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

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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.

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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 (MLC_{20}), 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 holoenzyme 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_{20} phosphorylation and force. However, other mechanisms have also been demonstrated to result in Ca^{2+} sensitization, including a direct phosphorylation of MLC_{20} 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 (PKGI), 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

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 analogue 8Br-cGMP did not produce a significant decrease in MLC_{20} phosphorylation, while 8Br-cGMP resulted in a dose-dependent decrease in the level of MLC_{20} phosphorylation in SMCs expressing a LZ+ MYPT. These results demonstrate that the expression of the COOH-terminal LZ of MYPT1 is required for PKGIα 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 MLC_{20} phosphorylation and force.

MYPT1 phosphorylation at Ser695 cannot be the sole mechanism by which cGMP stimulation mediates MLC_{20} 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, Ca^{2+} desensitization (MLC_{20} dephosphorylation at a constant [Ca^{2+}]) 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 Ca^{2+} sensitization [68, 69], cGMP-mediated activation 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 demonstrated. 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 NH_2-terminus LZ domain of PKGIα designed to disrupt the ability of PKGIα 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 PKGIα-MYPT1 signalling pathway is important for the maintenance of normal vascular tone and blood pressure. These results suggest that any decrease in PKGIα-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 PKGIα-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 activates 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 vasculature [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 reduction 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 vasodilatation; *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 demonstrated 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 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 [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 Gα 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 isoform. 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.

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