Homocysteine, Hydrogen sulfide (H$_2$S) and NMDA-Receptor in Heart Failure

Neetu Tyagi, Paras K Mishra and Suresh C Tyagi*
Department of Physiology & Biophysics, School of Medicine University of Louisville Louisville, KY 40202, USA

Received 4 August 2009; revised 25 November 2009

Mitochondrial mechanism of oxidative stress and matrix metalloproteinase (MMP) activation was unclear. Our recent data suggested that MMPs are localized to mitochondria and activated by peroxynitrite, which causes cardiovascular remodeling and failure. Recently, we have demonstrated that elevated levels of homocysteine (Hcy), known as hyperhomocysteinemia (HHcy) increase oxidative stress in the mitochondria. Although HHcy causes heart failure, interestingly, it is becoming very clear that Hcy can generate hydrogen sulfide (H$_2$S), if the enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CGL) are present. H$_2$S is a strong anti-oxidant and vasorelaxing agent. Paradoxically, it is interesting that Hcy, a precursor of H$_2$S can be cardioprotective. The CGL is ubiquitous, while the CBS is not present in the vascular tissues. Therefore, under normal condition, only half of Hcy can be converted to H$_2$S. However, there is strong potential for gene therapy of CBS to vascular tissue that can mitigate the detrimental effects of Hcy by converting it to H$_2$S. This scenario is possible, if the activities of both the enzymes (CBS and CGL) are increased in tissues by gene therapy.

**Keywords:** Mitochondrial matrix metalloproteinase, Myocyte mechanics, Calcium transient, Mitochondrial permeability, NMDA-R1, Hydrogen sulfide, Cystathionine β-synthase Cystathionine γ-lyase, Homocysteine, Hyperhomocysteinemia

Introduction

Although many studies have focused on the detrimental effects of homocysteine (Hcy), a non-protein sulphur containing amino acid formed during the metabolism of dietary methionine, very few studies have demonstrated the beneficial effects of Hcy. This review suggests that HHcy can be beneficial, if excess of Hcy can be converted to hydrogen sulfide (a potent antioxidant and vasorelaxing agent), which may decrease the blood pressure and mitigate the cardiovascular dysfunction.

Homocysteine and hydrogen sulfide in cardiac remodeling

H$_2$S plays a crucial role as a signaling molecule in gastrointestinal and nervous systems and is also established as a cardioprotective. However, its role in regulating the extracellular matrix (ECM) remodeling and apoptosis is still nebulous. Considering the tremendous therapeutic potential, H$_2$S gas has attracted the attention of biomedical research. It is a strong anti-oxidant and is generated endogenously by two important enzymes, namely cystathionine β-synthase (CBS) and cystathionine γ-lyase (CGL), which are involved in sulfur-containing amino acids (cysteine and homocysteine, Hcy) metabolism. Paradoxically, it is interesting that excess of Hcy, a precursor of H$_2$S can be cardioprotective. The CGL is ubiquitous, while the CBS is not present in the vascular tissues. Therefore, under normal condition, only half of Hcy can be converted to H$_2$S. However, there is strong potential for double gene therapy of CBS and CGL to vascular tissue (i.e. where the levels of CBS and CGL are low) that can mitigate the detrimental effects of Hcy by converting it to H$_2$S. This is possible, if the activities of both the enzymes are increased in every tissue by gene therapy (Figure 1).

During stress condition, several biochemical changes occur in heart and the levels of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) change to maintain homeostasis. The MMPs are a family of structurally and functionally-related zinc-dependent...
Homocysteine to H$_2$S

Homocysteine (Hcy)  
Cystathionine β synthase (CBS)  
Cystathionine γ lyase (CGL)  
Cysteine  
CBS: Cystathionine β synthase  
CGL: Cystathionine γ lyase  
H$_2$S: Heteronuclear spin-lattice relaxation  
H$_2$S: Cardiovascular modulator  
CBS: Brain, Kidney and Liver  
CGL: Ubiquitous

Figure 1—Mechanism of conversion of homocysteine (Hcy) to H$_2$S

Endopeptidases that play pivotal role in ECM remodeling through their proteolytic effect$^{11}$. The MMP-2 and MMP-9 have collagen-binding fibronectin type-II inserts in their catalytic domain, making them indispensable for ECM remodeling in the heart, where elastin: collagen ratio determines chronic heart failure (CHF)$^{12}$. The MMPs are found in latent state in the heart and maintain normal elastin: collagen ratio through matrix synthesis and degradation. However, when reactive oxygen and nitrogen species (ROS and RNS) engender oxidative stress, the MMPs get activated, leading to deposition of excess matrix protein (collagen), which in turn remodels ECM by changing the elastin: collagen ratio, causing fibrosis that ultimately leads to CHF$^{13,14}$. Endogenous inhibitors of MMPs called TIMPs modulate MMP activity and suppress ECM turnover$^{15}$. The TIMP-4 prevents the activation of MMP-2 and MMP-9 and is predominantly present in the cardiac tissue, hence also called cardiac-specific inhibitor of metalloproteinase (CIMP)$^{12}$. On the other hand, TIMP-1 and TIMP-3 increase with oxidative stress. The TIMP-1 has been implicated in cardiac fibrosis$^{16}$, while TIMP-3 induces apoptosis in the vascular smooth muscle cells$^{17}$.

The oxidative stress generated by ROS and RNS alarms not only MMPs and TIMPs, but also the downstreams β-integrin and ADAM-12$^{18}$. To simulate the condition of oxidative stress, we created aorta-venacava fistula (AVF) in the mice and assessed the change in the levels of MMPs, TIMPs, β-integrin and ADAM-12 to confirm that the heart was under oxidative and proteolytic stress. The change in the levels was recorded after treating the AVF mice with H$_2$S. Interestingly, we found that H$_2$S mitigated the oxidative and proteolytic stress in the AVF mice. Thus, we showed how H$_2$S inhibited fibrosis and apoptosis (that leads to CHF) by regulating MMPs, TIMPs, β-integrin and ADAM-12$^{18}$. Because H$_2$S is anti-oxidant and anti-oxidant inhibits MMP, therefore, we proposed a model for H$_2$S-dependent ECM remodeling, resulting into cardio-protection.

Homocysteine and NMDA-receptor in cardiac remodeling

The pathophysiology of CHF involves abnormalities in systolic and/or diastolic function and increases the propensity for reentry arrhythmias. The continued elevation of cardiac sympathetic drive contributes to myocardial toxicity, leading to the decline in cardiac contractility$^{19}$. An increase in glutamatergic activity on sympathetic regulation has been reported due to the upregulation of hypothalamic NMDA NR$_1$ receptor (NMDA-R1) subunits during CHF$^{20}$. Ischemia/reperfusion-induced arrhythmias are sensitive to NMDA-R1 blockade$^{21}$.

The hyperhomocysteinemia (HHcy) is a graded risk factor for CHF$^{22}$ and sudden cardiac death (SCD), resulting from coronary fibrous plaques$^{23-26}$. The Hcy induces interstitial cardiac fibrosis, leading to systolic/diastolic dysfunction$^{27}$. The antagonist to the N-methyl-D-aspartate receptor (NMDA-R) protects against Hcy-induced oxidative damage in the neurons$^{28}$ and protects against increase in the heart rate by NMDA-analog, suggesting that Hcy is an agonist to NMDA-R. The cardiomyocyte expresses NMDA-R. Furthermore, the activation of NMDA-R increases oxidative stress and calcium load in the mitochondria, leading to cell death in neonatal rat cardiomyocytes. However, the functional consequences of myocyte NMDA-R activation in HHcy are not well understood. The MMPs, the Zn-containing endopeptidase are involved in ECM turnover and induce structural remodeling, leading to arrhythmogenesis. The MMP activation in HHcy decreases collagen/elastin ratio, increases the deposition of interstitial collagen (fibrosis) between endothelium and myocytes and is arrhythmogenic. The NMDA-R antagonist inhibits MMP activation$^{29}$ and attenuates SCD.

Recently$^{28}$, the concept that “MMPs are not just for the matrix anymore” has been emerged. Intracellular localization of MMP has been suggested. The MMP-2 is synthesized by both cardiac myocytes and fibroblasts and is co-localized with contractile proteins, such as troponin I within myofilaments$^{30}$ and sarcomeres. Acute activation of MMP-2 leads to a
The presence of MMP has been shown in the cardiac mitochondria (mtMMP); however, the physiological consequences of MMP activation in the mitochondria are not well-understood. Although there is little information regarding the mechanisms by which MMP-2 disrupts mitochondria, it is well-recognized that ROS generated by mitochondria can drive both MMP-2 expression and activation. Such activation could result in a negative feedback mechanism that degrades mitochondrial membrane potential and impairs mitochondrial function. We have shown that Hcy-induced calpain protease activation induces membrane permeability transition (MPT) and the treatment with NMDA-R1 blocker MK-801 attenuates Hcy-induced MPT in HL-1 cardiomyocytes.

Several studies have documented the pathological role of Hcy on the vasculature and have suggested a strong correlation between Hcy and heart failure. The Hcy induces fibrosis and causes systolic/diastolic dysfunction, however, the direct impact of Hcy on the cardiomyocytes and its physiological consequences remain to be elucidated. We have also demonstrated that Hcy increases NMDA-R1 expression in the ventricular myocytes, causes activation of MMP in the myocyte mitochondria and induces MPT. In addition, Hcy decreases myocyte contractility and alters calcium transients, in part by decreasing the expression of Ca$^{2+}$ handling proteins SERCA-2a and NCX. These observations unequivocally support the notion that Hcy increases MMP-9 expression by agonizing NMDA-R1 in the myocyte mitochondria, which may be attributed to the increase in oxidative stress and calcium load in the mitochondria.

In a recent report, it has been suggested that overexpression of MMP-2 causes mitochondrial dysfunction by degrading the mitochondrial membrane potential. The Hcy is well associated with the mitochondrial abnormalities. Recently, we have presented the evidence that Hcy-induced calpain (calcium-dependent cysteine protease) activation induces MPT by agonizing NMDA-R1 in HL-1 cardiomyocytes. However, how the induction of MMP in the mitochondria leads to increase in MPT is to be elucidated.

It is known that intact mitochondrial Cxn43 preserves mitochondrial permeability transition pore (MPT) in the closed state and hence is cardioprotective. Based on our unpublished findings, we suggest that MMP activation in the mitochondria disrupts mitochondrial connexin 43 protein, leading to MPT. The Hcy causes decline in myocyte mechanical properties by agonizing NMDA-R1 and involves MPT. This may be attributed to the myocyte Ca$^{2+}$ overloading which can contribute to ATP depletion by activation of Ca$^{2+}$-dependent ATPases and by induction of the MPT, leading to the myocyte dysfunction.

**Figure 2**—Mitochondrial mechanism of MMP activation

![Figure 2](image-url)
We have shown that in HHcy the Ca\(^{2+}\) clearance rate is slower, which is reflected by a decrease in the expression of calcium handling protein SERCA-2a. The ER-stress caused by Hcy overloads the myocyte with Ca\(^{2+}\) which depletes ATP by the induction of MPT, leading to the decline in the myocyte contractility. The HHcy increases MMP expression in the myocyte mitochondria, alters Ca\(^{2+}\) homeostasis, and induces MPT, leading to the decline in the myocyte contractility by agonizing NMDA-R1. Further studies are needed to understand the mechanism of HHcy-induced MPT, leading to the myocyte dysfunction\(^{18,29}\).

**Restoration of contractility in hyperhomocysteinemia by cardiac-specific deletion of NMDA-R1**

The elevated plasma level of Hcy, a non-protein sulphur containing amino acid formed during the metabolism of dietary methionine is an independent risk factor for heart failure and for SCD. It induces interstitial cardiac fibrosis and endothelial-myocyte disconnection, leading to myocardial contraction dysfunction\(^{14,18}\).

Although NMDA-R1 expression has been widely studied in central nervous system, evidence for its expression in non-neuronal tissue is rare. The NMDA-R1 is expressed in cardiomyocytes and endothelial cells. The NMDA-R1 antagonist dizocilpine ameliorates stress-induced sudden death in cardiomyopathic hamster. Furthermore, the activation of NMDA-R1 increases oxidative stress and calcium load in the mitochondria, leading to cell death\(^{14,18}\).

Intracellular localization of MMP has been suggested\(^{29}\). The MMP-2 is synthesized by both cardiac myocytes and fibroblasts and is co-localized with contractile proteins, such as troponin I within myofilaments, leading to the decline in contractile performance. We have shown the presence of MMP in the mitochondria (mtMMP)\(^{29}\), however, the physiological consequences of MMP activation in the mitochondria are not well explored. The abnormal activation of MMP impairs the mitochondrial function. Very recently, by using pharmacological blocker for NMDA-R1, our lab has suggested that Hcy is agonistic to NMDA-R1 and causes decline in myocyte contractility, in part by inducing MMP-9 in the mitochondria\(^{29}\). However, these studies\(^{31}\) did not reflect the specific contribution of cardiomyocyte NMDA-R1 on contractile function in the setting of HHcy. Therefore, we have determined the mechanistic role of cardiac NMDA-R1 on the myocyte contractile function by the generation of cardiomyocyte-specific deletion of NMDA-R1\(^{18}\).

We have demonstrated that cardiomyocyte-specific deletion of NMDA-R1 attenuates the Hcy-induced increase in NMDA-R1 expression\(^{18}\). The Hcy causes the increase in ROS and NO levels in the myocyte mitochondria by agonizing NMDA-R1. The cardiac-specific KO of NMDA-R1 ameliorates Hcy-induced mitochondrial permeability transition and involves the decrease in MMP activation. The cardiac-specific ablation of NMDA-R1 mitigates Hcy-induced decline in contractile performance and prolongation of TPS-90 and TR. The Hcy causes decrease in contraction amplitude with the increasing concentration of calcium. These observations suggest that Hcy increases the generation of mitochondrial peroxynitrite (by increasing ROS and NO levels in the mitochondria), activates MMP-9 in the mitochondria, and causes decline in myocyte mechanics by altering calcium handling, leading to the functional uncoupling of myocyte contraction\(^{14,18,27}\).

The Hcy induces fibrosis and endothelial-myocyte disconnection, leading to the decrease in myocardial contractility, however, the direct impact of Hcy on the cardiac myocytes and the underlying mechanism for contractile dysfunction remain to be elucidated. The cardiomyocyte expresses NMDA-R and the activation of NMDA-R increases oxidative stress and calcium load in the mitochondria, leading to cell death. However, the physiological significance of cardiomyocyte NMDA-R and how the activation of NMDA-R alters the myocyte physiology in the setting of HHcy remain to be elucidated\(^{14,18,27}\).

Recently\(^{29}\), our lab has tried to determine the role of myocyte NMDA-R1 and the possible underlying mechanism in modulating the myocyte mechanics in HHcy. In that study, MK-801, the competitive pharmacological blocker of NMDA-R1 was administered i.p and the myocyte contraction was studied in HHcy. However, these observations ruled out the specific contribution of cardiomyocyte NMDA-R1 gene on the myocyte physiology in HHcy. Since the homozygous deletion of NMDA-R1 is embryonic lethal, cardiac-specific KO of NMDA-R1 was generated, and the underlying mechanism by which myocyte NMDA-R1 regulates the myocyte mechanics in HHcy was determined\(^{18}\).

The generation of peroxynitrite induces MMP-2 activation, leading to myocyte contractile dysfunction.
(Figure 2). We have reported that Hcy induces the generation of mitochondrial peroxinitrite, leading to the activation of MMP-9 in the myocyte mitochondria by agonizing the NMDA-R1. We presented the evidence that targeted deletion of NMDA-R1 causes decrease in Hcy-induced MPT and involves the decrease in MMP activation in the myocyte mitochondria. This is consistent with our earlier finding that Hcy agonizes NMDA-R1 and causes MPT by MMP activation

The cardiomyocyte-specific deletion of NMDA-R1 restores the Hcy-induced decline in contractile performance, in part by maintaining MPT and by decreasing in MMP activation. This is complementary to our recent finding that Hcy activates MMP-9 in the myocyte mitochondria induce MPT, leading to the decrease in myocyte contraction. This decrease in myocyte mechanics by Hcy involves the alteration in calcium transients. The Hcy prolongs the recovery of calcium transient by agonizing NMDA-R1, suggesting that rate of clearance of calcium is slow in HHcy myocyte, compared to control myocytes. The results suggested that cardiomyocyte-specific deletion of NMDA-R1 mitigates the Hcy-induced MMP activation in the myocyte mitochondria, in part by decreasing mitochondrial ROS and NO. The cardiac-specific KO of NMDA-R1 restores Hcy-induced decrease in myocyte contractile performance, in part by decreasing MMP activation and maintaining MPT. This is consistent with our earlier finding that Hcy-induced calpain (calcium-dependent cysteine protease) activation induces MPT by agonizing NMDA-R1 in HL-1 cardiomyocytes.

Conclusion

Although elevated levels of Hcy i.e., hyperhomocysteinemia (HHcy) cause myocyte dysfunction, this detrimental effect of Hcy can be converted to a beneficial effect, if CBS and CGL gene transfer is performed in the tissues or organ expressing low levels of CBS and CGL.

References

16. Lindsay M M, Maxwell P & Dunn F G (2002) TIMP-1, a marker of left ventricular diastolic dysfunction and fibrosis in hypertension. Hypertension 40, 136-141
21. D’Amico M, Filippo C & Di Rossi F (1999) Arrhythmias induced by myocardial ischemia-reperfusion are sensitive to...
ionotrophic excitatory amino acid receptor antagonists. *Eur J Pharmacol* 366, 167-174


28 Folbergrova J (1994) NMDA and not non-NMDA receptor antagonists are protective against seizures induced by homocysteine in neonatal rats. *Exp Neurol* 130, 344-350

