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Arrhythmia and neuronal/endothelial myocyte uncoupling in hyperhomocysteinemia*

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Abstract

Elevated levels of homocysteine (Hcy) known as hyperhomocysteinemia (HHcy) are associated with arrhythmogenesis and sudden cardiac death (SCD). Hcy decreases constitutive neuronal and endothelial nitric oxide (NO), and cardiac diastolic relaxation. Hcy increases the iNOS/NO, peroxynitrite, mitochondrial NADPH oxidase, and suppresses superoxide dismutase (SOD) and redoxins. Hcy activates matrix metalloproteinase (MMP), disrupts connexin-43 and increases collagen/elastin ratio. The disruption of connexin-43 and accumulation of collagen (fibrosis) disrupt the normal pattern of cardiac conduction and attenuate NO transport from endothelium to myocyte (E-M) causing E-M uncoupling, leading to a pro-arrhythmic environment. The goal of this review is to elaborate the mechanism of Hcy-mediated iNOS/NO in E-M uncoupling and SCD. It is known that Hcy creates arrhythmogenic substrates (i.e. increase in collagen/elastin ratio and disruption in connexin-43) and exacerbates heart failure during chronic volume overload. Also, Hcy behaves as an agonist to N-methyl-D-aspartate (NMDA, an excitatory neurotransmitter) receptor-1, and blockade of NMDA-R1 reduces the increase in heart rate-evoked by NMDA-analog and reduces SCD. This review suggest that Hcy increases iNOS/NO, superoxide, metalloproteinase activity, and disrupts connexin-43, exacerbates endothelial-myocyte uncoupling and cardiac failure secondary to inducing NMDA-R1.

Keywords

Heart failure; ECM; calcium channel; tachycardia; bradycardia; arrhythmia; LVH; peri-capillary fibrosis; MMP; TIMP; integrin; connexin; contraction; relaxation; neuronal endothelial myocyte coupling; NOS; sudden cardiac death; NMDA

Introduction

SCD is a major cause of mortality (Hiromasa *et al.*, 1988; Palakurthy *et al.*, 1989). Approximately 65% of SCD cases occur in patients with underlying acute or chronic heart disease. The incidence of SCD increases 2- to 4-fold in the presence of coronary disease and 6- to 10-fold in the presence of structural heart disease. Ventricular tachycardia (VT) leading to ventricular fibrillation (VF) is a primary cause of cardiac arrest and SCD. One of the challenges in preventing SCD lies in identifying individuals at highest risk for SCD within a

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lower-risk population (Podrid *et al.*, 2005). The identification of conventional risk factors for coronary artery disease and structural heart disease during progression to arrhythmogenesis and SCD can be very daunting. Although both ischemia as well as reperfusion induced arrhythmia, only reperfusion-induced arrhythmias were sensitive to NMDA-R1 blockade (D'Amico *et al.*, 1999). This may suggest that arrhythmias in high cardiac output are influenced by circulating factors and are mitigated by NMDA-R1 blockade. However, increased serum Hcy has been identified as a risk factor for SCD resulting from coronary fibrous plaques (Bollani *et al.*, 1999; Albert *et al.*, 2002; Burke *et al.*, 2002). Cardiac interstitial fibrosis is the result of HHcy and the combination of HHcy and hypertension (Miller *et al.*, 2002). Both cardiac arrhythmias and neurological disorders contribute to SCD, and the blockade of NMDA-R1 mitigates SCD (Folbergrova, 1994; Huang & Su, 1999; Matsuoka *et al.*, 2002; Simandle *et al.*, 2005). Since the induction iNOS increases SCD (Mungrue *et al.*, 2002), and Hcy increases iNOS, therefore, the use of iNOSKO mice to determine the contribution of inducible NO in Hcy-mediated E-M uncoupling (disruption of connexin-43 and fibrosis), and generation of arrhythmogenic substrate (Gutstein *et al.*, 2001; Kitamua *et al.*, 2003; Poelzing & Rosenbaum, 2004) is very important for the understanding of the mechanism of iNOS-NO-mediated cardiac dysfunction. Hcy increases mitochondrial oxidative stress and activates MMPs. The MMPs are activated in VT, VF, and SCD (Hoit *et al.*, 2002; Xu *et al.*, 2004; Mukherjee *et al.*, 2006). The metalloproteinases degrade connexin-43 (Hunt *et al.*, 2002), therefore, it is important to measure metalloproteinases and connexin alteration in Hcy-mediated E-M uncoupling in heart failure.

Homocysteine (Hcy) metabolomics

Elevated levels of Hcy known as hyperhomocysteinemia (HHcy) are a significant risk factor for cardiac arrhythmia and sudden cardiac death (SCD, Bollani *et al.*, 1999; Burke *et al.*, 2002). There are five ways by which homocysteine (Hcy) is accumulated in the plasma and tissues: 1) by a methionine rich protein diet; 2) by hyper de-methylation of methionine by methyl transferase (MT) during DNA/RNA methylation; 3) by hypo re-methylation of Hcy to methionine by MTHFR/vitamin b_{12}/folate deficiency; 4) by heterozygous/homozygous mutation in cystathione β synthase (CBS) activity, b_6, and transsulphuration deficiency; and 5) by renal disease and volume retention (Figure 1). Mammalian vascular cells are lacking the CBS (Finkelstein, 1990; 1998). Decrease in methionine-rich diet and treatment with vitamin b_{12}/folate reduce the levels of plasma Hcy and ameliorate vascular dysfunction, in part, by re-methylation of Hcy to methionine, however, the mechanisms of other genetic causes of HHcy are unknown. There are three ranges of hyperhomocysteinemia: moderate (16 to 30 μM), intermediate (31–100 μM), and severe (>100 μM) (Cheng & Kaplowitz, 2004). Extracellular thiols are oxidized, and only a fraction of total plasma homocysteine (Hcy) is in the reduced form *in vivo* and *in vitro*. Hcy at doses of 0.1–1.0 mM markedly inhibits endothelial cell growth over time *in vitro*; in contrast, vascular smooth muscle cells respond to similar concentrations of Hcy with an increase in cyclin D1 and cyclin A mRNA expression and a resulting marked increase in cell proliferation (Tsai *et al.*, 1994).

Importance of endothelium in the heart

Although the volume of capillaries may account to 16%, the endothelial cell volume is probably only 2–3%, whereas red blood volume is 6% and plasma volume 7%. The importance of a cell species cannot be judged simply based on cell volume. Nonetheless, sixteen percent of the myocardial mass is capillaries, including the lumen and endothelium (Hoppeler & Kayar, 1988). The capillary endothelium is embedded in the muscle, and plays a very important role in myocardial diastolic relaxation (Roberts & Waern, 1941; Henderson *et al.*, 1992; Smith *et al.*, 1992; Mebazaa *et al.*, 1995). Nitric oxide (NO) generation from the

endocardial endothelium contributes to myocyte contraction, relaxation, and heart rate (Brady *et al.*, 1994; Pinsky *et al.*, 1997). A gradient of NO concentration (i.e. high in endocardium and low in midmyocardium) has been depicted (30) that is consistent with the notion that there is more capillary endothelium in the endocardium than in epi- or mid-myocardium (Fukuchi *et al.*, 2001; Scarabelli *et al.*, 2001). The importance of endocardial endothelium in cardiac contraction/relaxation is illustrated in an experiment in which the responses to $CaCl_2$ and acetylcholine were attenuated in the endothelium-denuded myocardium (Wang & Morgan, 1992; Gattuso *et al.*, 1999; Tyagi *et al.*, 1999).

Endothelium-myocyte (E-M) coupling implies the E-M cell-cell connections, the thickness of the basement membrane between the E and M, and the efficiency of transport of endothelial-derived cardio-active agents to the cardiac muscle. Primarily there are three connexins in the heart, connexion-40 is in endothelium, connexion-43 and -45 are present in myocytes (Bastide *et al.*, 1993). The disruption of connexin-43 impairs cardiac electrical impulse. The accumulation of interstitial collagen between E and M increases distance from E to M, and interferes with cardiac diastolic relaxation. In addition, the increase in distance from E to M impairs endothelial-derived NO diffusion mechanism to the cardiac muscle (Moshal *et al.*, 2005).

Elevation of Hcy levels has been shown to increase [Ca2+]i

The treatment of spinal motorneurons with homocysteine elevated calcium, which resulted in cell death, this may contribute to SCD. Interestingly, increased levels of Hcy create myocardial conduction abnormalities and are associated with SCD (James *et al.*, 1974; Bollani *et al.*, 1999; Burke *et al.*, 2002). Hcy behaves as an agonist to NMDA-R1, and NMDA induces Ca2+ and K+ currents (Robinson *et al.*, 2005; Yang *et al.*, 2005). Treatment of spinal motor neurons with Hcy elevated [Ca2+]i which culminated in cell death (Adalbert *et al.*, 2002). Culturing embryonic cortical neurons and differentiated human neuroblastoma cells in folate-free medium increased Hcy, [Ca2+]i and reactive oxygen species (Ho *et al.*, 2003). Addition of 3-deazaadenosine (DZA), an inhibitor of SAHH and Hcy formation, abrogated the formation of Hcy and the increase in ROS (Ho *et al.*, 2003). Due to S-(1,2-dichlorovinyl)-L-Hcy, an analog of Hcy, Hcy has much more potent agonist at specific receptors, but a poor metabolic analogue, and therefore elevated [Ca2+]i nearly five fold (Vamvakas *et al.*, 1990). Results from our laboratory showed that Hcy-mediated cardiac contractile dysfunction and increase in [Ca2+]i were amplified by subphysiological levels of angiotensin II and endothelin-1 which did not normally elicit cardiac responses (Tyagi *et al.*, 1999; Mujumdar *et al.*, 2000). This suggested synergism between Hcy, angiotensin II and endothelin-1, causing cardiac dysfunction.

The MMP family includes gelatinases, collagenases, and membrane type (MT-MMP) (Rosenberg, 2002). The metalloproteinase family also includes a disintegrin metalloproteinase (ADAM) (Loechel *et al.*, 1998). These metalloproteinases are neutral proteases that act on the MVEC BM resulting in its degradation (Tyagi *et al.*, 1996). It has been shown that association of MMP-2 with integrin results in its disengagement from ECM and promotes cell death (Frisch *et al.*, 1994). MMPs are regulated by their interaction with tissue inhibitors of metalloproteinases (TIMPs) (Visse & Nagase, 2003). TIMPs inactivate MMPs by binding to their catalytic site. There are four TIMPs. In general, TIMP-4 inhibits MMP-2, and -7 and to a lesser extent, MMP-1, -3 and -9. TIMP-3 inhibits MMP-1, -3, -7 and -13. TIMP-1 and -2 inhibit a broad range of MMPs (Stamenkovic, 2003). Composition of extracellular matrix (ECM) in the BM is important for conductance of signals from endothelial to myocyte side. It is known that focal adhesion complex integrates incoming signals and orchestrates an intricate interrelationship between ECM, cytoskeleton and signaling cascades (Levkau *et al.*, 2002; Spragg & Kass, 2005). Connexin-43 −/− promotes

cardiac arrhythmia and SCD, in part, by inducing endothelial-myocyte uncoupling (Gutstein *et al.*, 2001; Kitamua *et al.*, 2003; Poelzing & Rosenbaum, 2004). In end-stage human heart failure connexin-43 is disrupted and metalloproteinases are activated (Hunt *et al.*, 2002). We showed that most of the MMPs in the heart are latent (Tyagi *et al.*, 1993) due to active-site Zn^{2+} coordination with constitutive NO in a ternary complex (MMP/NO/TIMP) in the basement-membrane-matrix of endothelium. Increased oxidative stress leads to generation of nitro-tyrosine residues in TIMP and liberates active MMP (Figure 2, Tyagi *et al.*, 2005). This, in turn, degrades the connective matrix. Since collagen turnover is faster than other ECM components, degraded matrix is replaced by oxidized collagen (fibrosis). Two detrimental consequences of this process are: 1) degradation of ultrastructural matrix which causes disconnection (i.e. degradation of connexin-43) of the endothelium from myocytes; and 2) accumulation of oxidized collagen, which impairs the delivery of metabolites to underlying muscle, causing uncoupling. Generalized MMP activation is implicated in development of VF, SCD and CHF (Hoit *et al.*, 2002; Xu *et al.*, 2004; Mukherjee *et al.*, 2006). Furthermore, increased oxidative-modification of collagen by cross-linking is associated with diastolic dysfunction in CHF (Mizushige *et al.*, 2000; Joseph *et al.*, 2002, 2003; Kass *et al.*, 2004). NMDA-R1 antagonist ameliorates MMP activation (Meighan *et al.*, 2006). TIMP-1 is induced in fibrotic myocardium (Lindsay *et al.*, 2002) and Hcy at micro-molar range induces TIMP-1 (Torres *et al.*, 1999). TIMP-4 is highly expressed in the heart and is decreased during cardiac failure (Tammalapalli *et al.*, 2001). Unlike humans, rodents do not have a typical interstitial collagenase (MMP-1). Instead, they have MMP-13 equivalent to MMP-1 (Vincenti *et al.*, 1998). MMP-2 degrades interstitial collagen as well as elastin (Senior *et al.*, 1991; Aimes & Quigley, 1995), and under pathophysiological conditions MMP-9 at 92 kDa (gelatinase b) is induced. In addition, MMP-2 (72 kDa, gelatinase a) is present in all species. ADAM-12 is increased and connexin-43 is degraded in CHF (Hunt *et al.*, 2002).

ECM remodeling and endothelial-myocyte uncoupling

ECM remodeling interferes with integrin-mediated cell survival signaling. This leads to formation of filamentous actin (F-actin), gaps between the cells, and disrupts endothelial-myocyte tight junctions (Tyagi, 1997; Tyagi & Hoit, 2002; Shastry & Tyagi, 2004; Lominadze *et al.*, 2006), resulting in E-M uncoupling. In the BM of capillary endothelium, the MMPs reside in the latent ternary (MMP/NO/TIMP) complex. During HHcy TIMPs are oxidized and MMPs are activated (Figure 2, Tyagi & Hayden, 2003). MMPs are collagenases as well as elastases, therefore, because elastin turnover is relatively slower than collagen, it is replaced by oxidized collagen (Rucklidge *et al.*, 1992; Mujumdar & Tyagi, 1999). This alters collagen/elastin ratio, disrupts connexin-43, and leads to accumulation of interstitial collagen (fibrosis) between the endothelium and myocyte. Fibrosis causes endothelial-myocyte disconnection (uncoupling) and attenuates NO diffusibility from endothelium to myocyte. The disruption of connex-in-43 and lack of constitutive NO create an environment for trigger activity.

Oxidative stress plays significant role in VT, arrhythmia, and SCD during CHF (Fukuda *et al.*, 2005; Wolin & Gupte, 2005). Previous studies from our laboratory showed that Hcy increases oxidative stress by generating ROS and nitrotyrosine (Mujumdar *et al.*, 2001; Tyagi *et al.*, 2005). In tissues NADPH oxidase is a primary source of ROS generation. Most of the peroxidase activity depends on the levels of thioredoxin, the tissue level of thioredoxin is decreased as a result of an increased oxidative stress (Yamamoto *et al.*, 2000; Shao *et al.*, 2002; Goth & Vitai, 2003; Hui *et al.*, 2004). In addition, the level of thioredoxin is a strong predictor of oxidative stress (Farina *et al.*, 2001; Wong *et al.*, 2002; Barr & Gedamu, 2003; Oh *et al.*, 2004). Hcy increases mitochondrial oxidative stress by decreasing redoxins, SOD, and increasing NADPH oxidase (Nonaka *et al.*, 2001; Tyagi *et al.*, 2005). In

normal myocardium, the MMPs active site is bound to NO (Tyagi & Hayden, 2003). During chronic increases in load (82 Cox *et al.*, 2002), and oxidative stress (Hunt *et al.*, 2002), MMPs are activated, causing a decrease in endothelial NO bioavailability (Mujundar *et al.*, 2001). To reduce the load by dilating the heart in the absence of endothelial NO, the latent resident MMPs are activated (Tyagi & Hayden, 2003). However, persistent activation of MMP (i.e. increase in MMP/TIMP ratio) leads to degradation of connexin-43 (Hunt *et al.*, 2002). Interestingly, matrix degradation and proteolytic shedding (activation) of NMDA receptor have been implicated by tPA, an active serine proteinase (Frey *et al.*, 1996). Although ligand-gated glutamate binds to NMDA-R1, activates K+/Na+ channels, and increases [Ca2+]i-mediated contraction (James *et al.*, 1974; Duchen, 2004; Robinson *et al.*, 2005; Yang *et al.*, 2005, 35–37, 85), the role of disruption of matrix-connection in creating pro-arrhythmic environment is unclear. Hcy activates NMDA-R1 (Chen *et al.*, 2005; Qureshi *et al.*, 2005) and decreases endothelial NO (Gu *et al.*, 2002), increases iNOS-NO, ROS contents, and generates peroxynitrite (Mujumdar *et al.*, 2001). The increase in superoxide, peroxynitrite and nitration of proteins (Mihm *et al.*, 2001) leads to cardiac arrhythmia and failure. The blocker of NMDA-R1 inhibits MMP activation (Meighan *et al.*, 2006) and mitigates SCD (Matsuoka *et al.*, 2002).

Hcy, NMDA-R1, iNOS/NO, arrhythmia and SCD

Constitutively, NO released from sympathetic and parasympathetic nerves moderate neuronal-myocyte coupling and cardiac rhythm (Nihei *et al.*, 2005), and NO released from endothelium regulates E-M coupling and cardiac diastolic relaxation (Brady *et al.*, 1994; Pinsky *et al.*, 1997). Interestingly, Hcy decreases both neuronal (Kim, 1999) and endothelial NO (Chen *et al.*, 2002) but increases inducible NO through iNOS (Welch *et al.*, 1998). The increase in iNOS induced sudden cardiomyocyte death and may lead to SCD (Mungrue *et al.*, 2002). NMDA is a major excitatory neurotransmitter, and NMDA-R1 is present in the mammalian central nervous system (CNS, Lalo *et al.*, 2006), in cardiomyocytes (Kraine *et al.*, 1998; Huang & Su, 1999) and the endothelial cells (Chen *et al.*, 2005; Qureshi *et al.*, 2005). The relative expression of the NMDA-receptor in heart, specifically expression in cardiomyocytes versus endothelial cells and neuronal tissue (sympathetic and parasympathetic nerve endings, for example) is in the order of neuronal >cardiomyocyte >endothelial cells. The NMDA-R1 increases neuronal membrane “excitability” resulting in the excitotoxic actions of NMDA (Fridman, 1999; Jara-Prado *et al.*, 2003). In addition, NMDA-R1 activation increases mitochondrial oxidative stress and [Ca2+]i (Duchen, 2004). The antagonist to the NMDA receptor protects against Hcy mediates oxidative toxic effects in neurons (Folbergrova, 1994), and protects against increase in heart rate by NMDA-analog (DiMicco & Monroe, 1996), suggesting that Hcy is an agonist to NMDA-receptor. In summary, activation of NMDA-R1 decreases constitutive NO, increases iNSO/NO/oxidative stress, creates arrhythmogenic condition and SCD.

Hcy, NMDA-R1, Connexin-43, collagen, elastin and arrhythmias

Paradoxically, Hcy overexpresses and nitrosylates connexin-43, which is then degraded in the mitochondria (Li *et al.*, 2002). Hcy decreases connexin expression and impaired EDHF-mediated vasodilatation (Heil *et al.*, 2004). Both developmental gap junction uncoupling and connexin-36 down regulation were prevented by the blockade of NMDA receptors (Arumugam *et al.*, 2005), suggesting that the connexins are functionally linked to NMDA-R1 (Grozdanvic *et al.*, 1998). In addition, mutations in connexin-43, elastin, and collagen genes are associated with long Q-T syndromes (Keating, 1995; Pavlovich, 1998; Gutstein *et al.*, 2001; Kaplan *et al.*, 2004). The overexpression of connexin-43 is associated with activation of MMP-2 and -9 (Zhang *et al.*, 2003). Although increased Hcy levels are

associated with arrhythmias and SCD, it is unclear whether Hcy mediates arrhythmia in parallel through MMP activation, collagen, elastin, and connexin degradation.

Heart rate inversely related to elastin/collagen ratio

An associative and not causative relationship between animal size and heart rate has been suggested. The higher the metabolic demand is, the higher the heart rate is. To determine whether normal heart rate depends on metabolic demand, we compared heart rate between mice and elephants (Webb *et al.*, 1998; Breukelman *et al.*, 2006; Gehlen *et al.*, 2006). The data suggests that the bigger the animal is, the lower the heart rate is. The cardiac elastin/collagen ratio increases as the size of animal increases (Figure 3), suggesting cardiac function follows the structure. These data also suggest that cardiac elastin/collagen ratio may contribute to cardiac rhythm.

Hcy increases heart rate

Twelve weeks of Hcy administration increases LV collagen expression and causes endocardial, peri-capillary and interstitial fibrosis (Miller *et al.*, 2002). The elastin/collagen ratio was robustly decreased in Hcy-treated animals. The chronic administration of Hcy increases the heart rate (HR). The withdrawal of Hcy reduces the Hcy-mediated increase in HR (Figure 3). These results suggest that Hcy decreases elastin/collagen and increases HR (Tyagi *et al.*, 1995; Sood *et al.*, 2002). However, the inverse relationship shown for the elastin/collagen ratio (Figure 3B) and heart rate (Figure 3A) for mice, rats, rabbits and humans may be entirely unrelated. Similarly, the observations that Hcy reduces the elastin/collagen ratio (Figure 3C) and increases heart rate (Figure 3D) may be associative and not causative.

Hcy increases intracellular [Ca2+]i

Treatment of primary MVEC and SMC with Hcy induces calcium transient. Hcy increases [Ca2+]i with an EC_{50} of 60 nM. The concentrations of Hcy in mammals are around 5–10 μM and the process may be saturated completely under physiological baseline conditions. This may enhance the sensitivity of these cells to subphysiological levels of angiotensin II which normally do not elicit responses in these cells, suggesting a role of Hcy in cardiac calcium-mediated contraction (Mujumdar *et al.*, 2000; Moshal *et al.*, 2005).

Summary

There is an inverse relationship between the increase in heart rate and cardiac elastin/collagen ratio in mice, rats, rabbits and humans. The peri-capillary fibrosis attenuates endothelial ability to relax the cardiac muscle, causing diastolic dysfunction. There is direct relationship between the Hcy administration and increase in systolic blood pressure. Hcy increases intracellular Ca^{2+} and mitochondrial oxidative stress. Hcy decreases eNOS and increases iNOS expression, generates super oxide and peroxynitrite. Hcy activates MMP. The cardiac connexin-43 is degraded in human heart end-stage failure. The response to $CaCl_2$ is enhanced in endocardial endothelial-denuded myocardium. Hcy damages the endocardial endothelial cells. In endocardial endothelial denuded myocardium Hcy induces contraction. The mechanism of Hcy mediated cardiac contraction and enhanced $CaCl_2$ sensitivity in endocardial-endothelium denuded myocardium may suggest Ca^{2+}-sensitive Hcy receptor (NMDA-R1, Chen *et al.*, 2005; Qureshi *et al.*, 2005) playing a significant role in Hcy mediated cardiac contractile dysfunction. Therefore, it is important to determine the role of NMDA-R1 in Hcy-mediated cardiac contraction, arrhythmia and failure (Figure 4).

Abbreviations

ADAM	a disintegrin and metalloproteinase
ADMA	asymmetric dimethyl arginine
AV	aortavenacava shunt
L-arg	L-arginine
BH_4	tetrahydrobiopterin
BM	basement membrane
CBS	cystathionine beta synthatase
CHF	chronic heart failure
DDAH	dimethyl arginine hydrolase
DZA	3-deazaadenosine
ECM	extracellular matrix
EDRF	endothelial-derived relaxing factor
EDHF	endothelial-derived hyperpolarizing factor
EE	endocardial endothelial
EET	epoxy-eicosatrienoic acid
E-M	endothelial-myocyte
eNOS	endothelial nitric oxide synthase
Hcy	homocysteine
HETE	20-hydroxyeicosatetraenoic acid
HHcy	hyperhomocysteinemia
LV	left ventricle
MK	MK-801
MMP	matrix metalloproteinase
MT-MMP	membrane type-MMP
MTHFR	methylene tetrahydrofolate reductase
MVEC	microvascular endothelial cells
NADPH	nicotinamide adenosine diphosphate
NE	norepinephrine
NMDA-R1	N-methyl-D-aspartate receptor-1
nNOS	neural nitric oxide synthase
PVC	premature ventricle contraction
Redox	reduction-oxidation
RNS	reactive nitrogen species
ROS	reactive oxygen species
SAHH	S-adenosyl-homocysteine hydrolase

SAM	S-adenosyl-methionine
SOD	superoxide dismutase
TIMP	tissue inhibitor of metalloproteinase
t-PA	tissue plasminogen activator
VF	ventricular fibrillation
VT	ventricular tachycardia
Q-RT-PCR	quantitative real time polymerase chain reaction
WT	wild type

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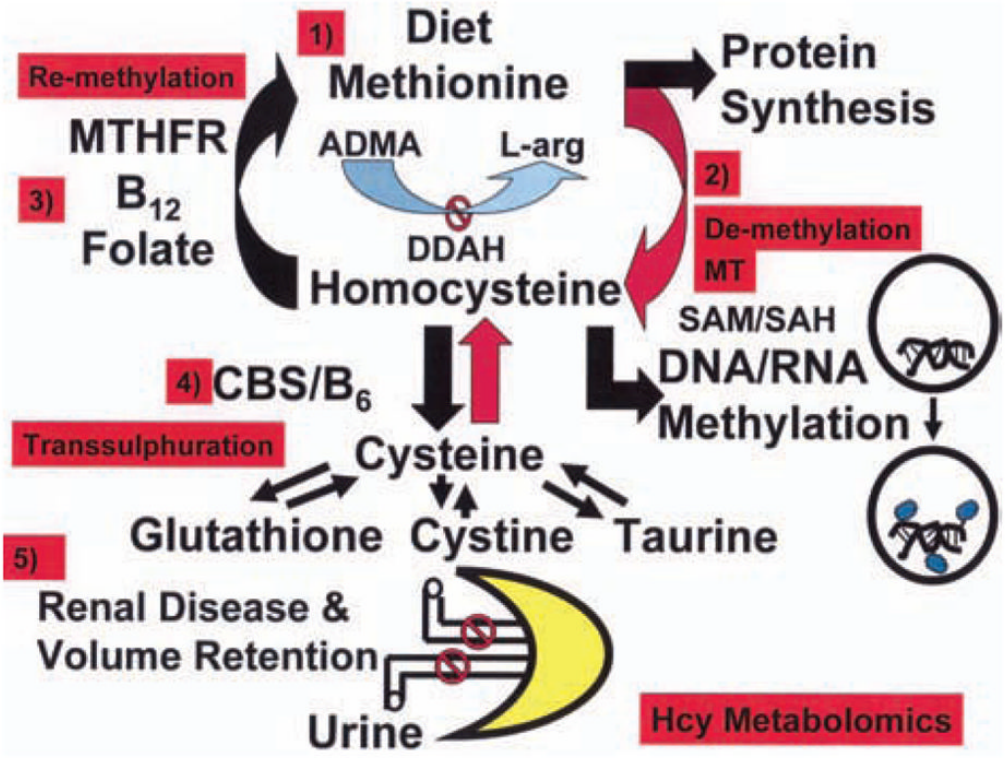

Figure 1.

Methionine rich protein diet increases Hcy levels. The hyper de-methylation of methionine by methyl transferase (MT) and SAHH activity during DNA/RNA methylation cause HHcy. The hypo re-methylation of Hcy to methionine by MTHFR/vitamin b_{12}/folate dependent pathways cause increase in Hcy levels. The heterozygous/homozygous in cystathione β synthase (CBS) activity, b_6, and transsulphuration deficiency exacerbate HHcy. The renal disease and volume retention increase plasma Hcy levels.

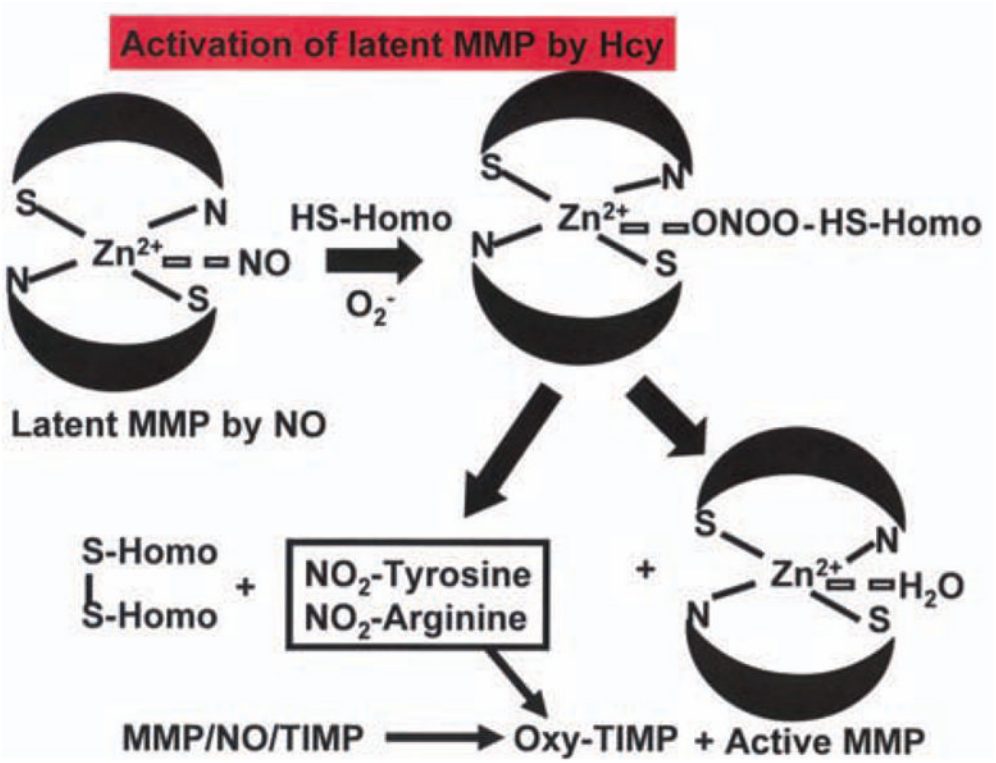

Figure 2.
Oxidative stress and increase ROS in HHcy decrease constitutive NO in ternary MMP/NO/TIMP complex and generate RNS and nitrotyrosine. This process oxidizes the TIMP and liberates active MMP.

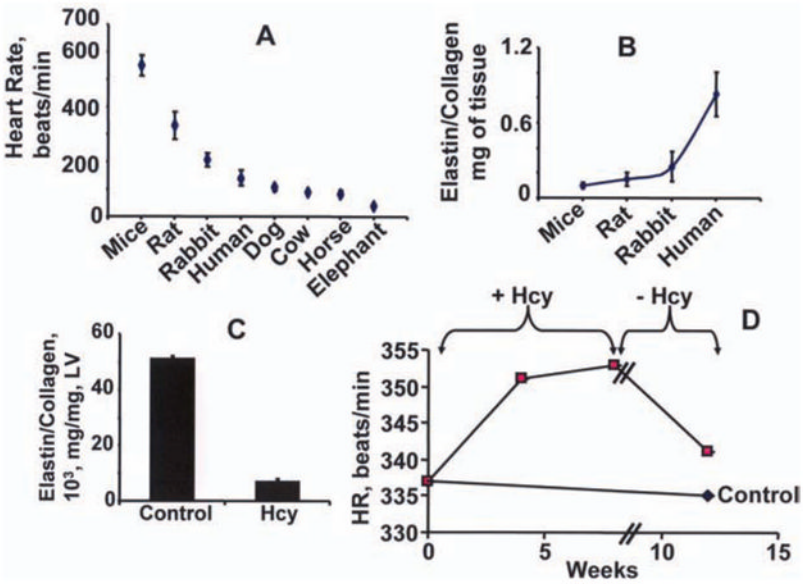

Figure 3.

Panel A shows comparative heart rates for animals of different sizes. There was a correlation between the size and the heart rate of mice, rat, rabbit, human, and dog (Henegar *et al.*, 2001; Cox *et al.*, 2002; Hunt *et al.*, 2002; Carroll & Tyagi, 2005; Moshal *et al.*, 2005), cow, horse and elephant (Webb *et al.*, 1998; Breukelman *et al.*, 2006; Gehlen *et al.*, 2006). **Panels B, C and D** show quantitative data. The data in panels B, C and D is from our previous reports (Tyagi *et al.*, 1995; Sood *et al.*, 2002). After chronic oral administration of Hcy (32 μmol/L) in drinking water for 12 weeks, the heart rate and blood pressure were measured by a PE-50 catheter in femoral artery of the rats (Sood *et al.*, 2002).

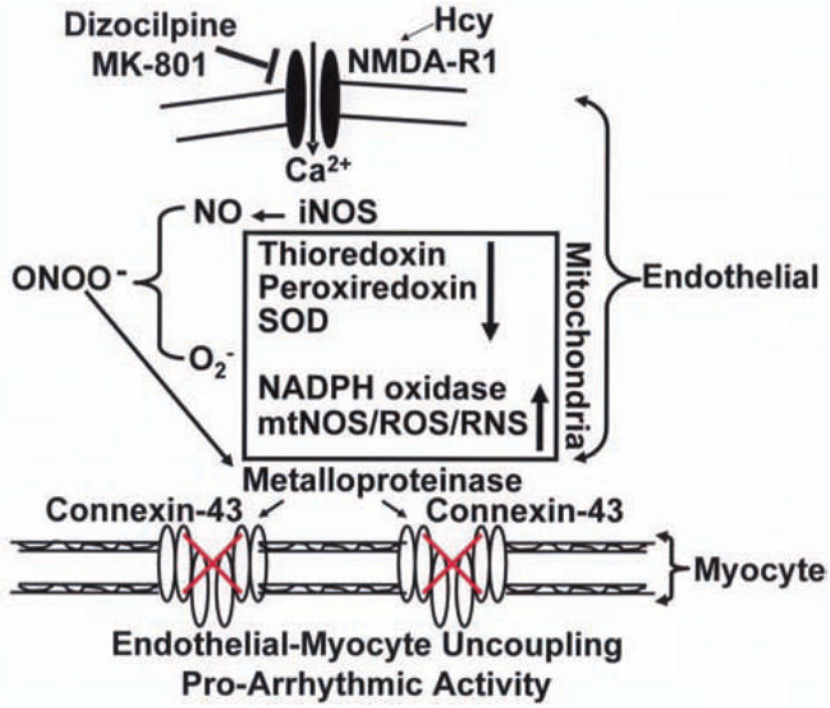

Figure 4.

Hcy increases iNOS/NO, superoxide, metalloproteinase activity, and disrupts connexin-43, exacerbates endothelial-myocyte uncoupling and cardiac failure secondary to inducing NMDA-R1. In mitochondria Hcy decreases thioredoxin, peroxiredoxin and SOD and increases NADPH oxidase increasing ROS and RNS.