The potential role of MLC phosphatase and MAPK signalling in the pathogenesis of vascular dysfunction in heart failure

Ozgur Ogut, Frank V. Brozovich*

Division of Cardiovascular Diseases, Mayo Medical School, Rochester, MN, USA

Received: July 30, 2008; Accepted: September 1, 2008

Abstract

The clinical syndrome of heart failure is associated with both a resting vasoconstriction and reduced sensitivity to nitric oxide mediated vasodilatation, and this review will focus on the role of myosin light chain (MLC) phosphatase in the pathogenesis of the vascular abnormalities of heart failure. Nitric oxide mediates vasodilatation by an activation of guanylate cyclase and an increase in the production of cGMP, which leads to the activation of the type I cGMP-dependent protein kinase (PKGI). PKGI then activates a number of targets that produce smooth muscle relaxation including MLC phosphatase. MLC phosphatase is a holoenzyme consisting of three subunits; a 20 kD subunit of unknown function, an ~38-kD catalytic subunit and a myosin targeting subunit (MYPT1). Alternative splicing of a 31 bp 3' exon generates MYPT1 isoforms, which differ by a COOH-terminus leucine zipper (LZ). Further, PKGI-mediated activation of MLC phosphatase requires the expression of a LZ MYPT1. Congestive heart failure is associated with a decrease in LZ MYPT1 expression, which results in a decrease in the sensitivity to cGMP-mediated smooth muscle relaxation. Beyond their ability to reduce afterload, angiotensin converting enzyme (ACE) inhibitors have a number of beneficial effects that include maintaining the expression of the LZ MYPT1 isoform, thereby conserving normal sensitivity to cGMP-mediated vasodilatation, as well as differentially regulating genes associated with mitogen activated protein kinase (MAPK) signalling. ACE inhibition reduces circulating angiotensin II and thus limits the downstream activation of MAPK signalling pathways, possibly preventing the alteration of the vascular phenotype to preserve normal vascular function.

Keywords: MYPT1 • nitric oxide • smooth muscle • vasoconstriction • vasodilatation • angiotensin

Clinical syndrome of heart failure

Patients with congestive heart failure (CHF) complain of reduced exercise capacity, dyspnea on exertion, orthopnea and lower extremity edema [22, 33]. These symptoms are secondary to a reduction in cardiac function, and to date, despite a number of advances in our understanding of the molecular mechanism(s) that result in CHF, the mortality for this disease is high and the only known cure is cardiac transplantation [14, 22, 33]. Thus, drug regimens are aimed at prolonging survival and reducing symptoms [14, 22, 33].

A number of abnormalities of cardiac muscle and the contractile proteins have been documented, and the cardiac phenotype in CHF is the subject of intense investigation (reviewed in [57, 65]. However, CHF is also characterized by an elevated baseline vascular tone and an impaired response to nitric oxide mediated vasodilatation [22, 52]. These changes in the vasculature are not compensatory, but rather impair the heart's ability to generate cardiac output sufficient to meet demand, and further exacerbate symptoms. The mechanism that leads to these changes in the vasculature is unknown, and whether changes in the contractile phenotype of vascular smooth muscle are responsible for these abnormalities had, until recently, not yet been the subject of investigation.

*Correspondence to: Frank BROZOVICH, Cardiovascular Diseases, Mayo Clinic, 200 1st Street SW, Rochester, MN 55905, USA.
Tel.: (507)284-3727
Fax: (507)538-6418
E-mail: brozovich.frank@mayo.edu
doi:10.1111/j.1582-4934.2008.00536.x
Neuroendocrine activation of the renin–angiotensin system (RAS) has been implicated in contributing to the abnormal vasoconstriction in CHF [33, 42]. However, recent studies have demonstrated that angiotensin II (Ang II) also modulates the endothelium and regulates vascular remodelling [16]. In cultured vascular smooth muscle cells (SMCs), Ang II activates nicotinamide adenine dinucleotide (NAD)/NADH oxidases and increases superoxide production, which results in enhanced nitric oxide catabolism and decreased availability [29]. Additionally, reactive oxygen species, superoxide anions and hydrogen peroxide, also stimulate hypertrophy and hyperplasia of vascular SMCs [44, 45, 47, 77]. Although decreased nitric oxide bioavailability would result in enhanced vascular tone, animal models and human studies have shown that both endothelium-dependent and -independent vasorelaxation are impaired in CHF [35, 37, 43].

**Regulation of smooth muscle contractility**

Activation of smooth muscle is dependent on the level of phosphorylation of the 20-kD regulatory myosin light chain (MLC20), which is determined by the relative activities of MLC kinase (MLCK) and MLC phosphatase [28, 56]. For years, the dogma was that activation and relaxation of smooth muscle was regulated by only the Ca$^{2+}$ dependence of MLCK, whereas MLC phosphatase was an unregulated housekeeping enzyme [30]. However, recent evidence indicates that the majority of signalling pathways for the regulation of vascular tone converge on MLC phosphatase [31, 69]. Thus, changes of vascular tone are critically dependent on the regulation of MLC phosphatase activity [31, 69].

MLC phosphatase isolated from smooth muscle is a homodimer consisting of three subunits (see [31] for review); an ~20-kD subunit, an ~38-kD catalytic subunit and a myosin targeting subunit (MYPT1) of 110–133 kD. MYPT1 isoforms can be generated by the inclusion or exclusion of a 41 aa central insert (CI) [67]. In addition to this CI, isoforms are also generated through alternative splicing of a 31 bp 3' exon; exon inclusion codes for a MYPT1 that lacks a COOH-terminus leucine zipper (LZ), while exon exclusion shifts the reading frame and codes for a LZ+ MYPT1 isoform [38]. Thus, there are four distinct MYPT1 isoforms differing due to the presence or absence of a CI and a LZ [31].

**Ca$^{2+}$ sensitization**

Agonist stimulation, compared to depolarization, has long been known to produce a higher force for a given Ca$^{2+}$ concentration [11], and this phenomenon has been coined agonist-induced Ca$^{2+}$ sensitization. The mechanism(s) leading to this sensitization of the contractile filaments have been the subject of several extensive reviews [68, 69]. Briefly, stimulation of G-protein coupled receptors activates a Rho kinase mediated pathway leading to the phosphorylation of MYPT1. MYPT1 phosphorylation at either Thr695 or Thr850 of the avian sequence [20] has been shown to inhibit MLC phosphatase activity [40], which results in an increase in MLC$^{2+}$ phosphorylation and force. However, other mechanisms have also been demonstrated to result in Ca$^{2+}$ sensitization, including a direct phosphorylation of MLC$^{2+}$ by both Zip-like kinase [55] and integrin-linked kinase [13]. Another mechanism for Ca$^{2+}$ sensitization involves CPI-17, a small protein which is a substrate for both Rho kinase and protein kinase C [41]; phosphorylated CPI-17 binds to the catalytic subunit of MLC phosphatase to inhibit the enzyme’s activity [18]. Whether any of these mechanisms contribute to the increase in vascular tone during CHF has yet to be investigated.

**Ca$^{2+}$ desensitization**

Nitric oxide is the classical agent to produce Ca$^{2+}$ desensitization [24, 71], and nitric oxide mediated, or flow-mediated, vasodilatation is a fundamental response of the vasculature [24]. In the vasculature, an increase in flow increases shear stress on endothelial cells, which stimulates nitric oxide production. Nitric oxide diffuses into the SMCs to activate the soluble pool of guanylate cyclase, thereby increasing the intracellular cGMP concentration. cGMP then activates type I cGMP-dependent protein kinase (PKG), which subsequently acts on the maxi K$^+$ channel to produce a hyperpolarization [2], the sarcoplasmic reticulum (SR) and voltage-dependent Ca$^{2+}$ channels to decrease Ca$^{2+}$ flux [23, 64], and also activates MLC phosphatase [71]. In addition, PKGI-dependent pathways for vasodilatation may include a phosphorylation of telokin [39, 73, 76] and HSP20 [63]. Thus, either endothelial dysfunction leading to a decrease in nitric oxide production, or a defect in a more distal part of the signalling pathway, may lead to SMC dysfunction and impaired flow-mediated vasodilatation. A decrease in sensitivity to nitric oxide mediated vasodilatation would result in a decreased vessel radius at any level of flow, which would contribute to the mechanism responsible for the resting vasoconstriction associated with CHF.

**MYPT1 isoforms and the sensitivity to cGMP**

A number of groups have demonstrated that the sensitivity to cGMP-mediated SMC relaxation correlates with the relative expression of LZ+/LZ– MYPT1 isoforms [38, 46, 58, 59, 79], suggesting that the relative expression of LZ+/LZ– MYPT1 isoforms determines the sensitivity of the smooth muscle to nitric oxide mediated vasodilatation [71]. Although there is strong evidence that the relative expression of LZ+/LZ– MYPT1 isoforms correlates with the sensitivity to cGMP-mediated relaxation, we have also demonstrated a direct casual relationship between LZ+/LZ– MYPT1 expression and the resultant sensitivity to cGMP-mediated smooth muscle relaxation [32]. For these studies, 4 recombinant adenoviruses containing the four endogenous avian MYPT1 isoforms (CI+/LZ+, CI+/LZ–, CI–/LZ+, CI–/LZ–) were prepared and transfected into cultured chicken gizzard SMCs. After
adeno virus infection, the exogenous MYPT1 subunit replaced the endogenous MYPT1 isof orm in the MLC phosphatase holo-enzyme. In cells over-expressing LZ- MYPT1 isof orms, the non- hydrolysable cGMP analogue 8Br-cGMP did not produce a signifi- cant decrease in MLC20 phosphorylation, while 8Br-cGMP resulted in a dose-dependent decrease in the level of MLC20 phospho-phylation in SMCs expressing a LZ+ MYPT1. These results demonstrate that the expression of the COOH-terminal LZ of MYPT1 is required for PKGI to activate MLC phosphatase during cGMP-mediated smooth muscle relaxation [32, 71].

cGMP and MYPT1 phosphorylation
As discussed above during agonist stimulation, a Rho kinase mediated MYPT1 phosphorylation at Thr696 of the mammalian sequence inhibits MLC phosphatase activity [68, 69]. During cGMP stimulation, several groups have demonstrated that MYPT1 is phosphorylated at Ser695 of the mammalian sequence [51, 75]. Phosphorylation of MYPT1 at Ser695 does not alter MLC phosphatase activity [51], but rather, decreases MYPT1 phosphorylation at Thr696 [51, 75]. Thus a cGMP-induced MYPT1 phosphorylation at Ser695, and a resulting decrease in MYPT1 phosphorylation at Thr696 would disinhibit MLC phosphatase and return its activity to baseline, resulting in a decrease in MLC20 phosphorylation and force.

MYPT1 phosphorylation at Ser695 cannot be the sole mecha- nism by which cGMP stimulation mediates MLC20 dephosphorylation [70]. Others have demonstrated that cGMP stimulation modulates the phosphorylation of CPI-17 [3, 19] as well as telokin [39, 73, 76], which result in an increase in MLC phosphatase activity. More importantly, the Ser695 phosphorylation does not increase MLC phosphatase activity [51], but rather decreases the level of Thr696 phosphorylation to disinhibit and return phosphatase activity to baseline levels [51, 75]. However, Ca²⁺ desen- sitization (MLC20 dephosphorylation at a constant [Ca²⁺]) occurs during all types of smooth muscle activation, including those that do not elicit MYPT1 phosphorylation at Thr696 [17, 55, 69, 70]. Thus similar to Ca²⁺ sensitization [68, 69], cGMP-mediated activa- tion of MLC phosphatase activity cannot be universally explained by changes in MYPT1 phosphorylation [70].

Vascular function and cGMP signalling
Changes in vascular function are associated with changes in blood pressure, but until recently, whether changes in vascular function produce hypotension and/or hypertension had not been demon- strated. Coffman's group [9], in an elegant series of experiments, demonstrated that peripheral and renal type 1 angiotensin II (AT1) receptors have equal and additive contributions to the regulation of blood pressure in mice. These studies demonstrated that mice lacking both renal and peripheral AT1 receptors (AT1 KO) had relative hypotension compared to wild type (WT) mice. Further, the blood pressure of mice lacking only renal AT1 receptors or only lacking peripheral AT1 receptors were equal and intermediate compared to WT and AT1 KO animals.

Further, Mendelsohn's group has demonstrated the importance of the interaction between PKGI-α and MYPT1 in blood pressure homeostasis [49]. These investigators generated mice with mutations in the NH₂-terminus LZ domain of PKGI-α to disrupt the ability of PKGI-α to interact with MYPT1. Compared to WT ani- mals, the mice were hypertensive and the smooth muscle was less sensitive to both ACh and cGMP-mediated relaxation. Mendelsohn's work further demonstrates that the PKGI-α-MYPT1 signalling pathway is important for the maintenance of normal vascular tone and blood pressure. These results suggest that any decrease in PKGI-α- MYPT1 signalling (i.e. a decrease in LZ+ MYPT1 expression), would result in a decrease in the activity of MLC phosphatase. The decrease in MLC phosphatase activity then produces an increase of vascular tone, which results in hypertension. On the other hand, an increase in PKGI-α-MYPT1 signalling (i.e. an increase in LZ+ MYPT1 expression) increases MLC phosphatase activity, which decreases vascular tone and blood pressure.

Captopril therapy and MYPT1 expression in HF
Ang II is synthesized locally by the endothelium and it results in vasoconstriction through its effects on both the endothelium and vascular smooth muscle [53, 54]. Additionally, Ang II acti- vates both NF-κB, which increases the expression of interleukin (IL)-6 and tumour necrosis factor (TNF)-α to induce a pro-inflammatory state at the level of the vascular intima [16, 61, 74], and membrane oxidases (NADH/NADPH oxidases), which generate reactive superoxide anions to decrease nitric oxide bioavailability [78]. Hence in the setting of CHF, the unique ability of angiotensin converting enzyme (ACE) inhibitors to counter these deleterious effects of Ang II could help explain their ability to reduce cardiovascular morbidity and mortality [8, 22, 33, 61]. Moreover, despite only modest blood pressure reduc- tion with ACE inhibitors [22], the same improvement in survival has not been observed with vasodilators; i.e. prazosin [50], suggesting that there are incremental benefits to ACE inhibitors in addition to relaxing smooth muscle to produce vasodilata- tion; i.e. modulating the relative expression of the LZ+ MYPT1 isoform to preserve normal sensitivity to nitric oxide mediated vasodilatation.

In an animal model of CHF, Abassi et al. [1] have demonstrated that angiotensin receptor blockade normalizes the impaired vasodilatory responses to ACh. These investigators also demon- strated that the production of nitric oxide was normal [1], which suggests that a defect in nitric oxide mediated vasodilatation is at the level of the smooth muscle. These data could suggest that neuronal mechanism activation may alter MYPT1 LZ+-/LZ⁻ isoform expression, and ACE inhibitor therapy could prevent and/or reverse the change in LZ+/LZ⁻ MYPT1 expression.
To explore these questions, we used a rat infarct model of CHF [12, 25, 26, 62, 72]. In our studies [5, 36], between 2 and 4 weeks following left anterior descending coronary artery (LAD) ligation, there was a significant decrease in the expression of the LZ+ MYPT1 isoform in arterial smooth muscle, and the decrease in LZ+ MYPT1 isoform expression produced a decrease in the sensitivity to cGMP-mediated smooth muscle relaxation [5].

Captopril has been shown in the rat infarct model to normalize haemodynamic parameters and reduce infarct size [62]. In clinical use, captopril has been demonstrated to prevent the progression of heart failure and improve survival in human beings after acute myocardial infarction [61]. In our studies [5], we demonstrated that ACE inhibition, but not prazosin therapy, both preserved the normal level of MYPT1 LZ+ isoform expression and maintained the normal sensitivity to cGMP-mediated smooth muscle relaxation [5]. These data demonstrate that ACE inhibition maintains the normal vascular phenotype, and preserves the normal vasodilatory response to nitric oxide. Compared to other forms of therapy, the ability of ACE inhibitors to alter LZ+ MYPT1 isoform expression may explain why this drug therapy improves survival in persons with heart failure [8, 22, 33, 61].

**Captopril therapy and gene expression**

As discussed above between 2 and 4 weeks following a myocardial infarction (MI), two fundamental vascular responses change: there is a significant decrease in both the expression of the LZ+ MYPT1 and the sensitivity to cGMP-mediated vasodilatation [5]. However, captopril treatment of rats following an MI preserves both normal LZ+ MYPT1 isoform expression and sensitivity to cGMP [5]. Thus, we reasoned that between 2 and 4 weeks following an MI, a change in gene expression could contribute to the vascular abnormalities associated with CHF. We analysed gene expression using the rat genome microarray (Affymetrix) and then confirmed differential gene expression with real-time PCR. We found that captopril therapy differentially regulated gene expression [4]: at 2 weeks after infarct, the expression of three genes (MIR16, Agt, Cxcl12) was increased with captopril therapy and then subsequently fell to the control level at 4 weeks. For seven genes (Taok1, Raf1, IL-1β, Fmr1, Rock2, Baat, Gls2), there was no difference between control and captopril treatment at 2 weeks, but at 4 weeks captopril depressed the expression of these genes. Captopril's ability to modulate expression of several of these genes could be linked to the mechanism explaining the vascular abnormalities associated with CHF.

The decrease in Rho kinase (Rock2) expression with captopril therapy is consistent with ACE-inhibition decreasing systemic vascular resistance. Ang II stimulation of the AT1 receptor leads to a G-protein-dependent activation of the Rho/Rho kinase signalling cascade, which leads to an inhibition of MLC phosphatase and vasoconstriction (reviewed in [68, 69]). Thus, the decrease Ang II as a result of captopril therapy would decrease Rho/Rho kinase signalling and decrease afterload, and further a decrease in Rho kinase expression would enhance the decrease in afterload produced by this signalling pathway.

At 2 weeks after infarction with captopril, there was increased expression of the membrane interacting protein of RGS16 (MIR16), a membrane glycerophosphodiester phosphodiesterase involved in modulating G-protein-mediated signalling [80]. A decrease in both the duration and intensity of signalling by the G-protein subunits of the AT1 receptor is mediated by RGS binding to the Gαi subunit [48]. Therefore, a loss of RGS activity would remove its inhibitory effect on the AT1 receptor, resulting in increased stimulation by Ang II [48]. Captopril attenuated the loss of MIR16 expression, which would restore the inhibition of the RGS on Ang II signalling.

ACE inhibition also suppressed the expression of both Taok1, a rat homologue of the MAPK kinase kinase (MAP3K), a known activator of p38 MAPK [6] and also Raf-1, an activator of p42/44 MAPK [60]. Activation of both p38 MAPK and p42/44 MAPK signalling leads to the activation of PHAS-1 and a resulting release of a eukaryotic initiation factor-4E (eIF4E) from PHAS-1, which has been demonstrated to initiate translation for vascular SMC hypertrophy [47]. Additionally, eIF4E controls translation efficiency by regulating nuclear mRNA export, mRNA stability, and the preferential loading of mRNAs onto ribosomes [10]. We also found that one of the differentially expressed genes (LOC297481) between 2 and 4 weeks is an EST with sequence homology to eIF4E, which is consistent with a MAPK-mediated pathway effecting LZ+ MYPT1 expression.

Multiple cytokines are up-regulated by p38 MAPK, including TNF-α, IL-1α/β, IL-6 and IL-10 [21, 64]. Both IL-1β and TNF-α can also activate p38 MAPK [27], which results in a positive feedback cascade of Ang II-mediated p38 MAPK signalling [64]. Our data indicated that at 2 weeks after infarction, IL-1β expression was increased in both captopril and placebo-treated groups. However at 4 weeks after infarction, captopril suppressed IL-1β gene expression, while IL-1β production remained elevated with placebo-treatment. Captopril's ability to decrease IL-1β would also contribute to the suppression of p38 MAPK signalling, since IL-1β creates a positive feedback loop by both activating p38 MAPK and up-regulating the AT1 receptor [27]. Captopril's ability to suppress IL-1β expression at 4 weeks after MI could be produced by the suppression Taok1 expression, and these data could suggest that the levels of Taok1 and IL-1β may be biomarkers for vascular dysfunction.

**Conclusions**

Vascular dysfunction is a known complication of heart failure. These abnormal responses of the vasculature include both a resting vasoconstriction and decrease in sensitivity to nitric oxide mediated vasodilatation. Data suggest that the decrease in sensitivity to nitric oxide mediated vasodilatation can be attributed to both
a decrease in nitric oxide due to endothelial dysfunction, as well as a defect at the level of the smooth muscle evident by the decrease in the expression of the LZ+ MYPT1 isof orm. The decrease in LZ+ MYPT1 expression produces a decrease in sensitivity to nitric oxide mediated vasodilatation, and the resulting decrease in response to nitric oxide will blunt flow-mediated vasodilatation, which would contribute to a resting vasoconstriction.

Both p38 MAPK and p42/44 MAPK signalling activate a number of transcription factors [15, 34, 46, 60] and homeobox genes [7]. We have demonstrated that treatment with the ACE inhibitor, captopril, alters the vascular phenotype to preserve normal LZ+ expression and sensitivity to cGMP-mediated vasodilatation [5, 36]. A reduction in expression of these MAPK signalling pathways could alter transcription to maintain LZ+ MYPT1 expression.

Nonetheless, data suggest a potential role of MAPK signalling in the pathogenesis of vascular dysfunction associated with CHF. Along with the pleomorphic effect of MAPK in triggering vascular SMC hypertrophy and proliferation, the ability of Ang II to modulate gene expression augments vascular tone. Hence with rational drug design, blocking the MAPK cascade could reverse the vascular dysfunction in patients with heart failure.

Acknowledgements

This study was supported by NIH grants HL69894 and HL64137 (to F.V.B.) and HL78845 (to O.O.).

References

1. Abassi ZA, Gurbanov K, Mulroney SE, Potlog C, Opgenorth TJ, Hoffman A, Haramati A, Winaver J. Impaired nitric oxide-mediated renal vasodilation in rats with experimental heart failure: role of angiotensin II. Circulation. 1997; 96: 3655–64.
2. Allioua A, Tanaka Y, Wallner M, Hofmann F, Ruth P, Meera P, Toro L. The large conductance, voltage-dependent, and calcium-sensitive K channel, Hslo, is a target of cGMP-dependent protein kinase phosphorylation in vivo. J Biol Chem. 1998; 273: 32995–6.
3. Bonnevier J, Arner A. Actions down-stream of cyclic GMP/protein kinase G can reverse protein kinase C-mediated phosphorylation of CPI-17 and Ca2+ sensitization in smooth muscle. J Biol Chem. 2004; 279: 28998–9003.
4. Chen FC, Brozovich FV. Gene expression profiles of vascular smooth muscle show differential expression of mitogen-activated protein kinase pathways during captopril therapy of heart failure. J Vasc Res. 2008; 45: 445–54.
5. Chen FC, Ogut O, Rhee AY, Holt BD, Brozovich FV. Captopril prevents myosin light chain phosphatase isoform switching to preserve normal cGMP-mediated vasodilatation. J Mol Cell Cardiol. 2006; 41: 488–95.
6. Chen Z, Raman M, Chen L, Lee SF, Gilman AG, Cobb MH. TAO (thousand-and-one amino acid) protein kinases mediate signaling from carbachol to p38 mitogen-activated protein kinase and ternary complex factors. J Biol Chem. 2003; 278: 22278–83.
7. Clempus RE, Griending KK. Reactive oxygen species signaling in vascular smooth muscle cells. Cardiovasc Res. 2006; 71: 216–25.
8. Cohn JN, Johnson G, Ziesche S, Cobb F, Tristani F, Smith R, Dunkman WB, Loeb H, Wong ML, Bhat G, Goldman S, Fletcher RD, Doherty J, Hughes CV, Carson P, Cintron G, Shabetai R, Haakenson C. A comparison of enalapril with hydralazine isosorbide dinitrate in the treatment of chronic congestive heart failure. New Engl J Med. 1992; 325: 303–10.
9. Crowley SD, Gurely SB, Oliverio MI, Pazmino AK, Griffiths R, Flannery PJ, Spurney RF, Kim HS, Smithies O, Le TH, Coffman TM. Distinct roles for the kidney and systemic tissues in blood pressure regulation by the renin-angiotensin system. J Clin Invest. 2005; 115: 1092–9.
10. Cumkovic B, Topisirovic I, Borden KL. Controlling gene expression through RNA regulation: the role of the eukaryotic translation initiation factor eIF4E. Cell Cycle. 2007; 6: 65–9.
11. DeFeo TT, Morgan KG. Calcium-fore relationships as detected with aequorin in two different vascular smooth muscles of the ferret. J Physiol. 1985; 369: 269–82.
12. Delp MD, Duan C, Mattson JP, Musch TI. Changes in skeletal muscle biochemistry and histology relative to fiber type in rats with heart failure. J Appl Physiol. 1997; 83: 1291–9.
13. Deng JT, Van Lierop JE, Sutherland C, Walsh MP. Ca2+-independent smooth muscle contraction – A novel function for integrin-linked kinase. J Biol Chem. 2001; 276: 16365–73.
14. Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the VESNARINone trial (VEST). Circulation. 2001; 103: 2055–9.
15. Dodeler F, Schulze-Koops H. The p38 mitogen-activated protein kinase signaling cascade in CD4 T cells. Arthritis Res Ther. 2006; 8: 205.
16. Dzau VJ. Theodore Cooper Lecture: Tissue angiotensin and pathobiology of vascular disease: a unifying hypothesis. Hypertension. 2001; 37: 1047–52.
17. El-Toukhy A, Given AM, Ogut O, Brozovich FV. PH-1 interacts with the catalytic subunit of myosin light chain phosphatase to produce a Ca2+-independent increase in MLC20 phosphorylation and force in avian smooth muscle. FEBS Lett. 2006; 580: 779–84.
18. Eto M, Ohmori T, Suzuki M, Furuya K, Morita F. A novel protein phosphatase-1 inhibitory protein potentiated by protein kinase C. Isolation from porcine aorta media and characterization. J Biochem. 1995; 118: 1104–7.
19. Ettcr EF, Eto M, Wardle RL, Brautigan DL, Murphy RA. Activation of myosin light chain phosphatase in intact arterial smooth muscle during nitric oxide-induced relaxation. J Biol Chem. 2001; 276: 34681–5.
20. Feng JH, Ho M, Ichikawa K, Isaka N, Nishikawa M, Hartshorne DJ, Nakano T. Inhibitory phosphorylation site for Rho-associated kinase on smooth muscle myosin phosphatase. J Biol Chem. 1999; 274: 37385–90.
lates NADH and NADPH oxidase activity in Angiotensin II stimu-

Gong MC, Cohen P, Kitazawa T, Ikebe M, Francis GS, Cohn JN.

Heart failure: mechanisms of cardiac and vascular dysfunc-

Kaibuchi K. Myosin phos-

the role of myosin light chain

with drug treatment in heart failure: what have trials taught? Am J Cardiol. 2003; 91: 121–2.

Li PF, Dietz R, von Harsdorf R. Reactive oxygen species induce apoptosis of vascu-

Lu Y, Zhang H, Gokina N, Mandaal M, Sato O, Ikebe M, Osol G, Fisher SA.

Uterine artery myosin phosphatase iso-

Lyle AN, Griendling KK. Modulation of vascular smooth muscle signaling by reactive oxygen species. Physiology (Bethesda). 2006; 21: 269–80.

Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular sys-

Miller RR, Awan NA, Maxwell KS, Mason DT. Sustained reduction of cardiac imped-

Negrao CE, Hamilton MA, Fonarow GC, Hage A, Morighici JD, Middlekauff HR.

Nakamura K, Koga Y, Sakai H, Homma K, Ikebe M. cGMP-dependent relaxation of smooth muscle is coupled with the change in the phosphorylation of myosin phos-

Nikolic SD, Forman R, LeJemtel TH. Contractile proteins in the infarcted overloaded

Ventricular function and con-

Reactive oxygen species induce apoptosis of smooth muscle cell. FEBS Lett. 1997b; 404: 249–52.

Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherosclerosis: part I: oxidative stress and atherosogenesis. Circulation. 2002a; 105: 393–8.

Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherosclerosis: part II: AT(1) receptor regulation. Circulation. 2002b; 105: 530–6.

Nirito N, Ikebe M. Zipper-interacting protein kinase induces Ca2+-free smooth muscle contraction via myosin light chain phosphorylation. J Biol Chem. 2001; 276: 29567–74.

Ogut O, Brozovich FV. Determinants of the contractile properties in the embryonic chicken gizzard and aorta. Am J Physiol. 2000; 279: C1722–32.

22. Francis GS, Cohn JN. Heart failure: mechanisms of cardiac and vascular dysfunction and the rationale for pharmacologic intervention. FASEB J. 1990; 4: 3068–75.

23. Fukao M, Mason HS, Britton FC, Kenyon JL, Horowitz B, Keef KD. Cyclic GMP-dependent protein kinase activates cloned BKCa channels expressed in mammalian cells by direct phosphorylation at serine 1072. J Biol Chem. 1999; 274: 10927–35.

24. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980; 288: 373–6.

25. Geenen DL, Malhotra A, Liang D, Scheuer J. Ventricular function and contractile proteins in the infarcted overloaded rat heart. Cardiovasc Res. 1991; 25: 330–6.

26. Geenen DL, Malhotra A, Scheuer J. Regional variation in rat cardiac myosin isoenzymes and ATPase activity after infarction. Am J Physiol. 1989; 256: H745–50.

27. Gong Y, Valbracht J, Lotz M. Selective activation of the mitogen-activated protein kinase subgroups c-Jun NH2 terminal kinase and p38 by IL-1 and TNF in human articular chondrocytes. J Clin Invest. 1996; 98: 2425–30.

28. Gong MC, Cohen P, Kitazawa T, Ikebe M, Masuo M, Somiya AP, Somiya AV. Myosin light chain phosphatase activities and the effects of phosphatase inhibitors in tonic and phasic smooth muscle. J Biol Chem. 1992; 267: 14662–8.

29. Griendling KK, Minieri CA, Oliverenshaw JD, Alexander RW. Angiotensin II stimu-

lates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res. 1994; 74: 1141–8.

30. Hartshorne DJ. Biochemistry of the Contractile Process in Smooth Muscle. In: Johnson LR editor. Physiology of the Gastrointestinal Tract. New York: Raven Press; 1987. pp. 432–82.

31. Hartshorne DJ, Ito M, Erdidi F. Myosin light chain phosphatase: subunit composi-

tion, interactions and regulation. J Muscle Res Cell Motil. 1998; 19: 325–41.

32. Huang QQ, Fisher SA, Brozovich FV. Unzipping the role of myosin light chain phosphatase in smooth muscle cell relaxation. J Biol Chem. 2004; 279: 597–603.

33. Jessup M, Brozena S. Heart failure. N Engl J Med. 2003; 348: 2007–18.

34. Johnson GL, Lapradat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science. 2002; 296: 1911–2.

35. Kaiser L, Spickard RC, Olivier NB. Heart failure depresses endothelium-dependent responses in canine femoral artery. Am J Physiol. 1989; 256: H962–7.

36. Karim SM, Rhee AT, Given AM, Faux MD, Hoit BD, Brozovich FV. Vascular reactivity in heart failure. Role of myosin light chain phosphatase. Circ Res. 2004; 95: 612–8.

37. Katz SD, Biasucci L, Sabha C, Strom JA, Jondeau G, Galvao M, Solomon S, Nikolid SC, Forman R, LeJemtel TH. Impaired endothelium-mediated vasodilatation in the peripheral vasculature of patients with congestive heart failure. J Am Coll Cardiol. 1992; 19: 918–25.

38. Khatri JJ, Joyce KM, Brozovich FV, Fisher SA. Role of myosin phosphatase isoforms in cGMP-mediated smooth muscle relaxation. J Biol Chem. 2001; 276: 37250–7.

39. Kromov AS, Wang H, Choudhury N, McDuffie M, Herring BP, Nakamoto R, Owens GK, Somiya AP, Somiya AV. Smooth muscle of telokin-deficient mice exhibits increased sensitivity to Ca2+ and decreased cGMP-induced relaxation. Proc Natl Acad Sci USA. 2006; 103: 2440–5.

40. Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng JH, Nakano T, Okawa K, Iwamatsu A, Kaibuchi K. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science. 1996; 273: 245–8.

41. Kitazawa T, Eto M, Woodsome TP, Kaibuchi K. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science. 1996; 273: 245–8.

42. Konstam MA. Improving clinical outcomes with drug treatment in heart failure: what have trials taught? Am J Cardiol. 2003; 91: 9–14.

43. Kubo SH, Rector TS, Bank AJ, Williams RE, Helfetz SM. Endothelium-dependent vasodilation is attenuated in patients with heart failure. Circulation. 1991; 84: 1589–96.

44. Li PF, Dietz R, von Harsdorf R. Differential effect of hydrogen peroxide and superox-

idion on apoptosis and proliferation of vascular smooth muscle cells. Circulation. 1997a; 96: 3602–9.

45. Li PF, Dietz R, von Harsdorf R. Reactive oxygen species induce apoptosis of vascular smooth muscle cell. FEBS Lett. 1997b; 404: 249–52.
57. Palmiter RA, Solaro RJ. Molecular mechanisms regulating the myofilament response to \( \text{Ca}^{2+} \): implications of mutations causal for familial hypertrophic cardiomyopathy. Basic Res Cardiol. 1997; 92: 63–74.

58. Payne MC, Zhang HY, Proscodico T, Joyce KM, Koga Y, Ikebe M, Fisher SA. Myosin phosphatase isoform switching in vascular smooth muscle development. J Mol Cell Cardiol. 2006; 40: 274–82.

59. Payne MC, Zhang HY, Shirasawa Y, Koga Y, Ikebe M, Benoit JN, Fisher SA. Dynamic changes in expression of myosin phosphatase in a model of portal hypertension. Am J Physiol. 2004; 286: H1801–10.

60. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev. 2001; 22: 153–83.

61. Pfeffer MA, Braunwald E, Moje LA, Basta L, Brown EJ, Cuddy TE, Davis BR, Getelman EM, Goldman S, Flaker GC, Klein M, Lamas GA, Packer M, Rueloue J, Rutherford J, Wertheimer JH, Hawkins CM. Effect of captopril on mortality and morbidity in patients with left ventricular enlargement trial. New Engl J Med. 1992; 327: 669–77.

62. Pfeffer MA, Pfeffer JM, Fishbein MC, Fitcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. Circ Res. 1979; 44: 503–12.

63. Rembold CM, Foster DB, Strauss JD, Wingard CJ, Van Eky JE. cGMP-mediated phosphorylation of heat shock protein 20 may cause smooth muscle relaxation without myosin light chain dephosphorylation in swine carotid artery. J Physiol. 2000; 524: 865–78.

64. Schmidt HH, Lohmann SM, Walter U. The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. Biochim Biophys Acta. 1993; 1178: 153–75.

65. Schoenfeld JR, Vasser M, Jhurani P, Ng P, Hunter JJ, Ross J Jr, Chien KR, Lowe DG. Distinct molecular phenotypes in murine cardiac development, growth, and hypertrophy. J Mol Cell Cardiol. 1998; 30: 2269–80.

66. Sekiguchi K, Li X, Coker M, Flesch M, Barger PM, Sivasubramanian N, Mann DL. Cross-regulation between the renin-angiotensin system and inflammatory mediators in cardiac hypertrophy and failure. Cardiovasc Res. 2004; 63: 433–42.

67. Shimizu H, Ito M, Miyahara M, Ichikawa K, Okubo S, Konishi T, Naka M, Tanaka T, Hirano K, Hartshorne DJ, Nakano T. Characterization of the myosin-binding subunit of smooth muscle myosin phosphatase. J Biol Chem. 1994; 269: 30407–11.

68. Somlyo AP, Somlyo AV. Signal transduction by G-proteins, Rho-kinase and protein kinase C: implications of mutations of telokin in muscular dys trophy. Cardiovasc Res. 2004; 63: 433–42.

69. Somlyo AP, Somlyo AV. Ca\(^{2+}\) sensitivity of smooth muscle and nonmuscle myosin II: modulated by G-proteins, kinases, and myosin phosphatase. Physiol Rev. 2003; 83: 1325–58.

70. Somlyo AV. Cyclic GMP regulation of myosin phosphatase: a new piece for the puzzle? Circ Res. 2007; 101: 645–7.

71. Surks HK, Mochizuki N, Kasai Y, Georgescu SP, Tang KM, Ito M, Lincoln TM, Mendelsohn ME. Regulation of myosin phosphatase by a specific interaction with cGMP-dependent protein kinase \( \text{I}_\alpha \). Science. 1999; 286: 1583–7.

72. Symons JD, Stebbins CL, Musch TI. Interactions between angiotensin II and nitric oxide during exercise in normal and heart failure rats. J Appl Physiol. 1999; 87: 574–81.

73. Walker LA, MacDonald JA, Liu XP, Nakamoto RK, Haystead TA, Somlyo AV, Somlyo AP. Site-specific phosphorylation and point mutations of telokin modulate its \( \text{Ca}^{2+}\)-desensitizing effect in smooth muscle. J Biol Chem. 2001; 276: 24519–24.