaaagccagctccatgata-3'; anti-sense primer 5'-tcaaggtccattttcatc-3' for pig and sense primer 5'-cgaagcggagacagataaga-3', anti-sense primer 5'-gtgtgcacagcgagcagga-3' for rat, then quantified as previously described (Khatri et

al., 2001) with minor modifications. cDNAs derived from purified RNA pools from endothelial cells (EC) and smooth muscle cells (SMC) from adult Wistar rat mesenteric arteries were provided by Dr. An Huang (Sun et al., 2011). MYPT1, CPI-17, PDE5, eNOS, Kir2.1, and smooth muscle α -actin message were quantified by real-time PCR using Taqman probes (Table 1) and Taqman Fast Advanced Master mix (Life Technologies) using one ng input cDNA. Relative expression was calculated as 2^{-C_t} normalized to smooth muscle α -actin.

Sequencing and Bioinformatical analysis of pig MYPT1

Pig MYPT1 PCR products were gel purified, sequenced and aligned with pig genomic sequence on the UCSC and Ensembl genome browsers (<http://genome.ucsc.edu>; <http://useast.ensembl.org>). Sequences were aligned using Clustal Omega from EMBL-EBI (<http://www.ebi.ac.uk/Tools/msa/clustalo/>).

Statistical analysis

Data are expressed as mean $\pm$ SEM or as fold change. Non-parametric Wilcoxon signed ranks test was used for comparing the differences between related samples of non-occluded and collateral-dependent groups and Mann-Whitney test was used for analysis of the independent samples of sedentary and exercise-trained pigs. $P < 0.05$ was considered statistically significant.

Results

Myosin phosphatase isoforms in the pig

Pig (*Sus scrofa*) genomic sequence in the publicly accessible genome browsers identified the MYPT1 gene with high conservation with other mammals (XM_003355700.2). This sequence aligned with other mammal MYPT1 sequences through exon 23. Poor alignment of exons 24–26 suggested error in sequencing or gene annotation. A cDNA of MYPT1 derived from pig aortic endothelial cells also only contained the first 17 exons (Hirano et al., 1999). To identify the 3' end of the pig MYPT1 transcript containing the alternative exon, PCR primers were designed based on the known pig 5' sequence and conserved mouse 3' sequence (Fig. 1 and Method). Sequencing of PCR products obtained from bladder and aorta cDNAs identified the E24 alternative exon and flanking sequence (Fig. 1 B and C). The E24 sequence is also present in the human genomic sequence though it has yet to be identified as an alternative exon. The pig and mouse MYPT1 E24 sequence differ by 2 nt (Fig. 1C). The nt difference at position 4 of the exon (A vs. G) is identical in human and rat, while the difference at position 7 (T vs. G) is unique to pig and since the full genomic sequence is not available, could represent an artifact of PCR. The complete sequences of other pig MP subunits M21, CPI-17, PP1c, and other gene products examined in this study are available in the genome browsers.

MYPT1 isoform expression in pig coronary arteries and arterioles

PCR with primers flanking the alternative exon yielded MYPT1 exon-included (LZ-) and exon-skipped (LZ+) splice variants in pig epicardial arteries and resistance arterioles (Fig. 2A). MYPT1 splice variant ratios were not changed in epicardial arteries or arterioles from

the chronically occluded left circumflex artery, and were not different between exercise-trained and sedentary pigs (Fig. 2A, n=2 each). In the resistance arterioles, the E24+ (LZ-) splice variant was predominant (90±4%, n=8). In contrast in the epicardial coronary arteries, the E24- (LZ+) isoform was predominant (78±3%), similar to the pig aorta and the large arteries of other species (Khatri et al., 2001; Payne et al., 2004). As MYPT1 is expressed in vascular endothelial and smooth muscle cells (Hirano et al., 1999; Kim et al., 2012; Ogut and Brozovich, 2008; Verin et al., 2000), we used cDNAs from RNA pools purified from the different cell types within rat small mesenteric arteries (Sun et al., 2011) to measure MYPT1 in ECs vs. SMCs. Approximately 83% of MYPT1 transcripts derive from the SMC population and 17% from the EC population. In the ECs MYPT1 was predominately of the E24- variant (73%), while in SMC MYPT1 was 89% E24+ (Fig. 2B), confirming that the E24+ variant derives from the SMCs in the small arteries. Corresponding well with the mRNA expression data, the LZ- motif of MYPT1 protein was 5.3 fold more abundant in the coronary arterioles as compared to the epicardial coronary arteries normalized to β -actin (Fig. 2C).

MP subunit levels are not different while eNOS and Kir2.1 are increased in arterioles

MP subunits MYPT1 and CPI-17 measured by real-time PCR were not different between arterioles and epicardial arteries (Fig. 3). PDE5, which catabolizes cGMP and is a target of the Sildenafil class of drugs, was also not different between arterioles and epicardial arteries. In contrast, Kir2.1 and eNOS were 6.6 times and 3.9 times more abundant, respectively, in arterioles vs. epicardial arteries (Fig 3). All values were normalized to smooth muscle α -actin which was not different between groups (epicardial arteries Ct= 24.94 ± 0.49, arterioles Ct=24.65 ± 0.73, mean ± SEM, n = 4; p > 0.05). Normalization of the data to β -actin yielded similar results (not shown).

Effect of coronary occlusion and exercise training on gene expression in epicardial arteries and arterioles

Transcript levels were compared in epicardial arteries and arterioles from the collateral-dependent LCX territory vs. the non-occluded control LAD territory of sedentary and exercise-trained pigs and normalized to smooth muscle α -actin. Smooth muscle α -actin levels were not different between groups. In the epicardial arteries there were no significant differences in the levels of eNOS, Kir2.1, MYPT1, CPI-17 or PDE5 amongst the four groups: sedentary non-occluded LAD, sedentary collateral-dependent LCX, exercise non-occluded LAD and exercise collateral-dependent LCX (data not shown). Nor was there any significant difference when comparing coronary arteries from exercise-trained vs. sedentary pigs, or non-occluded (LAD) vs. collateral-dependent (LCX) arteries. In resistance arterioles there was no significant change in the levels of these transcripts when the four groups were compared. However, when grouped, CPI-17 mRNA was significantly increased 1.2 fold in collateral-dependent arterioles as compared with non-occluded control arterioles (Fig. 4A), and PDE5 mRNA was increased significantly increased 1.7 fold in arterioles from exercise-trained pigs as compared to arterioles from sedentary pigs (Fig. 4B).

Discussion

Myosin phosphatase expression in coronary arteries vs. arterioles

Here we show that the pig coronary arterioles express nearly exclusively the E24-included isoform of MYPT1 coding for the LZ⁻ isoform. The larger epicardial arteries express predominately the E24-skipped isoform coding for the LZ⁺ isoform. This is similar to other vascular beds such as the mesenteric, renal and others where the smaller resistance arteries express the MYPT1 E24⁻ isoform and other components of the fast muscle gene program (DiSanto et al., 1997; Karim et al., 2004; Lu et al., 2008; Payne et al., 2004; Shiraishi et al., 2003) (reviewed in (Fisher, 2010)). A prior study measured MYPT1 protein in epicardial vs. small (diameter 500–700 μ m) pig coronary arteries (Ying et al., 2012). Consistent with the current study, the LZ⁺ isoform of MYPT1 (coded by E24⁻ splice variant) was less abundant in the smaller coronary arteries. However, the prior study did not measure LZ⁺/LZ⁻ ratios, and the current study examined much smaller vessels (diameter 125 μ m) more representative of resistance vessels. The prior study also reported that total MYPT1 protein was lower in smaller vs. larger coronary arteries, while in the current study there was no difference in mRNA as measured by quantitative PCR. The MYPT1 E24⁺ (LZ⁻) isoform expressed in the resistance arterioles is the isoform that is not activated by NO/cGMP in contrast to the MYPT1 E24⁻/LZ⁺ isoform expressed in the conduit arteries (Huang et al., 2004; Khatri et al., 2001; Payne et al., 2006), but the functional significance of this in vivo with respect to control of the coronary or any other circulation has yet to be established.

eNOS mRNA was 3.9 times more abundant in the resistance arterioles as compared to the epicardial conduit arteries. As eNOS is primarily expressed in the EC fraction of small arteries (Sun et al., 2011), the higher level of eNOS in the smaller arteries could reflect the lesser contribution of EC to the pool of RNA in the larger vessels. A prior study observed no difference in eNOS protein abundance in pig coronary arteries varying in size from >300 to 50 μ m internal diameter, while arterioles less than 50 μ m had 50% less eNOS protein (Laughlin et al., 2003; Woodman et al., 2001). The reason for the disparity between the two studies, other than the obvious difference of measuring mRNA vs. protein, is not clear. The real-time PCR assay used here is more quantitative than the Western blot used in the prior study. Different normalizers were used in the two studies. In the current study, the same relative difference was observed when normalized to smooth muscle α -actin or β -actin, while the prior study used GAPDH. A study quantifying mRNA and protein from the same animals is required to resolve these differences, a technically difficult feat due to the small size of the samples.

Kir2.1 expression in large vs. small coronary arteries

Kir2.1 is the main member of the Kir2.x family expressed in smooth muscle cells (reviewed in (de Boer et al., 2010)) and is required for Kir currents and contributes to K⁺-induced dilation (Chrissobolis and Sobey, 2003; Park et al., 2005; Zaritsky et al., 2000). In pig coronary arteries, the inward rectifier K⁺ current density was 25-fold greater in smooth muscle cells isolated from fourth-order branches vs. the main LAD artery (Quayle et al., 1996). The 6.6-fold greater abundance of Kir2.1 mRNA in pig arterioles vs. epicardial arteries observed in the current study is consistent with these functional studies, and is

consistent with K⁺ serving as an important metabolic vasodilator in the coronary and cerebral circulations (Park et al., 2005; Rosenblum, 2003; Zaritsky et al., 2000). This 6.6-fold greater expression of Kir2.1 in the coronary resistance arterioles vs. epicardial arteries is quite similar to the 6.1-fold higher expression of Kir2.1 in third-order vs. superior mesenteric arteries (Kim et al., 2005). Kir2.1 is expressed in ECs (Fang et al., 2005) but is predominately smooth muscle in origin (de Boer et al., 2010). Nonetheless a direct comparison of Kir2.1 expression levels in purified SMCs from conduit arteries vs. resistance arterioles is needed to support the premise that functional differences in Kir currents are generated by regulated expression of Kir2.1. The control mechanisms for the differences in gene expression between conductance and resistance artery smooth muscle remains to be defined.

Effects of exercise and coronary occlusion on MP and associated gene expression in pig coronary arteries and arterioles

Prior studies have shown that exercise training increases calcium sensitivity of endothelin-1 (ET-1) triggered force production in collateral-dependent resistance arterioles in this model of coronary occlusion (Robles et al., 2011; Xie et al., 2013). Based on pharmacological inhibitors, this appeared to be mediated by PKC signaling, of which the inhibitory subunit of MP, CPI-17, is a primary target (reviewed in (Eto, 2009)). In other contexts, the regulated expression of CPI-17 correlates with agonist-dependent calcium sensitization (Kitazawa and Kitazawa, 2012; Su et al., 2013; Woodsome et al., 2001). In the current study there was a small (1.2-fold) but significant increase in CPI-17 mRNA in arterioles from the collateral-dependent territory (LCX). Thus, further study is indicated to determine if changes in the expression or activity of this inhibitory subunit of MP underlie increased sensitivity to ET-1 or other vasoconstrictors signaling through PKC.

With regards to EDRF/NO mediated vasodilation, prior studies have been conflicting. One study showed that exercise in this pig model enhanced endothelial (NO)-dependent relaxation of collateral-dependent arterioles concordant with normalization of an ~25% decrease in eNOS mRNA (Griffin et al., 2001). However, a subsequent study showed no difference in NO-dependent vasodilation between non-occluded and collateral-dependent arterioles of exercise and sedentary pigs, while eNOS and p-eNOS (s1179) protein levels were increased with occlusion, independent of exercise (Xie et al., 2012). In the current study, there was no significant effect of exercise training or coronary occlusion on eNOS mRNA levels. Unlike studies of altered blood flow in the mesenteric circulation (Payne et al., 2004; Zhang and Fisher, 2007; Zhang et al., 2009), a shift in MYPT1 isoforms from E24+ (LZ-) to E24- (LZ+), predicted to increase sensitivity to NO/cGMP, was not observed. There was a significant 1.7 fold increase in PDE5 mRNA with exercise in the coronary arterioles but not in the larger coronary arteries. Induction of PDE5 has been observed in rat models of mesenteric occlusion where functional studies suggested that it attenuates increased NO signaling (Zhang et al., 2009). PDE5 is also induced in heart and lung in response to chronic pressure overload (Oishi et al., 2007; Vandewijngaert et al., 2013), and in resistance pulmonary arteries to hyperoxia (Farrow et al., 2008), and underscores the complex changes in gene expression that determine signaling through the NO/cGMP signaling pathway. To the best of our knowledge PDE5 inhibitor drugs have not

been tested for salutary effects on coronary blood flow in this model of chronic coronary occlusion. In other models, PDE5 inhibitors have been shown to increase coronary blood flow (de Beer et al., 2013; Houweling et al., 2012). In a pig model of myocardial infarction, PDE5 mRNA was reduced in coronary arterioles remote from the zone of infarction associated with attenuated coronary vasodilation to PDE5 inhibition, while the effect of exercise training was not investigated (Merkus et al., 2013).

Limitations

The small sample size and multiple comparators limit the power to detect small but significant changes in several of the gene products that showed trends. The costs of these large animal studies and limited amounts of arteriolar tissue are problematic. There was not sufficient sample to measure proteins in the coronary arterioles from the four groups, but generally we have found good correlation between the expression of MP subunit mRNA and protein, particularly in the chronic phase of disease models (Payne et al., 2004; Zhang and Fisher, 2007). The current study only examined a 22-week endpoint, while our studies in the mesenteric circulation observed dynamic changes in the expression of MP after artery ligation many of which had resolved by four weeks (Zhang and Fisher, 2007). Another difference in the models, in addition to the organ bed, is the gradual onset in this model vs. abrupt onset in the mesenteric artery ligation model. Lastly, in the current study selected gene targets were examined; a more complete analysis of the gene program would be informative but is precluded by the small size of the resistance arterioles.

Conclusions

Regulation of coronary blood flow occurs predominately at the level of the resistance arterioles of the micro-circulation. It is well appreciated that the function of the coronary micro-vasculature is distinct from that of the epicardial arteries and affected by exercise and chronic ischemia. The molecular bases of these differences are largely undefined no doubt due to the difficulty in molecular and biochemical characterization of the micro-vessels. The current study using highly sensitive PCR assays of select regulatory genes identifies differences in the expression of the MP regulatory subunit isoforms between the pig coronary resistance arterioles and epicardial arteries, and modest up-regulation of CPI-17 and PDE5 mRNA specifically in the coronary arterioles related to occlusion and exercise respectively. This provides a basis for further molecular and functional characterization of the coronary micro-vasculature under normal and disease conditions.

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Highlights

- Myosin phosphatase (MYPT1) splice variants were identified in the pig
- Differential expression of MYPT1, eNOS and Kir2.1 in coronary arterioles vs arteries
- Chronic coronary occlusion increase arteriolar CPI-17 gene expression
- PDE5 expression increased in exercise-trained pigs

Figure 1. Identification of the MYPT1 alternative Exon 24

A, MYPT1 genomic sequence alignments show conservation of exon-intron structure among pig, mouse and human (<http://genome.ucsc.edu>). The pig MYPT1 sequence and alignment is incomplete beyond exon 23. Black box indicates the 31nt alternative exon in mouse. B, Sequencing of RT-PCR products with primers as indicated identified exons 23–26 of pig MYPT1 with high conservation of exon sequences with other mammalian MYPT1. The alternative exon is filled. C, Alignment of mammalian MYPT1 31 nucleotide alternative exon 24 sequences using Clustal Omega from EMBL-EBI, * indicates identical nucleotide among all species.

Figure 2. MYPT1 isoforms in pig coronary arteries and resistance arterioles
A. MYPT1 E24+/- splice variants (coding for LZ-/LZ+ isoforms) were amplified by conventional PCR using a single pair of primers flanking the alternative exon and separated by gel electrophoresis. Band intensities were directly quantified and the percentage exon-included in each sample was calculated as E24+/total signal. Data is shown as the mean percentage exon included n= 2 each for coronary arteries and arterioles. Bladder and aorta are shown as standards for this assay. B. MYPT1 E24+/- splice variants from purified RNA pools derived from ECs and SMCs from rat mesenteric arteries, n = 2. C. Rabbit polyclonal antibody specific to the MYPT1 LZ- sequence was used to compare MYPT1 LZ- isoforms by Western blot in pig coronary arteries vs. arterioles, n= 2 each. Antibodies against GAPDH and β-actin were used as internal controls.

Figure 3. Myosin phosphatase subunits and other transcripts in pig coronary arteries and resistance arterioles

eNOS, Kir2.1, MYPT1, CPI-17 and PDE5 were measured in pig coronary arteries and resistance arterioles by real time PCR. Data is expressed as fold difference using 2^{-Ct} with normalization to smooth muscle α -actin and then to the epicardial coronary artery. eNOS and Kir2.1 were 3.9-fold and 6.6-fold higher in the resistance arterioles, respectively, while the other transcripts were not different between groups. Data is shown as Mean $\pm$ SEM, n=4. *, P<0.05.

Figure 4. Effect of coronary occlusion and exercise training on the expression of myosin phosphatase subunits and other transcripts in epicardial coronary arteries and coronary resistance arterioles

eNOS, Kir2.1, MYPT1, CPI-17 and PDE5 were measured in pig coronary resistance arterioles by real time PCR as above 22 weeks after placement of an ameroid constrictor around the left circumflex artery (LCX) and 14 weeks after randomization to sedentary or exercise-trained (treadmill: 5 days/week) groups. (A) CPI-17 mRNA is significantly increased by 1.2 fold in collateral-dependent arterioles vs. the non-occluded group. (B) PDE5 mRNA is significantly increased by 1.7 fold in arterioles from exercise-trained pigs as compared to sedentary pigs. Data is expressed as fold change using 2^{-Ct} with normalization to smooth muscle α -actin and then to the non-occluded LAD arteriole group (A) or the sedentary arteriole group (B). n=8. *, P<0.05.

Table 1

Gene	HGNC name	Taqman Assay ID	Assay Location	Product Length, bp
Smooth muscle α -actin	ACTA2	Ss04245588_m1	165	84
MYPT1	PPP1R12A	Ss03372900_m1	2092	72
CPI-17	PPP1R14A	Ss03384219_u1	348	58
PDE5A	PDE5A	Ss03376347_u1	656	99
eNOS	NOS3	Ss03383840_u1	3452	77
Kir2.1	KCNJ2	Ss03382889_u1	1153	79

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