Acute Inhibition of Myoglobin Impairs Contractility and Energy State of iNOS-Overexpressing Hearts

Carsten Wunderlich, Ulrich Flögel, Axel Gödecke, Jacqueline Heger, Jürgen Schrader

Abstract—Elevated cardiac levels of nitric oxide (NO) generated by inducible nitric oxide synthase (iNOS) have been implicated in the development of heart failure. The surprisingly benign phenotype of recently generated mice with cardiac-specific iNOS overexpression (TGiNOS) provided the rationale to investigate whether NO scavenging by oxymyoglobin (MbO₂) yielding nitrate and metmyoglobin (metMb) is involved in preservation of myocardial function in TGiNOS mice. ¹H nuclear magnetic resonance (NMR) spectroscopy was used to monitor changes of cardiac myoglobin (Mb) metabolism in isolated hearts of wild-type (WT) and TGiNOS mice. NO formation by iNOS resulted in a significant decrease of the MbO₂ signal and a concomitantly emerging metMb signal in TGiNOS hearts only (ΔMbO₂: −46.3±38.4 μmol/kg, ΔmetMb: +41.4±17.6 μmol/kg, n=6; P<0.05) leaving contractility and energetics unaffected. Inhibition of the Mb-mediated NO degradation by carbon monoxide (20%) led to a deterioration of myocardial contractility in TGiNOS hearts (left ventricular developed pressure: 78.2±8.2% versus 96.7±4.6% of baseline, n=6; P<0.005), which was associated with a profound perturbation of cardiac energy state as assessed by ³¹P NMR spectroscopy (eg, phosphocreatine: 13.3±1.3 mmol/L (TGiNOS) versus 15.9±0.7 mmol/L (WT), n=6; P<0.005). These alterations could be fully antagonized by the NOS inhibitor 5-s-ethylisothiourea. Our findings demonstrate that myoglobin serves as an important cytoplasmic buffer of iNOS-derived NO, which determines the functional consequences of iNOS overexpression. (Circ Res. 2003;92:lll–lll.)

Key Words: inducible nitric oxide synthase ■ myoglobin ■ cardiac contractility and energetics ■ magnetic resonance spectroscopy

Nitric oxide (NO) is a pleiotropic signaling molecule synthesized from the amino acid l-arginine by a family of enzymes termed nitric oxide synthase (NOS). The constitutive isoforms of NOS, namely neuronal (nNOS) and endothelial (eNOS) normally generate small amounts of NO modulating a variety of physiological functions such as vasodilator tone, platelet aggregation, and regulation of myocardial contractility. Another isoform of NO synthase is termed inducible NOS (iNOS)—and releases larger quantities of NO.¹ In the latter scenario, NO may switch from friend to foe, being beneficial as a second messenger and for immunological self-defense, but having potentially toxic effect on the host organism by inhibiting cell respiration or promoting apoptosis (see review²).

Proinflammatory cytokines have been shown to potently stimulate iNOS expression in cardiomyocytes,³ and both systemic as well as cardiac cytokine activation have been observed in patients with advanced heart failure.⁴⁻⁵ Finkel et al⁶ were the first to demonstrate that cytokine-induced cardiodepression is mediated, at least in part, through NO. Consistently, various clinical⁷,⁸ and experimental⁹,¹⁰ investigations reported on significant iNOS expression and activity in heart failure of different origin, although conflicting results have been published.¹¹ These findings led to the concept that iNOS-derived NO is causally related to contractile dysfunction in heart failure. However, cardiac-specific iNOS overexpression in a mouse model (TGiNOS), recently generated in our laboratory, resulted in a 40-fold increased in vivo NOS activity but only minor changes of myocardial function.¹² We have postulated that this lack of phenotype may have been caused by myoglobin (Mb), which was recently reported to effectively scavenge NO within the cardiomyocyte by continuous breakdown to nitrate and metmyoglobin.¹³ Because myoglobin is present in high concentrations (~200 μmol/kg wet weight¹⁴,¹⁵) in the cardiac cytosol, this NO-degrading reaction may play a pivotal role in preserving myocardial function in TGiNOS hearts in vivo.

The goal of the present study was to uncover the mechanism that enables iNOS-overexpressing hearts to maintain normal function. We therefore combined the transgenic with a pharmacological approach and subjected isolated hearts of TGiNOS mice and wild-type (WT) mice to 20% carbon monoxide for acute Mb inhibition (thereby also blocking the Mb-mediated detoxification of NO). Hemodynamic and contractile parameters together with ³¹P nuclear magnetic reso-
Subsequently, 200 and 15.8 fixed to the steady flow at which the hearts had stabilized (16.4 minutes after the onset of cardiac pacing, coronary perfusion was pacing (500 bpm) was initiated and continued throughout. Left pressure, coronary flow, coronary venous PO \(_2\), and left ventricular probe of a Bruker DRX 400 WB NMR spectrometer. Perfusion in perfusion buffer, and transferred into a heated (37°C) 10-mm size, 1K; zero filling to 2K; exponential weighting resulting in a accumulation (acquisition time, 42 ms; sweep width, 12136 Hz; data size, 1K; zero filling to 2K; exponential weighting resulting in a 40-Hz line broadening; chemical shifts were referenced to the H\(_2\)O resonance at 4.8 ppm).

### Materials and Methods

#### Animals

Transgenic mice were generated as described previously.\(^{12}\) Animal body weights ranged from 23 to 32 g, and heart weights ranged from 110 to 150 mg, with no significant differences between the two groups.

#### Langendorff Experimental Setup for NMR Measurements

The preparation of murine hearts and retrograde perfusion at 100 mm Hg constant pressure with modified Krebs-Henseleit buffer (KHB) containing (in mmol/L) NaCl 116, KCl 4.6, MgSO\(_4\) 1.1, NaHCO\(_3\) 24.9, CaCl\(_2\) 2.5, KH\(_2\)PO\(_4\) 1.2, glucose 8.3, pyruvate 2.0, and EDTA 0.5, gassed at 95% O\(_2\)/5% CO\(_2\) (carbogen), pH 7.4, were performed essentially as described elsewhere.\(^{16}\) In brief, mice were anesthetized by an intraperitoneal (IP) injection of urethane (1.5 g/kg) and heparinized with 250 U IP. Hearts were rapidly excised and placed in ice-cold KHB for preparation of the aortic arch. After aortic cannulation, hearts were perfused in a nonrecirculating Langendorff mode at constant pressure (100 mm Hg). For NMR measurements, hearts were placed inside a 10-mm NMR tube, immersed in perfusion buffer, and transferred into a heated (37°C) 10-mm probe of a Bruker DRX 400 WB NMR spectrometer. Perfusion pressure, coronary flow, coronary venous PO\(_2\), and left ventricular developed pressure (LVDP) were recorded continuously, the latter via a homemade fluid-filled balloon inserted into the left ventricle. All signals were recorded using a personal computer with dedicated software (EMKA Technologies). Statistical analysis was based on data measured in steady-state conditions.

#### Protocol

Incipiently, all hearts were perfused at constant coronary pressure (100 mm Hg) with KHB equilibrated with carbogen (95% O\(_2\)/5% CO\(_2\)). After the hearts were stabilized inside the magnet, cardiac pacing (500 bpm) was initiated and continued throughout. Left ventricular end-diastolic pressure was set to 5 mm Hg. Thirty minutes after the onset of cardiac pacing, coronary perfusion was fixed to the steady flow at which the hearts had stabilized (16.4±2.3 and 15.8±2.6 mL·min\(^{-1}\)·g\(^{-1}\) in WT and TGiNOS, respectively, with no significant differences between the groups). After the switch to constant flow, baseline data were recorded. Perfusion was then turned to buffer gassed at 75% O\(_2\)/20% N\(_2)/5% CO\(_2\) (Figure 1).

Subsequently, 200 μmol/L L-arginine was added and kept in the perfusion medium until the end of the experiment. After 20 minutes, N\(_2\) was replaced by the same aliquot of carbon monoxide, which quantitatively blocks the oxygen-binding site of Mb and to demonstrate complete inhibition of the oxygen-binding site of Mb via appearance of the MbCO signal.

To exclude any affects due to the altered oxygen content of the perfusion buffer, we necessarily had to reduce the oxygen concentration in a previous step (75% O\(_2\)/20% N\(_2)/5% CO\(_2\) ). The conducted protocol (Figure 1) enabled us to study exclusively the alterations induced by the administration of CO.

In each section of the protocol, \(^1\)H NMR and \(^3\)P NMR spectra of both strains were taken. In each phase, the coronary venous effluent was collected to measure the release of nitrite, nitrate, and adenosine.

#### NMR Spectroscopy

Spectra were recorded by a Bruker DRX 400 WB NMR spectrometer, operating at frequencies of 400 MHz for \(^1\)H and 161.97 MHz for \(^3\)P. Shimming was done on the free induction decay of the water signal. A line width at half height of 15 Hz could be routinely obtained.

\(^1\)H NMR

Selective excitation of the Mbo\(_2\) and metMb resonances at \(-2\) to \(-4\) ppm was performed by using a 13T; pulse sequence as previously described in detail.\(^{13}\) The delay for binomial H\(_2\)O suppression was set to 166 μs, resulting in maximal excitation of the region of interest. A 45-degree pulse was used; 16K transients were averaged for a typical \(^1\)H NMR spectrum requiring 15 minutes of signal accumulation (acquisition time, 42 ms; sweep width, 12136 Hz; data size, 1K; zero filling to 2K; exponential weighting resulting in a 40-Hz line broadening; chemical shifts were referenced to the H\(_2\)O resonance at 4.8 ppm).

\(^3\)P NMR

Transients (n=240) were accumulated with a 75-degree flip angle, a repetition time of 1 second, a spectral width of 5682 Hz, a data size of 4K, zero filling to 8K, and exponential weighting resulting in a 10-Hz line broadening (4 minutes of signal accumulation). Chemical shifts were referenced to the phosphocreatine (PCr) resonance at \(-2.52\) ppm.

For absolute quantification, baseline spectra were related to HPLC and SDS/PAGE data for ATP and Mb, respectively, obtained from mice hearts of each strain snap-frozen in a separate series of experiments (n=8 in each group) under basal conditions, assuming 100% NMR visibility of the particular compounds. Relative peak areas were obtained by integration after baseline correction and converted to concentrations as described before.\(^{16}\) PCr and metMb concentrations were determined from the PCr/ATP and Mbo\(_2/\)metMb ratios, respectively, measured by NMR spectroscopy. Cytosolic free ADP was derived from the creatine kinase equilibrium as previously described.\(^{16}\) The respective creatine concentrations were calculated from [Cr]=[Cr]\(_{t=0}\)−[PCr], supposing total creatine to be constant (21.3±2.3 and 20.7±2.6 mmol/L for the WT and the TGiNOS group, respectively, as determined by HPLC). Calculated values for free energy of ATP hydrolysis (ΔG\(_{\text{ATP}}\)), AMP, and intracellular pH (pHi) were derived from established relations.\(^{16}\)

#### Measurement of Adenosine

Adenosine concentration in samples of the coronary venous effluent was determined via HPLC analysis according to published protocols.\(^{16}\) Gradient elution of the samples was performed using a C\(_{18}\) reverse-phase column (150x3.9 mm particle size 10 μm, Waters), and absorbance of column eluate was continuously recorded at λ=254 nm (Linear Instruments Uvis 200).
Determination of Nitrite and Nitrate

Nitrate and nitrite levels of the coronary venous effluent were determined by chemiluminescence with an NO analyzer (NOA280, Sievers Inc). Nitrite was converted to NO by potassium iodide in acetic acid, and total NOx (NO3 and NO2) was reduced to NO with VCl3 according to the manufacturer’s instructions.

Materials

All compounds used in this study were either analytical grade or of the highest purity available. Heparin was obtained from Hoffmann-LaRoche (Grenzau, Germany). Urethane was purchased from Sigma (Deisenhofen, Germany). All other reagents were obtained from Merck (Darmstadt, Germany).

Data Analysis

All results are expressed as mean±SD. For multiple comparisons, ANOVA was applied, followed by the Bonferroni correction. Differences were assumed to be significant with a probability value of less than 0.05.

Results

Figure 2 illustrates the temporal changes in LVDP and coronary perfusion pressure (CPP) of constant volume-perfused hearts paced at 500 bpm. No significant differences between WT and TGiNOS hearts could be detected during perfusion with carbogen buffer or during perfusion with buffer gassed at 75% O2/20% N2/5% CO2. Because L-arginine, an essential substrate for NOS, was reported to be drastically reduced in cardiomyocytes of iNOS-overexpressing hearts, we supplemented the perfusion medium with in vivo–like L-arginine concentrations (200 μmol/L). As shown in Figure 2B, CPP, a measure of coronary resistance in constant volume-perfused hearts, significantly decreased in TGiNOS hearts only (100.2±6.1 versus 118.7±13.4 mm Hg in WT, n=6; P<0.05). Replacing N2 by CO elicited a marked decline in myocardial contractility of TGiNOS hearts (78.2±8.2% of baseline), but cardiac function in WT controls was unaltered (96.7±4.6% of baseline, n=6; P<0.005; Figure 2A). The subsequent intracoronary infusion of 100 μmol/L ETU completely abolished the CO-induced cardiodepression in TGiNOS hearts (97.0±3.4% of baseline), whereas ETU had no significant effect on cardiac contractility in WT hearts (99.5±2.0% of baseline).

Myocardial energy state was continuously monitored by means of 31P NMR spectroscopy; respective data are summarized in Figures 3A and 3B. Note that the initial lowering of the oxygen concentration (75% in equilibrating gas phase) did not significantly alter PCr or ATP levels of isolated hearts. Subsequent L-arginine administration did not affect cardiac energy state; however, upon additional application of CO, both PCr and ATP levels were markedly diminished in TGiNOS hearts compared with littermate controls (PCr: 13.3±1.3 versus 15.9±0.7 mmol/L; ATP: 5.6±0.4 versus 7.2±0.6 mmol/L, n=6; P<0.005; Figures 3A and 3B). This perturbation of cardiac energy status was also reflected by a significant rise of cytosolic free AMP (690.6±142.4 versus 289.6±87.0 nmol/L in WT, n=6; P<0.05) and a pronounced decrease in the free energy for ATP hydrolysis (ΔGATP: −57.3±1.5 (TGiNOS) versus −59.7±1.7 kJ/mol (WT), n=6; P<0.05). The coinfusion of 100 μmol/L ETU com-
pletely abrogated the energy depletion of TGiNOS hearts (PCr: 15.6±2.0 mmol/L; ATP: 7.3±0.3 mmol/L, n=6; Figures 3A and 3B).

$^1$H NMR spectroscopy enabled us to directly assess the tissue concentration of both [MbO$_2$] and [metMb]. As depicted in a representative recording (Figure 4A) and quantitatively illustrated in Figure 4B, supplementation of in vivo-like L-arginine concentrations caused a significant decrease of the MbO$_2$ signal in TGiNOS hearts only. Concomitantly, a new signal emerged at $\sim$3.8 ppm (Figure 4A), which could be unequivocally assigned to metMb by comparison with published data.$^{13,20}$ From the data summarized in Figure 4B, it is evident that in TGiNOS hearts the MbO$_2$ level decreased by the same extent as did the metMb concentration increase ($\Delta$MbO$_2$: $-46.3\pm38.4$ μmol/kg; $\Delta$metMb: $+41.4\pm17.6$ μmol/kg, n=6; $P$,0.05). When NOS was inhibited with 100 μmol/L ETU, neither a decrease in MbO$_2$ (209.5±36.7 versus 201.2±29.7 μmol/kg under control conditions) nor an increase in metMb levels (0.6±0.3 versus 0.5±0.4 μmol/kg under baseline conditions) could be detected. In WT controls, the metMb signal was not detectable under any condition (Figure 4A). Perfusion with CO-containing medium resulted in the appearance of the MbCO signal at $-2.3$ ppm, whereas no signals for MbO$_2$ and metMb were detectable, demonstrating the complete inhibition of Mb (Figure 4). As in previous experiments, lowering PO$_2$ to 75% in equilibrating gas phase did not result in Mb desaturation.$^{21}$

To further explore cardiac NO catabolism, we measured the two major oxidation products of NO, nitrate (NO$_3^-$) and nitrite (NO$_2^-$) in the coronary venous effluent. In the presence of L-arginine, nitrate release by TGiNOS hearts was dramatically elevated (30.5±10.2 nmol · min$^{-1}$ · g$^{-1}$, n=6; $P$,0.005) above background (5.6±2.3 nmol · min$^{-1}$ · g$^{-1}$; Figure 5A). In contrast, nitrate release by WT hearts did not increase under all conditions tested. As illustrated in Figure 5B, a 7-fold rise in nitrite release by TGiNOS hearts during perfusion with CO was detectable whereas nitrite release by WT hearts remained unchanged (0.47±0.12 nmol · min$^{-1}$ · g$^{-1}$ (TGiNOS) versus 0.03±0.01 nmol · min$^{-1}$ · g$^{-1}$ (WT), n=6; $P$,0.005). Because it is well known that adenosine formation serves as a sensitive indicator for even small perturbations of cardiac energetic homeostasis,$^{22}$ we have also measured the release of this nucleoside into the coronary venous effluent. In TGiNOS hearts, adenosine release was only elevated in the presence of CO, whereas no changes were observed in WT hearts (2.7±0.4 versus 1.0±0.3 nmol · min$^{-1}$ · g$^{-1}$, n=6; $P$,0.005, Figure 5B).

**Discussion**

We have previously reported that cardiac-specific iNOS overexpression in a transgenic mouse model (TGiNOS) results in massive formation of endogenous NO. Surprisingly, this does not result in severe cardiac dysfunction.$^{12}$ We now provide experimental evidence that the maintenance of car-
Cardiac function is ensured by the continuous degradation of NO by MbO₂ to nitrate and metMb. Thus, the presence of Mb is crucial for the detoxification of NO in cardiac muscle and thereby determines the functional consequences of iNOS overexpression.

Cardiac-specific iNOS overexpression under control of the α-MHC promoter resulted in a homogenous expression of iNOS in the cytosol. In vivo iNOS activity was increased at least 40-fold compared with WT hearts. In the present study, we have supplemented the perfusion medium with in vivo-like L-arginine concentrations (200 μmol/L), because extracellular arginine was found to be necessary for iNOS activity in view of drastically reduced intracellular levels of this amino acid in TGiNOS hearts. Consequently, normal iNOS activity is reflected by the substantial release of the NO oxidation product NO₃⁻ to coronary effluent (Figure 5). This endogenous NO generation caused pronounced vasodilation in transgenic hearts without altering cardiac contractility or energetics, which is consistent with our previous observation. ¹¹H NMR spectroscopy of TGiNOS hearts revealed that the increased release of NO was paralleled by the appearance of the metMb signal at −2.38 ppm whereas the MbO₂ signal at −2.7 ppm was decreased by the same extent. These changes were completely abolished by the NOS inhibitor ETU, which demonstrates the specificity of the observed changes. It is important to note that under these experimental conditions, NO released from transgenic hearts was found to be almost completely converted to nitrate, which is the reaction product of NO with transition metals or oxyhemoproteins like MbO₂. Because hearts were perfused with a saline hemoglobin-free medium, Mb appears to be the most likely candidate for intracellular NO degradation. The emerging metMb signal together with the markedly increased NO₃⁻ release provides strong evidence for an Mb-mediated response.

Figure 4. A, Representative ¹¹H NMR spectra of WT and TGiNOS hearts showing L-arginine–induced alterations in cardiac Mb metabolism. B, Illustration of the quantitative changes of [MbO₂] and [metMb] in isolated hearts of TGiNOS mice detected by ¹¹H NMR spectroscopy. Note: during myoglobin blockade by 20% carbon monoxide, neither MbO₂ nor metMb was detectable (n.d.). Values are mean±SD, n=6. *P<0.05. MbO₂ indicates oxymyoglobin; metMb, metmyoglobin; and MbCO, carboxymyoglobin.
oxidative NO breakdown by reaction of MbO2 to metMb (Figure 6). Thereby, the NO scavenging by Mb attenuates the effect of the increased intracellular NO formation on cardiac function and energetics in TGiNOS hearts.

Further support for this hypothesis can be drawn from experiments in which cardiac Mb was acutely inhibited by CO. Because CO binds more tightly to the heme group of Mb than O2 does,24 it displaces O2 at the prosthetic group and prevents the reaction between MbO2 and NO. Hence, the pharmacological intervention with CO blocked the rapid Mb-mediated NO degradation, resulting in higher levels of bioactive NO, which was associated with a marked impairment of cardiac contractile force. Again, ETU completely antagonized both the functional and energetic impairment of TGiNOS hearts, which supports the notion that NO accounts for the observed alterations.

The adverse effect of high cardiac levels of exogenously applied18 or endogenously formed13,25 NO has been demonstrated in several studies. The underlying mechanisms for the negative inotropic effect of NO are still a matter of debate, and diverse reactions have been implicated to mediate this effect. It has been shown that NO interacts with the mitochondrial electron transport chain, myocardial creatine kinase, and soluble guanylate cyclase, all of which have been described to contribute to NO-mediated cardiac impairment.25–29 In our experiments, the significant fall in energy content of TGiNOS hearts during Mb blockade suggests NO interference with the heme enzymes of respiratory chain as a
major determinant in NO-mediated cardiodepression. Chemically, NO closely resembles O2, and it has been repeatedly shown that by competitive interaction with molecular oxygen NO is capable to inhibit cell respiration reversibly, thereby impairing bioenergetics (see review3). In the latter scenario, NO may also increase leakage of superoxide from the electron transport chain to promote the formation of peroxynitrite, which in a recent study was demonstrated to be a major contributor to cytokine-induced myocardial dysfunction.30 It should be noted, however, that in contrast to NO-induced effects, peroxynitrite-mediated alterations are largely irreversible.31,32 Considering the functional and energetic recovery of TGiNOS hearts during ETU application, peroxynitrite is rather unlikely to mediate the described changes in contractility and energetics.

The almost complete conversion of NO to nitrate in iNOS-overexpressing hearts reflects the substantial capacity of Mb for NO degradation. When exposing these hearts to CO, thereby blocking the Mb breakdown cycle, the 7-fold increase in nitrite release most likely reflects the increased half-life of bioactive NO within the heart, because the degradation of NO to NO2 by reaction with physically dissolved oxygen (Figure 6) has been shown to be at least 20-fold slower than the Mb-mediated NO breakdown.33,34 However, the unchanged nitrate release of CO-perfused TGiNOS hearts demonstrates that NO3− is still the major degradation product of NO, which may be attributed to the interaction of NO with other oxyhemoproteins such as cytochromes, which are abundantly present in the mitochondria of the cell.

A limitation of the present study is that all experiments have been performed on isolated hearts. However, we deliberately used this reductionist approach and perfused hearts without red blood cells to exclude interfering side reactions between nitric oxide and hemoglobin. Only by this experimental setup were we able to exclusively study the specific role of myoglobin within iNOS-overexpressing hearts. Intrinsically, this ex vivo approach cannot exclude that additional NO-neutralizing mechanisms may exist in the blood-perfused heart that are lacking in the saline-perfused preparation.

Recently, Mungrue et al35 reported a mouse model for conditional cardiac-specific iNOS expression using a tetracycline-responsive transcriptional activator to be associated with a high incidence of sudden cardiac death together with cardiac fibrosis, hypertrophy, and dilation. These findings are in sharp contrast to the benign cardiac phenotype of our mouse model with massive cardiac iNOS overexpression. In vitro, iNOS activity in our transgenic model is approximately 100- to 120-fold higher than the induced activity in the conditional model. This is accompanied by elevated cardiac and systemic NO3− levels, indicating elevated NOS activity in vivo. It is noteworthy that Mungrue et al were unable to measure any of these changes and therefore failed to provide biochemical evidence of iNOS activity in vivo. Because, in our view, NO can be ruled out to explain these discrepant findings, the pronounced cardiac phenotype of the conditional model could be due to transactivator-induced alterations of cardiac gene expression36 or caused by the reporter gene (LacZ) coexpression.

In the present and our previous study, enhanced iNOS activity in TGiNOS hearts was characterized by a 6-fold increase in nitrate release from the isolated heart whereas plasma NO3− concentration was doubled compared with WT hearts. Because in several experimental models of severe cardiac dysfunction the changes in plasma NO3− levels were reported to be lower or in the same range,37,38 the preservation of the contractile performance in TGiNOS hearts is particularly striking. In our transgenic mouse model, both iNOS and myoglobin are localized in the cytosolic compartment so that the NO synthesized can be immediately oxidized by the avid reaction with oxyhemoglobin and potential targets are protected. In this context, the reduction of contractility observed in eNOS-overexpressing hearts39 is most likely related to the close proximity of eNOS and important effector molecules such as the β-adrenergic receptor or the L-type Ca2+ channel at the plasma membrane. Colocalization of distinct NOS isoforms with different target structures may therefore critically determine the resulting functional effects.40 It is therefore tempting to speculate that because of the high capacity of Mb to inactivate NO, it prevents spillover of NO from one
compartment to another, thereby resulting in a more localized action of NO.

In summary, our results reveal that Mb-mediated NO degradation is a crucial factor that determines the benign phenotype of iNOS-overexpressing hearts. Considering the large amount of Mb in the heart (∼200 μmol/kg wet weight4,13), Mb can account for substantial cytosolic NO buffering. Therefore, Mb may be regarded as a molecular firewall (Figure 6) protecting NO-sensitive cell components (eg, mitochondrial cytochromes) against potentially noxious consequences of increased endogenous NO production.

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