



Published in final edited form as:

*J Cell Mol Med.* 2008 December ; 12(6A): 2158–2164. doi:10.1111/j.1582-4934.2008.00536.x.

## The Potential Role of MLC Phosphatase and MAPK Signaling in the Pathogenesis of Vascular Dysfunction in Heart Failure

Ozgur Ogut and Frank V Brozovich

Division of Cardiovascular Diseases, Mayo Medical School, Rochester, MN 55906

### Abstract

The clinical syndrome of heart failure is associated with both a resting vasoconstriction and reduced sensitivity to NO mediated vasodilatation, and this review will focus on the role of MLC phosphatase in the pathogenesis of the vascular abnormalities of heart failure. NO 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 (PKG1). PKG1 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 kDa subunit of unknown function, a ~38 kDa catalytic subunit and a myosin targeting subunit (MYPT1). Alternative splicing of a 31 bp 3' exon can generate MYPT1 isoforms, which differ by a COOH-terminus leucine zipper (LZ). Further, PKG1 mediated activation of MLC phosphatase requires the expression of a LZ+ MYPT1. CHF 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 MAPK signaling. ACE inhibition reduces circulating angiotensin II and thus limits the downstream activation of MAPK signaling 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 (Francis & Cohn, 1990; Jessup & Brozena, 2003). 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 (Deswal et al., 2001; Francis & Cohn, 1990; Jessup & Brozena, 2003). Thus, drug regimens are aimed at prolonging survival and reducing symptoms (Deswal et al., 2001; Francis & Cohn, 1990; Jessup & Brozena, 2003).

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 (Palmiter & Solaro, 1997; Schoenfeld et al., 1998). However, CHF is also characterized by

an elevated baseline vascular tone and an impaired response to NO-mediated vasodilatation (Francis & Cohn, 1990; Negrao *et al.*, 2000). 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.

Neuroendocrine activation of the renin-angiotensin system (RAS) has been implicated in contributing to the abnormal vasoconstriction in CHF (Jessup & Brozena, 2003; Konstam, 2003). However, recent studies have demonstrated that angiotensin II (Ang II) also modulates the endothelium and regulates vascular remodeling (Dzau, 2001). In cultured vascular smooth muscle cells (SMCs), Ang II activates NAD/NADH oxidases and increases superoxide production, which results in enhanced NO catabolism and decreased availability (Griendling *et al.*, 1994). Additionally, reactive oxygen species, superoxide anions and hydrogen peroxide, also stimulate hypertrophy and hyperplasia of vascular SMCs (Li, Dietz & von Harsdorf, 1997a, b; Zafari *et al.*, 1998). Although decreased NO 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 (Kaiser, Spickard & Olivier, 1989; Katz *et al.*, 1992; Kubo *et al.*, 1991).

## Regulation of Smooth Muscle Contractility

Activation of smooth muscle is dependent on the level of phosphorylation of the 20 kDa regulatory myosin light chain (MLC<sub>20</sub>), which is determined by the relative activities of MLC kinase (MLCK) and MLC phosphatase (Gong *et al.*, 1992; Ogut & Brozovich, 2000). For years, the dogma was that activation and relaxation of smooth muscle was regulated by only the Ca<sup>2+</sup> dependence of MLCK whereas MLC phosphatase was an unregulated housekeeping enzyme (Hartshorne, 1987). However, recent evidence indicates that the majority of signaling pathways for the regulation of vascular tone converge on MLC phosphatase (Hartshorne *et al.*, 1998; Somlyo & Somlyo, 2003). Thus, changes of vascular tone are critically dependent on the regulation of MLC phosphatase activity (Kitazawa *et al.*, 1992; Hartshorne *et al.*, 1998; Solaro, 2000; Somlyo & Somlyo, 2003).

MLC phosphatase isolated from smooth muscle is a holoenzyme consisting of three subunits (see Hartshorne, Ito & Erdődi, 1998) for review); an ~20 kDa subunit, an ~38 kDa catalytic subunit and a myosin targeting subunit (MYPT1) of 110 – 133 kDa. MYPT1 isoforms can be generated by the inclusion or exclusion of a 41 aa central insert (Shimizu *et al.*, 1994). In addition to this central insert (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 (Khatri *et al.*, 2001). Thus, there are four distinct MYPT1 isoforms differing due to the presence or absence of a CI and a LZ (Hartshorne, Ito & Erdődi, 1998).

## Ca<sup>2+</sup> Sensitization

Agonist stimulation, compared to depolarization, has long been known to produce a higher force for a given Ca<sup>2+</sup> concentration (DeFeo & Morgan, 1985), and this phenomenon has been coined agonist induced Ca<sup>2+</sup> sensitization. The mechanism(s) leading to this sensitization of the contractile filaments have been the subject of several extensive reviews (Somlyo & Somlyo, 2000; Somlyo & Somlyo, 2003). 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 (Feng *et al.*, 1999) has been shown to inhibit MLC phosphatase activity (Kimura *et al.*, 1996), which results in an increase

in MLC<sub>20</sub> phosphorylation and force. However, other mechanisms have also been demonstrated to result in Ca<sup>2+</sup> sensitization, including a direct phosphorylation of MLC<sub>20</sub> by both Zip-like kinase (Niuro & Ikebe, 2001) and integrin linked kinase (Deng *et al.*, 2001). Another mechanism for Ca<sup>2+</sup> sensitization involves CPI-17, a small protein which is a substrate for both Rho-kinase and protein kinase C (Kitazawa *et al.*, 2000); phosphorylated CPI-17 binds to the catalytic subunit of MLC phosphatase to inhibit the enzyme's activity (Eto *et al.*, 1995). Whether any of these mechanisms contribute to the increase in vascular tone during CHF has yet to be investigated.

### Ca<sup>2+</sup> Desensitization

Nitric oxide (NO) is the classical agent to produce Ca<sup>2+</sup> desensitization (Furchgott & Zawadzki, 1980; Surks *et al.*, 1999; Lincoln *et al.*, 2001), and NO-mediated, or flow-mediated, vasodilatation is a fundamental response of the vasculature (Furchgott & Zawadzki, 1980). In the vasculature, an increase in flow increases shear stress on endothelial cells, which stimulates NO production. NO diffuses into the smooth muscle cells to activate the soluble pool of guanylate cyclase, thereby increasing the intracellular cGMP concentration. cGMP then activates type I cGMP-dependent protein kinase (PKG1), which subsequently acts on the maxi K<sup>+</sup> channel to produce a hyperpolarization (Alioua *et al.*, 1998), the SR and voltage dependent Ca<sup>2+</sup> channels to decrease Ca<sup>2+</sup> flux (Fukao *et al.*, 1999; Schmidt, Lohmann & Walter, 1993), and also activates MLC phosphatase (Surks *et al.*, 1999). In addition, PKGI dependent pathways for vasodilatation may include a phosphorylation of telokin (Khromov *et al.*, 2006; Walker *et al.*, 2001; Wu *et al.*, 1998) and HSP20 (Rembold *et al.*, 2000). Thus, either endothelial dysfunction leading to a decrease in NO production, or a defect in a more distal part of the signaling pathway, may lead to smooth muscle cell dysfunction and impaired flow-mediated vasodilatation. A decrease in sensitivity to NO-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 smooth muscle cell relaxation correlates with the relative expression of LZ+/LZ- MYPT1 isoforms (Khatri *et al.*, 2001; Lu *et al.*, 2008; Payne *et al.*, 2006; Payne *et al.*, 2004; Zhang & Fisher, 2007), suggesting that the relative expression of LZ+/LZ- MYPT1 isoforms determines the sensitivity of the smooth muscle to NO mediated vasodilatation (Surks *et al.*, 1999). 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 (Huang, Fisher & Brozovich, 2004). 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 adenovirus infection, the exogenous MYPT1 subunit replaced the endogenous MYPT1 isoform in the MLC phosphatase holoenzyme. In cells over-expressing LZ- MYPT1 isoforms, the non-hydrolysable cGMP analog 8Br-cGMP did not produce a significant decrease in MLC<sub>20</sub> phosphorylation, while 8Br-cGMP resulted in a dose dependent decrease in the level of MLC<sub>20</sub> phosphorylation in SMCs expressing a LZ+ MYPT1. These results demonstrate that the expression of the COOH-terminal LZ of MYPT1 is required for PKGI $\alpha$  to activate MLC phosphatase during cGMP mediated smooth muscle relaxation (Surks *et al.*, 1999; Huang *et al.*, 2004).

**cGMP and MYPT1 Phosphorylation**—As discussed above during agonist stimulation, a Rho kinase mediated MYPT1 phosphorylation at Ser696 of the mammalian sequence inhibits MLC phosphatase activity (Somlyo & Somlyo, 2000; Somlyo & Somlyo, 2003). During cGMP stimulation, several groups have demonstrated that MYPT1 is phosphorylated at Ser695 of the

mammalian sequence (Nakamura et al., 2007; Wooldridge et al., 2004). Phosphorylation of MYPT1 at Ser695 does not alter MLC phosphatase activity (Nakamura *et al.*, 2007), but rather, decreases MYPT1 phosphorylation at Ser696 (Nakamura et al., 2007; Wooldridge et al., 2004). Thus a cGMP induced MYPT1 phosphorylation at Ser695, and a resulting decrease in MYPT1 phosphorylation at Ser696 would disinhibit MLC phosphatase and return its activity to baseline, resulting in a decrease in MLC<sub>20</sub> phosphorylation and force.

MYPT1 phosphorylation at Ser695 cannot be the sole mechanism by which cGMP stimulation mediates MLC<sub>20</sub> dephosphorylation (Somlyo, 2007). Others have demonstrated that cGMP stimulation modulates the phosphorylation of CPI-17 (Bonnievier & Arner, 2004; Etter et al., 2001) as well as telokin (Khromov et al., 2006; Walker et al., 2001; Wu et al., 1998), which result in an increase in MLC phosphatase activity. More importantly, the Ser695 phosphorylation does not increase MLC phosphatase activity (Nakamura *et al.*, 2007), but rather decreases the level of Ser696 phosphorylation to disinhibit and return phosphatase activity to baseline levels (Nakamura et al., 2007; Wooldridge et al., 2004). However, Ca<sup>2+</sup> desensitization (MLC<sub>20</sub> dephosphorylation at a constant [Ca<sup>2+</sup>]) occurs during all types of smooth muscle activation, including those that do not elicit MYPT1 phosphorylation at Ser696 (El-Toukhy et al., 2006; Niiro & Ikebe, 2001; Somlyo & Somlyo, 2003; Somlyo, 2007). Thus similar to Ca<sup>2+</sup> sensitization (Somlyo & Somlyo, 2000; Somlyo & Somlyo, 2003), cGMP mediated activation of MLC phosphatase activity cannot be universally explained by changes in MYPT1 phosphorylation (Somlyo, 2007).

## Vascular Function and cGMP Signaling

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 demonstrated. Coffman's group (Crowley *et al.*, 2005), 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 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 $\alpha$  and MYPT1 in blood pressure homeostasis (Michael *et al.*, 2008). These investigators generated mice with mutations in the NH<sub>2</sub>-terminus LZ domain of PKGI $\alpha$  designed to disrupt the ability of PKGI $\alpha$  to interact with MYPT1. Compared to WT animals, 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 $\alpha$ -MYPT1 signaling pathway is important for the maintenance of normal vascular tone and blood pressure. These results suggest that any decrease in PKGI $\alpha$ -MYPT1 signaling (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 $\alpha$ -MYPT1 signaling (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 (Nickenig & Harrison, 2002a, b). Additionally, Ang II activates both NF- $\kappa$ B, which increases the expression of IL-6 and TNF- $\alpha$  to induce a proinflammatory state at the level of the vasculature (Dzau, 2001; Pfeffer

*et al.*, 1992; Warnholtz *et al.*, 1999), and membrane oxidases (NADH/NADPH oxidases), which generate reactive superoxide anions to decrease NO bioavailability (Zalba *et al.*, 2001). 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 (Cohn *et al.*, 1992; Francis & Cohn, 1990; Jessup & Brozena, 2003; Pfeffer *et al.*, 1992). Moreover, despite only modest blood pressure reduction with ACE inhibitors (Francis & Cohn, 1990), the same improvement in survival has not been observed with vasodilators; i.e., prazosin (Miller *et al.*, 1977), suggesting that there are incremental benefits to ACE inhibitors in addition to relaxing smooth muscle to produce vasodilatation; i.e., modulating the relative expression of the LZ+ MYPT1 isoform to preserve normal sensitivity to NO mediated vasodilatation.

In an animal model of CHF, Abassi *et al.* (Abassi *et al.*, 1997) have demonstrated that angiotensin receptor blockade normalizes the impaired vasodilatory responses to ACh. These investigators also demonstrated that the production of NO was normal (Abassi *et al.*, 1997), which suggests that a defect in NO mediated vasodilatation is at the level of the smooth muscle. These data could suggest that neuroendocrine 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 (Delp *et al.*, 1997; Geenen *et al.*, 1991; Geenen, Malhotra & Scheuer, 1989; Pfeffer *et al.*, 1979; Symons, Stebbins & Musch, 1999). In our studies (Chen *et al.*, 2006; Karim *et al.*, 2004), between 2 and 4 weeks following 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 (Chen *et al.*, 2006).

Captopril has been shown in the rat infarct model to normalize hemodynamic parameters and reduce infarct size (Pfeffer *et al.*, 1979). In clinical use, captopril has been demonstrated to prevent the progression of heart failure and improve survival in humans after acute myocardial infarction (Pfeffer *et al.*, 1992). In our studies (Chen *et al.*, 2006), 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 (Chen *et al.*, 2006). These data demonstrate that ACE inhibition maintains the normal vascular phenotype, and preserves the normal vasodilatory response to NO. 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 humans with heart failure (Cohn *et al.*, 1992; Francis & Cohn, 1990; Jessup & Brozena, 2003; Pfeffer *et al.*, 1992).

## Captopril Therapy and Gene Expression

As discussed above between 2 and 4 weeks following an 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 (Chen *et al.*, 2006). However, captopril treatment of rats following an MI preserves both normal LZ+ MYPT1 isoform expression and sensitivity to cGMP (Chen *et al.*, 2006). 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 analyzed 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 (Chen & Brozovich, 2008): at 2 weeks post 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 7 genes (Taok1, Raf1, IL-1 $\beta$ , 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 signaling cascade, which leads to an inhibition of MLC phosphatase and vasoconstriction (reviewed in (Somlyo & Somlyo, 2000; Somlyo & Somlyo, 2003)). Thus, the decrease Ang II as a result of captopril therapy would decrease Rho/Rho kinase signaling and decrease afterload, and further a decrease in Rho kinase expression would enhance the decrease in afterload produced by this signaling pathway.

At 2 weeks post-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 signaling (Zheng *et al.*, 2003). A decrease in both the duration and intensity of signaling by the G protein subunits of the AT1 receptor is mediated by RGS binding to the  $G\alpha$  subunit (Mehta & Griendling, 2007). Therefore, a loss of RGS activity would remove its inhibitory effect on the AT1 receptor, resulting in increased stimulation by Ang II (Mehta & Griendling, 2007). Captopril attenuated the loss of MIR16 expression, which would restore the inhibition of the RGS on Ang II signaling.

ACE inhibition also suppressed the expression of both Taok1, a rat homolog of the MAPK kinase kinase (MAP3K), a known activator of p38 MAPK (Chen *et al.*, 2003) and also Raf-1, an activator of p42/44 MAPK (Pearson *et al.*, 2001). Activation of both p38 MAPK and p42/44 MAPK signaling 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 (Lyle & Griendling, 2006). Additionally, eIF4E controls translation efficiency by regulating nuclear mRNA export, mRNA stability, and the preferential loading of mRNAs onto ribosomes (Culjkovic, Topisirovic & Borden, 2007). 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 upregulated by p38 MAPK, including TNF- $\alpha$ , IL-1 $\alpha/\beta$ , IL-6, and IL-10 (Force *et al.*, 2004; Sekiguchi *et al.*, 2004). Both IL-1 $\beta$  and TNF- $\alpha$  can also activate p38 MAPK (Geng, Valbracht & Lotz, 1996), which results in a positive feedback cascade of Ang II-mediated p38 MAPK signaling (Sekiguchi *et al.*, 2004). Our data indicated that at 2 weeks post-infarction, IL-1 $\beta$  expression was increased in both captopril and placebo-treated groups. However at 4 weeks post-infarction, captopril suppressed IL-1 $\beta$  gene expression, while the increase in IL-1 $\beta$  production remained elevated with placebo-treatment. Captopril's ability to decrease IL-1 $\beta$  would also contribute to the suppression of p38 MAPK signaling, since IL-1 $\beta$  creates a positive feedback loop by both activating p38 MAPK and up-regulating the AT1 receptor (Geng, Valbracht & Lotz, 1996; 1992). Captopril's ability to suppress IL-1 $\beta$  expression at 4 weeks post-MI could be produced by the suppression Taok1 expression, and these data could suggest that the levels of Taok1 and IL-1 $\beta$  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 NO mediated vasodilatation. Data suggest that the decrease in sensitivity to NO mediated

vasodilatation can be attributed to both a decrease in NO 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 isoform. The decrease in LZ+ MYPT1 expression produces a decrease in sensitivity to NO-mediated vasodilatation, and the resulting decrease in response to NO will blunt flow-mediated vasodilatation, which would contribute to a resting vasoconstriction.

Both p38 MAPK and p42/44 MAPK signaling activate a number of transcription factors (Dodeller & Schulze-Koops, 2006; Johnson & Lapadat, 2002; Lyle & Griendling, 2006; Pearson et al., 2001) and homeobox genes (Clempus & Griendling, 2006). 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 (Chen et al., 2006; Karim et al., 2004). A reduction in expression of these MAPK signaling pathways could alter transcription to maintain LZ+ MYPT1 expression. Nonetheless, data suggest a potential role of MAPK signaling 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.

## Acknowledgments

This study was supported by NIH grants HL69894 and HL64137 (to FVB) and HL78845 (to OO).

## Literature Cited

- Abassi ZA, Gurbanov K, Mulrone 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;96:3655–3664. [PubMed: 9396468]
- Alioua A, Tanaka Y, Wallner M, Hofmann F, Ruth P, Meera P, Toro L. The large conductance, voltage-dependent, and calcium-sensitive K<sup>+</sup> channel, Hslo, is a target of cGMP-dependent protein kinase phosphorylation *in vivo*. *J Biol Chem* 1998;273:32950–32956. [PubMed: 9830046]
- Bonnevier J, Arner A. Actions downstream of cyclic GMP/protein kinase G can reverse protein kinase C-mediated phosphorylation of CPI-17 and Ca<sup>2+</sup> sensitization in smooth muscle. *J Biol Chem* 2004;279:28998–29003. [PubMed: 15123611]
- 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;45:445–454. [PubMed: 18418003]
- Chen FC, Ogut O, Rhee AY, Hoit BD, Brozovich FV. Captopril prevents myosin light chain phosphatase isoform switching to preserve normal cGMP-mediated vasodilatation. *J Mol Cell Cardiol* 2006;41:488–495. [PubMed: 16815432]
- 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;278:22278–22283. [PubMed: 12665513]
- Clempus RE, Griendling KK. Reactive oxygen species signaling in vascular smooth muscle cells. *Cardiovasc Res* 2006;71:216–225. [PubMed: 16616906]
- Cohn JN, Johnson G, Ziesche S, Cobb F, Francis G, 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;325:303–310. [PubMed: 2057035]
- Crowley SD, Gurley 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;115:1092–1099. [PubMed: 15841186]
- Culjkovic B, Topisirovic I, Borden KL. Controlling gene expression through RNA regulons: The role of the eukaryotic translation initiation factor eIF4E. *Cell Cycle* 2007;6:65–69. [PubMed: 17245113]

- DeFeo TT, Morgan KG. Calcium-force relationships as detected with aequorin in two different vascular smooth muscles of the ferret. *J Physiol* 1985;369:269–282. [PubMed: 4093883]
- 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;83:1291–1299. [PubMed: 9338439]
- Deng JT, Van Lierop JE, Sutherland C, Walsh MP.  $Ca^{2+}$ -independent smooth muscle contraction - A novel function for integrin-linked kinase. *J Biol Chem* 2001;276:16365–16373. [PubMed: 11278951]
- 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;103:2055–2059. [PubMed: 11319194]
- Dodeller F, Schulze-Koops H. The p38 mitogen-activated protein kinase signaling cascade in CD4 T cells. *Arthritis Res Ther* 2006;8:205. [PubMed: 16542479]
- Dzau VJ. Theodore Cooper Lecture: Tissue angiotensin and pathobiology of vascular disease: A unifying hypothesis. *Hypertension* 2001;37:1047–1052. [PubMed: 11304501]
- El-Toukhy A, Given AM, Ogut O, Brozovich FV. PHI-1 interacts with the catalytic subunit of myosin light chain phosphatase to produce a  $Ca^{2+}$  independent increase in MLC<sub>20</sub> phosphorylation and force in avian smooth muscle. *FEBS Lett* 2006;580:5779–5784. [PubMed: 17022978]
- 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;118:1104–1107. [PubMed: 8720121]
- Etter EF, Eto M, Wardle RL, Brautigam DL, Murphy RA. Activation of myosin light chain phosphatase in intact arterial smooth muscle during nitric oxide-induced relaxation. *J Biol Chem* 2001;276:34681–34685. [PubMed: 11461918]
- Feng JH, Ito 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;274:37385–37390. [PubMed: 10601309]
- Force T, Kuida K, Namchuk M, Parang K, Kyriakis JM. Inhibitors of protein kinase signaling pathways: Emerging therapies for cardiovascular disease. *Circulation* 2004;109:1196–1205. [PubMed: 15023894]
- Francis GS, Cohn JN. Heart failure: Mechanisms of cardiac and vascular dysfunction and the rationale for pharmacologic intervention. *FASEB J* 1990;4:3068–3075. [PubMed: 2210153]
- 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;274:10927–10935. [PubMed: 10196172]
- Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288:373–376. [PubMed: 6253831]
- Geenen DL, Malhotra A, Liang D, Scheuer J. Ventricular function and contractile proteins in the infarcted overloaded rat heart. *Cardiovasc Res* 1991;25:330–336. [PubMed: 1832082]
- Geenen DL, Malhotra A, Scheuer J. Regional variation in rat cardiac myosin isoenzymes and ATPase activity after infarction. *Am J Physiol* 1989;256:H745–H750. [PubMed: 2522283]
- Geng 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;98:2425–2430. [PubMed: 8941662]
- Gong MC, Cohen P, Kitazawa T, Ikebe M, Masuo M, Somlyo AP, Somlyo AV. Myosin light chain phosphatase activities and the effects of phosphatase inhibitors in tonic and phasic smooth muscle. *J Biol Chem* 1992;267:14662–14668. [PubMed: 1321813]
- Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 1994;74:1141–1148.
- Hartshorne, DJ. Biochemistry of the Contractile Process in Smooth Muscle. In: Johnson, LR., editor. *Physiology of the Gastrointestinal Tract*. New York: Raven Press; 1987. p. 432–482.
- Hartshorne DJ, Ito M, Erdidi F. Myosin light chain phosphatase: Subunit composition, interactions and regulation. *J Muscle Res Cell Motil* 1998;19:325–341. [PubMed: 9635276]
- 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. [PubMed: 14530290]



- Jessup M, Brozena S. Heart failure. *N Engl J Med* 2003;348:2007–2018. [PubMed: 12748317]
- Johnson GL, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* 2002;298:1911–1912. [PubMed: 12471242]
- Kaiser L, Spickard RC, Olivier NB. Heart failure depresses endothelium-dependent responses in canine femoral artery. *Am J Physiol* 1989;256:H962–H967. [PubMed: 2705566]
- Karim SM, Rhee AY, Given AM, Faulx MD, Hoit BD, Brozovich FV. Vascular reactivity in heart failure. Role of myosin light chain phosphatase. *Circ Research* 2004;95:612–618.
- Katz SD, Biasucci L, Sabba C, Strom JA, Jondeau G, Galvao M, Solomon S, Nikolic SD, Forman R, LeJemtel TH. Impaired endothelium-mediated vasodilatation in the peripheral vasculature of patients with congestive heart failure. *J Am Coll Cardiol* 1992;19:918–925. [PubMed: 1552112]
- Khatri JJ, Joyce KM, Brozovich FV, Fisher SA. Role of myosin phosphatase isoforms in cGMP-mediated smooth muscle relaxation. *J Biol Chem* 2001;276:37250–37257. [PubMed: 11486008]
- Khromov AS, Wang H, Choudhury N, McDuffie M, Herring BP, Nakamoto R, Owens GK, Somlyo AP, Somlyo AV. Smooth muscle of telokin-deficient mice exhibits increased sensitivity to  $Ca^{2+}$  and decreased cGMP-induced relaxation. *Proc Natl Acad Sci* 2006;103:2440–2445. [PubMed: 16461919]
- 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;273:245–248. [PubMed: 8662509]
- Kitazawa T, Eto M, Woodsome TP, Brautigan DL. Agonists trigger G protein-mediated activation of the CPI-17 inhibitor phosphoprotein of myosin light chain phosphatase to enhance vascular smooth muscle contractility. *J Biol Chem* 2000;275:9897–9900. [PubMed: 10744661]
- Konstam MA. Improving clinical outcomes with drug treatment in heart failure: What have trials taught? *Am J Cardiol* 2003;91:9D–14D.
- Kubo SH, Rector TS, Bank AJ, Williams RE, Heifetz SM. Endothelium-dependent vasodilation is attenuated in patients with heart failure. *Circulation* 1991;84:1589–1596. [PubMed: 1914099]
- Li PF, Dietz R, von Harsdorf R. Differential effect of hydrogen peroxide and superoxide anion on apoptosis and proliferation of vascular smooth muscle cells. *Circulation* 1997a;96:3602–3609. [PubMed: 9396461]
- Li PF, Dietz R, von Harsdorf R. Reactive oxygen species induce apoptosis of vascular smooth muscle cell. *FEBS Lett* 1997b;404:249–252. [PubMed: 9119073]
- Lu Y, Zhang H, Gokina N, Mandala M, Sato O, Ikebe M, Osol G, Fisher SA. Uterine artery myosin phosphatase isoform switching and increased sensitivity to SNP in a rat L-NAME model of hypertension of pregnancy. *Am J Physiol* 2008;294:C564–C571.
- Lyle AN, Griendling KK. Modulation of vascular smooth muscle signaling by reactive oxygen species. *Physiology (Bethesda)* 2006;21:269–280. [PubMed: 16868316]
- Mehta PK, Griendling KK. Angiotensin II cell signaling: Physiological and pathological effects in the cardiovascular system. *Am J Physiol* 2007;292:C82–C97.
- Michael SK, Surks HK, Wang Y, Zhu Y, Blanton R, Jamnongjit M, Aronovitz M, Baur W, Ohtani K, Wilkerson MK, Bonev AD, Nelson MT, Karas RH, Mendelsohn ME. High blood pressure arising from a defect in vascular function. *Proc Natl Acad Sci* 2008;105:6702–6707. [PubMed: 18448676]
- Miller RR, Awan NA, Maxwell KS, Mason DT. Sustained reduction of cardiac impedance and preload in congestive heart failure with the antihypertensive vasodilator prazosin. *N Engl J Med* 1977;297:303–307. [PubMed: 889599]
- 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 phosphatase. *Circ Research* 2007;101:712–722.
- Negrao CE, Hamilton MA, Fonarow GC, Hage A, Moriguchi JD, Middlekauff HR. Impaired endothelium-mediated vasodilation is not the principal cause of vasoconstriction in heart failure. *Am J Physiol* 2000;278:H168–H174.
- Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: Part I: Oxidative stress and atherogenesis. *Circulation* 2002a;105:393–396. [PubMed: 11804998]
- Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: Part II: AT(1) receptor regulation. *Circulation* 2002b;105:530–536. [PubMed: 11815439]

- Niiri N, Ikebe M. Zipper-interacting protein kinase induces  $\text{Ca}^{2+}$ -free smooth muscle contraction via myosin light chain phosphorylation. *J Biol Chem* 2001;276:29567–29574. [PubMed: 11384979]
- Ogut O, Brozovich FV. Determinants of the contractile properties in the embryonic chicken gizzard and aorta. *Am J Physiol* 2000;279:C1722–C1732.
- Palmiter KA, 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. [PubMed: 9202846]
- Payne MC, Zhang HY, Prosdocimo T, Joyce KM, Koga Y, Ikebe M, Fisher SA. Myosin phosphatase isoform switching in vascular smooth muscle development. *J Mol Cell Cardiol* 2006;40:274–282. [PubMed: 16356512]
- 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–H1810.
- 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;22:153–183. [PubMed: 11294822]
- Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ, Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker GC, Klein M, Lamas GA, Packer M, Rouleau J, Rouleau JL, Rutherford J, Wertheimer JH, Hawkins CM. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction - Results of the survival and ventricular enlargement trial. *New Engl J Med* 1992;327:669–677. [PubMed: 1386652]
- Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. *Circ Research* 1979;44:503–512.
- Rembold CM, Foster DB, Strauss JD, Wingard CJ, Van Eyk 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;524:865–878. [PubMed: 10790164]
- Schmidt HH, Lohmann SM, Walter U. The nitric oxide and cGMP signal transduction system: Regulation and mechanism of action. *Biochim Biophys Acta* 1993;1178:153–175. [PubMed: 7688574]
- Schoenfeld JR, Vasser M, Jhurani P, Ng P, Hunter JJ, Ross J Jr, Chien KR, Lowe DG. Distinct molecular phenotypes in murine cardiac muscle development, growth, and hypertrophy. *J Mol Cell Cardiol* 1998;30:2269–2280. [PubMed: 9925364]
- 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;63:433–442. [PubMed: 15276468]
- 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;269:30407–30411. [PubMed: 7982954]
- Somlyo AP, Somlyo AV. Signal transduction by G-proteins, Rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. *J Physiol* 2000;522:177–185. [PubMed: 10639096]
- Somlyo AP, Somlyo AV.  $\text{Ca}^{2+}$  sensitivity of smooth muscle and nonmuscle myosin II: Modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev* 2003;83:1325–1358. [PubMed: 14506307]
- Somlyo AV. Cyclic GMP regulation of myosin phosphatase: A new piece for the puzzle? *Circ Research* 2007;101:645–647.
- 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;286:1583–1587. [PubMed: 10567269]
- 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;87:574–581. [PubMed: 10444615]
- Walker LA, Macdonald JA, Liu XP, Nakamoto RK, Haystead TAJ, 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;276:24519–24524. [PubMed: 11346659]
- Warnholtz A, Nickenig G, Schulz E, Macharzina R, Brasen JH, Skatchkov M, Heitzer T, Stasch JP, Griendling KK, Harrison DG, Bohm M, Meinertz T, Munzel T. Increased NADH-oxidase-mediated

- superoxide production in the early stages of atherosclerosis: Evidence for involvement of the renin-angiotensin system. *Circulation* 1999;99:2027–2033. [PubMed: 10209008]
- Wooldridge AA, MacDonald JA, Erdodi F, Ma C, Borman MA, Hartshorne DJ, Haystead TA. Smooth muscle phosphatase is regulated *in vivo* by exclusion of phosphorylation of threonine 696 of MYPT1 by phosphorylation of Serine 695 in response to cyclic nucleotides. *J Biol Chem* 2004;279:34496–34504. [PubMed: 15194681]
- Wu X, Haystead TA, Nakamoto RK, Somlyo AV, Somlyo AP. Acceleration of myosin light chain dephosphorylation and relaxation of smooth muscle by telokin. Synergism with cyclic nucleotide-activated kinase. *J Biol Chem* 1998;273:11362–11369. [PubMed: 9556631]
- Zafari AM, Ushio-Fukai M, Akers M, Yin Q, Shah A, Harrison DG, Taylor WR, Griendling KK. Role of NADH/NADPH oxidase-derived H<sub>2</sub>O<sub>2</sub> in angiotensin II-induced vascular hypertrophy. *Hypertension* 1998;32:488–495. [PubMed: 9740615]
- Zalba G, San Jose G, Moreno MU, Fortuno MA, Fortuno A, Beaumont FJ, Diez J. Oxidative stress in arterial hypertension: Role of NAD(P)H oxidase. *Hypertension* 2001;38:1395–1399. [PubMed: 11751724]
- Zhang H, Fisher SA. Conditioning effect of blood flow on resistance artery smooth muscle myosin phosphatase. *Circ Research* 2007;100:730–737.
- Zheng B, Berrie CP, Corda D, Farquhar MG. GDE1/MIR16 is a glycerophosphoinositol phosphodiesterase regulated by stimulation of G protein-coupled receptors. *Proc Natl Acad Sci* 2003;100:1745–1750. [PubMed: 12576545]